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Marie Guérin

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Caractérisation et mise en évidence des effets fonctionnels de la consommation de fruits tropicaux lactofermentés

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Résumé

La fermentation lactique est une méthode qui améliore la conservation des fruits et à un effet sur leurs propriétés sensorielles et nutritionnelles. La littérature indique une augmentation ou un maintien de l'activité antioxydante pendant la fermentation, contrairement à d'autres procédés de conservation. Cette observation peut s'expliquer par la libération de composés phénoliques, mais aussi par la production d'exopolysaccharides (EPS). Cette thèse visait à caractériser les modifications de composition des fruits résultant de la fermentation lactique et à identifier les effets fonctionnels d'une alimentation enrichie en fruits tropicaux fermentés. Ananas, mangue et papaye, sous forme de jus ou de purée, ont été fermentés avec différentes souches de *Lactiplantibacillus plantarum*, *Weissella cibaria* ou *Leuconostoc pseudomesenteroides*, puis conservés plusieurs semaines. Ces trois fruits diffèrent dans leur composition en mono et disaccharides, acides organiques, vitamine C, composés phénoliques et caroténoïdes. La mangue, peu acide (pH 4,2), voit son pH diminuer significativement pendant la fermentation puis la conservation, du fait de la consommation de glucose et fructose au profit d'une production des acides lactique et acétique. La papaye voit son pH diminuer significativement de 4,9 à 3,4 pendant la fermentation, sans que des changements notables de composition en sucres simples soient observés. En revanche, le glucose et le fructose de l'ananas, dont le pH est de 3,4, sont nettement consommés pendant la fermentation. Selon les souches, la production est orientée vers le lactate ou un mélange lactate-acétate. La consommation des acides organiques, citrate et malate, est notable. Une orientation du flux carboné vers l'acétate et probablement ses dérivés tels l'acétoïne est observée. *Ltp. plantarum* 17a et *W. cibaria* 21 et 64 sont favorables à une meilleure préservation de la vitamine C. L'activité antioxydante mesurée par le test du DPPH est plus élevée pour l'ananas et la papaye par comparaison à la mangue. Sa dégradation évolue de la même façon pour la purée de papaye non fermentée et fermentée au cours de la conservation. La formation d'EPS est fortement dépendante du fruit fermenté et de la souche utilisée. Les EPS ne pouvant être séparés des fibres des fruits en milieu liquide, leur quantification directement dans les fruits n'est pas possible. Pour pouvoir disposer de données quantitatives, une méthode a été mise au point en milieu solide. Une différence de production d'un facteur 5 selon la souche a été montrée. Sur ananas, la production d'EPS est stimulée par l'ajout de saccharose, alors que sur mangue, elle est augmentée à un pH de 6. Toutefois, d'autres facteurs interviennent puisque la production d'EPS reste difficile à induire lors de la fermentation de papaye. La fonctionnalité du jus d'ananas lactofermenté a été évaluée *in vivo* chez la souris (modèle « high-fat diet », HFD). La prise de poids en six semaines a été plus élevée lorsque de l'eau sucrée était proposée, en lien avec une augmentation du volume ingéré, par rapport à un jus fermenté ou non. La glycémie et la cholestérolémie étaient négativement impactées chez les souris HFD ayant pris de l'eau sucrée enrichie en bactéries ou du jus non fermenté, comparativement au témoin (eau), et contrairement au jus fermenté. Si la perméabilité intestinale ne semblait pas affectée, l'équilibre des flores lactiques du microbiote intestinal était modulé par l'ingestion de jus fermenté par rapport à de l'eau. Au plan métabolique, les souris HFD ayant pris du jus d'ananas fermenté montrent très peu de différences avec les individus témoins ayant consommé de l'eau. L'ingestion de jus d'ananas fermenté permet de limiter les effets métaboliques délétères du régime HFD, contrairement à de l'eau sucrée, avec ou sans bactéries ajoutées. Ces travaux montrent que la production d'EPS au cours de la fermentation ne peut expliquer les effets fonctionnels, mais ouvrent des perspectives pour optimiser la production des EPS à partir de fruits.

Mots clés : bactéries lactiques, ananas, mangue, papaye, exopolysaccharides, métabolisme énergétique.

Abstract

Lactic acid fermentation (LAF) improves sensory quality, safety, and nutritional properties of fruits. LAF maintains or increases antioxidant activity, contrarily to other food transformation process. This observation can be explained by the release of phenolic compounds, but also by the production of exopolysaccharides (EPS). This thesis aimed to characterize the modifications of fruit composition resulting from LAF and to identify functional effects on health of a diet enriched with fermented tropical fruits. Three fruit, pineapple, mango and papaya, were fermented with different strains of *Lactiplantibacillus plantarum*, *Weissella cibaria* or *Leuconostoc pseudomesenteroides*, and stored for several weeks. The composition of these fruit differs for mono and disaccharides, organic acids, vitamin C, phenolic compounds and carotenoids. During fermentation and subsequent storage, the pH of the low acidic mango fruit (pH 4.2) decreased. Glucose and fructose were consumed, leading to the production of both lactic and acetic acid. Papaya fruit pH was reduced from 4.9 to 3.4 during LAF, but we could not detect major changes in sugar composition. During LAF of pineapple juice, whose pH is 3.4, glucose and fructose were converted into lactate or a mix lactate-acetate in a strain-dependent way. Consumption of the two organic acids citrate and malate was significant. A rerouting of carbon flux towards acetate, and probably derived compounds such as acetoin, was observed. Vitamin C was better preserved when fruit were fermented with the strains *Ltp. plantarum* 17a or *W. cibaria* 21 or 64. The antioxidant activity measured by the DPPH test was higher for pineapple and papaya compared to mango. DPPH reduction evolved in the same way for unfermented and fermented papaya purees during storage. EPS production was strongly dependent on the fermented fruit and the strain used. As EPS cannot be separated from dietary fibers from fruit for analytical purposes, a method was developed to quantify EPS production. The method relies on the use of fruit-based media solidified with agar before recovery of EPS from bacteria grown on its surface. Thank to this quantification method, a 5-fold difference of EPS production level was shown depending on the strain. Addition of sucrose to pineapple stimulated EPS production, whereas a similar effect was observed on mango when pH was increased to 6. However, other unknown factors limited EPS production on fruit, since EPS production on papaya purée was difficult to induce. The functional effects of lactofermented pineapple juice ingestion were studied in mice fed a high-fat diet (HFD). As expected, weight gain over six weeks was higher, in association with an increase of ingested volume, when sweetened water was proposed to mice, compared to fermented or unfermented juice. A negative effect on blood glucose and blood cholesterol was observed for HFD mice fed with the bacterium enriched sweetened water or unfermented juice, compared to the control (water), and in contrast to the fermented juice. While no change of intestinal permeability was detected, lactic acid bacterium composition of intestinal microbiota was modulated when fermented juice was proposed instead of water. At the metabolic level, HFD mice that ingested fermented pineapple juice showed very few differences with the control HFD animals that consumed water. Metabolic disorders induced by HFD were not increased with fermented pineapple juice ingestion contrarily to sweetened water, with or without added bacteria. This work shows that functional effects cannot be attributed to the production of EPS during fermentation but opens perspectives to optimize EPS production from fruit.

Keywords: lactic acid bacteria, pineapple, mango, papaya, exopolysaccharides, energetic metabolism.

Publications et communications scientifiques

Publication d'articles de revue

Garcia, C.; **Guérin, M.**; Souidi, K.; Remize, F. Lactic fermented fruit or vegetable juices: Past, present and future. *Beverages*, **2020**, 6, 8.

Guérin, M.; Silva, C. R.-D.; Garcia, C.; Remize, F. Lactic acid bacterial production of exopolysaccharides from fruit and vegetables and associated benefits. *Fermentation*, **2020**, 6(4), 115.

Nabot, M.; **Guérin, M.**; Sivakumar, D.; Remize, F.; Garcia, C. Variability of bacterial homopolysaccharide production and properties during food processing. *Biology*, **2022**, 11, 171.

Publication des travaux de recherche

Seke, F.; Manhivi, V.E.; **Guérin, M.**; Shoko, T.; Akinola, S.A.; Garcia, C.; Remize, F.; Slabbert, R.M.; Sivakumar, D. Antioxidant activities of co-encapsulated Natal plum (*Carissa macrocarpa*) juice inoculated with *Ltp. plantarum* 75 in different biopolymeric matrices after *in vitro* digestion. *Foods*, **2022**, 11, 2116.

Guérin, M.; Remize, F.; Bringart, M.; Planesse, C.; Rondeau, P.; Robert-Da Silva, C.; Garcia, C. Effect of pineapple fermented juice consumption on energetic metabolism of mice. Soumis à publication dans *Molecular Nutrition and Food Research* le 05 octobre 2022.

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Liste des abréviations

ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
APRIFEL	Agence Pour la Recherche et l'Information en Fruits Et Légumes
ARS	Agence Régionale de Santé
ATP	Adénosine 5'-triphosphate
AVC	Accident Vasculaire Cérébral
B.	<i>Bifidobacterium</i>
CAE	Citric Acid Equivalent
CFU	Colony Forming Unit
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CYROI	Cyclotron Réunion Océan Indien
DAAF	Direction de l'Alimentation, de l'Agriculture et de la Forêt
DPPH	2,2-diphényl-1-picrylhydrazyl
e.g.	Par exemple
EPS	Exopolysaccharides
FAO	Food and Agriculture Organization of the United Nations
FDGDON	Fédération Départementale des Groupements de Défense contre les Organismes Nuisibles
FW	Fresh Weight
GAE	Gallic Acid Equivalent
GRAS	Generally Recognized As Safe
HePS	Hétéropolysaccharides
HFD	High-Fat Diet
HoPS	Homopolysaccharides
HPLC	High Performance Liquid Chromatography
i.e.	C'est-à-dire
INSERM	Institut National de la Santé et de la Recherche Médicale
kDa	Kilodalton
L.	<i>Lactococcus</i>
LAB	Lactic Acid Bacteria
LAF	Lactic Acid Fermentation
Lb.	<i>Lactobacillus</i>
Lc.	<i>Leuconostoc</i>
Lcb.	<i>Lactocaseibacillus</i>
Ltp.	<i>Lactiplantibacillus</i>
Lvb.	<i>Levilactobacillus</i>
MF	Matière Fraîche
MRS	De Man, Rogosa and Sharpe
MS	Matière Sèche
MSS	Matière Sèche Soluble
OMS	Organisation Mondiale de la Santé
ONUAA	Organisation des Nations Unies pour l'Alimentation et l'Agriculture

ORS	Observatoire Régional de la Santé
P.	<i>Pediococcus</i>
PNNS	Plan National Nutrition Santé
ROS	Reactive Oxygen Species (espèces réactives de l'oxygène)
TA	Titratable Acidity
TCC	Total Carotenoid Content
TMS	Tétraméthylsilane
TSS	Total Soluble Solids
UFC	Unité Formant Colonie
UMR	Unité Mixte de Recherche
UPLC	Ultra Performance Liquid Chromatography
W.	<i>Weissella</i>
WHO	World Health Organization

Préambule

Ces travaux de thèse ont pu être réalisés grâce à l'obtention d'une Allocation Régionale de Recherche (ARR) financée par la Région Réunion et grâce au fonds européen de développement régional (FEDER PO 2014-2020).

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En complément, une mission d'une durée d'un mois a été effectuée au sein du laboratoire du Pr. Dharini SIVAKUMAR à la Tshwane University of Technology (TUT) située à Pretoria en Afrique du Sud. Cette mobilité a été financée par le PHC-Protea 2019-2020 et réalisée grâce au programme Erasmus +. L'objectif de cette mobilité était d'étudier l'effet de la co-encapsulation de composés bioactifs issus d'un jus de prune de Natal enrichi avec différents biopolymères et contenant ou non des bactéries lactiques.

INTRODUCTION GENERALE

Les politiques de santé publique, au travers du Plan National Nutrition Santé (PNNS) notamment, préconisent une consommation augmentée de fruits et légumes pour contribuer à lutter contre l'apparition de l'obésité et des maladies métaboliques. L'obésité est reconnue comme une maladie chronique depuis 1997 par l'Organisation Mondiale de la Santé (OMS) et est associée à différentes anomalies métaboliques telles qu'une intolérance au glucose, une résistance à l'insuline, une inflammation, une dyslipidémie, une hypertension artérielle au sein du syndrome métabolique (Scheen & Van Gaal, 2005). Ces anomalies constituent un risque cardiovasculaire majeur. La région Réunion est particulièrement affectée par les problèmes d'obésité et de maladies métaboliques associées. En 2019 à La Réunion, 44 % de la population de l'île est en surcharge pondérale et 16 % est en situation d'obésité (ORS Réunion, 2022). Concernant les maladies métaboliques, 10 % des Réunionnais de 15 ans et plus sont diabétiques, contre 7 % en Métropole. Les données obtenues par standardisation par âge et par sexe de la population française montrent que la proportion de patients pris en charge est deux fois plus élevée au niveau régional qu'au niveau national. La Réunion est de ce fait la région française la plus concernée par le diabète pris en charge (ORS Réunion, 2022). La lutte contre l'obésité et les pathologies associées est ainsi devenue une priorité de santé publique pour l'Agence Régionale de Santé (ARS Réunion).

En 2019 à La Réunion, les taux de couverture en produits frais sont de 71 % pour les légumes et 60 % pour les fruits et la production en fruits et légumes couvre environ 50 % du marché alimentaire comprenant les produits frais, transformés et congelés (ORS Réunion, 2022). D'après l'Observatoire Régional de la Santé (ORS), moins de 10 % de la population consomme au moins cinq portions de fruits et légumes à La Réunion en 2019, soit deux fois moins que la population métropolitaine (ORS Réunion, 2022). L'élaboration de produits alimentaires innovants à base de fruits et légumes pourrait être un vecteur efficace pour favoriser leur consommation.

La composition nutritionnelle des fruits et légumes leur confère des effets bénéfiques liés à la présence de fibres, micronutriments, minéraux et composés phytochimiques tels que les vitamines, les caroténoïdes, les composés phénoliques et les phytostérols. La composition des fruits tropicaux présents sur l'île de La Réunion est relativement bien documentée. Les fruits apparaissent comme une ressource riche en micronutriments aux capacités antioxydantes et anti-inflammatoires comme les polyphénols et les caroténoïdes (dos Santos Pereira et al., 2018; Godoy & Amaya, 1995; Septembre-Malaterre et al., 2016; Zhang et al., 2015). Ces composés phytochimiques sont capables de moduler les processus métaboliques et sont reconnus pour leur effet protecteur envers les maladies chroniques, en particulier les pathologies cardiovasculaires et métaboliques (Masibo & Qian, 2008). Toutefois, les fruits présentent des teneurs en sucres simples élevées, ce qui contrebalance partiellement leurs effets bénéfiques pour la santé. Au niveau intestinal, la consommation de fruits et légumes, riches en fibres et glucides résistants aux enzymes digestives humaines mais métabolisables par les bactéries du côlon, favorise la croissance de certaines espèces bactériennes. Ainsi, la consommation d'un régime strictement végétarien ou l'ajout de fibres au régime alimentaire conduit à une modification de la composition du microbiote (Senghor et al., 2018).

La fermentation lactique permet de prolonger la durée de conservation des aliments tout en améliorant la sûreté sanitaire par inhibition de la croissance de micro-organismes pathogènes (Di Cagno et al., 2011). Lors de la fermentation, les bactéries lactiques vont utiliser les sucres et acides organiques du substrat comme source de carbone pour leur développement, modulant l'acidité du

milieu et réduisant la teneur en sucres de la matrice (Mousavi et al., 2011). L'utilisation de « starters », ou bactéries sélectionnées et ajoutées pour initier la fermentation, permet d'orienter la qualité du produit obtenu.

La fermentation lactique présente aussi des bénéfices potentiels sur la santé par l'effet de l'apport d'organismes probiotiques sur le microbiote. L'OMS définit les probiotiques comme des micro-organismes vivants ayant des effets bénéfiques sur la santé de l'hôte quand ils sont ingérés en proportion adéquate. Ils semblent jouer un rôle dans les fonctions immunologiques, digestives et respiratoires. Outre un effet des bactéries vivantes, certaines souches de bactéries lactiques vont pouvoir synthétiser pendant la fermentation des molécules à activité antioxydante, parmi lesquelles les exopolysaccharides (EPS) (Hur et al., 2014). Ces composés vont également contribuer à augmenter la viscosité des aliments (Di Cagno et al., 2011). Ils vont ainsi améliorer leurs propriétés rhéologiques et texturales (Karovičová & Kohajdová, 2003). Des données de la littérature montrent aussi que la fermentation lactique de légumes entraîne une dépolymérisation ou une libération des composés phénoliques bioactifs et donc une amélioration de l'activité antioxydante (Hur et al., 2014). Ainsi, l'exploitation de la fonction de biopréservation des bactéries lactiques peut contribuer à favoriser la consommation de fruits tropicaux par la population, tout en conservant ou en améliorant les propriétés nutritionnelles et sensorielles des produits (**Figure 1**) (Fessard et al., 2017; Swain et al., 2014).

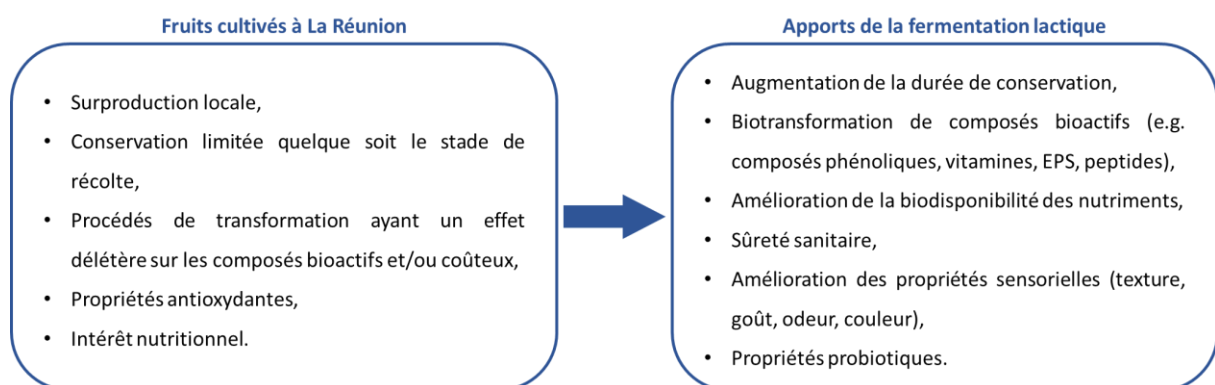


Figure 1 : Avantages de la fermentation lactique des fruits cultivés à La Réunion.

La fermentation lactique de fruits tropicaux est une voie à explorer pour diversifier l'offre de produits alimentaires d'origine végétale, dans un contexte d'amélioration des bénéfices nutritionnels. D'après ces éléments, cette thèse s'articule autour de deux principaux objectifs :

- (1) Explorer la formation de composés d'intérêt nutritionnel, dont les EPS, pendant la fermentation lactique ;
- (2) Préciser l'impact sur la santé de la consommation de fruits tropicaux lactofermentés.

La perspective à l'échelle du territoire serait le développement de produits lactofermentés afin de proposer des produits innovants et d'améliorer la qualité de l'offre alimentaire. Cette thèse constitue une opportunité d'ouvrir de nouvelles voies de valorisation des ressources naturelles de l'île par l'utilisation d'espèces bactériennes isolées à partir de fruits et légumes de La Réunion pour la fermentation de fruits locaux, à savoir l'ananas Queen Victoria, la mangue Cogshall et la papaye Solo. Outre le bénéfice potentiel en termes de santé pour la population réunionnaise, le développement d'activités de transformation alimentaire sur l'île pourrait contribuer à la dynamique économique. Un

accroissement de la transformation des fruits produits localement contribuerait également à réduire les pertes en fruits lors des pics de production.

Dans un premier temps, une étude bibliographique permettra d'argumenter quant à la démarche de ces travaux de thèse et aux hypothèses posées. Dans un deuxième temps, l'étude expérimentale exposera la méthodologie scientifique choisie et les résultats obtenus. Dans un troisième temps, l'ensemble du travail de thèse et les perspectives envisagées seront discutés.

Étude bibliographique

PARTIE 1 : Valorisation des fruits tropicaux et intérêt de la fermentation lactique

1.1. Les fruits tropicaux de La Réunion

1.1.1. La production fruitière

La production fruitière à La Réunion était estimée en 2018 à 35 100 tonnes dont 14 300 tonnes d'ananas et 1 191 tonnes de mangue (**Figure 2**) (DAAF, 2019). En 2020, l'île exportait 3 486 tonnes de fruits dont 2 080 tonnes d'ananas et 1 272 tonnes réparties entre la mangue, le fruit de la passion et le litchi. La production fruitière locale couvre 58 % du marché du frais et 43 % du marché global, i.e. fruits frais, transformés, congelés (Agreste La Réunion, 2021).

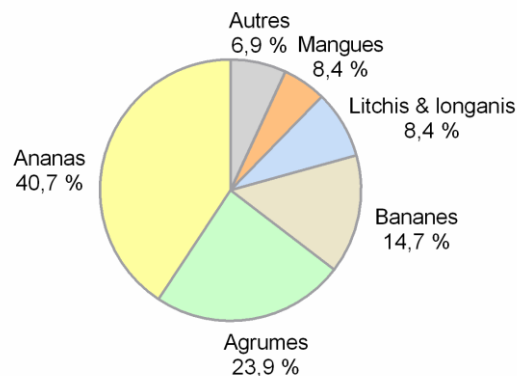


Figure 2 : Production fruitière en 2018 à La Réunion (d'après les données publiées par DAAF, 2019).

L'ananas (*Ananas comosus*) est un des fruits les plus consommés dans le monde. Sa production est la plus importante au Costa Rica, au Brésil, en Thaïlande, aux Philippines, en Chine et en Inde. À La Réunion, la variété d'ananas Queen Victoria est majoritairement produite bien que la variété Maingard soit également présente (Aubert, 1976). L'ananas est un fruit non climactérique, qui doit donc être récolté à maturité. Les ananas sont essentiellement consommés comme produit frais, prédécoupés et en jus. Les fruits de la variété Queen Victoria ont une saveur sucrée appréciée par les consommateurs. Bien que l'ananas puisse être cultivé toute l'année, les variations climatiques liées à la période de production influencent les constituants du fruit (Awasthi et al., 2022). Les jus d'ananas sont caractérisés par un pH de 3,5 en moyenne. Cette acidité inhibe la croissance de bactéries pathogènes, e.g. *Salmonella* ou *Escherichia coli* (Leneveu-Jenvrin et al., 2020). La localisation des cultures d'ananas à La Réunion et le climat influencent le pH et l'acidité titrable du jus de fruit obtenu. C'est ainsi que le pH des jus d'ananas est plus élevé pour les fruits récoltés dans le Nord et dans le Sud de l'île par comparaison avec l'Ouest et l'Est. D'autres facteurs impactent le pH des fruits parmi lesquels un fort rayonnement solaire, des températures chaudes et une évapotranspiration élevée (Leneveu-Jenvrin et al., 2020).

Les vergers de mangue (*Mangifera indica* L.) s'étendent du Nord-Ouest au Sud de La Réunion en passant par la côte Ouest. Ces zones présentent un climat plus favorable à leur production. Cette culture est répartie sur approximativement 329 hectares et concerne près de 80 exploitations. La production de mangue est la cinquième production fruitière la plus importante de l'île. Sur le marché réunionnais, la mangue Cogshall ou « mangue Américaine » et la mangue José représentent 80 % de

la production locale de mangue (Vincenot & Normand, 2009). Une cinquantaine de variétés sont néanmoins retrouvées à La Réunion, parmi lesquelles Caro, Carotte, Heidi, Kensington Pride, Lise, Maison Rouge, Nam Doc Mai, Orphée, Tommy Atkins et Violette. La récolte de ces fruits a lieu majoritairement de décembre à février (Vincenot & Normand, 2009). La période de floraison de la mangue Cogshall s'étale durant l'hiver austral, de juillet à septembre. La période de production de cette variété est qualifiée de précoce. La mangue José est caractérisée par plusieurs périodes de floraison et sa récolte peut être, par conséquent, précoce, de saison ou tardive et s'étend de novembre à avril (Vincenot & Normand, 2009). La récolte de la mangue est d'autant plus complexe que la maturité des fruits au sein d'un même arbre est hétérogène. La période de floraison et les conditions environnementales sont variables selon les branches d'un même arbre (Joas et al., 2012). L'exposition au soleil ou non lors de la croissance de la mangue Cogshall ne modifie cependant pas significativement son pH, entre 3,5 et 5,5 (Joas et al., 2012; Mashitoo et al., 2021). Le stade « point jaune » correspond au début de la crise climactérique caractérisée par une production élevée d'éthylène qui va influencer la maturation du fruit. La récolte de la mangue à ce stade permet de contrecarrer la maturation hétérogène présente au sein d'un même arbre (Joas et al., 2012).

La papaye (*Carica papaya*) Colombo et la papaye Solo sont les variétés cultivées à La Réunion. Ce fruit est principalement cultivé dans l'Ouest mais il est présent sur toute l'île à basse altitude. La papaye peut être consommée verte, en préparations salées, ou mûre. Elle atteint sa maturité lorsque sa peau initialement verte devient jaune sur environ un tiers du fruit, son pH est alors voisin de 5 (ARS Réunion, 2021; Mashitoo et al., 2021). Ce fruit est climactérique. Bien que son pic de production s'étende de janvier à avril, il est disponible toute l'année (ARS Réunion, 2021). Les données de production de papaye à La Réunion ne sont pas disponibles.

La consommation de ces fruits est contrainte par leur durée de vie courte. Les fruits frais sont des tissus vivants, sensibles aux paramètres extérieurs et aux micro-organismes causant des altérations organoleptiques et sanitaires. Les pertes de fruits s'avèrent élevées en saison et limitent les rentes générées par ces cultures (Porat et al., 2018). D'après l'Organisation des Nations Unies pour l'Alimentation et l'Agriculture (ONUAA ; Food and Agriculture Organization of the United Nations, FAO), les pertes en fruits et légumes le long de la chaîne logistique représentent entre 45 et 55 % des fruits et légumes produits dans le monde avec 23 % de perte au moment de la production, des manipulations et de la conservation post-récolte de ces denrées dans les pays développés (FAO, 2011; Porat et al., 2018).

Les cultures fruitières à La Réunion sont fortement impactées par l'invasion de différentes espèces de mouches de fruits. La mangue est particulièrement touchée suite à l'invasion par la mouche orientale des fruits (*Bactrocera dorsalis*) en avril 2017 (ANSES, 2019). D'après la Fédération Départementale des Groupements de Défense contre les Organismes Nuisibles de La Réunion (FDGDON), les pertes varient selon les zones et les variétés mais étaient de 20 % au minimum en 2018 (FDGDON Réunion, 2019). Il est possible de réduire les infections, et donc de limiter les pertes, en récoltant les fruits au stade vert-mature ou point-jaune où le taux d'infestation est de 10 % (Grechi et al., 2022). La papaye est également touchée par la mouche orientale des fruits mais de manière moins importante. Cette problématique a conduit les autorités à interdire l'export des mangues, ce qui engendre une augmentation des pertes en fruits lors des périodes de forte production. Un essor de la transformation locale permettrait de pallier cet inconvénient.

1.1.2. Composition des fruits tropicaux

1.1.2.1. Généralités

La composition des fruits tropicaux, en particulier les concentrations en sucres, acides organiques, caroténoïdes, vitamine C et polyphénols varient selon le type de fruit, la variété et l'environnement et sont détaillés dans le **Tableau 1**.

La valeur énergétique de la mangue peut être élevée, en conséquence de sa teneur en sucres. L'ananas présente une valeur énergétique moindre, résultant de sa teneur élevée en saccharose, mais supérieure à l'orange ou la pomme. C'est la papaye qui dispose de la valeur énergétique la plus faible. De plus, ce fruit est riche en vitamine C et en caroténoïdes.

1.1.2.2. Composés liés aux propriétés antioxydantes

Certains micronutriments sont reconnus pour leur rôle antioxydant et sont particulièrement présents dans certains fruits tropicaux.

1.1.2.2.1. Les caroténoïdes

Les pigments sont particulièrement présents dans les fruits tropicaux. La famille des caroténoïdes renferme des pigments allant du rouge au jaune en passant par l'orange, précurseurs de vitamines pour certains. Les caroténoïdes sont des lipides et la plupart ont une structure de type $C_{40}H_{56}O_n$ (avec n allant de 0 à 6). Ils se divisent en deux grandes classes :

- Les carotènes qui sont des caroténoïdes non-oxygénés de structure hydrocarbonée (i.e. $n = 0$),
- Les xanthophylles qui sont oxygénés (i.e. $n > 0$).

Plus de 750 caroténoïdes différents ont été identifiés et environ 40 sont consommés de manière significative, les plus abondants dans l'alimentation humaine étant le β -carotène, le lycopène, la lutéine, la β -cryptoxanthine, l' α -carotène et la zéaxanthine (Desmarchelier & Borel, 2017).

Les fruits tropicaux se révèlent plutôt riches en caroténoïdes, en particulier le fruit de la passion, la papaye ou la mangue dont la teneur en caroténoïdes dépasse les 2 mg/100 g de matière fraîche. La mangue Cogshall présente les teneurs les plus remarquables en caroténoïdes, celles-ci sont en effet près de cinq fois supérieures à celles de la mangue José. Les teneurs en caroténoïdes dans les papayes Colombo et Solo sont en revanche assez proches. Les caroténoïdes sont essentiellement sous forme de β -carotène dans ces fruits, bien que du lycopène soit aussi présent dans la papaye et de la cis/trans-violaxanthine dans la mangue Cogshall (Da Silva et al., 2014; Rosalie et al., 2018). À titre de comparaison, l'orange présente au maximum 0,5 mg de caroténoïdes pour 100 g de matière fraîche.

Tableau 1 : Données nutritionnelles de fruits transformés sous forme de morceaux, pulpe ou jus (éq. : équivalent, MF : matière fraîche, MS : matière sèche).

Fruit	Variété	Origine	Valeur énergétique (KJ/100 g MF)	Fibres alimentaires (g/100 g MF)	Sucres (g éq. glucose/100 g MF)	Saccharose (g/100 g MF)	Fructose (g/100 g MF)	Glucose (g/100 g MF)	Polyphénols totaux (mg éq. ac. gallique/100 g MF)	Flavonoïdes totaux (mg éq. quercétine /100 g MF)	Caroténoïdes (µg éq. β-carotène /100 g MF)	Vitamine C (mg éq. ac. ascorbique/100 g MF)	Références
Ananas	Queen Victoria	Réunion	304,0	1,4	14,3	9,6	2,8	2,5	33,0	2,0	52,6	28,9	(CIQUAL-ANSES, 2020a; Septembre-Malaterre et al., 2016)
	Indéterminée	Brésil							38,1			13,0	(Almeida et al., 2011)
	Perola	Brésil		0,8	6,9	5,4	0,8	0,6				24,2	(Cordenunsi et al., 2010)
	Golden Ripen	Chine			6,8	5,1	1,0	0,7					(Zhou et al., 2021)
Banane	Cavendish	Réunion		2,6	23,9				174,4	9,9	26,6	4,7	(Septembre-Malaterre et al., 2016)
Fruit de la passion	Indéterminée	Réunion			13,7				286,6	70,1	3829,2	44,4	(Septembre-Malaterre et al., 2016)
Mangue	Cogshall	Réunion		1,6	13,2				49,0	5,3	2806,8	18,2	(Septembre-Malaterre et al., 2016)
	José	Réunion	376,0		26,3	6,6	8,7	3,2	41,1	6,4	572,6	6,0	(CIQUAL-ANSES, 2020b; Septembre-Malaterre et al., 2018)
	Tommy Atkins	Brésil			6,2	4,0	0,4	0,1					(Cruz et al., 2012)
	Indéterminée	Équateur							60,0			19,0-20,0	(Vasco et al., 2008)
	16 variétés	Inde				0,0-14,0	0,1-2,2	0,5-3,0	338,7-861,4		2130,0-8030,0	20,8-56,2	(Megha et al., 2022)
Orange	Indéterminée	Indéterminée	192,0	2,7	7,6	3,5	2,1	2,0	48,9		5-237	47,5	(Aprifel, n.d.; CIQUAL-ANSES, 2020)
Papaye	Solo	Réunion		1,7	15,7				41,3	1,7	1573,4	84,9	(Septembre-Malaterre et al., 2016)
	Colombo	Réunion	169,0		7,7	< 0,2	3,6	4,1	33,4	1,5	1922,4	51,5	(CIQUAL-ANSES, 2020c; Septembre-Malaterre et al., 2018)
	Indéterminée	Brésil							53,2			8,6	(Almeida et al., 2011)
	Sel-42	Turquie			8,1	0,4	3,5	4,1	171,7			71,7	(Kelebek et al., 2015)
	Tainung	Turquie			7,2	0,5	2,9	3,7	119,8			67,3	(Kelebek et al., 2015)
Pomme	Pink Lady	Indéterminée	254,0	2,9	12,3	4,6	6,6	1,1				2,1	(CIQUAL-ANSES, 2020)
	Golden	Indéterminée	232,0	2,5	11,3	2,8	6,9	1,6				1,3	(CIQUAL-ANSES, 2020)

1.1.2.2.2. La vitamine C

La vitamine C, ou acide ascorbique, est connue pour ses propriétés antioxydantes et son rôle dans le métabolisme. Il est de coutume d'associer la vitamine C aux agrumes mais le **Tableau 1** montre que les fruits tropicaux sont également une source importante de vitamine C. La papaye réunionnaise présente une teneur en vitamine C environ 2 fois plus importante que l'orange. Ce fruit se révèle également comme ayant la teneur en vitamine C la plus élevée des fruits tropicaux présentés dans ce tableau. L'ananas et la mangue présentent aussi des teneurs en vitamine C intéressantes, dans une gamme proche de celle de l'orange, et peuvent être considérées comme étant riche en vitamine C par comparaison à la pomme. Une forte variabilité de la teneur en vitamine C est cependant observée selon l'origine et la variété de ces fruits. La teneur en vitamine C évolue aussi pendant la maturation des fruits et est généralement moins élevée pour les fruits matures, en partie parce qu'elle est impliquée en tant que coenzyme dans différentes voies de synthèse dont celles de l'éthylène, de l'oxalate et du tartrate (Maldonado-Celis et al., 2019).

1.1.2.2.3. Les composés phénoliques

Les composés phénoliques constituent une classe de micronutriments dont les propriétés antioxydantes ne sont plus à démontrer. Les propriétés antioxydantes des fruits tropicaux dépendent de leur composition en composés phénoliques. La concentration et la composition varient selon le degré de maturité et la variété végétale (**Tableau 1**), le climat, la nature du sol, la situation géographique avec l'influence du climat et les conditions de stockage (Haminiuk et al., 2012). Les composés phénoliques sont classés en deux familles selon leur structure chimique (**Figure 3**) (Lopez-Corona et al., 2022) :

- Les flavonoïdes ont deux noyaux phényles liés à un noyau pyrane. Cette famille est composée de six sous-familles, i.e. les flavanones, les isoflavones, les flavanols, les anthocyanidines, les flavonoles et les flavones qui diffèrent par la structure du cycle central.
- Les non-flavonoïdes comprennent quatre sous-familles, i.e. les coumarines, les stilbènes, les tannins (hydrolysables, complexes ou condensés/proanthocyanidines) et les acides phénoliques (hydroxybenzoïques, hydroxycinnamiques, hydrohyphenylacétiques).

Les flavonoïdes sont les principaux composés bioactifs contenus dans les fruits. Ils peuvent être ou non associés à un ou plusieurs sucres. Leur capacité antioxydante résulte de leur degré de glycosylation. Plus la chaîne glucidique est longue, plus leur capacité antioxydante va être réduite. Les flavonoïdes sont principalement retrouvés sous forme de glycosides mais sont également présents sous forme d'esters et plus rarement sous forme libre (Haminiuk et al., 2012). Les acides phénoliques font également partie des composés phénoliques alimentaires majoritaires. Ils sont présents sous forme liée et possèdent un groupe carboxylique. Ils s'organisent en deux classes : les acides hydroxybenzoïques, e.g. les acides gallique, vanillique, ellagique et syringique, et les acides hydroxycinnamiques, e.g. l'acide férulique, l'acide *p*-coumarique et l'acide caféique. (Haminiuk et al., 2012). Ces composés phénoliques sont bénéfiques lorsqu'ils sont ingérés selon leur biodisponibilité.

Leur biodisponibilité va diminuer si leur structure est celle d'un polymère à haut poids moléculaire et augmenter s'ils sont présents sous forme d'une molécule phénolique simple (Haminiuk et al., 2012).

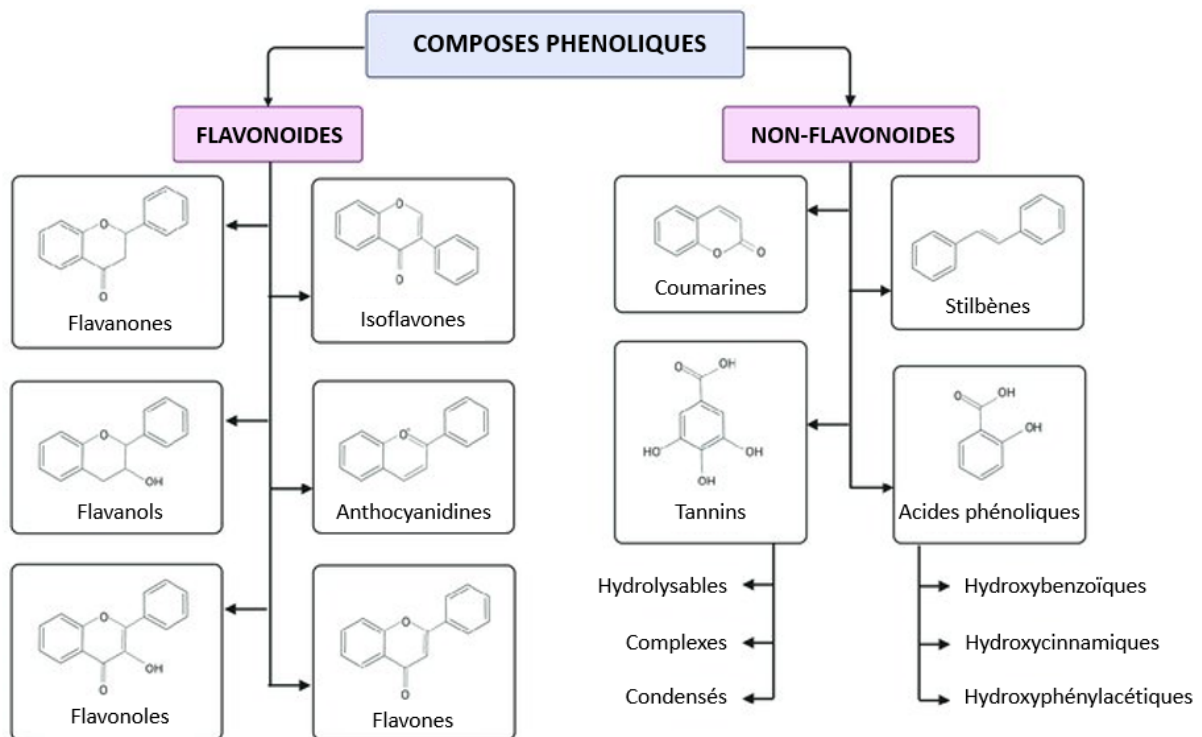


Figure 3 : Classification des familles de composés phénoliques (adapté de Lopez-Corona et al., 2022).

Au niveau des fruits réunionnais, la concentration en polyphénols totaux est équivalente pour l'ananas Queen Victoria et la papaye Solo tandis qu'elle est 1,5 fois plus élevée dans la mangue Cogshall. Au-delà de ces variations quantitatives, la structure des composés phénoliques présents dans ces fruits diffère.

Les composés phénoliques majoritaires de la mangue regroupent des composés appartenant aux classes des flavonoïdes (catéchines, quercétine, kaempférol, rhamnétine, anthocyanine) et des non-flavonoïdes (mangiférine et acides hydroxybenzoïques (acides gallique, protocatechique, benzoïque, allagique, gallate de propyle et méthyle) (Masibo & Qian, 2008). Des composés appartenant à la classe des flavonoïdes (rutine) et des non-flavonoïdes (acides hydroxycinnamiques (acides férulique, caféique, et *p*-coumarique) et acide *p*-hydroxybenzoïque)) sont aussi retrouvés dans la papaye (Dotto & Abihudi, 2021; Gayosso-García Sancho et al., 2011; Rivera-Pastrana et al., 2010). L'ananas contient principalement des acides phénoliques (acides *p*-coumarique, férulique et caféique) (Steingass et al., 2015).

Dans les fruits, les polyphénols sont retrouvés en proportion variable dans la peau, la pulpe et les graines. Une forte diminution de la concentration en flavonoïdes est observée après retrait de la peau, cette partie contenant une importante proportion de ces composés (Haminiuk et al., 2012).

1.1.2.3. Composés liés aux propriétés sensorielles

Bien que leur teneur en protéines et lipides ne soit pas nulle, les fruits sont connus pour être riches en sucres, ces derniers étant à l'origine de la valeur énergétique élevée de ces aliments et leur conférant une saveur particulièrement sucrée. Les fruits de La Réunion ne dérogent pas à la règle et présentent une concentration en glucides particulièrement élevée comparé aux mêmes fruits produits dans d'autres régions du monde et comparé aux deux fruits les plus consommés au niveau mondial, l'orange et la pomme (Leneveu-Jenvrin et al., 2020). Parmi les fruits tropicaux, la mangue José se révèle très riche en sucres avec une teneur 1,8 fois plus élevée que la papaye Solo ou l'ananas Queen Victoria. Ce n'est toutefois pas le cas de la mangue Cogshall et de la papaye Colombo, beaucoup moins sucrées.

L'acidité des fruits dépend de la concentration en ions H^+ libres (pH) et en acides organiques dont la concentration diffère selon les matrices végétales (Lee et al., 2013). Si l'ananas est un fruit acide dont le pH est voisin de 3,5, ce n'est pas le cas de la mangue et de la papaye dont le pH à maturité est plutôt voisin de 4-5. La perception de l'acidité étant contrebalancée par la sucrosité, l'ananas demeure un fruit agréable à déguster (Marsh et al., 2006). Lors de la maturation des fruits, la teneur en acides organiques diminue (Lee et al., 2013). Les acides organiques métabolisés par les cellules végétales sont utilisés pour le métabolisme énergétique et la synthèse des acides aminés. Ils peuvent également servir de substrat pour la synthèse de métabolites secondaires comme les pigments. La chair des fruits contient majoritairement des acides malique et citrique mais peut aussi comprendre des acides isocitrique, tartrique et quinique (Famiani et al., 2015; Matheyambath et al., 2015). La teneur en acide citrique diminue au cours de la maturation de la mangue Cogshall et, cet acide constituant 90 % de la concentration en acide organique total, induit une diminution de la teneur en acide organique total (Joas et al., 2012; Léchaudel & Joas, 2006).

Sucres et acides modulent aussi la perception aromatique des fruits. La concentration en composés aromatiques augmente lors de la maturation des fruits. Pendant la maturation de la mangue Cogshall une augmentation de la teneur en aldéhydes est observée, indiquant que la synthèse de ces arômes survient pendant la première phase du processus climactérique. La concentration en composés aromatiques dépend du stade de récolte et de la variété de mangue. Ce fruit contient principalement des terpènes dont le δ -3-carène qui est le composé majoritaire dans la plupart des variétés (Joas et al., 2012). La qualité aromatique de la papaye est complexe, plus de 120 molécules volatiles ont été mises en évidence dans la pulpe, et ce fruit est diversement apprécié par les consommateurs. Les terpènes représentent la classe de molécules majoritaires avec le linalol qui est le composé le plus abondant dans la papaye Solo. La présence des acides butanoïque et octanoïque, ou de butanoate de méthyle est plutôt associée à des saveurs indésirables (Brewer et al., 2021). Dans l'ananas, la qualité aromatique dépend de la teneur en esters, notamment le butanoate de méthyle et l'héxanoate de méthyle (Mohd Ali et al., 2020).

1.1.3. Conservation et transformation

En raison de la courte durée de conservation des fruits et légumes, il est nécessaire d'avoir recours à des procédés permettant d'étendre la disponibilité de ces produits. Un fruit est rapidement altéré après sa récolte à cause du développement de levures et moisissures ainsi qu'en raison de son métabolisme endogène, e.g. l'oxydation enzymatique ou la fermentation cellulaire, et de réactions chimiques. Ces phénomènes sont d'autant plus marqués lorsque le fruit est soumis à des

manipulations : altérations de structure par voie mécanique et mise en contact de substrats avec les enzymes (Leneveu-Jenvrin et al., 2020).

Dès la découpe ou le pressage surviennent des altérations microbiennes et physiologiques (Leneveu-Jenvrin et al., 2020). Les fruits sont sujets à un brunissement enzymatique dû à l'oxydation des composés phénoliques par la peroxydase et la polyphénol oxydase, à la peroxydation des lipides membranaires, à la dégradation des anthocyanes, à la production d'espèces réactives de l'oxygène (ROS, « reactive oxygen species ») et à la diminution de leur capacité antioxydante (Leneveu-Jenvrin et al., 2020). Le brunissement enzymatique et la destruction des caroténoïdes induisent une modification de l'apparence et de la saveur des fruits (Leneveu-Jenvrin et al., 2020). Une augmentation de la concentration en composés volatiles, résultant du métabolisme cellulaire ou d'une production par des levures est observée. Elle altère les caractéristiques organoleptiques (Leneveu-Jenvrin et al., 2020).

Les procédés de transformation couramment appliqués inactivent les micro-organismes et les enzymes présents dans les fruits, entraînant ainsi une amélioration de la durée de conservation de ces produits. Les basses températures permettent de diminuer la vitesse des réactions enzymatiques et chimiques, ainsi que le développement microbien. Les procédés de conservation par modification de la température, e.g. chauffage, réfrigération ou surgélation, ou par déshydratation ont un impact négatif sur les micronutriments et les composés phytochimiques naturellement présents dans les fruits (Nicoli et al., 1999; Oboh, 2005). Des technologies innovantes appliquées à des jus de fruits permettent en revanche d'étendre la durée de conservation des fruits tout en préservant leur fraîcheur (Leneveu-Jenvrin et al., 2020). Ces procédés incluent le chauffage ohmique, le chauffage par micro-ondes, la thermosonication, les champs électriques pulsés, le traitement par la lumière, le dioxyde de carbone supercritique et la haute pression hydrostatique. Ces traitements nécessitent toutefois un investissement financier conséquent dû à l'équipement requis. Cela représente une limite majeure à leur acquisition par les entreprises (Leneveu-Jenvrin et al., 2020).

À La Réunion, les fruits tropicaux sont valorisés par des industries agroalimentaires locales qui les transforment. Parmi elles, Royal Bourbon Industries est un des transformateurs majeurs de l'océan Indien et commercialise des confitures et gelées, des desserts de fruits, des fruits tropicaux surgelés et des jus et nectars (Albius, Ceres, Kayamba). Les fruits locaux sont également proposés sous forme de confits, pâtes de fruit, gâteaux, glaces et sont distribués par Soleil Réunion, La Confiserie d'Émilie ou encore Adélis (Figure 4).



Figure 4 : Différents produits transformés à base de fruits tropicaux de La Réunion.

Une alternative à l'utilisation des traitements physiques de transformation des fruits est la fermentation lactique. Ce procédé biologique de conservation permet d'étendre significativement la durée de consommation des végétaux par acidification du milieu et synthèse de composés

défavorables à la croissance d'autres espèces microbiennes, en limitant les conséquences défavorables au plan nutritionnel observées pour d'autres traitements (Lai et al., 2013; Li et al., 2019).

Les traditions culinaires de La Réunion, bien que majoritairement issues d'Afrique, d'Inde et de Chine, ne contiennent pas de produits à base de fruits ou légumes fermentés. En Afrique, les aliments végétaux fermentés sont principalement des céréales et tubercules, e.g. gari à base de manioc ou injera éthiopien à base de teff. En Inde, légume-feuilles et radis fermentés sont fabriqués, e.g. gundruk et sinki, ainsi que des pousses de bambou tendres fermentées (Swain et al., 2014). En Asie, les produits céréaliers lactofermentés sont principalement des pains cuits à la vapeur ou des crêpes à base de riz ou de blé, tandis que les légumes fermentés comprennent le kimchi, le dakguadong, le jiang-gua, le soidon et la choucroute, à base de légumes divers, moutarde brune, concombres et pousses de bambou (Ashaolu & Reale, 2020).

1.2. Fermentation lactique

1.2.1. Les bactéries lactiques, agents de fermentation

La fermentation lactique est un procédé de transformation utilisé pour maintenir ou augmenter la qualité sanitaire et les propriétés nutritionnelles et sensorielles des aliments, tout en étendant leur durée de conservation (Fessard et al., 2017). Les bactéries lactiques forment un groupe hétérogène caractérisé par son caractère anaérobie, i.e. n'ayant pas besoin d'oxygène pour se développer, et fermentent les sucres principalement en acide lactique. Deux voies métaboliques principales pour la fermentation des hexoses sont décrites (**Figure 5**) (Gänzle, 2015) :

- La fermentation homolactique utilise la glycolyse, i.e. voie d'Embden-Meyerhoff, pour former du pyruvate qui sera ensuite réduit en acide lactique par une lactate déshydrogénase ;
- La fermentation hétérolactique emprunte des réactions associées à la voie des pentoses phosphates, pour former du dioxyde de carbone, du lactate, de l'acétate et de l'éthanol.

Toutefois, cette dichotomie est réductrice et incomplète. Les bactéries homofermentaires utilisent principalement le glucose du milieu et l'utilisation des disaccharides est réprimée. Le pyruvate est un nœud métabolique central, pouvant conduire à la formation de formate, éthanol, acétate ou acétoïne en plus du lactate. Dans le cas d'un métabolisme hétérofermentaire, le fructose et le saccharose sont préférés au glucose. Le fructose est principalement converti en mannitol par les *Leuconostoc*, mais pas par les *Weissella*, et le nœud métabolique central chez les bactéries hétérofermentaires est l'acétyl-phosphate. La fermentation hétérolactique produit un seul adénosine triphosphate (ATP) par glucose, contre le double en homolactique. La formation d'acétate peut alors permettre d'améliorer le rendement en ATP.

Enfin, certaines bactéries lactiques comme les *Leuconostoc* peuvent utiliser le citrate en complément des sucres, générant les intermédiaires pyruvate et oxaloacétate, convertis selon les conditions en différents produits (lactate, acétate, succinate, éthanol, acétoïne) (Bintsis, 2018; Marty-Teyssset et al., 1996). Le malate est converti par certaines bactéries en acide lactique et dioxyde de carbone : cette voie requiert la présence d'une malate perméase et d'une décarboxylase.

Les équilibres sont assurés par l'état énergétique, l'activité des différentes enzymes et l'état rédox intracellulaire, dirigeant le métabolisme vers des produits plus ou moins oxydés selon le besoin de régénération des cofacteurs. À titre d'exemple, dans du jus de mûre, dont le pH est de 2,65,

Lactiplantibacillus plantarum utilise préférentiellement l'acide malique et le duo glucose-fructose. Si le pH initial est ajusté à 3,5, ce sont les acides citrique et quinique qui sont majoritairement consommés, en plus de l'acide malique (Wei et al., 2018).

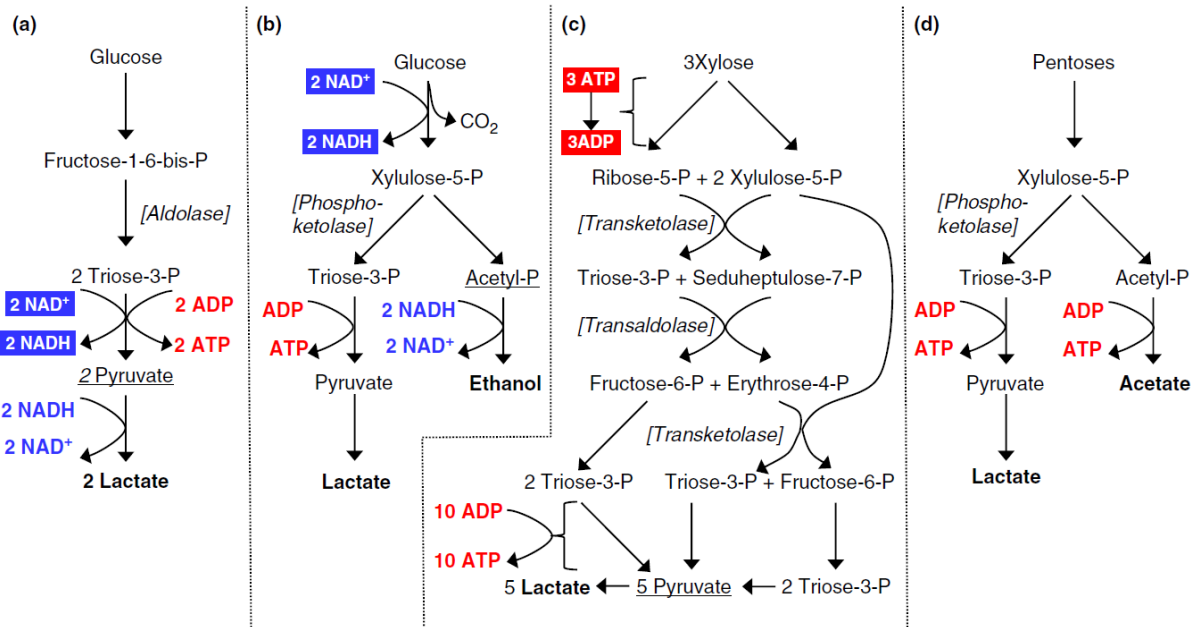


Figure 5 : Voies de fermentation des glucides par les bactéries lactiques (Gänzle, 2015). Les principaux produits finaux du métabolisme apparaissent en gras ; les points d'embranchement du métabolisme ou "interrupteurs métaboliques" sont soulignés. La formation de cofacteurs réduits (en blanc sur fond bleu) ou oxydés (en bleu) est indiquée si elle se produit en amont ou en aval des points d'embranchement du métabolisme. Le rendement net en ATP de la voie métabolique apparaît en rouge. (a) Métabolisme homofermentaire des hexoses par la voie d'Emden-Meyerhoff. (b) Métabolisme hétérofermentaire des hexoses par la voie de la phosphocétolase. (c) Métabolisme homofermentaire des pentoses par la voie des pentoses phosphates. (d) Métabolisme hétérofermentaire des pentoses par la voie de la phosphocétolase.

Alors que dans les fermentations de produits laitiers et viandes, ce sont des bactéries homofermentaires qui sont retrouvées, les produits fermentés à base de produits végétaux comprennent généralement un mélange de bactéries homo et hétérofermentaires.

La majorité des bactéries lactiques utilisées pour la fermentation appartient au phylum des Firmicutes, à la classe des Bacilli et à l'ordre des Lactobacillales (Figure 6) (Mora-Villalobos et al., 2020). L'ordre des Lactobacillales comprend six familles : Aerococcaceae, Carnobacteriaceae, Enterococcaceae, Lactobacillaceae, Leuconostocaceae et Streptococcaceae. Il inclut plus de 30 genres bactériens ainsi que plus de 300 espèces (Mora-Villalobos et al., 2020). Parmi celles-ci, les espèces *Ltp. plantarum* et *Weissella cibaria/confusa* et les genres *Leuconostoc*, *Lactobacillus*, *Enterococcus* et *Pediococcus* sont retrouvés dans les fruits et légumes fermentés (Di Cagno et al., 2013). Dans le phylum Actinobacteria, dans la famille des Bifidobacteriaceae, le genre *Bifidobacterium* fait aussi partie des bactéries lactiques (Mora-Villalobos et al., 2020).

Les *Lactiplantibacillus* sont homofermentaires, alors que les Leuconostocaceae, qui comprennent les *Leuconostoc* et les *Weissella*, sont hétérofermentaires.

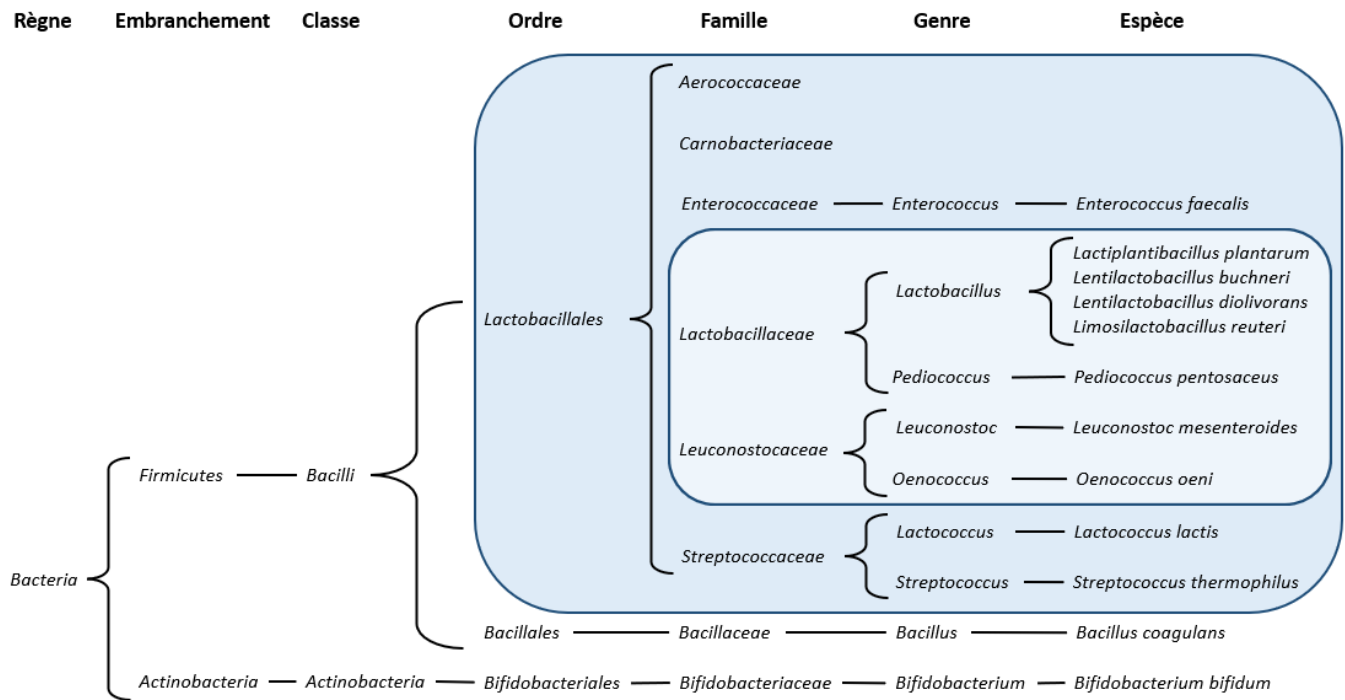


Figure 6 : Classification phylogénétique des bactéries lactiques (d'après Sauer et al. (2017), adapté à la nomenclature publiée par Zheng et al. 2020).

1.2.2. Obtention des aliments lactofermentés

Les bactéries lactiques sont exploitées dans l'industrie agroalimentaire notamment pour la fermentation de produits alimentaires à base de fruits ou légumes. Parmi eux, la fermentation de chou permet d'obtenir des produits traditionnels commercialisés, à savoir la choucroute et le kimchi coréen (Karovičová & Kohajdová, 2003). La choucroute est obtenue par fermentation avec des bactéries lactiques hétérofermentaires, i.e. les *Leuconostoc*, et homofermentaires, i.e. les *Lactobacillus* et les *Pediococcus* (Karovičová & Kohajdová, 2003). Des jus de légumes fermentés à base de chou, betterave, carotte, céleri et tomate sont expérimentés (Karovičová & Kohajdová, 2003) (Tableau 2). Le plus souvent, les souches utilisées sont des lactobacilles et appartiennent aux espèces *Ltp. plantarum*, *Lactobacillus bavaricus*, *Lactobacillus xylosus*, *Lactobacillus bifidus*, *Levilactobacillus brevis* (Karovičová & Kohajdová, 2003). Plus rarement, d'autres genres sont utilisés comme pour la fermentation de smoothies avec une combinaison de souches de *Ltp. plantarum*, *W. cibaria*, *Lactiplantibacillus pentosus* et *Pediococcus pentosaceus* (Di Cagno et al., 2011).

Tableau 2 : Exemples d'études sur la capacité de bactéries lactiques à fermenter des fruits ou légumes.

Matrice végétale	Bactéries lactiques	Référence
Smoothies à base de fruits et légumes	Combinaison de souches de <i>Lactiplantibacillus plantarum</i> , <i>Weissella cibaria</i> , <i>Ltp. pentosus</i> et <i>Pediococcus pentosaceus</i>	(Di Cagno et al., 2011)
Jus de cerise	Inoculation individuelle de souches de <i>Ltp. plantarum</i> , <i>Levilactobacillus spicheri</i> , <i>Limosilactobacillus fermentum</i> ou <i>Lmb. reuteri</i>	(Filannino et al., 2015)
Jus de mûre	Inoculation individuelle de souches de <i>Ltp. plantarum</i> , <i>Lactobacillus acidophilus</i> ou <i>Lacticaseibacillus paracasei</i>	(Kwaw et al., 2018)
Jus de grenade	Inoculation individuelle de souches de <i>Lb. acidophilus</i> , <i>Ltp. plantarum</i> , <i>Lb. delbrueckii</i> ou <i>Lcb. paracasei</i>	(Mousavi et al., 2011, 2013)
Jus de jicama (tubercule), de courge cireuse et de carotte	Inoculation individuelle de souches de <i>Ltp. plantarum</i> ou <i>Lb. acidophilus</i>	(Do & Fan, 2019)
Jus de pastèque	Inoculation individuelle de souches de <i>Ltp. plantarum</i> , <i>Lcb. rhamnosus</i> , <i>Lcb. casei</i> , <i>Lvb. brevis</i> ou <i>P. pentosaceus</i>	(Mandha et al., 2021)
Jus de papaye	Inoculation individuelle de souches de <i>Lb. acidophilus</i> ou <i>Ltp. plantarum</i>	(W. Chen et al., 2018)
Jus de citron doux	Souche de <i>Ltp. plantarum</i>	(Hashemi et al., 2017)
Jus de figue de Barbarie	Souche de <i>Lmb. fermentum</i>	(Panda et al., 2017)
Jus d'agrumes	Souche de <i>Lmb. fermentum</i>	(Yu et al., 2015)
Jus de figue de Barbarie	Souche de <i>Ltp. plantarum</i>	(Verón et al., 2019)
Jus de tomate	Inoculation individuelle de souches de <i>Lcb. casei</i> ou <i>Ltp. plantarum</i>	(Liu et al., 2018)
Jus de pomme de cajou	Inoculation individuelle de souches de <i>Ltp. plantarum</i> ou <i>Lcb. casei</i>	(Kapasob et al., 2017)
Jus de pomme de cajou	Inoculation individuelle de souches de <i>Lb. acidophilus</i> , <i>Lcb. casei</i> , <i>Ltp. plantarum</i> , <i>Leuconostoc mesenteroides</i> ou <i>Bifidobacterium longum</i>	(Kapasob et al., 2018)
Jus de myrtille des marais	Inoculation individuelle de souches de <i>Ltp. plantarum</i>	(Wei et al., 2018)
Jus de grenade	Inoculation individuelle de souches de <i>Ltp. plantarum</i>	(Di Cagno et al., 2017)
Jus de pomme	Inoculation individuelle de souches de <i>Lb. acidophilus</i> , <i>Lcb. rhamnosus</i> , <i>Lcb. casei</i> ou <i>Ltp. plantarum</i>	(Chen et al., 2019)
Jus de pomme, carotte, tomate, concombre et aubépine	Inoculation individuelle de souches de <i>Ltp. plantarum</i> , <i>Lcb. casei</i> , <i>Lb. acidophilus</i> , <i>Lmb. fermentum</i> ou <i>Lcb. rhamnosus</i>	(Cui et al., 2019)
Jus de sureau	Inoculation individuelle de souches de <i>Ltp. plantarum</i> , <i>Lcb. rhamnosus</i> et <i>Lcb. casei</i>	(Ricci et al., 2018)
<i>Suansun</i> (pousse de bambou aigre)	Inoculation de souches de <i>Leuconostoc citreum</i> et/ou <i>Ltp. plantarum</i>	(Lu et al., 2022)
Boisson à base de jus d'orange et d'extrait de malt	Souche de <i>Weissella cibaria</i>	(Rolim et al., 2019)

Dans ces études, les boissons lactofermentées sont obtenues à partir de fruits ou légumes d'abord fermentés dont le jus est ensuite extrait, ou par transformation de la matrice végétale en jus ou purée, puis fermentation (**Figure 7**) (Karovičová & Kohajdová, 2003). Une pasteurisation des jus de fruits est généralement réalisée avant de procéder à la fermentation lactique, afin d'éliminer les levures naturellement présentes, très adaptées à la fermentation de ces matrices, qui domineraient l'écosystème (Chaudhary, 2019). La pasteurisation préalable de jus de fruits ou légumes favorise l'implantation des « starters » sélectionnés (Di Cagno et al., 2013). Pour une initiation efficace de la fermentation lactique, l'inoculation est effectuée en utilisant des bactéries viables à une population

minimale de 10^6 unités formant colonie (UFC)/g (Gobbetti et al., 2010). Pour favoriser la fermentation, les produits sont ensuite incubés de 25 à 30 °C pendant 15 à 48 h (Di Cagno et al., 2013; Fessard et al., 2017). Les préparations fermentées sont enfin réfrigérées afin de prolonger la durée de conservation des aliments et la viabilité des souches bactériennes utilisées lors de la fermentation (Chaudhary, 2019).

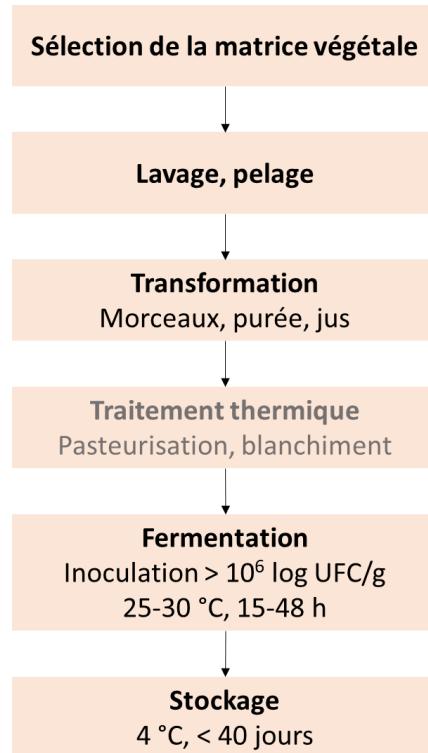


Figure 7 : Diagramme de fabrication d'un produit fermenté (d'après Di Cagno et al., 2013 ; Fessard et al., 2017 ; Gobbetti et al., 2010).

Partant du constat que de nombreuses études se rapportent à des jus de fruits ou légumes lactofermentés en soulignant les bénéfices sur la santé, nous avons rédigé un article de revue intitulé « **Lactic fermented fruit or vegetable juices: Past, present and future** » publié dans le journal *Beverages*. Cet article permet de faire un état de l'art des travaux réalisés sur la fermentation lactique de matrices végétales transformées en jus. Il présente les boissons fermentées traditionnelles ainsi que les nouvelles tendances liées à l'intérêt grandissant des consommateurs pour les aliments fonctionnels. Les espèces bactériennes majoritairement utilisées sont décrites ainsi que l'impact de différents procédés de stabilisation appliqués lors de la préparation des boissons. Les procédés permettant d'optimiser l'inoculation des bactéries lactiques pour assurer leur survie sont introduits. L'effet de la consommation de produits fermentés par des « starters » sur le microbiote est expliqué ainsi que les changements nutritionnels et les effets fonctionnels liés à l'activité des bactéries lactiques. L'effet probiotique et les bénéfices-santé des bactéries lactiques sont exposés.

Review

Lactic Fermented Fruit or Vegetable Juices: Past, Present and Future

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Abstract: Numerous traditional low-alcohol fermented beverages produced from fruit or vegetables are described around the world. Fruit and vegetables and lactic fermented products both present nutritional benefits, which give reasons for the recent expansion of non-dairy lactic fermented juices on the market. In addition, fruit and vegetable juices are new carriers for probiotic bacteria. Specific phenotypic traits of lactic acid bacteria (LAB) are required so that LAB can effectively grow in fruit or vegetable juices, increase their safety and improve their sensory and nutritional quality. From the diversity of microbiota of spontaneous fermentations, autochthonous starters can be selected, and their higher performance than allochthonous LAB was demonstrated. Achieving long-term storage and constant high quality of these beverages requires additional processing steps, such as heat treatment. Alternatives to conventional treatments are investigated as they can better preserve nutritional properties, extract bioactive compounds and promote the growth and metabolism of LAB. Specific processing approaches were shown to increase probiotic viability of fruit and vegetable juices. More knowledge on the metabolic activity of lactic acid bacterium consortium in fruit or vegetable juices has become a bottleneck for the understanding and the prediction of changes in bioactive compounds for functional beverages development. Hopefully, the recent developments of metabolomics and methods to describe enzymatic machinery can result in the reconstruction of fermentative pathways.

Keywords: probiotic; starter; nutritional quality; microbiota; processing

1. Introduction

Traditional fermented foods and beverages have been made for centuries in many regions of Europe, Asia, America and Africa. These foods are produced by ethnic people using their native knowledge from locally available raw materials. Based on the raw material employed, nine groups of fermented foods can be distinguished: fermented cereals, vegetables, legumes, roots/tubers, milk, meat and fish products, miscellaneous fermented products and alcoholic beverages [1]. Nowadays, fermented foods and beverages are obtained through spontaneous fermentation or by starter or probiotic addition. Addition of active bacterial cultures can be considered by the consumer as an added value and a marker of high quality. Historically, the use of probiotics in dairy beverages has been widely investigated, whereas it was less documented in other matrices.

Dietary guidelines around the world recommend increasing the intake of fruit and vegetables for the prevention of chronic diseases. Fruit and vegetables are naturally rich in carbohydrates, dietary fibers, vitamins minerals, polyphenols and phytochemicals [2]. Single-fruit or blend purées, smoothies or juices, that can be probiotic enriched or fermented, are a way to promote the consumption of fruit and vegetables.

Lactic acid bacteria (LAB) metabolize different substrates, leading to biochemical changes in composition [3]. Lactic acid fermentation is recognized to improve nutritional properties, flavor, and health-related aspects of food products [4]. This process improves the organoleptic properties of food matrixes and their acceptability on top of shelf-life properties [4,5].

Domestication of microorganisms induced genetic changes which reflect adaptation to the food matrix [6–8]. Lactic acid fermentation of fruit is less widespread than milk fermentation and probably requires a specific adaptation of LAB. Some research has been conducted to investigate the suitability of various fruit and/or vegetable beverages as raw substrates to the lactic acid fermentation. The most common genera used were *Lactobacillus*, *Leuconostoc*, *Weissella* and *Bifidobacterium*, obtained commercially or isolated from plants. These bacteria share the ability to produce lactic acid, and in a strain-dependent way, aromatic compounds, exopolysaccharides, bacteriocins, and enzymes implied in the increase in food safety and shelf life [9]. However, each species presents its own characteristics and produces miscellaneous effects depending on the substrates and process applied.

Several factors must be considered for the selection of candidate LAB for fermented fruit or vegetable juice elaboration. LAB capacity to grow and acidify the juice must be ensured, together with the increase in safety by elimination of pathogens and absence of biogenic amine production. The effects of lactic acid fermentation on sensory and nutritional quality should be determined [10]. Starter strains must also satisfy several criteria such as good yields and viability for starter production or manufacturing, administration and application. Survival and colonization in the host is a prerequisite for LAB to be considered as probiotic [11].

In this article, we examine the literature on traditional fruit and vegetable fermented beverages and new trends, with regards to the characteristics of LAB diversity, the impact of processing steps, and the reputed nutritional and health benefits. Recent achievements with “omic” tools are shown to be powerful to increase knowledge for the successful development of promising functional beverages.

2. Actual Market

The global functional beverage market is a growing sector of the food industry as modern health-conscious consumers show an increasing desire for foods that can improve well-being and reduce the risk of disease. It was reported that the market of functional foods was the fastest-growing sector, with an expected annual growth of 10% compared to 2% to 3% for the food industry in 2008 [12]. Within the range of new functional foods, an important segment is composed of beverages elaborated from cereal, legume, tuber and fruit. The functional beverage market was distinctly the fastest growing segment at the same time, evolving and growing at different rates both within and across Countries, with a noticeable annual growth of 14% in particular in the U.S. between 2002 and 2007 [12]. These products have become significant in the context of a greater prevalence of lactose intolerance, and the preference for low cholesterol products, leading consumers to avoid dairy beverages [13].

The position of lactic fermented beverages benefits from the presence of probiotics bacteria and it is to highlight that among probiotic foods, the ones containing fruit ingredients are increasingly preferred by consumers [14]. These drinks meet a growing demand for less sugary products. Especially as the reaction against sugar is driving a change in flavor preferences across Europe, with sour, bitter or tangy flavor being more popular. A telling example is the 1st price of innovation from the last SIAL world food exhibition in 2018 being awarded to a Greek fruit and water kefir [15].

Further, the application of fruit as substrates for lactic fermentation has the advantage of the incorporation of flavors and nutrients specific to each type of fruit, resulting in products with differential sensory and physicochemical characteristics, appreciated by consumers. A comparison of beverage formulations made from different fermented Brazilian fruits, with the addition or not of whey, shows that the addition of dairy ingredients is not desirable, and that the consumers preference is lactose-free fermented fruit juices [16].

We explored the scientific literature on fermented beverages by bibliometrics on the Science Direct database to quantify the interest for this topic during the last 20 years. A low number of

scientific publications for fermented beverages until 2005 (Figure 1) was observed, but then the interest in exploration of this process was progressively and noticeably growing. The topic exceeded the threshold of 250 original research articles per year in 2011, 500 in 2016, and is now above 700 while the count reached 937 for the total publications. A focus on fruit and vegetable fermented beverages showed a moderate scientific production reaching only 100 original research articles per year in 2012 but then the number of publications was doubled in 2017 and is now 384 for the total publications.

Scientific interest is growing for beverages such as fermented fruit and vegetable juices, as they seem to be new adapted carriers of probiotic bacteria, winning consumer preference and leading to developing products with a specific taste.

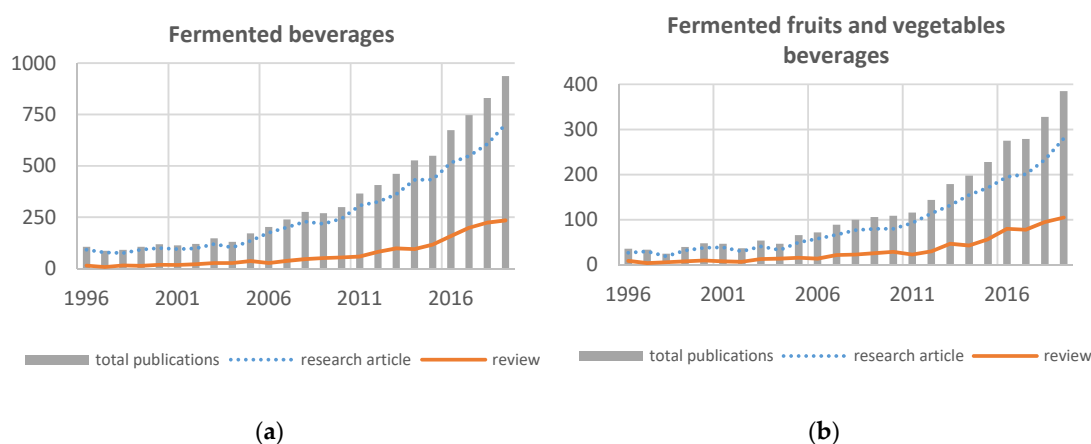


Figure 1. Publication trends per year on fermented beverages (a) and fruit and vegetable fermented beverages (b) according to the Science Direct database.

3. Traditional Lactic Fermented Beverages

The elaboration of fermented beverages with health-promoting properties from milk, cereals and other substrates is traditional for centuries in many regions of Asia, Africa, Europe, the Middle East and South America. An important class of non-dairy fermented beverages was those made from cereals, popular in tropical regions and in Africa in particular [17]. Traditional African fermented products include fermented milks, sour porridges, and alcoholic and non-alcoholic beverages. African traditional fermented beverages are mainly made with cereals of cassava, maize, sorghum and millet. These products are fermented using mainly LAB, such as *Lactobacillus* sp., *Bacillus* sp., *Pediococcus* sp. and *Weissella* sp., and yeasts [11].

Moreover, some lactic fermented beverages are traditionally produced from fruit or vegetables in specific areas, such as Turkey [18,19] or Albania and Kosovo [20]. Most fruit-based non-alcoholic or low-alcoholic traditional beverages are made by complex microbiota associating yeasts, LAB and acetic acid bacteria. This applies to water kefir or sugary kefir.

Water kefir is made from water, sugar and dried fruit, fermented with kefir grains containing a microbial consortium surrounded by a polysaccharidic matrix, named kefiran. This consortium is steady or stable for years [21]. In commercial water kefir grains available on market, *Zymomonas mobilis* was shown to be the dominating bacteria, representing up to 87% of bacteria [13]. Water kefir microbiota examination is recent, compared to milk kefir. Research mainly focused on grains, and few data are available on possible changes concerning the ecosystem diversity over fermentation and storage of the products [22,23]. However, the microbial diversity relies more upon grain origin and between grain and beverage, rather than changes over fermentation time [22–24].

Kombucha is a fermented black tea beverage in which sugar has been added. Like water kefir, it is fermented through a complex ecosystem made of yeasts, acetic acid bacteria and LAB. But this consortium is embedded in a cellulosic mat and the fermentation is dominated by yeasts, especially

Zygosaccharomyces or *Candida* and *Lachancea* depending on Kombucha origin, and acetic acid bacteria [17,25]. Acetic acid bacteria represent 60% to more than 90% of bacteria [17]. *Lactobacillus*, *Leuconostoc* and *Lactococcus* are present in the first fermentation stages, although at low levels (<5%). However, by the end of fermentation process, during which pH decreases, *Lactobacillus* species may represent up to 10% of bacteria and reach 30% in the mat pellicle [17].

Other traditional fermented beverages made from fruit and vegetables can be found in Turkey. They are named *Shalgam* juice and *Hardaliye* [18]. *Shalgam* juice is made from a mixture of black or purple carrot (*Daucus carota*) slices, turnip (*Brassica rapa*) slices and wheat flour in salted water. After fermentation and filtration, a red-colored cloudy beverage is obtained. This juice has a sour taste and is rich in minerals (Ca, K and Fe), vitamins (A, C and B group vitamins) and polyphenols. Its microbiota is mainly composed of yeasts and *Lactobacillus* (89.63%), *Leuconostoc* (9.63%) and *Pediococcus* (0.74%). *Hardaliye* is prepared from grapes, mustard seeds and cherry leaves and is fermented mainly by *Lactobacillus* spp. Also, in Turkey, *Gilaburu* juice is made using water and European cranberry bush (*Viburnum opulus* L., *gilaburu* in Turkish), a red-colored fruit with an astringent taste. The LAB identified include *Lactobacillus* spp. and *Leuconostoc* spp. [26].

Soymilk is traditionally made in Asia from a water extract of soybeans which is fermented using five LAB species, namely *Lactobacillus casei*, *Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium longum*. This product is used as an antioxidant, a probiotic, in treatment of breast cancer and to maintain intestinal environment [9].

Sauerkraut juice is obtained by white cabbage fermentation using the same process as for making sauerkraut meal. This beverage is consumed in Germany, Ukraine, Romania, Serbia and other regions in the Black Sea. The LAB involved in this process are *Lactobacillus brevis*, *Lactobacillus sakei*, *Lactobacillus plantarum* and *Leuconostoc mesenteroides* [26].

4. Recently Developed Lactic acid Fermented Juices

4.1. Overview

Beyond products rooted in tradition, the development of new lactic fermented fruit or vegetable juices has recently generated interest. These products have been shown to have considerable market value, because of the combination of nutritional advantages of the raw materials and benefits resulting from lactic fermentation [27–33]. Some attempts to develop lactic fermented fruit or vegetable juices have been made on various substrates. To achieve these fermentations, water kefir grains have been occasionally used. However, most studies were performed with *Lactobacillus* spp., and some with *Bifidobacterium* spp., *Leuconostoc* spp. or *Weissella* spp.

To be successful, the manufacturing process of these beverages requires a mild pasteurization and inoculation with selected starters [34]. Pasteurization reduces microbial population in the juice, possibly inactivates foodborne pathogens, and hence favors the implantation of the starter. To obtain a well-accepted beverage, the sensory characteristics have to be carefully examined, by adjustment of the mix of fruit or vegetables, to avoid an unpleasant acidic or astringent beverage, and by the choice of starters to avoid undesirable compounds, flavors or biogenic amines.

The ability of starter strains to grow in the juice is an important parameter to consider for successful fermentation or to expect some probiotic effects [14,30,35–39]. Long-term survival of bacteria in the fermented juice and putative probiotic properties of starters in the beverages are regarded as advantages [40,41]. For instance, a 1000× population decrease was observed after four weeks of storage in fermented tomato juice for *L. plantarum*, whereas a similar effect was observed for *L. acidophilus* in red beet juice and *Lactobacillus delbrueckii* in cabbage juice [37–39]. Some factors, such as low temperature and the presence of sugars, favor survival in an acidic medium [42,43].

Therefore, it is recommended to isolate autochthonous starters from fruit or vegetable carposphere or from spontaneous fermentations as those isolates exhibit specific phenotypic traits [28,44–48]. For instance, *Lactococcus lactis* isolates from dairy environment greatly differ in their genes from environmental strains [49], showing domestication of strains. As a consequence, their phenotype reflects their adaptation to a specific niche [50,51]. Similar exploitation of genomic data,

through comparative genomics, have been performed with *L. plantarum* and *Fructobacillus* spp. [52], [53].

From literature data, it appears that the bacterial strain-food material combination determines the bacterial growth characteristics, sensory properties of the fermented food and changes in bioactive compounds. The nature, content and structure of bioactive compounds is specific to each raw material. These features can be modulated by processing and microbial metabolism.

4.2. Juices Stabilization Treatments

4.2.1. Conventional Thermal Treatment of Juices

Fruit and vegetable juices are generally preserved by physical, chemical or biological processes prior to consumption and utilization. These processing steps aim to ensure safety and to maintain the quality of products which can be affected by enzyme activity or chemical reactions. The efficacy of processing is impacted by the complexity of composition of juices and their microbial diversity, which trigger the choice of process and process parameters. Thermal and non-thermal processes with various intensities are the most commonly used methods in the processing of fruit and vegetable juices prior to their fermentation.

Table 1 provides a comprehensive summary of the outputs of processing treatments applied to fruit and vegetable juices prior to fermentation, or cold storage of treated and fermented juices. Processing treatments comprise mainly heat treatments, but also treatments with alternative technologies.

Table 1. Process parameters for lactic fermentation of fruit and vegetable juices.

Processing Conditions	Raw Material	Outputs	Reference
Thermal Treatments Prior to Fermentation			
Sterilization (120 °C/5 min); fermentation (30 or 37 °C) <i>Lactobacillus fermentum</i> and <i>Lactobacillus casei</i> ; cold storage (4 weeks)	Watermelon and tomato	Different survival of bacteria during the cold storage period depending on fermentation temperature	[54]
Homogenization; heating (100 °C/5, 30, 60, 90 and 120 min); fermentation (<i>Bifidobacterium brevis</i> , <i>Bifidobacterium longum</i> , and <i>Bifidobacterium infantis</i>)	Fresh tomato	No heating impact on pH, Brix, acidity of juice; redness increases until 60 min heating; heating increases lycopene content; fermentation did not affect lycopene content	[55]
Commercial tomato juice (pasteurized); fermentation (<i>Lactobacillus acidophilus</i> LA39, <i>L. casei</i> A4, <i>Lactobacillus. delbrueckii</i> D7, <i>Lactobacillus plantarum</i>); storage (4 °C/4 weeks)	Commercial tomato juice	Viability of <i>L. acidophilus</i> and <i>L. delbrueckii</i> did not decrease during cold storage of fermented juice (10 ⁶ to 10 ⁸ CFU/mL)	[39]
Extraction; autoclave sterilization (121 °C/15 min); fermentation; cold storage for 4 weeks	Red beets	The fermented juice contained a significant load of beneficial viable cells for LAB (10 ⁹ CFU/mL)	[37]
Centrifugal juice extractor; pasteurization (75 °C/5 min); fermentation (25 °C/48 h) by commercial water kefir preparation of 10 ⁹ UFC/mL containing (<i>Lactobacillus</i> , <i>Lactococcus</i> , <i>Leuconostoc</i> and <i>Saccharomyces</i>)	Carrots, fennels, melons, onions, tomatoes	Thermal treatment reduced all microbial groups at levels below the detection limits; physicochemical and organoleptic attributes of fermented juice were acceptable; changes in antioxidant activities	[56]
Pre-treatments (filtration connected or not with thermal treatment and/or enzymatic processing) of juices prior to fermentation	Pomegranate	A short pasteurization at 95 °C/30–60 s eliminated all microorganisms found in juice → Preservation of bioactive molecules + less anthocyanin degradation + improved antioxidant activities; clarification was related to reduction of phenol content and lower antioxidant activity	[57]
Combination between Thermal and Non-Thermal Treatments			
Drying of raw material (40 °C/3 days); sterilization of powder suspension (121 °C/15 min); fermentation by <i>Bacillus amyloliquefaciens</i> and <i>Lactobacillus brevis</i> separately; light illumination (LED, sunlight and dark)	Blueberry	Light illumination enhanced metabolic activity + maximal LAB growth + highest TPC and flavonoids; blue LED mediated fermentation → an extract with antibacterial activity only; Red LED mediated fermentation → extract with antioxidant (phenols and flavonoids) and cytotoxic activities	[58]

Ultrasonicated homogenization (376 W/cm ² , 500 W, 10 min); of <i>L. casei</i> ; fermentation (24 h; 10 41 °C); cold storage (4 °C/42 days)	Pineapple	Ultrasonication of pineapple juice improved LAB viability and prevented browning; fermented juice exhibited acceptance for color and sensory evaluation	[59]
Heating (95 °C/1 min); High Hydrostatic Pressure: HHP (500 MP/2 min; 25 °C); fermentation of <i>L. casei</i> (10 ⁵ CFU/mL)	Litchi	HHP fermented juice exhibited better color, flavor and overall acceptance than heated one; both HHP and Heated fermented litchi juice showed enhanced cell viability	[60]
Bleaching (100 °C/2 min), squeezing, filtering; thermal processing TP (100 °C/30 s); HHP (300, 400, 500 MP/15 min; 25 °C); storage (20 °C) until use; fermentation (<i>Lactobacillus rhamnosus</i> (30 °C/18h), <i>Gluconacetobacter xylinus</i> (30 °C/24 h; 160 rpm))	Yacon, Litchi and Longan (YLL)	TP (100 °C/30 s) and HHP (500 MPa/15 min) of juice complied with aseptic standards and ensured microbial safety; TP reduced the taste score of juice and reduced flavor molecules (alcohols, esters,...); HPP preserved the nutritional and aroma characteristics of juice; fermentation of HHP juice improved the taste (aminoacids, carbohydrates,...) and flavors of YLL juice; HHP cofermentation improved the quality of YLL juice	[61]

The aim of the High-Temperature, Long-Time treatment (HTLT) application is the inactivation of native microflora prior to fermentation. For example, Koh et al. [55] and Sivudu et al. [54] worked on the effect of HTLT of tomato juice alone or mixed to be subject to the fermentation by bifidobacterial species. Authors reported that these conditions of pre-treatment did not seem to affect the physicochemical properties of vegetable juice and were able to maintain viability of LAB cells.

Thermal treatment was evaluated as function of its intensity and time. For example, HTLT ($T \geq 80$ °C and holding time > 30 s) could reduce or inactivate some enzymes, whose activities result in undesirable changes in sensory quality attributes and nutritive value of the products [62], such as polyphenoloxidase, peroxidase, pectin esterase and polygalacturonase [63]. High temperature treatment might present some drawbacks as it affects many antioxidant compounds, thus reducing their beneficial health effects and deteriorate the quality attributes and physicochemical properties.

4.2.2. Alternative Physical Treatment

Many studies were carried out on the potential of use of Ohmic Heating (OH) based on electrical current passage in the food processing. This process provides uniform and rapid heating of foods, with a beneficial effect on the nutritional and organoleptic properties of processed products [64]. According to literature reviews [65–67], OH is a promising alternative to conventional thermal technologies for juice processing with a maximum retention of functional components and the destruction of foodborne microorganisms by electroporation mechanism. A nutritional advantage consisting in the limitation of xanthophyll losses to 20%–30% was reported by Achir et al. in ohmic heated citrus fruit juices, compared losses of 40%–70% with conventional heating [68].

It seems that no studies or at least a few have focused on the OH treatment for fermented fruit or vegetable-based juices. Taking into account the findings reported by literature, we can assume that OH treatment can be effectively used to pasteurize fresh fruit or vegetable juices with minimal sensory and functional deterioration prior to their fermentation. Some studies [69] reported that a combination of OH with moderate electric field is desirable in the food fermentation process to shorten the lag-period and to promote the growth and metabolism of fermentation microorganisms under optimized conditions compared with conventional fermentation. The combination effect of OH and Ultrasound Irradiation deals in improvement of substrate bioavailability to fermentation process in general.

Another promising non-conventional thermal treatment of fruit and vegetable juices is the microwave heating (MWH). This way presents some benefits, like the reduced processing time, high power efficiency and a good process control for reducing the loss of juices quality as well as destroy harmful pathogenic bacteria [70]. Overall, the effectiveness of MWH as compared to the traditional treatment is confirmed by: the high total phenols and flavonoid compounds in watermelon, black jamun, litchi, grapefruit and pineapple juices [71]; the preservation of physicochemical properties in tomato juice [72] and many juice-blends [73]; the significant retention of ascorbic acid and the preservation of color and rheological properties in guava nectar [74]. The study of Pérez-Grijalva [75] highlights the potential application of microwaves and ultrasound to increase the functional value of blackberry juice. Authors reported that microwaves in combination with ultrasound technologies present an alternative in fruit juices processing when the antioxidant activity is high, and the microbial quality is good. Some drawbacks related to MWH include the formation of colored decomposition products (browning) in beetroot juice [76] and the decrease in pH and color values in pomegranate juice.

Thermosonication is another good alternative technique to replace the conventional heat treatment process. It has potential in enhancing quality, safety and yield extraction of fruit and vegetable juices [77]. This process is able to inactivate the enzymes and destroy the microorganisms at lower temperature and shorter time to give lower losses of ascorbic acid, total phenolics, flavonoids and flavonols [78]. Pulsed electric field treatment is also an alternative, tested on whole citrus fruit (orange, pomelo, and lemon) in aqueous media to increase yield extraction and phenolic content of final juice [79].

In order to avoid oxidative damage of the probiotics and reduce their sensitivities to reactive oxygen species in fruit and vegetable juices, the modification of the product environment was suggested by the literature data. This modification deals to control the composition of the atmosphere by raising the CO₂ content in the headspace [12].

Low-dose irradiation (≤ 3 kGy) used for treatment of juices widely extended their shelf life. Compared to non-irradiated samples, γ -irradiation (2.0 kGy) provided antioxidants content in grape juice blends and maintained the quality of products. This mild irradiation reduced fungi and yeasts during storage at room temperature of juice blends [80].

4.2.3. Pre-Treatments Combined with Fermentation

A comparison of heating and high hydrostatic pressure (HHP) treatment on litchi juice was conducted to ensure the microbial safety of this substrate before its fermentation by *L. casei* [78] (Table 1). The results showed a good ability of HHP to preserve color, flavor acceptance and antioxidant activity of fermented litchi juice. Authors suggested that HHP and heating process exhibited the potentiality to improve the viability probiotic cells and extend these quality attributes after the storage of 4 weeks at 4 °C.

The work of Mestry et al. [81] focused on the study of formulation and spray drying of a non-dairy fermented beverage. The mixture of watermelon-carrot juice 70:30 was fermented at 37 °C with *L. acidophilus*. It was found that high temperature of air coupled with longer exposure time at lower feed flowing have a negative impact by reducing viability of probiotic cells, lycopene and β -carotene contents of juice powder. The increase in atomization pressure enhanced the cohesiveness but decreased flowability of the dried powder because the particle size was lowered. Chen et al. [61] were interested in the study of the effect of HHP treatment on the quality of fruit juice and of the co-fermentation of *Lactobacillus rhamnosus* and *Gluconacetobacter xylinus* on the physicochemical properties of yacon-litchi-longan juice. Authors reported that by comparison to thermal processing, the HHP treatment prior to co-fermentation can be used to improve the quality of yacon-litchi-longan juice, especially the flavor and aroma.

The ultrasonic processing was used on pineapple juice as an alternative treatment for the incorporation of probiotics *L. casei* [59]. According to the authors, sonicated pineapple juice was shown to be a suitable substrate for *L. casei* cultivation and for the development of an alternative non-dairy probiotic beverage.

Light is widely used in photobioreactors because some microorganisms need light for growth and metabolites production. Fermentation of blueberry fruit powder by *Bacillus amyloliquefaciens* and *L. brevis* under different light sources illumination (sunlight, LED lights, darkness) showed that fermented extracts have significant antibacterial and antioxidant activities [58].

Among other technological methods to prevent clouding of juices before its lactic fermentation is clarification by using membranes filtration (microfiltration, ultrafiltration...) or clarifying agents (gelatin, bentonite...). Clarification of pomegranate juice, which has a high content of biological active compounds, reduced the phenolic content and the associated antioxidant activity [57].

4.3. Microbiota of Traditional and Recently Developed Lactic Acid Fermented Beverages

The lactic acid fermentation process was used for centuries but still fermentations using plant materials as main substrates are underexplored compared to dairy-based food fermentations, except for a few notable examples which have been subjected to investigation on microbiota.

In water kefir commercial grains, *Z. mobilis* was shown to be the dominating bacteria, representing up to 87% of bacteria [13]. LAB identified in water kefir grains are *Leuconostoc citreum*, *L. mesenteroides*, *L. brevis*, *Lactobacillus buchneri*, *L. casei* subsp. *casei*, *L. casei* subsp. *rhamnosus*, *Lactobacillus diolivorans*, *Lactobacillus fermentum*, *Lactobacillus harbinensis*, *Lactobacillus hilgardii*, *Lactobacillus hordeii*, *Lactobacillus kefirifaciens*, *Lactobacillus kefirii*, *Lactobacillus lactis*, *Lactobacillus mali*, *Lactobacillus nagelli*, *Lactobacillus paracasei*, *Lactobacillus parafarraginis*, *Lactobacillus perolens*, *L. plantarum*, and *Lactobacillus satsumensis* [24,82]. In commercial grains analyzed by metabarcoding, *Lactobacillus* accounted for 12%–25.4% of bacteria, whereas *Leuconostoc* represented 0.4% to 25.6%.

These genera represented between 6.0 and 8.5 log CFU/mL, whereas yeasts and molds accounted for 5.6 to 7.3 log CFU/mL and acetic acid bacteria for 6.0 to 8.3 log CFU/mL [22]. From isolation of 453 bacteria from three water kefir from different origins [83,84] showed the most abundant species were *L. hordei* and *L. nagelii*, representing ca. two third of bacteria, the last third being *L. citreum* or *L. mesenteroides*. The presence of *Bifidobacteria* at low levels has been reported [13,84]. Interestingly, the microbial composition of fermentates diverged from water kefir grains, showing an adaptation of the cultures to the medium and environment [13]. Interactions between microorganisms, especially regarding nitrogen sources, has a key role to explain the balance between microflora [82,85].

In Kombucha, the most frequently encountered species are *L. kefirifaciens* subsp. *kefirgranu*, *Bifidobacteria* and *Weissella* [25]. *Pediococcus pentosaceus* [86] has also been identified in Kombucha.

Turkish *Shalgam* microbiota is mainly composed of yeasts and *Lactobacillus* (89.63%), *Leuconostoc* (9.63%) and *Pediococcus* (0.74%). More specifically, *L. plantarum* and *L. paracasei* subsp. *paracasei* are the most frequent species, followed by *L. brevis* and *L. fermentum*. At the beginning of fermentation, *L. mesenteroides* subsp. *mesenteroides*, *P. pentosaceus*, *L. buchneri*, *Lactobacillus pentosus* and *L. delbrueckii* subsp. *delbrueckii* were present [87,88]. Hardaliye LAB composition comprises *L. paracasei* subsp. *paracasei* and *L. casei* subsp. *pseudoplantarum*, *Lactobacillus pontis*, *L. brevis*, *Lactobacillus acetotolerans*, *Lactobacillus sanfranciscensis*, and *Lactobacillus vaccinoferus* [18].

Sauerkraut fermentation is driven by *L. mesenteroides*, *L. brevis*, *P. pentosaceus*, and *L. plantarum* [44,89]. However, large scale isolate analyses have revealed new species and succession of species during the process [90]. Heterolactic species, *L. mesenteroides*, *Weissella* spp. and *L. citreum*, dominated the beginning of fermentation. Thereafter, *Lactobacillus curvatus*, then *L. plantarum* became dominant [90]. In fermented carrot juice, *Leuconostoc*, with species *L. mesenteroides* and *Leuconostoc pseudomesenteroides* which tolerates better low pH, was present at all stages of fermentation, in a range 0.3% to 24.6% depending on sample origin [91]. *Lactobacillus* was the dominant genus, and its relative abundance reaches 72% after 30 days of fermentation. The *Lactobacillus* phylogenetic groups detected in the fermented carrot juices comprised *L. brevis*, *L. casei*, *Lactobacillus coryniformis*, *L. plantarum*, *L. sakei*, a subpart of the *Lactobacillus salivarius* group, and *L. vaccinoferus*. *Lactococcus* and *Weissella* were found in ca. two third of samples, but with abundances up to 50% and 40% respectively [91].

The recent investigations for the development of lactic acid fermented fruit or vegetable juices particularly focused on LAB growth and effect on health-related properties (Table 2). Different substrates, water kefir, vegetable or fruit juices, treated in most studies to inactivate indigenous flora prior to fermentation, were used. To perform fermentation, the range of bacterial species is more restrictive than the ones which are found in spontaneous fermentation. The main LAB species belong to *Leuconostoc*, *Lactobacillus*, *Weissella*, *Enterococcus* and *Pediococcus* genera. They were isolated from raw or spontaneously fermented vegetables and fruit (Table S1). The most frequent species is *L. plantarum*.

Eventually, strains commercially used for fermentation of juices are mainly from the species *L. plantarum*, *Lactobacillus bavaricus*, *Lactobacillus xylosum*, *Lactobacillus bifidus* and *L. brevis*. These LAB earned the “generally regarded as safe” (GRAS) status and are therefore considered safe for human consumption [4].

Table 2. Lactic fermented fruit or vegetable juices recently developed.

Raw Material	Pre-Fermentative Treatment	Microorganism	Nutritional Impact	Reference
Water kefir beverages				
Cocoa pulp	-	Kefir grains	-	[92]
Kiwifruit, quince, pomegranate, grape, apple, cactus pear	Heat treatment 75 °C 5 min	Kefir grains	Decrease in DPPH ¹ activity	[93]
Carrot, fennel, melon, onion, tomato, strawberry	Heat treatment 75 °C 5 min	Kefir grains	-	[56]
Vegetable-based beverages				
Tomato juice	Pasteurization (commercial)	<i>L. acidophilus</i> , <i>L. casei</i> , <i>L. delbrueckii</i> , <i>L. plantarum</i>	-	[39]
Tomato juice	Sucrose addition, Heat treatment 80 °C 5 min	<i>L. plantarum</i> , <i>W. cibaria/confusa</i> , <i>L. brevis</i> , <i>P. pentosaceus</i> , <i>Lactobacillus</i> sp., <i>Enterococcus faecium/faecalis</i>	Lower decrease in ascorbic acid content, glutathione content and total antioxidant activity in juices started with <i>L. plantarum</i> compared to other conditions	[28]
Red beetroot juice				
	Heat treatment 121 °C 15 min	<i>L. acidophilus</i> , <i>L. casei</i> , <i>L. delbrueckii</i> , <i>L. plantarum</i>	-	[37]
Cabbage juice	Heat treatment 121 °C 15 min	<i>L. plantarum</i> , <i>L. casei</i> , <i>L. delbrueckii</i>	-	[38]
Celery and beetroot juice	Heat treatment 120 °C 15 min	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	-	[94]
Carrot	Heat treatment 80 °C 20 min	<i>B. lactis</i> , <i>B. bifidum</i>	Decrease of 10%–20% of β -carotene and 5%–25% of α -carotene	[95]
Bitter gourd, bottle gourd, carrot	Heat treatment 80 °C 20 min	<i>L. plantarum</i>	-	[96]
Fruit-based juices				
Pineapple	Filtration 0.22 μ m, heat treatment 75 °C 15 s	<i>L. plantarum</i> , <i>L. rossiae</i>	Slight increase in antioxidant DPPH activity for started juices	[97]
Pineapple	Sonication	<i>L. casei</i>	-	[59]
Pineapple, papaya, mango, green tea, black tea	Commercial pasteurized pineapple juice; 75 °C 5 min for papaya and mango;	<i>W. cibaria</i> , <i>W. confusa</i> , <i>W. paramesenteroides</i> , <i>W. soli</i> , <i>L. mesenteroides</i> , <i>L. pseudomesenteroides</i> , <i>L.</i>	Increase in total phenolic content and DPPH activity with several strains on papaya and pineapple	[5]

	infusion 5 min in boiling water for tea	<i>citreum</i> , <i>Leuconostoc lactis</i> , <i>L. paraplantarum</i> , <i>L. plantarum</i>		
Pomegranate	Heat treatment 80 °C 5 min	<i>L. plantarum</i> , <i>L. acidophilus</i> , <i>L. paracasei</i> , <i>L. delbrueckii</i>	Increase in DPPH radical scavenging activity; decrease in anthocyanin content; increase in antioxidant activity more marked with <i>L. acidophilus</i> than with <i>L. plantarum</i>	[98,99]
Pomegranate	Filtration 0.22 µm	<i>L. plantarum</i>	Total phenolic compounds concentration and free radical scavenging activity were better maintained in fermented juice than in control; fermented juice modulated the synthesis of the immune mediators into peripheral blood mononuclear cells	[100]
Pomegranate	-	<i>L. acidophilus</i> , <i>L. plantarum</i> , <i>B. longum</i> subsp. <i>infantis</i> , <i>B. bifidum</i>	Identification of catechin and α -punicalagin in fermented juices; increase in antioxidant activity and concentration of phenolic derivatives during simulated gastrointestinal digestion of fermented juices; fermentation increased bioaccessibility of phenolic compounds	[101]
Pomegranate	Heat treatment 70 °C 5 min	<i>L. plantarum</i> , <i>L. acidophilus</i> , <i>L. delbrueckii</i>	Higher antioxidant activity and total phenolic compounds in juices fermented with <i>L. plantarum</i> compared to unfermented	[102]
Noni	Heat treatment 121 °C 15 min	<i>L. casei</i> subsp. <i>casei</i> , <i>B. longum</i> , <i>L. plantarum</i>	Antioxidant activity of juice fermented with <i>B. longum</i> was better maintained than with other strains and remained similar to initial non fermented juice	[103]
Cherries, tomato, blackberry, prune, white grape juice and aloe vera Kiwifruit, fennels, spinach, papaya, white grape juice and aloe vera	Heat treatment 80 °C 10 min	<i>L. plantarum</i> , <i>L. pentosus</i> , <i>Lactobacillus</i> sp.	Better maintain of radical scavenging activity and total phenolic compound concentration in started juices	[104]
Pear	-	<i>L. acidophilus</i>	Significant increase in α -glucosidase inhibition for most samples of fermented juice	[105]
Cherry juice and broccoli purée	Cherry juice was heat treated at 121 °C for 10 min	<i>L. plantarum</i>	Fermented cherry juice and broccoli puree were enriched in phenolic derivative with high human bioavailability and biological activity	[31]
Coconut water	-	<i>L. plantarum</i> , <i>B. animalis</i> subsp. <i>lactis</i>	-	[106]
Apple, grape or orange juices	Heat treatment 80 °C 20 min	<i>L. acidophilus</i> , <i>L. casei</i> , <i>L. rhamnosus</i> , <i>L. paracasei</i>	-	[14]

Mango	Addition of 50 mg/L potassium metabisulfite and pectinolytic enzymes, pasteurization	<i>L. acidophilus</i> , <i>L. delbrueckii</i> , <i>L. plantarum</i> , <i>L. casei</i>	-	[107]
Cactus pear	Heat treatment 70 °C 5 min	<i>L. mesenteroides</i>	Increase in free radical scavenging activity; started fermented juice markedly inhibited the inflammatory status of Caco-2/TC7 cells; started fermented juice scavenged the reactive oxygen species generated by H ₂ O ₂ on Caco-2 cells; fermented juices variously affected the immunomodulatory activity towards anti- and pro-inflammatory cytokines	[108]
Cactus pear	Dilution 2:1 in water	<i>L. fermentum</i>	No change in total phenolic content during fermentation; decrease in antioxidant activity during fermentation	[109]
Cactus pear	Heat treatment 64 °C 30 min	<i>L. plantarum</i>	Maintain of health-promoting properties in fermented cactus pear juice; fermented juice markedly promoted antioxidant mechanisms in yeast cells; significant decrease in the body weight gain and amelioration of the insulin resistance, hyperglycemia, and hyperlipemia after feeding obese mice with fermented juice	[110]
Carambola, guava, mango, pitaya	Heat treatment 80 °C 5 min	<i>L. casei</i> , <i>S. thermophilus</i> , <i>L. bulgaricus</i>	-	[16]
Sweet lemon juice	Heat treatment 80 °C 5 min	<i>L. plantarum</i>	No effect of fermented juice on ascorbic acid; decrease in total phenolic compound concentration; higher antioxidant activity (DPPH and FRAP ²) in fermented juice during 28 days of storage compared to unfermented juice	[111]
Cashew apple juice	Heat treatment 70 °C 15 min	<i>L. plantarum</i> , <i>L. casei</i> , <i>L. acidophilus</i>	Decrease in hydrolysable tannin content during fermentation; juice fermented with <i>L. plantarum</i> retained better free radical scavenging activity (DPPH and ABTS ³) than with other strains	[112]
Sohiong juice	Heat treatment 70 °C 5 min	<i>L. acidophilus</i> , <i>L. plantarum</i>	-	[113]
Apple, carrot, tomato, cucumber, haw (40:25:15:15:5)	Heat treatment 80 °C 20 min	<i>L. plantarum</i> , <i>L. casei</i> , <i>L. acidophilus</i> , <i>L. fermentum</i> , <i>L. rhamnosus</i>	-	[114]

¹: DPPH (1, 1-diphenyl-2-picrylhydrazyl), ²: FRAP Ferric ion Reducing Antioxidant Power, ³: ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)]

4.4. Directing Fermentation

Controlled lactic acid fermentation of fruit and vegetables appeared as a way to develop functional food with standardized and reproducible quality using characterized bacterial strains. This can be achieved using autochthonous, isolated from and re-used on the same raw matrix, or allochthonous, isolated from certain raw matrices but used to ferment various products, starters (Table 3) [10].

Table 3. Properties of autochthonous and allochthonous strains.

Autochthonous Strains	Allochthonous Strains
Highest viscosity of the juice [10]	
Highest values of ascorbic acid, glutathione and total antioxidant activity during storage [10]	
Rapid decrease in pH [28]	Longer latency phases of growth and acidification [10]
Marked consumption of fermentable carbohydrates [28]	
Inhibition of <i>Enterobacteriaceae</i> and yeasts [28]	
Positively affected firmness and color [28]	

A study done by Di Cagno et al. [28] compared autochthonous *L. plantarum* starters to allochthonous strains isolated from green olives during fermentation of tomato juice. The results showed that allochthonous strains presented longer latency phases of growth and acidification and that autochthonous strains conferred the highest viscosity to the juice. Indeed, after fermentation at 25 °C for 17 h autochthonous strains grew from approximately 7.0 to 9.6 log CFU/mL and allochthonous strains reached approximately 8.5 log CFU/mL. Compared to spontaneously fermented pineapple juice, started juice using autochthonous *L. plantarum* and *Lactobacillus rossiae* had up to 10⁶-fold more LAB, a lower number of yeasts, better preservation of the natural colors, and better overall acceptability [10]. Moreover, juices fermented with autochthonous strains maintained the highest values of ascorbic acid, glutathione and total antioxidant activity during storage [10]. In another study, peeled and cut carrots, French beans and marrows fermented with autochthonous starters isolated from these matrices, namely *L. plantarum*, *L. mesenteroides* and *P. pentosaceus* showed a rapid decrease in pH, marked consumption of fermentable carbohydrates, and inhibition of *Enterobacteriaceae* and yeasts. These results were not obtained using allochthonous starters belonging to the same species. There are also differences between autochthonous and allochthonous strains regarding the concentration of vitamin C, color indexes, firmness and sensory properties. Indeed, autochthonous strains dominated the microbiota and positively affected firmness and color [10]. Moreover, matrices fermented with the autochthonous starter had significantly higher total concentration of vitamin C compared to those fermented with the allochthonous starter [115].

These few examples demonstrate the advantages of the selection of autochthonous LAB to perform lactic acid fermentation.

4.5. Nutritional Changes and Functional Effects Mediated by LAB Activity

Originally devised as a means of food preservation, lactic acid fermentation, by using active microorganisms induces changes in the product physicochemical composition. The liquid phase fermentation occurring in beverages leads to rapid bioconversion, together with the direct release of the metabolites into the culture medium by comparison with solid-state fermentations [116]. The LAB strains selected for fermentation possesses functional properties such as degradation of toxic and anti-nutritional compounds, generation of bioactive molecules, enhancement of the bioavailability of nutritive compounds and may be considered as probiotics [33].

Lactic acid is recognized as the main metabolite of LAB and acidification is one of the desirable effects of their growth. As a consequence, the pH dropped along the time due to the increase in lactic acid content in fermented beverages. It is observed that the stoichiometric ratio between glucose, fructose and malic acid consumed, and lactic acid and acetic acid synthesized was almost respected

in fermented pomegranate juice by *L. plantarum* [100,117]. The LAB metabolism of carbohydrates varies however from strain to strain and depends on the substrate and even on the fermentation time (Table S2). Fructose appeared to be the most consumed sugar during fermentation of Brazilian cuapussu by *L. casei* (84.8%), followed by sucrose (62.1%) and glucose (34.5%) [118]. Glucose and sucrose were the main carbon and energy sources for *Bifidobacterium* growth in carrot juice [95] and for *Lactobacillus* in pomegranate juice [98].

A study comparing *Lactobacillus* strains in apple, orange, and grape juices showed that either *L. paracasei* or *L. plantarum* were able to decrease glucose, malic, tartaric and citric acids when these acids are available while they were able to increase lactic and succinic acids [119]. *L. casei* was reported to use quinic acid for compound synthesis and reductions in citric acid levels were reported during the fermentation of pomegranate juice, while *L. plantarum* might be able to metabolize this acid from catechol [98,119]. During elderberry juices fermentation, both malic and citric acids were extensively metabolized by different *Lactobacillus* spp. while glucose and fructose were almost unaffected suggesting a switch in the microbial metabolism with the use of organic acids instead of sugars in an environment considered as hostile [120]. Combination with treatment by pectinolytic enzymes on wild berries, used in industry for increasing yield and clarify the juice, led to apparent contradictory effects [121]. Where chokeberry fermentation with different *L. plantarum* strains led to significant reduction of total sugars concentrations and decreased sugar/acid ratio, the same protocol for sea buckthorns led to an increase in pH and sugar/acid ratio. It seems due to a higher content (or lower reduction) of quinic acid and to the presence of galacturonic acid derived from pectin degradation during enzymatic incubation.

Besides the production of organic acids, LAB also have the ability to contribute to the production of several important bioactive compounds through fermentation. Fruit and vegetable juices are valuable sources of vitamins, minerals and antioxidant molecules providing a suitable growth substrate for LAB in parallel with a strong health appeal. Beverages fermentation with various strains of LAB has been largely reported to increase the antioxidant activity [100,111,117,118,122–125] sometimes together with an increase in total phenols and flavonoids content [120,126] (Table 2). This is the case for many and varied matrices: pomegranate, blueberry, mulberry, cupuassu (*Theobroma grandiflorum*), elderberry, *Momordica charantia* L. and to date, only prickly pear beverages among all lactic fermented beverages was not linked to an increased antioxidant activity by comparison with non-fermented preparation [109]. It is suggested that modification in juice polyphenol profile could be linked to this increase in radical scavenging activity (Table 4). This is in line with data relative to mulberry juice [124]. A total of 11 phenolic acids, three anthocyanins and five flavonoids were identified in mulberry and it was observed that the concentration of all the phenolic compounds quantified in the fermented sample increased after fermentation either by *L. paracasei*, *L. plantarum* or *L. acidophilus*. The correlation analysis additionally demonstrated that flavonols and anthocyanins were mostly responsible for the increased in ABTS assay (2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) scavenging activity while phenolic acids and flavonols were responsible for DPPH assay 2,2-diphenyl-1-picrylhydrazyl) scavenging activity and reducing power capacity of the fermented juice. The conversion of complex phenolics into free forms, decarboxylation and depolymerization of high molecular weight phenolic compounds by LAB enzyme are also observed in *Momordica charantia* L. or cherry juices, leading to the conversion of dihydrocaffeic acid from caffeic acid or catechol from protocatechuic acid by different *Lactobacillus* subspecies, and the release of numerous flavonols, anthocyanins and phenolics acids according to the initial matrix [123,124,127]. *Lactobacillus* spp. used for cherry juice and broccoli puree fermentation exhibited strain-specific metabolism of phenolic acids, including the release of different hydroxybenzoic acids, hydroxycinnamic acids, and hydroxycinnamic acid derivatives. In this case, *L. plantarum* also exhibited the most extensive conversion of protocatechuic, caffeic and p-coumaric acids. *L. reuteri* did not affect the profile of phenolic acids as it was the only strain capable of hydrolysing chlorogenic acid [119]. Some of the released compounds during fermentation, such as dihydrocaffeic acid or pyrogallol, are strong antioxidants and can explain the enhanced antioxidative properties observed in fermented juices. Furthermore, the stability of polyphenols is pH-dependent. Hence, the decrease

in pH due to lactic acid released might stabilize the phenolic compounds of the fermented beverages [124].

The refinement of polyphenols by specific microorganisms may participate in the elaboration of fruit and vegetable juices offering optimized nutritional profiles. Polyphenolic compounds are active against reactive oxygen species (ROS) by scavenging unpaired electrons, and numerous studies attempt to show their interest to limit the risk of various chronic diseases associated with oxidative stress [128,129]. Some of the released metabolites along lactic fermentation are able to exert biological activities more relevant to human health in respect to their parent phenolic compounds. For instance, dihydrocaffeic acid was reported to be a more potent antioxidant than caffeic acid and can bioaccumulate inside the endothelial cells employing an intracellular antioxidant activity [130,131].

Phenolic compounds are considered as good regulators of carbohydrate metabolism through the inhibition activities of α -glucosidase and α -amylase due to their chelating, structure altering, and biological function-limiting capacity to the enzymes [132,133]. Further, antioxidant-rich plant foods have shown an ability to inhibit α -glucosidase and α -amylase, in addition to pepsin and pancreatic lipase [134–136]. The observed potent inhibitory activities limit or delay starch digestion and absorption, and were connected to subsequently reduce postprandial glycemic response in vivo [135]. Therefore, beverages with a refined polyphenol profile would constitute a promising opportunity for the development of functional foods relevant to diabetes management.

Table 4. Review of the recent studies on changes in polyphenol profile with fermentation of fruit and vegetables by LAB.

Juices	Compounds Metabolized	Compounds Released	LAB Species	Ref.
Apple	QA, Q, Q-3-gal, Q-3-glc, phlorizin	5-O-caffeoylquinic acid, quercetin, phloretin	<i>L. plantarum</i>	[125]
Broccoli	CGA, QA	PA, sinapic acid	<i>L. reuteri</i>	[31]
	Sinapic acid	PA	<i>L. plantarum</i> or <i>L. mesenteroides</i>	[137]
Black chokeberry	CA, CGA, neochlorogenic acid	Anthocyanins: C-3-gal, C-3-glc, C-3-xyl	<i>L. plantarum</i>	[121]
Cherry	PTA, CA	catechol, DCA	<i>Lactobacillus</i> spp.	[31]
Elderberry	PTA, CA	DCA, catechol, flavonols glycosides (Q-3-rut, Q-3-glc) and anthocyanins (C-3-samb, C-3-glc)	<i>L. plantarum</i> , <i>L. rhamnosus</i> and <i>L. casei</i>	[138]
Momordica charantia L.	CA, p-CA, p-HBA, vinylphenol, catechol, PTA, BA, vanillin	DCA, phloretic acid, vinylcatechol, pyrogallol	<i>L. plantarum</i>	[123]
Mulberry		Flavonols (catechin, rutin, morin, quercetin, kaempferol) Anthocyanins (C-3-glc, C-3-O-rut, esculin) Phenolic acids (gallic acid, syringic acid, vanillic acid, quinic acid, ferulic acid, cinnamic acid, PTA, CGA, CA, p-HBA, p-CA)	<i>L. plantarum</i> , <i>L. acidophilus</i> or <i>L. paracasei</i>	[124]
Sea buckthorn	Q-3-glc, I-3-glc	PTA	<i>L. plantarum</i>	[121]

Abbreviations: CA, caffeic acid; CGA, chlorogenic acid; PTA, protocatechuic acid; QA, quinic acid; PA, phloretic acid; DCA, dihydrocaffeic acid; BA, benzoic acid; p-HBA, p-hydroxybenzoic acid; p-CA, p-coumaric acid; Q, quercetin; I, isorhamnetin, C, Cyanidin; gal, galactoside; glc, glucoside; rut, rutoside; xyl, xyloside; gal, galactoside; samb, sambubioside.

The presence of antinutritive compounds in some plant foods such as tannins, saponins, oxalates, phytic acid or phytates can contribute to bitter taste and mainly to a lower nutrient absorption or utilization. Lactic acid fermentation is a long-established processing method to reduce toxicity in tubers and cereals, like cassava or sorghum [139–141]. On beverages, a study on *Momordica charantia* juices fermented with *L. plantarum* showed a significant decrease in total saponin content compared with fresh juices and that might be correlated with the enzyme activities of *L. plantarum* (such as β -glucosidase) which are responsible for hydrolyzing momordicoside to aglycones [123].

Some of the LAB strains are able to produce exopolysaccharide (EPS), which interacts with the water molecules and control the rheological properties and physical stability of foods. The typically branched heteropolysaccharides are synthesized intracellularly by some strains of *Lactobacillus*, *Lactococcus*, *Streptococcus*, and *Bifidobacterium* spp. The homopolysaccharides, including glucans, fructans and galactans, are obtained via the action of an external enzyme of some strains of *Lactobacillus*, *Leuconostoc*, *Streptococcus* and *Weissella* [142]. An EPS production resulted in an increased viscosity of fermented litchi juice, along with fructose production, reflecting that EPS synthesis [60]. In pureed carrots, the formation of low-branched dextran was correlated with thickness, while the production of β -glucan was correlated with elasticity perception. *Weissella confusa* and *L. lactis* strains (low-branched dextran-producers) produced pureed carrots with a thick texture and a pleasant odor and flavor [143]. It was also reported that EPS are contributors to improved sensorial acceptance for fermented products [144].

Antioxidant properties were reported for EPS produced by strains of *L. plantarum*, *L. lactis* subsp. *lactis* and *L. paracasei* subsp. *paracasei*. Some specific EPS also exhibit significant in vitro immunomodulatory properties [145–147] which is of interest for functional beverages development since it was reported that EPS produced by *Weissella cibaria* and *L. plantarum* are resistant to the gastrointestinal tract conditions [148].

5. Probiotication

5.1. Probiotic Characteristics and Health Benefits

LAB comprise a significant component of the human gut flora and have beneficial roles in the gastrointestinal tract. They may be considered as probiotics, that are living microbial food supplements, which beneficially affect the host by improving the intestinal microbial balance [11]. The *Lactobacillus* genus bacteria are able to compete with pathogens for adhesion sites, to antagonize pathogens or to modulate the host's immune response, which will improve nutrition, soothe intestinal disorders, improve the immune system, optimize gut ecology and finally promote overall health [11]. The *Bifidobacterium* genus are also interesting for fermentation due to their ability to colonize the intestinal tract and control undesirable intestinal bacteria [149]. According to literature data and the suggestions of the Food and Drug Administration (FDA) / World Health Organization (WHO), in order to fulfill health benefits of probiotic beverages, the strains should have the ability to survive at unfavorable conditions at a concentration of at least 6–7 log CFU/mL and also maintain activity in the final product at the end of the shelf life [150,151].

The LAB population in recently developed lactic fermented fruit or vegetable juices was above the limit of 6–7 log CFU/mL, required for probiotic products, at the end of fermentation and even after a storage period of fruit and vegetable beverages. The criteria for a LAB to be used as probiotic include the abilities to withstand into a foodstuff at high cell counts, and remain viable throughout the shelf-life of the product; be nonpathogenic and nontoxic; withstand transit through the GI tract; adhere to the intestinal epithelium cell lining and colonize the lumen of the tract; produce antimicrobial substances towards pathogens; stabilize the intestinal microflora and be associated with health benefits [152]. Basic relevant criteria for LAB selection to develop functional fruit and vegetable beverages are resumed in Figure 2. Scientific investigation has supported the important role of probiotics as a part of a healthy diet for human as well as for animals. Some of the beneficial effects of LAB consumption include improving intestinal tract health; enhancing the immune system; synthesizing and enhancing the bioavailability of nutrients; reducing risk of certain cancers and cardiovascular diseases [152–155]. Therefore, LAB use is of interest for functional food development.

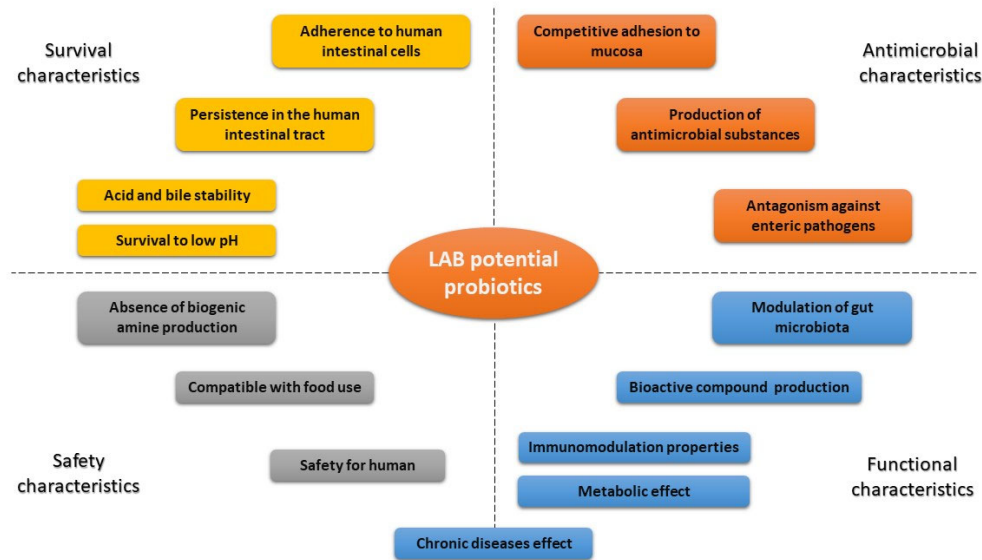


Figure 2. Probiotic strains characteristics of selection for fermented fruit and vegetable beverages.

Only a few studies to date investigated the potential effects of fruit and vegetable juices as carrier for probiotics. In an attempt to increase flavanone bioavailability, orange juices were orally administered to human volunteers together with microencapsulated *B. longum* within a protein-carbohydrate-oil emulsion. Flavanone bioavailability, however, assessed by 0–24 h urinary excretion, was similar when the juices were consumed with and without acute probiotic intake [156]. Tomato and feijoa juices were compared as fermentable carriers of *L. plantarum*. The fermented tomato juice showed the largest improvement to intestinal barrier integrity. However, once digested, it did not increase the trans-epithelial electrical resistance on Caco-2 cells, although *L. plantarum* adhered well to the cells [157]. Blueberry juices mixed with dry probiotic tablets (*Bifidobacterium infantis*, *Bifidobacterium animalis* and *L. acidophilus*) were used for supplementation in rats Non-Alcoholic SteatoHepatitis (NASH) models () and significantly reduced metabolic factors, hepatic inflammation and apoptosis connected to NASH progression by affecting the SREBP-1c/PNPLA-3 pathway via PPAR- α [158].

5.2. Optimized Inoculation to Ensure LAB Viability

Viability and metabolic activity of the bacteria are important features of probiotic inclusion in beverages. For this purpose, LAB should be first inoculated in adequate amounts to provide benefits to the host and viability should be ensured throughout the shelf life. Different strategies were assessed to optimize living LAB in juices (Table 5).

5.2.1. Immobilization

The encapsulation approach to extend shelf life of lactic acid fermented juices and maintain cell viability of bacterial strains under storage conditions was discussed specially for fruit juice, wherein LAB may show some sensitivity to acidic conditions due to the typically low pH, between pH 2.5 and 3.7. The microencapsulation of *L. casei* with sodium alginate by emulsion system showed difference in viability according to the fruit juice. A 100% rate of viability in pineapple juice and 91% in orange juice was found whereas no viability was found at the end of the storage period in raspberry juice, because of the absorption of high concentrations of anthocyanin inside microcapsules [159].

The stability of free and entrapped *L. rhamnosus* in alginate and silica-coated alginate carriers was compared in apple juice during cold storage [160]. Encapsulation being more efficient for LAB viability with core-shell alginate-silica beads in both the filtrate and inside the carriers.

Pineapple juice was proved to be a suitable carrier for encapsulated probiotic cell viability whereas microcapsules and free cells were not stable in strawberry-apple juice and died after 14 days of storage at 22 °C [161].

Viability of encapsulated *L. casei*, *L. plantarum* and *L. fermentum* cells was higher than free cells in tomato and carrot juices over a period of cold storage, indicating better cell protection using alginate coated chitosan beads even if the sensory quality and the turbidity of vegetable juices changed [162].

Viable count of encapsulated *L. plantarum* in grapes, orange, and watermelon juices was evaluated as 7 log CFU/mL or more throughout the storage whereas, the viable counts of free cells were remarkably dropped to 2–3 log CFU/mL during the storage [163]. Authors reported that the encapsulation of probiotics in alginate beads can protect the inside cells from oxygen and high concentrations of substrates or products, and unfavorable conditions like low pH, in fruit juices.

Microencapsulation by spray drying using maltodextrin or inulin as encapsulating agents affects the survival rate, as highlighted in passion fruit juice added with *B. animalis* ssp. *lactis*. During storage at 25 °C, the maltodextrin encapsulated samples showed lower viability, showing that inulin may offer better protection to the lactic bacteria at this temperature. No difference was detected at 4 °C [164].

Comparison of beverages containing commercial pectin or pectin extracted from passion fruit pulp as probiotic carrier for *L. rhamnosus* showed no differences according to the carrier, with 100% survival rates when the juices were not fermented. In fermented beverage survivals were 73% for commercial pectin and 60% when CP and PE for pectin extracted, respectively [165].

Vivek et al. [166] conducted a study to characterize and evaluate the physical stability of spray dried probiotic Sohiong fruit powder during storage. The determination of moisture isotherms at various temperature and relative humidity were made. It was found that spray drying technique played a role to preserve and maintain *L. plantarum* cells to be viable (10^6 cells/g) after long period of storage.

The suitability of entrapping *L. acidophilus* on the k-carrageenan to perform tomato juice fermentation was evaluated [167]. Authors showed that the unfavorable conditions in tomato juice could be overcome and certain benefits on cell viability were provided by LAB immobilization in the fermented juice.

Finally, a study investigated the impact of the probiotic addition methodology on the quality parameters of orange juice [168]. The direct addition of the commercial culture appeared to be the most suitable methodology, resulting in products with physical and chemical characteristics and sensory acceptance similar to those of the puree product, and improved the volatile compounds profile contrary to the addition of the activated probiotic culture or encapsulated probiotic culture.

5.2.2. Antioxidants

Damage to cell membranes due to oxidation might occur during long-term storage and antioxidants addition was tested to examine their influence on LAB viability in fruit juices. In a model juice made with sucrose, sodium citrate and citric acid powder, the addition of green tea extract, vitamin C, or grape seed extract showed better survival of LAB bacteria (*L. rhamnosus*, *L. paracasei*, *B. lactis*) after 6 weeks of storage, than the addition of vitamin B2, vitamin B3, vitamin B6 or vitamin E [169]. The effect of melon juice supplementation with phenolic compounds in the form of herbal extract, was also studied on the viability of six probiotic-type LAB [170]. This study highlighted the synergistic interaction between antioxidant compounds of melon juice and herbal phenolic extract to improve the survivability of probiotic LAB under cold storage for 4 days.

5.2.3. Co-Inoculation

As yeast also played an important role in the production of many traditional fermented foods and beverages, it was supposed they might positively stimulate the abundance of LAB in fermented environments. This novel technological approach was tested on non-edible *Prunus mahaleb* allowing to efficiently reduce sourness making *P. mahaleb* beverage suitable for consumption. Co-inoculation

by *Saccharomyces cerevisiae* and *L. plantarum* resulted in a high cell count of approximately 7–8 log CFU/mL after 4 days of fermentation. However, strains were both slowed down from day 8 to the end of fermentation, contrary to fermentation by *L. plantarum* alone [171].

Yeast use for co-inoculation seems to be conditioned to strain compatibility, since the addition of *Williopsis saturnus* did not bring any effect on the survivability nor on substrate transformation by bifidobacteria, leading to early death of the yeast on durian pulp puree. This is in contrast to the enhanced viability of *L. casei* and sugar metabolism (fructose and glucose) as well as the various volatile compounds (alcohols, esters and aldehydes) produced that may improve the aroma profiles of the fermented durian pulp puree [172].

Table 5. Process parameters for probiotication of fruit and vegetable juices.

Processing Conditions	Raw Material	Mains Findings	Reference
Bleaching (60 °C/20 min), extraction, clarification (muslin cloth); autoclave sterilization (121 °C/15 min); encapsulation of <i>Lactobacillus</i> spp and <i>S. cerevisiae</i> using alginate coated chitosan beads; cold storage (6 weeks)	Tomato and carrot	Survivability of encapsulated probiotic cells in a blended juice of tomato-carrot fruit was improved; overall sensory acceptance (taste profile, mouthfeel, acidity, flavor, aroma, color)	[54]
Filter sterilization; immobilization of <i>L. acidophilus</i> in an alginate CaCl ₂ beads	Grapes, orange, watermelon	Immobilized cultures remained viable over a long period of time; probiotically drinks were potentially inhibiting the pathogenic growth	[163]
Model juice (sucrose, sodium citrate, citric acid powder, distilled water); water bath pasteurization (85 °C/10 min); enrichment by antioxidants (grape seed and green tea extracts) and vitamins (B, C); probiotication by (<i>L. rhamnosus</i> , <i>B. lactis</i> , <i>L. paracasei</i>); storage (6 weeks)	Model juice	Turbidity and viscosity of model juice increases → do not support growth of probiotics; combined effect of probiotics and bioactive ingredients → improve cells viability on model juice	[169]
Sterilized filtration (<i>stainless steel strainer</i>) of juice pulp; polyphenol-fortified melon juice; inoculation by LAB probiotics and incubation (4 °C/4 days)	Melon	Herbal extract based additive → increases antioxidant activity; delivering viable LAB + survivability and growth into refrigerated enriched juice	[170]
Electrospraying assisted microencapsulation of <i>L. rhamnosus</i> → Silica-coated alginate beads; freeze drying (85 °C; 0.2 mbar; 20 h) of microcapsules; addition of these microcarriers to pasteurized apple juice	Apple	Silica-coated beads diminished the leakage of <i>L. rhamnosus</i> when compared to alone alginate beads → More protection and survival	[160]
Inoculation <i>L. acidophilus</i> of juice mixture (70:30 v/v); air spray drying (T _{air} : 120–160 °C; flow rate: 25 mL/min); with maltodextrin	Watermelon and carrot	Higher T _{air} + longer time + lower flow → Reducing of cell viability, lycopene and β-carotene of dried juice (powder); increased atomization pressure → Better cohesiveness but less flowability of juice powder	[81]
Ultrasonic (90%/9.95 min), pectinase (0.05% w/w) extraction of juice; sonicated pasteurization (15 min); fermentation by <i>L. plantarum</i> ; Anticaking agent + maltodextrin; spray drying (concurrent, 120 °C, 201 mL/h); storage (25 °C, 50% RH)	Sohiong fruit (Indian blackberry fruit)	A decreasing trend of probiotics viability during storage → This might be the result of stress during spray drying + thermal shock, oxygen and humidity exposure at ambient storage; dried probiotic juice could be maintained up to 36 days without packaging	[166]
Mixing (45 s); short pasteurization (95 °C/15 s); skin and seeds removal (mesh screen); juice was kept (88 °C/2 min); <i>L. acidophilus</i> cell immobilization k carrageenan beads; free and cell-immobilized fermentation of tomato juice; cold storage (4 °C/10 weeks)	Fresh raw tomato	Fermentation was improved by immobilized cells → protection effect against adverse environment of tomato juice; immobilization increased growth and viability of <i>L. acidophilus</i> during fermentation and storage compared to the free cells; high overall palatability score (before and during cold storage) of juice	[167]

6. Perspectives Related to LAB Physiological Traits and Metabolism of Phenolic Compounds

Fruit and vegetable juices contain a large diversity of carotenoids and phenolic compounds. These compounds are involved in antioxidant activity and their bioconversion by LAB has been demonstrated in many fruit and vegetables.

The effect of processing, including lactic acid fermentation, on antioxidant compound composition is a key stone to understanding health-promoting properties of fermented fruit and vegetable beverages [33,57]. Antioxidant compounds include carotenoids, flavonoids such as anthocyanins, procyanidins, and phenolic acids, including condensed and hydrolyzable tannins. Many of these compounds are glycosylated and the glycosidic group nature and position influences their absorption and biological activity. The enzymatic machinery of LAB involved in metabolization of these compounds is still poorly characterized [33,157,173–176].

Only recent studies investigated the use of metabolomics to decipher changes in beneficial bioactive compounds resulting from lactic fermentation of fruit and vegetable juices [101,177,178]. The development of analytical methods and data analyses may be used to predict the nutritional (and sensory) quality of fermented foods. Interestingly, Tomita et al. (2017) [179] discriminated vegetable (leaves and roots) juices fermented with different *Lactobacillus* strains on the basis of fermentative metabolites and low-abundance metabolites.

Recent developments into comparative genomics, functional genomics, transcriptomics and metabolomics open new horizon for tailoring LAB strain selection for specific ecological niches or metabolism of phenolic compounds. Reconstruction of fermentative metabolic pathways have been successfully applied for *L. mesenteroides* [180]. This approach can be implemented together with the collection of transcriptional datasets to increase knowledge on metabolic pathways and identification of specific traits relevant for the adaptation of LAB to the ecological niche, and for phenolic compound metabolism [181]. Functional genomics, transcriptomics and metabolomics would then contribute to exploit the potential of fruit and vegetables for human health.

7. Conclusions

Fruit and vegetable juices are suitable substrates for lactic acid fermentation or as vectors for probiotic bacteria. The combination of active LAB with fruit and vegetable properties presents numerous nutritional advantages. Moreover, synergistic effects can be expected resulting from LAB metabolism. The advantages of the combination cover lactose-free beverages, specific taste, production of EPS, decrease in sugar content and production of bioactive compounds. LAB consumption can improve intestinal tract health, enhance the immune system, synthesize and enhance the bioavailability of nutrients and reduce the risk of certain cancers and cardiovascular diseases. LAB fermented fruit and vegetable beverages may provide a new asset for metabolic disease management.

Processing of juice, especially with alternative technologies to heat treatment, is not only beneficial as a pre-fermentative microbial decontamination treatment but can affect LAB activity and resulting biochemical modifications. Alternatives to conventional heat treatments and pre-treatments of beverages contribute to preserving nutritional properties, reducing oxidative damages of LAB, increasing functional value and promoting the growth of fermentation microorganisms. Encapsulation or addition of antioxidants has an effective role in maintaining probiotic LAB viability.

Future challenges come from a rational tailored selection of strain—substrate—processing in order to increase the nutritional, sensory and microbiological quality of resulting beverages. To that aim, “omic” approaches open new perspectives.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1. Table S1: Species of lactic acid bacteria, which were isolated from raw or spontaneously fermented vegetables and fruit; Table S2: Review of the recent studies on sugars and organic acids metabolism by LAB along fermentation of plant beverages.

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1.2.3. Adaptation des bactéries lactiques aux fruits

La fermentation lactique des aliments peut se faire de manière spontanée ou induite par utilisation de « starters » (Di Cagno et al., 2008). La fermentation lactique spontanée est caractérisée par un écosystème contenant des bactéries lactiques homofermentaires ou hétérofermentaires, d'autres bactéries et possiblement de levures. Elle peut se produire lorsque les conditions d'anaérobiose, d'activité de l'eau, de concentration en sel et de température sont favorables (Di Cagno et al., 2013). La fermentation spontanée peut aussi être déclenchée par « back slopping » où une matrice préalablement fermentée spontanément sert à inoculer la nouvelle matrice à fermenter. Cette technique est traditionnellement utilisée lors de la fermentation de la choucroute afin de favoriser la fermentation par le développement des souches les mieux adaptées et ainsi réduire le temps de fermentation (Di Cagno et al., 2013). Toutefois, la fermentation spontanée peut ne pas inhiber suffisamment la croissance de micro-organismes pathogènes et entraîner des modifications indésirables et non prévisibles des propriétés sensorielles, nutritionnelles et rhéologiques. Ces facteurs ont contribué à privilégier l'induction de la fermentation par des « starters ». Les « starters » sont des bactéries sélectionnées et ajoutées pour initier la fermentation. Ils permettent d'améliorer de manière reproductible la qualité des produits fermentés (Di Cagno et al., 2013).

Les bactéries lactiques sélectionnées sont historiquement utilisées pour la fermentation de lait mais leur emploi s'est diversifié pour obtenir divers aliments fermentés à partir de céréales, fruits et légumes, légumineuses, racines et tubercules, viandes ou poissons (Di Cagno et al., 2008; Tamang et al., 2016). Les « starters » peuvent avoir une origine autochtone ou allochtone (Di Cagno et al., 2013). Les cultures autochtones sont isolées à partir d'une matrice et utilisées pour la fermentation de cette même matrice tandis que les cultures allochtones sont isolées d'une certaine matrice et utilisées pour fermenter une autre matrice. La majorité des « starters » commerciaux sont des souches issues de produits laitiers (Di Cagno et al., 2013). S'ils sont utilisés pour fermenter des fruits, ce sont des « starters » allochtones.

La sélection de « starters » se fait suivant plusieurs critères, parmi lesquels l'adaptation et la tolérance à l'environnement, le taux de croissance et la production d'acides à partir des substrats carbonés (Juturu & Wu, 2018).

Une fois inoculées, les bactéries lactiques produisent de l'acide lactique en quantité importante, ce qui provoque une acidification du milieu et contribue à éliminer ou inhiber des organismes pathogènes (Juturu & Wu, 2018). L'abaissement de pH par la fermentation lactique dans les fruits, déjà acides, présente un intérêt moindre pour la sûreté des aliments par comparaison aux légumes ou produits laitiers (Karovičová & Kohajdová, 2003).

La viabilité et l'activité métabolique des micro-organismes sont influencées par la température, l'acidité du milieu, la teneur en oxygène, la présence de substances antimicrobiennes et le manque de nutriments (Gobbetti et al., 2010). La température est un paramètre impactant la croissance bactérienne et l'activité enzymatique et elle peut être adaptée à la bactérie (Hur et al., 2014). La teneur élevée en sucres dans les fruits fournit aux bactéries des substrats carbonés en abondance pour leur croissance, mais peut conférer un niveau de stress osmotique non négligeable. La tolérance des bactéries à de fortes concentrations en composés phénoliques est aussi un facteur de sélection pour la fermentation de matrices végétales au regard de leur abondance dans les fruits (Di

Cagno et al., 2013). Toutefois, le stress acide conféré aux bactéries par les fruits est le paramètre non maîtrisable le plus impactant pour l'activité des « starters ».

La présence d'acides organiques, tartrique, citrique et malique le plus souvent, est responsable des pH bas dans les fruits. Ce niveau d'acidité des fruits représente un facteur de stress pour les micro-organismes. Par exemple, des souches de *Lb. acidophilus*, *Ltp. plantarum*, *Lb. delbrueckii* et *Lacticaseibacillus* inoculées individuellement se sont mieux développées dans un bouillon De Man, Rogosa and Sharpe (MRS), sélectif des bactéries lactiques, que dans des jus de grenade. La différence de pH entre les milieux, à savoir un pH 5,6 pour le milieu MRS contre un pH initial de 3,1 pour le jus de grenade qui induit un stress acide, explique les différences de croissance (Mousavi et al., 2011). L'activité métabolique des bactéries a été alors fortement impactée : il en a résulté une diminution de la vitesse de consommation de sucre et de production d'acide. L'utilisation des acides organiques par les bactéries lactiques vise en partie à réduire ce stress acide du milieu (Papadimitriou et al., 2016). La même étude a montré que la croissance et l'activité métabolique des bactéries peut reprendre dans le jus de grenade après une phase d'adaptation, conduisant ainsi à une augmentation du pH par consommation de l'acide citrique (Mousavi et al., 2011).

Le maintien du pH intracellulaire nécessite de l'énergie, sous forme d'ATP (Chaudhary, 2019). Les acides organiques sous forme non ionisée entrent dans les cellules microbiennes par diffusion, ils se retrouvent dans un milieu neutre et donc sont ionisés. La cellule doit excréter des protons par transport actif pour maintenir son pH intracellulaire et consomme ainsi de l'ATP (Guan & Liu, 2020). Cette utilisation énergétique est aux dépens d'autres fonctions cellulaires et peut ainsi provoquer un stress, voire la mort des cellules (Chaudhary, 2019). La majorité des bactéries est capable de s'adapter et de survivre lors de la diminution du pH du milieu dans lequel elles se développent. Une tolérance à l'acidité est alors induite grâce à des mécanismes de résistance, e.g. altération des membranes cellulaires et régulation du métabolisme (Guan & Liu, 2020). L'acquisition de la résistance au stress acide par les bactéries lactiques est nécessaire pour leur survie après ingestion dans le tractus gastro-intestinal, au vu de l'abondance des acides gastriques présents (Guan & Liu, 2020; Wang et al., 2018).

Des souches de *Ltp. plantarum*, *Lb. acidophilus* et *Lacticaseibacillus casei* qui survivent bien dans des milieux acides ont été décrites (Chaudhary, 2019). La tolérance des souches au stress acide présente toutefois une diversité au niveau intra-espèce (Fessard & Remize, 2019). Ainsi, il est légitime de penser que les « starters » autochtones vont être plus facilement adaptés à la matrice végétale. L'isolement de souches à partir des microbiotes des fruits permet de collecter des bactéries adaptées *a priori* à ces matrices.

Une étude réalisée au sein du laboratoire QualiSud a permis d'établir les conditions optimales de culture de 77 souches de bactéries lactiques isolées de fruits et légumes de La Réunion (Fessard & Remize, 2019). Treize espèces ont été collectées parmi cinq genres bactériens. Pour chacun des isolats, la tolérance aux stress acide et oxydant et les paramètres de croissance bactérienne, dont la température optimale, ont été évalués. Les souches bactériennes *Ltp. plantarum* 17a et *W. cibaria* 21 et 64 ont montré une résistance aux stress acide et oxydant. La souche *Ltp. plantarum* 75 est également résistante au stress oxydant et a montré une meilleure croissance dans un milieu MRS à pH 6,5, avec la souche *W. cibaria* 64, par comparaison aux autres souches testées. La température optimale de croissance des isolats se situe entre 25 et 30 °C pour les espèces de *Leuconostoc* et est supérieure à 30 °C pour les espèces de *Lactiplantibacillus* et *Weissella*. Les souches

Leuconostoc pseudomesenteroides 56 et *W. cibaria* 21 et 64 ont induit la synthèse d'EPS (Fessard & Remize, 2019).

D'après ces caractéristiques, les souches de *Ltp. plantarum* 17a et 75, *Lc. pseudomesenteroides* 56 et *W. cibaria* 21 et 64 ont été sélectionnées pour les fermentations lactiques des fruits réalisées pendant cette thèse.

1.2.4. Modification de la qualité sensorielle des jus de fruits par fermentation lactique

1.2.4.1. La qualité sensorielle

La qualité sensorielle d'une boisson est définie par (Pinto et al., 2022) :

- L'aspect général, en particulier la couleur ;
- Les propriétés gustatives, à savoir la saveur, la persistance en bouche et l'arrière-goût ;
- Les propriétés olfactives résultant des arômes ;
- Les propriétés tactiles, à savoir la sensation en bouche ;
- L'absence d'odeurs et saveurs défavorables.

Les attributs négatifs d'une boisson, e.g. la décoloration, la mousse, la sédimentation, le gaz, l'odeur désagréable, l'amertume et l'astringence, entraînent son rejet par les consommateurs. Le principal intérêt des consommateurs lors de l'achat de boissons fonctionnelles est l'acceptation sensorielle, bien qu'ils soient informés de leur potentiel bénéfique sur la santé (Pinto et al., 2022).

Peu d'effets notables sur l'aspect visuel de jus de fruits ayant subi une fermentation lactique ont été observés. À noter toutefois que la différence de couleur et l'indice de brunissement résultant de l'effet du stockage des produits fermentés sont limités par la fermentation lactique, contrairement aux produits non fermentés ou ceux ayant subi une pasteurisation douce ou un traitement par champs électriques pulsés (Di Cagno et al., 2011). Les effets de la fermentation lactique sur les autres attributs de la qualité sensorielle vont être détaillés.

1.2.4.2. Sucrosité

La sucrosité des produits fermentés dépend non seulement de la teneur en sucres mais aussi de celle en acides organiques (Marsh et al., 2006). La composition en sucres des fruits est modifiée par la fermentation lactique. Ce procédé modifie également les teneurs en acides.

La concentration en glucose a diminué d'environ 25 % en 72 h de fermentation de jus de grenade, et celle du fructose d'environ 15 % (Mousavi et al., 2011). Dans des smoothies à base de fruits et légumes fermentés avec une combinaison de souches de *Ltp. plantarum*, *W. cibaria*, *Ltp. pentosus* et *P. pentosaceus*, la consommation de glucose et de fructose était seulement de 7-9 % et 3-4 % respectivement, alors qu'une consommation de plus de 20 % de l'acide malique était observée (Di Cagno et al., 2011). Toutefois, la quantité de données disponibles est relativement faible, la plupart des études limitant l'analyse au suivi de la teneur en matière sèche soluble.

La fermentation de jus de grenade a montré une forte production d'acide lactique et une production moindre d'acides formique, propionique et acétique par les souches de *Ltp. plantarum* et *Lb. acidophilus* (Mousavi et al., 2013). La fermentation lactique de jus de cerise a également entraîné une production majoritaire d'acide lactique (Filannino et al., 2015). *Ltp. plantarum* a accumulé 130 mM de lactate contre 87, 65 et 27 mM pour *Lvb. spicheri*, *Limosilactobacillus fermentum* et *Lmb. reuteri*,

respectivement. La fermentation de jus de sureau par des souches de bactéries lactiques a induit une production élevée d'acide lactique (Cirlini et al., 2020). Ce procédé a entraîné une métabolisation élevée des acides malique et citrique tandis que les concentrations en glucose et en fructose n'ont pas évolué. Cela suggère un changement dans le métabolisme des bactéries lactiques avec l'utilisation des acides organiques plutôt que des sucres (Cirlini et al., 2020).

1.2.4.3. Modulation de la flaveur

La flaveur regroupe les sensations olfactives et gustatives résultant notamment des arômes, qui peuvent provenir de la synthèse de composés dans les cellules des fruits ou être synthétisés par les micro-organismes (Hadj Saadoun et al., 2021; Pinto et al., 2022).

La capacité de synthèse de composés aromatiques est directement liée aux voies métaboliques des protéines, des glucides et des lipides des bactéries lactiques lors de leur croissance (Hadj Saadoun et al., 2021; Kaprasob et al., 2017). Ces micro-organismes vont produire différents métabolites selon les composés nutritionnels disponibles dans le substrat. Les bactéries lactiques synthétisent des composés aromatiques et leurs précurseurs, e.g. l'acide acétique, les esters, les cétones, les alcools et les terpènes, et influencent ainsi favorablement la saveur des jus fermentés (Pinto et al., 2022; Szutowaska, 2020). Des notes florales et fruitées peuvent être associées à des concentrations élevées en alcools, cétones, terpènes et aldéhydes et une odeur douce par une forte concentration en esters (Hadj Saadoun et al., 2021; Szutowaska, 2020).

Des smoothies de fruits et légumes fermentés par des souches de *Ltp. plantarum*, *W. cibaria*, *Ltp. pentosus* et *P. pentosaceus*, ont montré une meilleure acceptabilité par les consommateurs en termes de couleur, saveur, sensation sucrée et texture (Di Cagno et al., 2011). Un jus à base de jicama (tubercule), de courge cireuse et de carotte fermenté individuellement par des souches de *Ltp. plantarum* et *Lb. acidophilus* s'est révélé bien accepté avec un score entre cinq et huit sur une échelle hédonique à neuf points. La couleur et la texture étaient particulièrement appréciées (Do & Fan, 2019). Une analyse sur des jus de pastèque fermentés individuellement avec *Ltp. plantarum*, *Lacticaseibacillus rhamnosus*, *Lcb. casei*, *Lvb. brevis* et *P. pentosaceus* a montré un effet sur les propriétés sensorielles propre à chaque souche. Les jus inoculés avec les souches de *Levilactobacillus brevis* et *P. pentosaceus* étaient préférés par comparaison avec les autres boissons fermentées proposées. Les principaux termes utilisés pour les caractériser étaient « goût de pastèque », « goût naturel », « sucré » et « couleur de pastèque » (Mandha et al., 2021).

La fermentation des jus de pomme, carotte, tomate, concombre et aubépine par la souche hétérofermentaire de *Lmb. fermentum* a révélé une production élevée d'acide acétique associée à une saveur aigre plaisante (Cui et al., 2019). Cependant, à forte concentration l'acide acétique entraîne des effets non désirables aux niveaux sensoriel et qualitatif, d'où la nécessité de contrôler cette production (Karovičová & Kohajdová, 2003).

Un jus de pomme fermenté par différentes souches de *Lactobacillus* a montré la formation de nouveaux composés aromatiques, tels que des cétones et de l'acétaldéhyde, et l'augmentation significative des esters et des alcools. Ces composants sont responsables de la saveur finale des jus fermentés (Chen et al., 2019). Lors d'une analyse sensorielle, un jus de grenade fermenté a été préféré à un jus non fermenté. La fermentation a en effet induit la formation de composés volatiles favorables, tels que des alcools, des cétones, des alcènes, des terpènes et des dérivés du benzène, et la diminution de composés indésirables que sont les aldéhydes (Di Cagno et al., 2017). La fermentation de jus de

pomme, carotte, tomate, concombre et cendres et de jus de pomme de cajou par des souches de *Lcb. casei*, *Lcb. rhamnosus*, *Ltp. plantarum* ou *Lb. acidophilus* a également généré des composés volatils à différentes étapes de la fermentation (Cui et al., 2019; Kaprasob et al., 2017).

Au sein du laboratoire QualiSud, du jus d'ananas a été fermenté avec les souches *W. cibaria* 64 ou *Lc. pseudomesenteroides* 12b. Les produits fermentés ont développé une odeur et un goût de yaourt par comparaison avec le jus témoin non fermenté (Fessard et al., 2017). La fermentation par utilisation de la souche *W. cibaria* 64 n'a pas entraîné de différence notable d'autres caractéristiques sensorielles par rapport au jus d'ananas non fermenté. En revanche, le jus fermenté avec la souche *Lc. pseudomesenteroides* 12b a induit un goût plus doux, acide, fort et pétillant. Ce jus a montré moins de caractéristiques du jus d'ananas, d'où une moins bonne appréciation générale par comparaison avec le jus fermenté avec la souche *W. cibaria* 64 et le jus témoin non fermenté. Lors de leur analyse sensorielle, les jus fermentés ont obtenu, sur une échelle de qualité hédonique de 0 à 10, des scores compris entre 5,1 et 5,5, contre 6,9 pour le jus non fermenté (Fessard et al., 2017).

1.2.4.4. Modulation de la texture

Certaines souches de bactéries lactiques sont capables de sécréter des polysaccharides extracellulaires, les EPS (Szutowaska, 2020). Ces composés contribuent à l'acceptabilité des produits fermentés par les consommateurs car ils améliorent les propriétés rhéologiques de ces aliments (Caggianiello et al., 2016; Liu et al., 2011; Lynch et al., 2018). Ces composés modifient la viscosité, la texture, la sensation en bouche et la stabilité des aliments (Ray et al., 2022).

Ces effets ont été démontrés en boulangerie et sur les produits laitiers. Les EPS ont la faculté de se lier à l'eau et d'interagir avec divers constituants du lait, notamment les micelles de caséines, et d'ainsi renforcer la fermeté du réseau des caséines (Lynch et al., 2018; Ripari, 2019). Dans de la purée de carotte, l'utilisation de souches de *W. cibaria* et *Leuconostoc lactis* productrices d'EPS pour la fermentation a modifié la viscosité et l'élasticité des matrices (Juvonen et al., 2015). Une augmentation de la viscosité de smoothies de fruits et légumes a été montrée comme la conséquence de la synthèse d'EPS et de protopectinases par les souches de *W. cibaria* utilisées lors de la fermentation.

La modification des propriétés rhéologiques par les EPS dépend de leur concentration, leur composition, leur taille, leur structure et leur charge (Ripari, 2019).

La synthèse d'EPS par les souches *W. cibaria* 21 et 64 et *Lc. pseudomesenteroides* 56 utilisées lors de ces travaux de recherche a été démontrée préalablement (Fessard & Remize, 2019).

Outre leur rôle en rhéologie, les EPS présentent des effets sur la santé qui seront décrits dans la **partie 2.2.2.4**.

PARTIE 2 : Évolution des composés bioactifs lors de la fermentation lactique et effets santé liés à la consommation de fruits lactofermentés

2.1. Évolution de la composition en molécules bioactives pendant la fermentation lactique

2.1.1. Composés phénoliques

La fermentation lactique permet de stabiliser ou d'améliorer la capacité antioxydante des fruits et légumes.

Une des hypothèses proposée est que cette aptitude est attribuée aux bactéries lactiques. Leurs systèmes antioxydants enzymatiques ou non enzymatiques leur permettent de limiter la formation d'espèces réactives de l'oxygène (ROS, « reactive oxygen species ») à des concentrations non délétères pour elles-mêmes (Hur et al., 2014). En cas d'exposition à des ROS, les bactéries vont déclencher des mécanismes pour piéger les ROS et lutter contre les dommages oxydants. De plus, les bactéries lactiques sont capables de libérer des composés phénoliques simples par hydrolyse enzymatique de composés phénoliques polymérisés (Hur et al., 2014).

La présence d'enzymes capables d'agir sur les composés phénoliques est essentiellement décrite chez les lactobacilles (Hur et al., 2014). Une tanase, hydrolysant les tannins et esters d'acide gallique ainsi qu'une β -glucosidase qui métabolise les isoflavones glycosylés en aglycone ont été caractérisées chez *Ltb. plantarum* (Kostinek et al., 2005).

L'action de ces enzymes provoque la libération d'aglycones ou de monomères de polyphénols. Cela engendre une meilleure biodisponibilité, c'est-à-dire une capacité accrue à être assimilés par l'homme, améliorant ainsi l'activité antioxydante *in situ* (Filannino et al., 2014).

De nombreuses études relatent des modifications de composition en composés phénoliques pendant la fermentation lactique. Il peut s'agir d'une diminution de la teneur en acide protocatéchique, qui est décarboxylé en catéchol dans du jus de cerise par *Ltp. plantarum* (Filannino et al., 2015). Dans du jus de mûre, *Ltp. plantarum*, *Lb. acidophilus* et *Lactocaseibacillus paracasei* ont induit une augmentation de la concentration totale en composés phénoliques, anthocyanes et flavonoïdes (Kwaw et al., 2018). En revanche, la concentration en anthocyanes a diminué suite à la fermentation de jus de grenade par *Ltp. plantarum* et *Lb. acidophilus* (Mousavi et al., 2013).

A La Réunion, la fermentation de purée de papaye avec les souches *Ltp. plantarum* 75, *W. cibaria* 64 et *Lc. pseudomesenteroides* 56 a induit une diminution de la concentration en gallate de gallocatéchine et une augmentation de la teneur en catéchine par comparaison à la purée non fermentée (Mashitoo et al., 2021). L'utilisation des souches *W. cibaria* 64 et *Ltp. plantarum* 75 pour la fermentation de purée de mangue Cogshall a entraîné l'augmentation de gallocatéchine et des acides protocatéchique, syringique, chlorogénique et ellagique (Mashitoo et al., 2021). Ces souches ainsi que la souche *Lc. pseudomesenteroides* 56 ont induit une augmentation de la concentration en acide *p*-coumarique. La fermentation des purées de mangue par la souche *Lc. pseudomesenteroides* 56 a résulté en l'augmentation de la concentration en acide gallique tandis que l'utilisation des souches *W. cibaria* 64 et *Ltp. plantarum* 75 a induit une diminution de cette concentration. La concentration en quercétine aglycone a augmenté pour les souches *Lc. pseudomesenteroides* 56 et *Ltp. plantarum* 75 et a diminué pour la souche *W. cibaria* 64 par comparaison à la purée de mangue non fermentée. La

souche *W. cibaria* 64 a augmenté la concentration en mangiférine tandis que les souches *Lc. pseudomesenteroides* 56 et *Ltp. plantarum* 75 ont diminué cette concentration dans les purées de mangue (Mashitoa et al., 2021).

L'utilisation d'espèces de *Lactobacillus*, notamment *Ltp. plantarum*, pour la fermentation des fruits et légumes est avantageuse grâce à leur influence sur le métabolisme des composés phénoliques (Filannino et al., 2015).

2.1.2. Vitamines

La fermentation lactique de fruits et légumes a une influence variable sur la concentration en vitamine C (Szutowska, 2020). Des smoothies de fruits et légumes fermentés par une combinaison de souches de *Ltp. plantarum*, *W. cibaria*, *Ltp. pentosus* et *P. pentosaceus* ont montré une stabilisation de la concentration en acide ascorbique (Di Cagno et al., 2011). Différents jus fermentés à base de citron doux, de figue de Barbarie ou de papaye ont montré une diminution de la concentration en acide ascorbique dès les premières heures de fermentation tandis qu'une fermentation de 12 h de jus d'agrumes a permis de maintenir cette concentration à la teneur retrouvée dans le même jus pasteurisé (Chen et al., 2018; Hashemi et al., 2017; Panda et al., 2017; Yu et al., 2015). Une stabilisation de la concentration en vitamine C est aussi observée dans des jus fermentés de tomate (Liu et al., 2018). La fermentation de jus de pomme de cajou par des souches de *Ltp. plantarum* ou de *Lacticaseibacillus casei* a induit une augmentation de cette teneur après 12 h de fermentation et elle est restée stable après 48 h pour la boisson fermentée avec la souche de *Ltp. plantarum* (Kaprasob et al., 2017).

Certaines souches de bactéries lactiques et de bifidobactéries sont capables de convertir des composés bioactifs et nutriments apportés par l'alimentation en vitamine K ou en vitamines hydrosolubles du groupe B, e.g. la thiamine (B1), la riboflavine (B2), la niacine (B3), la pyridoxine (B6), les folates (B11) et la cyanocobalamine (B12) (Szutowska, 2020). Certaines bactéries lactiques utilisent des vitamines du groupe B et entraînent ainsi une diminution de leur concentration. La synthèse des vitamines du groupe B est toutefois observée chez *Ltp. plantarum*, *Limosilactobacillus reuteri*, *Lb. acidophilus* et *Bifidobacterium longum* pendant la fermentation (Szutowska, 2020). La fermentation du jus de pomme de cajou par des souches de *Lb. acidophilus*, *Lcb. casei*, *Ltp. plantarum*, *Lc. mesenteroides* et *B. longum* a entraîné une diminution de la concentration en thiamine dans chacune des boissons fermentées et une augmentation de la riboflavine, de la nicotinamide et de la cyanocobalamine dans les jus fermentés avec les souches de *Lb. acidophilus* et *Lcb. casei* (Kaprasob et al., 2018).

2.1.3. Bactériocines

Les bactériocines sont des peptides antimicrobiens de faible poids moléculaire, compris entre 5 et 10 kDa, synthétisés par les bactéries à Gram positif, à Gram négatif et les archées. Ces composés inhibent la croissance d'autres micro-organismes, y compris les pathogènes d'origine alimentaire (Juturu & Wu, 2018; Karovičová & Kohajdová, 2003). La capacité des bactéries à produire des bactériocines peut être profitable au niveau industriel car elles peuvent potentiellement être utilisées comme conservateurs dans les produits (De La Fuente-Salcido et al., 2015; Juturu & Wu, 2018). Pour cette raison, les bactériocines produites par les bactéries lactiques ont été étudiées (De La Fuente-Salcido et al., 2015; Karovičová & Kohajdová, 2003).

Ces peptides sont non toxiques pour l'humain (Santesteban-López et al., 2022). Les bactériocines sont capables de renforcer l'immunité innée sans provoquer de réponse pro-inflammatoire et protègent ainsi l'organisme des infections bactériennes (Juturu & Wu, 2018).

La production de bactériocines est généralement liée à la croissance des bactéries lactiques avec une phase de croissance exponentielle suivie par une phase stationnaire. L'activité des bactériocines peut cependant ne pas être élevée, malgré une forte croissance bactérienne (Abbasiliasi et al., 2017). Les bactériocines synthétisées par les bactéries à Gram positif sont classées en 12 groupes selon la similarité de leur structure, leur proximité phylogénétique et leur séquence de motifs consensus. Celles synthétisées par les bactéries à Gram négatif sont réparties en deux groupes, les colicines et les microcines, tout comme celles produites par les archées, les halocines et les sulfobactériocines (Juturu & Wu, 2018; Santesteban-López et al., 2022).

Le type et la concentration de bactériocines produites dépendent de la souche bactérienne utilisée, de la composition du milieu (sources carbonées, sources azotées, sucres, vitamines, présence d'agents tensioactifs), des conditions de fermentation (pH, température, agitation et ventilation) et du mode de fermentation (batch, fed-batch et fermentation continue) (Abbasiliasi et al., 2017; Santesteban-López et al., 2022). La production de bactériocines est stimulée favorablement par l'utilisation de glucose comme source de carbone. La température optimale pour cette production peut différer de celle optimale à la croissance bactérienne (Abbasiliasi et al., 2017).

La nisine est une bactériocine produite par *Lactococcus lactis* subsp. *lactis*, une bactérie à Gram positif. Elle est qualifiée de « Generally Recognized As Safe » (GRAS) et est la seule bactériocine autorisée comme conservateur (E234) dans l'Union Européenne (Juturu & Wu, 2018; Santesteban-López et al., 2022). La nisine intervient en inhibant la formation du lipide II, intermédiaire final de la synthèse des peptidoglycanes. La conséquence est la déstructuration de la paroi cellulaire, la lyse cellulaire (Juturu & Wu, 2018; Santesteban-López et al., 2022). La nisine est principalement produite lors de la phase de croissance exponentielle de la souche bactérienne productrice et cette production chute lors de la phase de croissance stationnaire (Abbasiliasi et al., 2017; Juturu & Wu, 2018). Sa synthèse peut être augmentée par ajout d'une source d'azote au substrat. La nisine Z, un variant de la nisine A et la gallidermine sont des bactériocines démontrant une réponse immunitaire améliorée (Juturu & Wu, 2018). Les bactéries lactiques utilisent préférentiellement le glucose comme source carbonée pour produire de la nisine Z. Cependant, une concentration en glucose supérieure à 25 g/L inhibe cette production. Ce phénomène résulte de la saturation du transport intracellulaire du glucose et d'une diminution de l'absorption du glucose (Abbasiliasi et al., 2017).

Hormis la nisine, de nombreuses autres bactériocines sont décrites chez les bactéries lactiques, parmi lesquelles *Ltp. plantarum* subsp. *plantarum* (Juturu & Wu, 2018), *W. paramesenteroides* (Pal & Ramana, 2010), *W. hellenica* (Woraprayote et al., 2015), *W. cibaria* (Srionnual et al., 2007) et *Lc. carnosum* (Wan et al., 2015), pour ne citer que quelques exemples.

2.1.4. Acides aminés et amines biogènes

Les acides aminés libres peuvent aussi être décarboxylés par les bactéries lactiques pour former des amines biogènes (Ogrodowczyk & Drabińska, 2021). Ces amines peuvent être bénéfiques à l'hôte qui va ingérer l'aliment fermenté en améliorant son immunité, en augmentant son activité vasculaire et en régulant son activité mentale (Ray et al., 2022). Une ingestion excessive de ces composés peut cependant provoquer des effets psychoactifs et/ou vasoactifs. Les amines biogènes sont donc toxiques

pour les humains à forte concentration, notamment l'histamine et la tyramine (Karovičová & Kohajdová, 2003; Ray et al., 2022). Elles peuvent également conférer un goût désagréable au produit alimentaire lorsqu'elles sont présentes en concentration élevée (Ray et al., 2022). Aucune étude n'a rapporté d'intoxication alimentaire liée à la formation d'amines biogènes par les bactéries lactiques (Fessard & Remize, 2017). Les bactéries lactiques possédant des enzymes décarboxylases, e.g. *Lc. mesenteroides*, sont plus susceptibles d'entraîner la formation d'amines biogènes tandis que cette production est limitée pour les souches bactériennes possédant des enzymes amines-oxydases, e.g. *Lcb. casei* subsp. *casei*, *Ltp. plantarum* et *Lactilactobacillus curvatus* (Ray et al., 2022). La formation des amines biogènes est influencée par différents facteurs, e.g. la température, le pH, l'accès à l'oxygène et la concentration en chlorure de sodium.

Une production d'amines biogènes, e.g. la putrescine, la tyramine, l'histamine et la cadavérine, a été montrée au cours de la fermentation de la choucroute (Karovičová & Kohajdová, 2003).

Une étude réalisée préalablement au sein de l'UMR QualiSud a montré que les souches bactériennes utilisées pendant cette thèse, i.e. *Ltp. plantarum* 17a et 75, *Lc. pseudomesenteroides* 56 et *W. cibaria* 21 et 64 n'ont pas induit de production d'amines biogènes à partir de la lysine, l'ornithine, la tyrosine et l'histidine. De plus, les gènes de la décarboxylase de l'histidine, de la tyrosine et de l'ornithine n'ont pas été détectés (Fessard & Remize, 2019).

2.1.5. Exopolysaccharides produits par les bactéries lactiques

Les EPS possèdent des activités antibactérienne, antioxydante, immunomodulatrice et anticancéreuse. Ils favorisent la colonisation intestinale des bactéries lactiques par adhésion aux cellules épithéliales intestinales ou par formation d'une occlusion protectrice, i.e. les biofilms. Les EPS sont également impliqués dans la protection contre le stress extérieur (Ray et al., 2022; Szutowaska, 2020).

La production d'EPS lors de la fermentation de jus de fruits pourrait modifier la structure et la fonction du microbiote intestinal après ingestion (Ray et al., 2022). Deux types d'EPS peuvent être produits, des homopolysaccharides (HoPS) et/ou des hétéropolysaccharides (HePS). Les HoPS sont composés d'un seul type de monosaccharides, i.e. le glucose, le fructose ou le galactose, tandis que les HePS en contiennent plusieurs (Ray et al., 2022).


La production d'EPS dépend de la souche bactérienne utilisée lors de la fermentation et de la composition du substrat, e.g. sa concentration en saccharose, et des prétraitements appliqués lors de la préparation des matrices (Oleksy & Klewicka, 2018; Ray et al., 2022). Il a été montré sur un milieu MRS enrichi en saccharose que toutes les espèces de *Leuconostoc* et toutes les souches de *W. confusa* et *W. cibaria* testées en produisent (Fessard et al., 2017; Kavitate et al., 2020; Maina et al., 2008). *Ltp. plantarum* ne produirait pas d'HoPS mais des HepS (Imran et al., 2016).

L'exploitation de la capacité de production d'HoPS par les bactéries lactiques représente un fort intérêt. Ces composés peuvent être synthétisés à des concentrations de l'ordre du g/L et ainsi avoir un effet plus élevé lors de leur consommation par comparaison avec les HePS présents à des teneurs de l'ordre du mg/L (Lynch et al., 2018). La rédaction d'un article de revue intitulé « **Variability of bacterial homopolysaccharide production and properties during food processing** » publié dans le journal *Biology* permet de faire un état de l'art des HoPS produits selon les espèces bactériennes et de l'intérêt de leur utilisation dans l'industrie agroalimentaire. Cet article expose la variabilité structurelle

et de taille des HoPS et les gènes impliqués dans leur production. L'effet de la composition du substrat et des traitements appliqués sur la synthèse des HoPS est également développé, ainsi que l'influence de ces paramètres sur les matrices alimentaires.

Review

Variability of Bacterial Homopolysaccharide Production and Properties during Food Processing

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Simple Summary: Bacteria can produce diverse homopolysaccharides (HoPSs), which are polymers of glucose, fructose or galactose. The synthesis of these compounds is catalyzed by glycosyltransferases. The range of HoPS sizes is very large and mostly depends on the carbon source in the medium and the catalyzing enzyme; however, factors such as nitrogen nutrients, pH, water activity, temperature and duration of bacterial culture also impact size and yield. The sequence of the polymerization enzyme influences the structure of the HoPS, by modulating the type of linkage between monomers, both for the linear chain and for the ramifications. HoPSs' size and structure influence its rheological properties. As a consequence, the choice of catalyzing enzyme and the modulation of environmental factors open ways to guide the production of specific HoPSs in foods by bacteria. This approach presents many advantages to propose applications of bacterial HoPS to texture foods, either by *in situ* or *ex situ* production, but requires a better knowledge of HoPS production conditions.

Abstract: Various homopolysaccharides (HoPSs) can be produced by bacteria: α - and β -glucans, β -fructans and α -galactans, which are polymers of glucose, fructose and galactose, respectively. The synthesis of these compounds is catalyzed by glycosyltransferases (glycansucrases), which are able to transfer the monosaccharides in a specific substrate to the medium, which results in the growth of polysaccharide chains. The range of HoPS sizes is very large, from 10^4 to 10^9 Da, and mostly depends on the carbon source in the medium and the catalyzing enzyme. However, factors such as nitrogen nutrients, pH, water activity, temperature and duration of bacterial culture also impact the size and yield of production. The sequence of the enzyme influences the structure of the HoPS, by modulating the type of linkage between monomers, both for the linear chain and for the ramifications. HoPSs' size and structure have an effect on rheological properties of some foods by their influence on viscosity index. As a consequence, the control of structural and environmental factors opens ways to guide the production of specific HoPS in foods by bacteria, either by *in situ* or *ex situ* production, but requires a better knowledge of HoPS production conditions.

Keywords: exopolysaccharide; texture; rheology; natural polymers



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1. Introduction

Homopolysaccharides (HoPSs) or homoglycans are polymeric compounds that have the particularity of being composed of a single type of monosaccharide. They include starch, cellulose, pullulan, glucan and inulin, which do not contain functional groups other than the hydroxyl group, contrarily to chitosan, alginate or carrageenans [1]. They can either be linear, such as amylose, or ramified, such as amylopectin. These compounds are produced by animals, plants, algae, fungi and bacteria.

Bacterial HoPS can be water soluble and produced in foods, especially fermented foods, in which they contribute to the functional properties. They belong to the family of exopolysaccharides (EPSs), which means that they are produced extracellularly. As such, their production has been mainly studied from lactic acid bacteria (LAB), but others, such as *Bacillus*, acetic acid bacteria, *Streptomyces*, *Zymomonas*, *Pseudomonas* or *Paenibacillus*, can produce HoPSs [1]. One particularity of bacterial HoPSs is that they can be produced at a high level, up to 70 g L⁻¹ when conditions are optimized in *Bacillus subtilis* Natto [2,3].

Many studies investigated EPSs' bioactivities, and it is now recognized that EPSs exert anticancer, immunoregulatory (heteropolysaccharides), antidiabetic, antioxidant, anti-inflammatory, hypolipidemic and hypoglycemic effects (for a review see [1]). The observed effects change according to the producing strain and the subsequent nature of the produced EPS. As such, dextran obtained from the fermentation with *Weissella* spp. strains demonstrated antioxidant potential, just like heteropolysaccharides (HePS) from the same species [4,5], and antifungal activity but no antibacterial activity against standard bacterial strains, as opposed to mannan produced from *Weissella confusa*, for example [6,7]. In addition, as dietary soluble fibers, some are investigated for prebiotic effect [8]. Apart from bioactivity, HoPSs are produced for industrial applications: cosmetics, tissue engineering, packaging or edible coating, agriculture as biosurfactants and petroleum industry [9]. In the food industry, they play a role in product texture and water retention. They have been extensively studied in dairy and bakery products and to a minor extent in beverages [10–13]. The long duration of fermentation is a positive factor that allows bacteria to produce EPS [14].

Naturally occurring polymers are receiving renewed attention as highly biocompatible and environmentally friendly materials. The HePS from LAB have long been characterized since they play an important role in the rheology, texture and “mouthfeel” of fermented milks and were studied for some other fermented products. HoPS have long been evaluated to a lesser extent; however, recently, the most notable advances in LAB-EPS research have been related to HoPSs as they could be considered as a promising alternative to conventional additives given their higher level of synthesis [15].

From an applied point of view, an improvement of food functional properties can be expected from HoPS production through inoculation of selected strains and process optimization. Hence, this review aims to draw a clear picture of the possibilities of modulation of EPS levels in fermented foods from the variability of bacterial sources and through the adjustment of environmental conditions.

2. Variability in Structure and Size

2.1. Different Families of Homopolysaccharides

HoPSs from bacteria are mainly formed from sucrose, by polymerization of either glucose or fructose. The enzymes involved belong to the hexosyltransferase family. The synthesis depends on the involved enzyme, which triggers the type of link between hexoses.

Four types of bacterial HoPSs (Figure 1) are described and divided into subclasses based on the type of linkage and the position of the carbon involved in the linkage: α -glucans (dextran, alternan, reuteran and mutan), β -glucans, β -fructans (levan, inulin) and α -galactans [16]. These four groups contain polysaccharides with different types of linkage, molecular weights or lengths and levels of ramification.

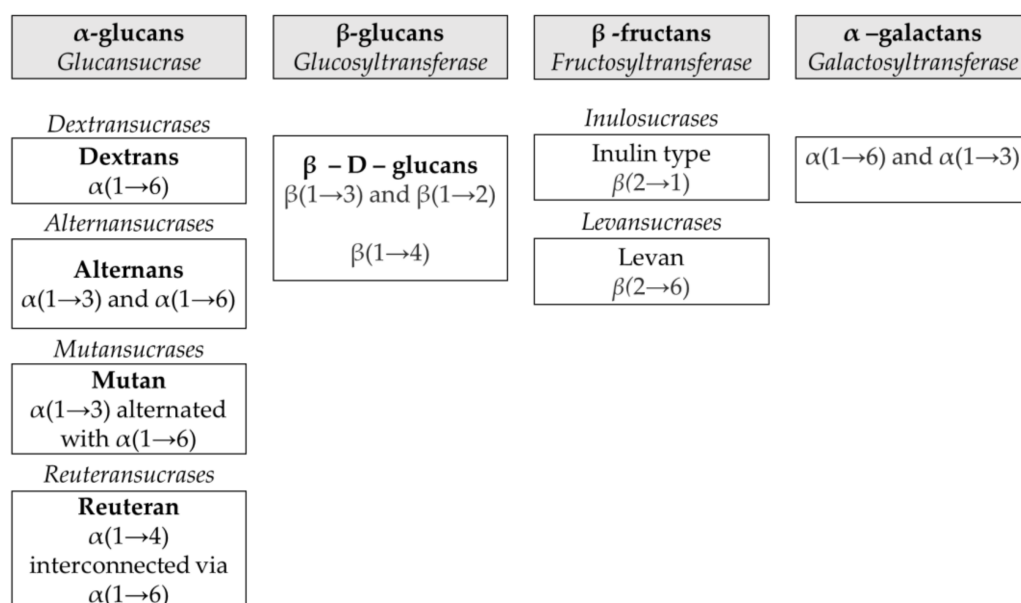


Figure 1. Classification of bacterial homopolysaccharides according to the enzymes involved and their linear chain linkages.

Generally, the molecular weights of HoPSs range from 4.0×10^4 to 1.8×10^9 Da. Some molecular weights are described for reuteran (2.8×10^7 Da), levan (2.0×10^6 Da) or inulin-like fructan (1.0×10^7 Da) [17,18]. The size of dextrans varies between 10^4 Da for *Acetobacter tropicalis* to 10^9 Da for *Oenococcus kitaharae* [19,20]. The size of the EPS molecule may depend on the carbon source in the culture medium (Table 1). Polak-Berecka et al. [21] showed that the molecular weight of dextrans synthesized by *Lactobacillus rhamnosus* grown on five carbon sources, i.e., glucose, galactose, sucrose, maltose or lactose, were around 5.0×10^5 , 3.9×10^6 , 11.1×10^6 , 1.9×10^5 and 7.0×10^6 Da, respectively.

Table 1. Size and linkages according to the producing strain. HoPS: homopolysaccharide; Glc: glucose units; Fru: fructose units.

Strain	HoPS	Molecular Weight Range (Da)	Linkage	Reference
<i>Leuconostoc mesenteroides</i> TDS2-19	Dextran	9×10^7	$\alpha(1\rightarrow6)$ Glc linear	[22]
<i>Leu. mesenteroides</i> RTF10	Dextran	4×10^8	$\alpha(1\rightarrow6)$ Glc and branched	[23]
<i>Latilactobacillus sakei</i> MN1	Dextran	2×10^8	$\alpha(1\rightarrow6)$ Glc and partially branched in the O-3 position by a single α -glucopyranose unit (between 8.5% and 10.3%)	[24]
<i>Leu. mesenteroides</i> NRRL	Dextran	6×10^5	95% $\alpha(1\rightarrow6)$ Glc and 5% $\alpha(1\rightarrow3)$ Glc	[16]
<i>Acetobacter tropicalis</i>	Dextran	1×10^4 to 4×10^4	$\alpha(1\rightarrow6)$ Glc linear	[19]
<i>Limosilactobacillus reuteri</i> ML1	Reuteran		$\alpha(1\rightarrow6)$ Glc and 4,6-disubstituted α -glucosyl units at the branching points	[17]
<i>Levilactobacillus brevis</i> TMW 1.2112	β -Glucan		$\beta(1 \rightarrow 3)$ Glc ramified with β -Glc residues at position O2	[25]
<i>Lim. reuteri</i> 121	Levan	2×10^5 to 2×10^6	98% $\beta(2\rightarrow6)$ Fru and 2% $\beta(1\rightarrow2)$ and $\beta(2\rightarrow6)$ Fru branched	[18]
<i>Bacillus subtilis</i> Natto	Levan		$\beta(2\rightarrow6)$ fructofuranoside	[3]
<i>Lactobacillus rhamnosus</i>	Inulin	11×10^6	$\beta(2\rightarrow1)$ Fru glycosidic and Fru branched at the $\beta(2\rightarrow6)$ position	[26]
<i>Lactobacillus gasseri</i> DSM 20604	Inulin	6×10^6	$\beta(2\rightarrow1)$ Fru glycosidic	[27]
<i>Weissella confusa</i> KR780676	Galactan		$\alpha(1 \rightarrow 6)$ galactose	[28]

2.2. α -Glucans

The α -glucans are usually classified into four groups depending on the type of links and sequence in the HoPS: dextrans, alternans, reuterans and mutans.

Dextrans are mainly composed of a linear chain, e.g., 95% in *Leuconostoc mesenteroides* or fully linear for *A. tropicalis* [19,29]. Dextrans can be linked by $\alpha(1\rightarrow6)$ glycosidic bonds and branched at the carbon atom at position 3 or, less frequently, at positions 2 and 4 [30]. Alternan contains in nearly equal proportion $\alpha(1\rightarrow3)$ and $\alpha(1\rightarrow6)$ linkages, which alternate on the chain with some degree of $\alpha(1\rightarrow3)$ branching.

Glucansucrases gather dextransucrase (EC 2.4.1.5) and alternansucrase (EC 2.4.1.140). The enzymes transfer a d-glucosyl residue from sucrose to a glucan chain and release d-fructose. Those enzymes belong to the glycosyl hydrolase (GH) 70 family and descriptions can be found on the CAZy (Carbohydrate-Active Enzyme; www.cazy.org, accessed on 25 October 2021) and BRENDA (The Comprehensive Enzyme Information System; www.brenda-enzymes.org, accessed on 25 October 2021) databases. Until 2016, GH70 enzymes were only described in lactic acid bacteria (LAB) [31].

Dextransucrase is largely described in *Leu. mesenteroides* and in others LAB such as *Latilactobacillus curvatus*, *Leuconostoc citreum*, *Leuconostoc lactis*, *Limosilactobacillus reuteri*, *Liquorilactobacillus hordeii*, *O. kitaharae*, *Pediococcus pentosaceus*, *Weissella cibaria* and *W. confusa*. *A. tropicalis* and *Streptococcus mutans* also produce a dextransucrase [19]. It mainly catalyzes the formation of $\alpha(1\rightarrow6)$ linkages, plus possibly side-chains linked with $\alpha(1\rightarrow2)$, $\alpha(1\rightarrow3)$ or $\alpha(1\rightarrow4)$ linkages. The size and structure of dextrans are mainly affected by the sequence and structure of dextransucrase. Mutagenesis has shown dextransucrase regions involved in dextran size and other amino acids of the protein sequence implied in branching [32,33].

Alternansucrase is produced by *Leu. mesenteroides* and *Leu. citreum*. As for dextransucrase, the role of certain amino acids of the enzyme is of high importance regarding the size of the polysaccharide and the type of linkages. For instance, mutation of L940 increases the percentage of $\alpha(1\rightarrow6)$ linkages and L940W leads to absence of linkages of the $\alpha(1\rightarrow3)$ type and more oligo- than polysaccharides [34]. Similarly the nature of amino acids involved in acceptor sub-sites was shown to modify the linkage type [35].

Reuteransucrase, responsible for reuteran synthesis, differs from dextransucrase as it produces a HoPS containing mainly $\alpha(1\rightarrow4)$ linkages, interconnected via $\alpha(1\rightarrow6)$ bridges [17]. Slight differences in the sequence, as weak as a triple amino acid mutation, can lead to differences of HoPS structure from 70% $\alpha(1\rightarrow4)$ linkages to 80% $\alpha(1\rightarrow6)$ linkages [17]. Reuteransucrases are produced by *Lim. reuteri* [36], for instance, the reuteran from the probiotic bacterium *Lim. reuteri* 121 contains $\alpha(1\rightarrow4)$ and $\alpha(1\rightarrow6)$ glycosidic linkages in proportions of 58% and 42%, respectively [36].

Mutans are polymers consisting of glucose residues containing more than 50% of $\alpha(1\rightarrow3)$ glucoside linkages, mainly associated with $\alpha(1\rightarrow6)$ linkages. They are produced by *Lim. reuteri*, *Leu. mesenteroides* and several strains of *Streptococcus* [30].

The properties of dextran, regarding rheology, depend on its structure and size. The shorter the linear chain, the lower the viscosity [19,20]. Other factors, such as pH, the presence of maltose or temperature, play a role in the structure and size of the produced HoPS dextran [37–39].

2.3. β -Glucans

Most β -glucans from bacterial origin are unbranched glucose polymers linked via $\beta(1\rightarrow3)$ glycosidic bonds. In some cases, trisaccharide repeats linked by $\beta(1\rightarrow3)$ form a linear chain, and ramifications are formed from $\beta(1\rightarrow2)$ glucose units. They are produced by *Levilactobacillus brevis*, *Pediococcus claussenii* [40], *Pediococcus parvulus*, *Oenococcus oeni* [41] and other *Pediococcus* or *Lactobacillus* species [42,43]. The enzyme is a 1,3- β -glucan synthase (EC 2.4.1.34), which uses intracellular UDP-glucose as a substrate.

In addition, acetic acid bacteria, such as *Acetobacter* and *Gluconobacter*, produce long-chains of $\beta(1\rightarrow4)$ glucan, called bacterial cellulose, and adopt a ribbon-like secondary structure [44].

Viscosity of β -glucans is directly related to their molecular weight, molecular structure, solubility in water and food matrix [45]. The impact of the linkage type on the litheness of the polysaccharidic chain is recognized: $\beta(1\rightarrow4)$ bonds result in stiffer chains compared to $\alpha(1\rightarrow4)$ or $\beta(1\rightarrow3)$ bonds [46].

2.4. Fructans

Inulin is composed of branched $\beta(2\rightarrow1)$ fructose linkages whereas levan has $\beta(2\rightarrow6)$ or $\alpha(2\rightarrow1)$ and $\alpha(2\rightarrow6)$ fructose linkages.

Bacterial inulin is obtained from inulosucrase (EC 2.4.1.9) activity. It catalyzes the transfer of a fructose residue from sucrose to form a polysaccharide chain connected by $\beta(2\rightarrow1)$ fructosyl linkages, resulting in the synthesis of high-molecular-weight inulin polymers. Inulosucrase is found in *Bacillus* sp., *Leu. citreum*, *Lactobacillus johnsonii*, *Lactobacillus gasseri*, *Lim. reuteri*, *Paenibacillus macerans*, *W. confusa* and *W. cibaria* [27].

Levansucrase (EC 2.4.4.10) catalyzes the transfer of D-fructosyl residues from fructose to yield $\beta(2\rightarrow6)$ osidic bonds, which characterize levan. Many bacteria produce this enzyme, such as *Bacillus licheniformis*, *B. subtilis*, *Fructolactobacillus sanfranciensis*, *Gluconoacetobacter* sp., *Gluconobacter oxydans*, *L. gasseri*, *Leu. mesenteroides*, *Lim. reuteri*, *Paenibacillus polymyxa*, *Pseudomonas* spp. and *Zymomonas mobilis*. *L. reuteri* 121 is known to produce a linear levan [47]. *Leu. mesenteroides* NRRL B-512F produces levansucrase in addition to dextransucrase. Levansucrase is responsible for at least 25% of the reducing sugars released when grown in the presence of sucrose. This corresponds to a significant level of glucose and explains why fructose repression of dextransucrase is observed in *Leu. mesenteroides* [48].

2.5. α -Galactans

Galactans are a relatively less abundant class of polysaccharides, and their main structure is characterized by a chain of galactose units linked by $\alpha(1\rightarrow6)$ and $\alpha(1\rightarrow3)$ bridges [49]. *W. confusa* KR780676 produces a linear galactan containing $\alpha(1\rightarrow6)$ -linked galactose units [28].

3. Gene Regulation and Variability of HoPS Production Level

The bacterial genes responsible for EPS production and the promotion of various secondary functional features to the bacteria have been explored. Studies helped to specifically identify the genes that are crucial in the production process of bacterial HoPSs. Depending on their great structural diversity, EPSs can be produced by microorganisms via different pathways. HoPSs are unique in that they are synthesized by extracellular glycosaccharides using mostly sucrose as the glycosyl donor (fructose or glucose). Described in the genera *Weissella*, *Leuconostoc*, *Lactobacillus* and *Pediococcus*, the synthesis pathway of HoPSs generally occurs extracellularly [50]. HoPSs are produced by transglycosylases (glycansucrases), which are able to use the energy of the osidic bond of sucrose to catalyze the transfer of a corresponding glycosylated fraction. The transfer of monosaccharide in a specific substrate (e.g., sucrose) to the medium results in the growth of polysaccharide chains [51]. Most of the HoPS producing-LAB harbor only one glucansucrase gene; however, some LAB genomes exhibit more than one gene encoding hexosyltransferases and are, thus, able to synthesize different HoPSs. For instance, the *Leu. mesenteroides* NRRL B-512F strain coding both a glucansucrase and a fructansucrase can produce levan in addition to the dextran usually produced [52].

The subsequent production of HoPSs by LAB can be regulated by the production level and the activity of these enzymes. Of note, the production of dextransucrase is dependent on the presence of sucrose for all species, except some *Streptococcus* spp. On the contrary, reuteransucrase is constitutively expressed whatever the sugar [53]. The optimum sucrose level varies depending on the strain, generally between 2% and 5% for dextransucrase but up to 10% for reuteransucrase. On the other hand, pH of the medium does not influence

the level of production of the enzyme but could act on its stability [37]. Divalent cations, Ca^{2+} , Mg^{2+} and Mn^{2+} and dextrans activate and stabilize the enzyme of *Leu. mesenteroides*.

The production of EPSs can be influenced by changing the nature or quantity of substrates or available nutrients as well as by changing the pH, water activity, temperature and oxygen concentration of the culture medium [36]. The structure of the EPSs and their content vary according to the sources of carbon, nitrogen, phosphorus or sulfur. Sucrose is the most commonly used substrate for the synthesis of HoPSs and often the best substrate for HoPS production yield. LAB are sensitive to pH and the nature and amount of sugars. These factors must be taken into consideration for the production of EPSs in fruit and vegetable products with respect to their composition [52]. The specificity of carbon substrate differs from species to species. The temperature and yield depend on the different strains used (Table 2).

Regulation at constant pH favors better HoPS yields, because glycohydrolases are activated when acidification occurs due to lactate production, around pH 5. When the maximum concentration of lactate is reached in *L. rhamnosus* culture, the polysaccharide yields decrease due to enzymatic digestion. It usually corresponds to 24 to 48 h of fermentation [21]. The study by Polak-Berecka et al. [26] showed that culture conditions have a strong effect on the production of EPSs by *L. rhamnosus* and scaling from flask to fermenter can increase EPS biosynthesis by 175.8% in commercial production processes.

The viscosity of aqueous solutions is partly linked to HoPS level, molecular weight, degree of chain stiffness, radius of gyration of the molecule and the presence of ionizable groups that confer polyelectrolytic behavior to the polymer [54]. Looijesteijn et al. [55] reported that the main factor in the viscosity intensifying ability of EPS was the molecular weight. In this study, *Lactococcus lactis* subsp. *cremoris* strains NIZO B40 and NIZO B891 produced EPS with lower molecular weight in a medium with a low glucose content. The chemical composition of these polymers remained unchanged, but the rheological properties of the solution changed.

Table 2. Influence of culture conditions and strains on HoPS production yield.

Strain	MW (Da)	Optimum Temperature (°C)	pH	Medium Composition	Yield (g L ⁻¹)	Application	Reference
<i>Leu. mesenteroides</i> TDS2-19	8.8×10^7	25	6.8	MRS ¹ medium	71.23	Used in food industries as an emulsifier or as part of starter culture	[22]
<i>Leu. mesenteroides</i> RTF10	4.4×10^8	30	4.8	CDM ² 0.8% sucrose	1.25	Used as adjuvant and stabilizer in food industries	[23]
<i>Lat. sakei</i> MN1	1.7×10^8	30		MRS 2% sucrose	1.72	Used for biofilm formation	[24]
<i>W. cibaria</i> MG1	7.2×10^8	30	4.0–4.1	MRS 10% sucrose		Used in bakery for sorghum bread	[7]
<i>Lim. reuteri</i> ML1		35	4.7	Sucrose (purified enzyme)	5.12		[17]
<i>Lim. reuteri</i> 121		37	4.8	MRS medium sucrose	5.2		[56]
<i>Lim. reuteri</i> VIP	1.0×10^7	37	3.6	MRS 10% sucrose		Used in bakery for sorghum bread	[7]
<i>B. subtilis</i> Natto		37	5.6–5.8	SM ³ sucrose 20%	70.60	Used in pharmaceutical industries as a commercial spore, on alginate matrix for repeated production of levan	[3]
<i>Weissella hellenica</i> SKkimchi3	20.3×10^4	20	5	MRS sucrose 30%	74.00		[2]
<i>Lim. reuteri</i> 121	2.0×10^6	37	4.5–5.5	In vitro enzyme			[18]
<i>L. rhamnosus</i>	11.1×10^6	37	5.0	YNB ⁴	2.10		[26]
<i>Lim. reuteri</i> Y2	8.9×10^6	37	3.7–3.8	MRS 2.5% sucrose or raffinose			[7]
<i>L. gasseri</i> DSM 20604	5.8×10^6	35	5.5	sucrose	53.00		[27]

¹ MRS is composed of sucrose, yeast extract, beef extract, anhydrous sodium acetate, ammonium citrate; ² CDM, chemically defined medium; ³ SM, Spizizen medium; ⁴ YNB, yeast nitrogen base.

4. Effect of Food Composition and Processing

In the case of EPS synthesis occurring during food processing, the production yield will be influenced by the food environment on top of the experimental conditions of fermentation (Figure 2).

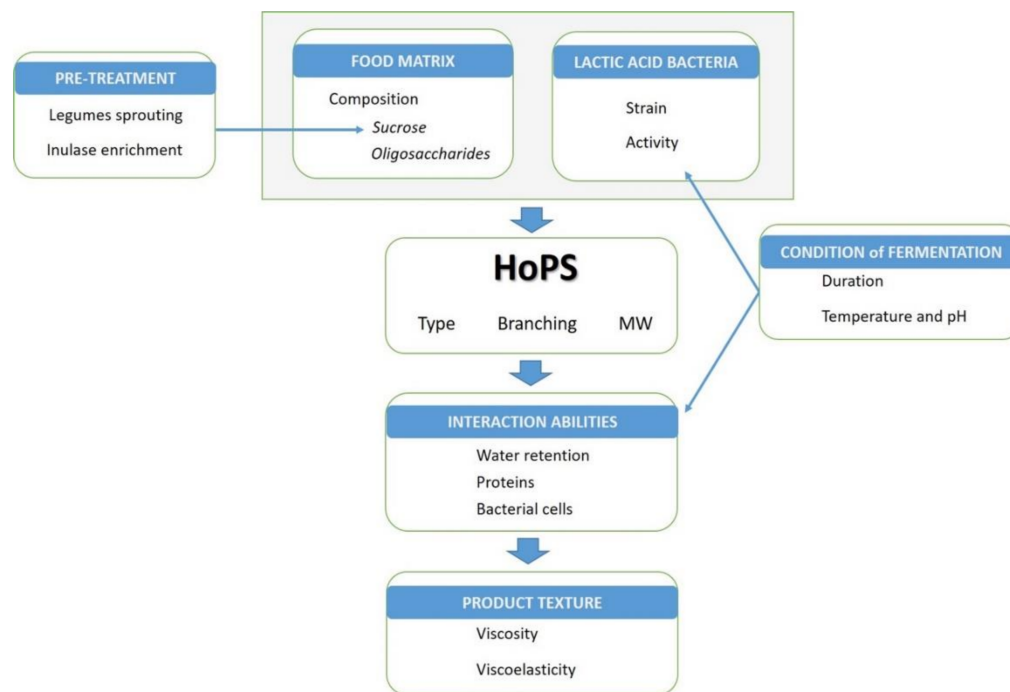


Figure 2. Relation between food matrix, processing parameters and product technological parameters.

4.1. Role of the Matrix Composition

The importance of the choice of the substrate was shown for HoPS production in cereals, influencing both the level of production or the structure of the HoPS [57]. Thereby, during sourdough production based on four different flours (buckwheat, quinoa, sorghum and teff) with a dextran-producing *W. cibaria* strain, EPS and oligosaccharide concentrations were observed to be inversely related. The highest dextran content was formed in sourdoughs prepared with buckwheat (4.2 g EPS kg⁻¹ sourdough) and quinoa (3.2 g EPS kg⁻¹ sourdough). Furthermore, the level of maltose in flour was found to be a key point for high-molecular-weight EPS synthesis. Maltose was found to be higher in wheat and lowest in buckwheat, resulting in the lowest levels of high-MW dextran in wheat sourdoughs [57,58].

Factors such as monosaccharide composition, type of linkage, side chains, molecular weight and their interaction with other constituents (mainly casein and ions) also affect the rheological functions of EPS lactobacilli in fermented milk products [59].

4.2. Addition of Ingredients

It is known that sucrose is first used by LAB for growth during the exponential phase and then can be used for dextran production during the stationary phase by EPS-producing strains liberating fructose. Sucrose addition was tested on fava bean flour fermented with *Leuconostoc* spp. and *Weissella* spp. to evaluate its potential in EPS production [60]. It was observed that the addition of sucrose strongly induced dextran and glucan production when compared to control fava bean dough fermented with the same starter. Glucan contents were found to be higher than dextran contents in all cases, with 1.86% to 3.67% and 2.57% to 4.33% in sucrose-enriched doughs versus 0.32% to 0.82% and 0.11% to 0.74% in control dough, respectively, as low- and high-branched dextrans were both included in glucans. Interestingly, the higher production of glucan noticed for *Leuconostoc* spp. compared to

Weissella spp. in sucrose-enriched doughs could be related to the low-branched dextran preferentially produced by *Weissella* spp. while *Leuconostoc* spp. tend to produce more than one type of dextran.

Sucrose addition was also tested on legume-based sourdough as a potential approach to improve the use of legume flour in bakery products. The addition of 2% of sucrose during fermentation at a temperature of 30 °C allowed the production of linear dextran with 2.6% $\alpha(1\rightarrow3)$ linked branches by a *W. confusa* strain isolated from spontaneous fermentation and promoted the technological properties of legume-based sourdough [61].

Strategies to promote sugar availability in tubers also include enzyme activity, e.g., inulinase was added in Jerusalem artichoke for simultaneous saccharification and fermentation with *Bacillus velezensis* LT-2. The maximum EPS yield (9.25 g L⁻¹), benefiting from a shortened fermentation period by 26.67% and a significantly associated reduced cost, was obtained with the inulinase dosage of 18 U g⁻¹ sugar. This condition provided sufficient monosaccharides while exhibiting no substrate inhibition and affected the bacterial genes' regulation in the following aspects: impaired fructose inhibition, accelerated the synthesis of EPS nucleotide-sugars precursors, induced the transcription of the EPS synthetic gene cluster and strengthened the electron transport respiratory chain and transporter system [62].

The influence of fat content on EPS production was tested in meat fermented products with the aim of producing fat-reduced sausages. For a final fat content ranging between 17% and 33%, the EPS production observed in spreadable raw sausages ranged between 0.46 and 1.03 g kg⁻¹ for the HoPS-producing strains *Latilactobacillus sakei* 1.411 and *Lat. curvatus*, 1.1928 resulting in reduced hardness and higher softness. However, no significant differences of HoPS production levels were noted for the different fat contents [63].

4.3. Pretreatments

Some pretreatments are considered to modulate the level of EPS production in the food matrix. This is the case with sprouting, which was recently tested for lentil flour [64]. The dextran levels synthesized *in situ* by a *W. confusa* strain were found to be slightly higher in sprouted lentil sourdoughs than in those with unsprouted lentils, with 9.7% and 9.2% w/w flour weight, respectively.

It appears that other pretreatments influence the EPS production yield as is the case for thermal treatment. The heat intensity applied upstream of fermentation seems to be a determining factor, as demonstrated in a lupin-based milk alternative, where a more intensive heat treatment (ultra-high temperature) resulted in higher amounts of HePSs and better rheological and textural properties than pasteurization [65].

Nonthermal technology such as ultrasound was also used to shorten fermented products' fermentation time. This technology allows to shorten the adaptation phase of microorganisms in the growth curve and enhance the membrane permeability, therefore allowing a mass transfer. This technic showed a tendency to improve the EPS production rate in kefir [66].

4.4. Conditions of Fermentation

The role of fermentation conditions on *in situ* EPS production levels was shown on sucuk sausage, the most consumed fermented meat product in Turkey [67]. A similar tendency for the EPS production levels with both *Latilactobacillus plantarum* and *Leu. mesenteroides* strains was observed when fermentation temperatures of 14 to 18 °C and durations of 8 to 16 days were compared. The EPS production increased significantly with the prolongation of ripening period and fermentation temperature, from 4.68 to 18.96 mg EPS kg⁻¹ dry matter, in a conversely proportional way to pH. This can be related with the temperature requirement and growth-associated dependency of EPS production depending on the mesophilic or thermophilic nature of the strain.

4.5. Traditional (Spontaneous) vs. Inoculated

Lactic acid bacteria EPSs from spontaneously fermented wheat bran sourdough have been studied under the prism of the production by dominant isolates [68,69]. However, the diversity or the level of EPS production in spontaneous fermented food in comparison with inoculated fermented food has not been characterized, and the question remains of interest for functional food optimization.

5. Technological Impact of EPSs in Foods, Depending on Matrix and Processing Conditions

The combination of the endogenous factors of the matrix and the exogenous factors relative to the applied processes greatly influences the synthesis and the properties of EPSs in foods and, ultimately, the quality of the final product (Table 3).

Table 3. Influence of some food pretreatment and processing condition on EPS production and properties.

EPS	Strain	Processing Conditions	Effects	Reference
Dextran	<i>W. cibaria</i> MG1	Different fermented flours (buckwheat, quinoa, sorghum and teff) used as the basis for bread recipes using sourdough	Yield depended on the substrate and was highest in buckwheat and quinoa sourdough. The level of maltose in flour influences the MW of synthesized EPSs in sourdoughs (lower maltose in buckwheat resulting in the most high-MW dextran). Bread rheological properties were influenced by the flour matrix in relation to the variability of HoPS: reduction of crumb hardness in buckwheat (−122%), teff and quinoa breads; reduction of the staling rate in buckwheat and teff breads.	[57]
Dextran	<i>W. confusa</i> VTT E-90392	<i>In situ</i> EPS production in wheat sourdoughs, 10% enriched with sucrose or unenriched. Sourdoughs were used in baking at 43% of the dough weight.	<i>W. confusa</i> efficiently produced polymeric dextran (11–16 g/kg DW) from the added sucrose in wheat sourdough without strong acid production. The produced dextran significantly increased the viscosity of the sourdoughs. Application of dextran-enriched sourdoughs in bread baking provided mildly acidic wheat bread with improved volume (up to 10%) and crumb softness (25–40%) during 6 days of storage.	[70]
Dextran	<i>W. confusa</i> SLA4	Lentil flour sprouting	Increase slightly the dextran synthesis in comparison to nonsprouted lentil sourdoughs (9.7% and 9.2% w/w flour weight, respectively).	[64]
EPS	Kefir starter culture	Ultrasonic sound waves	The treatment allows kefir production in a shorter time by affecting the growth rate and lactic acid and EPS production rate.	[66]
EPS	<i>Latilactobacillus plantarum</i> 162R and <i>Leu. mesenteroides</i> N6	Ripening period and fermentation temperature	Increase of EPS production level, associated with hardness reduction of the fat-reduced products and lower loss and storage moduli, when the ripening period was prolonged and the fermentation temperature was higher.	[67]
Dextran Fructan, glucan Dextran	<i>Latilactobacillus curvatus</i> TMW 1.624 <i>Ligilactobacillus animalis</i> TMW 1.971 <i>Lim. reuteri</i> TMW 1.106	<i>In situ</i> production of various EPSs compared to the addition of hydroxypropyl-methylcellulose (HPMC)	Only HPMC and the dextran of <i>Lat. curvatus</i> TMW 1.624 retained water. The moisture content, baking loss and crumb firmness were improved the most by dextran of <i>Lat. curvatus</i> TMW 1.624. Structure analysis revealed that this dextran had the highest molecular weight of the analyzed EPSs (118–242 MDa) and was branched in position 3 (8–9%). A structure–function relation was suggested in which high weight, average molar mass and branching at position 3 of the glucose monomer foster a compact conformation of the molecule, enabling an increased water-binding capacity and promoting superior structural effects in gluten-free breads.	[71]

Table 3. Cont.

EPS	Strain	Processing Conditions	Effects	Reference
Dextran	<i>Leuconostoc pseudomesenteroides</i> DSM 20193 and <i>W. confusa</i> E3403	<i>In situ</i> production in legume protein-rich foods (fava bean protein concentrate)	Stabilization, prevention of protein aggregation. Improvement in rheological and textural parameters in sucrose-added pastes after fermentation. <i>W. confusa</i> exhibited a higher viscosity index and a more rigid character of formed gel values than <i>Leu. pseudomesenteroides</i> (1441 and 766 g sof viscosity index, 0.39 and 0.23 of relative viscoelasticity index $\tan \delta$, respectively). The content of 35%, w/w of quinoa flour in water was determined as optimal regarding the viscosity parameter. The viscosity and water holding capacity increased during fermentation with <i>W. confusa</i> , as the consequence of the EPS synthesized, contrary to what observed for other strains (0.7 Pa s and 98% observed for <i>W. confusa</i> versus 0.2 Pa s and 69–70% for the other tested strains, respectively).	[72]
Dextran	<i>W. confusa</i> DSM 20194, compared to probiotic strains (<i>Lat. plantarum</i> T6B10, <i>L. rhamnosus</i> SP1)	Quinoa flour subjected to desaponification and gelatinization prior to fermentation		[73]

5.1. Applications of EPSs

There are many potential industrial applications for LAB-originated EPSs based on their role as gelling, thickening, emulsifying, stabilizing, water-binding and viscosifying agents. EPS technological features are traditionally useful in fermented dairy foods manufacture such as yogurt, milk-based desserts, kefir, cultured cream or cheese. They can act as thickeners and texturizers conferring advantageous properties during the process. Their structure allows them to bind water to stabilize and increase the viscosity of the final product, to interact with milk proteins and micelles to improve the firmness of the casein network and at the same time to decrease syneresis [74].

HePSs, composed of several carbohydrate moieties, play an important role in dairy products as they can be produced from various sugars and notably lactose in the absence of sucrose [75,76]. It was shown that HePSs produced by utilizing different carbon sources vary only in the fraction of different sugars, while the composition remained the same, indicating that sugar type has no effect on the composition of HePSs despite its capacity to process the sugars into various monomer units [77].

HoPSs on the other hand, consisting mainly of glucose or fructose, are effectively used in cereal- and legume-flour-based products as a natural alternative to commercial hydrocolloids for the enhancement of both gluten-containing and gluten-free cereal- and legume-based products [71]. The exploitation of HoPS production is also particularly interesting in yogurt-like beverages or cheese alternatives aiming to mimic fermented dairy products, because the polysaccharides formed improve the textural and sensory properties and extend the shelf life of these plant-based products [74].

5.2. In Situ Production versus Ex Situ Addition

LAB-derived EPSs can be added as additives in foods or formed *in situ*. EPSs can be considered for addition into food products during processing as a biopolymer, as an alternative that is easier to control than *in situ* production by LAB culture. The addition of adapted amounts of polysaccharide at a specific time-point during food processing can lead to the desired functional and physical characteristics of the end product. This use of dextran is currently allowed by the European Commission at levels of up to 5% in bakery products [78] provided it is listed as an additive in the ingredients on packaging. For instance, the *ex situ* production and the use of bacterial EPSs as ingredients enables wider application of this category of EPSs since, in this form, the polymer can be added to the food in greater quantities than it would be if synthesized *in situ* [79].

Regarding the *ex situ* addition, hydrocolloids such as xanthan or hydroxypropyl-methylcellulose are commonly used in gluten-free formulations in order to improve the viscoelastic properties of doughs. Indeed, gluten-free products are characterized by low water absorption, changes in crumb characteristics, decreased bread volume and poor

stability. The efficiency of *in situ* HoPS production on the structure and quality of gluten-free bread enhancement has been studied by comparing buckwheat and rice flour mix and supplemented with different *Lactobacillus* EPSs (levan and dextran) or hydroxypropylmethylcellulose by Rühmkorf et al. [71]. In this study, all supplements increased the specific volume and reduced the crumb hardness noticeably. However, the improvement in moisture content, baking loss and crumb hardness were higher with dextran produced by the *L. curvatus* TMW 1.624 strain. This specific dextran was found to have a higher water retention capacity and molar mass than other analyzed EPSs. A structure–function relation was suggested in which high weight, average molar mass and branching at position 3 of the glucose monomer foster a compact conformation of the molecule and higher water binding capacity, conferring a high efficiency to this *in situ* HoPS. It goes in the direction of more efficiency for EPSs formed *in situ* compared to that of those produced *ex situ* and then added externally, as previously reported for bread [80]. In addition, the *in situ* production of EPS is generally considered as a promising alternative to conventional additives [75]. *In situ* production of EPS by EPS-producing strains present many advantages since it has less impact on production cost than addition, isolation and purification of EPS for use as an ingredient, and it fits the consumer demand for products with fewer additives and “clean labeled” products.

5.3. Texture

Independently of their origin, EPSs have been classified based on their functionality. It has been established that the intrinsic properties of EPSs (e.g., composition, branching, charge, molar mass) drive inter-macromolecular associations through hydrogen bonds, electrostatic or ionic forces. These factors are a key point for protein interaction and complexations with bacterial cells that are of great importance in rheological properties of fermented products, pro- and prebiotics interactions and biofilm formation [81]. Based on this statement, a web-based platform-independent database of bacterial exopolysaccharides provides access to detailed structural, taxonomic, growth conditions, functional properties and genetic and bibliographic information for EPS (<http://www.epsdatabase.com>) (accessed on 21 December 2021) in order to provide a fast access to functional EPS properties.

5.3.1. Rheological Properties

The comparison of bread recipes using sourdough obtained from different fermented flours (buckwheat, quinoa, sorghum and teff) with a dextran-producing *W. cibaria* strain has shown an impact of the flour matrix on the bread's rheological properties. For instance, the crumb hardness was reduced in buckwheat (−122%), teff and quinoa breads whereas a significant reduction of the staling rate was observed in buckwheat and teff breads in relation with the variability of HoPS production [57].

5.3.2. Viscosity

Specific spatial properties of the macromolecular conformation of EPSs are reflected in the intrinsic viscosity value $[\eta]$. This hydrodynamic parameter is influenced by the chain length, the backbone stiffness, the charge and the molecular weight and can be considered a key parameter describing the thickening capacity of a polysaccharide. Intrinsic viscosity depends on polymer size and conformation as well as on polymer–solvent interactions. As $[\eta]$ describes the volume occupied by a single polymer chain, a high $[\eta]$ usually represents large molecules and stiff chains, whereas low $[\eta]$ indicates small molecules and flexible chains [82]. In solution, HoPSs have generally low $[\eta]$. As such, levans have a very low $[\eta]$ ($>0.5 \text{ dL g}^{-1}$) even at high molecular weights, and this can be related to their compact and spherical molecular structure [83]. Less compact HoPSs such as dextran have an $[\eta]$ between 0.1 and 1.5 dL g^{-1} [84,85]. By comparison, polyionic EPSs such as xanthan have a more extended structure due to their charge repulsion and a greater $[\eta]$ (up to 76 dL g^{-1}) as ordered secondary structure can also affect $[\eta]$ [86].

The impact of *in situ* produced EPS on acidification, rheology and texture was evaluated in legume protein-rich foods by comparing two strains of *Leuconostoc pseudomesenteroides* and *W. confusa*. Along with a higher amount of dextran, a clear improvement in rheological and textural parameters was observed in 5% sucrose-added fava bean pastes after fermentation [72]. Higher viscosity index and viscoelasticity values (firmness, consistency, cohesiveness) were observed, conferring an elastic-paste structure due to the presence of dextran that could interact with proteins and further strengthen the gel structure, compared to pastes fermented with the same starter having a liquid structure. The benefit of *in situ* dextran production in fava bean protein is emphasized, since the observed properties could not be mimicked by simply mixing dextran, organic acids and the protein concentrate.

Several studies following the effect of sucrose supplementation in sterile skim milk textural properties demonstrated that milk solidification depended on the concentration of sucrose added with EPSs produced by *Weissella* sp. TN610, *W. hellenica* SKkimchi3 and *Leu. pseudomesenteroides* PC strain from pickled Chinese cabbage [2,87,88]. In the case of EPS from *Leu. pseudomesenteroides*, good pseudoplastic rheological properties and an increased viscosity according to the EPS concentration were observed. It was observed that the viscosity was influenced by process parameters such as temperature and pH. Indeed, at the same shear rate, viscosity decreased with increasing temperature maybe in relation to an increase in molecular flexibility and loosened polymer structure at higher temperatures [88]. It was also observed that the viscosity of EPS decreased as pH increased from 3.0 to 9.0, in line with previous studies on *Sporidiobolus pararoseus* EPSs [89].

5.3.3. Syneresis

The application of EPSs in the dairy industry is of high interest, especially for beverages and fermented milk products with the aim of reducing the amount of added milk solids. EPSs are able to texturize these products by improving viscosity without affecting aroma or taste and by reducing syneresis occurring along the fermentation process. However, these properties have only been connected to HePSs so far since they are characterized by high intrinsic viscosities linked to their $\beta(1\rightarrow4)$ linkages, leading to the formation of EPS solutions with high consistency, and by branching degree, which is directly related to the polymer stiffness [90].

6. Conclusions

The properties of HoPSs in food products open new opportunities for functional food development through their textural effects. As such, their use in plant-based foods aiming to mimic fermented dairy products or in cereal and legume flours as a natural alternative to additives, arouses growing interest. While they are composed of a single type of monosaccharide, the diversity of structures observed for these molecules is large, with a manifold combination of basic units and linkages leading to multiple linear or ramified chains, and various molecular weights. These factors on top of the yield of production strongly influence the HoPS characteristics.

A majority of the data concerns the culture media, and further investigations will be required before being able to predict the *in situ* HoPS production level, structure and functional effects according to the food matrixes and the sequence of the gene encoding glycosyl hydrolase. Nevertheless, production in foods can be controlled by the bacterial strain and the optimization of incubation parameters for bacterial growth and glycosyl hydrolase enzymes' stability. Sugar availability as a substrate and enzyme activity are crucial for HoPS production in order to control the composition and environment of the food and to optimize the production.

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2.2. Bénéfices de la consommation de fruits lactofermentés dans la lutte contre les désordres métaboliques

2.2.1. Désordres métaboliques et contexte réunionnais

Le syndrome métabolique caractérise un groupe d'altérations telles que l'obésité et la stéatose hépatique, augmentant le risque de diabète, de maladies cardiovasculaires et de carcinogenèse. Son développement est caractérisé par des facteurs génétiques et environnementaux (Ahn et al., 2013).

A La Réunion, plus de 40 % de la population est en surcharge pondérale en 2019, dont 16 % en situation d'obésité. Les femmes sont près de deux fois plus concernées par l'obésité que les hommes (ORS Réunion, 2022). L'obésité est caractérisée par une accumulation excessive de masse grasse et à une modification du tissu adipeux. Elle est induite par un déséquilibre entre les apports et les dépenses énergétiques. Le surpoids et l'obésité sont responsables de 44 % des cas de diabète de type 2 et 23 % des cas de maladies cardiaques et constituent la cinquième cause de mortalité dans le monde selon l'OMS (INSERM, 2017). L'obésité est liée à une accumulation excessive d'acides gras sous forme libre dans le foie conduisant à une stéatose hépatique ou stéatohépatite non-alcoolique (Choi et al., 2020). L'accumulation de graisse peut être causée par une résistance à l'insuline. Cette résistance entraîne l'activation des lipases hormono-sensibles dans les adipocytes, entraînant ainsi la libération de triglycérides dans le sang sous forme d'acides gras. L'accumulation de graisse dans le foie est due au caractère insulino-résistant du tissu adipeux qui ne peut plus capter le glucose. Ce dernier est donc capté par le foie où il est transformé en lipides puis stocké (Choi et al., 2020). L'obésité augmente également le risque d'hypertension artérielle, d'athérosclérose, i.e. dépôt d'une plaque majoritairement composée de lipides sur la paroi des artères, de dyslipidémie, de maladie rénale chronique, de cancers et de maladies respiratoires, de troubles hormonaux et de maladies articulaires (INSERM, 2017). Les changements alimentaires favorisant une consommation calorique excessive ainsi qu'une sédentarité accrue, i.e. un manque d'activité physique et une réduction des dépenses énergétiques, influencent fortement l'apparition de l'obésité. Cependant, la susceptibilité des individus à l'obésité est promue par des prédispositions génétiques avec un risque deux à huit fois plus élevé d'un individu à être obèse si des membres de sa famille le sont eux même. L'identification de gènes impliqués dans la prise de poids, l'obésité sévère et/ou les complications liées à l'obésité a montré qu'associés à des facteurs externes, e.g. un déséquilibre énergétique, ces gènes agissent sur la masse et la composition corporelles (INSERM, 2017). Outre l'alimentation et l'activité physique, l'horloge biologique augmente le risque de surpoids. Elle régule les fonctions de l'organisme et du métabolisme sur 24 h et est perturbée par l'insuffisance de sommeil, l'irrégularité des repas ou le travail nocturne. L'obésité est également influencée par le stress, certains médicaments, des virus, la composition du microbiote intestinal, l'exposition à des polluants et des facteurs de risque prénatal, e.g. tabagisme, surpoids maternel, diabète maternel et déficit ou excès de croissance du fœtus (INSERM, 2017).

D'après l'enquête européenne sur la santé réalisée en 2019, 10 % des Réunionnais de plus de 15 ans sont diabétiques ainsi que 27 % des personnes âgées de plus de 65 ans, cette dernière proportion est plus de 20 % plus élevée que celle de France métropolitaine (INSERM, 2019; ORS Réunion, 2022). La proportion de femmes prises en charge pour le diabète est de 54 % à La Réunion, soit approximativement deux fois plus que les femmes résidant en France métropolitaine (ORS Réunion, 2022). Sur l'île, 9 % de la population générale est prise en charge pour leur diabète, de type 1 et type 2 confondus, avec une augmentation de 3 % en moyenne estimée par an. Une surfréquence est observée au niveau régional concernant la surcharge pondérale, l'obésité et le diabète pris en charge par comparaison avec le territoire national (INSERM, 2019; ORS Réunion, 2022). En 2019, 4 % des Réunionnais diabétiques pris en charge ont subi des complications, e.g. accident vasculaire cérébral (AVC), infarctus, cardiopathie ischémique, insuffisance rénale chronique, dialyse, stéatose hépatique et/ou amputation (Figure 8). A La Réunion, 49 % des patients en insuffisance rénale chronique terminale en 2020 sont diabétiques. Cette fréquence est plus élevée que sur le territoire métropolitain (INSERM, 2019; ORS Réunion, 2022). Une altération de la perméabilité des muqueuses intestinales est liée au degré de stéatose et d'accumulation de graisse dans le foie (Lin et al., 2019). Entre 2015 et 2017, le diabète est directement responsable de 5 % des décès à La Réunion, soit un taux de mortalité trois fois supérieur à celui de la Métropole (ORS Réunion, 2022). De même que pour l'obésité, l'augmentation de la prévalence du diabète de type 2 est liée aux déséquilibres nutritionnels, i.e. une alimentation trop grasse et/ou trop sucrée, et à la sédentarité des individus (INSERM, 2019).

Parmi les 79 900 patients pris en charge pour leur diabète en 2019

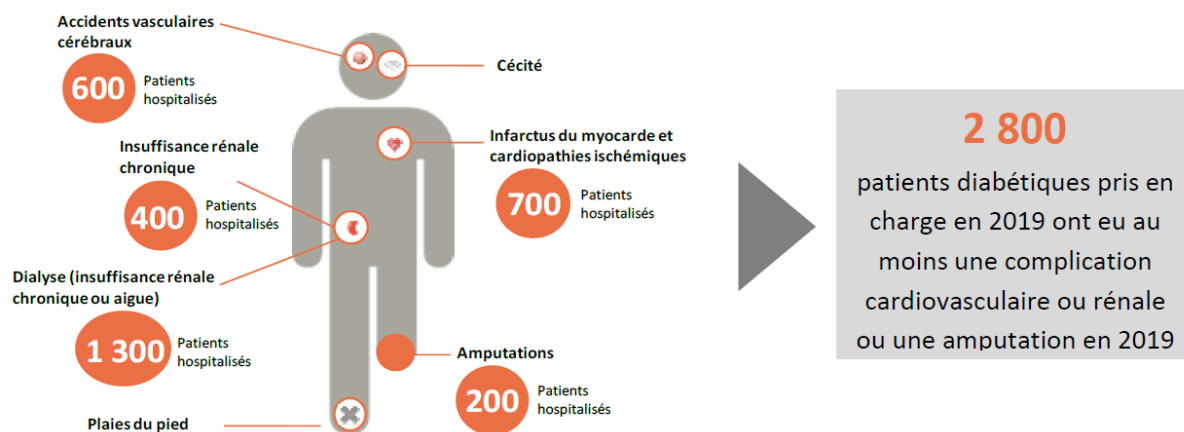


Figure 8 : Complications survenues en 2019 à La Réunion chez les patients diabétiques pris en charge (fréquence arrondie à la centaine près) (ORS Réunion, 2022).

Le diabète de type 2 affecte 90 % des patients diabétiques. Il s'agit d'une maladie métabolique chronique caractérisée par une hyperglycémie. Le diabète est diagnostiqué lorsque la glycémie à jeun excède 1,26 g/L, la concentration d'une personne saine étant de 1 g/L (INSERM, 2019). Cela va engendrer une résistance à l'insuline, une hormone dont le rôle est de faciliter le transport du glucose aux cellules. La sensibilité des cellules à l'insuline produite par le pancréas diminue, la sécrétion d'insuline devient insuffisante et engendre une accumulation du glucose dans le sang (INSERM, 2019). Le diabète de type 2 peut également être associé à une dysbiose intestinale, un stress oxydant et des désordres neurologiques et comportementaux. Cette maladie peut porter atteinte au cœur, aux yeux, aux reins, aux vaisseaux sanguins et aux nerfs (Zepeda-Hernández et al., 2021). Le développement de solutions thérapeutiques constitue un enjeu majeur dans le traitement du diabète. Le microbiote

intestinal joue un rôle dans la survenue du diabète de type 2. Un axe de recherche consiste donc à développer des approches nutritionnelles visant à rééquilibrer le microbiote des patients (INSERM, 2019).

Une grande majorité, i.e. 98 %, des espèces bactériennes du microbiote intestinal sont classées en quatre phyla : *Firmicutes*, *Bacteroidetes*, *Proteobacteria* et *Actinobacteria* (Figure 9) (Lin et al., 2019). Entre 500 et 1 000 espèces bactériennes sont présentes dans le tractus gastro-intestinal. La diversité bactérienne peut être modifiée par de nombreux facteurs tels que l'âge de l'individu, l'alimentation, des traitements médicamenteux ou des pathologies telles que l'obésité et le diabète de type 2 (Dave et al., 2012; Hopkins et al., 2002; Le Chatelier et al., 2013). Les individus présentant une adiposité globale élevée, une résistance à l'insuline et une dyslipidémie sont caractérisés par une faible richesse bactérienne alors que les individus sains présentent une diversité bactérienne plus importante (Le Chatelier et al., 2013). Des variations de composition du microbiote intestinal jouent un rôle important dans la pathogenèse de l'obésité. Un déséquilibre du microbiote intestinal, ou une dysbiose, influence l'apparition de désordres métaboliques chez l'Homme, i.e. l'obésité, l'inflammation chronique et la résistance à l'insuline (Cabello-Olmo et al., 2019; Lin et al., 2019). Un ratio élevé entre les Firmicutes et les Bactéroïdes est lié à une augmentation du poids des individus. Une diminution de ce ratio est liée à une amélioration de la glycémie, une réduction de l'accumulation de graisse et à une diminution de la masse corporelle. Le microbiote intestinal joue donc un rôle majeur dans la régulation du métabolisme énergétique (Lin et al., 2019).

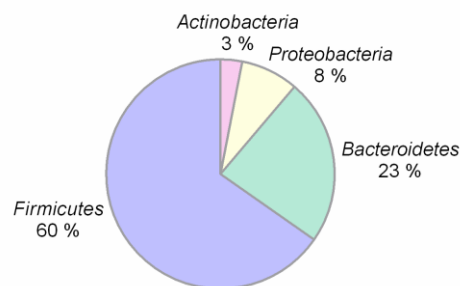


Figure 9 : Classification du microbiote intestinal (d'après Lin et al., 2019).

2.2.2. Effets de la consommation de fruits lactofermentés

Outre une consommation de légumineuses supérieure sur l'île par rapport au territoire national qui se rapproche des recommandations nutritionnelles, les habitudes alimentaires locales tendent plutôt vers une consommation élevée en protéines animales et en aliments gras, sucrés et salés par comparaison à la France métropolitaine. La prise quotidienne de boissons sucrées industrielles s'élève à 13 % chez les Réunionnais et il a été constaté que moins de 10 % de la population consomme cinq portions de fruits et légumes par jour (ORS Réunion, 2022). Les bénéfices de cette catégorie d'aliment sont nombreux et il est important de favoriser leur consommation. Dans cette optique, une stratégie consiste à diversifier l'offre alimentaire relative à ces produits. Au vu des nombreux effets de la fermentation lactique sur les composés nutritionnels décrits précédemment, la lactofermentation constitue une piste tangible pour le développement de produits alimentaires à base de fruits, destinés à la prévention des maladies métaboliques sur le territoire réunionnais. Les bénéfices sont envisagés à plusieurs niveaux.

2.2.2.1. Sur la prise alimentaire

Les fruits sont riches en fibres alimentaires résistantes à la digestion enzymatique et non-absorbées dans l'intestin. La consommation d'un régime riche en fibres solubles et insolubles améliore la santé au niveau gastro-intestinal et réduit les risques d'apparition des maladies cardiovasculaires, du diabète de type 2, des pathologies liées à l'obésité (Bosch-Sierra et al., 2019). Cette consommation est liée à la sensation de satiété et, par conséquent, à une potentielle prise alimentaire. Ne pas ressentir cette sensation de satiété va conditionner un apport alimentaire calorique chez les individus et aura donc un impact sur la prévalence de l'obésité dans le monde. Le type de fibres et les propriétés physicochimiques, e.g. hydratation, viscosité, taille des particules et fermentescibilité, vont influencer l'effet de leur consommation sur la sensation de satiété. Les fibres contribuent à l'équilibre énergétique (Bosch-Sierra et al., 2019). La consommation de boissons enrichies en fibres visqueuses augmente la satiété de manière dose-dépendante en ralentissant la vidange gastrique et le transit intestinal. Cela réduit l'absorption intestinale du glucose et module la sécrétion des hormones gastro-intestinales impliquées dans la régulation de l'appétit, i.e. le glucagon-like peptide 1, le peptide YY et la ghréline. La consommation de fibres diminue également la glycémie postprandiale et la réponse insulinémique (Bosch-Sierra et al., 2019).

2.2.2.2. Sur l'action des enzymes digestives

Des études *in vitro* ont montré que les composés phénoliques isolés ou issus de fruits et légumes sont capables d'inhiber l'activité d'enzymes digestives comme la lipase pancréatique, l' α -amylase, l' α -glucosidase (He et al., 2007; Tan et al., 2017; Zhang et al., 2011). L'activité inhibitrice des polyphénols contre les enzymes glycolytiques est liée à leur structure, qui leur permet d'interagir avec l'enzyme ou le substrat de la réaction. Les groupements hydroxyles semblent jouer un rôle important dans cette activité puisque les acides phénoliques avec plus d'un groupement hydroxyle, comme les acides caféique ou protocatéchiq, ont un pouvoir inhibiteur supérieur aux acides phénoliques moins polaires possédant un ou deux groupements méthoxy, comme les acides *p*-coumarique, syringique ou vanillique (Alexandre et al., 2022).

L'inhibition des enzymes de digestion des glucides conduit à une diminution de la concentration en glucose au niveau intestinal et de son absorption et limite donc l'hyperglycémie post-prandiale. Cette capacité pourrait donc représenter un atout dans la lutte contre les maladies métaboliques en modérant l'hyperglycémie post-prandiale de façon ciblée, contrairement à des inhibiteurs de synthèse entraînant des effets secondaires (Zhang et al., 2011). Des travaux récents menés au sein de notre équipe de recherche ont montré que la fermentation lactique permet d'accroître cette activité inhibitrice de l' α -glucosidase dans les purées de mangue et de papaye par rapport à des purées non fermentées (Mashitola et al., 2021; Mashitola et al., 2021).

2.2.2.3. Sur la biodisponibilité des molécules à activité antioxydante des fruits

Les composés tels que les composés phénoliques, les caroténoïdes, les tocophérols et l'acide ascorbique ont une forte activité antioxydante conférée par leur structure chimique, et sont susceptibles de prévenir des dommages oxydatifs moléculaires et cellulaires induisant diverses pathologies dont le diabète de type 2 et les maladies cardiovasculaires. La capacité antioxydante, c'est-à-dire la capacité à neutraliser les radicaux libres et leur propagation, est modulée selon le groupe fonctionnel attaché à un aglycone basique pour les polyphénols, c'est le cas des groupements hydroxyles des acides phénoliques (Hur et al., 2014). La métabolisation de ces molécules par

l'organisme influence fortement leur capacité redox. Ainsi à titre d'exemple, l'ingestion en quantité importante de flavonoïdes n'induit pas une augmentation de l'activité antioxydante (Haminiuk et al., 2012). D'une manière générale, l'activité antioxydante sera modulée par la biodisponibilité des molécules à partir de la matrice alimentaire.

La bioaccessibilité et la biodisponibilité sont définies par deux critères (Haminiuk et al., 2012) :

- (1) La proportion de composés libérés par la matrice alimentaire après digestion et qui devient donc disponible pour l'absorption intestinale ;
- (2) La proportion de composés absorbés retrouvée dans la circulation sanguine qui devient disponible pour une utilisation métabolique et qui agit au niveau du site d'action.

2.2.2.3.1. Les polyphénols

Sous forme libre et solubles dans la phase aqueuse des digestats gastro-intestinaux, ils sont absorbés par transport actif ou par diffusion passive (**Figure 10**). Ils sont ensuite distribués dans l'organisme où s'exercent leur actions bénéfiques, par inhibition de l'oxydation des lipoprotéines de basse densité (LDL), du cholestérol et des liposomes (Lin et al., 2019).

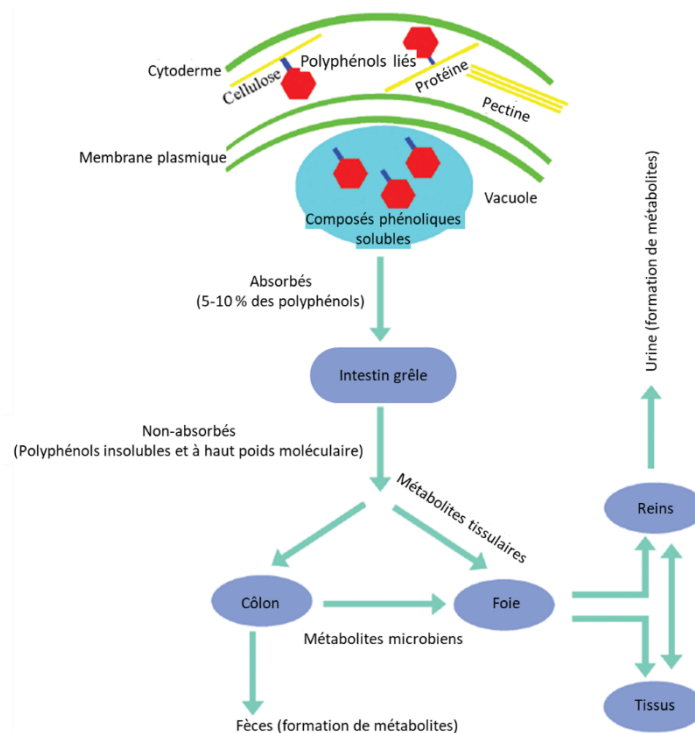


Figure 10 : Voie métabolique des polyphénols apportés par l'alimentation chez l'Homme (Lin et al., 2019).

Les polyphénols non-bioaccessibles constituent 90 à 95 % des polyphénols apportés par l'alimentation. Ils sont liés aux protéines, à la cellulose, à la pectine et à d'autres macromolécules de la paroi cellulaire par des liaisons éther, ester ou carbone-carbone (Lin et al., 2019). Les polyphénols de haut poids moléculaire sont principalement transformés par le microbiote intestinal mais peuvent aussi être absorbés par le tractus gastro-intestinal. Un très grand nombre de métabolites dérivés de polyphénols apportés par l'alimentation sont ainsi retrouvés dans l'urine et les fèces (Lin et al., 2019).

Les polyphénols alimentaires sous forme de polymères, glycosylés ou estérifiés, doivent être hydrolysés par des enzymes digestives ou bactériennes pour pouvoir être absorbés. Leur structure chimique et leurs interactions avec les protéines et les fibres de la matrice alimentaire influencent leur libération. Au niveau intestinal, l'absorption peut être réduite et dépend du type de sucres liés aux polyphénols et de leur degré de glycosylation. Il a été montré que la réduction de l'interaction entre les polyphénols et les polymères glucidiques augmente la biodisponibilité des premiers. Certains composés phénoliques peuvent cependant atteindre le côlon et ainsi contribuer à un environnement antioxydant (Haminiuk et al., 2012; Lin et al., 2019).

La modification par la transformation des composés antioxydants naturellement présents dans les fruits peut avoir des répercussions fonctionnelles après ingestion. Les étapes unitaires les plus délétères sont les étapes impliquant une séparation physique (raffinage, épluchage), en lien avec les concentrations plus élevées en phytonutriments dans l'épiderme, tandis qu'une bonne stabilité dans la matrice végétale a été observée lors des traitements thermiques couramment appliqués en agroalimentaire (Renard et al., 2020). Durant la fermentation lactique, il a été observé une conversion des formes conjuguées des composés phénoliques en leurs formes libres, améliorant ainsi leur absorption et leurs effets (Hur et al., 2014).

2.2.2.3.2. Les caroténoïdes

Ce sont des molécules lipophiles qui suivent la voie d'absorption des lipides. Une fois libérés de leur matrice alimentaire et solubilisés dans des gouttelettes lipidiques, ils sont incorporés aux micelles mixtes de sels biliaires pour permettre leur absorption par transporteurs ou par diffusion passive (Kopec & Failla, 2018). La biodisponibilité de ces molécules est complexe et dépend du type de caroténoïde lui-même (polarité, nombre de doubles liaisons conjuguées, isomérie), de la matrice alimentaire (association avec des protéines, présence de lipides, caractéristiques structurales, traitements appliqués) et de paramètres liés à l'individu (statut nutritionnel et pathologique, âge, facteurs génétiques) (Shilpa et al., 2020; Stephenson et al., 2021).

Des données suggèrent un effet lié à la présence de composés phytochimiques des fruits (flavones, phytostérols, polyphénols, acides gras oméga-3, tocophérols) et de cations divalents sur l'absorption de ces molécules. Les traitements technologiques appliqués en impactant la structure au niveau de la pulpe, des cellules ou des composants cellulaires, influencent aussi la biodisponibilité des caroténoïdes comme cela a été décrit sur des produits à base de carotte, tomate et agrumes (Kopec & Failla, 2018).

Par comparaison avec de nombreux procédés de transformation tels que le blanchiment, la cuisson vapeur, le séchage ou la cuisson par friture, la fermentation lactique semble préserver ou augmenter les teneurs en β -carotène après une période de conservation. Cet effet peut en partie être expliqué par une libération accrue du β -carotène plutôt que par une synthèse de caroténoïdes. En effet, la fermentation lactique entraîne des changements structuraux de la matrice végétale qui touche aux particules de la pulpe ou aux composants cellulaires végétaux, et améliore l'extractibilité des caroténoïdes (Mapelli-Brahm et al., 2020).

La fermentation lactique influence aussi la configuration géométrique des caroténoïdes. Une augmentation de teneur en lycopène a ainsi été observée sur de la purée de tomate lactofermentée. Cette augmentation est accompagnée d'un ratio cis/trans-lycopène plus élevé, en faveur de l'isomère le plus biodisponible, à savoir le cis-lycopène (Bartkiene et al., 2013; Cooperstone et al., 2015).



2.2.2.4. Sur la génération d'exopolysaccharides bioactifs

La rédaction d'un article de revue intitulé « **Lactic acid bacterial production of exopolysaccharides from fruit and vegetables and associated benefits** » publié dans le journal *Fermentation* permet de faire un état de l'art des EPS produits lors de la fermentation lactique en abordant notamment les souches bactériennes productrices, les EPS obtenus, leur structure et leur classification et leur synthèse selon la composition du substrat et les paramètres d'incubation. L'influence de cette production sur la qualité des produits alimentaires et les bénéfices sur la santé sont développés.



Review

Lactic Acid Bacterial Production of Exopolysaccharides from Fruit and Vegetables and Associated Benefits

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Abstract: Microbial polysaccharides have interesting and attractive characteristics for the food industry, especially when produced by food grade bacteria. Polysaccharides produced by lactic acid bacteria (LAB) during fermentation are extracellular macromolecules of either homo or hetero polysaccharidic nature, and can be classified according to their chemical composition and structure. The most prominent exopolysaccharide (EPS) producing lactic acid bacteria are *Lactobacillus*, *Leuconostoc*, *Weissella*, *Lactococcus*, *Streptococcus*, *Pediococcus* and *Bifidobacterium* sp. The EPS biosynthesis and regulation pathways are under the dependence of numerous factors as producing-species or strain, nutrient availability, and environmental conditions, resulting in varied carbohydrate compositions and beneficial properties. The interest is growing for fruits and vegetables fermented products, as new functional foods, and the present review is focused on exploring the EPS that could derive from lactic fermented fruit and vegetables. The chemical composition, biosynthetic pathways of EPS and their regulation mode is reported. The consequences of EPS on food quality, especially texture, are explored in relation to producing species. Attention is given to the scientific investigations on health benefits attributed to EPS such as prebiotic, antioxidant, anti-inflammatory and cholesterol lowering activities.

Keywords: food rheology; prebiotic; health benefits

1. Introduction

Lactic acid fermentation (LAF) is a liable method to improve the shelf-life of fruit and vegetables along with nutritional and sensory qualities. Bacterial strains belonging to *Lactobacillus*, *Leuconostoc*, *Weissella* and *Bifidobacterium* genera are commonly involved in food LAF. These lactic acid bacteria can produce lactic acid and other organic acids, carbon dioxide, aromatic compounds, exopolysaccharides (EPS), bacteriocins and enzymes, all involved in the modification of quality of fermented foods. It has also been shown that lactic acid bacteria (LAB) are able to modulate the composition in phenolic compounds and enhance antioxidant activity [1]. Several studies have suggested that lactic acid fermented fruit juices, which exhibit a specific phenolic composition and contain EPS, could constitute a prebiotic food and have an influence on the structure and function of the gut microbiota [2,3]. The ability of LAB to modulate nutritional status of plant-based foods depends on bacterial strains and matrix used for LAF [4]. Among the compounds produced during LAF, EPS have the ability to change rheological properties of food such as viscosity [5], which contributes to the sensory acceptability and

stabilization of the products [2]. In the food industry, hydrocolloids gather polysaccharides, which improve textural stability of suspensions, rheological properties of foods, pastry and cake texture and shelf-life by swelling in aqueous medium. It has been shown that EPS produced by *Lactobacillus*, *Leuconostoc* and *Weissella* strains have hydrocolloid properties [6] and would deserve to be tested as an innovative alternative for food formulation.

Up to 70% of the energy of microbial cell can be used to produce EPS. However, this energy consumption is balanced by the protective effect of EPS against stress by the formation of a protecting barrier [7]. The cell is therefore protected from temperature or osmolarity shifts and from toxins and antibiotics. Both quantity and quality of EPS produced depend on sugar availability, presence of micronutrients and fermentation conditions [8].

LAB polysaccharides have interesting and attractive characteristics, including antioxidant activities, and generate an increasing interest for their possible use in the field of food and pharmaceutical industries. This review aims at exploring EPS production during fruit and vegetable LAF, the contribution of EPS-producing LAB species and the associated nutritional and sensory benefits that can result from this production.

2. EPS Classification

EPS produced by LAB are classified according to their chemical composition and their biosynthesis mechanism. Homopolysaccharides (HoPS) are produced in the extracellular medium from one type of monosaccharide, whereas heteropolysaccharides (HePS) are produced intracellularly from several monosaccharides and are then exported from the cell [2,8–11].

2.1. Homopolysaccharides

HoPS can be characterized according to the type of monosaccharide composing their structure. Glucan, namely dextran, contains only glucose, whereas fructans, namely levan and inulin, contain only fructose and polygalactan contains only galactose [8]. As HoPS are composed of a single monosaccharide, they can be further characterized according to their glycosidic bonds (Figure 1), branching, chain length, molecular weight, and polymer structure [12].

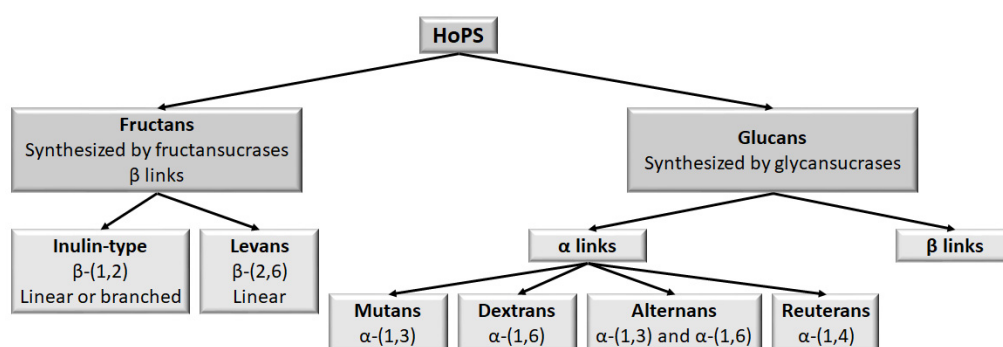


Figure 1. Homopolysaccharides (HoPS) classification according to their glycosidic linkages (adapted from [10]).

LAB producing HoPS belong to *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus* and *Weissella* genera. For instance, synthesis of a dextran-type glucan by a *Leuconostoc pseudomesenteroides* strain isolated from juçara palm fruit was confirmed using nuclear magnetic resonance (NMR) spectroscopy. More precisely, a Fourier transform-infrared (FT-IR) spectroscopy analysis identified functional groups and characterized covalent bonds of the produced EPS [13].

HoPS molecular weight ranges between 10^4 Da and 10^8 Da and is bacterial strain-dependent [8]. Indeed, dextrans from *Leuconostoc mesenteroides* and *Weissella cibaria* have a molecular weight between

6.2 and 7.1×10^6 Da and between 5×10^6 and 4×10^7 Da, respectively. HoPS production yield can reach up to 10 g/L of bacterial culture [2].

HoPS classification takes into account the carbon atoms involved in α or β linkages (Figure 1). α -D-glucans include dextrans, mutans, reuterans and alternans and are produced by *Lactobacillus*, *Leuconostoc* and *Streptococcus* species. *Pediococcus* species produces β -glucans [14]. More precisely, dextrans are composed of a main chain of repeated glucosyl units with α -(1 \rightarrow 6) linkages and of simple units or side chains with α -(1 \rightarrow 2), α -(1 \rightarrow 3) or α -(1 \rightarrow 4) linkages [6,15]. They form compacted branched structures regardless of the number of branch chains, which explain their effect on viscosity when present at high concentrations. *Lactobacillus fermentum*, *Lactobacillus sakei*, *Lactobacillus hilgardii*, *Lactobacillus parabuchneri* and *Lactobacillus curvatus* produce dextrans which are mainly composed of α -(1 \rightarrow 6) glycosidic bonds and ramified at the carbon atom in position 3. Dextrans confer different rheological properties depending on their solubility and structural heterogeneity. They are commonly used as plasma substitutes, thickening agents and emulsifiers. Mutans are generally linked by α -(1 \rightarrow 3) bonds. They are characterized by their ability to contribute to microbial adhesion [12].

The main chain of fructans contains fructosyl fragments with β -(2 \rightarrow 6) or β -(2 \rightarrow 1) linkages [6]. Fructans gather inulins and levans which are synthesized by *Lactobacillus*, *Leuconostoc*, *Streptococcus* and *Weissella* species [10]. Levans are produced by some *Lactobacillus reuteri* and *Lactobacillus sanfranciscensis* strains and contained predominantly β -(2 \rightarrow 6) linkages [6]. Inulins are composed of β -(2 \rightarrow 1) linkages and ramifications at position β -(2 \rightarrow 6) [12].

2.2. Heteropolysaccharides

HePS consist of repeated units with different degrees of polymerization and are composed of two to eight different monosaccharides such as glucose, rhamnose, galactose, fructose or mannose. HePS are produced by *Lactobacillus*, *Lactococcus*, *Streptococcus* and *Bifidobacterium* species [8]. HePS structure depends on the number and type of monosaccharides and on the kind of linkages involved [2]. They can be linear or branched with α and β linkages [8]. HePS produced by *Lactobacillus* species are composed of repeated units of seven monosaccharides with glucose, galactose and rhamnose as main sugars. The molecular weight of HePS ranges from 10^4 to 10^6 Da [10].

Unlike HoPS, HePS are produced at small concentrations, typically from 50 to 200 mg/L of bacterial culture [2,8,12].

3. EPS Production

3.1. Effect of the Substrate Composition

A method commonly used to demonstrate EPS production by LAB is to grow them on solid MRS medium supplemented with sugars such as sucrose, maltose, fructose, glucose or lactose and to characterize EPS produced according to the appearance of the colonies. Indeed, colonies with HoPS have a viscous appearance whereas those with HePS have a shiny aspect [2]. EPS production can also be determined by evaluating their production in a liquid medium supplemented with various nitrogen and carbon sources and vitamins. Moreover, for EPS production in foods, the influence of sugars already present in the studied matrix must be taken into account [2].

A specific substrate is required for EPS synthesis and allows the action of specific enzymes on oligosaccharide carbon sources. Sucrose is the most commonly used substrate for HoPS synthesis. EPS production can also be influenced by modifying the nature or/and the amount of nutrients available as well as pH, water activity and oxygen concentration in the culture medium [12]. Indeed, the structure of EPS and their content vary according to carbon, nitrogen, phosphorus or sulphur sources [15] and their biosynthesis depends also on the carbohydrate source [16].

For instance, a more than five-fold increase in HePS production was achieved by optimization of glucose, yeast extract and ammonium sulphate content in growth media of two *Lactobacillus plantarum* strains [16].

LAB are sensitive to pH and nature or amount of sugars, these factors should be considered for EPS production in fruit and vegetable products in regards to their composition. Sugar content is highly variable in these matrixes, and approximatively ranges from 0.8 in citrus to 19.6 g/100 g in black grape within fruits, and from traces in baby spinach to 6.8 g/100 g in beetroot within vegetables. The main sugars consist mostly in fructose, glucose or sucrose as it was found for litchi and red pepper, black grape and black radish, or pineapple and carrot, respectively. Apart from these major sugars, some products also contain compounds in smaller amounts, such as galactose or maltose in peach [17]. As for the pH, going from around 2.3 in citrus and 3.5 in pineapple to around 6 in cantaloupe melon and carrot [18,19]. Both pH and main sugars variation could lead to a wide diversity of EPS production from fruit and vegetables.

3.2. Effect of Bacterial Strain and Incubation Parameters on EPS Production

Apart from pH and sugar content, the type of bacteria influences HoPS production levels. Yu et al. [20] showed that only one strain of *W. cibaria* screened from kimchi can achieve a significant EPS production (up to 9.8 g/L) in a dose-dependent way in response to a high sucrose supplementation in the growing media. Similarly, different strains from the same species, *W. cibaria* MG1, MG7 and F33 isolated from cereal environments, showed approximately 2.8-fold variance in EPS production in sucrose-MRS broth [21].

Fessard and Remize [22] demonstrated the impact of temperature on EPS production using various LAB isolated from tropically grown fruits and vegetables. *L. pseudomesenteroides/mesenteroides*, *Leuconostoc lactis* and *Weissella cibaria/confusa* isolates were able to produce EPS at 30 °C but not at 37 °C [22]. Similarly, dextran production by *L. lactis* AV1n could be observed with growth media containing sucrose (but not glucose, fructose or maltose) and this production occurred at 20 and 30 °C but not at 37 °C. Dextran content was higher at 30 °C than at 20 °C, with 4.15 g/L and 2.96 g/L, respectively. Dextran production decreased to 0.41 g/L when the LAB was grown at 37 °C. These results demonstrated that dextran production by *L. lactis* AV1n was the highest at 30 °C [23]. Turkish-type fermented sausages, named *Sucuk*, were prepared using *Lb. plantarum* 162 R, *L. mesenteroides* N6 or a mixture of both strains. These LAB were chosen for their ability to produce EPS. An increase in temperature or fermentation time resulted in a rise in EPS production. Under the same fermentation conditions, EPS production was higher for *L. mesenteroides* compared to *Lb. plantarum* and the mix of both strains. Increases in temperature and incubation time led to an increase in hardness, gumminess, and chewiness and to a decrease in adhesiveness [24]. It therefore appears that among LAB species, the capacity and yields of EPS production would be a relevant parameter to consider, in order to select bacterial species and strains that match the desired properties of fermented fruits and vegetable products.

The addition of H₂O₂ and CaCl₂ to the media led to an overproduction of HePS from *Lactobacillus rhamnosus* ZY [25]. Indeed, initial EPS production under anaerobic conditions at 37 °C was measured at 342.8 mg/L and increased to 567 mg/L following incubation with 3 mM H₂O₂ for 24 h and up to 2203 mg/L following incubation with 10 mM CaCl₂ for 12 h. EPS concentration was further increased by incubation with both H₂O₂ and CaCl₂ for 12 h reaching 2498 mg/L, which represents a 9.5-fold increase when compared to the initial condition. These results suggested a stimulation of EPS production to protect *Lb. rhamnosus* ZY against the cytotoxic oxidative effect of H₂O₂ [25]. Regarding monosaccharide composition on overall cell content, higher total carbohydrate concentrations were measured with 3 mM H₂O₂ but a decrease in total protein content and the molecular weight were observed when CaCl₂ was present in the growth medium. Further analysis revealed that EPS produced by *Lb. rhamnosus* ZY were composed of rhamnose, galactose, glucose, mannose, fucose and fructose and that EPS composition varied depending on culture conditions [25].

3.3. HoPS Production Pathway

Biosynthesis of HoPS, described in *Weissella*, *Leuconostoc*, *Lactobacillus* and *Pediococcus* genera, is performed by extracellular enzymes, glucansucrases or fructansucrases (Figure 2) [6]. Fructansucrase and glucansucrase transfer a monosaccharide from a specific substrate to the growing polysaccharide chains [26]. These enzymes belong to the glycosyltransferase (GTF, E.C. 2.4x.y) group and catalyze the hydrolysis of sugars, the resulting monosaccharide residues being attached to a glycan acceptor chain. These enzymes can be categorized into transglucosidases (E.C. 2.4.1.y; glucan-synthesizing dextran-sucrases, mutansucrases, and reuteransucrases) and transfructosidases (E.C. 2.4.1.y or 2.y; fructan-catalyzing transfructosidases levansucrases and inulosucrases). These proteins are encoded by the *gtf* and the *ftf* genes, respectively [12]. Glucansucrases are responsible for glucans and fructans synthesis and belong to the α -amylase superfamily as part of the glycosides hydrolases (GH), in clan GH-H [2,11].

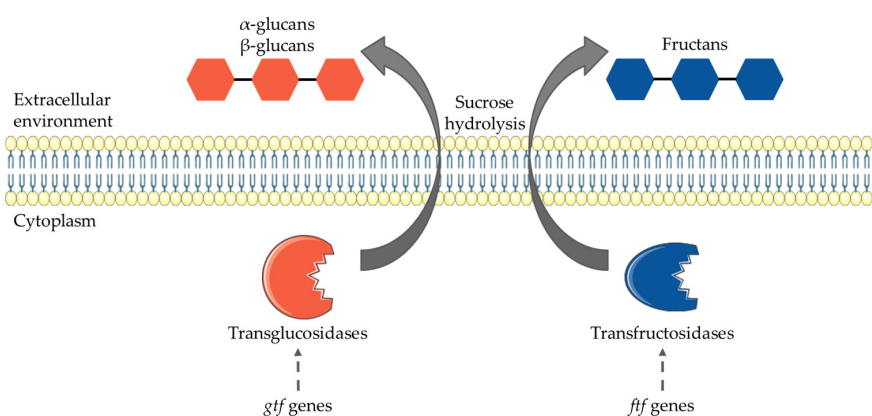


Figure 2. HoPS biosynthesis pathway

Glucansucrases are characterized by their ability to cleave the α -glycosidic linkage between a glucose moiety and another monosaccharide moiety using a catalytic (β/α)8-barrel domain which determines the reaction specificity [27]. Moreover, small differences in the amino acid sequence can influence the product specificity. Regarding the three-dimensional structures of glucansucrases, the catalytic core contains three domains to which two extra domains are attached, namely IV and V domains. Some of the domains are made up from two discontinuous segments of the polypeptide chain, resulting in a U-shaped structure [27].

3.4. HePS Production Pathway

HePS synthesis involves several steps: sugar transportation into the cytoplasm of bacterial cells, sugar nucleotide and sugar-1-phosphate (sugar nucleotide donor substrate) synthesis, repeating unit synthesis, polymerization of the sugar repeated units, and lastly, EPS exportation from the cell (Figure 3) [8,12]. During this last step, EPS can be secreted out as a slimy polymer or by adhering to the cell wall to form a capsule [12].

Enzymes, transporters and regulators are encoded by genes of plasmid replication origins in mesophilic *Lactobacillus* strains and of chromosomal replication origins in the thermophilic ones [12].

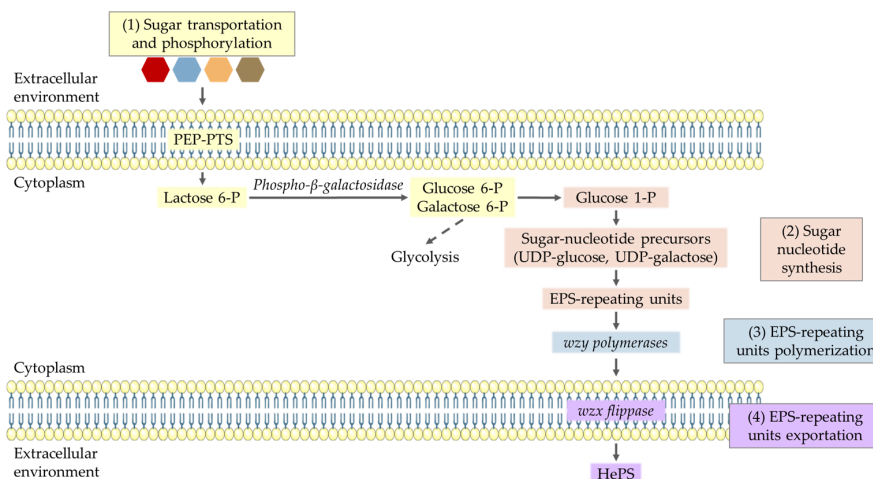


Figure 3. HePS biosynthesis pathway. EPS: exopolysaccharides; HePS: heteropolysaccharides; P: phosphate PEP-PTS: phosphoenolpyruvate-phosphotransferase system; UDP: uridine diphosphate.

Transportation and phosphorylation of sugars mainly occur using the phosphoenolpyruvate-phosphotransferase system (PEP-PTS) in which two protein groups link sugar residues to the carriers and are in charge of the intermembrane transport and sugar phosphorylation [11]. Regarding sugar nucleotide formation, lactose-6-phosphate is hydrolyzed by phospho- β -galactosidase to produce glucose-6-phosphate and galactose-6-phosphate. Likewise, lactose is hydrolyzed by β -galactosidase to produce glucose and galactose. Then, galactose-6-phosphate and glucose-6-phosphate are transformed into glucose-1-phosphate, which will later be converted into UDP-glucose, UDP-galactose and dTDP-rhamnose. The glucose-6-phosphate branch-point is involved into carbon distribution between metabolic pathways. The role of NAD⁺/NADH balance as a regulation factor for carbon distribution into pathways was proposed and engineering of intracellular redox level was showed to be a potent way to increase HePS production level [28].

Sugar-nucleotide precursors, such as UDP-glucose and UDP-galactose, formed intracellularly, polymerize to form HePS. These precursors do not only serve for EPS production and are also used for various intracellular polysaccharides synthesis. The synthesis of precursors leading to HePS is controlled by glycosyltransferase genes which are located in an operon with genes involved in regulation, chain length determination, polymerization and cell export. Diversity of HePS produced by LAB is due to the variability of glycosyltransferase genes in the operon [2]. Indeed, overexpression of a complete *eps* gene cluster in *Lactococcus lactis* was shown to induce higher EPS production levels. Likewise, a slight increase in EPS production was observed when the NIZO B40 priming glycosyltransferase gene *epsD* was overexpressed [28].

Finally, the formation, transportation and polymerization of EPS-repeating units involve the Wzx/Wzy pathway. The repeating units are polymerized to long-chain HePS by several polymerases, namely Wzy, and exported from the cell by a flippase, namely Wzx (Figure 3) [11].

4. Enzyme Activities, Genes Involved and HoPS Synthesis Pathway Regulations

4.1. Dextranucrase Activity and In vitro Enzyme Properties

Several LAB selected for their ability to produce EPS showed a dextranucrase activity. These strains belong to *Weissella*, *Leuconostoc* and *Streptococcus* genera. *Weissella* species were able to ferment cellobiose, fructose, galactose, maltose, ribose, and sucrose whereas *Leuconostoc* species fermented melibiose and arabinose. Regarding dextranucrase activity, *Leuconostoc* isolates demonstrated the highest activities, followed by *Weissella* and *Streptococcus* isolates, respectively [29].

For further analyzes, the most promising strains were selected and identified as *W. confusa* KIBGE-IB38 and *W. confusa* KIBGE-IB39 [29]. To improve dextranucrase activity, the authors modified

one fermentation parameter at a time. They demonstrated a maximum enzyme production of 1.508 dextransucrase units·mg⁻¹ after 8 h of incubation. For the *W. confusa* strains tested, higher enzyme activity was reached at 25 °C and was not altered up to 30 °C. An increase in the pH of the medium resulted in an increase in enzyme activity. Indeed, this activity was enhanced at pH 7.5 and decreased at higher pH as well as at pH values of 6.0 and 6.5. Optimization of fermentation conditions improved dextransucrase activity and thus dextran production by *W. confusa* [29].

Bounaix et al. [30] characterized dextransucrase production by dextran-producing *W. cibaria* strains isolated from sourdough. They evaluated the production level of dextransucrase using sucrose or glucose as the carbon source and showed that dextransucrase activity was 1.5-fold higher when glucose was used instead of sucrose. Using electrophoretic analyses and zymograms, the authors determined a soluble dextransucrase at 180 kDa regardless of the carbon source used (sucrose or glucose) [30]. Using a recombinant *W. cibaria* DSRWC glucansucrase (rDSRWC), Kang et al. [31] assessed optimal parameters for its activity and showed the highest dextran production at pH 5.2 and 30 °C. However, 62% of the original activity remained at temperatures down to 20 °C. Likewise, 80% of the original activity remained within a pH range of 5.0–6.0 but a rapid decrease occurred outside this pH range [31].

Amari et al. [32] have studied the complete sequence of a dextransucrase from *W. confusa* LBAE C39-2 and *W. cibaria* LBAE K39 strains isolated from traditional French wheat sourdoughs. When dextransucrase gene from *W. confusa* LBAE C39-2 was cloned and expressed in *Escherichia coli*, results showed 75% of residual activity at pH 4.5–6 and 60% at pH 4.0, suggesting that this enzyme is active in an acidic environment. Using sucrose as a substrate, dextransucrase activity was enhanced at a temperature range of 35 to 40 °C. This study also illustrated that the enzyme remains during storage at 4 °C when conserved in 20 mM sodium acetate at pH 5.4 with about 10% activity loss after 6 days and 35% after 20 days of storage. The molecular weight of produced EPS was 2 × 10⁶ Da. Further analysis revealed that the dextran obtained was composed of α-(1 → 6)-linked D-glucopyranosyl units with α-(1 → 3)-linked branches [32].

Dextransucrases isolated from *Lb. hordei* TMW 1.1822 allowed dextran production only when sucrose was present [33]. The authors confirmed a pH-dependent release of dextransucrases in LAB and optimal activities at pH 4.0–4.5. This study suggested that controlling the pH during EPS production can be an asset to enhance production of dextrans with varying properties of dextransucrase. Maximum dextran production was obtained when *Lb. hordei* TMW 1.1822 was grown at pH 4.0 and 4.5 or when dextransucrases were recovered at these pH values. However, high concentrations of dextrans can be produced at pH 5.0 to pH 6.0 if the enzymes were recovered at pH 4.5, reducing the denaturation and the loss of transglycosylation activity of dextransucrases at a non-optimum pH. A pH increase resulted in an increase in molecular mass (and thus functional properties) of the dextrans produced by *Lb. hordei* TMW 1.1822. Likewise, the activity of a dextransucrase from *W. cibaria* RBA 12 isolated from pumelo pulp was influenced by the pH of the reaction mixture. In fact, a higher activity was observed at pH 5.4 and a decreased above this value. In addition, dextransucrase activity seems to be influenced by the temperature, as a loss of activity was observed at temperatures beyond 40 °C [34].

Taken together, these data indicate that an exhaustive comprehension of the parameters influencing biosynthesis pathways for each selected strain is needed for efficient EPS production in fermented fruit and vegetables. Fermented conditions should be optimized as, given the notable low pH, fruit and vegetables' natural environment could be considered as unfavorable according to the strain. Their possibility of modulation could be limited by sensorial aspect and consumer acceptability of the final products.

4.2. Genes Involved in Production of HoPS

Several LAB strains, such as *Lb. plantarum*, *L. pseudomesenteroides/mesenteroides* and *W. cibaria*, were isolated from Turkish sourdough to determine their EPS production and the EPS genes involved were screened. All strains possessed *eps* genes required for EPS biosynthesis (Table 1) [35].

Most LAB producing HoPS harbor only one glucansucrase gene; however, some LAB genomes have more than one gene encoding the enzyme and are thus able to synthesize different HoPS. For instance, the *L. mesenteroides* NRRL B-512F strain coding both a glucansucrase and fructansucrase can produce levan in addition to dextran usually produced [2].

Table 1. Review of the various genes involved in HoPS production by lactic acid bacteria (LAB).

Gene	Function	EPS	LAB Species	Ref.
<i>gtf</i>	Glucansucrase gene	Dextran	<i>Lb. plantarum</i> , <i>Lb. curvatus</i> , <i>Lb. rossiae</i> , <i>Lb. sanfranciscensis</i> , <i>Lb. brevis</i> , <i>Lb. paralimentarius</i> , <i>W. paramesenteroides</i> , <i>L. mesenteroides</i> , <i>L. pseudomesenteroides</i> , <i>W. cibaria</i> , <i>W. confusa</i>	[35,36]
<i>fff</i>	Fructansucrase	Levan	<i>W. confusa</i>	[37]
<i>lev</i>	Levansucrase gene	Levan	<i>Lb. paraplantarum</i> , <i>Lb. sanfranciscensis</i> , <i>Lb. paralimentarius</i> , <i>W. paramesenteroides</i> , <i>L. mesenteroides</i> , <i>L. pseudomesenteroides</i>	[35]
<i>dsr</i>	Gene of extracellular Dsr enzyme	Dextran	<i>Streptococcus</i> , <i>Leuconostoc</i> and <i>Lactobacillus</i> strains	[23]
<i>dps</i>	Transmembrane glycosyltransferase (Gtf) gene	β -glucans	<i>Pediococcus</i> spp.	[14]

Leuconostoc spp. produces insoluble glucans. More precisely, *L. mesenteroides* NRRL B-1118 (ATCC 8293) generates insoluble dextran. Côté et al. [38] cloned the *DsrI* gene (referring to dextransucrase insoluble product) from *L. mesenteroides* NRRL B-1118 [38]. Besrou-Aouam et al. [23] characterized the *dsrLL* gene encoding DsrLL of *L. lactis* AV1n. The analysis revealed 97–99% identity of *dsrLL* with homologous genes from *L. lactis*, *L. garlicum* or *L. mesenteroides*. Dsr coding genes are also found in the chromosomes of *Streptococcus*, *Leuconostoc* and *Lactobacillus* species. The DsrLL enzyme is composed of 1500 amino acids. A signal peptide sequence in the N-terminal region of the protein allows translocation of the enzyme through the cell membrane leading to the protein secretion extracellularly where the Dsr acts. Further analysis showed that the DsrLL enzyme was bound to the cell wall, suggesting that this enzyme synthesizes dextran attached to the cell surface to protect the bacterial cell from extracellular stress [23]. Expression analysis revealed that *dsrLL* transcription was not modulated by carbon source neither by growth temperature. An increase in EPS production level according to the presence of sucrose or low temperature probably resulted from post-transcriptional regulations, such as a higher turn-over of Dsr protein at lower temperatures [23].

The glucansucrase DSRWC was isolated from *W. cibaria* CMU from human saliva. The corresponding gene, namely *dsrWC*, encodes an extracellular enzyme that produces glucans with only α -(1 → 6) linkages [31].

W. confusa MBF8-1 strain isolated from a homemade Indonesian fermented soybean product has at least two *gtf* genes encoding glucansucrase enzymes. These genes, *gtf8-1A* and *gtf8-1B*, encode Gtf8-1A and Gtf8-1B glucansucrases which may then be exported by the secretory (Sec)-dependent pathway [36].

Pediococcus species EPS production is linked to the presence of plasmids containing a glycosyltransferase gene, namely *dps*. This gene leads to Gtf production which polymerizes UDP-glucose into β -glucans [14].

Approximately 150 glucansucrase genes have been sequenced and about a third correspond to functional genes [27]. Sequence analysis of glucansucrase encoding genes from *Lactobacillus*, *Leuconostoc*, *Weissella* and *Pediococcus* species confirmed that these LAB can be clustered according to the specificity of the EPS glycosidic bonds, rather than the 16S rRNA taxonomy (Figure 4).

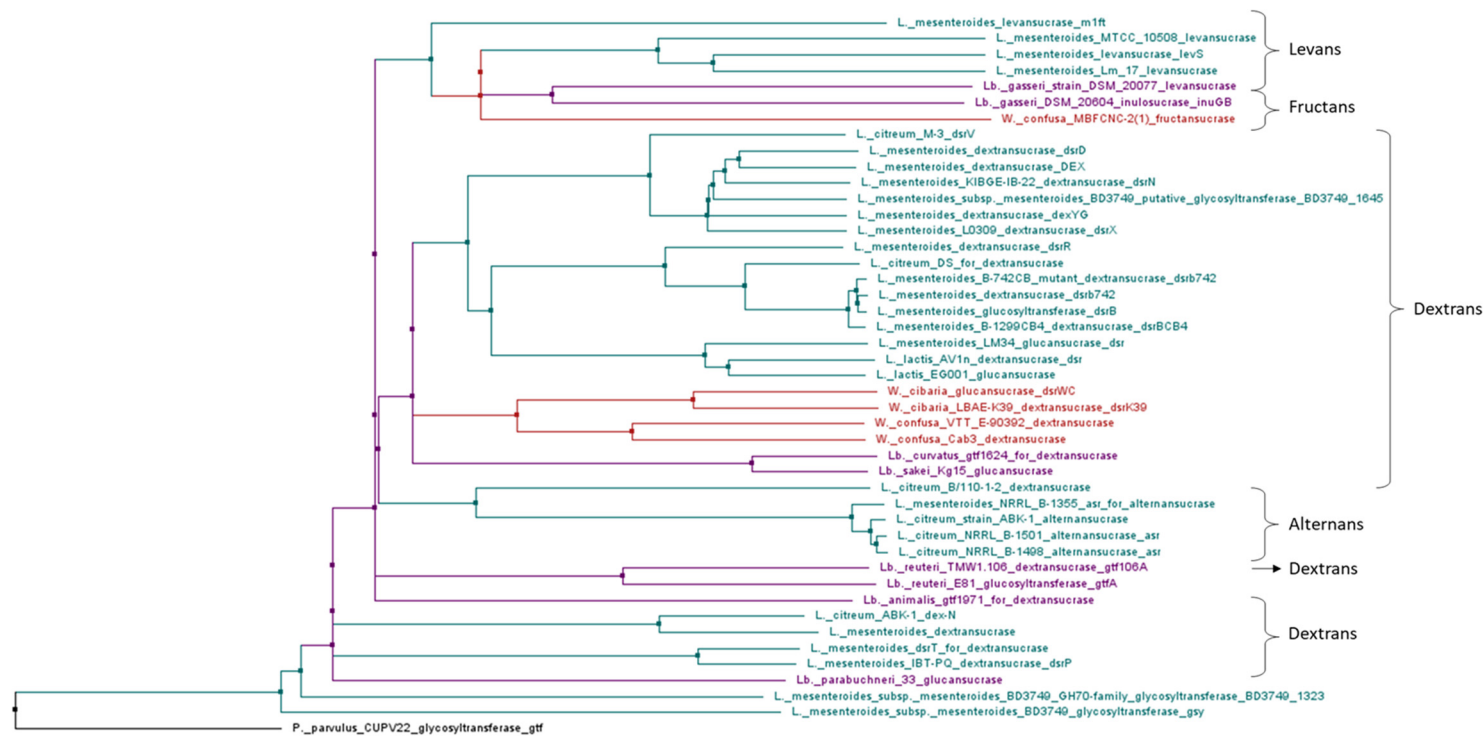


Figure 4. Phylogenetic tree of genes involved in glucansucrases synthesis. NCBI database was used to identify genes involved in glucansucrases synthesis. Only those with a complete coding sequence from “start” to “stop” were selected. Next, multiple sequence alignment was inferred with Clustal Omega program. Finally, the phylogenetic tree was calculated using Jalview software. *Leuconostoc* spp. sequences are shown in blue, *Lactobacillus* spp. sequences in purple and *Weissella* spp. sequences in red. Further details regarding these genes’ identification are developed in the Supplementary Materials (Table S1).

5. LAB Producing EPS

Various LAB strains can be isolated from fruit and vegetables and/or perform spontaneous fermentation [39–41], but only a few were demonstrated to be able to produce EPS, with variable characteristics according to the strain as listed in Table 2.

Table 2. Characteristics of exopolysaccharide (EPS) produced by LAB.

LAB Species	EPS Structure	Production Improvement Factor	Observed Effect on the Product	Ref.
<i>L. mesenteroides</i>	Levan-type and fructans	Sucrose	Strong acidity of smell and taste, poor sweetness, enhanced thickness	[6,42]
<i>L. pseudomesenteroides</i>	Dextrans with α -(1 → 4) linkages	Sucrose		[6,42]
<i>L. lactis</i>	Dextrans, mainly glucopyranose units with α -(1 → 6) linkages and side chains made of a α -glucopyranose unit			[23]
<i>W. cibaria</i>	Dextrans with α -(1 → 6) linkages and α -(1 → 3) linked branches, or Fructans			[43,44]
<i>Lb. plantarum</i>	HePS containing fructose, arabinose, galactose, glucose, and mannose- α -D-glucan with α -(1 → 6) linkage and α -(1 → 3) branching	Glucose		[45–47]
<i>Lb. fermentum</i>	β -glucan and two HePS composed of glucose and galactose	Glucose		[48]
<i>Lb. rhamnosus</i>	EPS containing rhamnose, glucose and galactose			[49]
<i>Pediococcus</i> spp.	β -glucans composed of D-glucose with β -(1 → 3) linkages and β -(1 → 2) branches		Enhance viscosity, modulated mouthfeel	[14]
<i>Bifidobacterium</i> spp.	HePS containing galactose, glucose and rhamnose			[50]

5.1. *L. mesenteroides* and *L. pseudomesenteroides*

Leuconostoc were isolated from fruit and vegetables traditionally fermented in Romania, green tomato, cauliflower, and carrot. Production by *L. mesenteroides/pseudomesenteroides* was evaluated at 15 g/L. Results also showed that colonies belonging to *Leuconostoc* spp. have a mucoid appearance on sucrose-supplemented MRS [51]. Juvonen et al. [6] confirmed an important EPS production by *L. pseudomesenteroides* when sucrose was present in the medium. This strain produced dextran with 14% α -(1 → 4) linkages. Moreover, adjustments of saccharose concentration in fermented carrot puree suggested that *L. mesenteroides* also produces levan-type fructan. However, total concentration of dextran and fructan was not in line with the sucrose consumption observed for the strain. The authors hypothesized that this *L. mesenteroides* strain could use sucrose as an energy source or for production of a dextran with more linkages [6]. *L. mesenteroides* also produced lactic acid, acetic acid, and mannitol. Sensory analysis carried out on carrot puree fermented with *L. mesenteroides* showed a strong acidity of smell and taste and poor sweetness. Results indicated a positive correlation ($R^2 > 0.77$) between concentrations of lactic acid plus acetic acid and the perceived acidity and a negative correlation ($R^2 < -0.89$) with sweetness. Regarding the appearance of these purees, fermentation with *L. mesenteroides* enhanced the matrix thickness [6]. This bacterial species is used in the food industry because of its ability to produce dextran up to 20 g/L [2].

Recently, Abdalrahim et al. [42] showed that *L. pseudomesenteroides* and *L. mesenteroides* isolated from fermented fruits and vegetables and from dairy products (potato, gawava, molokhai, kiwi, labneh) produced EPS from sucrose. EPS concentrations of the seven tested strains of *L. pseudomesenteroides/mesenteroides* ranged from 18.08 to 61.9 g/L [42].

5.2. *Leuconostoc lactis*

EPS production of *L. lactis* AV1n isolated from Tunisian avocado yielded 2.25 g/L. Further characterization showed that EPS produced were α -glucans composed of glucose units. More precisely, these HoPS contain a main chain of glucopyranose units with 35% of α -(1 \rightarrow 6) linkages with side chains made of a α -glucopyranose unit, allowing their classification as dextran-type HoPS [23]. *L. lactis* was occasionally isolated from cabbage, especially kimchi. The genome of the strain CCK940 producing EPS was sequenced, confirming the presence of genes possibly encoding enzymes of EPS synthesis [22,52].

5.3. *Weissella cibaria*

W. cibaria is a bacterial strain able to produce dextran up to 36 g/L which is of great interest at the agri-food level [2]. A *W. cibaria* strain was isolated from cabbage to assess its EPS production. Results showed dextran production of 2.4 g/dL linked to a dextransucrase activity. Dextran exhibited a linear structure with consecutive α -(1 \rightarrow 6) linkages and 3.4% of α -(1 \rightarrow 3) linked branches. Moreover, a gel permeation chromatography analysis determined the molecular weight of this dextran at approximately 2×10^6 Da [43].

W. cibaria/confusa strains isolated from spontaneously fermented Malian sour milk or cassava produced glucan-type EPS in culture medium supplemented with sucrose [44]. From 123 strains assayed all producing dextran, 18 additionally produced fructan.

5.4. *Lactobacillus* Species

Lactobacillus sp. are less efficient producers of EPS compared to other LAB, because they produce mainly HePS known for having a small production yield, which make their production uneconomical when used for the food industry. EPS produced by these LAB are mainly synthesized during the exponential growth phase and there is a decrease in their concentration at the end of the fermentation, which suggests that EPS could be used as alternative carbon sources [12].

Lactobacillus strains isolated from human intestinal microbiota were grown in MRS-C agar (de Man, Rogosa, Sharpe supplemented with 0.25% L-cysteine) for 5 days to provide enough EPS for further characterization. Identified species include *Lb. plantarum*, *Lactobacillus casei*, *Lb. rhamnosus* and *Lactobacillus vaginalis*. All EPS isolated from these strains were composed of galactose and glucose and 52% of them contained rhamnose. This latter monosaccharide content is higher than the concentration found from bacterial strains isolated from food. The major monosaccharide was strain-dependent but glucose, and rhamnose when present, were generally the most abundant. Screening of genes involved in EPS synthesis confirmed that EPS produced by *Lactobacillus* were HePS [50].

5.4.1. *Lactobacillus plantarum*

Lb. plantarum is an EPS-producing LAB with various functions well investigated. *Lb. plantarum* strains are tolerant to bile and gastric juices, are able to adhere to the intestinal epithelium and have antimicrobial properties [9].

The EPS produced by *Lb. plantarum* NTU 102 contained fructose, arabinose, galactose, glucose, and mannose, and are, therefore, HePS [9,45]. EPS produced by *Lb. plantarum* YO175 and OF101 isolated from a Nigerian fermented food had a molecular weight of 1.2×10^6 and 4.4×10^5 Da, respectively [53]. Chemical composition revealed a total carbohydrate content of 87.1% for EPS-YO175 and 80.6% for EPS-OF101. More precisely, these EPS were composed of glucose and galactose for YO175 and glucose for OF101. Further analyses showed that these EPS have an important antioxidant capacity [53].

A *Lb. plantarum* strain isolated from Turkish sourdough showed an average EPS production of $1153.8 \mu\text{g}/10^7$ cells at 30 °C [35]. Two strains of *Lb. plantarum*, NTMI05 and NTMI20, isolated from cow milk for their ability to produce EPS, showed EPS yields of 197 mg/L and 187 mg/L, respectively [16]. The greatest production was obtained when glucose (20 g/L) was used as the carbon source in the growth media. The production was also improved for the two studied strains by the addition of yeast

extract. This effect was explained by yeast extract composition which includes amino acids, peptides, carbohydrates, and salts. In addition, a high EPS production was observed at an incubation time of 72 h, which corresponds to the most suitable period for enzymatic activity and polysaccharide metabolism rate. Analysis of EPS chemical composition indicated a total carbohydrate content of 95.45% for *Lb. plantarum* NTMI05 and 92.35% for *Lb. plantarum* NTMI20, the absence of protein or nucleic acid and a production of lactic acid of 14 mg/mL for NTMI05 and 11 mg/mL for NTMI20. Monosaccharide composition analysis of EPS produced by these strains revealed the presence of galactose [16].

It has been shown that EPS produced in response to environmental conditions, such as temperature, pH and light, by *Lb. plantarum* and *W. cibaria* are resistant to gastric and intestinal digestions and are also able to promote beneficial intestinal bacteria [2,3].

Characterization of an α -D-glucan isolated from *Lb. plantarum* DM5 showed that it comprises glucose monomers with an average molecular mass of 1.11×10^6 Da. They are linked through 86.5% α -(1 \rightarrow 6) linkage and 13.5% α -(1 \rightarrow 3) branching [47].

5.4.2. Lactobacillus fermentum

Lb. fermentum strains have been isolated from the spontaneous fermentation of 'Almagro' eggplants [41,54] and have been assayed for their functional and technological properties [48]. EPS production by *Lb. fermentum* 139, *Lb. fermentum* 263 and *Lb. fermentum* 296 was 47.4 mg/L, 55.1 mg/L and 55.6 mg/L, respectively. *Lb. fermentum* Lf2 is able to produce EPS to levels of 1 g/L. This production can be further improved by modifying the medium composition and culture conditions. Indeed, the highest influence on EPS production was observed by changing the proportions of the nitrogen and type of carbon sources as well as the pH. EPS production at 300 and 600 mg/L from *Lb. fermentum* increased yogurt hardness and consistency without altering sensory and syneresis properties. EPS produced by *Lb. fermentum* consist of a β -glucan and two HePS composed of glucose and galactose. *Lb. fermentum* MC3 was studied to evaluate its ability to produce EPS using various carbohydrate sources. Supplementation of the growth medium with different sugars significantly improved EPS production, with the highest yield at 178.2 mg/L when glucose was added [48].

5.4.3. Lactobacillus rhamnosus

Lb. rhamnosus strains interest for fruit juice LAF was studied through their viability in acidic conditions [55–57], allowing to survive in fruit juice and gastrointestinal tract. Interestingly, the *Lb. rhamnosus* 9595 strain was described to produce 2.7 g/L of EPS, which is one of the highest concentrations reached by a *Lactobacillus* strain. The EPS obtained contained rhamnose, glucose and galactose [49].

5.5. Other LAB Species

5.5.1. *Pediococcus* spp.

Pediococcus pentosaceus is the species of the genus the most frequently found in fermented vegetables [58,59]. *P. damnosus*, *P. parvulus* and *P. pentosaceus* can cause "ropiness" of wine, which is hypothesized to increase resistance to low pH, high ethanol content and sulfur dioxide [14].

Pediococcus species involved in winemaking can synthesize EPS such as β -glucans of 500–2000 kDa composed of D-glucose with β -(1 \rightarrow 3) linkages and β -(1 \rightarrow 2) branches made up of single units [14]. Genes encoding EPS synthesis are located on a plasmid. These EPS enhance the beverage viscosity, affect filtration, and modulate the mouthfeel from a concentration as low as 20–30 mg/L, and production above 100 mg/L results in an unpalatable wine.

5.5.2. *Bifidobacterium* spp.

Bifidobacterium animalis, *Bifidobacterium longum* and *Bifidobacterium pseudocatenulatum* isolated from human intestinal microbiota and grown in MRS-C agar (de Man, Rogosa, Sharpe supplemented with 0.25% L-cysteine) produced EPS composed of galactose and glucose [50]. Some of the EPS also

contained rhamnose in higher proportion compared to those produced by LAB isolated from food. The identification of genes involved in synthesis of EPS produced by *Bifidobacterium* sp. confirmed HePS production [50].

Bifidobacterium sp. ability to survive in acidic environment is a crucial parameter for fruits LAF. One of the more acid resistant strains among *Bifidobacterium* species, *Bifidobacterium animalis*, has been investigated for stability in an orange, grape, passion fruit mixed juice [60] and showed a lower viability compared to milk and at room temperature. Therefore, these data of cultures alone do not give an accurate enough prediction of the strain versatile functionality in adverse conditions and should be more investigated. Recently, *B. animalis* subsp. *lactis* B94 was specifically selected due to its better growth and viability in Durian pulp fermentation and inoculated in combination with other LAB in order to extend this fruit preservation and to reduce the spoiling and wasting of large volume of Durian fruit [61]. The use of *Bifidobacterium* spp. as probiotics in non-dairy beverages is of interest for lactose-intolerant people, and research towards their incorporation in fruit or vegetable based foods is wide [62].

6. Production of EPS during Lactic Acid Fermentation of Fruit and Vegetables and Consequences on Food Quality

The production of EPS by LAB is widely considered for fermented milk-based products, where they can contribute to the texture and physical stabilization, while not being considered as food additives. The effect of EPS production in fruit and vegetable products was recently examined to achieve consumer sensory satisfaction while avoiding the use of hydrocolloid additives in industrial foods.

Depending on their rheological properties, EPS can be used as emulsifiers or stabilizers in the food industry [9,47]. EPS produced by LAB led to a modification of rheological properties linked to their composition, structure, size, and charge [6,10]. For example, β -glucans highly promoted viscosity and elasticity of food matrix regardless of their concentration, thanks to their linear structure and to their production in an acidic environment. On the contrary, bacterial strains producing dextran led to a moderate acid formation and decrease in pH [6]. Enhancing rheological properties of fermented products is an asset for consumer consumption by improving organoleptic quality, appealing appearance and pleasant mouthfeel [10], which increased the gustatory sensations of the consumers [12].

Zheng et al. [5] underlined an EPS production in fresh litchi juices fermented with *Lb. casei* at $5 \log$ CFU/mL, reaching 7.07 g/L after 18 h of fermentation. Although EPS have not been characterized, this production resulted in an improvement of the viscosity of this matrix which increased from 4.7 mPas initially to 84.8 mPas after fermentation. Moreover, fructose concentration was also higher after fermentation, which showed that enzymes partially involved in EPS production were glucansucrases [5].

In another study, EPS production in vegetable matrixes was assessed in agar medium containing 30% of carrot puree and supplemented with 5% sucrose [6]. EPS production assayed for 37 LAB strains was positive for *Lactobacillus* spp. except *Lb. plantarum* strains, several *L. mesenteroides* strains and one *W. cibaria* strain. According to these results *L. mesenteroides* strains producing EPS were selected for carrot puree fermentation and the presence of water-soluble low branching dextrans was analyzed using an enzymatic method. Results showed a dextran concentration of 0.2% for two strains (out of six) and production of fructan-type levans for one of them. On the other hand, viscosity increased for fermented carrot purees since *L. mesenteroides* increased the viscosity from 50% to 270% compared to non-fermented samples. Likewise, fermentation changes the texture of purees by thickening through EPS production [6].

Similarly, Han et al. [63] showed EPS production by a *L. mesenteroides* strain used for fermentation of a tomato juice supplemented with 15% sucrose. Monosaccharides of the EPS produced by *L. mesenteroides* were identified using high-performance anion-exchange chromatography equipped with a pulsed amperometric detector (HPAEC-PAD) and the only monosaccharide present was glucose [63]. Dextran concentration obtained was 32.15 g/L, which approximately corresponds to

60% of glucosyl units of metabolized sucrose. This increased dextran concentration was consistent with the observed increases of bacterial growth and viscosity. Viscosity increased up to 451 CP (centipoises) during fermentation with *L. mesenteroides*, which is a modification of the matrix commonly observed during dextran production. The analysis of sugars in the tomato juice showed that after 48 h of fermentation, sucrose concentration initially at 15.10% decreased to 4.11% whereas fructose concentration increased from 1.11% to 4.93%. Observation of fructose content increase, derived from sucrose, is characteristic of dextran synthesis by this LAB [63].

EPS solubility is associated with their molecular weight and branching percentage. The porous matrix structure formed by polysaccharides chains is linked to their solubility and water holding capacity as large amounts of water can be held by hydrogen linkages. Some electrostatic interaction capacities were also described with charged proteins, resulting in the modification of the product elasticity [10].

7. Health Benefits of EPS

Besides technological and organoleptic properties, the EPS produced by LAB are associated to various biological properties, such as prebiotic, antioxidant, anti-inflammatory, cholesterol lowering capacities (Figure 5), and health benefits [64]. The studies conducted on EPS produced by LAB, showed a direct influence on biological potential of the chemical nature of EPS, whether it be monosaccharide composition, glycosidic linkage or chemical modifications [65]. Non-dairy sources, including fruit and vegetables, are nowadays targeted to explore their health promoting effects, as novel EPS are identified from different resources with outstanding benefits.

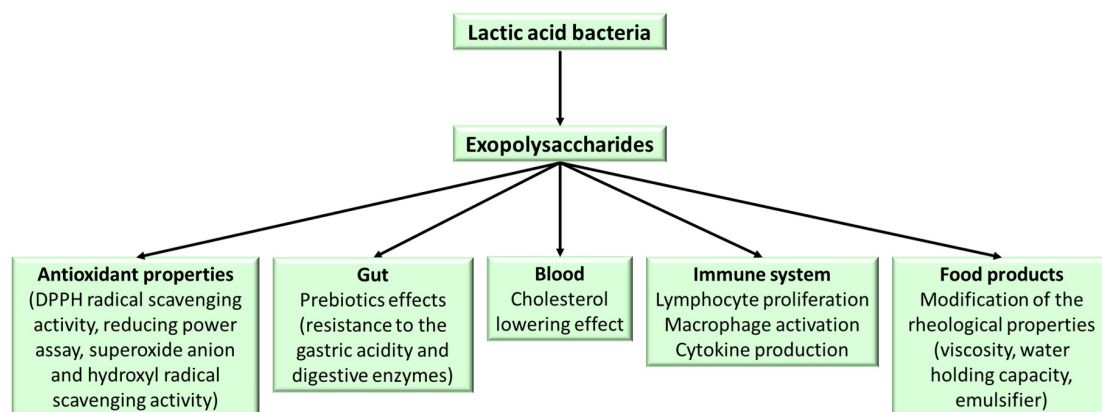


Figure 5. General representation of the properties attributed to EPS produced by LAB.

7.1. Prebiotic Properties

Gut microbiota play a major role in immunity against pathogens by contributing to the immune barrier role of intestinal mucosa [66–68]. The composition of intestinal microbiota is under the influence of multiple factors, including diet, that be linked to changes in microbial diversity [69,70]. Probiotics, but also prebiotics allowing the growth of specific microorganisms, including EPS, are widely studied in order to restore a balance in the intestinal microbiota of patients [9,69].

Das et al. [47] have shown that α -D-glucan produced by *Lb. plantarum* DM5 stimulates *B.infantis* and *Lb. acidophilus* growth, but also its own growth with a population increase from 6.09 to 9 log₁₀ CFU/mL in vitro. However, the growth of non-probiotic strains, *E. coli* and *Enterococcus aerogenes*, was not stimulated. An important criterion for prebiotic selection is their resistance to gastric acidity and digestive enzymes, so that they can reach the colon and be fermented by probiotic bacteria, including hydrolysis which allows absorption. This study showed that EPS produced by *Lb. plantarum* DM5 were resistant to gastric juice of pH 1–4 and that an increase in pH decreased the percent hydrolysis of glucan-DM5 from 0.39% to 0.19%. Regarding the intestinal fluid, the hydrolysis percentage of

glucan-DM5 was 0.21% which demonstrates this EPS high resistance to harsh intestinal environment. The hydrolysis ratio of glucan-DM5 by α -amylase increased with pH and incubation time from 0.09% to 0.22% after 1 h of incubation at pH 5–8 and from 0.13% to 0.32% after 5 h of incubation at pH 5–8. Inulin, a standard prebiotic used in this study, showed maximum hydrolysis of 19.13% at pH 8 after 5 h of incubation whereas this value was 0.32% for glucan-DM5 under the same conditions, showing significantly higher α -amylase resistance by glucan-DM5 (99.8%) compared to inulin. All together, these results suggest that α -D-glucan from *Lb. plantarum* DM5 will be accessible to the probiotic bacteria as a carbon source in the gastrointestinal tract [47]. As α -glucans are not degraded in the human digestive tract, they can reach the colon to be fermented by colonic bacteria. Therefore, produced HoPS can be considered as beneficial fibers with an extended liberation of glucose. Prebiotic properties of HoPS produced through glucansucrases have been assessed using in vitro fermentation of gut and colon models. Results have shown that only short oligosaccharides exert prebiotic abilities [27].

EPS are involved in mechanisms of adhesion to surfaces or to other organisms [8]. For fermented products, EPS drives an increase in the retention time in the gastro-intestinal tract resulting in an increased colonization by EPS-producing LAB [8]. In addition, EPS can alter the surface of bacterial cells by inhibiting their ability to bind to the surface of cells or to modulate expression of genes involved in biofilm formation, thus promoting their formation [9]. Resistance to antibacterial compounds is a thousand times higher for microorganisms producing biofilms [8].

Dextran produced by *L. mesenteroides* XG5 from homemade wine have been investigated for its ability to promote bacterial growth [71]. Supplementation of the growth media with this EPS resulted in a significant stimulation of growth of *Bifidobacterium* and *Lactobacillus* species. EPS produced by *L. mesenteroides* XG5 were not digested in human saliva. Likewise, simulated small intestinal juice did not affect these EPS; however, these molecules were substantially degraded during fecal fermentation. These results suggested that EPS produced by *L. mesenteroides* XG5 are resistant to gastro-intestinal digestion and are then utilized by the fecal microbiota. During human fecal fermentation, XG5 EPS significantly enhanced *Bifidobacterium* growth but not *Lactobacillus* growth. These EPS also enhanced the contents of short-chain fatty acids. The EPS enriched the cecum microbiota of C57BL/6J mice, leading to an increase in *Firmicutes* and a decrease in the *Bacteroidetes* to *Firmicutes* ratio. These microbiota modifications could be an asset for type 1 diabetes patients. These results suggested that EPS produced by *L. mesenteroides* XG5 could be used as prebiotics given their ability to stimulate probiotic growth [71].

7.2. Antioxidant Activity

The ability to exert antioxidant activity is widespread in LAB-derived EPSs. Imran et al. [16] evaluated the antioxidant activity of EPS produced by *Lb. plantarum* NTMI05 and NTMI20 by measuring free radical scavenging activity. The results showed an improvement of this activity with 500 μ g/mL of EPS with a capacity of 96.62% and 91.86%, respectively, for these two strains. A hypothesis suggested that antioxidant activity could be influenced by the presence of functional groups such as hydroxyl groups [9].

An EPS composed of glucose and rhamnose sugar units produced by *W. cibaria* strain GA44 demonstrated moderate but significant antioxidant capacities in a dose-dependent manner in various in vitro models such as DPPH radical scavenging activity, reducing power assay, superoxide anion and hydroxyl radical scavenging activity [53]. Similarly, strong and concentration-dependent antioxidant activities were found for EPS from *Lb. plantarum* C88 and for HoPS from *L. pseudomesenteroides* JF17 [13,72].

Another study characterizing antioxidant properties of an EPS produced by *Lactococcus lactis* strain EPS-1 showed an important antioxidant capacity. This EPS was mainly composed of fructose and rhamnose. EPS-1 was also able to inhibit hydroxyl and superoxide radical activities. These results suggested that EPS-1 produced by *Lactococcus lactis* could reduce lipid peroxidation of cell membranes [73].

EPS from LAB have been studied for their health-benefit properties. It follows that their chemical structure and molecular weight (MW) are relevant characteristics to define their health-promoting functions and activities [74].

7.3. Anti-Inflammatory Activity

EPS produced by LAB are able to stimulate dendritic cells and other antigens presenting genes thanks to Toll-like receptors (TLRs) and to modulate the inflammatory response by leading to the production of pro-inflammatory cytokines [45].

Anti-inflammatory properties have been observed using in vitro and in vivo approaches for EPS. Decreased expression of pro-inflammatory markers has been shown in a rat model of inflammation [75]. Lipopolysaccharide-stimulated RAW 264.7 cells also exhibited lower contents of TNF α , IL-1b and IL-6 [76].

EPS produced by *Lb. plantarum* NTU 102 promote proliferation of RAW 264.7 macrophages at a concentration below 5 μ g/mL but a cytotoxic effect was observed at higher concentrations. Moreover, macrophages treatment with these EPS allow phagocytosis. It was also showed that EPS produced by *Lb. plantarum* NTU 102 are able to induce a pro-inflammatory response by producing cytokines, such as IL-6, TNF- α and IL-1 β , in a dose-dependent manner in treated macrophages [45].

7.4. Cholesterol-Lowering Activity

Recently, studies have investigated the promising cholesterol-lowering effect related to LAB-derived EPS. A high molecular weight EPS of glucomannan nature from *Lb. plantarum* BR2 showed a strong ability to adsorb cholesterol, exhibiting 47.5% of cholesterol lowering ability on in vitro assimilation procedure [77]. Similarly, an EPS consisting of mannose, glucose and galactose produced by *Enterococcus faecium* K1, an isolate from indigenously fermented milk product *kalarei*, also displayed a 49% cholesterol-lowering property [78].

In vivo, the effect of water-soluble EPS from kefir was determined in C57BL/6J mice by Lim et al. [79]. The EPS supplementation had significant anti-obesity effect by reducing HFD-induced body weight gain, adipose tissue weight, and plasma very-low-density lipoprotein cholesterol concentration compared to the control group in this study.

Current hypotheses on the mechanism involved in EPS cholesterol-lowering effect considers elimination of bile, cholesterol assimilation and conversion, coprecipitation effect and short fatty acids promotion [26].

8. Conclusions

EPS production by LAB involved in fruit and vegetable fermentation is gaining interest because of both effects on sensory characteristics and potential positive health effects.

The impact of EPS of food texture can be seen as a strategy to limit the use of additives, but requires a production level of several g/L. To that aim, the production of HoPS is more favorable than HePS as several LAB species, such as *L. pseudomesenteroides*, *L. mesenteroides*, *W. cibaria* and *W. confusa* produce these compounds at high levels. Moreover, production level of EPS can be enhanced through adjustment of fermentation medium composition and incubation parameters. However, this optimization should not decrease the general sensory quality of fruit or vegetable fermented foods.

EPS produced during fermentation of fruit or vegetables can exert prebiotic activity, but also antioxidant, anti-inflammatory and cholesterol-lowering activity. Most of previous results were obtained in vitro, on cell models or in mice, and will require further investigations to determine if the observed effects can be expected from human diet modifications.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2311-5637/6/4/115/s1>, Table S1: Detailed information corresponding to the gene sequences used to achieve the phylogenetic tree of genes involved in glucansucrases synthesis.

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2.2.2.5. Sur le microbiote intestinal

Au cours de leur progression dans le tube digestif, les composés phénoliques ne sont que partiellement absorbés dans l'intestin grêle. La majorité d'entre eux atteint le côlon où ils sont exposés à l'action des enzymes du microbiote, qui, en hydrolysant les liaisons glycosidiques, décomposent l'hétérocycle des polyphénols et produisent des glycanes utilisés à leur tour comme nutriments par les micro-organismes intestinaux (Heleno et al., 2015; Selma et al., 2009). En complément de cette fonction de nutriment, les polyphénols d'origine alimentaire ont un effet sur le microbiote intestinal en régulant la proportion de bactéries commensales et de probiotiques, en inhibant la croissance de micro-organismes pathogènes et en améliorant les interactions entre l'hôte et les micro-organismes. L'ensemble de ces mécanismes conduit à moduler la composition du microbiote et peut conduire à des effets bénéfiques pour l'organisme, comme par exemple une perte de poids (Lin et al., 2019). Chez des patients obèses présentant un syndrome métabolique, la consommation de polyphénols issus du raisin a conduit à une augmentation du nombre de bactéries productrices de butyrate, un acide gras à chaîne courte reconnu pour son rôle anti-inflammatoire (Moreno-Indias et al., 2016). Les polyphénols peuvent également jouer le rôle de prébiotique et modifier l'architecture du microbiote intestinal en privilégiant la croissance d'espèces spécifiques comme *Akkermensia* spp., *Faecalibacterium* spp. ou encore *Roseburia* spp., parmi les plus productrices d'acides gras à chaîne courte (Tomás-Barberán et al., 2016).

Ce rôle de prébiotique est aussi attribué aux fibres telles que la pectine, l'inuline ou les β -glucanes, qui constituent des substrats utilisés de manière sélective par les micro-organismes de l'hôte. L'activité des bactéries intestinales sur les fibres dépend de leur degré de polymérisation, de leur poids moléculaire, de leur longueur de chaîne et du nombre de ramifications (Hamaker & Tuncil, 2014). Les fibres peuvent être classées au regard de leur sensibilité à la fermentation, généralement les fibres solubles sont les plus fermentescibles du fait d'une viscosité plus élevée que les fibres insolubles (McRorie & McKeown, 2017). Au niveau de l'intestin terminal, un régime alimentaire à base de produits végétaux, riches en fibres et glucides accessibles au microbiote, est corrélé à l'abondance des genres bactériens *Roseburia*, *Lachnospira* et *Prevotella* capables de métaboliser ces substrats. Ce régime entraîne également une synthèse accrue de métabolites, notamment des acides gras à chaîne courte, aux effets bénéfiques probables pour la santé de l'hôte (De Filippis et al., 2016; Lin et al., 2019).

La fermentation bactérienne altère significativement la structure des caroténoïdes ingérés. Des métabolites de caroténoïdes sont présents dans le côlon mais il est difficile d'en différencier l'origine entre fermentation colique et synthèse *de novo* et le bénéfice de ces métabolites dans le tractus digestif n'est pas encore établi (Schmidt et al., 2021). L'ingestion de caroténoïdes entraîne la production endogène d'acide rétinoïde qui pourrait avoir une action sur la muqueuse intestinale et réguler ses fonctions de barrière en modifiant l'expression des protéines de jonctions serrées, ainsi que sur le microbiote (Farré et al., 2020; Zhou et al., 2020).

L'apport de prébiotiques, probiotiques et synbiotiques est parfois préconisé pour leurs effets anti-diabétiques dans le traitement du diabète de type 2, afin également de diminuer les effets secondaires médicamenteux (diarrhée, nausée, risque hypoglycémique) (Cabello-Olmo et al., 2019; Zepeda-Hernández et al., 2021). Les bactéries lactiques constituent une composante importante de la flore intestinale humaine et ont des rôles bénéfiques dans le tractus gastro-intestinal. Elles peuvent être considérées comme des probiotiques qui ont un effet bénéfique sur l'hôte en améliorant l'équilibre microbien intestinal. Les probiotiques sont des micro-organismes vivants qui, lorsqu'ils sont

ingérés en quantité suffisante, exercent des effets positifs reconnus sur la santé, au-delà des effets nutritionnels traditionnels (World Health Organization: Food and Agriculture Organization of the United Nations, 2006). Les bactéries lactiques, qu'elles soient vivantes ou inactivées, sont capables d'agir avec le microbiote intestinal et peuvent aussi à ce titre être qualifiées de postbiotiques ayant des effets positifs en tant que micro-organismes inanimés ou médiés par certaines de leurs fractions ou composants cellulaires (Salminen et al., 2021; Zepeda-Hernández et al., 2021).

La fermentation des prébiotiques par les probiotiques et les micro-organismes du microbiote augmente la concentration en acides gras à chaîne courte (Zepeda-Hernández et al., 2021). La modulation du microbiote par les probiotiques, les prébiotiques et, par extension, les synbiotiques (compléments composés d'un probiotique et d'un prébiotique), aurait une influence sur sa dysbiose et son homéostasie et pourrait ainsi contribuer à la gestion de maladies chroniques telles que l'obésité et le diabète de type 2 (Zepeda-Hernández et al., 2021). Les mécanismes impliqués incluent la réduction du stress oxydant, l'inhibition des cytokines pro-inflammatoires, l'augmentation de la production d'acides gras à chaîne courte, le contrôle de la glycémie, la modulation de la perméabilité du tractus intestinal et la production de peptides bioactifs (Figure 11) (Zepeda-Hernández et al., 2021).

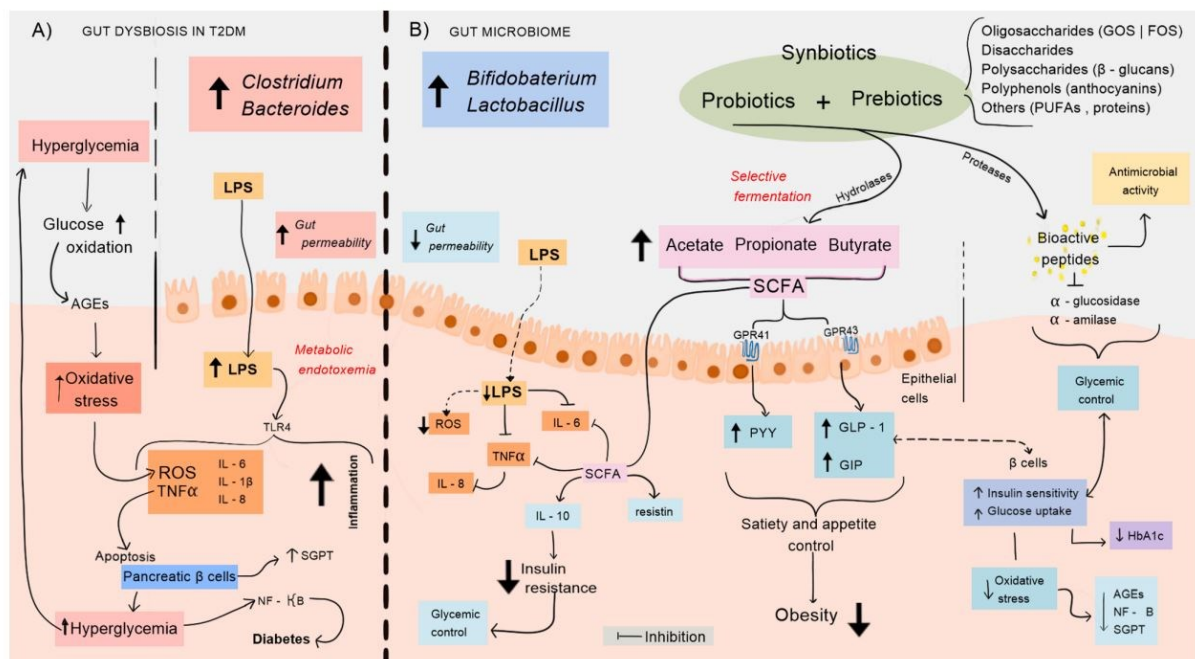


Figure 11 : Mécanismes d'action des probiotiques et des prébiotiques dans le traitement du diabète de type 2 (Zepeda-Hernandez et al., 2021).

Au niveau intestinal, l'effet combiné des fibres alimentaires et des bactéries lactiques permet d'améliorer la santé au niveau du côlon (Karovičová & Kohajdová, 2003). Des études suggèrent que des jus de fruits fermentés par des bactéries lactiques, pourraient constituer un aliment prébiotique, du fait de la présence de polyphénols, et exercer une influence sur la structure et la fonctionnalité du microbiote (Tomás-Barberán et al., 2016). Les bactéries lactiques appartenant aux genres *Lactobacillus* et *Bifidobacterium* contenues dans des jus de fruits fermentés peuvent également être considérées comme des probiotiques grâce à leur effet sur l'équilibre du microbiote intestinal (Di Cagno et al., 2013). Lors de la fermentation par les bactéries lactiques, les EPS produits en réponse aux conditions

environnementales, e.g. température et pH, par *W. cibaria* et *Ltb. plantarum* sont résistants aux digestions gastrique et intestinale. Ces composés sont également capables de favoriser la croissance de bactéries intestinales bénéfiques (Caggianiello et al., 2016; Liu et al., 2011; Lynch et al., 2018).

Les composés fortement antioxydants, comme la vitamine C, peuvent affecter le microbiote en fonction de leur concentration dans l'intestin et de la tolérance à l'oxygène des espèces présentes. La présence d'oxygène dans l'intestin est responsable de dysbiose par la réduction de groupes bactériens bénéfiques strictement anaérobies et au contraire favorise la croissance d'*Enterobacteriaceae* pathogènes (Pham et al., 2021; Steinert et al., 2020). La préservation de teneurs élevées en vitamine C dans les fruits lactofermentés pourraient ainsi permettre d'améliorer la composition du microbiote.

OBJECTIFS DU TRAVAIL DE THESE

L'étude bibliographique réalisée a montré que les fruits tropicaux peuvent être considérés comme des sources en caroténoïdes, vitamine C et composés phénoliques et présentent un intérêt au niveau nutritionnel et sensoriel. Les matrices végétales ont une durée de conservation limitée par les altérations microbiennes et physiologiques survenant après la récolte et lors de leur manipulation. Cette durée peut être améliorée par la fermentation lactique. Ce procédé de transformation permet également de maintenir ou augmenter la qualité nutritionnelle et sensorielle des matrices alimentaires. L'utilisation de « starters » d'origine autochtone est préférée pour induire la fermentation lactique afin de développer des produits alimentaires de manière reproductible. Ce procédé engendre une réduction qui serait mineure de la concentration en sucres présents dans le fruit. La production d'acide lactique et d'autres acides organiques lors de la fermentation entraîne une acidification du milieu contribuant à inhiber la croissance de micro-organismes pathogènes, mais influence aussi les propriétés sensorielles. La synthèse de composés aromatiques par les bactéries lactiques influence la saveur des produits fermentés. La fermentation lactique peut aussi améliorer les propriétés rhéologiques, e.g. la viscosité, la texture et la sensation en bouche, des aliments fermentés grâce à la sécrétion d'EPS, ce qui favorise l'acceptabilité de ces produits par les consommateurs. Les EPS possèdent également des propriétés antimicrobienne, antioxydante et immunomodulatrice. La production de ces composés est dépendante de la souche bactérienne et de la composition du substrat utilisé lors de la fermentation. La modification de l'activité antioxydante lors de la fermentation est liée à l'action d'enzymes bactériennes qui vont hydrolyser les composés phénoliques et améliorer ainsi leur biodisponibilité. La fermentation de fruits tropicaux pourrait donc conduire à l'obtention d'aliments capables de se conserver plus longtemps que les fruits, avec des propriétés organoleptiques originales et un intérêt nutritionnel.

Notre hypothèse est que la lactofermentation de fruits tropicaux permet, grâce au métabolisme des bactéries lactiques isolées de produits végétaux, de synthétiser des composés bénéfiques tels que les EPS, ou d'améliorer la bioactivité et la biodisponibilité des antioxydants naturellement présents, par dépolymérisation, isomérisation ou hydrolyse des polyphénols et caroténoïdes.

La prévalence de désordres métaboliques est particulièrement élevée à La Réunion avec une proportion de la population atteinte d'obésité, de diabète ou encore de maladies cardiovasculaires supérieure à celle de la population de la France métropolitaine. La prévention des maladies métaboliques consiste à favoriser la consommation de fruits et légumes. Dans ce cadre, la fermentation lactique présente un fort intérêt pour le développement de produits alimentaires à base de fruits tropicaux de La Réunion. Les modifications de composition nutritionnelle dues à la fermentation lactique suggèrent que la consommation de préparations fermentées aurait un impact au niveau fonctionnel. Les bénéfices sont considérés sur la prise alimentaire, l'action des enzymes digestives, la biodisponibilité des molécules antioxydantes des fruits, la formation d'EPS et le microbiote intestinal.

Afin de contribuer à la valorisation des ressources réunionnaises et à la prévention des désordres métaboliques sur le territoire régional, cette thèse visait à répondre à la problématique suivante : Dans quelle mesure les modifications de composition dues à la lactofermentation appliquée à des fruits tropicaux de La Réunion peuvent-elles contribuer à améliorer la qualité de l'offre alimentaire et impacter le métabolisme énergétique et la colonisation du tractus gastro-intestinal ?

Ainsi les travaux de recherche visaient à répondre à deux objectifs principaux :

- (1) Étudier les composés impactés par la fermentation lactique et leur évolution au cours de la fermentation et du stockage et explorer la formation de composés d'intérêt nutritionnel, les EPS ;**
- (2) Évaluer si les effets fonctionnels liés aux transformations lors de la fermentation lactique sont détectables chez le mammifère nourri avec un régime hypercalorique, si ces effets sont avérés au niveau du métabolisme énergétique et l'effet de la consommation des préparations fermentées sur la colonisation intestinale.**

Pour répondre au premier objectif, les fruits sélectionnés, à savoir l'ananas Queen Victoria (*Ananas comosus*), la mangue Cogshall (*Mangifera indica*) et la papaye Solo (*Carica papaya L.*), ont été utilisés sous forme de jus ou purées comme substrats et fermentés en monoculture par des souches bactériennes des espèces *Ltb. plantarum*, *W. cibaria* et *Lc. pseudomesenteroides* isolées dans des écosystèmes végétaux dont les caractéristiques technologiques ont été déterminées au sein du laboratoire QualiSud (Fessard & Remize, 2019). Une meilleure connaissance des composés dont la composition est modifiée par la fermentation pourrait contribuer à évaluer la possibilité d'effets fonctionnels et leur nature. Le comportement des souches sélectionnées a été déterminé d'après leur capacité à transformer des molécules à capacité antioxydante, à fermenter les sucres en acides organiques et à produire des EPS dans les différents fruits.

Concernant le second objectif, le modèle de souris nourries avec un régime riche en graisse a été choisi. Ce volet de l'étude a pu être mené suite à un avis favorable du comité d'éthique de La Réunion (dossier #20934_2019011015097480_v4). Les effets de l'ingestion de jus d'ananas Queen Victoria fermenté ont été étudiés sur le métabolisme énergétique au niveau circulant et des organes cibles, i.e. foie, tissu adipeux, intestin, ainsi que sur la composition intestinale en bactéries lactiques.

Dans le cadre d'une mission effectuée à la Tshwane University of Technology (TUT) située à Pretoria en Afrique du Sud, l'intérêt de la co-encapsulation a été étudié sur des composés bioactifs issus d'un jus de prune de Natal enrichi avec différents biopolymères, i.e. maltodextrine, isolat de protéine de pois et mucilage de psyllium, et inoculé ou non avec la souche bactérienne de *Ltp. plantarum* 75.

Étude expérimentale

PARTIE 1 : Conservation de jus d’ananas Queen Victoria, de purée de mangue Cogshall et de purée de papaye Solo lactofermentés

Manuscrit en préparation : « Changes during storage of lactic acid fermented tropical fruit ».

1.1. Introduction

Intake of fruit and vegetables has a beneficial effect on health resulting from the presence of fibers and micronutrients, such as vitamins, minerals, and phenolic compounds. Indeed, they offer antiproliferative, anti-inflammatory and antioxidant activities (Septembre-Malaterre et al., 2016). Tropical fruits are known as relevant sources of phenolic compounds with antioxidant activity. In the case of fruit from Reunion Island, it has been demonstrated that amounts of carbohydrates, vitamin C, carotenoids and phenolic compounds varied depending on the fruit, environmental stimuli and cultivation mode (Septembre-Malaterre et al., 2016).

Because of the perishability of fresh tropical fruit, it is often necessary to apply a process extending their shelf-life. Thermal processing such as cooking, boiling, pasteurization and sterilization are known to deteriorate vitamins and phenolic compounds present in these fruits, leading to a decrease in antioxidant activity (Nicoli et al., 1999). To preserve this property, alternative processing deserves to be considered. Lactic acid fermentation (LAF) is one of the oldest, most economical and most valuable option. This technique is liable to maintain or increase the nutritional, sensory and shelf-life properties of food (Fessard et al., 2017). LAF of fruit benefits from fruit nutritional properties, presence of viable bacteria and modification of fruit composition by bacteria.

Depending on their fermentative metabolism, lactic acid bacteria (LAB) produce lactic acid, acetic acid, ethanol and carbon dioxide in diverse proportions. In fruit, it has been shown that *Lactiplantibacillus plantarum* metabolizes organic acids preferably to sugars, as an adaptive response to acid stress (Filannino et al., 2013). In most studies, bacterial growth, pH and titratable acidity (TA) are used to assess fermentation efficacy. Determination of total soluble solids (TSS) or reducing sugar content are used to estimate carbohydrate consumption. However, little is known on which carbon sources are preferentially used, especially for strains belonging to species other than *Ltp. plantarum*. Given the impact of sugar content in fruit for diet and health, a better knowledge on the carbon central metabolism by LAB in fruit condition would explain a possible role of LAF to decrease sugar level.

Moreover, the effect on antioxidant activity and phenolic composition of LAB previously isolated from fruit and vegetables has been investigated. Fermentation started with strains belonging to *Ltp. plantarum* and *Leuconostoc mesenteroides* species has shown to increase vitamin C concentration, phenolic content, and antioxidant activity in several foods such as tomato juice, orange juice, blueberry juice (Bartkiene et al., 2013; Li et al., 2021; Quan et al., 2022). This enhancement is due to the release of bioactive compounds by LAB. The diversity of the observed changes of composition can be explained by the variety of substrates, the fermentation protocols and the LAB used. Previous studies with strains isolated in Reunion Island on pineapple juice have shown that the strains were able to grow properly in these acidic food matrices and to modulate antioxidant activity (Fessard et al., 2017). In addition, depending on the strain, sensory characteristics were modified, leading to spicy or milky descriptors depending on the strain used. Therefore, these LAB have a strong potential to be used as starters for fermentation of tropical fruits from Reunion Island.

The aim of this study was to evaluate the changes of tropical fruit purées occurring during LAF and subsequent storage using autochthonous strains. Pineapple, papaya and mango were chosen as they present different composition and acidity. These tropical fruits were fermented using independently five strains from the species *Ltp. plantarum*, *Weissella cibaria* or *Leuconostoc pseudomesenteroides*.

1.2. Materials and Methods

1.2.1. Bacteria

Five bacterial strains were used: *Ltp. plantarum* (17a and 75), *W. cibaria* (21 and 64) and *Lc. pseudomesenteroides* (56). Bacteria were reactivated by suspension in 9 mL of De Man, Rogosa and Sharpe (MRS) broth and incubation for 48 h at 30 °C and 100 RPM. Then, 100 µL of each pre-culture were transferred in new 9 mL MRS broth tubes and incubated for 48 h at 30 °C and 100 RPM.

1.2.2. Preparation and fermentation of pineapple juices, mango purees, and papaya purees

'Queen Victoria' pineapple (*Ananas comosus*), 'Cogshall' mango (*Mangifera indica* L.), and 'Solo' papaya (*Carica papaya*) grown in Reunion Island were used for this study. Fruits were harvested at the appropriate stage to ensure optimum eating quality. Separately, pineapples, papayas and mangos were washed, peeled, and cut. The pineapple juice was extracted using a Philips MicroMasticating juice extractor (Philips France Commercial, Suresnes, France), filtered and bottled. Papaya and mango were blended in puree using Grindomix GM 200 (Retsch, Éragny, France) and bottled. Then, juices and purees were pasteurized in a water bath so that a temperature of 82 °C was maintained for 10 min in the center of bottles containing 800 mL of fruit preparation, then cooled to room temperature within 2 h. The subsequent steps were performed in sterile conditions. From *Ltp. plantarum* (17a and 75), *W. cibaria* (21 and 64) and *Lc. pseudomesenteroides* (56) independent bacterial cultures, optical density was measured at 600 nm for each strain. Each bacterial culture was centrifuged at 10,000 x g for 5 min at 10 °C and the cell pellet was washed twice with sterile distilled water and re-suspended in sterile distilled water. From this suspension, the volume of inoculation was calculated in order to have an initial optical density of 0.05 DO per mL of juice or puree, corresponding to 1.10^7 cells/mL according to McFarland standards, for each bacterial culture. Each strain was inoculated separately in pineapple juice, or papaya or mango puree. For each fruit, fermentation was performed in triplicate for each strain. Fermented and non-inoculated (control) preparations were incubated at 30 °C for 48 h, then maintained at 4 °C for 26 days. Finally, they were stored at -20 °C until analysis.

1.2.3. Fermentation and storage monitoring

1.2.3.1. Bacterial growth

Bacterial enumeration was performed on MRS agar. Briefly, successive dilutions of juices or purees were prepared into sterile peptone water solution and dropped on MRS plates before incubation at 30 °C for 48 h. Afterwards, bacterial population was calculated from colony counting. To evaluate if the preparations were not contaminated, cell counts were also performed on Chloramphenicol Glucose Agar (CGA) plates to assess yeasts and mold population. Each test was performed in triplicate for each sample.

1.2.3.2. Acidity

The pH of fermented and non-inoculated (control) foods was measured using the Eutech pH2700 (EUTECH Instruments, Illinois, USA). The titratable acidity (TA) was obtained using a pH meter equipped with an automatic titrometer (TitroLine Easy, SI Analytics, Thermo Fisher Scientific, Massachusetts, USA) connected to a sodium hydroxide solution NaOH 0.05 M. Briefly, 100 µL of juice or 0.1 g of puree were mixed with 10 mL of distilled water, then NaOH 0.05 M was added until pH 8 is reached. TA was expressed as mg citric acid equivalent (CAE) for 100 g of juice or puree, calculated as follow:

$$TA = (V_{\text{NaOH}} \times C_{\text{NaOH}} \times 100) / P \times (M / n)$$

with:

- V_{NaOH} corresponding to the amount of sodium hydroxide used (mL)
- C_{NaOH} corresponding to the concentration of sodium hydroxide solution
- P corresponding to the amount of sample used (g)
- M corresponding to the molecular mass of citric acid (192.12 g.mol⁻¹)
- n corresponding to the stoichiometric factor (3 for citric acid)

1.2.3.3. Total soluble solids (TSS) content

The Brix degree of fermented and non-inoculated (control) juices and purees was measured using the ATAGO PAL-3 pocket refractometer (Atago USA Inc., Washington, USA) to analyze whether fermentation changes the total soluble solids content of the foods.

1.2.4. Color parameters

Color parameters L*, a* and b* were measured with a spectrophotometer CM 3500d (Minolta®, Carrières-sur-Seine, France) directly on samples of juice or puree.

1.2.5. DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical scavenging activity

Free radical-scavenging activity of fermented and control juices and purees was assessed through DPPH assay. This test was performed as described by (Fessard et al., 2016). DPPH solution was prepared at a concentration of 0.2 mM in methanol. Gallic acid was used as a standard in the range 0 to 12.5 mg/L. A volume of 50 µL of standard solution or 10-fold diluted sample and 150 µL of DPPH solution were mixed in a 96-well plate and incubated at room temperature for 20 min in the dark. Then, the absorbance was measured at 517 nm using a microplate reader (Infinite M200 PRO, Tecan, Männedorf, Switzerland). Results were expressed as a percentage of inhibition of DPPH reduction of sample compared to control by applying the following formula:

$$\% \text{ of DPPH reduction inhibition} = [(OD \text{ Control} - OD \text{ Sample}) / OD \text{ Control}] \times 100$$

From the standard curve, the EC50 (concentration required to obtain a 50 % antioxidant effect) was determined and the linear regression equation from % DPPH as a function of gallic acid concentration was used to determine the antioxidant activity as mg/L gallic acid equivalent (GAE), which was calculated as follows:

$$\text{DPPH GAE} = \% \text{ DPPH} \times \text{EC50} / 50 \% \times d$$

with:

- % DPPH corresponding to the percent of DPPH reduction inhibition
- EC50 corresponding to the concentration to obtain % DPPH of 50 (mg GAE/L)
- d corresponding to the dilution factor (10)

1.2.6. Vitamin C content

Ascorbic acid content was determined by titrimetry. A volume of 200 μL of juice or 0.2 g of puree were dissolved in 10 mL of a twice diluted solution of metaphosphoric acid 20 g/L and trichloroacetic acid 40 g/L in distilled water. Solutions were titrated with a solution of 2,6-dichlorophenol-indophenol 250 mg/L and sodium bicarbonate 210 mg/L in distilled water until a pink colored was observed. Vitamin C contents were expressed as mg ascorbic acid equivalent/100 g of fresh weight, using a calibration curve obtained from a standard solution of ascorbic acid (Sigma-Aldrich, Saint-Quentin Fallavier, France).

1.2.7. Total carotenoid content (TCC)

To determine total carotenoid content (TCC), 1 mL of juice or 1 g of puree was homogenized with 10 mL of extracting solvent (Hexane/Acetone/Ethanol, 50:25:25), sonicated for 30 min (frequency: 35 kHz, power: 80 %, function: sweep), and centrifuged at 4700 $\times g$ for 10 min at 5 °C. Then, supernatant was transferred in tubes protected from light and the absorbance was measured at 450 nm using Spectronic Helios Alpha UV-Vis spectrophotometer (Thermo Scientific, Massachusetts, USA).

1.2.8. Organic acid and sugar content

Organic acids and sugars were extracted using 80 % ethanol. Briefly, 15 mL of ethanol 80 % was mixed with 1 g of sample, the mixture was sonicated for 1 h, and centrifugated at 2800 $\times g$ for 5 min at 4 °C. Samples were then evaporated using a rotavapor (Laborota 400, Heidolph Instruments, Schwabach, Germany) and extracts were collected in 5 mL of ultrapure water and filtered before injection.

Organic acid content was determined by a High-Performance Liquid Chromatography (HPLC) with conductivity using AS11-HC column (Dionex). The mobile phase, at a flow rate of 0.6 mL.min⁻¹ consisted of ultrapure water (eluent A), KOH 0.1 M (eluent B) and KOH 0.002 M (eluent C). A volume of 0.02 mL for each sample was injected and run together with external standards.

Sugar content was assessed using an Ultra Performance Liquid Chromatography UPLC–1290 System Infinity II (Agilent, Santa Clara, CA, USA) equipped with a refractometer detector. A SHODEX SH1011 column 300 \times 8 mm (Tokyo, Japan) was used with an isocratic system of water with H₂SO₄ 0.01 % and a flow rate of 0.7 mL/min. Temperature was set at 30 °C, injection volume at 10 μL and refractometric detection at 210 and 245 nm. External calibration was established for each standard for concentrations from 0 to 10 g/L. Results were expressed in g/L.

1.2.9. Statistical analysis

Experiments were performed in triplicate. Data are presented as mean values \pm standard deviation (SD). Using XLSTAT software, statistical analysis was performed by using Two-way ANOVA. Significant differences were considered for p values < 0.001 .

1.3. Results

1.3.1. Mango fermentation and storage

Mango exhibited an initial pH of 4.2. During fermentation, pH decreased to 3.5 - 3.6, whereas it was not modified for the control condition (**Figure 1a**). A transient pH increase was observed after 7 days, then pH values reached 3.2 - 3.3 after 28 days, whatever the strain. Titratable acidity (TA) did not follow exactly the same trend (**Figure 1b**). For the two *Ltp. plantarum* strains and *W. cibaria* 64, TA increased gradually during fermentation and storage, whereas the increase occurred mainly during fermentation for *W. cibaria* 21 and *Lc. pseudomesenteroides* 56. After inoculation, bacterial population was in the range 6.0 log colony forming units (CFU)/mL to 7.6 log CFU/mL (**Figure 1c**). It increased during fermentation and afterwards, to reach 13.5 log CFU/mL to 15.7 log CFU/mL after 7 days. During further storage, a significant population decrease was observed for *Ltp. plantarum* 75 and *W. cibaria* 64. Both pH and population values indicated an efficient fermentation of mango within two days and denoted further evolutions of the product during refrigerated storage.

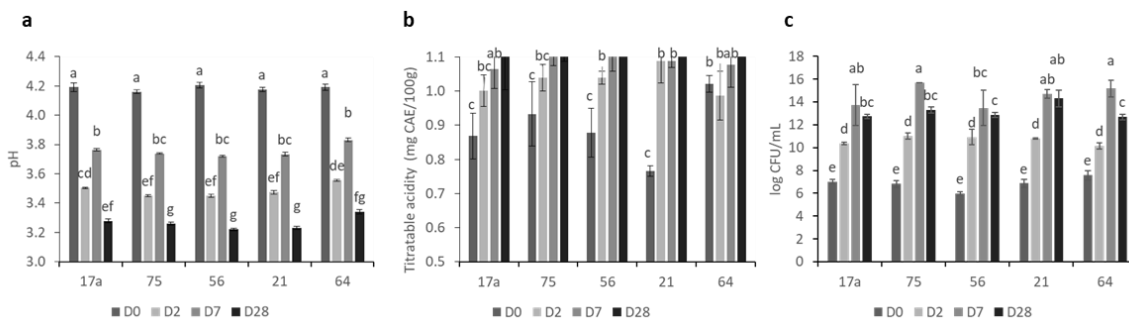


Figure 1. Evolution of (a) pH, (b) titratable acidity (TA) and (c) bacterial population (as log CFU/g) during mango puree fermentation and refrigerated storage. D0: day 0; D2: day 2; D7: day 7; D28: day 28. Different letters represent significant differences (p -value < 0.001) according to ANOVA.

Color parameters did not change during fermentation and storage (data not shown). Antioxidant activity of mango (diluted 10-fold) was weak (**Table 1**). Antioxidant activity expressed as mg GAE/L ranged between 11.3 and 22.3 at day 0. Vitamin C content decreased by 20 % to 40 % during fermentation and subsequent storage but the change was not significant, and no difference was observed between the control condition and the fermented samples. TCC was not modified whatever the strain.

Total soluble solids (TSS) were not modified during fermentation and storage, though a slight decrease was observed for all strains (**Table 1**). Investigation of mono and disaccharides and organic acid content was performed to understand the modifications observed for pH and TA, together with the absence of change in TSS (**Table 2** and **Table 3**). More than 80 % of glucose and more than 53 % of fructose were consumed. A preference for glucose was noticed. Surprisingly, sucrose content increased. Other sugars could result in the same HPLC retention time, for instance mannose and xylose,

which are poorly separated from sucrose under our conditions. However, this peak was not present on day 0 samples, meaning a production during fermentation. One hypothesis could be the synthesis of unidentified polysaccharides, but *Ltp. plantarum* 17a and 75 strains do not produce EPS. Another possibility could be the hydrolysis during fermentation of polysaccharides releasing a sugar with the same retention time than sucrose.

All the citrate present was consumed by the strain *W. cibaria* 64, whereas citrate consumption was not detected for other strains. After 7 days, acetate was the main organic acid produced, and lactate cannot be quantified due to overlap with acetate peak. Tartrate content increased by 30 % for two strains, *Lc. pseudomesenteroides* 56 and *W. cibaria* 21.

1.3.2. Papaya fermentation and storage

LAF of papaya puree resulted in a rapid decrease of pH, from 4.9 to 3.5 - 3.7 within 2 days (**Figure 2a**). The decrease was further observed to reach values of 3.3 - 3.5 after 7 days, then remained stable until 28 days. During the same period of time, TA significantly increased for *Ltp plantarum* 17a and 75 and *Lc. pseudomesenteroides* 56 strains (**Table 1**). Bacterial population increased during fermentation and for the 5 following days (**Figure 2b**). TSS was not modified during the 28-day experiment (**Table 1**).

The color parameters of the puree slightly changed: for the control condition, L* decreased from 53.6 to 50.5, a* increased from 36.8 to 39.8 and b* decreased from 50.5 to 48.4 (**Figure 2c**). However, those changes did not appear significant with p-values of 0.011, 0.015 and 0.030, respectively for L*, a* and b*. For fermented purees, the values L*, a* and b* were not modified, for *W. cibaria* 21 and 64 and *Lc. pseudomesenteroides* 56 strains or changed in the opposite way of the control for *Ltp. plantarum* 17a and 75.

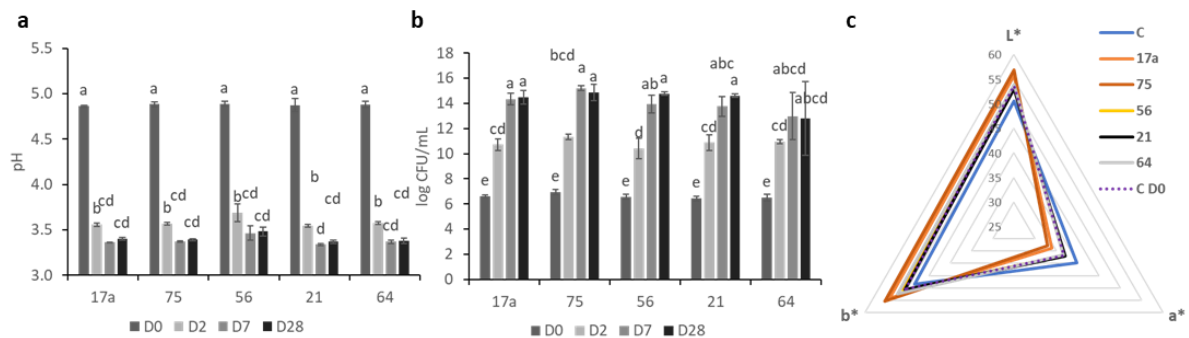


Figure 2. Evolution of (a) pH, (b) bacterial population (as log CFU/g) and (c) color parameters L*, a* and b*, during papaya puree fermentation and refrigerated storage. D0: day 0; D2: day 2; D7: day 7; D28: day 28. Different letters represent significant differences (p -value<0.001) according to ANOVA.

Antioxidant activity was not different between conditions after 28 days, though the control condition exhibited at day 0 a lower value than others (**Table 1**). Initial antioxidant activity was 85.6 mg GAE/L for the control at day 0 and ranged between 107.3 mg GAE/L and 132.9 mg GAE/L for other conditions. After 28 days, it was 66.8 mg GAE/L for the control and between 66.7 mg GAE/L and 77.7 mg GAE/L for the fermented samples.

Vitamin C content decreased during the 28-day observation period (**Table 1**). It was better preserved for *Ltp. plantarum* 17a, *W. cibaria* 21 and 64, which exhibited different values compared to the control after 28 days. A global decrease of total carotenoid content was observed, but the conditions did not show differences after 28 days (**Table 1**).

The analysis of glucose, fructose and sucrose content failed to show a consumption of sugars within 7 days (**Table 2**). However, all the strains produced several g/L of lactate and acetate and tartrate content increased for *Lc. pseudomesenteroides* 56 and *W. cibaria* 64 strains (**Table 3**).

1.3.3. Pineapple fermentation and storage

After pineapple juice inoculation, pH decreased from 3.4 to 3.2 within 2 days, then to 2.9 within 28 days (**Figure 3a**). TA increased gradually and became different from the initial juice only after 28 days, for all strains (**Figure 3b**). Population of LAB increased during the 2-day fermentation and thereafter until 28 days (**Figure 3c**).

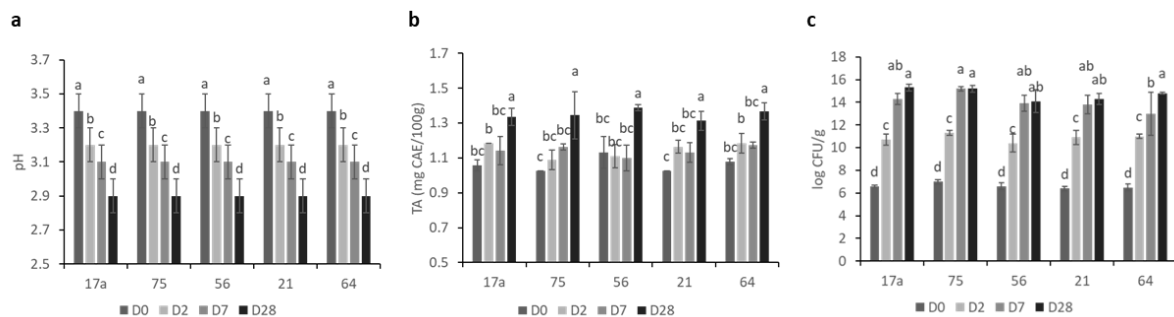


Figure 3. Evolution of (a) pH, (b) titratable acidity (TA) and (c) bacterial population (as log CFU/g) during pineapple juice fermentation and refrigerated storage. D0: day 0; D2: day 2; D7: day 7; D28: day 28. Different letters represent significant differences (p-value < 0.001) according to ANOVA.

TSS of pineapple juice decreased slightly during the 28 days of the experiment (**Table 1**). A significant decrease was noticed for *Ltp. plantarum* 75, *Lc. pseudomesenteroides* 56 and *W. cibaria* 64. Antioxidant activity decreased significantly only for the control condition. It was preserved in fermented juices. DPPH activity was 138.1 mg GAE/L for control condition at day 0 and 69.2 mg GAE/L for control condition at day 28. For fermented juices, it ranged between 139.4 mg GAE/L and 163.1 mg GAE/L at day 0 and between 82.9 mg GAE/L and 90.0 mg GAE/L at day 28. On a same day, there was no significant difference between conditions. Both vitamin C and TCC remained stable.

Examination of sugar content showed a 44 % - 68 % decrease of glucose and fructose concentration during fermentation and storage (**Table 2**). Sucrose concentration was not modified. Malate was consumed by *W. cibaria* 64. The same trend was observed for the juices fermented with the other bacteria, but the difference was not significant (**Table 3**) (p-value = 0.014). Citrate was possibly partly consumed but differences of concentrations were not significant, because of large standard deviation of values at day 0. A different pattern was observed for lactate and acetate production according to the strain. *Ltp. plantarum* 17a, *Lc. pseudomesenteroides* 56 and *W. cibaria* 21 strains produced only lactate whereas *Ltp. plantarum* 75 and *W. cibaria* 64 produced both lactate and acetate.

Table 1. Antioxidant activity, vitamin C and total carotenoid content of control and fermented fruit samples at the beginning (D₀) and at the end of the experiment (D_F corresponding to day 28). C: control, 17a and 75: *Lactiplantibacillus plantarum*, 21 and 64: *Weissella cibaria*, 56: *Leuconostoc pseudomesenteroides*, TA: titratable acidity, CAE: citric acid equivalent, FW: fresh weight, TCC: total carotenoid content. Different letters for the same fruit and same compound represent significant differences (p-value < 0.001) according to ANOVA.

Fruit	Condition	TA (mg CAE/100 g FW)		TSS (°Brix)		Antioxidant activity (%)		Vitamin C (mg/100 g FW)		TCC (mg/100 mL)	
		D ₀	D _F	D ₀	D _F	D ₀	D _F	D ₀	D _F	D ₀	D _F
Mango	C	0.95 ± 0.07 a	1.04 ± 0.14 a	17.5 ± 0.2 a	17.6 ± 0.2 ab	13 ± 12 a	0 ± 5 bc	26 ± 4 ab	19 ± 2 abc	3.1 ± 0.1 a	3.8 ± 0.3 a
	17a	0.87 ± 0.09 c	1.14 ± 0.08 a	17.7 ± 0.1 a	16.9 ± 0.1 b	9 ± 10 ab	0 ± 9 cd	22 ± 3 a	17 ± 3 ac	2.9 ± 0.5 a	4.0 ± 0.4 a
	75	0.93 ± 0.07 c	1.17 ± 0.01 a	18.0 ± 0.1 a	17.8 ± 0.1 a	10 ± 9 ab	9 ± 10 ab	27 ± 3 ac	16 ± 8 c	3.1 ± 0.6 a	3.6 ± 0.5 a
	56	0.88 ± 0.02 c	1.23 ± 0.04 a	17.9 ± 0.1 a	17.3 ± 0.1 ab	9 ± 8 ab	0 ± 6 ab	25 ± 2 a	17 ± 2 ac	3.4 ± 0.5 a	3.7 ± 0.3 a
	21	0.77 ± 0.02 c	1.22 ± 0.03 a	17.6 ± 0.4 a	17.5 ± 0.0 ab	7 ± 6 ab	2 ± 9 ab	26 ± 2 ac	19 ± 3 ac	2.9 ± 0.2 a	3.6 ± 0.5 a
	64	1.02 ± 0.01 b	1.13 ± 0.05 a	17.9 ± 0.2 ac	17.2 ± 0.1 ab	9 ± 7 ab	0 ± 8 d	27 ± 3 a	17 ± 2 ac	3.5 ± 0.5 a	4.0 ± 0.4 a
Papaya	C	0.90 ± 0.08 bcd	0.98 ± 0.14 abcd	11.3 ± 0.4 a	11.5 ± 0.3 a	45 ± 16 c	45 ± 22 c	52 ± 5 a	30 ± 1 ac	7.6 ± 0.5 a	6.7 ± 1.2 ae
	17a	0.86 ± 0.08 cd	1.14 ± 0.08 ab	12.0 ± 0.4 a	11.6 ± 0.3 a	56 ± 6 abc	51 ± 13 bc	62 ± 6 ab	59 ± 6 bd	9.9 ± 0.3 a	4.8 ± 1.3 bde
	75	0.88 ± 0.08 cd	1.11 ± 0.10 a	11.9 ± 0.5 a	11.9 ± 0.5 a	62 ± 10 abc	52 ± 10 bc	76 ± 13 b	39 ± 13 cd	9.3 ± 1.6 a	6.0 ± 2.6 ade
	56	0.79 ± 0.15 d	1.18 ± 0.11 a	10.1 ± 1.1 b	11.8 ± 0.3 a	69 ± 2 a	45 ± 15 c	76 ± 5 ab	25 ± 9 c	9.3 ± 0.7 a	7.3 ± 1.6 ade
	21	0.85 ± 0.15 abcd	1.22 ± 0.03 abc	11.8 ± 0.5 a	11.2 ± 0.9 a	56 ± 8 abc	47 ± 14 c	63 ± 16 ab	59 ± 4 b	9.9 ± 0.9 a	3.9 ± 0.9 be
	64	0.99 ± 0.03 bcd	1.13 ± 0.05 abc	11.5 ± 0.7 a	11.1 ± 0.7 a	65 ± 3 ab	50 ± 11 bc	70 ± 3 ab	59 ± 5 bd	10.2 ± 1.6 a	6.9 ± 2.3 ade
Pineapple	C	1.05 ± 0.02 bc	1.05 ± 0.02 bc	17.7 ± 0.1 a	17.6 ± 0.1 a	45 ± 7 ab	34 ± 4 c	27 ± 3 a	21 ± 5 a	12.2 ± 3.5 a	11.0 ± 3.3 ab
	17a	1.06 ± 0.03 bc	1.33 ± 0.05 a	17.5 ± 0.1 ad	17.1 ± 0.3 bd	49 ± 7 ab	40 ± 9 bc	21 ± 8 a	32 ± 5 a	8.9 ± 3.5 a	11.5 ± 2.6 a
	75	1.02 ± 0.01 c	1.34 ± 0.14 a	17.7 ± 0.1 a	17.3 ± 0.1 bd	45 ± 5 ab	43 ± 6 abc	36 ± 5 abc	26 ± 1 a	14.8 ± 0.9 a	11.2 ± 0.7 ab
	56	1.13 ± 0.09 bc	1.39 ± 0.02 a	17.6 ± 0.1 a	17.2 ± 0.1 bd	53 ± 11 a	41 ± 5 bc	29 ± 5 ac	21 ± 1 a	15.5 ± 1.2 a	11.9 ± 0.7 ab
	21	1.02 ± 0.01 c	1.31 ± 0.06 a	17.5 ± 0.1 ad	17.3 ± 0.1 ad	47 ± 5 ab	44 ± 5 abc	19 ± 5 a	19 ± 5 a	9.7 ± 4.1 ab	11.4 ± 0.3 ab
	64	1.05 ± 0.02 bc	1.37 ± 0.05 a	17.6 ± 0.1 a	17.2 ± 0.1 bd	50 ± 7 ab	43 ± 8 abc	38 ± 3 abc	27 ± 5 a	12.1 ± 3.1 a	11.9 ± 0.3 a

Table 2. Glucose, fructose and sucrose content of fruit samples at the beginning (D₀) and after 7 days (D₇ corresponding to 2 days of fermentation followed by 5 days of storage at 4°C). C: control, 17a and 75: *Ltp. plantarum*, 21 and 64: *W. cibaria*, 56: *Lc. pseudomesenteroides*, n.d.: not determined. Different letters for the same fruit and same compound represent significant differences (p-value < 0.001) according to ANOVA.

Fruit	Condition	Glucose (g/L)		Fructose (g/L)		Sucrose (g/L)	
		D ₀	D ₇	D ₀	D ₇	D ₀	D ₇
Mango	17a	15.7 ± 2.4 a	3.1 ± 0.8 b	20.4 ± 0.6 ab	8.4 ± 1.2 abc	2.7 ± 3.3 b	20.6 ± 1.4 a
	75	16.7 ± 1.7 a	1.4 ± 0.3 b	21.0 ± 0.5 a	6.6 ± 0.3 bc	0.0 ± 0.1 b	17.5 ± 0.4 a
	56	16.0 ± 4.2 a	2.2 ± 0.5 b	17.7 ± 4.0 abc	8.4 ± 0.2 abc	2.8 ± 4.8 b	20.5 ± 0.5 a
	21	18.6 ± 3.2 a	2.1 ± 0.8 b	20.3 ± 0.9 ab	6.4 ± 2.2 bc	0.1 ± 0.2 b	17.9 ± 2.5 a
	64	13.1 ± 11.7 a	1.0 ± 0.6 b	15.5 ± 13.7 abc	5.9 ± 2.4 c	5.9 ± 10.2 b	15.8 ± 3.3 a
Papaya	17a	n.d.	15.8 ± 3.3 a	n.d.	12.8 ± 2.0 a	n.d.	0.1 ± 0.1 a
	75	18.3 ± 0.1 a	n.d.	15.6 ± 0.1 a	n.d.	0.2 ± 0.1 a	n.d.
	56	14.2 ± 0.1 a	18.6 ± 3.7 a	11.7 ± 0.1 a	15.3 ± 4.8 a	0.2 ± 0.1 a	0.2 ± 0.1 a
	21	18.2 ± 8.1 a	18.7 ± 0.3 a	15.4 ± 9.5 a	15.2 ± 0.8 a	0.3 ± 0.3 a	0.2 ± 0.1 a
	64	14.6 ± 1.1 a	16.2 ± 0.1 a	11.6 ± 1.6 a	12.2 ± 0.1 a	0.1 ± 0.1 a	0.1 ± 0.1 a
Pineapple	17a	21.4 ± 0.7 a	10.7 ± 0.6 b	18.1 ± 1.1 ab	9.1 ± 0.6 bc	16.8 ± 0.2 a	16.6 ± 0.3 a
	75	24.9 ± 1.2 a	8.1 ± 7.0 b	21.9 ± 1.2 a	7.0 ± 6.1 c	18.3 ± 0.2 a	12.9 ± 11.1 a
	56	22.4 ± 7.9 a	10.0 ± 1.2 b	19.8 ± 7.2 a	8.5 ± 1.0 bc	18.6 ± 0.4 a	17.7 ± 0.7 a
	21	24.0 ± 0.9 a	13.3 ± 3.7 b	22.8 ± 2.4 a	11.6 ± 3.7 bc	19.6 ± 2.3 a	17.1 ± 1.7 a
	64	24.2 ± 1.7 a	12.4 ± 0.8 b	21.3 ± 1.8 a	10.5 ± 0.8 bc	17.8 ± 0.5 a	19.8 ± 0.6 a

Table 3: Organic acid content of fruit samples at the beginning (D₀) and after 7 days (D₇ corresponding to 2 days of fermentation followed by 5 days of storage at 4°C). C: control, 17a and 75: *Ltp. plantarum*, 21 and 64: *W. cibaria*, 56: *Lc. pseudomesenteroides*, n.d.: not detected; n.q.: not quantified. Different letters for the same fruit and same compound represent significant differences (p-value < 0.001) according to ANOVA.

Fruit	Condition	Lactate (g/L)		Acetate (g/L)		Malate (g/L)		Tartrate (g/L)		Citrate (g/L)	
		D ₀	D ₇	D ₀	D ₇	D ₀	D ₇	D ₀	D ₇	D ₀	D ₇
Mango	17a	0.1 ± 0.1 a	n.q.	0.5 ± 0.1 c	4.3 ± 0.8 ab	0.4 ± 0.1 a	0.1 ± 0.1 a	0.9 ± 0.1 abc	0.9 ± 0.1 ab	1.3 ± 0.1 a	1.3 ± 0.3 a
	75	0.1 ± 0.1 a	n.q.	0.5 ± 0.2 c	3.0 ± 0.5 b	0.3 ± 0.3 a	0.1 ± 0.1 a	0.9 ± 0.1 ab	0.8 ± 0.1 bcd	1.7 ± 0.2 a	1.0 ± 0.1 a
	56	0.3 ± 0.3 a	n.q.	0.3 ± 0.1 c	5.4 ± 1.1 a	0.4 ± 0.2 a	0.2 ± 0.1 a	0.6 ± 0.1 d	0.9 ± 0.1 abc	1.7 ± 0.3 a	1.6 ± 0.3 a
	21	0.1 ± 0.1 a	n.q.	0.5 ± 0.1 c	3.8 ± 0.9 b	0.3 ± 0.3 a	0.1 ± 0.1 a	0.7 ± 0.1 cd	1.0 ± 0.1 a	1.6 ± 0.1 a	1.0 ± 0.5 a
	64	0.3 ± 0.1 a	n.q.	0.2 ± 0.1 c	3.0 ± 1.3 b	0.3 ± 0.1 a	0.1 ± 0.1 a	0.7 ± 0.2 cd	0.8 ± 0.1 bcd	1.4 ± 0.5 a	n.d.
Papaya	17a	n.d.	n.q.	n.d.	3.4 ± 2.9 a	n.d.	0.1 ± 0.0 b	n.d.	0.5 ± 0.2 bc	n.d.	0.2 ± 0.0 b
	75	0.4 ± 0.1 a	n.d.	0.2 ± 0.1 a	n.d.	1.0 ± 0.0 a	n.d.	0.5 ± 0.0 bc	n.d.	1.8 ± 0.0 a	n.d.
	56	0.1 ± 0.1 a	n.q.	0.2 ± 0.1 a	4.0 ± 1.7 a	0.3 ± 0.0 b	0.1 ± 0.0 b	0.3 ± 0.0 c	0.8 ± 0.1 ab	0.3 ± 0.0 b	0.4 ± 0.4 b
	21	0.4 ± 0.4 a	n.q.	0.1 ± 0.1 a	4.5 ± 0.7 a	0.2 ± 0.3 b	0.1 ± 0.1 b	0.3 ± 0.0 c	0.7 ± 0.1 abc	0.9 ± 0.0 b	0.3 ± 0.2 b
	64	0.6 ± 0.4 a	n.q.	0.2 ± 0.1 a	3.5 ± 0.0 a	0.0 ± 0.0 b	0.1 ± 0.0 b	0.3 ± 0.0 c	1.0 ± 0.0 a	0.5 ± 0.2 b	n.d.
Pineapple	17a	0.3 ± 0.0 c	3.8 ± 0.6 a	0.1 ± 0.0 c	n.d.	1.9 ± 1.6 ab	0.1 ± 0.0 b	0.3 ± 0.0 b	0.7 ± 0.0 b	5.2 ± 4.4 a	3.0 ± 0.5 a
	75	0.2 ± 0.0 c	n.q.	0.0 ± 0.0 c	3.5 ± 0.1 b	2.7 ± 2.3 ab	0.1 ± 0.0 b	0.2 ± 0.0 a	0.5 ± 0.1 b	5.5 ± 7.6 a	3.0 ± 0.1 a
	56	0.2 ± 0.0 c	3.3 ± 0.5 b	n.d.	n.d.	1.3 ± 2.1 ab	0.1 ± 0.0 b	0.3 ± 0.1 b	0.3 ± 0.0 b	3.5 ± 6.0 a	2.5 ± 0.4 a
	21	0.4 ± 0.1 c	4.0 ± 0.5 a	0.0 ± 0.0 c	n.d.	2.9 ± 2.4 ab	0.1 ± 0.0 b	0.3 ± 0.0 b	0.3 ± 0.0 b	5.3 ± 7.3 a	3.2 ± 0.3 a
	64	0.3 ± 0.2 c	n.q.	n.d.	3.9 ± 0.2 a	3.7 ± 0.5 a	0.1 ± 0.0 b	0.2 ± 0.0 b	0.5 ± 0.0 b	10.4 ± 1.7 a	3.4 ± 0.2 a

1.4. Discussion

Mango, papaya and pineapple exhibit many composition differences.

Papaya is the less acidic fruit, with a pH of 4.9, mango is intermediately acidic with a pH of 4.2 and pineapple is acidic with a pH of 3.4. Fruits also differ in their concentration in sugars and organic acids.

Previous studies reported that mango contains 1 to 32 g/kg glucose, 1 to 8.7 g/kg fructose and 0 to 140 g/kg sucrose, and that citrate is the main organic acid (Cruz et al., 2012; Megha et al., 2022; Septembre-Malaterre et al., 2018). In our study, glucose content was ca. 17 g/L, fructose 19 g/L and sucrose 1.9 g/kg. It is reported that fructose is the main sugar in green mango and that its content decreases whereas that of sucrose increases after the climacteric peak (Maldonado-Celis et al., 2019). Our results suggest that the mango used were not fully ripe. Citrate was the main organic acid with concentration of ca. 1.5 g/L, followed by tartaric acid at 0.8 g/L.

Papaya reported sugar concentrations are glucose 32-41 g/kg, fructose 29-36 g/kg and sucrose < 5 g/kg, whereas citric acid is the main organic acid followed by malic acid (Organisation for Economic Co-operation and Development (OECD), 2010). We found 16.1 g/L glucose, 19.2 g/L fructose and 1.9 g/L sucrose, plus 1.5 g/L citric acid, 0.8 g/L tartaric acid and 0.4 g/L malic acid in 'Solo' cultivar papaya. Monosaccharides were at lower levels than reported.

Lastly, literature indicates that pineapple sugar concentrations are glucose 6-25 g/kg, fructose 8-28 g/kg and sucrose 51-96 g/kg, whereas malate content is around 2.5 g/kg and citrate 5 g/kg (Cordenunsi et al., 2010; Septembre-Malaterre et al., 2016). The concentrations obtained here were in the same range for glucose (23.4 g/L) and fructose (20.8 g/L) but much lower for sucrose (18.2 g/L), and similar for citrate (5.2 g/L) and malate (2.5 g/L). As for mango, the difference we obtained for sucrose remains questionable. Complementary analyses of sugars, especially sucrose, should be performed to check variability in Reunion 'Cogshall' mango and 'Queen Victoria' pineapple samples.

Vitamin C contents were consistent with those reported in other studies: 27.5 mg/100 g, 28.3 mg/100 g and 76.2 mg/100 g for mango, pineapple and papaya respectively. Values in (Septembre-Malaterre et al., 2016) were of 18.2 mg/100 g, 28.9 mg/100 g and 51.5 mg/100 g for the same fruit respectively. TCC of fruit were higher in this study than in (Septembre-Malaterre et al., 2016), but in the same range. For mango, it was 3.2 mg/100 g (for 2.8 mg/100 g in literature); for papaya 9.5 mg/100 g (for 1.9 mg/100 g in literature); and for pineapple 12 mg/100 g (for 0.05 mg/100 g in literature). Hence, papaya contains high levels of vitamin C and carotenoids, which make this fruit of interest for LAF. The levels of carotenoids found in pineapple were unusually high. In addition, antioxidant activity was the highest in pineapple (138-163 mg GAE/L), followed by papaya (100 mg GAE/L) and mango (11-22 mg GAE/L).

Taking into account those differences, it is not surprising that bacteria behave differently according to the fruit.

Whatever the fruit, the five selected strains grew well and acidified the medium. Growth was prolonged after 2 days, probably because the temperature decreased slowly when samples were transferred to the cold room and because bacteria were in growing phase after 2 days of incubation at 30 °C. Final pH was of the same order: 3.3-3.5 for mango and papaya and 2.9 for pineapple after 28 days. These values demonstrate a strong acidification ability of the strains. This could partly be

explained by their origin. *Ltp. plantarum* 17a was isolated from papaya pulp, whereas the other ones originated from acidic cabbage mixed with other vegetables, called *achard* in Reunion Island (Fessard & Remize, 2019). All the five strains exhibited a high growth ability and two strains, *Ltp. plantarum* 75 and *W. cibaria* 64, were showed to be able to grow at pH 3.

The color of fruit juice or puree was globally stable, including for the control condition, during storage, whereas browning was reported as an indicator of pineapple juice (Leneveu-Jenvrin et al., 2020) and mango cut (Leneveu-Jenvrin et al., 2021) spoilage. Pasteurization performed on juice and puree has probably inactivated polyphenol oxidase, responsible for browning.

A decrease of vitamin C content was expected during storage. It was observed for mango and papaya, but fermentation did not increase this degradation effect. It was better preserved in papaya fermented with *Ltp. plantarum* 17a or *W. cibaria* 21 or 64 strains. Similarly, TCC was stable in mango and pineapple and decreased slightly in papaya. The reduction of TCC in papaya was significant for *Ltp. plantarum* 17a and *W. cibaria* 21. Antioxidant activity decreased slightly during storage for the control condition and the effect of fermentation was weak. As a whole LAF was not detrimental to these bioactive compounds but did not increase their levels.

From previous studies on fruit juices, it is expected that TSS and reducing sugar content increase, and pH significantly decrease during storage (Ajibola et al., 2009; Rehman et al., 2014; Sohail et al., 2012). Different patterns of monosaccharide and organic acid consumption or production were observed with fermentation according to the fruit. A slight decrease of TSS was observed for mango and pineapple, but not for papaya. TSS decrease corresponded to fruit in which glucose and fructose were markedly consumed. However, TSS remained a poor indicator of sugar concentration variations. Whatever the fruit, pH decreased during fermentation and storage, and TA increased, but with different profiles. The analyses performed at days 0 and 7 give some explanations, but complementary analyses are required to clearly understand the observed changes. In mango, lactate and acetate were produced, with high levels of acetate. A production of tartaric acid was observed for *Lc. pseudomesenteroides* 56 and *W. cibaria* 21 strains, which are the ones exhibiting an early increase of TA. In papaya too, both lactate and acetate were produced, and tartaric acid was produced by *Lc. pseudomesenteroides* 56 and *W. cibaria* 64 strains. Both in mango and papaya, *W. cibaria* 64 consumed citrate. The source of carbon for acidification of papaya is not identified. It could be hypothesized that glucose was released from another polymeric compound during the course of fermentation and storage. But data on papaya composition are lacking to support this hypothesis. Extensive study of carbohydrate composition of papaya would help understanding. In pineapple, three strains produce mainly lactate, and two strains a mix of lactate and acetate. In addition to monosaccharide consumption, citrate was probably consumed, and malate was exhausted within 7 days for all five strains. The use of organic acids by LAB in conditions of acid stress is documented (Papadimitriou et al., 2016). In response to stress, LAB modify their metabolic pathways: inhibition of glycolytic pathway, selection of alternative fates of pyruvate, utilization of alternative carbon sources, activation of proteolytic system and catabolism of free amino acids. In particular, pyruvate metabolism is rerouted from lactate towards acetate, ethanol, carbon dioxide, butanediol, acetoin, and diacetyl (Figure 4). It was showed on pickle fermentation that *W. cibaria* produced 0.9 g/L lactic acid and 0.4 g/L acetic acid in three days, leading to an acidification to pH 3.2 (Xiang et al., 2020). Rerouting of pyruvate is clearly observed in our conditions for the three fruits, and more pronounced for pineapple from the changes in organic acid contents. This is also especially observed for *W. cibaria* 64 strain consuming

citrate. The description of yogurt olfactory notes in pineapple juice fermented with *W. cibaria* 64 strain (Fessard & Remize, 2017) strengthens this observation, as 2,3-butanediol, acetoin and diacetyl are compounds characterized by their buttery, dairy, milky odor (The Good Scents Company, n.d., 2016, 2017).

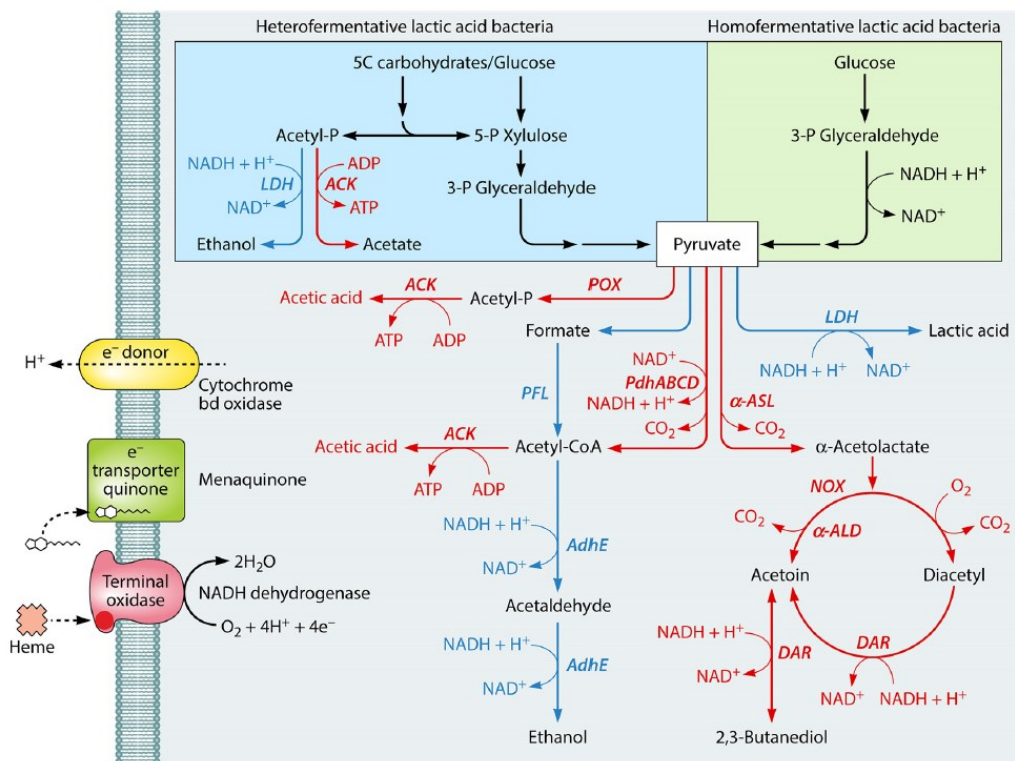


Figure 4. Schematic representation of changes of the carbohydrate metabolism, glycolysis, and fate of pyruvate in lactic acid bacteria (Papadimitriou et al., 2016). Colored arrows and enzymes indicate common reactions (black), those mainly induced during fermentation by unstressed cells (blue), and those induced in respiratory and/or environmentally stressed cells (red). LDH, lactate dehydrogenase; ACK, acetate kinase; POX, pyruvate oxidase; PFL, pyruvate formate lyase; PdhABCD, pyruvate dehydrogenase complex; α -ASL, α -acetolactate synthase; AdhE, alcohol dehydrogenase; NOX, NADH oxidase; α -ALD, α -acetolactate decarboxylase; DAR, diacetyl reductase.

In this study, we also pointed out differences between strains. The five strains belong to three species, among those *Ltp. plantarum* metabolism and fermentative activity on fruit is much more documented than for *Lc. pseudomesenteroides* and *W. cibaria*. *W. cibaria* 64 consumes citrate, *W. cibaria* 21 and *Lc. pseudomesenteroides* 56 produce tartaric acid, and those three strains produce EPS. *Lc. mesenteroides* was shown to produce erythritol and glycerol (Zaunmüller et al., 2006) and *Lc. citreum* produces mannitol (Sahin et al., 2021). Production of mannitol was also shown for *W. cibaria*, from glucose and fructose preferably to sucrose, from cabbage though mannitol dehydrogenase is absent from most *W. cibaria* strains (Choi et al., 2019; Gänzle, 2015). Hence, production of polyols by *Lc. pseudomesenteroides* and *W. cibaria* would deserve investigation. *Weissella* possess an enzymatic equipment which allow this bacterium to use a wide range of carbon sources (Hernández-Oaxaca et al., 2021). Moreover, *Lc. pseudomesenteroides* and *W. cibaria* produce EPS (Adesulu-Dahunsi et al., 2018; Farinazzo et al., 2022), used to protect bacterial cells from stress. A precise description of carbon source used and metabolite production from fruit by the strains used in

this study would bring knowledge on metabolic features of these species, found frequently in fermented foods.

1.5. Conclusion

This study has shown the efficiency of LAF on tropical fruits, with high bacterial growth despite different fruit compositions and acidic conditions. Likewise, apart from glucose and fructose which were the main sources on 'Cogshall' mango and 'Queen Victoria' pineapple, a consumption of oligosaccharides from 'Solo' papaya was hypothesized along with an increased acidification and a decreasing pH until a threshold. Interestingly, a mix of lactic and acetic acids seems to be produced during the fruit fermentation and other organic acids were consumed. A little decrease in antioxidant compounds happened along juice and purees storage however no deleterious effect was connected to LAF. The fermented fruit juices or purees revealed interesting compositional changes and nutritional properties, in particular the antioxidant properties. It would be interesting to evaluate the factors that influence metabolic pathways, especially in EPS synthesis for productive strains, and the gain of these fermented products on health.

PARTIE 2 : Étude de la production d'exopolysaccharides

2.1. Introduction

Certaines bactéries lactiques sont capables de produire des polysaccharides extracellulaires. Les exopolysaccharides (EPS) présentent un intérêt au niveau rhéologique pour les industries agroalimentaires grâce à leur capacité à influencer directement la texture des produits (Ripari, 2019). Ils ont également des propriétés antioxydantes (Lynch et al., 2018; Ray et al., 2022).

De nombreuses études présentent des données quantifiées de la production d'EPS à partir de cultures bactériennes liquides (Dueñas et al., 2003; Ispirli & Dertli, 2018; Khanh, 2018; Salazar et al., 2008). Les méthodes utilisées ne sont pas applicables aux jus et purées de fruits, car les fibres solubles et insolubles ne peuvent être séparées des EPS dans les milieux de culture liquide à base de fruit. De plus, la présence de sucres au sein de ces matrices interagit avec la quantification des EPS. Des essais pour contourner ce biais en ajoutant des étapes de centrifugation et filtration n'ont pas permis d'obtenir un résultat satisfaisant. Pour ces raisons, une méthode a été développée sur un milieu de culture solide, qui évite de récupérer les fibres du fruit. Pour ce faire, les bactéries sont cultivées à la surface de la gélose et les EPS produits sont récupérés par lessivage de la surface avant dosage.

Cette méthode a été testée sur MRS et sur les fruits d'intérêt, à savoir le jus d'ananas Queen Victoria, la purée de mangue Cogshall et la purée de papaye Solo. La capacité à produire des EPS au contact des différents fruits et les conditions optimales de production ont été déterminées. Le fait de pouvoir collecter les EPS produits sur fruits a permis d'envisager de déterminer leur structure. La souche bactérienne influence le type de monosaccharides présents, le type de liaisons, la taille et les ramifications des EPS. La structure des EPS peut également être modulée par la composition du milieu et les conditions de culture (Lynch et al., 2018). La structure des EPS produits sur milieux solides a été caractérisée par spectroscopie de Résonance Magnétique Nucléaire (RMN).

2.2. Résultats

2.2.1. Mise au point d'une méthode de quantification des EPS

La méthode développée sur milieu solide a été valorisée par la rédaction d'un manuscrit intitulé « **Quantification of bacterial exopolysaccharides: A new method for recovery from fermented fruit or vegetable beverages** » soumis à publication dans la revue *Beverages* le 10 novembre 2022. La production d'EPS a également été étudiée en faisant varier les conditions de culture, à savoir le pH et la concentration en saccharose, des milieux solides.

Quantification of bacterial exopolysaccharides: a new method for recovery from fermented fruit or vegetable beverages

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Abstract: Analysis of bacterial exopolysaccharides (EPS) produced in fruit or vegetable beverages interferes with plant dietary fibers. A method based on cultivation on a solid medium, simulating a fruit beverage, is proposed. EPS were recovered in a quantitative dependent manner and were quantified with the phenol-sulfuric acid colorimetric method. The protocol was assayed both on MRS medium and fruit-based media, with three bacterial strains from two species, *Leuconostoc pseudomesenteroides* 56 and *Weissella cibaria* 21 and 64. With that method, significantly different EPS production levels were obtained depending on the strain and on cultivation conditions, such as sucrose content modification and pH adjustment. This method opens new perspectives to pilot EPS production in lactic fermented fruit or vegetable beverages.

Keywords: fruit; pineapple; lactic acid bacteria; homopolysaccharide

1. Introduction

Bacterial homopolysaccharides are exopolysaccharides (EPS) extracellularly produced, essentially by lactic acid bacteria from different genera such as *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Oenococcus*, *Leuconostoc* and *Weissella*. Bacterial homopolysaccharides are composed of a single monosaccharide, as linear and ramified chains. Depending on the nature of the monosaccharide, they are named glucans or fructans. They also differ according to the nature of the link, α or β , between monosaccharides [1,2]. Those compounds are insoluble in water. They belong to dietary fibers (DF), together with resistant starch, pectins, cellulose, hemicellulose, and gums [3]. Production levels are typically of several g/L [4].

Bacterial EPS present numerous technological and functional properties. They can improve rheological properties and texture of foods and act as hydrocolloids. Additionally to their antioxidant activity, they exert biological effects such as anti-tumor and anti-inflammatory activities [2,5,6]. Moreover, they stimulate the growth of beneficial gut bacteria. For all that reasons, production of EPS in foods or in broth focused a lot of attention. Production and process conditions to obtain high yields of EPS implies to take into account the bacterial strain, both carbon and nitrogen sources, and temperature in a strain-dependent way [4,5]. Moreover, pH influences production and degradation of EPS, but also their structure and size. Hence, their industrial production is achieved from cultivation in broth, under regulation of environmental conditions. They can also be produced directly in foods in which they possibly modulate technological and sensory properties.

In all cases, their quantification is required to assess their production level, optimize cultivation conditions and correlate properties to concentrations.

Recovery of EPS from a culture can be achieved by removal of bacterial cells through centrifugation, then removal of undesirable compounds such as proteins, and further purification of EPS with precipitation, dialysis, filtration or chromatography techniques. Previous publications recommend to avoid presence of carbohydrate polymers in cultivation media because these compounds may hamper proper EPS analysis [7]. The main carbohydrate polymers in fruit and vegetable beverages are DF, which originate mostly from plants. A consensus definition has been established in 2016: “dietary fiber is composed of carbohydrate polymers, which are neither digested nor absorbed in the human intestine and pass into the large bowel, where they are partially or completely fermented by the colonic microbiota” [8]. Fruit and vegetables are rich in cellulose, hemicellulose and pectins, especially in their skin. In addition, fruit contain fructo-oligosaccharides and xylo-oligosaccharides, gums and mucilage [9]. For instance, mango pulp contains 7-15 g of DF for 100g, mostly as insoluble DF [10]. DF are classified according to their solubility in water, as a consequence of the AOAC methods for analysis [8,10]. In the AOAC 991.43 method, the sample is submitted to several hydrolysis steps, performed with α -amylase, protease and amyloglucosidase, then precipitated and filtered to recover two fractions, insoluble and soluble DF. Hence, the main drawback of actual methods for EPS recovery from fruit and vegetable juices is the impossibility to separate EPS from plant DF.

For subsequent quantification, the EPS fraction is usually dried into a powder that can be easily quantified by its weight. But this method remains imprecise because of possible impurities, and thus the method most frequently used is the determination of simple sugar content after complete hydrolysis of EPS [11]. Another method is based on the use of anthrone, which reacts with the dehydrated form of carbohydrate leading to a bluish-green complex. The major limitation of the anthrone method is its high detection level and usually can be accurately used only if concentration exceeds 10 mg/L [5,7]. Microscopy, either transmission electron microscopy or scanning microscopy, but also liquid chromatography and near-infrared spectroscopy, can be used to quantify EPS, but both require extensive lab-work and adapted equipment. Lastly, some studies describe the use of solid media for EPS recovery to perform structural characterization of EPS [12–14] or for visual estimation of EPS production [15].

From literature review, determination of bacterial EPS in fruit and vegetable beverages remains challenging because of interferences with naturally present DF during the analytical process. We propose here a simple method for recovery then quantification of bacterial EPS.

2. Materials and Methods

2.1. Bacterial strains

Three bacterial strains, previously isolated and characterized [16], were used: *Leuconostoc pseudomesenteroides* 56, *Weissella cibaria* 21 and *Weissella cibaria* 64.

2.2. Cultivation conditions

Lactic acid bacteria were reactivated and cultivated on MRS broth for 48h at 30°C.

2.3. Fruit preparation

Fresh pineapple (*Ananas comosus*, cv. Queen Victoria) juice was extracted after peeling of fruit. The collected juice exhibited a pH of 3.4, a total soluble solid (TSS) content of 17.7°Brix and a titratable acidity (TA) of 0.73 mg malic acid equivalent (MAE)/100g.

Mango (*Mangifera indica*, cv. Cogshall) and papaya (*Carica papaya*, cv. Solo) purées were prepared by mixing fruit after removal of peel, stone or seeds. Mango purée was characterized by a pH of 4.2, a TSS content of 17.5°Brix and a TA of 0.65 mg MAE/100g.

Papaya purée exhibited a pH of 4.9, a TSS content of 11.3°Brix and a TA of 0.63 mg MAE/100g. 93
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2.4. Screening for EPS production 95

Solid media were used in plates of diameter 5 cm. Each experiment was performed as triplicates. A negative control was performed without inoculation to check the absence of contamination. 96
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EPS production was detected after cultivation from a drop of 100 µL of active culture on MRS agar supplemented with 5 % sucrose, after 72h at 30°C. If specified, sucrose content was adjusted to 10 % or 20 %. For all experiments, pH was adjusted to 4.5, 5 or 6 with HCl 1 M prior to autoclaving. 99
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Alternatively, solid media were prepared by mixing either fresh pineapple juice, either mango or papaya purée diluted with water in a ratio 1:1, with 50 g.L⁻¹ agar. Bacteriological agar (reference 05040, Sigma-Aldrich, Saint-Quentin-Fallavier, France) was used. Initial pH of the media was conferred by the fruit juice, unless specified. To obtain an initial pH value of 4.5, 5 or 6 of the media, sodium hydroxide 2 M was used, prior to sterilization. If specified, sucrose was added to the media prior to sterilization, to obtain a concentration of 5%, 10% or 20 %, considering an initial sucrose content of pineapple juice, mango purée and papaya purée of 9.6 %, 6.5 % or 0 % respectively. If specified, peptone was added to the media prior to sterilization, to obtain a content of 2 % or 4%. Sterilized media were stored at 4°C for less than 72h before use. Plates were inoculated with 100 µL of active bacterial culture and incubated for 72h at 30°C. 103
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The colony was visually qualified as (-) non mucoid, (+/-) slightly mucoid, (+) mucoid, (++) strongly mucoid and (+++) very strongly mucoid. 114
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2.5. Recovery of EPS 116

Recovery of EPS was performed by washing the plate surface with 5 mL of ultrapure sterile water. Briefly, the plate was poured with 2 mL of water, submitted to manual agitation and the liquid was collected by pipetting, and the same steps were repeated with 3 mL of water. A centrifugation step of 10 min at 5000 × g was applied to remove cellular and gelose debris. The supernatant was stored at -20°C until use. 117
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2.6. Phenol-sulfuric colorimetric determination 122

EPS were analyzed according to the method of phenol-sulfuric reagent [11]. A volume of 500 µL of sample containing EPS was mixed with 500 µL of phenol 5% (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), then with 2.5 mL of 98% sulfuric acid (VWR Chemicals, Pennsylvania, USA). The mixture was heated at 95°C for 15 min. After cooling, OD was measured at 490 nm. The concentration was determined with D-glucose (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) as a standard, in the range 0 g/L to 0.5 g/L. The concentration of EPS was expressed as mg glucose equivalent per plate. 123
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2.7. Statistical analysis 130

Quantitative data were analyzed by ANOVA with Ryan-Einot-Gabriel-Welsch and Quiot (REGWQ) test, with XLSTAT 2016.1.1 software (Addinsoft, Paris, France). 131
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3. Results and discussion 133

3.1. Determination of EPS production on MRS sucrose 134

EPS production of the three lactic acid bacterium strains was first assessed on MRS medium containing sucrose at three different levels, and with initial pH value adjusted at 4.5, 5 or 6. A visual scoring of EPS production was applied and EPS were collected and analyzed with the method described. 135
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Production levels varied according to the strain, initial pH and sucrose content in the medium. Whatever the strain, the highest production was observed for MRS pH 6 and sucrose 20%, and the production was also high though significantly different for MRS pH 6 sucrose 10%. The lowest production levels were observed when low pH, 4.5 or 5, were combined with moderate sucrose levels, 5 % or 10 % (Figure 1). For pH values of 5 and 6, and whatever the sucrose level, the strain *L. pseudomesenteroides* 56 produced EPS at lower levels than the two other strains. Eventually, EPS production was strain-dependent, with differences between *L. mesenteroides* 56 and the two *W. cibaria* strains (p-value < 0.0001). The developed method allowed to differentiate between conditions and strains.

Coefficient of variation was acceptable but presented some discrepancies: it was below 25% for 16 conditions among 27. This means that the method could be improved to reduce variability of results. In existing methods, a further purification step was used to remove non-EPS compounds which could interfere with phenol-sulfuric colorimetric method [17]. Washing plate surface with water could solubilize mono- and di-saccharides from the cultivation medium. This hypothesis was confirmed through NMR analysis of water collected from MRS plate washing using the protocol hereby used (Joël Couprie, personal communication). Hence, an additional step designed specifically to remove monosaccharides and sucrose should be added to this method. Dialysis, gel filtration or EPS precipitation with ethanol would be relevant to that aim. The agar content of 5%, which was chosen very high, led to a strongly solid agar, facilitating surface scraping, and poorly sensitive to variations of medium composition, such as pH lowering.

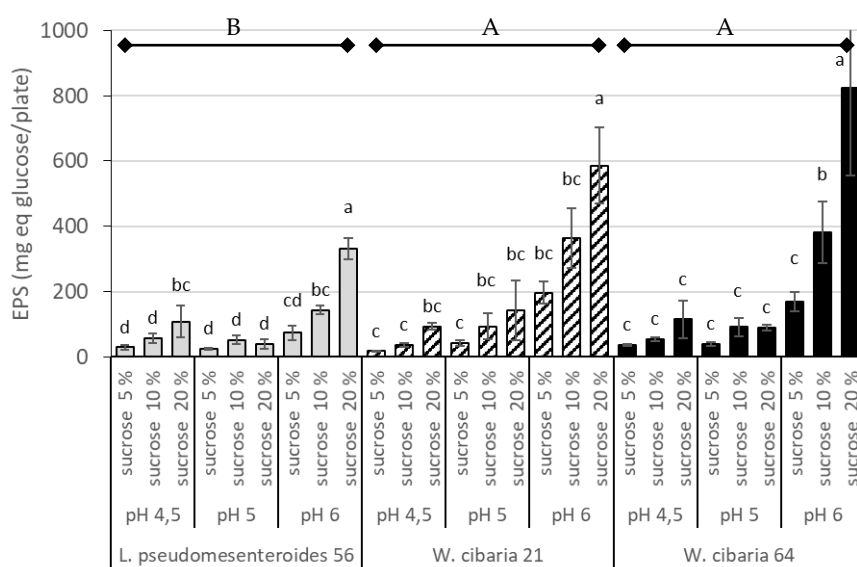


Figure 1. Production of EPS per MRS plate for the three strains (*W. cibaria* 21: grey bars, *L. pseudomesenteroides* 56: hatched bars, *W. cibaria* 64: black bars), according to initial pH and sucrose content of the medium. Different lowercase letters correspond to significant differences between conditions within each strain assays (p-value = 0.0002 for strain 21 and <0.0001 for 56 and 64). Different upper-case letters correspond to significant differences between strains (p-value < 0.0001).

A correlation was searched between quantitative EPS determination and visual score (Figure 2). The highest EPS content for each score respected the scoring order, but overlaps between EPS contents for different visual scores were observed. This observation pointed the limitations of visual scoring, which probably depended on the nature of EPS produced by the three strains, in terms of length and ramifications. Differences of EPS structure probably triggered differences in colony aspect. Indeed, viscosity of EPS solutions depends both on EPS concentration and on the length of branched chains [12,18,19]. More specifically, elasticity and thickness depend on the linkage types and proportion

observed in dextran. From colonies, ropiness, estimated from strand length formed after touching with a loop, and slime consistency, varying from liquid to compact, was dependent on the EPS type and possibly on chain length of α -(1,3)-linked branches [13].

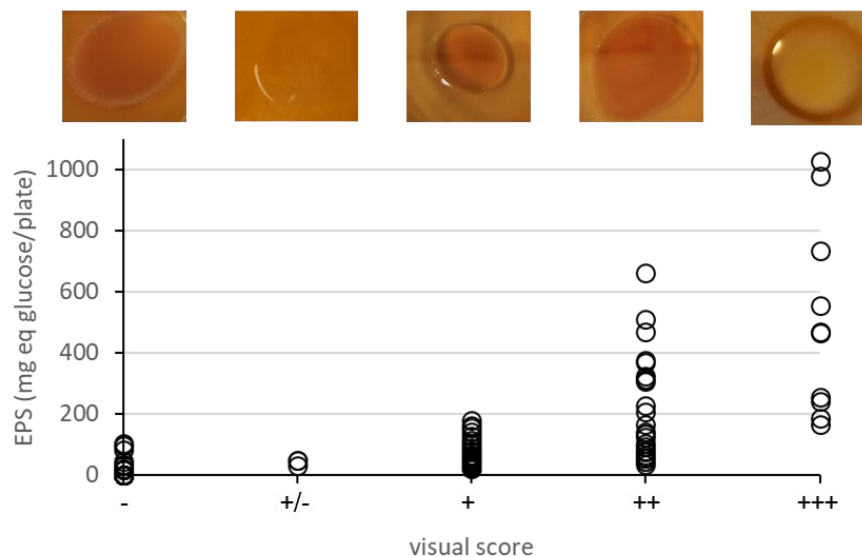


Figure 2. Correlation of EPS production, in mg glucose equivalent per plate, and visual score of EPS production on MRS plates, for the three strains 21, 56 and 64, three pH levels, 4.5, 5 and 6, and three sucrose content, 5%, 10% and 20%. Three replicates per condition were performed. Visual score of colonies was rated as (-) non mucoid, (+/-) slightly mucoid, (+) mucoid, (++) strongly mucoid and (+++) very strongly mucoid. Examples of visual scores are reported with pictures on top of the plot.

3.3. Visual assessment of EPS content on fruit-based media

A first assay was performed on fruit-based agar, with or without pH adjustment at 6, with or without sucrose addition to reach 20% (Table 1).

Table 1. Visual score of EPS production for the strains 56, 21 and 64, on fruit-based agar, with pH adjustment to 6 or sucrose addition to reach 20%.

Fruit	Modification of condition	<i>L. pseudomesenteroides</i> 56	<i>W. cibaria</i> 21	<i>W. cibaria</i> 64
pineapple	none	n.d. ⁽¹⁾	n.d.	n.d.
	pH adjusted to 6	+	+	+
	sucrose adjusted to 20 %	n.d.	n.d.	n.d.
	pH 6 and sucrose 20 %	++	++	++
mango	none	+	+	+
	pH adjusted to 6	+	++	++
	sucrose adjusted to 20 %	+	+	+
	pH 6 and sucrose 20 %	++	++	++
papaya	none	-	-	-
	pH adjusted to 6	-	-	-
	sucrose adjusted to 20 %	-	-	-
	pH 6 and sucrose 20 %	+/-	+/-	+/-

⁽¹⁾ n.d.: not determined

The three strains behaved similarly whatever the fruit. 219

Pineapple exhibited a low pH of 3.4. At that pH, the medium was semi-liquid as expected from agar gel properties, thus EPS production could not be determined. The adjustment of pH to 6 enabled EPS production. Production was further stimulated when sucrose was adjusted to 20 %, instead of 9.6 % for the fruit. 220
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On the contrary, mango resulted in EPS production for all tested conditions. A slight increase was observed when pH was adjusted from 4.2 to 6, and sucrose from 3.25 % to 20 %. 224
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Lastly, papaya medium resulted in a good growth of strains but did not favor EPS production. Papaya contains low levels of sucrose, mainly glucose and fructose [21,22]. Hence, EPS production cannot occur directly on fruit medium because of sugar substrate lack. Surprisingly, EPS production was not restored by sucrose addition to papaya purée. Papaya pH was close to 5 [23]. Adjustment of pH to 6, in combination to sucrose 20 %, resulted in a weak EPS production. It was previously reported that nitrogen availability could be critical for EPS production and that a correct nitrogen / sugar balance was required [5]. To test the hypothesis of a limitation of nitrogenous compounds for EPS production, peptone 2 % was added to the medium, but no EPS production was observed (data not shown). Papaya could contain a compound which exerted an inhibitory role for EPS production. 227
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From those results, we decided to apply the quantification method to several conditions of pineapple juice and mango purée, but not papaya purée. 238
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3.4. Determination of EPS content on pineapple juice agar 240

The quantitative method was applied to determine EPS production on pineapple-juice medium at two different sucrose levels, juice level estimated at 9.6 % and adjusted at 20 %, for three pH values, from 4.5 to 6, for the strains *L. pseudomesenteroides* 56 and *W. cibaria* 64 (Figure 3). 241
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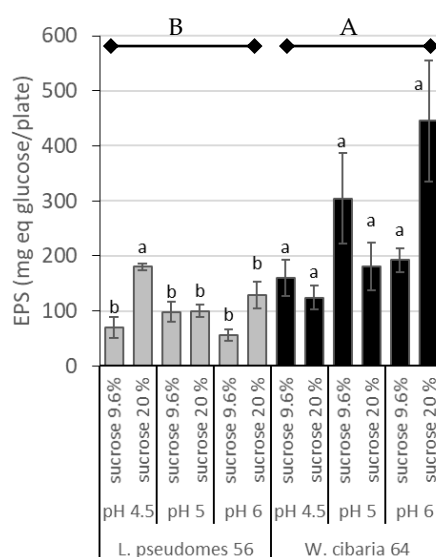


Figure 3. EPS production in pineapple juice agar medium, for the strains *L. pseudomesenteroides* 56 and *W. cibaria* 64, at initial pH of 4.5, 5 or 6 and sucrose level at 9.6 % (level in juice) or 20 %. Different lowercase letters correspond to significant differences between conditions within each strain assays (p-value = 0.007 for strain 56 and 0.304 for 64). Different uppercase letters correspond to significant differences between strains (p-value = 0.013). 263
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The mean production of EPS of the two strains differed significantly (p-value = 0.013). EPS production of *W. cibaria* 64 was higher than that of strain *L. mesenteroides* 56, as ob- 268
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served in MRS. EPS production of *L. mesenteroides* 56 was between 56 and 243 mg eq. glucose per plate. The highest level was obtained at pH 4.5 and sucrose 20 %. Though production was systematically higher for sucrose 20 % compared to 9.6 %, no significant sucrose content effect was detected. The medium pH did not show any effect in the range assayed. EPS production of *W. cibaria* 64 ranged between 159 and 345 mg eq. glucose per plate, and no effect of pH or sucrose level was detected in the conditions assayed. Comparatively to MRS, the production level was lower on pineapple juice. The inhibitory effect of pineapple acidity on EPS seemed relieved when pH was equal or above 4.5.

Variation coefficients obtained with the quantitative method were between 3 % and 27 %, with a majority of values below 20 %.

3.5. Determination of EPS content on mango purée agar

As differences in EPS production levels were detected for the two strains 56 and 64 on pineapple juice medium, contrarily to visual score, the three strains were assayed on mango purée agar. Two levels of pH were assayed, pH 5 and pH 6, and three levels of sucrose: 3.25 % corresponding to that of diluted mango purée, 10% and 20%. Eventually, addition of peptone 2% was performed for all conditions, and assays at pH 5 were also performed without peptone.

As shown in **Figure 5**, the production level of EPS differed between *L. pseudomesenteroides* 56 and the two other *W. cibaria* strains, 21 and 64. Production of EPS by strain 56 remained poor whatever the modification of condition, ie. pH modification, sucrose addition or peptone addition. The production of EPS by strain 21 was higher than for strain 56, and was significantly increased at pH 6, sucrose 20% and peptone 2%. EPS production from strain 64 showed a tendency to increase when sucrose content increased, though not significant. Peptone addition was ineffective. Compared to MRS, the production level of EPS was lower on mango purée. The maximum production level was achieved with *W. cibaria* at a pH of 6, with 20 % sucrose and 2 % peptone.

Only one third of EPS average values presented a variation coefficient below 25 %. As variation coefficients greatly varied according to the medium, ie. lowest on pineapple based agar and highest on mango based medium, the hypothesis of recovery of other compounds during sample preparation was strengthened. However, a variability of production by the strain cannot be excluded.

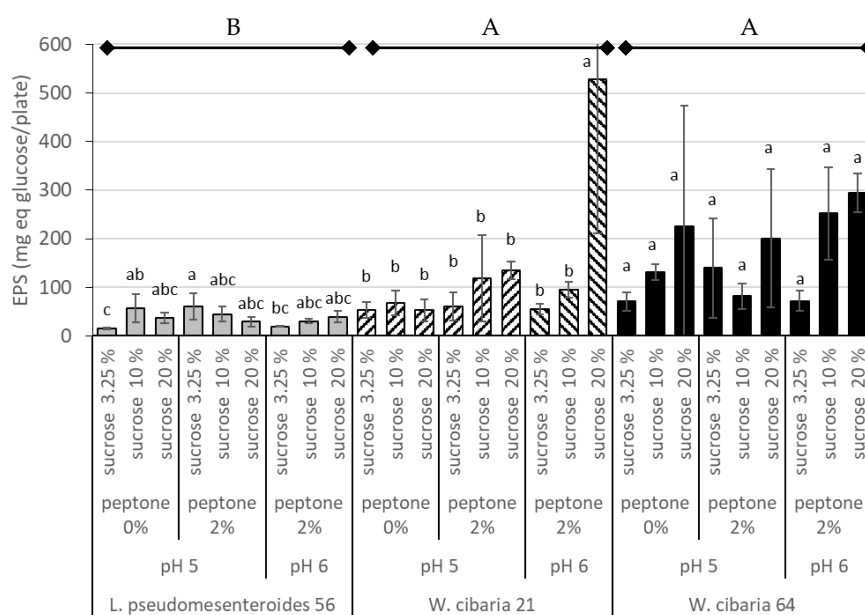


Figure 5. EPS production in mango purée agar medium, for the strains *L. pseudomesenteroides* 56, *W. cibaria* 21 and *W. cibaria* 64, at initial pH of 5 or 6 and sucrose level of 3.25 % (level in diluted purée), 10 % or 20 %, with or without peptone 2 %. Different lowercase letters correspond to significant differences between conditions within each strain assays (p-value = 0.028 for strain 56, 0.008 for strain 21 and 0.142 for 64). Different uppercase letters correspond to significant differences between strains (p-value = 0.0002).

This quantitative method opens possibilities to optimize EPS production from fruit or vegetable matrices with lactic acid bacteria. However, the method requires an improvement, as proposed above to separate simple sugars from EPS. In addition, the phenol sulfuric method used for sugar quantification, though the most widely used for that purpose and the most reliable, presents serious disadvantages: phenol is detrimental for environment and health, sulfuric acid is harmful for manipulators and molar absorption coefficient of colored compounds differ according to the carbohydrate [24,25]. Adjustments of the phenol-sulfuric method were proposed to obtain a constant response from different sugars, but remain based on the same chemical agents [26]. More recently, a method based on UV monitoring of furfurals, derived from sugars under acidic conditions, improved the response of sugars while not using phenol [25]. Eventually, for homopolysaccharides produced from bacteria, which are composed of a single type of monosaccharide, the use of a method specific for glucose or fructose, for instance enzymatic, would be the most relevant, provided free monosaccharides are efficiently removed before hydrolysis.

Production of EPS during lactic fermentation of fruit or vegetable beverages presents interests for texture properties, but also nutritional benefits [2,12,27]. Fermentation of carrot beverage with selected lactic acid bacteria, including *Leuconostoc* and *Weissella* strains, resulted in EPS production and consequently modified perceived texture and flavor to pleasant ones [12].

The choice of the strain to ferment fruit or vegetable matrices is crucial to control the quality of the product [28,29]. The strains used in this study were previously isolated from fruit. *L. pseudomesenteroides* is already known to produce dextran from sucrose, in which sugar residue linkages are α -(1,6) and with molecular mass above 10^6 Da [30,31]. Optimal production conditions in MRS were determined: 18% sucrose, 20°C and pH 7.3 in [32], and 10% sucrose, 25°C and pH 7 in [33]. *W. cibaria* also produces dextran with α -(1,6) linkages and molecular mass above 10^7 Da [34,35]. EPS production was studied in MRS

with an adjustment of conditions. Used sucrose levels were lower than in *L. pseudomesenteroides* studies, up to 10% at the maximum [36–38]. It appeared that depending on the study the optimal sucrose level was 4 %, 6 % or 8 %. Optimal pH values were between 6.2 and 7. Surprisingly, Yu *et al.* [37] found an optimal temperature of 22°C, which could be 10-fold lower at 30°C depending on other conditions, whereas Wongsupchachat *et al.* [36] found an optimal temperature of 37°C, in a range from 15°C to 37°C.

Hence, lowering temperature to 25°C could increase EPS production from *L. pseudomesenteroides* 56, and a range of temperature could be assayed for *W. cibaria* 21 and 64. Optimal pH were close to neutrality in the different studies. For an application to fermented fruit or vegetable, initial pH depends on the starting material. The moderate effect of pH, in the range 4.5 to 6, on EPS production in this study is advantageous for low acidic fruit, such as mango. An increase of initial pH of pineapple juice could be applied before lactic fermentation to stimulate EPS production. Pineapple already contains high concentrations of sucrose, which is convenient for EPS production. For mango, high maturity fruit could be selected for fermentation. Alternatively, sucrose could be added prior to fermentation. This was the case on carrot beverage which was supplemented with 5% sucrose [11]. However, in the perspective of production of lactic fermented beverages, addition of sucrose is detrimental for nutritional properties and should be avoided.

4. Conclusion

EPS characteristics which are close to that of naturally present DF in fruit prevent the analysis of lactic acid bacterium produced EPS in fruit juices. The developed method, based on a cultivation step on a plate followed by the recovery of EPS from the colony, allowed the quantitative determination of EPS production from fruit juice or purée. This method resulted in consistent EPS levels and was able to detect differences according to the used strain or medium composition. It could be improved to reduce variation coefficient by adding a purification step. It also could be adapted to the analysis of hetero-exopolysaccharides, produced at lower levels, by coupling with another quantification method such as HPLC. The availability of this method facilitates optimization of fermentation conditions of fruit and vegetables to obtain pleasant products and optimize EPS production.

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2.2.2. Détermination de la structure des EPS par spectroscopie RMN

2.2.2.1. Matériel et Méthodes

La production d'EPS a été induite pour *Lc. pseudomesenteroides* 56 et *W. cibaria* 64 sur une gélose MRS, sur un milieu solide à base de jus d'ananas Queen Victoria et sur un milieu solide à base de purée de mangue Cogshall diluée au 1/2 en ajustant ou non le pH à 6 et la concentration en saccharose à 20 %. Après une incubation à 30 °C pendant 72 h, les EPS produits sur les milieux solides ont été mis en suspension dans 5 mL d'eau ultrapure puis lyophilisés. Ensuite, 10 mg d'EPS lyophilisés ont été solubilisés dans 1 mL d'oxyde de deutérium D₂O (Eurisotop, France). Le standard interne utilisé est du tétraméthylsilane (TMS, Sigma-Aldrich, Allemagne). Des témoins ont été réalisés en appliquant la même méthode de récupération, i.e. lessivage avec 5 mL d'eau ultrapure, sur des géloses non inoculées.

La structure des EPS a été étudiée par utilisation d'un spectromètre RMN BRUKER Avance III – 600 MHz équipé d'une cryosonde ¹H/¹⁹F, ¹³C, ¹⁵N (Bruker, Allemagne). Les données ont été obtenues à une température de 323 K, soit 50 °C. Les spectres 1D ¹H ont été enregistrés avec 256 scans et une largeur spectrale de 10,8 parties par million (ppm). La fréquence porteuse était placée à 4,53 ppm pour le ¹H. Pour l'identification des sucres, des spectres 1D ¹³C, 2D COSY, 2D TOCSY, 2D NOESY, 2D ¹H-¹³C HSQC et 2D ¹H-¹³C HMBC ont également été enregistrés. Pour le ¹³C, la porteuse était placée à 77,5 ppm et la largeur spectrale était de 200 ppm (1D ¹³C) ou 100 ppm (expériences 2D ¹H-¹³C).

2.2.2.2. Résultats

Dans un premier temps, la présence de sucres résiduels issus du milieu de culture solide pouvant être solubilisés lors de la récupération des EPS produits a été évaluée. Les échantillons collectés à partir de géloses sans culture bactérienne ont été comparés à des standards de saccharose et de glucose (**Figure 12**). Dans tous les milieux témoins, le proton anomérique (H1 du glucose) du saccharose est détecté à 4,42 ppm et celui du glucose à 5,23 ppm. On note également dans tous les témoins la présence des signaux des autres protons du saccharose (entre 3,45 et 4,2 ppm). Les échantillons témoins des milieux MRS, à base de jus d'ananas Queen Victoria et à base de purée de mangue Cogshall contiennent donc tous majoritairement du saccharose et du glucose en quantité minimale. Les profils obtenus n'ont pas présenté de différences notables en fonction du milieu testé.

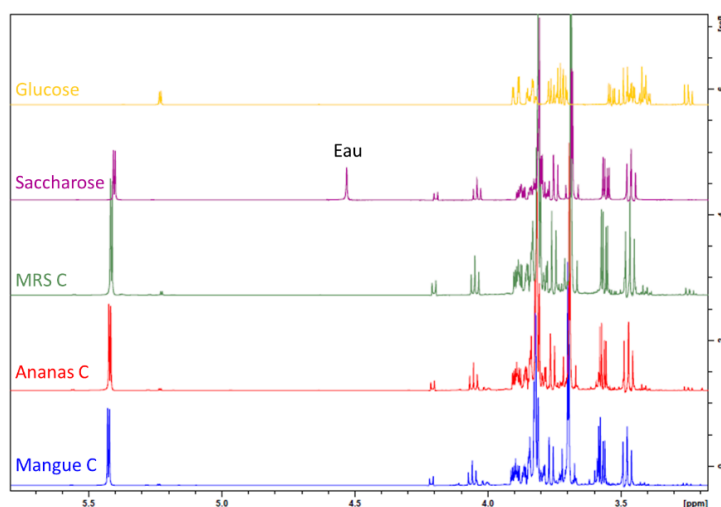


Figure 12 : Spectres RMN ¹H à 1 dimension de standards de glucose et saccharose et des échantillons témoins (C) par milieu.

La structure des EPS produits par les souches bactériennes d'intérêt sur des milieux MRS, à base de purée de mangue Cogshall et à base de jus d'ananas Queen Victoria a ensuite été analysée. Quel que soit le milieu étudié, les spectres RMN ^1H sont modifiés après culture de bactéries lactiques par comparaison au témoin (**Figure 13**).

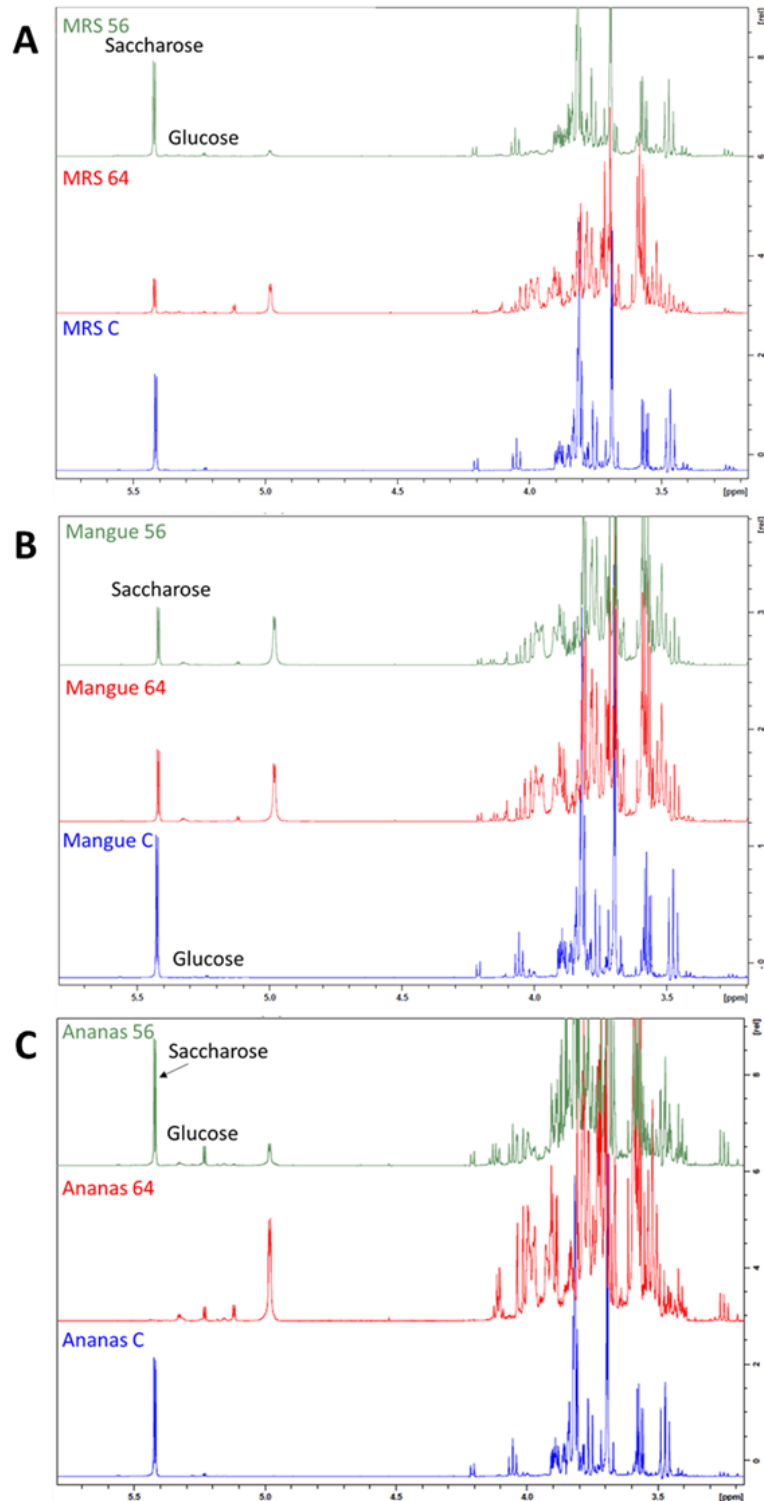


Figure 13 : Spectres RMN ^1H à 1 dimension des EPS produits sur milieu (A) MRS, (B) à base de purée de mangue Cogshall et (C) à base de jus d'ananas Queen Victoria. C : témoin, 56 : *Lc. pseudomesenteroides*, 64 : *W. cibaria*.

Sur le milieu de culture sélectif des bactéries lactiques (**Figure 13A**), l'apparition d'un proton anomérique est visible à 4,98 ppm pour les souches *Lc. pseudomesenteroides* 56 et *W. cibaria* 64. L'apparition d'un pic correspondant à un proton anomérique supplémentaire à 5,12 ppm par comparaison au profil de la souche *Lc. pseudomesenteroides* 56 indique la synthèse d'une unité supplémentaire par la souche *W. cibaria* 64, qui contient donc deux unités saccharidiques principales. De plus, l'utilisation de la souche *W. cibaria* 64 a induit une consommation importante du saccharose présent dans le milieu. La consommation du saccharose du milieu à base de purée de mangue Cogshall par les souches bactériennes est également notable (**Figure 13B**). L'inoculation des souches *Lc. pseudomesenteroides* 56 et *W. cibaria* 64 a induit l'apparition des deux mêmes protons anomériques, à 4,98 ppm et 5,12 ppm, que ceux observés avec le milieu de culture spécifique aux bactéries lactiques. L'inoculation de *W. cibaria* 64 sur un milieu à base de jus d'ananas Queen Victoria a entraîné la consommation de la totalité du saccharose présent dans le milieu (**Figure 13C**). Cette souche induit une synthèse d'exopolysaccharides élevée par comparaison à la souche *Lc. pseudomesenteroides* 56. L'unité saccharidique dont le proton anomérique résonne à 4,98 ppm apparaît largement majoritaire.

En conclusion, la diminution de la proportion de saccharose du milieu est liée à sa consommation par les souches de bactéries lactiques. L'influence de la souche bactérienne utilisée prédomine sur la production d'exopolysaccharides par comparaison à l'influence du fruit. La consommation de saccharose ainsi que l'utilisation d'unité monosaccharidique pour la synthèse d'EPS par la souche *W. cibaria* 64 est supérieure à celle de la souche *Lc. pseudomesenteroides* 56.

Afin d'identifier l'enchaînement des unités saccharidiques des EPS présentes, l'ensemble des ^1H et ^{13}C de l'unité saccharidique majoritaire ainsi que les H1, H2 et C1 de l'unité saccharidique minoritaire ont été attribués à l'aide des spectres 2D COSY, TOCSY et ^1H - ^{13}C HSQC. Les résultats sont reportés dans le **Tableau 3**.

Tableau 3 : Déplacements chimiques ^1H et ^{13}C des unités saccharidiques constituant les EPS produits par les souches *Lc. pseudomesenteroides* 56 et *W. cibaria* 64.

Unité saccharidique majoritaire			
Protons	δ (ppm)	Carbones	δ (ppm)
H1	4,98	C1	100,5
H2	3,58	C2	74,2
H3	3,73	C3	76,1
H4	3,52	C4	72,4
H5	3,91	C5	72,9
H6 ; H6'	3,77 ; 3,98	C6	68,4
Unité saccharidique minoritaire			
H1	5,12	C1	103,0
H2	3,55	C2	

La comparaison de ces valeurs avec la littérature permet d'identifier l'unité saccharidique majoritaire comme correspondant très probablement à un enchaînement de résidus glucosyl reliés par des liaisons glycosidiques α -(1 \rightarrow 6) alors que l'unité saccharidique minoritaire correspondant

probablement à un enchaînement de résidus glucosyl reliés par des liaisons glycosidiques α -(1→3) (Li et al., 2022; Purama et al., 2009; Yilmaz et al., 2022).

Ces données sont en accord avec la structure des EPS produits par *Lc. pseudomesenteroides* (Yang et al., 2018; Zhou et al., 2018) et *W. cibaria* (Kavitake et al., 2020). L'EPS majoritaire produit serait ainsi un dextrane avec un enchaînement de liaisons α -(1→6) et quelques branchements α -(1→3).

2.3. Discussion

La production d'EPS est régulée par différents facteurs, e.g. la nature du substrat, la température, le pH, le temps d'incubation et la source énergétique (Juvonen et al., 2015; Lynch et al., 2018; Ripari, 2019). Lors de cette étude, la production d'EPS par les souches bactériennes d'intérêt a pu être évaluée sur un milieu de culture sélectif des bactéries lactiques (MRS) et sur des milieux à base de jus d'ananas Queen Victoria, de purée de mangue Cogshall ou de purée de papaye Solo. Une production d'EPS plus faible a été obtenue pour les milieux à base de fruit par comparaison à celle obtenue sur milieu MRS. Cette méthode a été utilisée pour déterminer si des variations de la teneur en saccharose et du pH modifiaient la production d'EPS. La supplémentation en sources carbonées et azotées et en vitamines permet d'optimiser la production d'EPS. Elle peut cependant ne pas être induite malgré la modulation de ces facteurs. Selon la souche bactérienne utilisée, une différence de production d'un facteur 5 a pu être observée. L'ajout de saccharose a stimulé la production d'EPS sur un milieu à base de jus d'ananas tandis qu'un pH de 6 a permis de stimuler cette production sur un milieu à base de mangue. La supplémentation en peptone n'a pas induit de différence de production. En revanche, la production d'EPS sur un milieu à base de papaye n'a pas pu être induite par la modulation de la concentration en saccharose ni par l'alcalinisation du milieu, indiquant que d'autres facteurs propres à la papaye influencent la production d'EPS par les bactéries lactiques. Caractériser de manière approfondie la production d'EPS est donc nécessaire.

La production d'EPS est dépendante de la souche bactérienne utilisée et est donc dépendante de son adaptation aux conditions environnementales, sa consommation de sucres, sa régulation génétique et son activité enzymatique (Leroy & De Vuyst, 2016). La production d'EPS était plus élevée pour la souche de *W. cibaria* 64 par comparaison à la souche de *Lc. pseudomesenteroides* 56, quel que soit le milieu étudié.

La caractérisation des EPS produits effectuée par spectroscopie RMN a généré des spectres à une dimension permettant d'identifier les monosaccharides présents dans les extraits. Ces analyses ont également été effectuées sur des milieux sans culture bactérienne afin de pouvoir soustraire les résidus de sucres liés à la solubilisation du milieu lors de la récupération des EPS. Les analyses de nos échantillons par RMN ont montré une consommation du saccharose du milieu par les bactéries lactiques lors de la production d'EPS, résultant en l'apparition d'une ou deux unités monosaccharidiques. D'après la littérature, les souches étudiées ont induit la synthèse d'un dextrane avec un enchaînement de liaisons α -(1→6) et quelques branchements α -(1→3). Les spectres obtenus étaient similaires selon les souches bactériennes utilisées, quel que soit le milieu de culture. Des analyses RMN approfondies permettraient de déterminer plus précisément la structure des EPS et les interactions entre les molécules de carbone et d'hydrogène et différents atomes. Cela permettrait d'étudier leur position relative au sein de la structure des polysaccharides et de connaître la configuration du polymère. Il conviendrait de confirmer la nature des EPS produits à l'aide d'autres

techniques telles que la chromatographie en phase gazeuse couplée à la spectrométrie de masse et la spectroscopie infrarouge à transformée de Fourier. De plus, une chromatographie par filtration sur gel permettrait de déterminer la taille des différentes molécules. Des étapes de purification effectuée après la collecte des EPS produits sur milieu solide pourrait permettre d'éliminer les sucres du milieu pouvant interférer avec la quantification des EPS. Une précipitation des EPS à l'éthanol froid ou une dialyse permettrait par exemple d'exclure les mono et disaccharides.

PARTIE 3 : Effets fonctionnels du jus d'ananas Queen Victoria fermenté sur le métabolisme énergétique

3.1. Résumé des travaux effectués

À partir des données abondantes de la littérature sur la génération de molécules bioactives au cours de la fermentation lactique et des observations réalisées sur les jus et purées de fruits tropicaux lactofermentés dans les parties expérimentales précédentes, nous nous sommes interrogés sur le potentiel santé de ces produits dans un contexte réunionnais de forte prévalence de désordres métaboliques.

L'objectif était d'explorer les effets fonctionnels induits par la consommation de jus lactofermenté sur un organisme entier. Le jus d'ananas fermenté a été choisi comme modèle du fait de sa teneur initiale élevée en sucres, lesquels sont partiellement consommés pendant la fermentation, de sa forte activité antioxydante et de sa capacité à supporter la production d'EPS. Les souches bactériennes de *Ltp. plantarum* 75 et *Lc. pseudomesenteroides* 56 ont été choisies car elles conduisent à des modifications très différentes, en termes de production d'acides organiques et EPS notamment. Un modèle de souris C57BL/6J nourries avec un régime riche en graisse a été utilisé. Cinq groupes de souris se sont vu proposer pendant six semaines du jus d'ananas fermenté, du jus d'ananas non fermenté, de l'eau sucrée contenant des bactéries lactiques, de l'eau sucrée ou aucune de ces préparations (eau *ad libidum*). La glycémie, la triglycéridémie et la cholestérolémie ont été mesurées tandis que le foie, le tissu adipeux et l'intestin ont été collectés.

Dans un premier temps, la détermination de l'effet de ces jus sur l'activité de l' α -glucosidase a montré une inhibition de cette activité par le jus d'ananas Queen Victoria fermenté ou non.

Dans un second temps, les données issues du suivi des animaux ont montré que les quatre groupes consommant une boisson sucrée ont pris plus de poids que les souris du groupe non supplémenté. La consommation des eaux sucrées avec ou sans bactéries était supérieure à celle des jus d'ananas Queen Victoria fermentés ou non. Bien que la différence ne soit pas significative, le gain de masse grasse était modéré pour le groupe ayant consommé du jus fermenté. L'exploration du métabolisme glucidique a montré une glycémie à jeun après 6 semaines de supplémentation significativement plus élevée que celle du témoin pour les groupes ayant consommé du jus d'ananas et de l'eau sucrée contenant des bactéries lactiques mais pas du jus fermenté. Le test de tolérance à l'insuline montre une régulation similaire pour le groupe ayant consommé du jus fermenté et le témoin après 6 semaines de supplémentation. L'exploration du métabolisme lipidique a montré une modulation de la cholestérolémie médiée par la fermentation, par comparaison aux autres boissons sucrées. D'autre part, la supplémentation en boissons sucrées n'a pas induit d'altération de la perméabilité intestinale. De même, la population de bactéries lactiques est restée stable entre le groupe ayant consommé le jus d'ananas fermenté et celui ayant ingéré uniquement de l'eau. En revanche, un changement de la composition bactérienne a été observé.

En conclusion, les résultats de comparaison de l'effet de boissons chez le mammifère nourri en régime riche en graisse montrent des effets modérés. Le volume de consommation d'eau sucrée augmente au cours du temps et cette prise accentue les désordres sanguins, contrairement au jus d'ananas fermenté. En outre, la consommation de jus fermenté module la composition en bactéries lactiques du microbiote intestinal.

3.2. Résultats

Les résultats obtenus lors de cette étude sont valorisés par la rédaction d'un manuscrit intitulé « **Effect of pineapple fermented juice consumption on energetic metabolism of mice** » soumis à publication dans la revue *Molecular Nutrition and Food Research* le 05 octobre 2022.



Fermented pineapple juice consumption limits metabolic disorders associated to sugary drinks on high-fat diet-fed mice

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Keywords:	Bacterial colonization, Fruit, Lactic fermentation, Metabolic disorders, Beverages

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4 **high-fat diet-fed mice**
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42 **Abbreviations:** LAF, lactic acid fermentation; LAB, lactic acid bacteria; HFD, high-fat diet; OGTT, oral
43 glucose tolerance test; ZO-1, zonula occludens-1; F&V, fruit and vegetable; MRS, De Man, Rogosa and
44 Sharpe; TSS, total soluble solids; *Lc.*, *Leuconostoc*; *Ltp.*, *Lactiplantibacillus*; C, control, SW, sweetened
45 water; BSW, LAB in sweetened water; J, pineapple juice; FJ, fermented pineapple juice; TG,
46 triglycerides; REGWQ, Ryan–Einot–Gabriel–Welsch.
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59 **KEY-WORDS:** bacterial colonization; metabolic disorders; fruit; lactic fermentation; beverages
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ABSTRACT

Lactic acid fermentation modulates the composition of food, leading to changes in safety, sensory and nutritional properties. The effects of lactic fermented pineapple juice supplementation on energetic metabolism of high-fat diet (HFD) fed mice were compared with either water (control), sweetened water, bacteria in sweetened water and pineapple juice supplementation. Drink consumption and body weight were measured during the six weeks of experiment, whereas glycaemia and lipid content were determined at the beginning and at the end of the experiment. Total energy intake was similar between all groups though the volume of juice consumed was lower than that of sweetened waters. Weight gain was higher for mice provided with sugary drinks (5.65-7.74 g) compared to water (4.68 g). The fermented pineapple juice was less detrimental to blood carbohydrate regulation than other sugary drinks. Triglyceride and total cholesterol content were not modified following fermented juice or water consumption, contrarily to other sugary drinks. Whatever the drink, intestinal permeability was preserved. Lactic acid bacterium (LAB) population in feces was not affected by the beverage but species composition was modified. From a health perspective, fermented pineapple juice was preferable to other sugary drinks to limit metabolic disorders related to HFD.

1. Introduction

Unhealthy diet is one of the main risk factors responsible for obesity and related metabolic diseases. Fruit and vegetable (F&V) consumption can contribute to health enhancement.^[1] Indeed, they contain various micronutrients, such as vitamins, minerals and phenolic compounds, which offer anti-proliferative, anti-inflammatory and antioxidant properties.^[2] Among those, pineapple (*Ananas comosus*) has a high sugar content and presents a good balance of sugar with acidity, which contributes to its appreciated flavor.^[3] This fruit can be consumed either fresh or transformed mainly in juice, fresh-cut and cans. Pineapple global production was above 25 million tons in 2014, and this fruit is in the top 4 of tropical fruit in volume and value for export.^[4,5] Lactic acid fermentation (LAF) is a valuable method to extend shelf-life and to enhance nutritional and sensory properties of food, especially F&V, while preserving their micronutrients.^[6,7] Fermented F&V beverages are mostly developed with *Lactobacillus* starters, specially *Ltp. plantarum* species that demonstrated a short latency in acidic environment and strong acidification capacity.^[8] Applied to pineapple juice, LAF modulates sensory profile depending on the bacterial starter used, leading to either dairy flavors or spicy ones.^[8] Moreover, LAF can increase significantly total phenolic content, radical scavenging activity and reduces hemolysis of blood cells exposed to free radicals.^[8]

The positive effects on health of lactic fermented F&V result from the combination of F&V properties, in particular for their content in fibers and phenolics, and changes of composition due to LAF. They rely on different mechanisms, apart from a direct decrease of sugar content and increased safety due to inhibition of foodborne pathogens by acidity. Bacterial enzymes are able to separate phenolic compounds into monomers to improve their bioavailability and thus, induce beneficial effects.^[9] Hence, a decrease of glycaemia peak can be expected from the inhibition of digestive enzymes,^[10] by plant polyphenols, possibly of modified composition after LAF. In addition, a modulation of gut microbiota can be expected from ingestion of a high population of viable bacteria^[11] but also can result from bioactive metabolites from F&V.^[12] Numerous positive effects of

1
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3 bacterial metabolites are reported, among those lactate and exopolysaccharides, which exert an
4
5 antioxidant effect.^[11]
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8 Improvement of diet-induced metabolic syndrome in mice, as well as enrichment of gut
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10 microbial abundance, has been demonstrated with the consumption of fermented fruit beverages
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12 but using yeast probiotics combined with LAB, not making it possible to highlight the specific effects
13
14 of bacterial strains.^[13] A beneficial effect of the consumption of LAB fermented apple juices was only
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16 showed on streptozocin-induced diabetic mice.^[14,15] In this study, the aim was to determine if
17
18 chemical and microbial composition of fermented pineapple juice was able to induce early
19
20 observable effects on mouse metabolic disorder apparition. To achieve that, mice were fed with a
21
22 high fat diet (HFD) and were provided with different beverages, including water, sweetened water
23
24 and pineapple juice, fermented or not, during a six-week experiment.
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29 **2. Materials and methods**

30 31 **2.1. Microorganisms**

32
33 The bacterial strains used, *Leuconostoc pseudomesenteroides* 56 and *Lactiplantibacillus*
34
35 *plantarum* 75, previously isolated and genotyped, were reactivated then cultured in De Man, Rogosa
36
37 and Sharpe (MRS) broth and incubated for 48 h at 30 °C prior to inoculation of juice.
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42 **2.2. Preparation and fermentation of pineapple juice**

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44
45 Fresh pineapples (*Ananas comosus* var. 'Queen Victoria') were washed, peeled and cut. The
46
47 juice was extracted using Philips MicroMasticating juice extractor, filtered and bottled. Pineapple
48
49 juice was pasteurized in a water bath at 80 °C during 10 min, prior to cooling to room temperature
50
51 within 2 h. The following steps were performed under sterile conditions. From *Lc.*
52
53 *pseudomesenteroides* 56 and *Ltp. plantarum* 75 bacterial cultures, an inoculation level of 0.05
54
55 uOD/mL, corresponding to 1.10^7 bacteria/mL according to McFarland standards, was calculated for
56
57 each strain from turbidity at 600 nm. The adequate volume was collected and centrifuged at 5000 x g
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3 for 5 min at 10 °C. The cell pellet was washed twice and re-suspended in sterile distilled water. Each
4
5 bacterial culture was separately inoculated in pineapple juice. Inoculated and not inoculated juices
6
7 were incubated at 30 °C for 48 h. Then, the pineapple juices fermented with each strain were mixed
8
9 and maintained at 4 °C for 12 days. The level of inoculated LAB in pineapple fermented juice allows
10
11 to be above the amount of 1.10^{6-7} bacteria/mL described as having beneficial effect for probiotics
12
13 according to FDA/WHO.^[1] Following a ½ dilution in sterile water, juices were stored at -20 °C until
14
15 use. Beverages were thawed at 4 °C the day before administration.
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19 **2.3. Fermented pineapple juice characteristics**

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22 LAB enumeration was performed on MRS agar. Briefly, serial decimal dilutions of juices were
23
24 prepared into sterile peptone water solution and dropped on MRS plates before incubation at 30 °C
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26 for 48 h. Yeast and mold counts were performed on glucose chloramphenicol agar plates. Each
27
28 determination was performed in triplicate.
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33 Total soluble solids (TSS) of fermented and not-fermented juices were determined with
34
35 ATAGO PAL-3 pocket refractometer (Atago USA Inc., Washington, USA) and expressed as °Brix (\pm 0.1
36
37 °B).
38

39
40 The pH of fermented and not-fermented juices was determined with an uncertainty of 0.1
41
42 with a Eutech ph2700 apparatus (EUTECH Instruments, Illinois, USA).
43

44 **2.4. α -glucosidase inhibition assay of pineapple juices**

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46
47 The α -glucosidase inhibitory activity was determined according to Zhang et al. ^[16] and was
48
49 assayed with type I α -glucosidase from baker's yeast in a 0.1 M sodium phosphate buffer (pH 6.9). A
50
51 buffer solution containing 1 U/mL α -glucosidase solution was incubated with pineapple juice at 37 °C
52
53 under shaking during 2 min. Inhibition control was performed with 0.5 mg/mL voglibose solution. A 2
54
55 mM *p*-nitrophenol- α -D-glucopyranoside solution (12.5 %) was added as substrate and the
56
57 absorbance was monitored every minute for 20 min using a microplate reader (Infinite M200 PRO,
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59
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1
2
3 Tecan, Männedorf, Switzerland) to follow the released of the product colored compounds. The α -
4 glucosidase specific activity was calculated from the OD at 405 nm slope, divided by the enzyme
5 concentration, expressed as OD/(min.U).
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10 **2.5. Preparation of sweetened water containing LAB**

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13 Sucrose concentration in sweetened water was chosen to be similar to that of pineapple
14 juice, which exhibited 17.4 °Brix, diluted in water. Under sterile conditions, a solution at 87.2 g/L of
15 sucrose was prepared. Inoculation was performed as for pineapple juices using *Lc.*
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pseudomesenteroides 56 and *Ltp. plantarum* 75 bacterial cultures with an inoculation level of
0.05 uOD/mL. Sweetened water, with or without LAB, was stored at -20 °C until use. LAB
enumeration, TSS and pH were carried out as previously described.

2.6. Experimental parameters

2.6.1. Mouse model

Forty-six male C57BL/6J mice were used in this study. Animals were housed in a controlled environment, under constant temperature (21 °C \pm 2 °C), humidity (50 % \pm 5 %) and 12 h light-dark cycle. All experimental procedures were conducted in compliance with animal protocols approved by the French Ministry of Higher Education and Research (project # 019011015097480). After 5 weeks of acclimatization, mice (twelve weeks old and 25.3 g mean weight at the beginning of the experiment, Janvier Labs, Saint Berthevin, France) were randomly divided into one group of six mice (control -C- corresponding to water proposed *ad libitum*) and four groups of ten mice receiving *ad libitum* either sweetened water -SW-, LAB in sweetened water -BSW-, pineapple juice -J- or fermented pineapple juice -FJ-. Mice were housed by 5 apart from control group for which mice were divided into 2 groups of 3, and received food and water *ad libitum*. All groups were fed with a HFD corresponding to a 23.5 % fat semi pure rodent diet (SF04-001, Specialty Feeds, Glen Forrest, Western Australia) during the six-weeks experiment, leading to a pre-obesity state. Food and sugary beverage intakes were determined once-a-week and three times-a-week respectively, from the

1
2
3 difference between proposed and remaining quantities. The amount of HFD consumed also included
4
5 the food losses that may occur when mice consumed the solid diet. Energy intake was then
6
7 estimated per mouse and per day using the energetic value of each food, neglecting bacteria.
8
9 Digestible energy of HFD was 19.0 kJ/g according to the feed manufacturer. The used value for
10
11 pineapple juice was 1.2 kJ/mL and was obtained from the average of the values found in commercial
12
13 pineapple juices from Reunion Island taking into account dilution. For sweetened water, the energy
14
15 value of saccharose was used (1.5 kJ/mL). Total energy intake was calculated by addition of energy
16
17 intake from HFD and beverage consumption.
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21 Before the beginning of the experiment (baseline values) and at several evaluation points,
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23 body weight was measured, and blood samples and feces were collected. During this experiment,
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25 two mice of the SW group died on day 25 and one mouse of the BSW group died on day 29. At the
26
27 end of the experiment, mice were sacrificed by cardiac puncture under general anesthesia with
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29 isoflurane (2 %), and intestine, liver and adipose tissue were collected, weighted and/or measured,
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31 and kept for later analysis at -80 °C.
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34 35 36 **2.6.2. Assessment of blood parameters**

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38 After a 12 h fast, glycaemia was assessed from the tail of mice at the beginning and at the
39
40 end of the experiment using a commercial glucometer (OneTouch Verio Flex meter, OneTouch, USA).
41
42 An oral glucose tolerance test (OGTT) was performed on day 39 of the experiment after a 6 h fast.
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44 Glucose (30 % of body weight) was administered by gavage and blood sugar was measured at 0
45
46 (baseline), 15, 30, 45, 60, 90 and 120 min.
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50 At the beginning and at the end of the experiment, mice were fasted for 4 h and blood
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52 samples were collected by retro-orbital sampling and stored at -80 °C. Then, triglyceride (TG) and
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54 total cholesterol concentrations were determined using Triglycerides FS 5' and Cholesterol FS 10' kits
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56 (DiaSys Diagnostic Systems GmbH, Holzheim, Germany) according to manufacturer's instructions.
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59 60 **2.6.3. Measurement of zonula occludens-1 (ZO-1) in small intestines**

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3 ZO-1 levels were determined using a mouse Tight Junction Protein 1 (TJP 1) ELISA kit (Wuhan
4 Fine Biotech Co., Ltd, Wuhan, China). Briefly, 200 mg of small intestine (ileum) sample was mixed
5 with 500 μ L of PBS 1X buffer containing a protein kinase inhibitor, lysed and centrifuged. The
6 supernatant was collected, and the assay was performed following the manufacturer's instructions.
7
8 Results were normalized using total protein contents measured by bicinchoninic acid assay (BCA).
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10 Briefly, samples were incubated with the BCA reagent at 37 °C for 1 h and absorbance was read at
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2.6.4. Histopathological evaluation

The small intestine was rapidly removed after sacrifice, weighed and divided into duodenum, proximal jejunum, distal jejunum, and ileum (1:1:1:1 ratio) and flushed with cold saline solution. Proximal section of the duodenum was fixed in 4 % paraformaldehyde, embedded in paraffin, and dissected in five-micrometer sections using EpreDia™ HM 340E Electronic Rotary Microtome (ThermoFisher Scientific, Waltham, Massachusetts, USA). Then, the sections were stained with hematoxylin and eosin (H&E). Pictures were obtained using NanoZoomer S60 digital slides scanner (Hamamatsu Photonics, Hamamatsu City, Japan).

2.6.5. Assessment of fecal microbial population

LAB population was determined in the collected feces at the beginning, at half and at the end of the experiment. Serial dilutions of the samples were made in sterile 0.9 % sodium chloride and spread on MRS plates before incubation at 37 °C for 48 h. Each determination was performed in triplicate. Bacterial colonies were subcultured and isolates were preserved in Protect Microorganism Preservation System tubes (Technical Service Consultants Ltd, Lancashire, UK) at -80 °C.

For DNA extraction, InstaGene Matrix (Bio-Rad Laboratories, Marne-la-Coquette, France) was used according to supplier recommendations. Identification was performed on feces collected at D41 by sequencing of the region coding 16S RNA according to Fessard et al. (2017).^[17] The used primers were RD1m (5'-GGMTACCTTACGAYTTC-3') and FD1m (5'-AGAGTTTGATCCTGGCTCAG-3') (Eurogentec,

Seraing, Belgium). The reaction mixture contained 1X buffer, 0.2 mM dNTP, 0.3 μM per primer, 2.5 mM MgCl_2 , 0.05 $\text{U}\cdot\mu\text{L}^{-1}$ *Taq* polymerase (Eurogentec, Seraing, Belgium) and DNA solution. PCR was performed with Bio-Rad S1000 Thermal Cycler (Bio-Rad Laboratories, Marne-la-Coquette, France). PCR products were then separated by electrophoresis (30 min at 100 V) on 0.8 % agarose gel in 0.5X TAE. DNA was stained with ethidium bromide and detected using a UV-illuminator system. PCR product purification was performed using QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). Sequencing was achieved by Eurofins Genomics (Ebersberg, Germany) and the resulting sequences were compared with NCBI Nucleotide database with the BLASTN program. To characterize the genotype of bacterial strains a Rep-PCR method based on $(\text{GTG})_5$ primer (5'-GTGGTGGTGGTGGT-3') (Eurogentec, Seraing, Belgium) was performed according to Fessard et al. (2019).^[18] PCR products were then separated by electrophoresis (3 h at 50 V) on 0.8 % agarose gel in 0.5X TAE.

2.7. Statistics

To quantify the sample size, a power analysis was realized with an α -level of 0.05 and a β -level of 0.1 based on previous study investigating the effect of yeast fermented juice consumption on mice lipid metabolism.^[19] Sample size was adjusted to 10 animals per group for the anticipated animal losses. All values are expressed as mean \pm standard deviation. The statistical analysis was performed using XLSTAT (Addinsoft). ANOVA were performed with the Ryan–Einot–Gabriel–Welsch (REGWQ) test and comparison to control according to Dunnett's test. Differences were considered as significant for indicated p-values.

3. Results

3.1. Pineapple juice properties

Bacterial population increased from 5.7 ± 1.2 log of colony-forming unit (CFU)/mL to 11.8 ± 0.6 log CFU/mL between day 0 and day 7 after inoculation, confirming a proper fermentation of the juice (**Table 1**). On the same period, no yeast or mold were detected thus showing the absence of contamination. The pH values were not significantly modified during fermentation, as expected

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3 since the initial pH of pineapple was already low. TSS content was not significantly reduced by
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5 fermentation.
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8 **3.2. Effect of pineapple juices on a digestive enzyme activity**

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11 Pineapple juice, fermented or not, inhibited α -glucosidase, involved in carbohydrate
12
13 hydrolysis during digestion. The inhibition of the enzyme tended to decrease with dilutions of
14
15 pineapple juice, fermented or not (**Table 1**) (p-value < 0.0001).
16
17

18 **3.3. Drink consumption, energy intake and weight gain of mice**

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21 During the six-week experiment, solid food intake volume and energy were the same
22
23 regardless of the drink administered (**Table S1**). The volume of sugary drink consumption depended
24
25 on the beverage proposed. FJ and J consumptions were lower than SW and BSW consumption
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27 (**Figure 1A**). The drink consumption was stable for J and evolved upward for SW and BSW, or down
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29 for FJ leading to significantly different consumption of SW and BSW to that of FJ after 22 days and to
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31 that of J after 29 days.
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35 Energy intake of drinks represented 5.1 % to 10.7 % of the total energy intake for SW and
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37 BSW, whereas it was in the range 7.6 % - 9.3 % for J and 4.8 % - 7.2 % for FJ (**Figure S1**). The ratio of
38
39 energy provided by drink to food became different between water and other drinks after 11 days (p-
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41 value = 0.008), between SW, BSW plus J and FJ after 32 days (p-value = 0.001) and the difference
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43 increased (p-value = 0.0003) thereafter. However, the groups which consumed sugary drinks showed
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45 a total energy intake similar to that of the control group, because the energy provided by the drink
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47 remained below 11 % of the total energy intake (**Figure 1B**). The weight gain of mice, measured
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49 3-times a week, did not show statistical difference according to the groups receiving different sugary
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51 drinks (**Figure 1C**). The four groups gained more weight than the control group (water), though
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53 slightly significant (p-value = 0.025 after 39 days). Fat epididymal mass of the BSW group increased
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55 significantly compared to the control group, but this was not found for other groups receiving sugary
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57 dinks (**Table S2**). Fat mass of the FJ group was only 10.8 % above that of the control. This result
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3 suggests that weight gain was not primarily directed towards fat weight. Adipose tissue of the
4 control and FJ groups weighted 0.8 ± 0.3 g and 0.9 ± 0.2 g respectively at D41. Conversely, J
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6 consumption increased adipose tissue weight to 1.2 ± 0.4 g although not significant compared to the
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8 control group, and a significant increase of adipose tissue weight was obtained only for the BSW
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10 group compared to the control group. Weight of the liver was higher for all groups compared to the
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12 control.
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17 **3.4. Effect of fermented juice on carbohydrate metabolism**

20 Glycaemia significantly increased after a six-week supplementation of BSW and J, by 23.2 %
21 and 24.7 % respectively compared to the control group (**Figure 2A**). OGTT showed a similar
22 regulation for the FJ group compared to the control group (**Figure 2B**). This regulation appeared to be
23 less efficient for the other groups, especially for BSW and SW, which exhibited higher glycaemic
24 curves. A difference of the maximum of glycaemia reached at 15 min, was observed, with values of
25 23.3 mM for BSW and J, 21.8 mM for SW and 19.5 mM for the control. AUC increased by 45.0 % for
26 the BSW group, 18.6 % for the SW group, and 3.9 % for the J group compared to the control.
27 Although not significant (p -value = 0.154), our results showed a decrease of AUC for FJ group
28 compared to other groups (**Table 2**).
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41 **3.5. Sugary drink consumption effect on lipid metabolism**

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43 After ingestion of an HFD supplemented with sugary drinks, our results showed a TG
44 concentration at the end of the experiment (day 41) not significantly increased for groups
45 supplemented with sugary drinks compared to the control (**Figure 3A**). For an unknown reason,
46 groups C and SW exhibited a large discrepancy of TG content at day -1. This result is due to an intra-
47 group difference that could not be explained. Hence, the use of these values as references should be
48 done cautiously and do not allow us to precisely calculate TG evolution. Sugary drink consumption
49 induced a significant increase of total cholesterol concentration whatever the drink compared to the
50 control group at the end of the six-week supplementation experiment (**Figure 3B**). Indeed, blood
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3 cholesterol concentration ranged between 0.9 g/L and 1.1 g/L for the groups which consumed sugary
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5 drinks, versus 0.7 g/L for the control. A difference of cholesterol concentration between day 1 and
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7 day 41 was observed for SW, BSW and J groups, but not for the control and FJ group.
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10 **3.6. Sugary drink consumption effect on intestinal physiology**

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13 Obesity is correlated with an increased intestinal permeability due to a deficiency in proteins
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15 involved in tight junction formation and function, including the ZO-1 proteins.^[20] As we induced a
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17 pre-obesity state by HFD consumption, ZO-1 concentration was investigated (**Figure 4**). This
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19 parameter did not change regardless of the drink consumed (p-value = 0.076).
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23 Intestine weights were not significantly different between the five studied groups (**Table S2**).
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25 Histopathology of intestinal sections was performed using H&E staining (**Figure 5**). BSW group
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27 histological sections were not exploitable due to improper preparation and were not included in the
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29 analysis. No morphological differences were observed regardless of the sugary drink consumed
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31 compared to the control group. Measurements of the sections showed that crypts depth significantly
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33 increased following FJ consumption compared to SW (**Figure 6**). No significant differences were
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35 obtained regarding villus length and villus length/crypt depth ratio.
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39 **3.7. Evolution of lactic bacteria colonization following sugary drink consumption**

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42 Given that some of the groups of this study received daily LAB supplementation, we looked
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44 at this population size in the feces at the beginning of, at half and at the end of the six-week
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46 experiment (**Figure 7A**). For the control group receiving only a HFD, we observed an increase in LAB
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48 population during the experimentation, as well as for the SW group. However, for the group
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50 receiving LAB alone (BSW), the pineapple juice (J) and LAB and juice together (FJ), LAB population
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52 remained stable. LAB species identification was then performed for C and FJ groups (**Figure 7B**). The
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54 control group had a majority of *Lb. animalis/murinus* (28 identifications out of 43 isolates, i.e.,
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56 65.1 %) whereas the FJ showed a greater abundance of *Enterococcus faecalis* (34 identifications out
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58 of 49 isolates i.e., 69.4 %). *E. faecalis* was isolated on MRS agar: this culture medium is supposed to
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3 be selective to *Lactobacilli*, but the growth of *E. faecalis* was previously reported.^[21–23] Interestingly,
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5 the group receiving the fermented beverage showed an increased frequency of *Ltb. plantarum*
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7 detection compared to the control group (+ 13.1 %). The species *Lc. pseudomesenteroides* was not
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9 detected in any group.
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12 By comparing sequencing results with the profiles obtained by Rep-PCR(GTG)₅, we
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14 hypothesized that the P1 profile corresponded to *Lb. murinus/animalis* isolates (75.6 % in C and
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16 18.4 % in FJ by Rep-PCR), the P2 profile to *E. faecalis* isolates (24.4 % in C and 73.5 % in FJ by
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18 Rep-PCR), the P3 profile to *Ltp. plantarum* isolates (0.0 % in C and 6.1 % in FJ by Rep-PCR), and the P4
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20 profile to *Lb. reuteri* isolates (0.0 % in C and 2.0 % in FJ by Rep-PCR) (**Figure 7C**). The Rep-PCR profiles
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22 of strains 75 and 56 were not found in the analyzed isolates.
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26 Hence, the microbiota composition in mice was modified by fermented pineapple juice
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28 consumption, but microbiota was not colonized by the ingested bacteria.
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35 **4. Discussion**

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37 Extensive studies using models of HFD were conducted on pathobiology of obesity-related
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39 diseases since high energy diet results in obesity and associated metabolic disorders.^[24] Metabolic
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41 disorder symptoms cover intra-abdominal and intra-organ fat deposition, glucose intolerance, insulin
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43 resistance, peripheral inflammation, leptin signaling dysregulation, and increased gastrointestinal
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45 permeability.^[24] Addressing eating behavior by F&V consumption, specially through functional
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47 fermented food, can be an efficient prevention tool.^[6,25] In this study, mice were fed with a high-
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49 caloric diet during a six-week period to induce a pre-obesity state, the onset of metabolic disorders.
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51 No statistical differences were observed regarding food intake, but weight gain was slightly higher for
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53 mice supplemented with sugary drinks instead of water. Tough, the volume of sugary drink
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55 consumed varied depending on the beverage proposed. These variations may be due to a stronger
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57 appetite for sweetened waters suggesting a greater palatability compared to pineapple juices.
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3 Possibly, fibers contained in the juices provided satiety.^[26] Pineapple juices were prepared from both
4 the pulp and the core. Pineapple core is especially rich in dietary fiber: it was previously estimated at
5 64.19 g/100 g of dry weight (d.w.) with two third of insoluble dietary fiber, mainly cellulose and
6 hemicellulose, and one third of soluble dietary fiber.^[27,28] Consumption of high fiber content
7 enhances satiation and satiety. Fibers stimulate the release of gastric juice and satiety hormones.^[29]
8 They delay gastric emptying and gastric distension resulting in slower digestion and absorption in the
9 gastrointestinal tract.^[29–31] Soluble fibers possess a high water-binding capacity, have an important
10 swelling capacity, and stimulate satiety by activating vagal afferents.^[32] They are also more
11 susceptible to fermentation by colonic bacteria, generating short chain fatty acids whose affect
12 appetite regulation.^[33]

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As metabolic disorders linked to HFD lead to impaired carbohydrate and lipid metabolism, blood constants were investigated. Whatever the beverage, glycaemia of mice increased after six weeks of HFD. Compared to the control group having water, BSW and J group glycaemia was higher after 35 days. The FJ and control groups showed a similar regulation of glucose concentration according to the OGTT whereas the other groups showed a tendency to higher glycemic curves. All together these results suggest that FJ consumption, contrarily to other sugary drinks, does not increase glucose regulation disorders. Postprandial carbohydrate absorption depends on the amount of carbohydrate ingested and to their hydrolysis. The inhibition of hydrolytic enzymes can modulate digestion rate and exert an anti-hyperglycemic effect.^[34] Silva et al. (2018) suggested that polyphenols can bind and precipitate digestive enzymes and that polyphenol extract bioactivity depends on the polyphenol composition.^[35] Guaraná digests showed a dose-independent inhibitory activity on α -glucosidase, possibly influencing glucose metabolism and showing an anti-hyperglycemic potential.^[35] The activity of α -glucosidase was inhibited by both J and FJ. This inhibition could induce a slowing-down of carbohydrate digestion and a spreading of the glycemic curve in the postprandial state. It could result from polyphenol composition of the pineapple juice, but the impact of fermentation was not evidenced.^[36] Papaya (*Carica papaya*) purée fermented with

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3 *Lc. pseudomesenteroides* 56 or *Ltp. plantarum* 75 inhibited α -glucosidase activity by 37 % or 17 %,
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5 respectively.^[37] Similarly, fermented mango (*Mangifera indica* L. cv. 'Cogshall') purée was more
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7 inhibitory than the not-fermented one.^[38] The polyphenol composition of pineapple, different from
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9 that of papaya and mango, probably explained a more limited effect on α -glucosidase inhibition of
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11 lactic fermented pineapple.^[39] Probiotics can enhance fasting and postprandial blood glucose,
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13 glucose tolerance and hepatic glycogen synthesis.^[40] Hence, the observed effect of FJ on
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15 carbohydrate regulation could result from the presence of active bacteria.^[40,41] A causal link has
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17 been previously demonstrated between HFD diet and altered glucose regulation with higher AUC
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19 values for OGTT, in the same range as observed here.^[41] Our results are consistent with observations
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21 on diabetic mice receiving Aksu apple juice in addition of HFD diet, which showed a reduced fasting
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23 blood glucose level compared to mice receiving the not-fermented juice and the control group,
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25 although not significant.^[14]

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30 To deepen the study of energy metabolism, lipid variables were investigated through TG
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32 concentration as it is the storage form of carbohydrates and total cholesterol concentration as it is
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34 related to triglyceride transport. These circulating metabolites are dysregulated in case of a
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36 metabolic disorder. C57BL/6 mice fed with HFD did not show any change of TG and total cholesterol
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38 levels compared to mice with chow diet.^[41] TG and cholesterol levels did not change during the
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40 experiment for HFD mice supplemented with water. On the opposite, an increase of circulating lipid
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42 content was observed for mice supplemented with SW, BSW and J. FJ consumption maintained TG
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44 and cholesterol levels and adipose tissue weight. Concomitantly, a decrease in total bacteria
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46 population has been reported for HFD compared to low fat diet.^[42] Consumption of high caloric diets
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48 lead to shifts in the diversity of species belonging to the *Firmicutes* and *Bacteroidetes* phyla, reducing
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50 the microbiota diversity.^[43] Conversely, the administration of noni fruit phenolic extract restored gut
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52 microbiota structure.^[44] FJ consumption did not change LAB population but did change species
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54 composition in feces. Indeed, the control group mainly contained *Lb. animalis/murinus* whereas the
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56 FJ group had a greater abundance of *E. faecalis* and a higher proportion of *Ltb. plantarum*.

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3 These observations can be related to recent findings showing that streptozotocin-induced
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5 diabetic mice receiving fermented apple juice showed lower serum TG and total cholesterol levels
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7 than mice receiving a non-fermented juice, indicating a better blood lipid regulation in diabetic mice
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9 following FJ administration.^[14] Moreover, LAF has a beneficial health effect through microbiota
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11 interactions, promoting gastro-intestinal tract health, improving anti-inflammatory potential, and
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13 controlling oxidative stress.^[11,45] Interestingly, an increased abundance of *E. faecalis* was shown to
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15 promote a cholesterol-lowering effect.^[46] Also, HFD mice exhibited a proportion of *Firmicutes*
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17 reaching 62.56 %, among those *Lactobacillus* abundance increased, compared to the control group
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19 fed with a normal chow.^[14] *Lactobacillus* abundance increased further when fermented apple juice
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21 was consumed, possibly because of LAB added with the fermented juice.^[45]
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26 Intestinal permeability is impaired by metabolic disorders, as shown through a decrease in
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28 tight junction protein concentration or morphological changes of intestinal surface.^[47] HFD
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30 consumption is known to decrease ZO-1 concentration and to reduce the length of intestinal villi and
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32 the depth of intestinal crypts compared to mice fed with a regular diet.^[48-50] Tough it has been
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34 demonstrated that polyphenol extracts increased tight-junction protein concentration and thus,
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36 enhanced intestinal barrier integrity, we did not observed modifications of ZO-1 content by juice
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38 consumption compared to the control group.^[44,48,49] Administration of orange juice-derived
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40 nanovesicles to mice fed with a high-caloric diet resulted in an increase in villi size in mice and thus
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42 an improved absorptive area.^[51] This is consistent with the observation of a difference in crypt depth
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44 between SW and FJ, but morphological changes of the intestine were not significantly different
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46 between groups.
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51 Together our results show that fermented pineapple juice consumption does not increase
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53 metabolic disorders resulting from HFD, contrarily to other sugary drinks. Despite high amounts of
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55 sugar, fruit juices are rich in fibers and phenolic compounds.^[2] In addition, LAF has an impact on
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phenolic composition.^[8] The differences in health indicators observed between FJ and other sugary drink consumed possibly results from a combination of pineapple and LAF positive beneficial effects.

5. Statements

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The authors declare no conflict of interest.

All experiments were carried out in strict compliance with the protocol for the Animal Ethics Committee of CYROI Réunion Island who approved the *in vivo* protocol.

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16 **Authors contributions**

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19 M.G. contributed to the research project, performed the experiments, collected the data, performed
20
21 the statistical analysis and wrote the paper. M.B. supervised and performed the *in vivo* study. C.P.
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23 performed the *in vivo* study. P.R. supervised the *in vivo* study. F.R. supervised the research project,
24
25 performed the statistical analysis and wrote the paper. C.R.-D.S. conceptualized the research project,
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27 supervised the project, contributed to data analysis and wrote the paper. C.G. conceptualized the
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29 research project, supervised the project, contributed to data analysis and wrote the paper.
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3 **Tables**
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6 **Table 1.** Juice (J) and fermented juice (FJ) bacterial population, pH, total soluble solids (TSS) content
7 and inhibition of α -glucosidase activity (%). Inhibition by voglibose 0.5 mg/mL: 63.4 % \pm 1.5 %. Results
8 are presented as Mean \pm SD (CV%). Different letters correspond to significantly different values from
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13 two-way ANOVA, $p < 0.05$.
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	J		FJ	
	Day 0	Day 7	Day 0	Day 7
Bacterial population (log CFU/mL)	0.0 \pm 0.0	0.0 \pm 0.0	5.7 \pm 1.2	11.8 \pm 0.6
pH	3.4	3.4	3.5	3.3
TSS ($^{\circ}$Brix)	16.8	17.0	17.9	17.8
Activity inhibition (%)				
Dilution 1/5	76.0 \pm 5.2 a (21.5)		58.5 \pm 12.1 a (29.1)	
1/10	65.6 \pm 1.9 a (5.6)		49.8 \pm 17.5 a (34.9)	
1/20	60.2 \pm 12.3 a (30.9)		56.9 \pm 10.9 a (25.4)	
1/40	53.2 \pm 6.0 a (12.9)		45.4 \pm 2.0 a (3.6)	
1/80	53.0 \pm 3.3 a (7.0)		42.2 \pm 6.4 a (11.1)	

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Table 2. Values from OGTT after 39 days of experimentation. Control (C) corresponded to mice receiving only HFD and water *ad libitum*. SW: sweetened water, BSW: LAB in sweetened water, J: pineapple juice, FJ: fermented pineapple juice. Different letters correspond to significantly different values from two-way ANOVA, $p < 0.0001$.

Glycaemia (mmol/L) after administration of glucose 30 %				
	15 min	30 min	45 min	AUC (mmol.L ⁻¹ .min ⁻¹)
C	19.6 ± 2.9 b	14.6 ± 3.1 a	11.9 ± 1.1 a	457.5 ± 226.4 a
SW	21.6 ± 3.1 ab	15.6 ± 2.6 a	14.2 ± 1.9 a	542.7 ± 89.6 a
BSW	23.2 ± 2.7 a	17.6 ± 4.1 a	14.5 ± 3.7 a	663.5 ± 250.8 a
J	23.3 ± 2.1 a	16.2 ± 3.7 a	12.7 ± 2.0 a	475.4 ± 275.4 a
FJ	20.1 ± 1.3 b	14.2 ± 2.4 a	13.5 ± 1.4 a	424.2 ± 158.6 a

Figure caption

Figure 1. Evolution of sugary drink consumption (A), energy intake (B), and mice weight (C) during the experimentation. Control (C, (black curve with round symbols) corresponded to mice receiving only HFD and water *ad libitum*. SW: sweetened water (dashed blue curve with inverted triangle symbols), BSW: LAB in sweetened water (blue curve with triangle symbols), J: pineapple juice (dashed orange curve with square symbols), FJ: fermented pineapple juice (orange curve with diamond symbols). Different letters correspond to significantly different values at day 41 from two-way ANOVA, $p < 0.0001$.

Figure 2. Glycaemia at days 2 and 35 (A) and OGTT after 39 days of experimentation (B). Control (C, (black curve with round symbols) corresponded to mice receiving only HFD and water *ad libitum*. SW: sweetened water (dashed blue curve with inverted triangle symbols), BSW: LAB in sweetened water (blue curve with triangle symbols), J: pineapple juice (dashed orange curve with square symbols), FJ: fermented pineapple juice (orange curve with diamond symbols). Different letters correspond to significantly different values from two-way ANOVA, $p < 0.0001$.

Figure 3. Triglyceride (A) and total cholesterol (B) concentrations in blood at day -1 and day 41 for the five groups of mice. Control (C) corresponded to mice receiving only HFD and water *ad libitum*. SW: sweetened water, BSW: LAB in sweetened water, J: pineapple juice, FJ: fermented pineapple juice. Different letters correspond to significantly different values from two-way ANOVA, $p < 0.0001$.

Figure 4. Zonula occludens-1 (ZO-1) concentrations in the small intestine determined by ELISA for the five groups of mice. Control (C) corresponded to mice receiving only HFD and water *ad libitum*. SW: sweetened water, BSW: LAB in sweetened water, J: pineapple juice, FJ: fermented pineapple juice. Different letters correspond to significantly different values from two-way ANOVA, $p < 0.0001$.

Figure 5. Hematoxylin and eosin (H&E) staining for pathohistological evaluation of intestinal permeability. Structure presentation (A), Control (C) corresponded to mice receiving only HFD and

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3 water *ad libitum* (B). SW: sweetened water (C), J: pineapple juice (D), FJ: fermented pineapple juice
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5 (E). Se: serosa, Mu: muscularis externa, Su: submucosa, Lu: lumen, Cr: crypt, Vi: villus.
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8 **Figure 6.** Histological measurements of intestines. Purple bars values refer to the right y axis.

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10 Different letters within a measurement correspond to significantly different values from two-way
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12 ANOVA, $p < 0.05$.
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15 **Figure 7.** Lactic acid bacterium (LAB) population at days 0, 19 and 41 in mouse feces (A); proportion
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17 of bacterial species identified from isolates of control (C) and fermented juice (FJ) mouse group feces
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19 at day 41 (B); Rep-PCR profiles of LAB isolates at day 41 (C). Control (C) corresponded to mice
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21 receiving only HFD and water *ad libitum*. SW: sweetened water, BSW: LAB in sweetened water, J:
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23 pineapple juice, FJ: fermented pineapple juice. Species graphically represented the major ones for
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25 16S rRNA coding region sequencing. Different letters correspond to significantly different values from
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27 two-way ANOVA, $p < 0.0001$.
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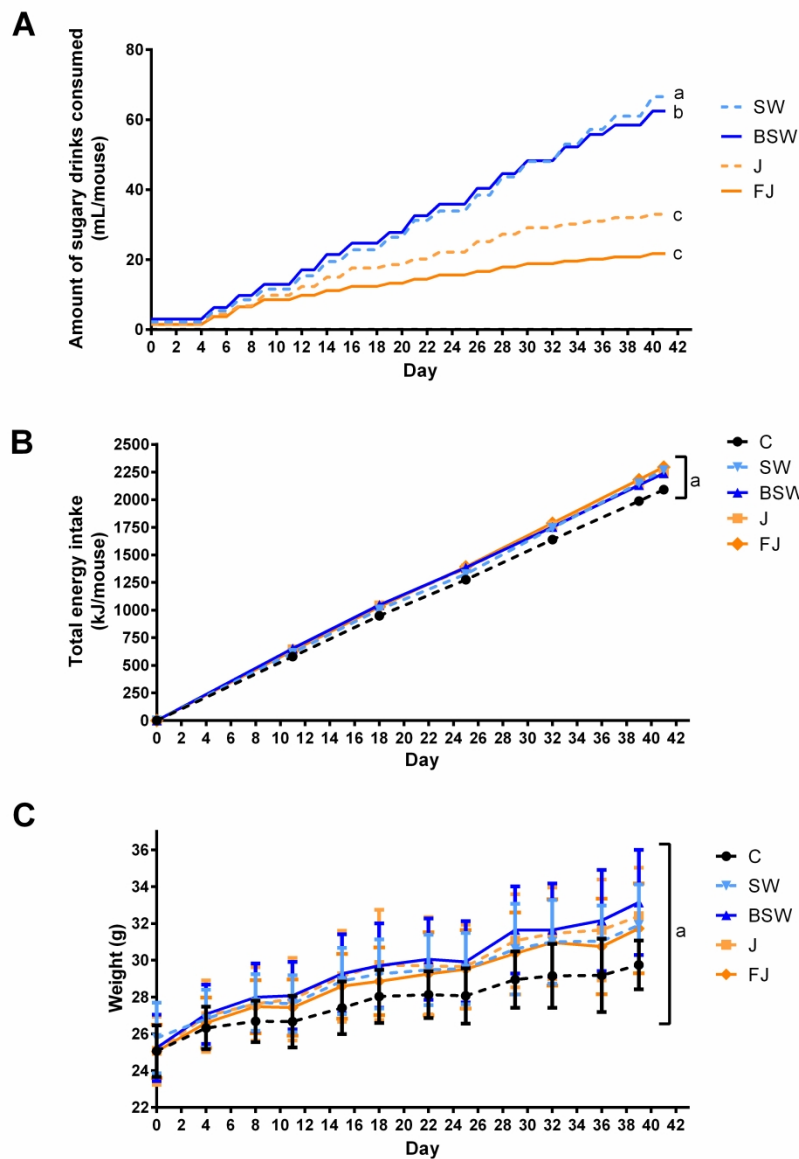


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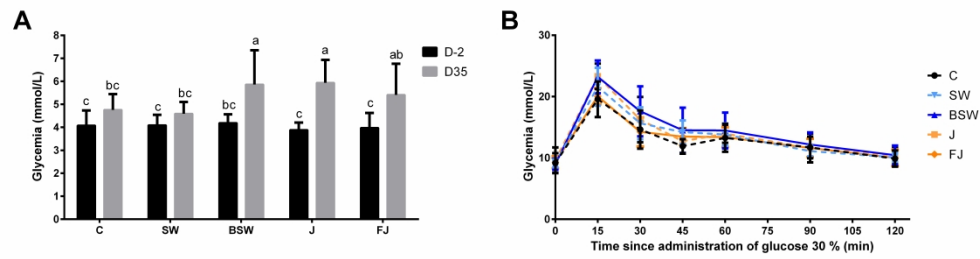


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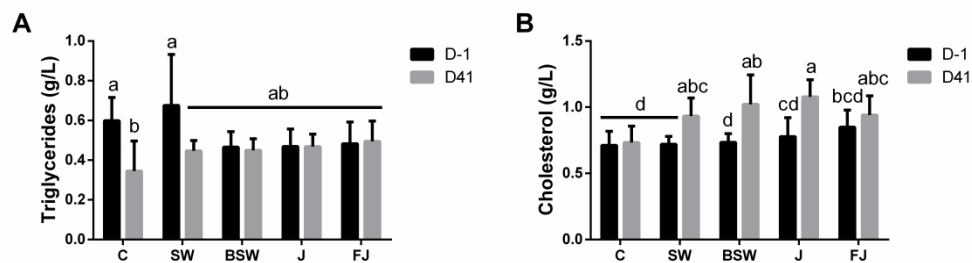


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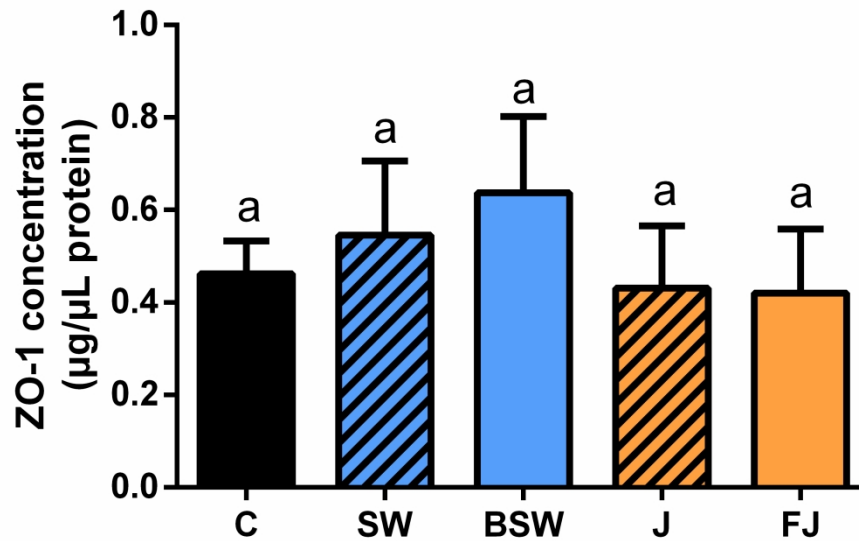


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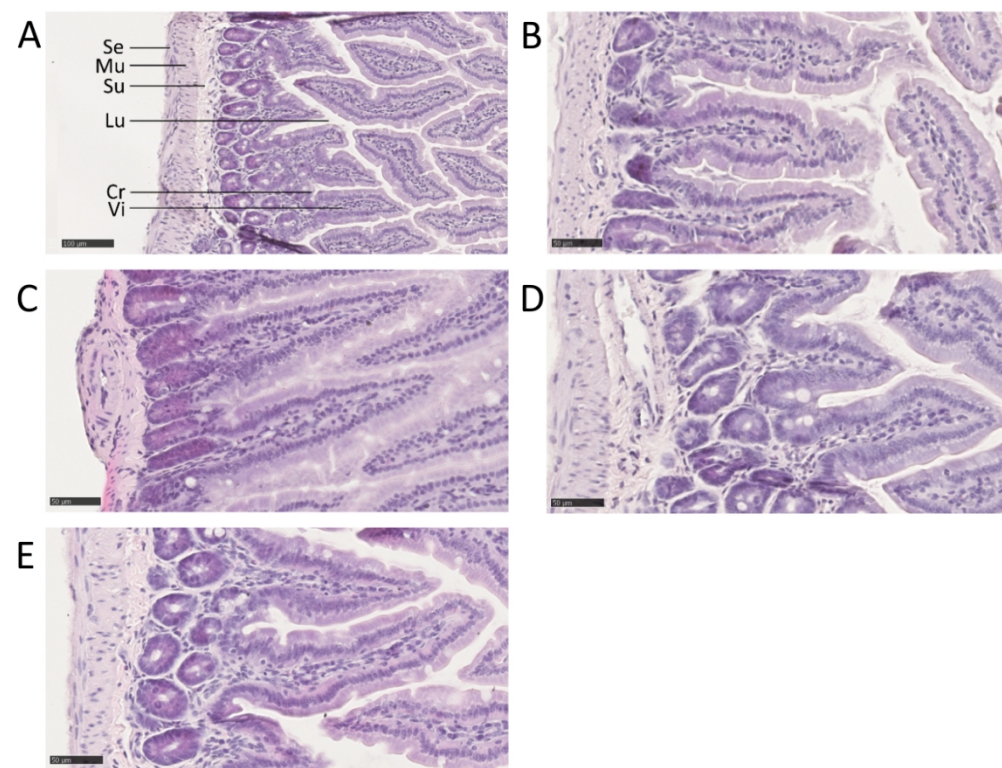


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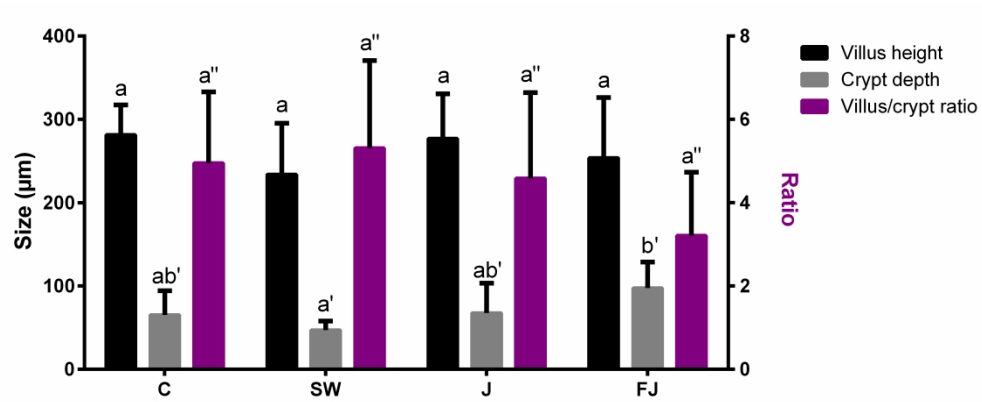


Figure 6. Histological measurements of intestines. Purple bars values refer to the right y axis. Different letters within a measurement correspond to significantly different values from two-way ANOVA, $p < 0.05$.

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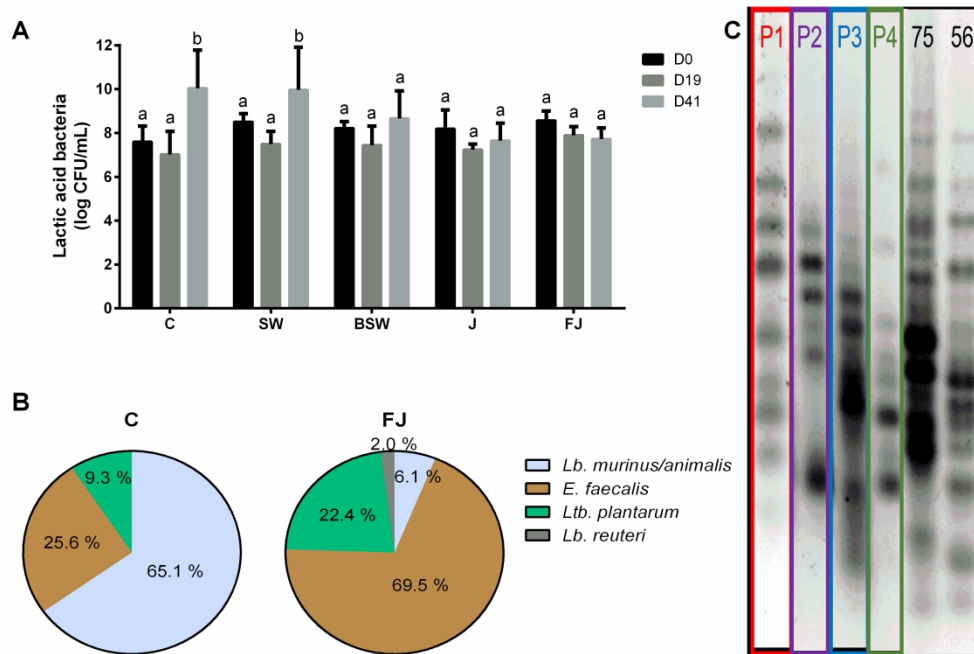
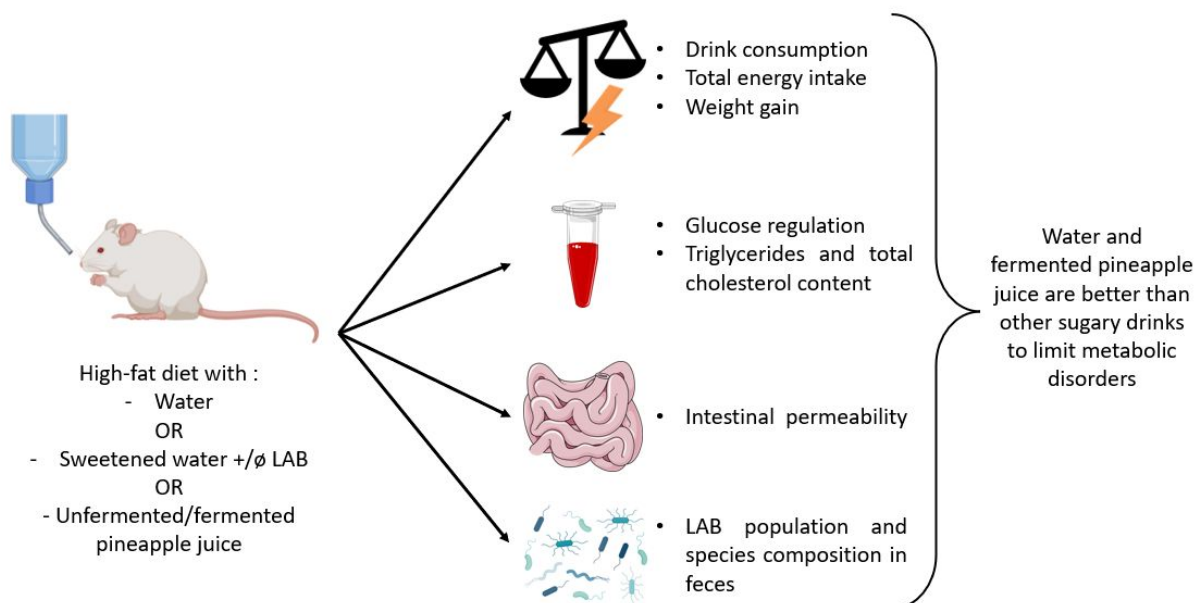


Figure 7. Lactic acid bacterium (LAB) population at days 0, 19 and 41 in mouse feces (A); proportion of bacterial species identified from isolates of control (C) and fermented juice (FJ) mouse group feces at day 41 (B); Rep-PCR profiles of LAB isolates at day 41 (C). Control (C) corresponded to mice receiving only HFD and water ad libitum. SW: sweetened water, BSW: LAB in sweetened water, J: pineapple juice, FJ: fermented pineapple juice. Species graphically represented the major ones for 16S rRNA coding region sequencing. Different letters correspond to significantly different values from two-way ANOVA, $p < 0.0001$.

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The effects of lactic fermented pineapple juice (FJ) supplementation on energetic metabolism of high-fat diet (HFD) fed mice were assessed. The FJ was less detrimental to blood carbohydrate regulation than other sugary drinks and triglyceride and total cholesterol content were not modified. Lactic acid bacterium (LAB) species composition in feces was modified. FJ was preferable to limit HFD-related metabolic disorders.

PARTIE 4 : Co-encapsulation de composés bioactifs issus d'un jus de prune de Natal enrichi avec différents biopolymères et contenant des bactéries lactiques

4.1. Résumé des travaux effectués

Lors du développement de produits fonctionnels contenant des micro-organismes vivants, la résistance aux conditions du tractus gastro-intestinal doit être prise en considération car ils peuvent être soumis à des conditions extrêmes liées à un milieu acide, en particulier dans l'estomac (Guan & Liu, 2020). Des études ont montré l'efficacité de la microencapsulation pour protéger les bactéries probiotiques en utilisant de la gomme arabique, de la maltodextrine ou des protéines. La combinaison de différentes substances permet d'augmenter les bénéfices de la microencapsulation c'est-à-dire la survie des bactéries, les qualités mécanique et morphologique (porosité) des produits alimentaires, et ainsi de développer un produit qui réponde aux attentes (Lotfipour et al., 2012; Rodrigues et al., 2020).

Par ailleurs, les composés alimentaires de type lipides, caroténoïdes et composés phénoliques peuvent aussi être encapsulés après lyophilisation ce qui permet d'obtenir des poudres, et ainsi permettre une stabilité des échantillons lors du stockage, et de prévenir leur détérioration au cours de la digestion (Afzaal et al., 2020; Phromthep & Leenanon, 2017). La microencapsulation présente donc un fort intérêt et permet d'augmenter les bénéfices dus à l'ingestion de composés bioactifs et de bactéries potentiellement probiotiques.

Les objectifs étaient d'étudier les effets de la microencapsulation avec différents biopolymères sur l'efficacité de l'encapsulation, la concentration en anthocyanes et composés phénoliques et sur l'activité antioxydante du jus de prune de Natal inoculé avec la souche *Ltp. plantarum* 75. Les composés phénoliques présents ont ensuite été identifiés et quantifiés. L'effet sur la viabilité cellulaire et sur la bioaccessibilité des composés phénoliques a été évaluée sur les produits encapsulés après une digestion gastro-intestinale *in vitro*.






Les résultats ont montré une meilleure efficacité de la co-encapsulation du jus de prune de Natal avec la maltodextrine, la protéine de pois et le mucilage de psyllium inoculé ou non avec la souche de *Ltp. plantarum*. Cette combinaison a entraîné une augmentation de la bioaccessibilité de la cyanidine 3-sambubioside, l'anthocyane majoritaire de la prune de Natal, et une augmentation des teneurs en acides protocatéchique et férulique après digestion gastro-intestinale des échantillons. La combinaison de ces biopolymères permet de protéger efficacement la souche *Ltp. plantarum* 75, les anthocyanes et les composés phénoliques lors de la simulation *in vitro* de la digestion gastro-intestinale.

4.2. Résultats

Les travaux effectués lors d'une mission à l'université de technologie de Tshwane (Tshwane University of Technology, Pretoria, Afrique du Sud) sont présentés dans un article intitulé « **Antioxidant activities of co-encapsulated Natal plum (*Carissa macrocarpa*) juice inoculated with *Ltp. plantarum* 75 in different biopolymeric matrices after *in vitro* digestion** » publié le 16 juillet 2022 dans *Foods*.

Article

Antioxidant Activities of Co-Encapsulated Natal Plum (*Carissa macrocarpa*) Juice Inoculated with *Ltp. plantarum* 75 in Different Biopolymeric Matrices after In Vitro Digestion

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Abstract: Biopolymeric systems that co-encapsulate probiotics and bioactive compounds ensure timely delivery in the gastrointestinal tract. Cyanidin 3-sambubioside is the dominant anthocyanin in Natal plum (*Carissa macrocarpa*). This study aims at the co-encapsulation of Natal plum (*Carissa macrocarpa*) juice inoculated with *Lactiplantibacillus plantarum* 75 (*Ltp. plantarum* 75) by freeze-drying using pea protein isolate, maltodextrin, and psyllium mucilage and evaluating their release in vitro. An encapsulation efficiency of >85% was noted in lactic acid bacteria (LAB) survival and anthocyanin content. Freeze-drying produced pinkish-red powder, rich in polyphenols and LAB (>6 Log CFU mL⁻¹) after 14 days of storage. Natal plum juice + maltodextrin + pea protein isolate + psyllium mucilage + *Ltp. plantarum* 75 (NMPeaPsyB) showed the highest LAB population (6.74 Log CFU mL⁻¹) with a survival rate of 81.9%. After digestion, NMPeaPsyB and NMPeaPsy had the highest LAB survival (>50%) at 67.5% and 67.5 ± 0.75%, respectively, and the highest bioaccessibility of cyanidin 3-sambubioside in Natal plum juice than the other co-encapsulation with other biopolymers. NM-PeaPsy and NMPeaPsyB showed phenolic stability in the gastric phase and controlled release in the intestinal simulated phase. The antioxidant activities had strong correlations with cyanidin 3-sambubioside. The results confirmed that microencapsulation is important for improving stability and allowing for the development of functional foods.

Keywords: indigenous fruit; anthocyanins; lactic acid bacteria; biopolymers; antioxidant power and scavenging activity; simulated gastrointestinal digestion

1. Introduction

Natal plum (*Carissa macrocarpa*) is a traditional fruit from southern Africa that has become popular due to its polyphenol content. Cyanidin 3-O-sambubioside, cyanidin 3-O-pyranoside, and cyanidin 3-O-glucoside were identified as the major polyphenols in Natal plum fruit extracts [1,2]. Cyanidin derivatives have been linked to various health benefits such as anti-influenza effects [3] and anti-diabetic properties [4]. The antioxidant properties of Natal plum are linked to cyanidin derivatives and other phenolic acids such as coumaric acid, syringic acid, dicaffeoyl tartaric acid, protocatechuic acid, caffeic acid, chlorogenic acid, ellagic acid, ferulic acid, and catechin [2]. The benefits of polyphenols have been found to be dependent not only on their intake but also on their bioaccessibility [5]. In our previous study, the bioaccessibility of cyanidin 3-sambubioside (C-3-S) in Natal

plum extract was reported to be 32.2% [2]. It is therefore critical to increase polyphenol bioaccessibility so that a significant amount of polyphenols reaches the colon, where they are depolymerized into simpler forms and improve body health.

Therefore, to improve the consumers' health, it is crucial to introduce the plant's bioactive compounds and probiotics into their diet. Furthermore, probiotics contribute to improved gastrointestinal health [6]. Combining bioactive compounds and probiotics, through food or supplements, is beneficial for enhancing their nutritional impact [6]. However, incorporating probiotics into foods presents certain concerns, as ingestion of probiotic bacteria-containing foods is ineffective without proper protection against adverse effects [6]. The bioaccessibility of probiotics is affected by high-temperature and high-acidity conditions [7]. Polyphenols and probiotics both require protection, and microencapsulation is one of the most effective ways to encapsulate sensitive chemicals in a coating or wall material. The wall material protects the sensitive compounds from unfavorable responses and controls their release in the gastrointestinal tract [7]. In the encapsulation process, selection of wall materials is important. Various wall materials (maltodextrin, guar gum, gum Arabic, and proteins) have been used to encapsulate polyphenols and probiotic bacteria [7]. Under common storage and gastrointestinal settings, the combination of materials with diverse qualities confers superior protective features and appropriate release characteristics [8].

Maltodextrins are polymers of saccharides that consist of glucose units, primarily linked by α -1,4 glucosidic bonds. Maltodextrin has provided excellent protection to anthocyanin in encapsulated blackcurrant [9] and purple sweet potato [10]. Maltodextrin protects encapsulated food ingredients from oxidation and boosts their stability [11]. Gandia-Herrero et al. [12] successfully used 20% of maltodextrin to encapsulate the bioactive pigment of opuntia fruits. However, it has been reported that maltodextrin has a high glycemic index value of 185–105 and should be consumed sparingly by diabetic and/or hypoglycemic individuals [13]; hence, it could be appropriate to use lower maltodextrin levels in meals and add other low glycemic index matrices during food processing. According to Fang and Bhandari [14], a single encapsulating matrix does not contain all of the needed features; hence, efforts to improve encapsulation properties have been made by combining proteins and polysaccharides. Polymer blends that potentially result in higher encapsulating efficiency and lower cost than individual biopolymers have piqued researcher curiosity.

The combination of diverse biopolymeric matrices also improves the morphological and mechanical qualities, which theoretically can be manipulated to produce food with customized properties [7]. Proteins such as pea protein isolates are also an intriguing wall material because of their low cost and food-grade variety. Psyllium mucilage is a plant-based arabinoxylan biopolymer and can boost the growth of probiotic bacteria in the gut and help with a variety of digestive issues [15]. Psyllium has also been suggested in combination with alginate for co-encapsulating probiotic bacteria, resulting in products with higher encapsulation yields and greater stability than other prebiotics [16]. Furthermore, psyllium enhances the stability of encapsulated components when the pH is lowered, which makes it useful in acidic products such as yogurt [17]. Therefore, it seems promising to co-encapsulate Natal plum juice inoculated with *Lactiplantibacillus plantarum* 75 using a combination of maltodextrin, pea protein and/or psyllium mucilage to improve phenolic bioaccessibility and lactic acid bacteria viability after storage and gastrointestinal digestion.

The purpose of this study was to study the effect of different compositions of biopolymers on the encapsulation efficiency, phenolic and anthocyanin content, and antioxidant activity of Natal plum juice inoculated with *Ltp. plantarum* 75 as well as to characterize and quantify the phenolics present in Natal plum juice and to determine the effects of encapsulating materials on cell viability and phenolic bioaccessibility when exposed to in vitro gastrointestinal conditions.

2. Materials and Methods

2.1. Plant Material and Reagents

Natal plum (*Carissa Macrocarpa*) fruits were harvested at the red stage of maturity between December 2020 and January 2021 (summer) from the Tshwane University of Technology, Pretoria campus (25°43'55.6'' S, 28°09' 52.3'' E) gardens, Pretoria, South Africa. After harvest, fruits were washed using tap water and deseeded and stored at −80 °C until further analysis.

All chemicals were purchased from Sigma Aldrich Co. (Johannesburg, Gauteng, South Africa). The chemical reagents, standards, and solvents used were of analytical grade.

2.2. Preparation of Culture

Ltp plantarum 75 (L75) was obtained from the culture collections held by the University of La Réunion and QualiSud, France. It was isolated from cabbage. *Ltp. plantarum* 75 cells were activated by adding 2 g of *Ltp. plantarum* 75 capsules to 100 mL of sterile MRS broth and incubating at 37 °C for 24 h, following Phromthep and Leenanon [18]. The *Ltp. plantarum* 75 cultures were then centrifuged (HermLe Z326k, HermLe Labortechnik, Wehingen, Germany) at 5000× *g* at 4 °C for 10 min. The pellets were harvested and centrifuged with phosphate buffer saline solution and thereafter suspended into 50 mL of phosphate buffer saline solution.

2.3. Development of Natal Plum Juice

The development of the co-encapsulated Natal plum juice inoculated with *Ltp. plantarum* 75 in different biopolymeric is illustrated in Figure 1 and was developed following a method described by Vanajakshi et al. [19] with some modifications. The Natal plum (100 g) fruit was homogenized using a blender, and 200 mL of water was added. Then, the mixture was homogenized again for 2 min. Ten formulations were obtained by blending the Natal plum juice with maltodextrin, maltodextrin and pea protein isolate, maltodextrin, pea protein isolate and psyllium mucilage and maltodextrin and psyllium mucilage with or without lactic acid bacteria (Table 1). The juice samples were then pasteurized at 70 °C in a water bath for 15 min. Each juice sample contained 200 mL pasteurized Natal plum juice, 200 mL of biopolymer dissolved in water, and 1 mL of lactic bacteria in an isotonic solution. The control juices did not have any biopolymers. The resulting Natal plum juice was then freeze-dried after 48 h of freezing using a Benchtop Freeze Dryer (VirTis Sp Scientific, Model # 2kBTES-55, Gardiner, NY, USA) at −47 to −53 °C. The freeze-dried powders were stored in brown bottles with screw caps and stored at 4 °C.

Table 1. Sample formulations.

	Sample	Code
1.	Natal plum (<i>Carissa macrocarpa</i>) juice	NM
2.	Natal plum (<i>Carissa macrocarpa</i>) juice + <i>Lactiplantibacillus plantarum</i> 75	NMB
3.	Natal plum (<i>Carissa macrocarpa</i>) juice + maltodextrin	NMa
4.	Natal plum (<i>Carissa macrocarpa</i>) juice + maltodextrin + <i>Ltp. plantarum</i> 75	NMaB
5.	Natal plum (<i>Carissa macrocarpa</i>) juice + maltodextrin + pea protein isolate	NMaPea
6.	Natal plum (<i>Carissa macrocarpa</i>) juice + maltodextrin + pea protein isolate + <i>Ltp. plantarum</i> 75	NMaPeaB
7.	Natal plum (<i>Carissa macrocarpa</i>) juice + maltodextrin + psyllium mucilage	NMaPsy
8.	Natal plum (<i>Carissa macrocarpa</i>) juice + maltodextrin + psyllium mucilage + <i>Ltp. plantarum</i> 75	NMaPsyB
9.	Natal plum (<i>Carissa macrocarpa</i>) juice + maltodextrin + pea protein isolate + psyllium mucilage	NMaPeaPsy
10.	Natal plum (<i>Carissa macrocarpa</i>) juice + maltodextrin + pea protein isolate + psyllium mucilage + <i>Ltp. plantarum</i> 75	NMaPeaPsyB

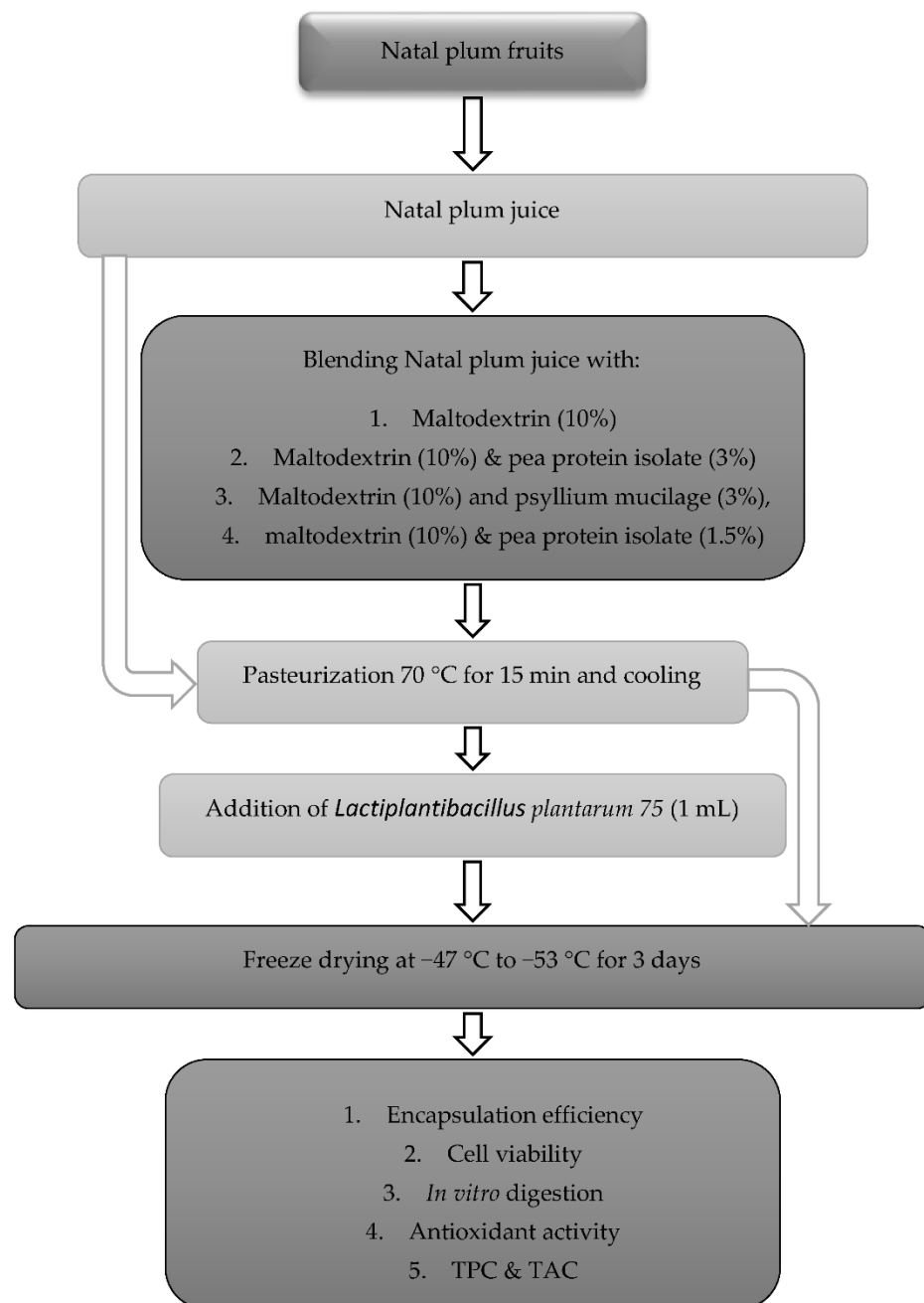


Figure 1. Schematic representation of the experimental design.

2.4. *In Vitro* Digestion of Natal Plum (*Carissa macrocarpa*) Juice Extract

To test the *in vitro* digestibility profile and stability of polyphenols and *Ltp. plantarum* 75 survival in co-microencapsulated powder. The co-microencapsulated powder samples were exposed to simulated gastric juice at pH 2.0 and intestinal juice at pH 7.7, as described by Chen et al. [20]. In addition, an enzyme-containing digestion blank without the material was stored at $-80\text{ }^{\circ}\text{C}$. According to Equation (1), we calculated the percentage release of the *Ltp plantarum* 75 and then the bioaccessibility (what was in the dialysis tube) using the following:

$$\text{Bioaccessibility}\% = \left(\frac{B_{SI}}{B_{ND}} \right) \times 100 \quad (1)$$

B_{SI} (mg kg^{-1}) is the phenolic compound content in the dialysis tube, and B_{ND} (mg kg^{-1}) is the phenolic compound content in the undigested sample.

2.5. Extraction of Phenolic Compounds in Natal Plum (*Carissa macrocarpa*) Juice Powder

Freeze-dried extracts of micro-encapsulated Natal plum juice biopolymer inoculated with or without *Ltp. plantarum* 75 was prepared using a method described by Seke et al. [2], with slight modifications. Each sample (100 mg) was extracted using 38% ethanol (10 mL) and ultrasonicated for 30 min at 40 °C and then centrifuged (Hermle Z326k, Hermle Labortechnik GmbH, Wehingen, Germany) at 1000× g for 30 min at 4 °C. The extracts were subjected to HPLC-DAD analysis for total phenolic content (TPC), total anthocyanin content (TAC), antioxidant capability, and different phenolic compounds.

2.6. Total Phenolic Content (TPC)

TPC of samples was determined using modified Folin–Ciocalteu method [1]. Encapsulated and un-encapsulated Natal plum juice extract (10 µL) was mixed with 100 µL of a 10% Folin–Ciocalteu reagent and 1 mL distilled water. After 8 min, 300 µL of 10% NaHCO₃ was added. The solutions were left to stand for 60 min in a dark room. The blank sample was also prepared, containing 10 µL methanol, 100 µL of 10% Folin–Ciocalteu reagent and 300 µL of 10% NaHCO₃. The absorbance was measured at 765 nm. The TPC was quantified using the gallic acid (GA) as standard, and the calibration curve was constructed. The phenolic content in the micro-encapsulated Natal plum juice extract containing biopolymers inoculated with or without *Ltp. plantarum* 75 was expressed in terms of the gallic acid equivalent mg GAE kg^{−1} DW.

2.7. Total Anthocyanin Content (TAC)

TAC was determined using the pH differential method described by Mendes et al. [21]. Encapsulated and un-encapsulated Natal plum juice extracts (10 µL) were diluted with 920 µL of 0.025 mol.L^{−1} KCl pH 1.0 and 0.4 mol.L^{−1} CH₃CO₂Na pH 4.5 buffers to achieve the same dilution. The absorbance was measured at 510 and 700 nm in both pH = 1.0 and pH = 4.5 buffers, and the results were expressed as mg Cyanidin 3-glucoside (Cyd-3-glu) 100^{−1} g dry weight of powder.

The encapsulation efficiency was calculated using Equation (2), as described by Mendes et al. [21], as follows.

$$EE\% = \frac{\text{Anthocyanin content in microbeads}}{\text{Anthocyanin content in the extract}} \times 100 \quad (2)$$

The lactic acid bacteria encapsulation efficiency was determined using a method described by Colín-Cruz et al. [8] and Maciel et al. [22]. The quantification of the viable bacteria was conducted by the pour plate method. The percentage (%) of the encapsulation efficiency was calculated using Equation (3).

$$EE\% = \frac{N}{N_0} \times 100 \quad (3)$$

where N is the number of viable cells (CFU g^{−1}) in the powder, and N_0 is the number of viable cells in the solution before the freeze-drying.

2.8. Viability of Lactic Acid Bacteria

To determine the viability of *Ltp. plantarum* 75 cells, the unencapsulated and encapsulated Natal plum juice powder was redissolved in sterile water. A 12-fold serial dilution was performed by using sterile peptone water and the pour plate method was adopted based on Güney and Güngörmüşler [23]. The cells were cultivated on the MRS-agar plates after 48 h of aerobic incubation at 37 °C to estimate the number of colony-forming units (CFU). The counts were expressed as CFU mL^{−1} DW.

2.9. Quantification of Targeted Phenolic Metabolites

Natal plum juice samples with the highest survival of *Ltp. plantarum* 75 after storage and after in vitro digestion were selected for phenolic acid characterization quantification, and the unencapsulated Natal plum juice samples were used as controls. The phenolic compounds of interest (anthocyanins and phenolic acids) were quantified by High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD) Model Flexar TM 89173-556 (PerkinElmer, Waltham, Massachusetts, USA), as described by Seke et al. [2]. Extracts from the different Natal plum juices (with and without lactic acid bacteria, before and after digestion) were filtered with PTFE membrane filters, followed by phenolic running on a Waters HSS T3 C18, 2.1100 mm, 1.7 mm column. Pure external standards were used for detection and quantification. Supplementary Table S1 provides the regression equations. Chromatograms were constructed at wavelengths 320 and 520 nm (phenolic acids, flavonoids, and anthocyanins), and the chromatograms are shown in Supplementary Figures S1–S4.

2.10. Determination of the Antioxidant Activity of Phenolic Extracts

The antioxidant activity of the Natal plum juice samples was determined using three different assays, namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) [1], and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay [2] and the Ferric Reducing Antioxidant Potential (FRAP) assay following a method described by Seke et al. [24]. The Natal plum juice samples with the best *Ltp. plantarum* 75 survival during storage and after in vitro digestion were selected for antioxidant activity assays compared to the unencapsulated Natal plum juice samples. DPPH was measured by taking the absorbance measurement of the extracts at 515 nm, and the results were represented as the antioxidant concentration required to reduce DPPH absorbance by 50% (IC₅₀). The decrease in absorbance at 734 nm at a concentration of 40 µL of the sample (ABTS+) was measured using a multiplate reader (EZ Read 2000; Biocrom Ltd., Cambridge, UK) to calculate the IC₅₀. The antioxidant capacity (FRAP assay) was expressed as Trolox-equivalent antioxidant capacity (mM TEAC g⁻¹).

2.11. Statistical Analysis

Data in this study were expressed as mean ± standard deviation of at least three independent replicates. The significant differences were determined at $p < 0.05$ using an analysis of variance (ANOVA) and Tukey's multiple range test. The nonlinear regression was used to calculate dose–response inhibition of DPPH, ABTS and FRAP scavenging activities. A Pearson's correlation analysis was performed on phenolic content, individual phenolic compounds, and antioxidant activities. Minitab Statistical Software for Windows (Minitab Statistical Software 20.3, Coventry, UK) was used to analyze the data.

3. Results and Discussion

3.1. Encapsulation Efficiency of *Ltp. plantarum* 75 and Anthocyanin in Natal Plum (*Carissa macrocarpa*) Juices-Biopolymer Powder

The encapsulation process allowed for a dark pink powder to be formed, with the encapsulation efficiency of anthocyanin ranging between 85% to 96%. The lowest encapsulation efficiency (EE) of 85% was observed for the Natal plum juice sample with maltodextrin (NMa). The addition of pea protein and psyllium mucilage to the Natal plum juice resulted in an increase in efficiency to 96% (Figure 2). The structures of the biopolymers added to the juice samples might have contributed to the higher efficiency of NmaPeaPsy (96%) and NmaPeaPsyB (95%). Covalently linked protein molecules to the carbohydrate chain act as good film-formers, which improve the encapsulation of the molecule and increase the stability of anthocyanins by making the flavylium cation less vulnerable to nucleophilic attack by water molecules [25]. A further possibility is that the complex nature created by the anthocyanins' flavylium cation in interaction with dextrin prevented the anthocyanins from transforming into a less stable form. Similar encapsulation efficiency ranges of 78.61%

and 92.98% have been reported for chokeberry fruit extracts encapsulated in maltodextrin, guar gum, gum Arabic, pectin, beta-glucan and inulin [26].

The lactic acid bacteria encapsulation efficiency of different encapsulated Natal plum juice samples is shown in Figure 2. The highest encapsulation efficiency (EE%) was observed for the Natal plum juice sample with a combination of maltodextrin, pea protein isolate and psyllium mucilage (NMaPeaPsyB) 95%. Natal plum juice containing maltodextrin (NMaB) had the lowest content (88%). Similar results were published by Colín-Cruz et al. [7], and the encapsulation efficiency of lactic acid bacteria was found to be 93.3% in whey protein concentrate and 89.4% in gum Arabic. Similar results have been reported by Colín-Cruz et al. [7], showing the encapsulation efficiency of lactic acid bacteria to be 93.3% in whey protein concentrate and 89.4% in gum Arabic.

Moreover, Bosnea, et al. [27] microencapsulated two different bacterial strains of *Lactobacillus paracasei* and *Lactobacillus paraplantarum* using complicated coacervation with whey protein isolate and gum Arabic and reported an encapsulation efficiency greater than 94.3%. Based on these findings, the combined use of maltodextrin, pea protein isolate, and psyllium mucilage as wall material could help deliver high-percent preservation and release of *Ltp. plantarum* 75.

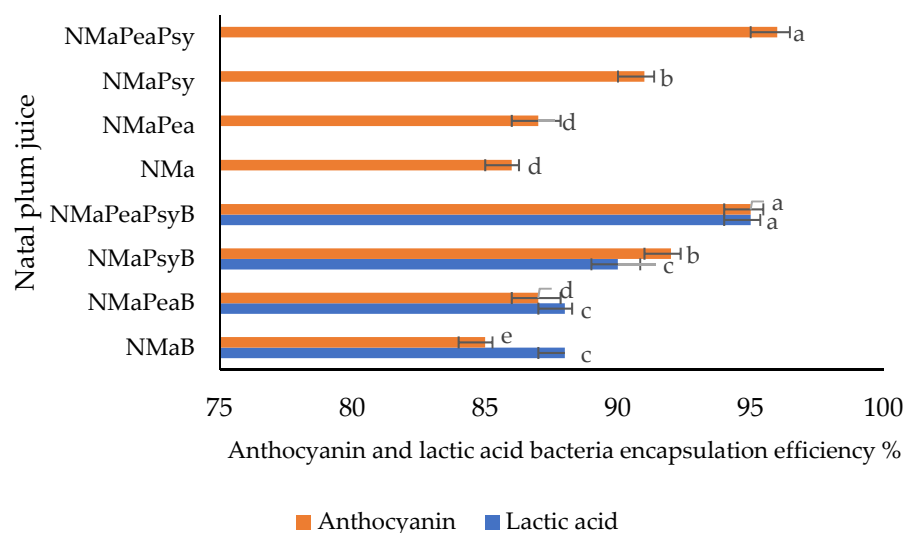


Figure 2. Lactic acid bacteria and anthocyanin encapsulation efficiency of co-encapsulated Natal plum (*Carissa macrocarpa*) juice. Error bars show the standard deviation value; bars with different letter are significantly different $p < 0.05$. Key: NMa: Natal plum (*Carissa macrocarpa*) juice + maltodextrin; NMaB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + *Ltp. plantarum* 75; NMaPea: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + pea protein isolate; NMaPeaB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + pea protein isolate + *Ltp. plantarum* 75; NMaPsy: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + psyllium mucilage; NMaPsyB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + psyllium mucilage + *Ltp. plantarum* 75; NMaPeaPsy: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + pea protein isolate + psyllium mucilage; NMaPeaPsyB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + pea protein isolate + psyllium mucilage + *Ltp. plantarum* 75.

3.2. Effect of Storage Time on the Survival of *Ltp. plantarum* 75 in Natal Plum (*Carissa macrocarpa*) Juices

Table 2 shows the surviving population of *Ltp. plantarum* 75 encapsulated and non-encapsulated Natal plum juice biopolymer matrices upon storage. Storage duration and encapsulation had a significant effect on the survival of *Ltp. plantarum* 75 ($p < 0.05$). After 14 days of storage, the final survival counts for NMB, NMaB, NMaPeaB, NMaPsyB, and NMaPeaPeaB samples were 3.48, 4.08, 4.32, 5.33 and 6.74 log CFU mL⁻¹, respectively. In all encapsulated samples, survival was higher than 50% until 14 days of storage than the control (NMB 48%). The low sugar content in NM with the biopolymer could preserve

the LAB. The present findings indicate that a synergistic effect is operating, whereby maltodextrin protects the pea protein and psyllium mucilage microencapsulants from fragmentation during storage while the pea protein and psyllium mucilage matrix protect the bacteria from membrane damage by maltodextrin. Ding and Shah [28] compared the survival rates of free and microencapsulated bacteria in orange and apple juices, demonstrating that encapsulation effectively protects against the acidic nature of these juices. Studies have shown that psyllium coating improves the viability of *Lb. acidophilus* during storage [29]. Accordingly, microencapsulation provides a suitable environment and a physical barrier to probiotic bacteria [28]. The findings of the present study also reviewed that after 14 days of storage, NMaPeaPeaB had a bacteria population $> 1 \times 10^6$ CFU mL⁻¹, which was above the standard concentration expected for LABs in the gut, which could suggest its functions as a probiotic. Since in this study no fermentation was performed, we assume that polyphenols had no direct effect on the survival of lactic acid bacteria. These results demonstrate that the bacterial culture used survived in the Natal plum juice supplemented with different biopolymers, with a greater survival rate for the NMaPeaPsyB sample.

Table 2. Survival of the bacterial growth and survival percentage in Natal plum (*Carissa macrocarpa*) juices over a 14-day storage period.

Sample	Day 0 Log CFU mL ⁻¹	Day 7 Log CFU mL ⁻¹	Day 14 Log CFU mL ⁻¹
NMB	7.25 ^a ± 0.21	4.81 ^b ± 0.12 (66.34%)	3.48 ^c ± 0.78 (48%)
NMaB	7.29 ^a ± 0.46	5.37 ^b ± 0.82 (73.66%)	4.08 ^b ± 0.23 (55.96%)
NMaPeaB	7.79 ^a ± 0.3	5.78 ^b ± 0.73 (74.19%)	4.32 ^b ± 0.69 (55.45%)
NMaPsyB	7.85 ^a ± 0.83	6.12 ^b ± 0.36 (77.96%)	5.33 ^b ± 0.52 (67.89%)
NMaPeaPsyB	8.92 ^a ± 0.74	7.16 ^b ± 0.87 (80.3%)	6.74 ^b ± 0.58 (75.5%)

Means followed by the same letter ± standard deviation within the row is not significantly different at $p < 0.05$. The numbers in brackets indicate the survival of microorganisms in percentages. Key: NMB: Natal plum juice + *Ltp. plantarum* 75; NMaB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + *Ltp. plantarum* 75; NMaPeaB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + pea protein isolate + *Ltp. plantarum* 75; NMaPsyB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + psyllium mucilage + *Ltp. plantarum* 75; NMaPeaPsyB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + pea protein isolate + psyllium mucilage + *Ltp. plantarum* 75.

3.3. Effect of In Vitro Digestion on the Survival of *Ltp. plantarum* 75 from Natal Plum Juices

The different Natal plum juice samples inoculated with *Ltp. plantarum* 75 were subjected to simulated gastrointestinal digestion, and the release rate was evaluated (Figure 3). The highest rate of release of *Ltp. plantarum* 75 from NMB during the gastric digestion phase (SGF) and intestinal digestion phase (SIF) was 5.76% and 80.65%, respectively.

NMaPeaPsyB, which was the best performing sample, released less than 50% of *Ltp. plantarum* 75 at the intestinal digestion. Co-encapsulation allowed for *Ltp. plantarum* 75 to be bioaccessible after intestinal digestion. The polysaccharides in the maltodextrin–pea protein isolate–psyllium matrix might have formed conjugates that prevented some soluble polysaccharides from dissociating in the GI tract, thus protecting *Ltp. plantarum* 75.

Huang et al. [30] reported that the buffering capacity of protein isolates such as pea protein isolate, and polysaccharides protect probiotic microorganisms from digestive stress. It is also possible that the physical–chemical characteristics of Natal plum juices delayed the release of probiotics, possibly because bioactive compounds interact with the polymers that form the capsules. To fully experience prebiotic and probiotic effects, the bacteria and polyphenols need to be protected so that they reach the colony where they will influence each other [31]. Our results indicated that co-encapsulation of prebiotics and probiotics with biopolymers enhanced the survival of *Ltp. plantarum* 75 during gastrointestinal digestion.

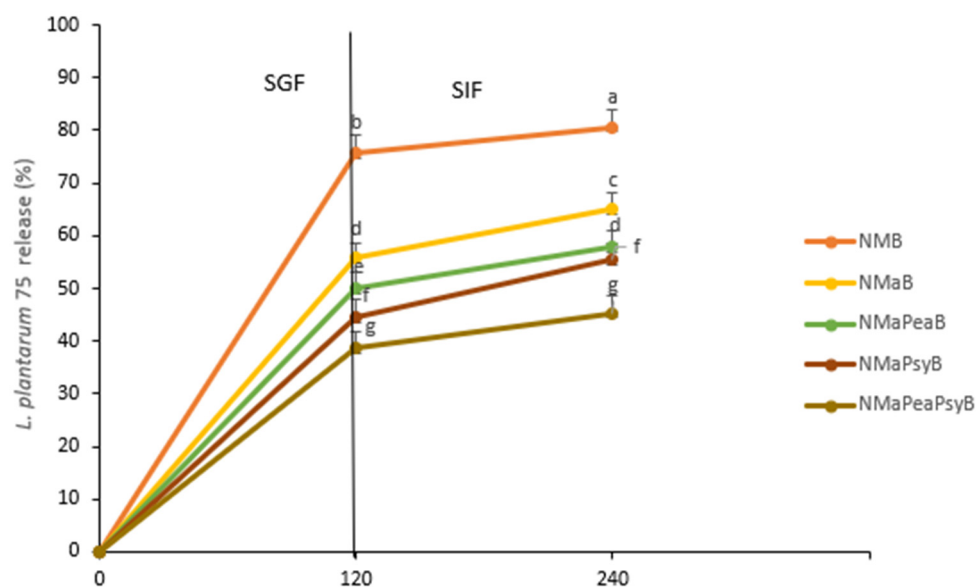


Figure 3. Release of *Ltp. plantarum* 75 in simulated gastric fluid (SGF) for 120 min, sequentially added to the simulated intestinal fluid (SIF) until complete 240 min of the assay, expressed as percentages (%), considering the initial number of viable cells in Natal plum (*Carissa macrocarpa*) juice. Error bars show the standard deviation value; bars with different letters are significantly different $p < 0.05$. Key: NMaB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + *Ltp. plantarum* 75; NMaPeaB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + pea protein isolate + *Ltp. plantarum* 75; NMaPsyB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + psyllium mucilage + *Ltp. plantarum* 75; NMaPeaPsyB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + pea protein isolate + psyllium mucilage + *Ltp. plantarum* 75.

3.4. Effect of In Vitro Digestion on the Total Phenolic Content (TPC) and Total Anthocyanin Content (TAC) from Natal Plum (*Carissa macrocarpa*) Juice

Table 3 shows the TPC and TAC of Natal plum juice-biopolymer extract. The highest TPC (859.52 ± 3.76 mg GAE kg^{-1} DW) was observed in NMaPeaPsy. The lowest TPC was found in NMB (836.53 ± 2.98 mg GAE kg^{-1} DW) and NM (838.38 ± 4.89) with no significant difference between them. The highest TAC was observed for NMaPeaPsy (54.32 ± 0.56 mg Cy-Glu 100^{-1} g DW) and NMaPeaPsyB (53.23 ± 0.68 mg Cy-Glu 100^{-1} g DW) while the least was observed for NM (40.27 ± 0.27 mg Cy-Glu 100^{-1} g DW) and NMB (40.03 ± 0.79 mg Cy-Glu 100^{-1} g DW).

After exposure to gastrointestinal conditions, both TPC and TAC reduced in all the samples. Natal plum encapsulated with different biopolymers showed bioaccessibility of TPC ranging from 37% to 55%. Of all biopolymer encapsulations with Natal plum, NM showed the least bioaccessible percentage (37%), while NMaPeaPsy had the highest of 55%. The bioaccessibility of TAC increased from 43% (NM) to 62% (NMaPeaPsy) after intestinal digestion. Therefore, co-encapsulation improved the bioaccessibility of TAC after exposure to gastrointestinal digestion. The findings of this study are consistent with those of Flores et al. [32], who investigated the in vitro release of encapsulated blueberry extracts and found a higher bioaccessibility of total phenol content (68%) after intestinal digestion. Saikia et al. [33] also found that the release of phenolic compounds from *Averrhoa carambola* pomace microencapsulated with maltodextrin by spray and freeze-drying was higher in the gastric-simulated medium (pH 2.0) than in simulated intestinal medium in both procedures (pH 7.7).

The microencapsulate behavior in simulated gastrointestinal media is influenced by the type and properties of the encapsulating covering material, as well as their tolerance or resistance to digestive enzymes and gastrointestinal circumstances such as pH range [34]. Polysaccharides such as psyllium mucilage have been shown to have numerous hydroxyl

groups (OH-) in their chemical structure, which improves the hydrophilic aspect of the molecule. The presence of hydroxyl groups in Natal plum juice biopolymer samples probably allowed for the creation of hydrogen bonds with water. In this way, hydroxyl groups help to produce the coating matrix by promoting contact between the wall material and the encapsulated compounds while also protecting the encapsulated substance [7]. Our results showed that encapsulated phenolic compounds are protected from the effect of changes in pH during digestion. The results of this study showed the significant potential of using a combination of maltodextrin, pea protein isolate and psyllium mucilage to encapsulate polyphenols and anthocyanins, allowing for targeted release.

Table 3. An evaluation of the effect of simulated in vitro digestion on the total phenolic, total anthocyanin content of Natal plum (*Carissa macrocarpa*) juices.

Total Phenolic Content (mg GAE kg ⁻¹ DW)	Undigested	Gastric Digested	Intestinal Digested	Bioaccessibility (%)
NM	838.38 ^a ± 4.89	545.36 ^b ± 2.48	310.24 ^c ± 2.74	37.00 ^d ± 0.47
NMB	836.53 ^a ± 2.98	555.55 ^b ± 3.31	311.17 ^c ± 1.29	37.19 ^d ± 1.23
NMa	840.46 ^a ± 4.46	639.61 ^b ± 2.19	417.47 ^c ± 2.19	49.67 ^c ± 0.75
NMaB	840.83 ^a ± 2.27	635.82 ^b ± 3.13	416.73 ^c ± 1.25	49.56 ^c ± 0.92
NMaPea	847.19 ^a ± 5.92	675.01 ^b ± 1.28	428.18 ^c ± 3.93	50.00 ^c ± 0.28
NMaPeaB	845.69 ^a ± 4.37	671.96 ^b ± 3.79	429.09 ^c ± 2.20	52.60 ^b ± 0.74
NMaPsy	847.25 ^a ± 5.36	688.75 ^b ± 1.75	432.71 ^c ± 3.15	50.00 ^c ± 0.93
NMaPsyB	845.88 ^a ± 4.28	681.96 ^b ± 3.38	431.39 ^c ± 2.17	52.60 ^b ± 0.84
NMaPeaPsy	859.52 ^{a±} 3.76	788.63 ^b ± 2.97	472.71 ^c ± 2.02	55.20 ^a ± 0.83
NMaPeaPsyB	858.73 ^{a±} 3.82	781.06 ^b ± 1.79	473.39 ^c ± 1.67	55.00 ^a ± 0.96
	Total anthocyanin content (mg Cy-Glu 100 ⁻¹ g DW)			
NM	40.27 ^a ± 0.27	39.29 ^b ± 0.37	17.54 ^c ± 0.28	43.55 ^d ± 0.78
NMB	40.03 ^a ± 0.79	39.93 ^b ± 0.53	17.38 ^c ± 0.30	43.41 ^d ± 0.34
NMa	44.27 ^a ± 0.34	39.23 ^b ± 0.07	24.27 ^c ± 0.75	54.82 ^c ± 0.88
NMaB	44.03 ^a ± 0.53	39.46 ^b ± 0.18	23.88 ^c ± 0.83	54.23 ^c ± 0.34
NMaPea	45.98 ^a ± 0.82	36.49 ^b ± 0.35	25.29 ^c ± 0.71	55.00 ^c ± 0.64
NMaPeaB	45.79 ^a ± 0.64	36.38 ^b ± 0.72	25.09 ^c ± 0.45	54.79 ^c ± 0.73
NMaPsy	46.86 ^a ± 0.84	36.02 ^b ± 0.86	27.29 ^c ± 0.21	58.23 ^b ± 0.58
NMaPsyB	46.73 ^a ± 0.37	36.78 ^b ± 0.42	26.69 ^c ± 0.89	57.11 ^b ± 0.39
NMaPeaPsy	54.32 ^a ± 0.56	36.75 ^b ± 0.38	32.79 ^c ± 0.41	60.36 ^a ± 0.82
NMaPeaPsyB	53.23 ^a ± 0.68	36.61 ^b ± 0.65	33.01 ^c ± 0.23	62.00 ^a ± 0.71

Means followed by the same letter ± standard deviation within the row are not significantly different at $p < 0.05$. Means followed by the same letter ± standard deviation within the bioaccessibility column are not significantly different at $p < 0.05$. Key: NM: Natal plum (*Carissa macrocarpa*) juice; NMB: Natal plum (*Carissa macrocarpa*) juice + *Ltp. plantarum* 75; NMa: Natal plum (*Carissa macrocarpa*) juice + maltodextrin; NMaB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + *Ltp. plantarum* 75; NMaPea: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + pea protein isolate; NMaPeaB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + pea protein isolate + *Ltp. plantarum* 75; NMaPsy: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + psyllium mucilage; NMaPsyB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + psyllium mucilage + *Ltp. plantarum* 75; NMaPeaPsy: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + pea protein isolate + psyllium mucilage; NMaPeaPsyB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + pea protein isolate + psyllium mucilage + *Ltp. plantarum* 75.

3.5. In Vitro Digestibility of Natal Plum (*Carissa macrocarpa*) Juice Polyphenols

The cyanidin 3-O-sambubioside (C-3-S) was the predominant anthocyanin in nondigested encapsulated and unencapsulated Natal plum juice extract. It reached the highest content (484 ± 4.23 mg kg⁻¹) with the encapsulated NMaPeaPsy while the least amount of content (464.5 ± 2.79 mg kg⁻¹) was found in the unencapsulated (NMB) (Table 4). Our previous study [24] showed that the bioaccessibility of a C-3-S from the Natal plum extract was 32.2%, and the encapsulation process improved the bioaccessibility of C-3-S in this study. The increase in protocatechuic acid and ferulic acid in the intestinal fraction corresponded with the breakdown of C-3-S, resulting in an increase in their bioaccessibility.

Table 4. Changes in phenolic components in Natal plum (*Carissa macrocarpa*) juice samples during gastrointestinal digestion for selected samples on a dry weight basis.

Phenolic Components (mg kg ⁻¹)	Undigested	Gastric	Intestinal	Bioaccessibility%
NM				
Cyanidin 3-sambubioside	467.00 ^a ± 3.87	329.40 ^b ± 1.00	196.54 ^c ± 2.71	42.00 ^l ± 0.58
Protocatechuic acid	32.30 ^a ± 1.03	16.01 ^c ± 0.63	19.92 ^b ± 0.87	61.50 ^f ± 0.62
Chlorogenic acid	47.99 ^a ± 0.29	22.27 ^b ± 0.29	15.38 ^c ± 0.83	32.10 ^m ± 0.23
Catechin	29.10 ^a ± 1.92	10.10 ^b ± 1.03	14.39 ^c ± 0.44	49.50 ^j ± 0.89
Quercetin-3-glucoside	53.37 ^a ± 0.21	38.19 ^b ± 0.82	24.01 ^c ± 0.39	44.00 ^k ± 0.49
Ferulic acid	27.99 ^a ± 0.79	15.09 ^c ± 0.22	19.28 ^b ± 0.87	68.80 ^b ± 0.75
Caffeic acid	25.75 ^a ± 1.28	19.05 ^b ± 0.68	12.65 ^c ± 0.79	49.10 ^h ± 0.49
p-Coumaric	28.07 ^a ± 0.49	12.40 ^b ± 0.65	17.28 ^c ± 0.18	61.60 ^f ± 0.28
Syringic Acid	15.40 ^a ± 4.27	7.23 ^b ± 1.80	9.47 ^c ± 1.03	61.50 ^f ± 0.68
Ellagic Acid	43.35 ^a ± 0.20	27.19 ^b ± 0.02	13.03 ^c ± 0.87	30.10 ^m ± 0.49
Dicaffeoylquinic acid	74.45 ^a ± 0.40	47.16 ^b ± 0.92	29.39 ^c ± 0.22	39.47 ^l ± 0.83
NMB				
Cyanidin 3-sambubioside	464.50 ^a ± 2.79	320.02 ^b ± 1.87	185.54 ^c ± 2.21	40.00 ^l ± 0.61
Protocatechuic acid	32.98 ^a ± 0.13	16.26 ^c ± 0.93	21.63 ^b ± 0.55	65.58 ^e ± 0.72
Chlorogenic acid	46.43 ^a ± 0.75	22.01 ^b ± 0.03	17.89 ^c ± 0.32	38.53 ^l ± 0.33
Quercetin-3-glucoside	54.27 ^a ± 0.38	38.39 ^b ± 0.21	22.01 ^c ± 0.19	40.50 ^l ± 0.58
Catechin	27.91 ^a ± 0.84	13.10 ^b ± 0.46	15.89 ^c ± 0.28	56.90 ^h ± 0.70
Ferulic acid	26.37 ^a ± 0.21	14.49.19 ^c ± 0.82	20.71 ^b ± 0.39	78.54 ^a ± 0.29
Caffeic acid	24.19 ^a ± 1.28	18.73 ^b ± 0.40	10.05 ^c ± 0.91	41.50 ^l ± 0.63
p-Coumaric acid	27.34 ^a ± 0.19	13.84 ^b ± 0.25	16.09 ^c ± 0.73	58.86 ^h ± 0.57
Syringic Acid	14.36 ^a ± 2.73	6.93 ^b ± 1.04	8.52 ^c ± 1.48	59.34 ^h ± 0.26
Ellagic Acid	42.73 ^a ± 0.72	25.22 ^b ± 0.23	12.74 ^c ± 0.59	29.80 ^m ± 0.38
Dicaffeoylquinic acid	72.28 ^a ± 0.39	47.23 ^b ± 0.62	27.09 ^c ± 0.53	37.47 ^l ± 0.49
NMaPeapsy				
Cyanidin 3-sambubioside	484.00 ^a ± 4.23	459.40 ^b ± 3.28	326.54 ^c ± 2.76	67.50 ^d ± 0.75
Protocatechuic acid	46.69 ^a ± 1.28	23.01 ^c ± 0.63	33.92 ^b ± 0.18	72.64 ^c ± 0.69
Chlorogenic acid	55.22 ^a ± 0.21	30.48 ^b ± 0.61	35.30 ^c ± 0.23	63.92 ^f ± 0.15
Catechin	32.89 ^a ± 1.03	16.10 ^b ± 0.92	20.31 ^c ± 0.68	61.70 ^f ± 0.49
Ferulic acid	28.75 ^a ± 0.13	19.09 ^c ± 0.41	24.28 ^b ± 0.87	84.40 ^a ± 0.51
Caffeic acid	46.47 ^a ± 1.70	35.04 ^b ± 0.83	27.81 ^c ± 0.92	59.80 ^g ± 0.27
Quercetin-3-glucoside	65.27 ^a ± 0.32	49.91 ^b ± 0.74	32.69 ^c ± 0.15	50.00 ⁱ ± 0.78
p-Coumaric	32.56 ^a ± 1.49	16.40 ^b ± 0.92	23.28 ^c ± 0.72	71.48 ^c ± 0.43
Syringic Acid	24.28 ^a ± 2.77	19.20 ^b ± 1.80	10.47 ^c ± 1.03	43.10 ^j ± 0.19
Ellagic Acid	53.35 ^a ± 1.20	38.19 ^b ± 0.92	28.74 ^c ± 0.81	53.87 ^h ± 0.95
Dicaffeoylquinic acid	72.34 ^a ± 1.44	42.84 ^b ± 1.59	39.09 ^c ± 0.76	54.00 ^h ± 0.70
NMaPeaPsyB				
Cyanidin 3-sambubioside	479.00 ^a ± 3.93	454.82 ^b ± 3.76	323.14 ^c ± 2.02	67.50 ^d ± 0.57
Protocatechuic acid	44.38 ^a ± 1.43	21.91 ^c ± 0.78	30.39 ^b ± 0.10	68.70 ^d ± 0.72
Chlorogenic acid	54.62 ^a ± 0.72	30.37 ^b ± 0.09	34.50 ^c ± 0.13	63.10 ^f ± 0.69
Quercetin-3-glucoside	64.87 ^a ± 0.45	49.03 ^b ± 0.32	36.01 ^c ± 0.90	55.50 ^h ± 0.44
Catechin	34.27 ^a ± 0.94	15.29 ^b ± 0.78	22.93 ^c ± 0.60	66.90 ^e ± 0.38
Ferulic acid	30.50 ^a ± 0.17	18.35 ^c ± 0.28	24.89 ^b ± 0.81	81.60 ^a ± 0.75
Caffeic acid	46.74 ^a ± 0.85	37.18 ^b ± 0.30	25.04 ^c ± 0.19	54.00 ^f ± 0.23
p-Coumaric	33.05 ^a ± 0.50	18.40 ^b ± 0.70	25.49 ^c ± 0.30	77.10 ^b ± 0.82
Syringic Acid	23.65 ^a ± 2.38	20.28 ^b ± 1.04	12.82 ^c ± 0.84	54.20 ^h ± 0.54
Ellagic Acid	52.95 ^a ± 1.74	36.90 ^b ± 0.59	30.04 ^c ± 0.62	57.00 ^h ± 0.93
Dicaffeoylquinic acid	74.94 ^a ± 1.12	42.04 ^b ± 0.75	37.59 ^c ± 0.28	50.10 ⁱ ± 0.47

Means followed by the same letter ± standard deviation within the row are not significantly different at $p < 0.05$. Means followed by the same letter ± standard deviation within the bioaccessibility column are not significantly different at $p < 0.05$. Key: NM: Natal plum juice; NMB: Natal plum juice + *Ltp. plantarum* 75; NMaPeaPsy: Natal plum juice + maltodextrin + pea protein isolate + psyllium mucilage; NMaPeaPsyB: Natal plum juice + maltodextrin + pea protein isolate + psyllium mucilage + *Ltp. plantarum* 75.

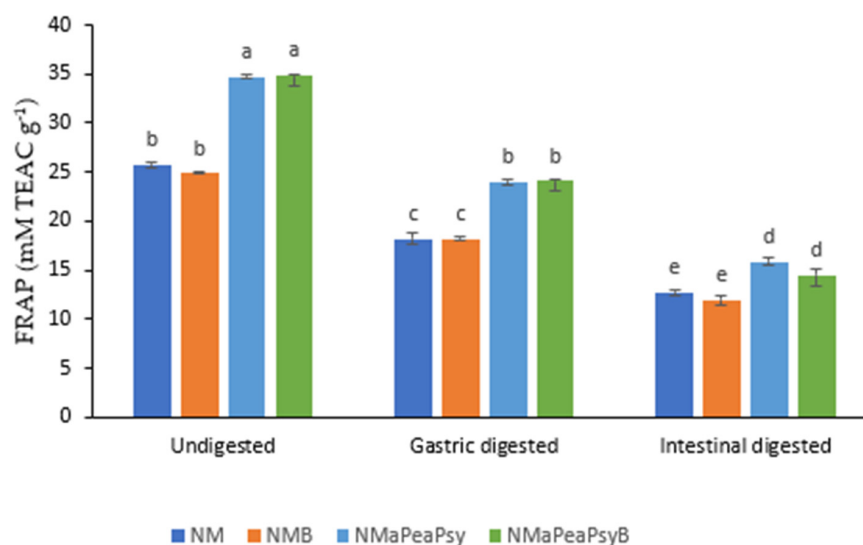
Ferulic acid and protocatechuic acid are byproducts of the degradation of cyanidin derivatives [24]. Thus, encapsulating the Natal plum juice resulted in improved phenolic acids bioaccessibility when compared to our previous study [2]. The bioaccessibility of phenolics during digestion depends on their stability when exposed to different pH, the structure of the compound, fruit matrix, solubility, and degradation, glycosylation, or esterification with other compounds [34]. According to Ortega et al. [35], the composition of the food matrix may influence the behavior of phenolic acids during digestion. The hydrolysis of gallotannins and ellagitannins could explain the increased amounts of ellagic acids after intestinal digestion [36]. pH 7 and bile salts may contribute to the loss of other phenolic acids in the intestinal phase, including chlorogenic acid, caffeic acid, and syringic acid [37]. Dietary polyphenols have been shown to aid in the maintenance of human health, particularly in the gut, by stimulating the growth of beneficial bacteria while inhibiting the growth of pathogenic bacteria, resulting in a prebiotic-like effect [38].

The majority of the ingested polyphenols do not reach the colon because polyphenols are poorly absorbed or metabolized by human enzymes during their passage through the upper gastrointestinal tract. Therefore, the results of this study showed that the encapsulation process improved the number of polyphenols that survived in the gastrointestinal digestion conditions and made them bioaccessible in the colon.

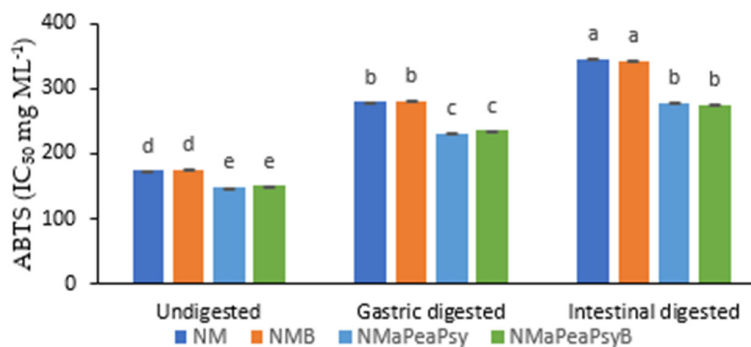
3.6. Effect of In Vitro Gastrointestinal Digestion on the Antioxidant Activities of Natal Plum (*Carissa macrocarpa*) Juice Phenolic Extract

Figure 4A–C shows the antioxidant activities of encapsulated and unencapsulated Natal plum juice extracts inoculated with or without *Ltp. plantarum* 75. Antioxidant activities (FRAP, ABTS and DPPH) decreased during digestion, and this could be attributed to phenolics being depleted after digestion. However, the encapsulated Natal plum juice possessed superior antioxidant activities compared to unencapsulated Natal plum juice. This can be attributed to the protective properties provided by the encapsulating wall material used. In addition, because of the potential of wall materials to donate electrons or hydrogen, they can participate in antioxidant processes, increasing the antioxidant activity of encapsulated food components [39]. The antioxidant properties of plant extracts are usually attributed to the presence of phenolic components. However, the changes that occur during gastrointestinal digestion can alter the chemical structure of the phenolic components, resulting in varying antioxidant capabilities [40]. A strong positive correlation exists between TPC and FRAP ($r = 0.95$), TAC and FRAP ($r = 0.94$), Cy-3-Sa ($r = 0.93$), syringic acid (0.85), and ellagic acid (0.74), which was significant at $p < 0.01$ (Figure 5).

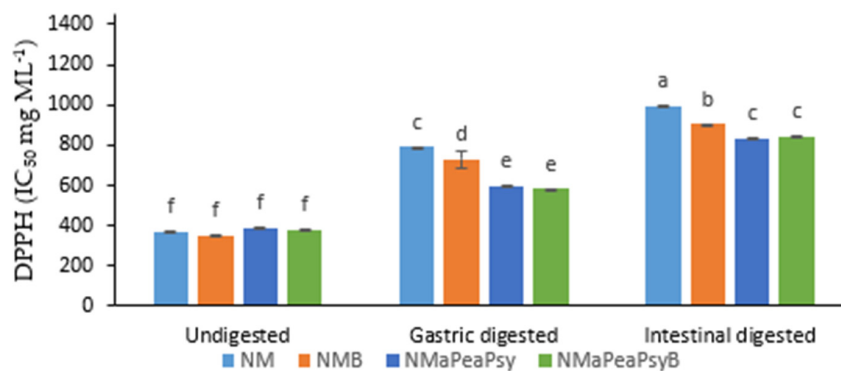
Similarly, a strong positive correlation between FRAP and TPC, TPC and anthocyanins has been observed previously by Seke et al. [2] and Manhivi et al. [41]. The antioxidant power (FRAP) also showed a positive correlation with all phenolic compounds identified in the Natal plum juice. The ABTS radical scavenging showed a strong positive correlation with TPC (0.87), ellagic acid (0.75), syringic acid (0.86), Cy-3-Sa (0.80) and chlorogenic acid (0.78). The DPPH radical scavenging activity showed a positive correlation with all the identified phenolic compounds, TPC and TAC. TPC (0.94), Cy-3-Sa (0.86), protocatechuic acid (0.86), ellagic acid (0.85), and TAC were found to have the strongest correlations with DPPH (0.87). Thus, these findings imply that phenolic components played an important role in the antioxidant activity of encapsulated Natal plum juice. Phenolics' antioxidant action is largely determined by their molecular structure. The CH₂CHCOOH grouping of hydroxycinnamic acids has been found to have a greater capacity to transfer a proton and to stabilize radicals later than the carboxyl (COOH) grouping of hydroxybenzoic acids [42]. Furthermore, flavonoids alone, depending on their structure, might operate as protons or electron donors, resulting in a strong association. The difference in activity could be attributable to the compounds' synergistic or antagonistic effects.



(A)

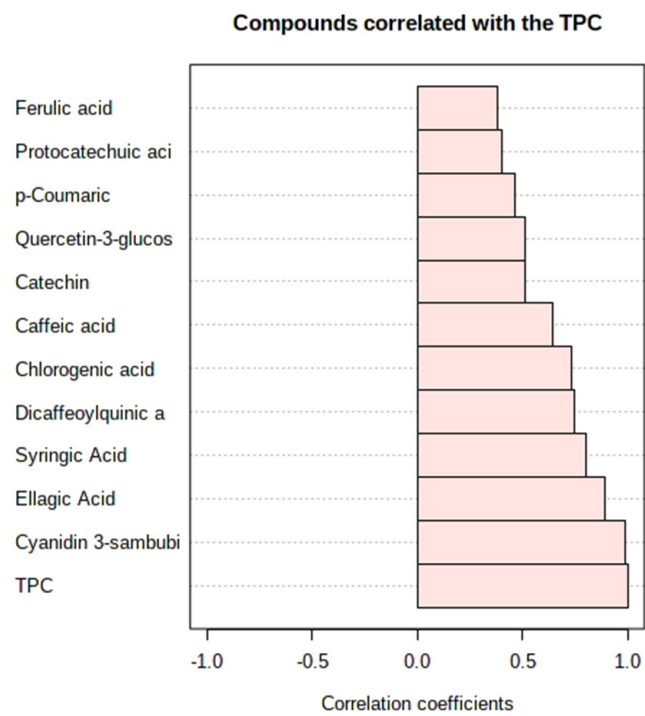


(B)

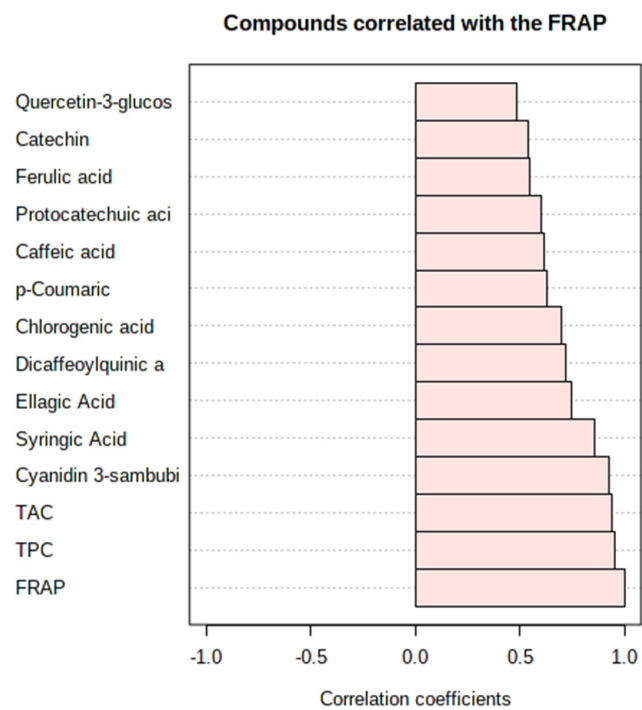


(C)

Figure 4. Effect of co-encapsulation of Natal plum (*Carissa macrocarpa*) juice on FRAP (A), ABTS (B) and DPPH (C) scavenging activity. Error bars show the standard deviation value, bars with different letters are significantly different $p < 0.05$. Key: NM: Natal plum (*Carissa macrocarpa*) juice; NMB: Natal plum (*Carissa macrocarpa*) juice + *Ltp. plantarum* 75; NMaPsy: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + psyllium mucilage; NMaPsyB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + psyllium mucilage + *Ltp. plantarum* 75; NMaPeaPsy: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + pea protein isolate + psyllium mucilage; NMaPeaPsyB: Natal plum (*Carissa macrocarpa*) juice + maltodextrin + pea protein isolate + psyllium mucilage + *Ltp. plantarum* 75.

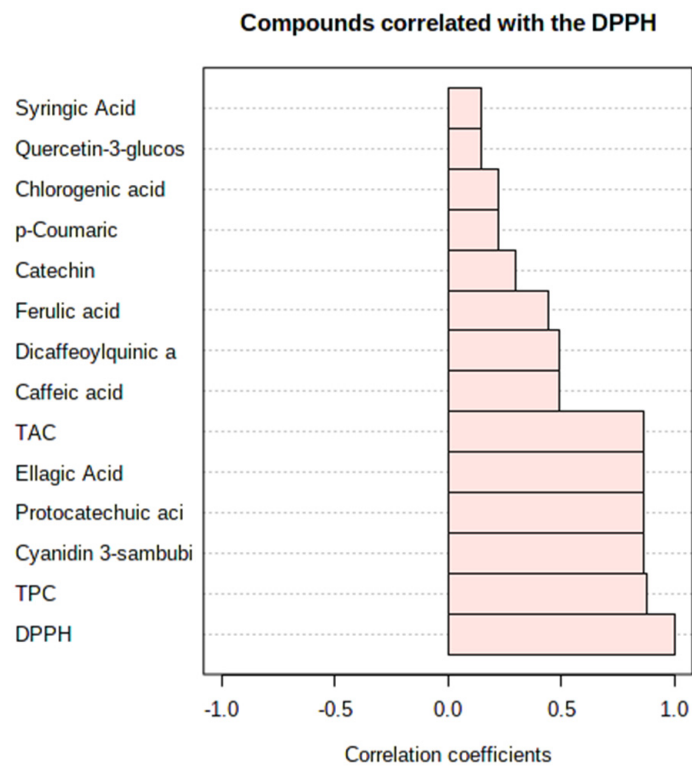


(A)

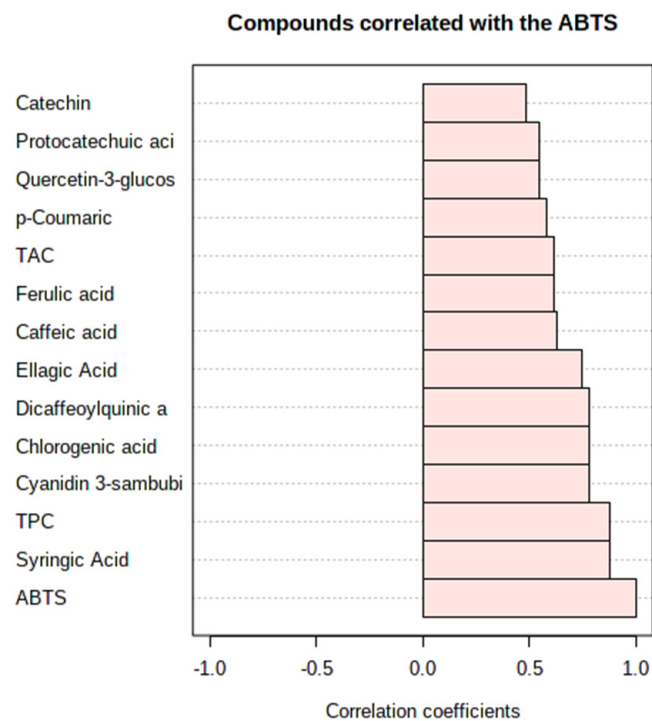


(B)

Figure 5. Cont.



(C)



(D)

Figure 5. Correlation of total phenolic content (A), FRAP antioxidant capacity (B), DPPH (C) and ABTS (D) radical scavenging capacities with phenolic compounds.

4. Conclusions

The bioaccessibility of cyanidin 3-sambubioside after intestinal digestion was highest in NMPeaPsy and NMPeaPsyB. The degradation of cyanidin 3-sambubioside correlated with the increase in protocatechuic acid and ferulic acid in the intestinal phase. A strong correlation was observed between cyanidin 3-sambubioside, protocatechuic acid, ellagic acid, syringic acid, chlorogenic acid, TPC and TAC against antioxidant (DPPH, ABTS, FRAP) activities. Moreover, the incorporation of maltodextrin, pea protein isolates and psyllium mucilage are promising since it provided greater protection to *Ltp. plantarum* 75, anthocyanins and phenolic compounds in vitro. Data suggest that the Natal plum juice powder can be a natural and functional colorant in the food industry. However, more research on cellular models such as Caco-2 as well as animal models is required to acquire qualitative data for human investigations.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/foods11142116/s1>. Figure S1: Chromatograms of Undigested Natal plum juice samples at 520 nm (anthocyanins); Figure S2: Chromatograms of Gastric digested Natal plum juice samples at 520 nm (anthocyanins); Figure S3: Chromatograms of Intestinal digested Natal plum juice samples at 520 nm (anthocyanins); Figure S4: Chromatograms of the identification of phenolic acids and flavonoids in Natal plum juice samples at 320 nm; Table S1: Retention time and regression equations for phenolic identification and quantification using HPLC-DAD.

Author Contributions: F.S.: PhD student who performed the experiments and gathered the data, conducted the statistical analysis, and wrote the first draft of the manuscript. V.E.M.: supervised the student on encapsulation and in vitro digestion methods, validated, and visualized the data and revised the first draft of the manuscript. M.G.: PhD student from the Univ de La Réunion who participated under the Erasmus+ programme and visited the Tshwane University of Technology, South Africa. T.S.: validated and visualized the identification and quantification of phenolic compounds. S.A.A.: validated and visualized the microbiology data and revised the first draft of the manuscript. C.G.: research collaborator. F.R.: provided the *Lactiplantibacillus plantarum* 75 and was a research collaborator. R.M.S.: co-supervisor and edited the manuscript. D.S.: grant holder, supervisor, conceptualized the research, edited and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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DISCUSSION GENERALE ET PERSPECTIVES

Le développement d'aliments fermentés représente un intérêt pour l'amélioration de leur conservation et de leurs propriétés nutritionnelles et sensorielles, pour valoriser les matières premières et pour contribuer au maintien d'une bonne santé. La souche bactérienne et le substrat adéquats pour procéder à la fermentation lactique doivent être sélectionnés selon le produit final souhaité. En effet, ces facteurs vont être responsables de la production et/ou de la modification de composés d'intérêt, sensoriel ou nutritionnel. Dans ce cadre, la fermentation lactique des fruits s'avère d'un intérêt particulier, du fait de leurs qualités sensorielles et nutritionnelles, mais aussi pour des raisons économiques au niveau de La Réunion.

Ces travaux de recherche visaient à répondre à deux objectifs principaux :

- (1) Étudier les composés des fruits impactés par la fermentation lactique et leur évolution au cours de la fermentation et du stockage et explorer la formation de composés d'intérêt nutritionnel, les EPS ;
- (2) Évaluer si les effets fonctionnels liés aux transformations lors de la fermentation lactique de fruit sont détectables chez le mammifère nourri avec un régime riche en graisse, si ces effets sont avérés au niveau du métabolisme énergétique et sur la colonisation intestinale.

Trois fruits tropicaux, à savoir l'ananas Queen Victoria, la mangue Cogshall et la papaye Solo, ont été choisis, sur la base de leur différence de composition et de leur production à La Réunion, puis transformés en jus ou purées. Les préparations ont ensuite été fermentées individuellement avec les souches *Ltp. plantarum* 17a ou 75, *Lc. pseudomesenteroides* 56 ou *W. cibaria* 21 ou 64. Ces cinq souches de trois espèces bactériennes ont été choisies du fait de leur origine, fruits ou légumes produits localement, mais aussi par leur aptitude de croissance et d'acidification de jus de fruits. L'évolution de la population de ces bactéries au sein des fruits a été étudiée pendant la fermentation et la période de stockage ainsi que leur effet sur des paramètres physico-chimiques et sur certains composés bioactifs. Parmi ceux-ci, la synthèse d'EPS par les souches bactériennes sélectionnées a été évaluée.

Quel que soit le fruit étudié, les souches bactériennes inoculées se sont favorablement développées et ont significativement acidifié les préparations, démontrant que ces bactéries sont métaboliquement actives. L'acidité est un paramètre à prendre en considération lors du développement de produits alimentaires car elle contribue à leur conservation et influence leur acceptabilité par les consommateurs. Cependant, l'acidité est contrebalancée par la sucrosité des fruits. La composition en molécules aromatiques influence aussi l'acceptabilité des produits.

La mangue Cogshall est récoltée en début de saison à La Réunion mais n'est pas exportable à cause de l'invasion par des mouches de fruit (ANSES, 2019). Ce fruit se situe dans une gamme intermédiaire à élevée pour ce qui est de sa valeur énergétique, richesse en caroténoïdes et vitamine C et son acidité est modérée (Joas et al., 2012; Rosalie et al., 2018; Septembre-Malaterre et al., 2016). Dans notre étude, les fruits utilisés étaient de maturité un peu faible, d'après les teneurs en sucres observées. Toutefois, la fermentation conduit à une diminution de 80 % du glucose et de 53 % du fructose, ce qui devrait être perceptible tant sensoriellement qu'au niveau de la valeur énergétique. Cette consommation des monosaccharides est conséquente par rapport à ce qui a été rapporté précédemment (Di Cagno et al., 2011; Mousavi et al., 2011, 2013). En parallèle, l'augmentation de la

teneur en acétate, lactate et tartrate pour certaines souches apporte une acidité nouvelle. La fermentation lactique suivie d'un stockage de plusieurs semaines n'a pas accentué la dégradation de la vitamine C, ni modifié celle des caroténoïdes. Les qualités nutritionnelles de la mangue semblent préservées à ce stade. De plus, nous avons montré une production d'EPS lors de la culture de *Lc. pseudomesenteroides* 56 et *W. cibaria* 21 et 64 sur des milieux solides à base de purée de mangue Cogshall. Une augmentation du pH initial à 6 augmente cette production pour les souches *W. cibaria* 21 et 64 et cette augmentation est accentuée si la teneur en saccharose augmente. Un pH plus élevé pourrait être atteint naturellement avec des mangues plus mûres, des pH de l'ordre de 5 ayant été observés au laboratoire sur cette variété de mangue (Leneveu-Jenvrin et al., 2021; Nabot, 2021, communication personnelle). De la même façon, la teneur en saccharose augmente avec la maturité du fruit. La fermentation lactique serait adaptée à la valorisation de fruits un peu trop mûrs. La mangue José, plus riche en sucre et moins acide que la Cogshall serait particulièrement adaptée à ce type de transformation. Mettre en place un test hédonique pour évaluer l'acceptabilité sensorielle de la purée fermentée serait maintenant nécessaire pour poursuivre le développement d'un produit.

La papaye Solo est considérée comme une source de caroténoïdes et vitamine C. Les concentrations obtenues dans notre étude sont en accord avec celles retrouvées dans la littérature (Septembre-Malaterre et al., 2016). Nous avons mis en évidence la formation des acides acétique et lactique lors de la fermentation lactique mais n'avons pas observé de consommation de fructose ni de glucose. Le malate et le citrate sont consommés par certaines souches. La papaye Solo peut être consommée verte ou mûre. Elle est peu caractérisée dans la littérature. Investiguer de manière approfondie la composition en composés phytochimiques permettrait de valoriser ce fruit, ainsi que la variabilité de la composition en sucres et acides organiques. Une fois la composition mieux caractérisée, l'impact de la fermentation lactique sur les composés d'intérêt pourra être reconsidéré. La papaye contient, entre autres, des acides aminés. L'acidification induite lors de la fermentation peut résulter en une diminution de l'utilisation des sucres par les bactéries lactiques qui vont alors préférentiellement métaboliser les acides aminés (Gänzle, 2015). Il faudrait mener des expérimentations permettant d'évaluer l'effet de la fermentation lactique sur les acides aminés présents et la source de carbone utilisée par les bactéries lactiques.

La papaye Solo présente un intérêt moindre pour la production d'EPS par comparaison avec la mangue Cogshall et l'ananas Queen Victoria. Les résultats obtenus lors de la fermentation de la papaye Solo montrent qu'il s'agit d'un bon substrat de fermentation permettant la croissance des bactéries lactiques étudiées. La papaye ne contient pas de saccharose, sa fermentation n'induit donc pas la formation d'EPS. Malgré un ajustement de la composition par ajout de saccharose et du pH, la synthèse de ces composés n'a pas été obtenue, quelle que soit la souche bactérienne utilisée. Ces observations suggèrent la présence d'un composé qui inhibe la production d'EPS lors de la fermentation de la papaye Solo. Sur ce fruit, l'inhibition de la synthèse d'EPS répond à des mécanismes inconnus. L'identification du ou des composé(s) de ce fruit en capacité d'inhiber cette synthèse pourrait faire l'objet d'une étude complémentaire. La composition de la papaye Solo pourrait être modulée pour ensuite évaluer la production d'EPS.

Nos résultats ont montré que le jus d'ananas Queen Victoria peut être utilisé comme substrat de fermentation malgré sa forte acidité. En effet, la croissance élevée des souches bactériennes utilisées a résulté en une forte acidification des préparations. La fermentation lactique du jus d'ananas Queen Victoria n'a pas induit de dégradation des composés bioactifs. Une autre étude effectuée au

sein du laboratoire QualiSud a démontré une augmentation de la concentration en polyphénols totaux dans du jus d'ananas fermenté avec la souche de *W. cibaria* 64 par comparaison avec du jus non fermenté. La capacité de piégeage des radicaux libres augmente également dans des jus d'ananas fermentés avec les souches *Lc. pseudomesenteroides* 56 et *W. cibaria* 64 (Fessard et al., 2017). Les différences constatées pourraient être dues aux variations de composition du fruit liées à la localisation géographique du cultivar et à la période de récolte. La synthèse d'EPS a pu être induite par les souches *W. cibaria* 21 et 64 et *Lc. pseudomesenteroides* 56 sur un milieu solide à base de jus d'ananas Queen Victoria. L'acidité de ce fruit a représenté une limite mais la modulation du milieu à un pH supérieur à 4,5 a résulté en la formation d'EPS.

Lors de notre étude, la fermentation lactique appliquée au jus d'ananas Queen Victoria, à la purée de mangue Cogshall et à la purée de papaye Solo a montré des effets variables selon le fruit puis selon la souche bactérienne utilisée. Au vu des différences de composition entre ces fruits, ce comportement des souches bactériennes apparaît cohérent. De plus, la fermentation lactique n'a pas eu d'effet délétère sur la capacité antioxydante ou sur les concentrations en composés bioactifs mesurés. La production d'EPS a pu être induite par les souches *Lc. pseudomesenteroides* 56 et *W. cibaria* 21 et 64 sur des milieux à base de jus d'ananas Queen Victoria ou de purée de mangue Cogshall. Il est à noter qu'une variation de la production d'EPS a été observée selon le nombre de générations des souches, suggérant une assez faible stabilité du phénotype de la production d'EPS, ce qui constitue un inconvénient majeur pour la production en conditions industrielles.

Les propriétés liées à la formation d'EPS présentent un intérêt dans le cadre du développement d'aliments fonctionnels. Aussi, quantifier et optimiser cette production au sein des fruits tropicaux fermentés est essentiel. Les EPS ont un impact sur les propriétés rhéologiques, ce qui permettrait d'améliorer l'acceptabilité des produits fermentés par les consommateurs. Il est important de déterminer la concentration en EPS permettant de bénéficier de leurs propriétés lors de la consommation des produits alimentaires fermentés. Ces composés ont en effet la capacité de moduler la structure du microbiote intestinal et pourraient ainsi avoir un impact sur le système immunitaire (Caggianiello et al., 2016). Ainsi, il serait intéressant d'approfondir la caractérisation et la quantification des EPS synthétisés afin de déterminer l'impact de cette production sur les propriétés rhéologiques des fruits fermentés et sur les propriétés biologiques qui en découlent, à savoir les activités prébiotiques, antioxydantes, anti-inflammatoires et hypocholestérolémiantes (Zannini et al., 2016).

D'après la littérature, les souches de *W. cibaria* et *Lc. pseudomesenteroides* produisent plusieurs g/L d'HoPS, de tailles moléculaires entre 10^4 et 10^8 Da. Nos résultats ont montré une production entre 56 et 243 et entre 159 et 345 mg d'équivalent de glucose par plaque de culture de milieu solide à base de jus d'ananas Queen Victoria pour *Lc. pseudomesenteroides* 56 et *W. cibaria* 64, respectivement. Sur un milieu solide à base de purée de mangue Cogshall, la production obtenue pour la souche *Lc. pseudomesenteroides* 56 variait de 16 à 61 mg d'équivalent de glucose par plaque de culture et entre 71 et 295 mg d'équivalent de glucose par plaque de culture pour la souche *W. cibaria* 64. D'après la littérature, les dextrans produits par les souches de *W. cibaria* et *Lc. pseudomesenteroides* ont des liaisons α -(1→4), α -(1→6) ou α -(1→3) (Lynch et al., 2018; Malang et al., 2015; Ripari, 2019). Les premiers essais effectués par spectrométrie RMN indiquent des ramifications différentes selon les souches *Lc. pseudomesenteroides* 56 et *W. cibaria* 64, mais nous ne disposons pas à ce stade d'information sur la taille et le nombre de ramifications. Ces deux éléments jouent un rôle sur les propriétés texturantes des EPS.

Les conséquences de la fermentation lactique appliquée à du jus d'ananas Queen Victoria ont été étudiées sur le métabolisme énergétique d'un modèle de souris nourries avec un régime riche en graisse. Notre hypothèse était que la lactofermentation des fruits permet de modifier ou de synthétiser des composés bioactifs conférant des bénéfices sur la santé. Bien que des études recensées lors de la rédaction d'un article de revue rapportent les effets bénéfiques potentiels de la lactofermentation sur la composition nutritionnelle des aliments (Garcia et al., 2020), nos travaux permettent d'évaluer les effets de fruits fermentés à l'échelle d'un organisme entier. La consommation de jus lactofermenté a entraîné une régulation du métabolisme glucidique identique à celle obtenue lors de la consommation d'eau. L'augmentation de la concentration en triglycérides et en cholestérol induite par la consommation d'un régime riche en graisse a été limitée par l'ingestion du jus d'ananas Queen Victoria lactofermenté. Il a cependant été démontré que la supplémentation en extrait de framboise chez des souris nourries avec un régime riche en graisse a eu un effet sur les lipides circulants (Tu et al., 2019). Aucune altération de la perméabilité intestinale n'a été observée au cours de notre étude. La consommation de jus lactofermenté a engendré un changement de la composition bactérienne dans les fèces. Une majorité de *Lactobacillus animalis/murinus* a été identifiée dans les échantillons du groupe témoin et les échantillons du groupe ayant consommé le jus d'ananas fermenté contenaient majoritairement *Enterococcus faecalis*. De manière intéressante, les échantillons des souris ayant consommé la boisson fermentée contenaient une proportion plus importante de *Ltp. plantarum* par rapport à celles ayant consommé de l'eau.

Ainsi, la consommation de jus d'ananas Queen Victoria lactofermenté semble limiter les dérégulations du métabolisme liées à la consommation d'un régime riche en graisse. Approfondir ces résultats permettrait de mieux comprendre cette régulation. Notre hypothèse est que la consommation du jus lactofermenté va influencer l'expression des gènes impliqués dans la synthèse lipidique au niveau du foie (Srebp2, Hmgcr). De manière plus précise, SREBP2 va induire la transcription de l'HMG-CoA réductase ce qui va entraîner la synthèse de cholestérol (Ahn et al., 2013). Étudier l'expression du gène de la Mucin-2 représente également un intérêt de par son implication dans la formation de mucus intestinal et son rôle de protection du tractus gastro-intestinal. L'accumulation excessive de graisse au niveau du tissu adipeux est liée à la libération de triglycérides dans le sang sous forme d'acides gras (Choi et al., 2020). Ayant nourries les souris de notre étude avec un régime riche en graisse et ayant constaté une accumulation de graisse limitée dans le tissu adipeux des souris ayant consommé du jus fermenté par comparaison avec les autres groupes ayant consommé une boisson sucrée, il serait intéressant de caractériser la composition en acides gras du tissu adipeux, par chromatographie en phase gazeuse par exemple. Enfin, la quantification d'ADN de gènes de différentes espèces bactériennes contenues dans les fèces des souris permettrait d'étudier la modification du microbiote de manière plus précise. L'abondance de différents phyla, à savoir *Bacteroidetes*, *Firmicutes* (*Lactobacillus*, *Clostridium*), *Actinobacteria* (*Bifidobacteria*), *Proteobacteria* (*Enterobacteria*) et *Akkermensia* serait mesurée. La proportion de ces espèces et genres bactériens de la flore intestinale étant influencée par le régime alimentaire. Le ratio *Bacteroidetes/Firmicutes* varie par exemple lors de la consommation de polyphénols (De Filippis et al., 2016; Tomás-Barberán et al., 2016).

À ce jour, il n'existe pas de produits à base de fruits lactofermentés commercialisés à La Réunion. Les fruits se dirigent naturellement vers une fermentation alcoolique. Pour initier la fermentation lactique, il est nécessaire de recourir à des procédés qui résultent en l'élimination des micro-organismes présents naturellement dans les fruits, comme la pasteurisation. Il est ensuite

possible d'orienter la transformation de ceux-ci par inoculation de souches bactériennes préalablement caractérisées. Nous avons vérifié lors de ces travaux de recherche que cela était bien réalisable par inoculation individuelle des souches *Ltp. plantarum* 17a et 75, *W. cibaria* 21 et 64 et *Lc. pseudomesenteroides* 56. Il serait important d'évaluer la possibilité de combiner ces souches, dès l'initiation de la fermentation ou avant le stockage, afin d'améliorer les effets obtenus lors d'une inoculation individuelle. Il est cependant nécessaire de mieux connaître le métabolisme de ces souches bactériennes afin d'obtenir une co-inoculation efficace. De nombreuses études portent sur l'effet des souches de *Ltp. plantarum* sur les fruits mais moins de données sont disponibles concernant les souches de *W. cibaria* et *Lc. pseudomesenteroides*.

Les bactéries lactiques et les composés bioactifs contenus dans les aliments fermentés sont soumis à des conditions hostiles lors de leur passage dans le tractus gastro-intestinal. Aussi, il est important d'étudier une méthode permettant leur protection lors du développement d'aliments fonctionnels. Dans ce cadre, la microencapsulation représente un fort intérêt et permettrait de bénéficier des bienfaits liés à la consommation d'aliments fermentés dont les composés bioactifs d'intérêt seraient préservés et la survie des souches bactériennes serait assurée lors de la digestion gastro-intestinale.

CONCLUSION GENERALE

Ces travaux de thèse avaient pour objectif de caractériser les modifications de composition des fruits résultant de la fermentation lactique et d'identifier les effets fonctionnels d'une alimentation enrichie en fruits tropicaux fermentés. Pour y répondre, des préparations d'ananas Queen Victoria, de mangue Cogshall et de papaye Solo ont été fermentées avec différentes souches de *Lactiplantibacillus plantarum*, *Weissella cibaria* ou *Leuconostoc pseudomesenteroides*.

Nos résultats ont montré que ces fruits peuvent être utilisés comme substrats de fermentation bien que leur composition en mono et disaccharides, acides organiques, vitamine C, composés phénoliques et caroténoïdes diffère. La synthèse des exopolysaccharides (EPS) était dépendante du fruit fermenté et de la souche utilisée. Une méthode a été mise au point en milieu solide pour quantifier ces EPS. Nos travaux ouvrent de nouvelles perspectives pour moduler la production d'EPS à partir de préparations lactofermentées d'ananas Queen Victoria et de mangue Cogshall.

L'effet de la consommation d'un jus d'ananas Queen Victoria lactofermenté *in vivo* sur un modèle de souris ayant consommé un régime riche en graisse a montré une régulation du métabolisme énergétique similaire à celle du groupe ayant reçu uniquement de l'eau *ad libitum*. L'ingestion de jus d'ananas Queen Victoria lactofermenté a modulé l'équilibre des flores lactiques du microbiote intestinal par comparaison à l'ingestion d'eau seule.

Mieux comprendre le métabolisme bactérien lors de la fermentation de fruits et mieux caractériser les changements du microbiote intestinal après une alimentation contenant des fruits fermentés sont les deux axes qui pourraient permettre une meilleure compréhension globale de la fermentation lactique et ses effets sur la santé.

La fermentation lactique de jus d'ananas Queen Victoria ou de purée de mangue Cogshall répond aux attentes nutritionnelles. Ce procédé mérite des investigations plus poussées, notamment au niveau sensoriel, en vue d'une valorisation économique.

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