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# Screening of lactic acid bacteria for their use as aromatic starters during fermentation of vegetables

Da Lorn

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# UNIVERSITÉ DE BOURGOGNE FRANCHE-COMTÉ

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Université de Bourgogne Franche-Comté /AgroSup Dijon

## DOCTORAL THESIS

Presented to obtain the degree of

Doctor of Philosophy in Agri-Food Biotechnology

Specialties: Biotechnology, Microbiology, Biochemistry

By **Da LORN**

Title:

### Screening of lactic acid bacteria for their use as aromatic starters during fermentation of vegetables

Thesis presented and defended in Dijon on December 15, 2020 in front of the jury composed of

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## ABSTRACT

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Lactic acid bacteria (LAB) have good growth capacities on various food matrices and produce very diverse enzymatic activities which are notably capable of positively modifying the organoleptic properties of fermented foods. Therefore, the selection of LAB starters possessing good metabolic abilities and interesting enzymatic activities towards plant matrices could improve the aroma profiles of fermented foods. The main objective of this study was to obtain a diversity of aroma profiles of fermented tomatoes by using biotechnological approaches. To achieve this, firstly, 200 LAB isolated from Cambodian and Vietnamese fermented foods were screened for their  $\beta$ -glucosidase activity and duplicate isolates identified through RAPD-PCR analysis were discarded. Thereby, 40 strains were found positive for  $\beta$ -glucosidase using *p*-nitrophenyl- $\beta$ -D-glucopyranoside as substrate. Among them, 14 displayed an activity greater than 10 nmol/min/mg dry cell. Thirteen were identified as *Lactobacillus (Lactiplantibacillus) plantarum* and one as *Lactobacillus (Lactiplantibacillus) pentosus*. Four strains of different phenotypes for  $\beta$ -glucosidase activity were tested for ADH activity. The highest reduction ability for hexanal and (*E*)-2-hexenal was obtained for *Lactobacillus (Limosilactobacillus) fermentum* V013-1A for which no  $\beta$ -glucosidase activity was detectable. The three other strains (*L. plantarum* C022-2B, C022-3B and V0023-4B2) exhibited a lower reduction ability and only for hexanal. Secondly, mashed tomatoes were fermented with these four strains individually to evaluate their ability to release volatile compounds from the tomato aroma precursors. Fifty-eight volatile compounds were identified and quantified by HS-SPME/GC-MS. Untreated tomatoes were rich in aldehydes. The tomatoes fermented with *L. plantarum* strains were rich in ketones whereas those with *L. fermentum* were rich in alcohols. However, for the generation of terpenoids that provide fruity and floral notes, our screening of  $\beta$ -glucosidase activity was not able to explain the differences among the strains. For ADH activity, *L. fermentum* exhibited high activity in fermentation as most of the target aldehydes and ketones disappeared and were replaced by their corresponding alcohols. *L. plantarum* strains exhibited a lower activity, but with an important substrate-selectivity diversity. A better knowledge of the functionality of each LAB strain in the food matrix will permit to predict and shape the aroma profiles of fermented food.

**Keywords:** Lactic acid bacteria, fermentation, tomato, aroma precursors,  $\beta$ -glucosidase, alcohol dehydrogenase, volatile compounds

## RESUME

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Les bactéries lactiques (LAB) ont de bonnes capacités de croissance sur différentes matrices alimentaires et produisent des activités enzymatiques très diverses qui sont notamment capables de modifier positivement les propriétés organoleptiques des aliments fermentés. Par conséquent, la sélection des ferments (starters) LAB possédant de bonnes capacités métaboliques et des activités enzymatiques intéressantes envers la matrice végétale pourrait améliorer les profils aromatiques des aliments fermentés. Le principal objectif de cette étude était d'obtenir une diversité de profils aromatiques des tomates fermentées en utilisant des approches biotechnologiques. Pour y parvenir, premièrement, 200 LAB isolées à partir d'aliments fermentés cambodgiens et vietnamiens ont été criblées pour leur activité  $\beta$ -glucosidase et les isolats en double identifiés par analyse RAPD-PCR ont été rejetés. Ainsi, 40 souches étaient positives pour la  $\beta$ -glucosidase en utilisant le *p*-nitrophényl- $\beta$ -D-glucopyranoside comme substrat. Parmi eux, 14 présentaient une activité supérieure à 10 nmol/min/mg de poids sec. Treize ont été identifiées comme *Lactobacillus (Lactiplantibacillus) plantarum* et une comme *Lactobacillus (Lactiplantibacillus) pentosus*. Quatre souches de phénotypes différents pour l'activité  $\beta$ -glucosidase ont été testées pour l'activité ADH. La capacité de réduction la plus élevée pour l'hexanal et le (*E*)-2-hexénal a été obtenue pour *Lactobacillus (Limosilactobacillus) fermentum* V013-1A pour laquelle aucune activité  $\beta$ -glucosidase n'était détectable. Les trois autres souches (*L. plantarum* C022-2B, C022-3B et V0023-4B2) présentaient une capacité de réduction plus faible et uniquement pour l'hexanal. Deuxièmement, les purées de tomates ont été fermentées avec ces quatre souches individuellement pour évaluer leur capacité à libérer des composés volatils à partir des précurseurs d'arômes de tomate. Cinquante-huit composés volatils ont été identifiés et quantifiés par HS-SPME/GC-MS. Les tomates non traitées étaient riches en aldéhydes. Les tomates fermentées avec les souches de *L. plantarum* étaient riches en cétones tandis que celles avec *L. fermentum* étaient riches en alcools. Cependant, pour la génération de terpénoïdes apportant des notes fruitées et florales, notre criblage de l'activité  $\beta$ -glucosidase n'a pas pu expliquer les différences entre les souches. Pour l'activité ADH, *L. fermentum* a montré une activité élevée dans la fermentation car la plupart des aldéhydes et cétones cibles ont disparu et ont été remplacés par leurs alcools correspondants. Les souches de *L. plantarum* ont montré une activité plus faible mais avec une importante diversité de sélectivité du substrat. Une meilleure connaissance de la fonctionnalité de chaque souche LAB dans la matrice alimentaire permettra de prédire et de façonner les profils aromatiques des aliments fermentés.

**Mots clés:** Bactérie lactiques, fermentation, tomate, précurseurs d'arômes,  $\beta$ -glucosidase, alcool déshydrogénase, composés volatils

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*“Life is beautiful not because of the things we see or do, but because of the people we meet.” Simon Sinek*

## ABBREVIATIONS

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### Screening of enzymatic activities

ADH: Alcohol dehydrogenases

EIA: Esculin Iron Agar

*p*NP: *p*-Nitrophenol

*p*NPG: *p*-Nitrophenyl- $\beta$ -D-glucopyranoside

NAD: Nicotinamide adenine dinucleotide

NAD<sup>+</sup> and NADH: oxidized NAD<sup>+</sup> and reduced NADH

*ee*: Enantiomeric excess (*ee* is a measurement of purity used for chiral substances.)

### Genotypic identification of LAB

DNA: Deoxyribonucleic Acid

PCR: Polymerase Chain Reaction

RAPD: Random amplified polymorphic DNA

16S rRNA: 16S ribosomal Ribonucleic Acid

### Volatile compound extraction

PDMS: Polydimethylsiloxane

PDMS/ DVB: Polydimethylsiloxane/Divinylbenzene

CAR/PDMS: Carboxen/ Polydimethylsiloxane

### Tomato treatment and fermentation

Glu: Glucosidase-treated tomatoes

MT: Mashed tomatoes

LA: Lactic-acid-treated tomatoes

LF1A: Fermented tomatoes with *Lactobacillus (Limosilactobacillus) fermentum* V013-1A

LP2B: Fermented tomatoes with *Lactobacillus (Lactiplantibacillus) plantarum* C022-2B

LP3B: Fermented tomatoes with *L. plantarum* C022-3B

LP4B: Fermented tomatoes with *L. plantarum* V0023-4B2

UT: Untreated tomatoes

### Bacterial genus

LAB: Lactic Acid Bacteria

*L.*: *Lactobacillus*



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## I. INTRODUCTION

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Consumers in industrialised countries nowadays demand for their daily food to be more 'natural', 'organic' and/or 'free from additives/preservatives'. This trend, in technical terms is called 'clean label'. Consumers are becoming more aware of health (e.g. diabetes, obesity) and sustainability (e.g. local food) (Asioli et al., 2017). They are cautious to how foods are made (e.g. ultra-processed, minimally processed) and to the list of ingredients on the food label. Ultra-processed foods and the E-numbers shown on food labels have been allegedly associated with negative health effects (Asioli et al., 2017). Meanwhile, lactic acid fermentation is a simple and natural process, which could be an approach to meet this demand. Lactic acid fermentation is caused by lactic acid bacteria (LAB). LAB are widely present in numerous fermented foods and beverages. LAB could contribute to the safety, shelf life, nutritional, and sensory quality of fermented foods through their metabolism. Homofermentative LAB produce lactic acid as the main end-product of carbohydrate fermentation, while heterofermentative LAB additionally produce other products such as acetic acid, CO<sub>2</sub>, ethanol, acetoin, and diacetyl (Gänzle, 2015; Leroy and De Vuyst, 2004). LAB can be alternative antimicrobial agents to replace chemical food additives (e.g. nitrite, sulphite) due to one or synergic effects (e.g. lowering pH, CO<sub>2</sub> gas atmosphere, bacteriocins). Some LAB improve the nutritional quality of fermented foods by increasing vitamins, minerals, dietary fibre, and antioxidants. LAB are also one of the most significant groups of probiotic organisms. Furthermore, some LAB can produce exopolysaccharides, aroma compounds, and important enzymes, which plays a significant role for sensory quality of fermented foods. Therefore, lactic acid fermentation with functional LAB is a promising 'clean label' approach to replace artificial additive in food products.

Consumers in Western countries are also questioning their meat consumption habits. High meat consumption has negative impacts on our health, including obesity, cardiovascular disease, diabetes, and on our environment, such as methane production, land degradation, air and water pollution, water shortages, loss of biodiversity, and bacterial resistance to antibiotic (Godfray et al., 2018). They also disquiet over the impact of intensive agriculture on the environment. Many French people have considerably reduced their meat consumption, resulting in an overall drop of 12% in meat consumption in France over the past 10 years (Tavoularis and Sauvage, 2018). In order to achieve the transition to a sustainable, affordable, and high-quality food system in the next decade, it is crucial to reduce meat consumption. Fruits and vegetables can be an excellent alternative. They provide the important components of a healthy diet and their sufficient daily consumption, which could help prevent major diseases

(e.g. cardiovascular diseases and certain cancers). A published WHO/FAO report recommends a minimum of 400 g of fruit and vegetables per day (excluding potatoes and other starchy tubers) (WHO, 2003). To increase the daily consumption of fruits and vegetables, lactic acid fermentation could be an eco-friendly approach to diversify fruit and vegetable supply. In Asia, lactic acid fermentation is one of the most popular techniques for processing and preserving fruits and vegetables. Almost all fruits and vegetables are lacto-fermentable. However, this process is roughly anecdotic for fruits and vegetables in Western countries (but including some popular examples like sauerkraut, olives, capers, turnip, and gherkins) (Di Cagno et al., 2013; Mäki, 2004; Tamang et al., 2016b).

Tomato fruit is the most popular vegetable in the world. Tomato was chosen for this study because of several reasons. Regular consumption of tomatoes and tomato products has been associated with positive effects on human health, due to mainly the presence of antioxidant and anti-inflammatory properties (Agarwal and Rao, 1998; Campbell et al., 2004; Clinton et al., 1996; Lavelli et al., 2000; Parfitt et al., 1994; Pinela et al., 2016; Rao and Agarwal, 1999; Weisburger, 1998). Tomato contains diverse aroma precursors such as fatty acids, carotenoids, terpenoids, and glycosides. It is one of the most studied in term of degradation of flavour precursors among fruits/vegetables, and is thus a good plant matrix for model studies. Furthermore, substantial amounts of overproduced tomatoes for fresh consumption are rejected and wasted each year due to unacceptable colour, shape, maturity, lesions, etc. In addition, since tomato is a versatile fruit/vegetable, huge amounts of tomatoes are consumed in the form of processed tomatoes. While the quantity of processed tomatoes has considerably increased, the quantity of tomato pomace has also increased significantly. The amount of related tomato pomace in the world is estimated at up to 50,000 tons per year (Simitzis and Deligeorgis, 2018). Many nutrients and bioactive compounds or flavour precursors still exist in the tomato pomace that remains unused. The unexploited tomato pomace can worsen the disposal problem and aggravate environmental pollution.

In developing countries like Cambodia and Vietnam, lactic acid fermentation is a cheap technique which could help for food preserving and processing (Nguyen et al., 2013; Peng et al., 2017). Most of fermented foods are produced in small-scales, homemade and spontaneously fermented by the microflora naturally present in the raw materials, ingredients, and/or the production environment. The quality of the final products depends on the microbial load and spectrum of the raw material, which may present a high risk of failure. The spontaneously fermented foods are unrepeatable and uncontrollable compared to a starter culture. In contrast, in Western countries, the addition of selected starter cultures to raw material has been a



breakthrough in the processing of fermented foods and in the large scale production. Starter cultures result in high degree of control over the fermentation process and standardisation of the final products. However, the biodiversity of industrial starters has become limited due to the basic selection criteria (e.g. rapid acidification and phase resistance), or the disappearance of certain strains during the propagation and freeze-drying. This often leads to a loss of the uniqueness of the original product and the characteristics that have made the product popular. Recently, food industries have sought strains possessing the desired properties and functionality of the final product. The natural and wild-type LAB isolated from complex ecosystems of traditional fermented foods have been extensively studied because they exhibit a diversity of metabolic activities that diverge strongly from industrial starters (Leroy and De Vuyst, 2004).

Even though lactic acid fermentation can satisfy the demand of Western consumers for a clean label, minimally processed foods, positive effects on health and sustainability, the sensory quality particularly the flavour is the main barrier to the acceptability of fermented fruits and vegetables. Therefore, the selection of LAB starters possessing good metabolic abilities and interesting enzymatic activities towards plant matrices could improve the aroma profiles of fermented foods. This requires to determine LAB starters according to their enzymatic activities in the matrix, which cannot be fully predicted in laboratory conditions.

## II. LITERATURE REVIEW

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### 1. Lactic acid bacteria

#### 1.1. Generalities

The term ‘lactic acid bacteria’ (LAB) describes as group of Gram-positive bacteria with lactic acid as the main product of carbohydrate fermentation that share metabolic and physiological characteristics (Beena Divya et al., 2012; Gänzle, 2015). The common characteristics of LAB are usually catalase-negative, non-spore forming, non-mobile, facultative anaerobes, fermentative, and acid-tolerant bacteria. LAB are either rod-shaped (bacilli) or spherical (cocci) (Hutkins, 2007). These bacteria inhabit in diverse habitats include human cavities (gastrointestinal tract, oral cavity, and respiratory tract), fruits, vegetable, meat, and dairy products. LAB are extensively used in various industrial applications including starter cultures in the dairy industry, probiotics in dietary supplements and bioconversion agents (Sun et al., 2014). LAB are divided in two distinct phyla namely Firmicutes and Actinobacteria. These two phyla are the most greatly studied and exploited. Within the Firmicutes phylum, the most essential genera of LAB are *Enterococcus*, *Lactobacillus* (*L.*), *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus* and *Weissella*, which all belong to the order *Lactobacillales* and are low-GC content organisms (31-49%). Within the Actinobacteria phylum, LAB belong to the *Bifidobacterium* genus, which have a high-GC content (58-61%) (Sun et al., 2014). Recently, International Journal of Systematic and Evolutionary Microbiology (IJSEM) published on Wednesday 15 April, 2020 a new classification scattering the species of the *Lactobacillaceae* family under *Lactobacillus*, *Paralactobacillus*, *Pediococcus* and 23 novel genera *Lactobacillus* taxonomy (Zheng et al., 2020) (<https://www.microbiometimes.com/the-lactobacillus-taxonomy-change-has-arrived-what-do-you-need-to-know/>).

LAB are widely present in numerous fermented foods and beverages since they are considered as nontoxic, food-grade microorganisms, and most of them have a ‘Qualified Presumption of Safety (QPS), a European equivalent of the Generally Recognized As Safe status (The European Food Safety Agency (EFSA, 2007)) (Capozzi et al., 2012). LAB could contribute to the safety, shelf life, sensory quality, and nutritional quality of a majority of fermented foods (Beena Divya et al., 2012). They are generally lacking catalase and need a fermentable carbohydrate for growth. LAB are classified as obligate homofermentative, facultative heterofermentative, and obligate heterofermentative (Gänzle, 2015). While homofermentative LAB convert glucose mainly to lactic acid, heterofermentative LAB convert

glucose to lactic acid, acetic acid, carbon dioxide, and ethanol (Figure 1). In addition, LAB produce other important substances during fermentation including aroma compounds (e.g. acetoin and diacetyl), exopolysaccharides (EPS), bacteriocins, B vitamins (mainly folate, riboflavin, and cobalamin) or low-calorie polyols (mannitol and sorbitol) as well as enzymes. Several enzymes produced by LAB play an important role in the sensory quality of fermented products such as proteases, lipases, pectinases,  $\beta$ -glucosidases, and alcohol dehydrogenases (Beena Divya et al., 2012).

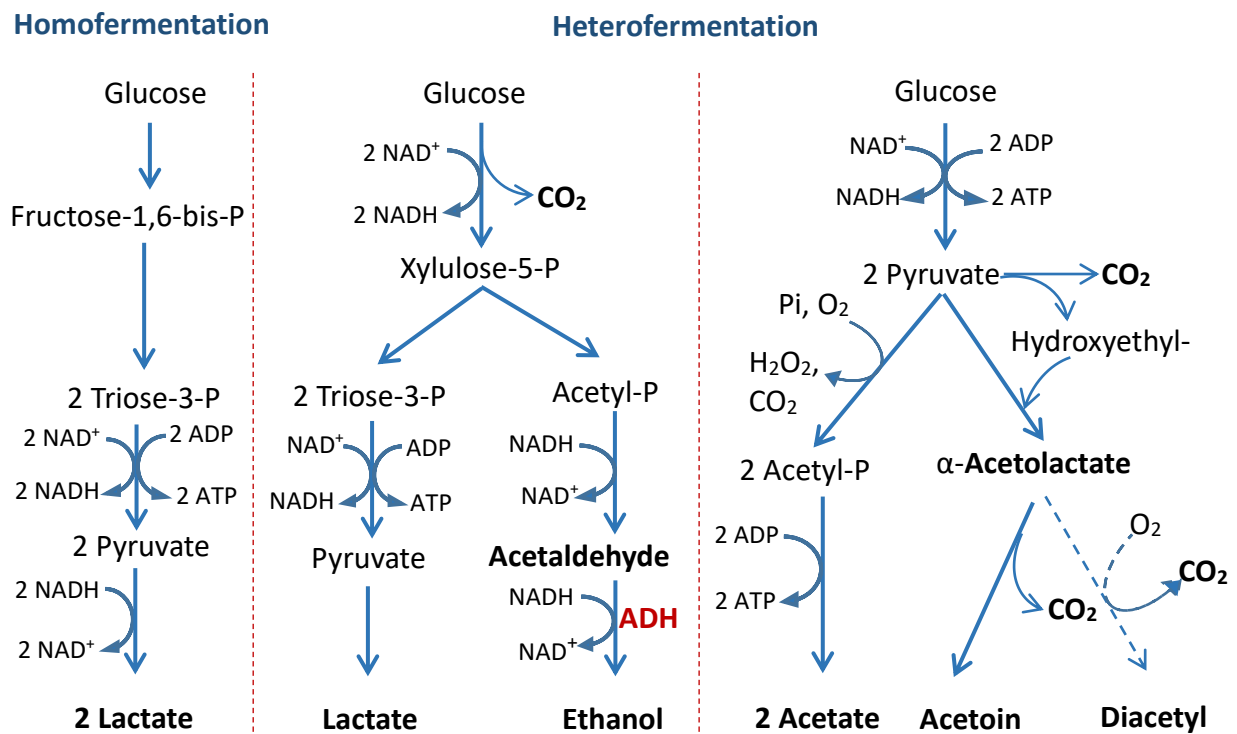


Figure 1. Overview on carbohydrate fermentation of lactic acid bacteria (Gänzle, 2015)

## 1.2. Impacts of LAB on human health

Lacto-fermented foods have unique functional properties imparting some health benefits to consumers. *Kimchi* is one of the most popular traditional fermented vegetables in Korea. *Kimchi* is composed mainly of baechu cabbage with other vegetables and LAB. *Kimchi* contained anti-inflammatory, antibacterial, antioxidant, anticancer, anti-obesity, probiotic properties, cholesterol reduction, and antiaging properties (Patra et al., 2016). *Weissella cibaria* 64 and *Leuconostoc mesenteroides* 12b were able to increase antioxidant activity in fermented pineapple juice (Fessard et al., 2017). In addition to these significant qualities, some LAB could produce exopolysaccharides (EPS) as well. The EPS can contribute to human health (Figure 2)

as prebiotics or due to antitumor, antiulcer, immunomodulating or cholesterol-lowering activities (De Vuyst and Degeest, 1999; Ruas-Madiedo et al., 2002).

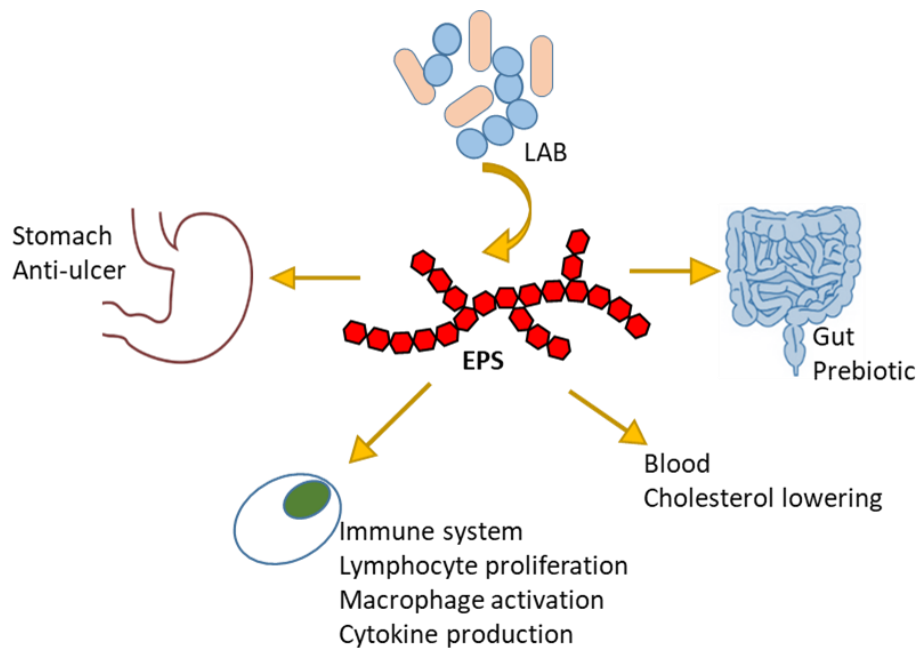


Figure 2. Schematic representation of the possible health-promoting properties of exopolysaccharides produced by lactic acid bacteria (Ruas-Madiedo et al., 2002)

The word probiotic means “for life”. In 2001, FAO/WHO defined probiotics as “live microorganisms which when administered in adequate amounts confer a health benefits on the host” (Hill et al., 2014). The health benefits of probiotics are to restore the natural balance of intestinal microbiota, to promote healthy intestinal function, to boost the immune system, to reduce bloating, gas, and intestinal discomfort (Figure 3). Due to the beneficial effects on human health, probiotics are popularly used in many functional foods and sold in capsule form (freeze-dried cell powders and tablets). The common foods includes dairy products, breakfast cereals, sausages and chocolates. LAB are indeed the most commercially important group of microorganisms as probiotics. *Lactobacillus* and *Bifidobacterium* are the most commonly used probiotics (Beena Divya et al., 2012; Zielińska and Danuta, 2018). Some popular commercial probiotic cultures include *L. acidophilus* NCFM (USA), *L. (Lacticaseibacillus* in the new taxonomy (Zheng et al., 2020)) *rhamnosus* HN001 (USA), *L. rhamnosus* R0011 (Canada), *L. (Lacticaseibacillus) casei* (Japan), *L. (Limosilactobacillus) fermentum* VRI003 (Australia) and *Bifidbacterium lactis* HN019 (USA), and *Bifidobacterium breve* (Japan) (Tamang et al., 2016a).

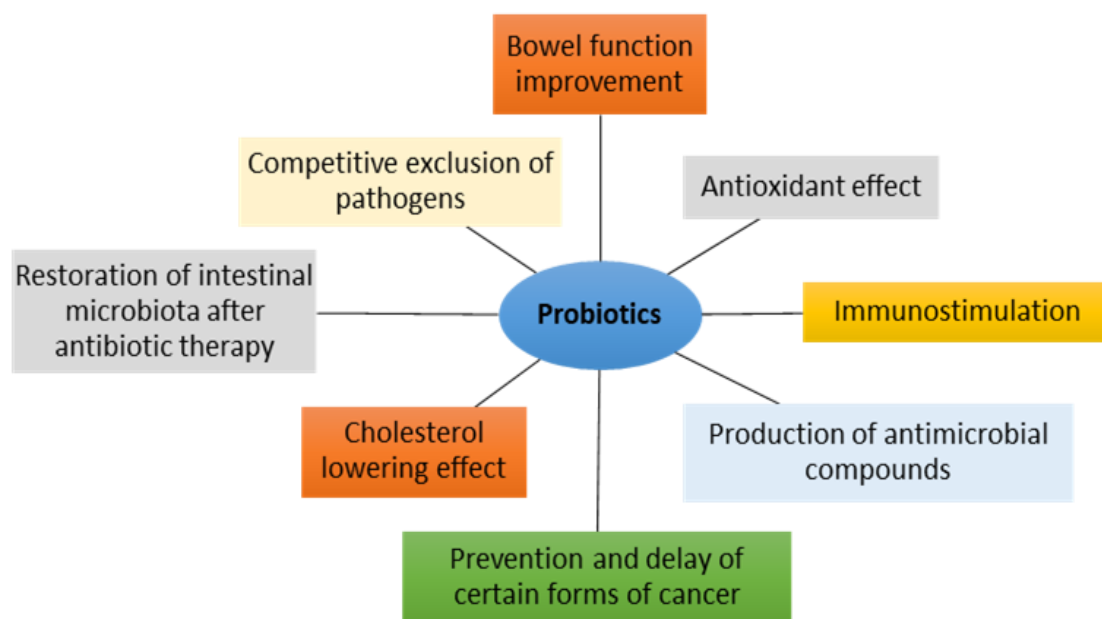


Figure 3. Mode action of probiotics (Beena Divya et al., 2012)

### 1.3. Biopreservation

LAB are important tools of biopreservation because of their ability to produce metabolic products with a strong antimicrobial effect against pathogenic microorganisms. Biopreservation indeed refers to the extended shelf life and enhanced safety of foods using their natural or controlled microflora (Gialamas et al., 2010). LAB might be an alternative antimicrobial agent to replace chemical food preservatives such as nitrite, sulphite, propionic acid, sorbic acid, and benzoic acid (Leroy and De Vuyst, 2004). LAB can inhibit pathogenic microorganisms by one or several synergic effects. In fact, there may be a competition for nutrients, lowering of pH, production of lactic acid, acetic acid, formic acid, phenyllactic acid, caproic acid, hydrogen peroxide, gas (CO<sub>2</sub>) composition of atmosphere or production of antimicrobial compounds such as bacteriocins, reuterin, and reuterincyclin (Gialamas et al., 2010; Leroy and De Vuyst, 2004). *L. (Lactiplantibacillus) plantarum* 21B isolated from sourdough, produced phenyllactic and 4-hydroxy-phenyllactic acids that are responsible for a broad antifungal activity (Lavermicocca et al., 2000). *L. plantarum* IB2 from *inziangsang* (Himalayan fermented leafy vegetable product) exhibited a bacteriocin against *Staphylococcus aureus* S1 (Tamang et al., 2009). *Pediococcus pentosaceus* CFF4 isolated from Cambodian fermented fish and shrimp, produced bacteriocins against *Listeria monocytogenes* NCTC 1199 and *Enterococcus faecalis* ATCC 29212 (Peng et al., 2017). *L. plantarum* H1.40 from *nem chua* (Vietnamese fermented meat), showed high antibacterial activity towards *Staphylococcus aureus*, *Bacillus cereus*,

*Listeria monocytogenes*, *Salmonella* Typhimurium and *Escherichia coli* (Nguyen et al., 2010; Phan et al., 2005).

## 1.4. Impacts of LAB metabolism on the organoleptic properties of fermented foods

### 1.4.1. Different sensory impacts

The major metabolites of LAB carbohydrate metabolism contribute not only to the food preservation, but also to the organoleptic properties of food. Lactic acid changes the texture (coagulation) and flavour (acid taste) of fermented foods. The minor metabolites such as diacetyl, acetoin, ethanol, acetic acid also play a significant role. For instance, acetoin and diacetyl have the desired contribution to wine flavour and dairy products, but it is considered a spoilage of beer (Gänzle, 2015). *L. casei* (7 strains), isolated from milk, had the ability to produce aroma compounds in Parmigiano Reggiano cheese. Among them, *L. (Lacticaseibacillus) paracasei* 4341 produced considerable amounts of the desired ketones, alcohols and acids (Bancalari et al., 2017). In addition, LAB's EPS can contribute to the texture (viscosity and firmness), mouth-feel, taste perception and stability of fermented dairy products (Duboc and Mollet, 2001). They are indeed considered as natural bio-thickener because they are produced in situ by LAB starters. The main bacterial genera including *Lactobacillus*, *Streptococcus*, *Lactococcus* and *Pediococcus* are able to produce EPS (De Vuyst et al., 2001; Ruas-Madiedo et al., 2002).

### 1.4.2. $\beta$ -Glucosidases

#### a. Classification of $\beta$ -glucosidases

$\beta$ -Glucosidases ( $\beta$ -D-glucoside glucohydrolases; EC 3.2.1.21) cleave  $\beta$ -D-glucosidic bonds of the various compounds by releasing glucose and aglycone (e.g. monoterpene, norisoprenoids, and benzene derivatives) as shown in Figure 4 (Ahmed et al., 2017).  $\beta$ -Glucosidases are ubiquitous in nature and can be found in bacteria, fungi, plants, animals, and humans (Krisch et al., 2010).  $\beta$ -Glucosidases among hydrolytic enzymes can be classified according to their substrate specificity or nucleotide sequence identity. According to their substrate specificity,  $\beta$ -glucosidases are categorised in three classes: aryl- $\beta$ -glucosidases (class 1) hydrolysing only aryl- $\beta$ -glucoside linkage, true cellobiases (class 2) hydrolysing only cellobiose, and broad substrate specificity enzymes (class 3) with various abilities for the cleavage of  $\beta$  (1-4),  $\beta$  (1-6),  $\beta$  (1-3),  $\alpha$  (1-4),  $\alpha$  (1-6), and  $\alpha$  (1-3) glycosidic bonds. On the other

hand, according to their nucleotide sequence identity,  $\beta$ -glucosidases are mainly in Glycoside Hydrolase (GH) family 1 and 3.  $\beta$ -Glucosidases belonging to the GH family 1 are reported in archaea, plants, and animals and the GH family 3 from bacteria, yeast, and fungi (Ahmed et al., 2017; Krisch et al., 2010; Singh et al., 2016). Generally, the  $\beta$ -glucosidases of the GH family 3 are localised in the form of extracellular or cell-bound enzymes. Microbial  $\beta$ -glucosidases are localised as intracellular, extracellular, or cell-bound enzymes (Ahmed et al., 2017). Studies have shown that LAB are able to produce  $\beta$ -glucosidases in the form of intracellular, extracellular, and cell-bound enzymes (Gueguen et al., 1997a; Michlmayr et al., 2010; Sestelo et al., 2004).

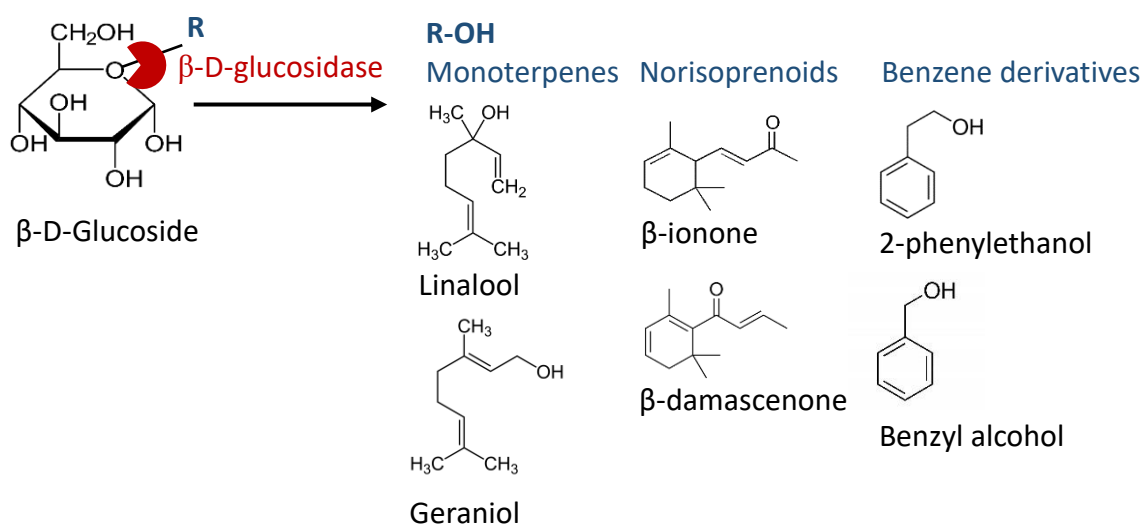


Figure 4. Hydrolysis of glycosidic aroma precursors by  $\beta$ -glucosidase

### b. Applications of $\beta$ -glucosidases

$\beta$ -Glucosidases can be applied in various biotechnological processes such as biofuel production, isoflavone hydrolysis, flavour enhancement, and alkyl/aryl  $\beta$ -D-glucoside and oligo-saccharides synthesis. In biofuel and ethanol production,  $\beta$ -glucosidases hydrolyse short oligosaccharide and cellobiose which have previously been degraded from cellulose by endoglucanase and exoglucanase into glucose units (Figure 5). Glucose can then be fermented to ethanol or other biofuel. In isoflavone hydrolysis, the majority of phenolic compounds (flavonoid, flavonone, flavones, and isoflavone) are naturally in glycoside form, which limits their absorption by the human gastrointestinal tract. The phenolic compounds are essential because of their biological activity as antioxidant, anticancer, anti-allergic, anti-inflammatory agents and so forth.  $\beta$ -Glucosidases are able to release aglycone from flavonoid glycosides. The

released aglycone can be easily absorbed, thereby increasing their biological potency to human health (Ahmed et al., 2017).

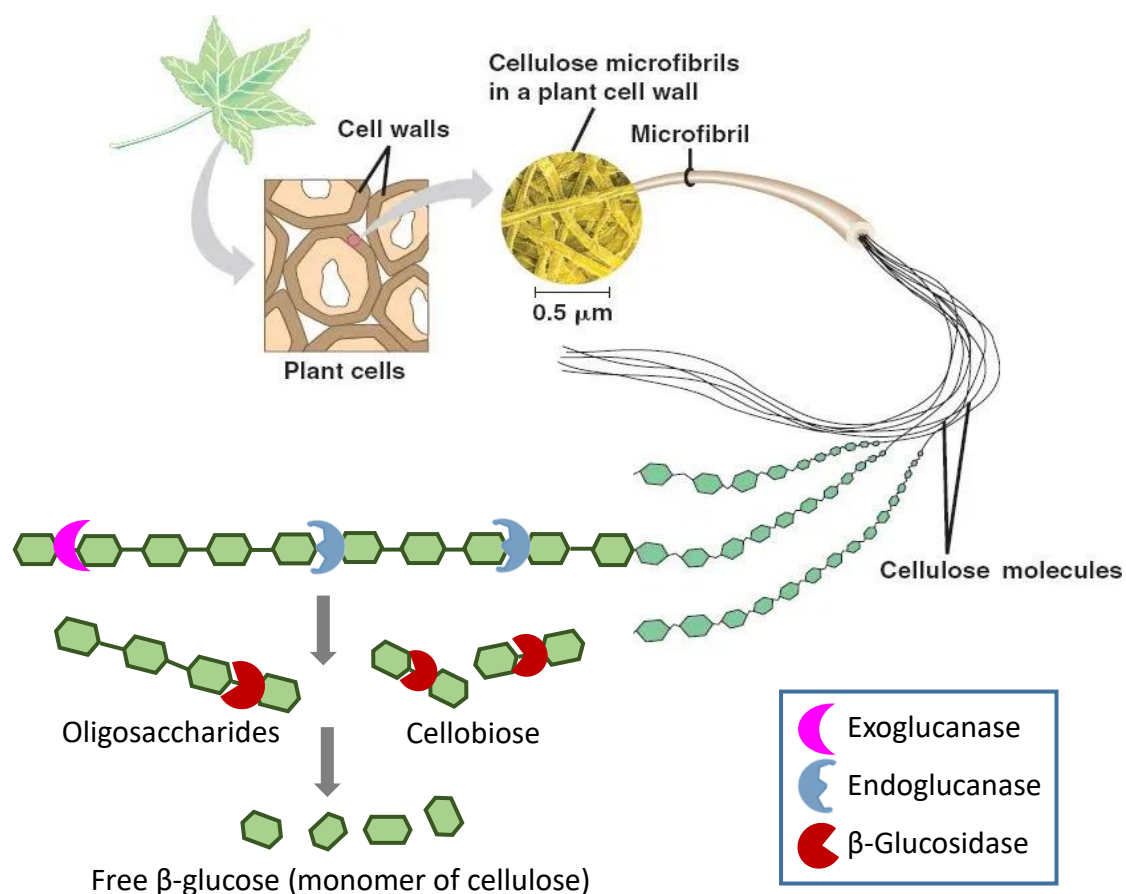


Figure 5. Diagrammatic overview of cellulose metabolism by cellulase system. During cellulose hydrolysis, cellulase along with exo and endoglucanase acts on the cellulosic fibers and hydrolysed it into the smaller sized oligosaccharides. These smaller molecules are finally utilised by β-glucosidase to release glucose (Singh et al., 2016).

In wine making, β-glucosidases play an important role as flavour enhancement. Yeast β-glucosidases (*Candida* spp.) improve the aromatic quality by releasing nerol, geraniol, linalool, terpinene, 2-phenylethanol, and benzyl alcohol from Muscat wine (Gueguen et al., 1996; Gueguen et al., 1997b; Gueguen et al., 1998). A similar study by Swangkeaw et al. (2009) showed that *Pichia anomala* β-glucosidases released glucosidically bound monoterpenes, which were the main contributors to the floral and fruity aroma of wines of Muscat-type varieties. In addition, wine treated with yeast β-glucosidases (*Saccharomyces* and non-*Saccharomyces*) increased C<sub>6</sub> compounds (hexanol, (*E*)-3-hexenol, (*Z*)-3-hexenol), terpenes (menthol), C<sub>13</sub>-norisoprenoids (β-damascenone), alcohols (phenylethanol, benzyl alcohol), and phenol (2-methoxy-phenol, phenol) (Wang et al., 2011). In fruit juices, β-glucosidases were



able to reduce the bitterness of citrus fruit juices by hydrolysing the naringenin to prunin (Krisch et al., 2010; Roitner et al., 1984). *Candida molischiana* 35M5N  $\beta$ -glucosidases increased significantly the release of aroma compounds such as linalool,  $\alpha$ - and  $\gamma$ -terpinene,  $\alpha$ -terpineol, 2-phenylethanol, and  $\alpha$ -pinene in an apricot fruit juice (Gueguen et al., 1996). In tea beverages,  $\beta$ -glucosidases increased the essential oil content by about 7-21% in a treated tea (Su et al., 2010). Beyond hydrolytic activity,  $\beta$ -glucosidase is known to have synthetic activity, namely transglycosylation and reverse hydrolysis resulting in the synthesis of a variety of oligosaccharides, aryl- and alkyl- $\beta$ -glycosides with a wide range of applications (Ahmed et al., 2017).

### c. Applications of LAB $\beta$ -glucosidases on lacto-fermented foods

Numerous studies have shown the ability and use of yeast  $\beta$ -glucosidases to improve the sensory quality of wines, fruit juices, and tea. However, it is only recently that a few studies have focused on the ability of LAB to produce  $\beta$ -glucosidases. *Leuconostoc mesenteroides* (Gueguen et al., 1997a), *L. plantarum* USC1 (Sestelo et al., 2004), *Lactobacillus* spp., and *Pediococcus* spp. (Grimaldi et al., 2005a) isolated from wines and fermented foods showed  $\beta$ -glucosidase activities. Likewise, Hernandez-Orte et al. (2009) found that *Oenococcus oeni*, *Lactobacillus (Levilactobacillus) brevis*, and *L. casei* from musts and wines were able to produce glycosidase activity. These LAB strains released terpenes, norisoprenoids, phenols, and vanillin in the conditions tested (model wine). Although only slight increases in these aroma compounds have been detected, the presence of LAB caused a broad change in the odour profile of the samples (Hernandez-Orte et al., 2009). In addition, Michlmayr et al. (2010) showed that *L. brevis* from a commercially malolactic starter culture exhibited high intracellular  $\beta$ -D-glucosidase,  $\beta$ -D-xylosidase, and  $\alpha$ -L-arabinosidase activities. Renchinkhand et al. (2015) found that six LAB strains of *kimchi* produced  $\beta$ -glucosidase activity. To my knowledge, no studies on LAB  $\beta$ -glycosidases in Cambodian and Vietnamese fermented foods.

LAB  $\beta$ -glucosidase activities can make a significant contribution to the dietary and sensory attribute of fermented foods (Figure 6). The main isoflavones in soybean are the  $\beta$ -glucosides genistein and daidzein, which primarily contribute to human health and disease prevention. The  $\beta$ -glucosidase activities of *L. acidophilus* B4496, *L. bulgaricus* CFR2028, *L. casei* B1922, *L. plantarum* B4495, *L. fermentum* B4655, and *L. rhamnosus* CRL981 hydrolysed glucoside isoflavones to genistein and daidzein at a significant level in the fermented soymilks (Marazza et al., 2009; Rekha and Vijayalakshmi, 2011). Similarly, Pyo et al. (2005) showed that soymilks fermented with *L. plantarum* KFRI 00144, *L. delbrueckii* sp. *lactis* KFRI 01181,

*Bifidobacterium breve* K-101, and *Bifidobacterium thermophilum* KFRI 00748 were able to increase 7.1-fold bioactive aglycones. Furthermore,  $\beta$ -glucosidases of *Lactobacillus* and *Bifidobacterium* species were able to hydrolyse glucoside malvidin and delphinidin (Ávila et al., 2009). In fact, the glucoside malvidin and delphinidin are anthocyanins belonging to the flavonoid family, which have great beneficial effects for humans. Anthocyanins are natural pigments imparting red, blue, and purple colours to many fruits, vegetables, flowers, and leaves. From a technological point of view, anthocyanins are also used as natural colorant or antioxidants in foods and beverages (Burton-Freeman et al., 2016). In addition,  $\beta$ -glucosidase activity of *L. plantarum*, *L. brevis*, and *Pediococcus pentosaceus* hydrolysed oleuropein (Ghabbour et al., 2011; Marsilio et al., 1996). Oleuropein is a phenolic glucoside responsible for the bitterness of unprocessed olives. Moreover,  $\beta$ -glucosidases of *Leuconostoc mesenteroides* hydrolysed the cyanogenic glucosides (linamarin, lotaustralin) presented in cassava pulp and might have been a potential interest in cassava detoxification (Gueguen et al., 1997).



Figure 6. Illustration of the application of LAB  $\beta$ -glucosidase activity on fermented foods. **A**, isoflavones in soymilk; **B**, oleuropein in olives; **C**, anthocyanins in red fruits; **D**, cyanogenic glucosides in cassava pulp; **E**, glycosides in wine

### 1.4.3. Alcohol dehydrogenases (ADHs)

#### a. Classification of ADHs

Alcohol dehydrogenases (ADHs, E.C. 1.1.1.x; x=1 or 2) are a group of oxidoreductases that catalyse the interconversion between alcohols and aldehydes or ketones depending on the presence of cofactor ( $\text{NAD}^+/\text{NADP}^+$ ) and other effectors in the medium (Figure 7a) (Gargouri et al., 2004; Hu et al., 2019). Based on cofactors, the most common ADHs are  $\text{NAD}^+$  and  $\text{NADH}$ -dependent enzymes. The  $\text{NAD}^+$  and  $\text{NADH}$ -dependent ADHs are further classified into zinc-dependent, short-chain, and iron-dependent types (Hu et al., 2019). ADHs are ubiquitous in nature and can be found in humans, plants, and microorganisms. In humans, ADHs serve to break down alcohols (ethanol) that would otherwise be toxic to aldehydes (acetaldehyde)

(Figure 7b (1)). In microorganisms, ADHs actually do the opposite. ADHs change aldehydes and ketones into alcohols that can be used for energy (Figure 7b (2)). The ADH reaction in yeast and LAB plays a quite critical role in sugar metabolism to regenerate cofactors (NADH and NAD<sup>+</sup>) through the reduction of acetaldehyde to ethanol (De Smidt et al., 2008; Gänzle, 2015).

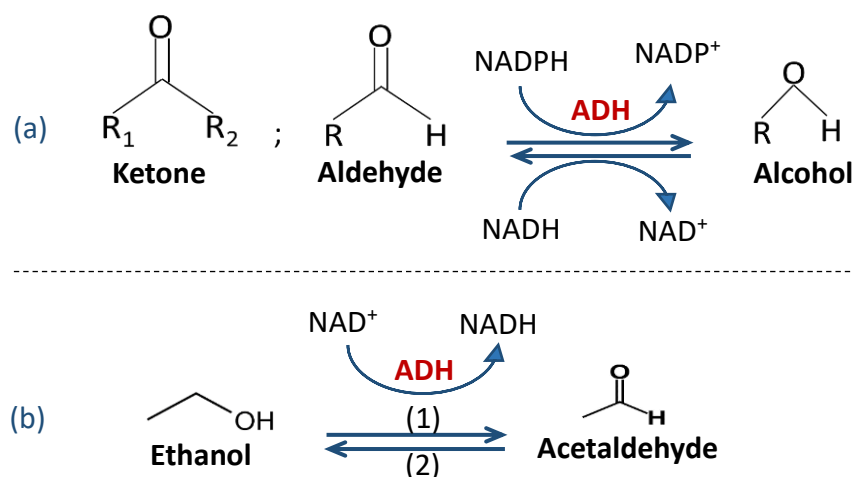


Figure 7. (a) General reaction scheme for the alcohol dehydrogenase (ADH)-interconverting aldehyde/ketone to alcohol; (b) Example of specific reaction of ethanol and acetaldehyde for ADH activity in human (1) and in microorganism (2)

## b. Application of ADHs in pharmaceuticals and fine chemicals

Microbial ADHs have generated considerable interest over the past decade as biocatalysts for the production of chiral pharmaceuticals and fine chemicals (Hu et al., 2019). For chiral pharmaceutical production, ADHs are used as intermediates for the production of antidepressants, anti-anxiety drugs, antiasthmatics, anticholesterol drugs, antihypertensive drugs, anticancer drugs, etc. For fine chemical production, ADHs are utilised for the preparation of  $\gamma$ -valerolactone (GVL) and (2*S*,3*S*)-2,3-butanediol. GVL has been identified as a potential intermediary for the fuel production. Targeted GVL production can be produced from methyl levulinate using ADHs. ADHs are able to reduce diacetyl to acetoin and then to three isomers of 2,3-butanediol, including meso-2,3-butanediol, (2*R*,3*R*)-2,3-butanediol, and (2*S*,3*S*)-2,3-butanediol (Figure 8) (Zheng et al., 2017). Díaz-rodríguez et al. (2014) employed ADHs from *Rhodococcus ruber* (*E.coli*/ADH-A) and *L. brevis* (LbADH) to synthesise a series of enantiopure hydroxyl esters and lactones, and enantioenriched GVL was obtained with >99% conversion and >99% *ee*.

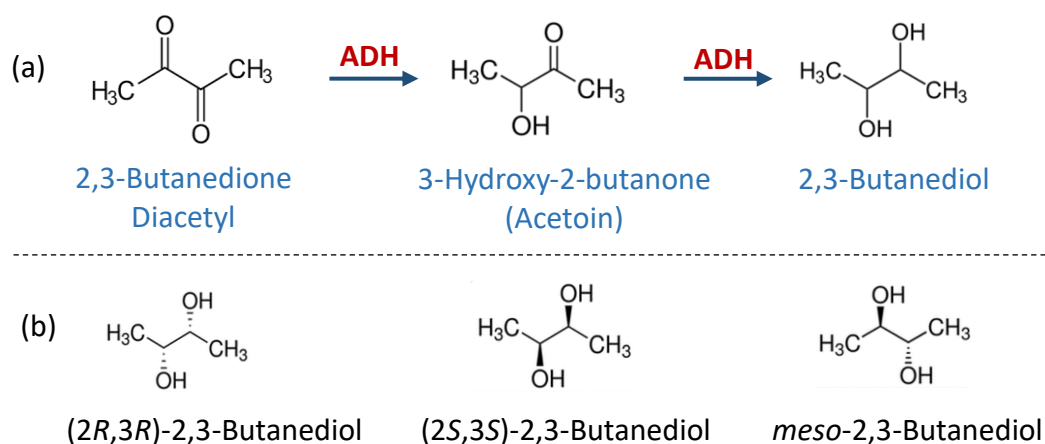


Figure 8. (a) Microbial reduction of diacetyl to acetoin and 2,3-butanediol with alcohol dehydrogenase (ADH) activity; (b) Stereoisomers of 2,3-butanediol (Kim et al., 2017)

### c. Application of ADHs in flavour

ADHs can contribute to the flavour development by interconverting aldehydes or ketones into alcohols. Aldehydes and alcohols are high value molecules widely employed in aromas and fragrances to impart both the green character and the impression of freshness (Speirs et al., 1998). ADH is the last enzyme involved in the LOX pathway, which acts by reducing C<sub>6</sub> or C<sub>9</sub> aldehydes to their corresponding alcohols. Alcohols seem to accumulate during the fruit ripening. For instance, hexanol and (*Z*)-3-hexenol level in tomato increased with ADH activity and reduced with low ADH activity. Fruits with a higher alcohol level were identified as having a more intense “ripe fruit” flavour (Gargouri et al., 2004). Furthermore, the corresponding alcohols can be an attribute for certain fermented products (e.g. cheese and legume-based foods) since they possibly increase the odour threshold, reducing the flavour intensity (Hu et al., 2019). Few studies on ADH activity of LAB have shown that they were able to reduce various aldehydes and ketones to alcohols. Hummel (1990) found that the purified ADH from *L. (Lentilactobacillus) kefir* DSM 20587 catalysed the enantioselective reduction of acetophenone to *R*(+)-phenylethanol. Haberland et al. (2002) studied whole resting cells of *L. kefir* DSM 20587 capable of reducing 2,5-hexanedione to (2*R*,5*R*)-hexanediol. Despite the fact that their encoding genes are generally present in LAB genomes, ADH activity has rarely been reported in LAB. Hu et al. (2019) cloned and recombinantly produced five novel ADH from *L. (Limosilactobacillus) reuteri* DSM20016 in *Escherichia coli* BL21 (DE3). Each purified ADH had a different substrate specificity. ADH-2 reduced isobutyraldehyde, valeraldehyde, and 3-methylbutanal, while ADH-4 reduced isobutyraldehyde, hexanal, and heptaldehyde (Hu et al.,

2019). ADH also plays a key role in flavour formation from amino acids due to its ability to reduce aldehydes to alcohols (Figure 9) (Liu et al., 2008).

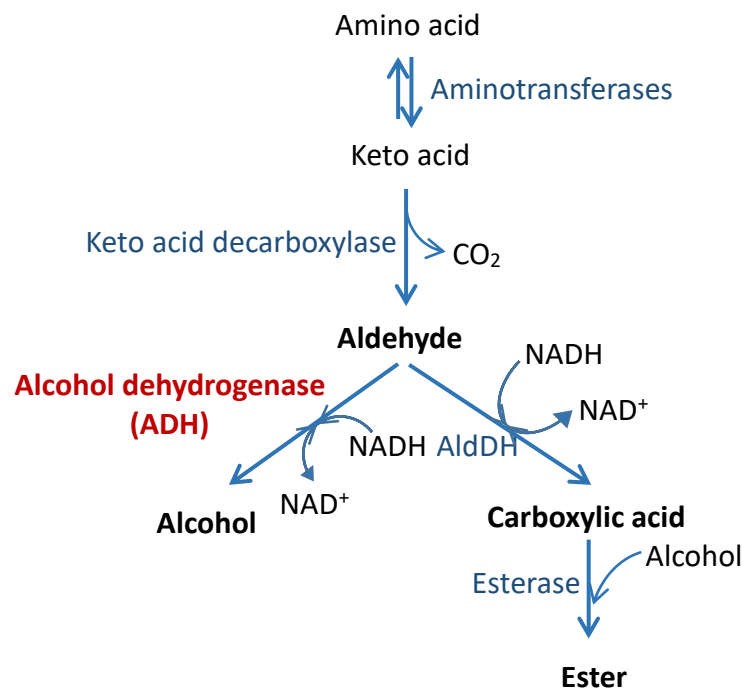


Figure 9. Flavour-forming pathways from amino acids (Liu et al., 2008)

#### 1.4.4. Pectinases

##### a. Classification of pectinases

Pectinases (pectinolytic enzymes) are an enzymatic group that catalyses the degradation of pectic substances, either through deesterification (esterases) or depolymerisation (hydrolases and lyases) reactions (Pedrolli et al., 2009; Singh et al., 2019). Pectic substances are polysaccharides present in the cell wall and the middle lamella, which contributes to the firmness and structural integrity of plant tissues (Gummadi and Panda, 2003). According to their mode of action on pectic substances, pectinases are categorised into esterases and depolymerases (Figure 10). Esterases act on pectin, depolymerases act on pectin, polygalacturonic acid, and rhamnogalacturonan. Esterases are further divided into two classes: (1) pectin methyl esterase (PME, E.C. 3.1.1.11) and (2) pectin acetyl esterase (PAE, E.C. 3.1.1.6). In contrast to esterases, depolymerases are much more complicated in subdivision due to their reaction to various pectin substances. For example, depolymerases acting on pectin are subdivided into: (1) polymethylgalacturanase (PMG, E.C. 3.2.1.1) and (2) pectin lyase (PL, E.C. 4.2.2.10). Depolymerases acting on polygalacturonic acid are further classified into: (1)

polygalacturonase (PG, E.C. 3.2.1.x; x=15, 67 or 82) and (2) pectate lyase (PGL, E.C. 4.2.2.x; x=2 or 9). Finally, depolymerases acting on rhamnogalacturonan are further classified into: (1) rhamnogalacturonase (RG, E.C. 3.2.1.171) and (2) rhamnogalacturonan endolyase (RGL, E.C. 4.2.2.23) (Pedrolli et al., 2009; Singh et al., 2019).

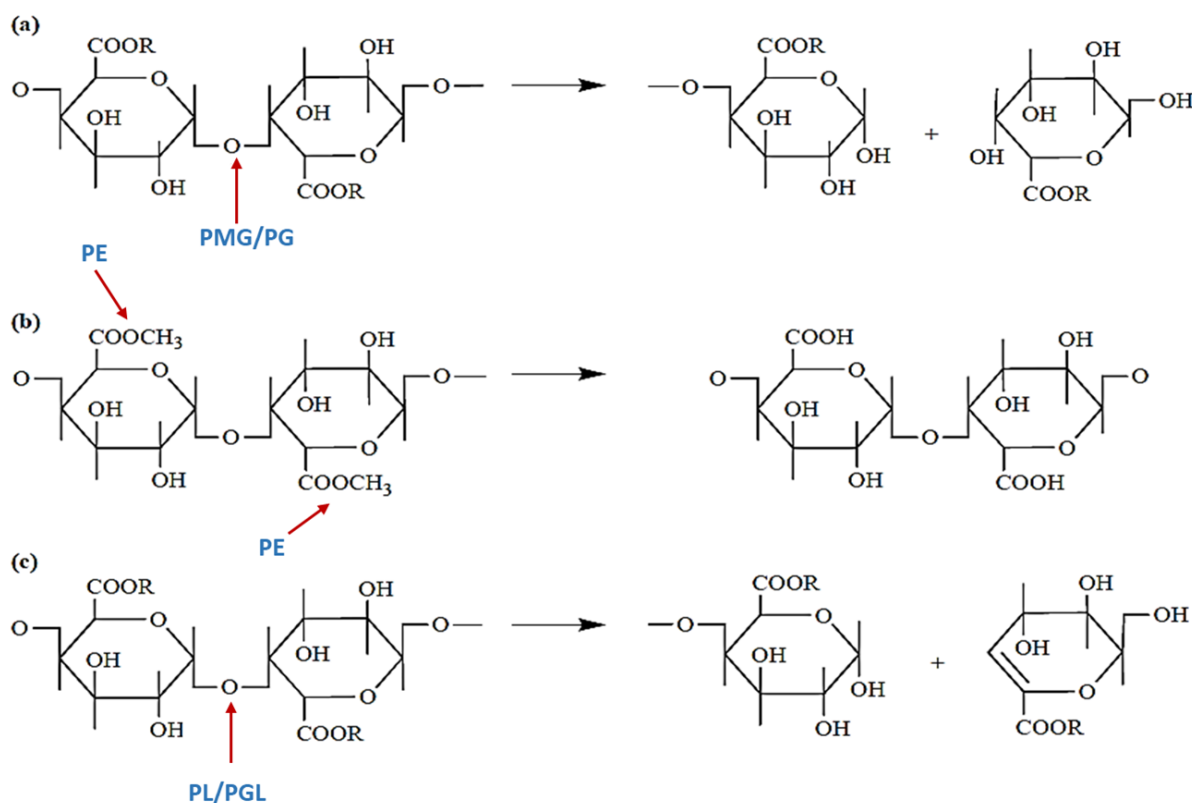


Figure 10. Mode of action of pectinases. (a) R=H for PG and CH<sub>3</sub> for PMG; (b) PE; and (c) R=H for PGL and CH<sub>3</sub> for PL. The arrow indicates the place where the pectinase reacts with the pectic substances. PMG, polymethylgalacturanases; PG, polygalacturonases, PE, pectinesterase; PL, pectin lyase (Pedrolli et al., 2009).

## b. Application of pectinases

Pectinases are one of the most widely distributed enzymes in bacteria, fungi, plants, and animals (Jayani et al., 2005). Pectinolytic enzymes are important for plants as they contribute to cell wall extension and softening of some plant tissues during maturation and storage (Jayani et al., 2005). Pectinases in fruits play natural role in the ripening process. However, microbial pectinases are the most extensively utilised in many processes due to their ease of multiplication and maintenance under controlled conditions. Various fungal and bacterial strains are used for pectinases production (Singh et al., 2019). In general, fungal-derived pectinases are acidic in nature, while bacterial strains secreted mainly alkaline enzymes. The most dominant resource of commercial pectinases is *Aspergillus niger*. There are other genera such as *Penicillium* sp.,

*Rhizomucor* sp., *Rhizopus* sp., *Saccharomyces* sp., and *Bacillus* sp. (Amin et al., 2019). Pectinases have been used in various industries including wine industry, food industry, fruit and vegetable processing, tea and coffee processing, processing of animal feed, textile industry, plant fibre processing, and treatment of industrial wastewater. In fruits and vegetables processing, pectinases play a key role in reducing viscosity, increasing yield, and clarifying (e.g. apple, pineapple, citrus, banana, papaya, carrot, and beet) juices. In winemaking, pectinases have a crucial function to facilitate extraction and filtration, intensify the flavour and colour, and maximise juice yield. In tea and coffee processing, pectinase treatment is extremely useful in accelerating the breakdown of pectin in the cell walls of tea leaves during fermentation. This treatment also eliminates the foam-forming property of instant tea powders. Last but not least, pectinases can also remove the mucilaginous coat from the coffee beans (Garg et al., 2016).

### **c. Pectinases of LAB**

Little work has focused on the pectinolytic enzymes of LAB, yet these enzymes may play an important role in fermented fruits and vegetables. Indeed, pectinases can make the plant tissues less rigid and release nutrients for the growth of bacteria (Juven et al., 1985; Vidhyasagar et al., 2013). LAB appear to produce pectinases in various food matrices. Juven et al. (1985) reported that *Leuconostoc mesenteroides* isolated from tomato juice produced pectinolytic enzymes. Pectinases caused a significant reduction in the viscosity of fermented tomato juice, thus improving juice yield. Furthermore, Vidhyasagar et al. (2013) showed that *Pediococcus pentosaceus* (VJ13, VJ41, and VJ56) and *Leuconostoc lactis* VJ52 of *idli batter* (Indian fermented rice cake), *L. (Lactiplantibacillus) pentosus* SJ65, and *L. plantarum* ssp. *argenteratensis* (SJ6, SJ33, SJ37, and SJ40) of fermented *uttapam batter* (Indian rice pancake) were able to degrade pectins. *Lactobacillus* spp., *Leuconostoc* spp., *Lactococcus* spp., *Pediococcus* spp., and *Streptococcus* sp. (cow raw milk), *Leuconostoc* sp. (black olives), and *Lactobacillus* spp., *Leuconostoc* sp., and *Pediococcus* spp. (green olives) synthesised polygalacturonases (Bekhouche et al., 2006). Likewise, Karam and Belarbi (1995) found that *L. casei* HNK10, *L. casei* L1-8, *L. plantarum* Lc5, and *Lactococcus lactis* NN01 of Algerian raw milk produced polygalacturonases and/or pectin esterases.

## 2. Spontaneously fermented foods of Southeast Asia

Non-dairy fermented foods are popular in Asia. Cambodia and Vietnam share very common fermented foods that are traditionally produced on a small scale using methods passed down from one generation to the next (La Anh, 2015). Most fermented foods result from spontaneous fermentation by indigenous species from raw materials, ingredients, equipment and handling and/or the production environment (Leroy and De Vuyst, 2004). Another process is known as “backslopping”. In backslopping, a small amount of previously fermented batch is used to inoculate a new batch. This results in the domination of the best adapted strains. These methods are cheap, but the fermented products are unrepeatable and uncontrollable compared to a “starter culture”. A starter culture consists of a large number of living microorganisms of a single strain or a mixed culture that initiates and accelerates the food fermentation. This technique can provide particular characteristics in a more controlled and predictable fermentation (Beena Divya et al., 2012). Traditional fermented foods in Cambodia and Vietnam are classified into short-term and long-term fermented foods (La Anh, 2015). Short-term fermented foods generally have a short fermentation time (1 to 3 days) and have a sour taste, known as lactic acid fermentation. The common lacto-fermented foods are fermented fruits/vegetables, shrimp, pork paste (e.g. *nem chua* in Vietnamese), and fish paste (e.g. *nem trey* in Khmer). Long-term fermented foods normally take 6 to 18 months of fermentation time and contain a high salt concentration (up to 33%). The most typical fermented foods are salty fermented fish, fish sauce, soybean paste and soy sauce (La Anh, 2015).

### 2.1. Fermented fruits and vegetables

*Chrouk* or *tram* (in Khmer) and *dua chua* (in Vietnamese) are the common names for lacto-fermented fruit- or vegetable-derived foods (Figure 11). The fermented fruits/vegetables are as simple as described in the following steps. Fruits or vegetables are washed, dried, and soaked in brine in a tight container. The brine solution is a combination of sea salt (2-7%) with/without table sugar and water, boiled, and cooled before pouring into a container. The container is stored at room temperature (20-37 °C) for 1-3 days to obtain optimum acidity which definitely depends on the product type and individual preferences (La Anh, 2015). The main function of salt during fermentation is to promote the growth of LAB over spoilage bacteria and to inhibit potential pectinolytic and proteolytic enzymes that can cause vegetable softening and further putrefaction. Salt also induces plasmolysis in plant cells, which creates anaerobic conditions around submerged products (Swain et al., 2014).





Figure 11. Fermented vegetables. **A**, fermented Thai round aubergine (*ca muoi*); **B**, fermented mustard green (*dua muoi*); **C**, fermented young melon; **D**, Fermented gherkins

The typical fruits used for fermentation in Cambodia and Vietnam are unripe mango, jackfruit, and papaya, while vegetables are numerous such as large petiole mustard, cabbage, cucumber, gherkins, lemon, bamboo shoot, lotus root, bean sprout, radish, carrot, eggplant, radish, leek, garlic and onion bulbs. Some important herbs and spices (garlic, black or red pepper and ginger) are often used as minor ingredients (La Anh, 2015). Another crucial ingredient is LAB. Nguyen et al. (2013) identified LAB in fermented mustard green and beet (*dua muoi*) and fermented Thai round aubergine (*ca muoi*). *L. fermentum* (56.6%), *L. pentosus* (24.4%), and *L. plantarum* (17.1%) were the main LAB species. The least abundant species were *Pediococcus pentosaceus* (1.0%) and *L. brevis* (0.5%). LAB species (<0.1%) included *L. paracasei*, *L. pantheris*, and *P. acidilactici* (Nguyen et al., 2013). To our knowledge, little research has focused on Cambodian fermented foods. The main species of fermented gherkins, fermented young melon, and fermented mustard cabbages were *L. fermentum* and *L. plantarum*, while the minor species were *L. reuteri*, *L. bulgaricus*, and *Pediococcus acidilactici* (unpublished Reasmey Tan research result).

## 2.2. Fermented soybeans

Soybeans are the most traditional fermented and processed legumes into recipes by Asian ethnic groups. Based on microorganisms and salty taste, fermented soybeans are divided into two categories. The first category is fermented soybeans which are only fermented by *Bacillus* (*B.*) spp. known as nonsalty fermented soybeans. *B. subtilis* is an important starter culture for many Asian fermented soybeans. The most common fermented soybeans are *natto* (Japan), *kinema* (India, Nepal, and Bhutan), *pepok* (Myanmar), *thua nao* (Thailand), and *sieng* (Cambodia and Laos). This type of fermentation gives rise to a stickiness characteristic. The second category is soybeans fermented with filamentous fungi, mainly *Aspergillus*, *Mucor*, and *Rhizopus*, namely salted fermented soybeans, for example *miso* (Japan), soy sauce (China),

*tuong* (Vietnam), etc. (Nagai and Tamang, 2010). A few fermented soybeans are shown in Figure 12.



Figure 12. Fermented soybeans. **A**, *natto*; **B**, *sieng*; **C**, *miso*; **D**, *thua nao* (Chukeatirote, 2015)

*Sieng* is a traditional and slightly salted fermented soybean, which is very popular in Cambodia. *Sieng* is consumed as seasonings or side dishes. Fermented soybeans are prepared by soaking the soybeans for a day. After 3-4 washes, soybeans are boiled in water until they become soft. *Sieng* can be fermented by spontaneous fermentation or using a starter culture. Spontaneous fermentation is made by spreading the cooked soybeans in shallow bamboo baskets and allow fermenting naturally for 2 days by microorganisms adhering the baskets and suspended in the air. Starter fermentation is prepared by sprinkling with powdered starter culture *Dombae* (in Khmer) or *Banh men* (in Vietnamese) on cooked soybeans and mixed well. The mixture is then stored in a dry cotton bag at a cool place for about 5-7 days until they are sticky and give the smell of decay. Finally, the sticky soybeans are soaked in salt and sugar solution in a new container for a few weeks. To our knowledge, no research has studied the diversity of *Sieng* microbiota, of which only *B. subtilis* was isolated (Sopheap et al., 2018).

### 2.3. Fermented fish and meat

Fermented fish products are widely consumed in Southeast Asia, but they are also found in other parts of the world. Fermented fish products have a unique aroma, flavour, and texture (Giyatmi and Irianto, 2017). Fermented fish products can be divided into two categories, salty and acidic tastes (Figure 13). Salted fermented fish products are generally used as seasoning or condiment, which provides a strong and complex umami taste. Their common forms are paste and sauce. Fish sauce is a fish-based sauce fermented in brine, which is also known as *nuoc mam* (in Vietnamese), *teuk Trey* (in Khmer), and *nam pla* (in Thai). Fish sauce is an essential ingredient in the cuisine of Southeast Asia, especially in Vietnam, Cambodia, Thailand, and Laos. Several microbial strains isolated from *nuoc mam* belong to genera *Micrococcus*, *Staphylococcus*, *Streptococcus*, and *Lactobacillus* (e.g. halophilic *L. plantarum*) (La Anh,

2015). Unlike *nuoc mam*, the main isolates in *teuk Trey* were spore-forming bacilli (*Bacillus*, *Virgibacillus*, *Lentibacillus*, *Lysinibacillus*, and *Clostridium*) and cocci (*Staphylococcus*, *Micrococcus*, and *Kocuria*) (Chuon et al., 2014). Another important fermented fish product in Cambodia is fish paste, known as *prahok* (in Khmer). The halophilic and halotolerant bacteria genera in *prahok* are *Staphylococcus*, *Tetragenococcus*, and *Clostridium*, while aerobic spore-forming bacilli (*Bacillus*, *Virgibacillus*, *Halobacillus*, and *Lysinibacillus*) (Chuon et al., 2014).



Figure 13. Fermented fish and meat. **A**, Vietnamese fermented raw pork (*nem chua*); **B**, Cambodian fermented raw fish (*nem trey*); **C**, Cambodian fermented fish (*prahok*); **D**, fish sauce (*nuoc mam*)

Unlike salted fermented fish, acidic fermented fish products contain less salt and taste sour. *Nem chua* (in Vietnamese) and *nem trey* (in Khmer) are traditional lacto-fermented sausages in Vietnam and Cambodia, respectively. They are consumed as an appetiser and prepared by a similar process, except that *nem chua* is made from pork and *nem trey* is made from fish. *Nem trey* is prepared by blending unboned fish with garlic, sugar, salt, sodium glutamate, and black/white peppers. Preparing *nem chua* requires more ingredients than *nem trey*. *Nem chua* is made by mixing finely ground lean pork (95% protein), sliced boiled pork rind (5% protein), powdered roasted rice, salt (2%, w/w), and spices (black/white pepper and garlic). The fish or pork paste is then shaped into small cubes or cylinders. The shaped pastes are covered with herbs (pieces of guava or Ming aralia leaves) and chili pepper for decoration and flavour. They are finally wrapped with banana leaves to prevent air penetration into the paste, thus creating a relatively anaerobic environment that promotes the growth of LAB. The sour taste and flavour begin to appear after 2 to 4 days depending on the room temperature (20-37 °C). Powdered roasted rice not only adds flavour to *nem chua*, but also acts as a carbohydrate source for the growth of indigenous LAB, thereby providing an acidic taste (La Anh, 2015). The final pH of *nem chua* is between 4.6-4.8, which is necessary to maintain the product at acceptable microbiological safety (La Anh, 2015). The cell density of LAB in *nem chua* is log

9±1.3 CFU/g. The dominant indigenous LAB species in *nem chua* were *L. plantarum*, *L. brevis*, *L. farciminis*, and *Pediococcus pentosaceus* (La Anh, 2015).

### 3. Tomato

#### 3.1. Tomato consumption and production

Tomato (*Solanum lycopersicum*) is the most popular vegetable in the world (WorldAtlas, published June 19, 2018), which can be consumed fresh or processed. There are now more than 500 varieties of tomatoes (<https://www.planetoscope.com/fruits-legumes/1266-production-mondiale-de-tomates.html>). Eurofresh Distribution, published on September 7, 2016, reported that global tomato production was around 130 million tons, of which 88 million tons were for the fresh market and 42 million tons for processing. The top five largest tomato producers were China, the EU, India, the US, and Turkey. Fresh tomatoes can be used in salads, hot/cold dishes, soups, juices, smoothies, etc. Processed tomatoes include canned and sun-dried tomatoes, juice, ketchup, paste, mash, sauce, and soup (Di Cagno et al., 2009a; Shi and Maguer, 2005). While the quantity of processed tomatoes has considerably increased, the quantity of tomato pomace has also increased significantly. Tomato pomace is a by-product of tomato with a mixture of tomato peels, seeds, and small amounts of pulp that remain after processing the tomato for juice, paste, and/or ketchup. The tomato pomace represents approximately 4% of the fruit weight. The amount of related wastes in the world is estimated at up to 50,000 tons per year (Simitzis and Deligeorgis, 2018). Unused waste can worsen the disposal problem and aggravate environmental pollution. However, an important amount of tomato pomace has been destined for poultry, dairy cows, and small ruminants (Ventura et al., 2009). The tomato pomace contained (by dry weight) about 59% fibre, 26% total sugars, 19% protein, 8% pectin, 6% total fat, and 4% minerals (Valle and Torija, 2006). Furthermore, tomato by-products can serve as functional food ingredients. Kalogeropoulos et al. (2012) showed very intriguing results between tomato by-products and unprocessed tomatoes. The tomato by-products contained significantly lower amount of lycopene, but higher amounts of  $\beta$ -carotene, tocopherols, phytosterols, and polyphenols (mainly flavonoids and terpenes) compared to unprocessed tomatoes. Their fatty acid profile and total polyphenols were similar. Due to the essential compounds remaining in the tomato pomace, it could therefore be a promising raw material for lactic acid fermentation.

### 3.2. Tomato bioactive compounds and human health

Tomatoes and tomato-based products are important sources of health-promoting components of the world's population. The main components in tomatoes (Figure 14) are carotenoids such as  $\beta$ -carotene and mostly lycopene.  $\beta$ -Carotene is converted into vitamin A or retinol (the active form of vitamin A) in the body. Tomatoes and tomato-based products are the primary sources of dietary lycopene (about 80%). In addition to carotenoids, tomatoes contain vitamins (ascorbic acid and tocopherols), phenolic compounds including hydroxycinnamic acids (mainly caffeic acid and its ester chlorogenic acid), flavonoids (naringenin and rutin), and other bioactive compounds (glycoalkaloids and lectins). They also provide essential minerals such as K, Mn, Ca, Cu, and Zn (Leonardi et al., 2000; Pinela et al., 2016; Sahlin et al., 2004; Sass-Kiss et al., 2005).

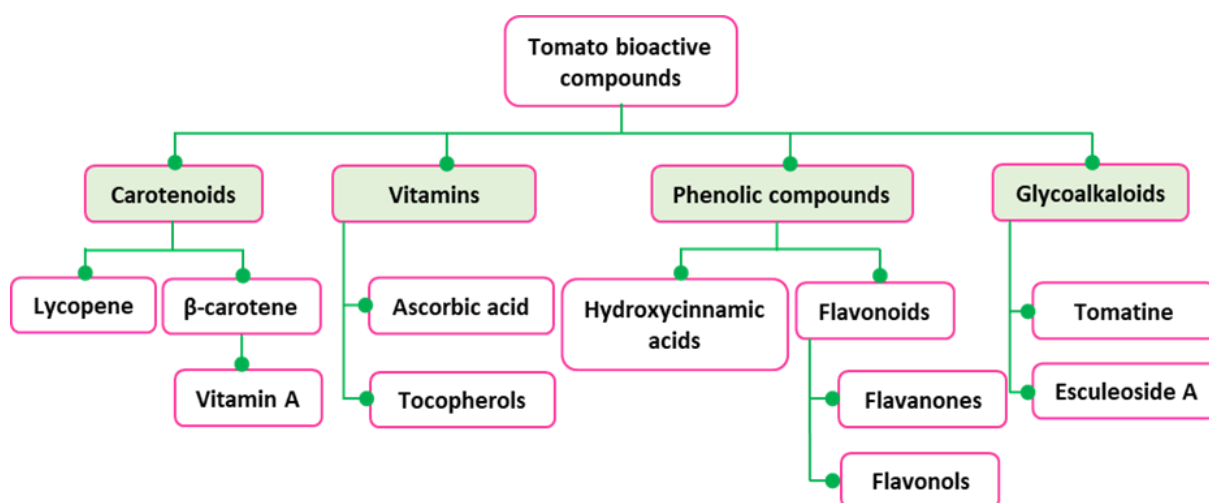


Figure 14. Groups of bioactive compounds of tomatoes (Pinela et al., 2016)

It should also be mentioned that the way food is prepared and consumed has a huge impact on our health. For instance, the beneficial actions of most carotenoids such as those of tomatoes, corn, carrots, etc., might be improved by cooking them, especially in oil (Sass-Kiss et al., 2005). Regular consumption of tomatoes and tomato products has been associated with several positive effects on human health, due to mainly the presence of antioxidant and anti-inflammatory properties. Studies have shown the benefits of tomato bioactive compounds, either isolated or in combined extracts including anti-carcinogenic, anti-mutagenetic, anti-cholesterolemic, anti-diabetic, cardioprotective, and hepatoprotective effects among other health benefits (Agarwal and Rao, 1998; Campbell et al., 2004; Clinton et al., 1996; Lavelli et al., 2000; Parfitt et al., 1994; Pinela et al., 2016; Rao and Agarwal, 1999; Weisburger, 1998).

Indeed, the bioactive compounds are capable of acting as free radical scavengers of reactive oxygen and nitrogen species (ROS/RNS). ROS and RNS include free radicals and other non-radical reactive substances, also called oxidants. ROS and RNS are generated as a normal part of human metabolism and their production can be promoted by external factors (smoking, radiation, drugs, pesticides, etc.) (Carocho and Ferreira, 2013). The accumulation of these species in the body gives rise to a phenomenon known as oxidative stress. In fact, the oxidative stress results from an imbalance between generation and neutralisation of reactive species in the cells. The production of ROS and RNS during oxidative stress and inflammatory processes is extensively associated with the development and progression of chronic diseases including cancer, diabetes, cardiovascular diseases, and other disorders associated with aging. The tomato bioactive compounds can neutralise the generated reactive species, thus preventing associated diseases (Pinela et al., 2016).

### 3.3. Aroma precursors in tomato

Aroma compounds are formed in the intact tomato fruit during ripening as well as upon tissue disruption (e.g. cutting, chewing, and cooking). When cell disruption occurs, previously compartmentalised enzymes and substrates meet, thus forming new volatiles (Baldwin et al., 2000). During processing, saturated and unsaturated C<sub>6</sub> and C<sub>9</sub> aldehydes and alcohols are released from fatty acids by the endogenous enzymes of tomatoes through the lipoxygenase (LOX) pathway. This LOX pathway is explained in more detail below. Meanwhile, terpene and carotenoid derivative compounds can be released from glycosides by endogenous tomato glycosidases activities (Servili et al., 2000). Over 400 volatile compounds (VCs) have been identified in tomatoes including aldehydes, alcohols, ketones, terpenes, and furans. Only 29 VCs present in concentrations over 1 ppb and about 16 compounds indicate a significant contribution to tomato aroma as shown in [Table 1](#) (Buttery and Ling, 1993; Klee, 2010; Wang et al., 2016). These VCs can be released from various aroma precursors ([Figure 15](#)) such as amino acids, fatty acids, carotenoids, terpenoid, and glycosides (Baldwin et al., 2000).

Table 1. Sixteen volatile compounds present in fresh ripe tomatoes with their precursors, concentrations, odour thresholds, log odour units, and odour descriptions (Buttery and Ling, 1993; Klee, 2010; Wang et al., 2016)

Volatile compound	Precursor	Concentration (ppb in water)	Odour threshold (ppb in water)	Log odour units	Odour description
<b>Aldehydes</b>					
(Z)-3-Hexenal	Fatty acids	12 000	0.25	4.7	Tomato, green
Hexanal	Fatty acids	3100	4.5	2.8	Green, grassy
3-Methylbutanal	Branched chain amino acid	27	0.2	2.1	Musty
(E)-2-hexenal	Fatty acids	270	17	1.2	Green
(E)-2-heptenal	Fatty acids	60	13	0.7	Green
2-Phenylacetaldehyde	Phenylalanine	15	4	0.6	Floral, alcohol
<b>Ketones</b>					
$\beta$ -Ionone	Carotenoids	4	0.007	2.8	Fruity, floral
1-Penten-3-one	Fatty acids	520	1	2.7	Fruity, floral, green
$\beta$ -Damascenone	Carotenoids	1	0.002	2.7	Fruity
6-Methyl-5-hepten-2-one	Carotenoids	130	50	0.4	Fruity, floral
<b>Alcohols</b>					
(Z)-3-Hexenol	Fatty acids	150	70	0.3	Green
2-Phenylethanol	Phenylalanine	1900	1000	0.3	Nutty, fruity
3-Methylbutanol	Branched chain amino acid	380	250	0.2	Earthy, musty
<b>Nitrogen-and oxygen-containing compounds</b>					
1-Nitro-2-phenylethane	Phenylalanine	17	2	0.9	Musty, earthy
<b>Esters</b>					
Methyl salicylate	Phenylalanine	48	40	0.008	Green
<b>Sulfur-containing heterocyclic compounds</b>					
2-Isobutylthiazole	Branched chain amino acid	36	3.5	1.0	Tomato wine, green

### 3.3.1. Amino acids

A few aroma compounds considered relevant for tomato aroma are derived from amino acids (alanine, valine, leucine, isoleucine, and phenylalanine). They can be divided into phenolic and branched-chain compounds (Figure 15). One of the most important phenolic compounds is 2-phenylethanol derived from phenylalanine. 2-phenylethanol was previously described as having a positive effect on tomato flavour, increasing the floral aroma and the perception of sweetness (Baldwin et al., 2008). The branched-chain compounds such as 2-methylbutanal, 3-methylbutanal, 3-methylbutanol, and 2-isobutylthiazole are also important since they are considered to participate in tomato aroma (Buttery et al., 1988).

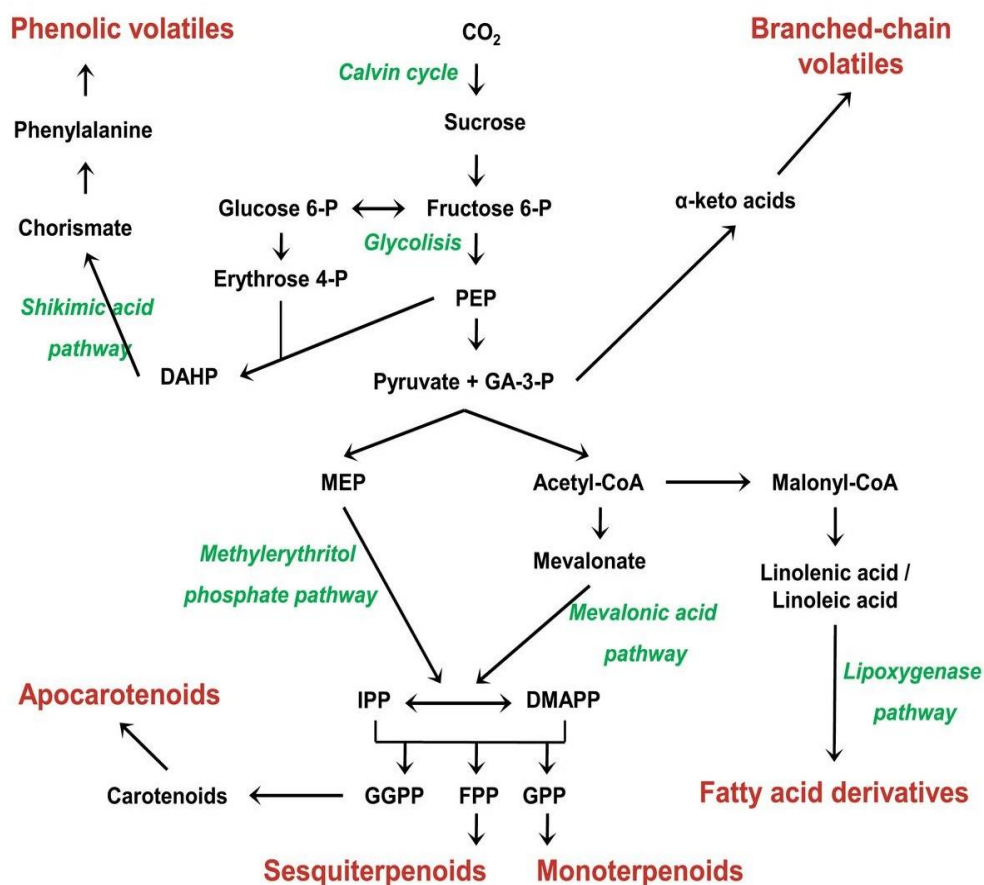


Figure 15. Biosynthetic pathways of the most relevant classes of volatiles in tomatoes. Volatile classes are highlighted in **bold**; metabolic pathways are represented in *italics*. Abbreviations: DAHP, 3-deoxy-D-arabino-heptulosonate 7-phosphate; DMAPP, dimethylallyl diphosphate; FPP, farnesyl diphosphate; GA-3-P, glyceraldehyde-3-phosphate; GGPP, geranyl diphosphate; IPP, isopentenyl diphosphate; MEP, 2-C-methyl-d-erythritol 4-phosphate; PEP, phosphoenolpyruvate (Rambla et al., 2014).

### 3.3.2. Fatty acids

Fatty acids are precursors of essential aromas responsible for the fresh, green, and fruity notes of fruits and vegetables. The degradation of fatty acids occurs primarily by three oxidative pathways: (1)  $\beta$ -oxidation, (2) oxidation by the LOX pathway, and (3) autoxidation.  $\beta$ -Oxidation typically takes place in intact tissues during the fruit and vegetable ripening process. This can lead to a variety of volatile compounds such as saturated and unsaturated lactones, esters, alcohols, ketones, and acids. In contrast, volatile aldehydes and alcohols are typically produced by the LOX pathway and the autoxidation. These aldehydes and alcohols are responsible for fresh and green sensorial notes. The LOX pathway generally occurs in fruits and vegetables during the ripening process, after tissue damage or in response to stress. The LOX pathway is a multi-enzymatic system in which polyunsaturated fatty acids are converted



into aldehydes and alcohols by sequential action of lipoxygenase, hydroperoxide lyase, and alcohol dehydrogenase. The biosynthetic pathway of volatiles derived from fatty acids is illustrated in Figure 16. Linoleic and linolenic acids are typically oxidised into 9-, 10- or 13-hydroperoxides depending on the specificity of the LOX (EC 1.13.11.12) catalyst. These compounds are then cleaved by hydroperoxide lyase (HPL) into mainly C<sub>6</sub>, C<sub>9</sub>, and C<sub>10</sub> aldehydes. These aldehydes can be subsequently reduced to corresponding alcohols by ADH (EC 1.1.1.1) (Akacha and Gargouri, 2015; Christensen et al., 2007).

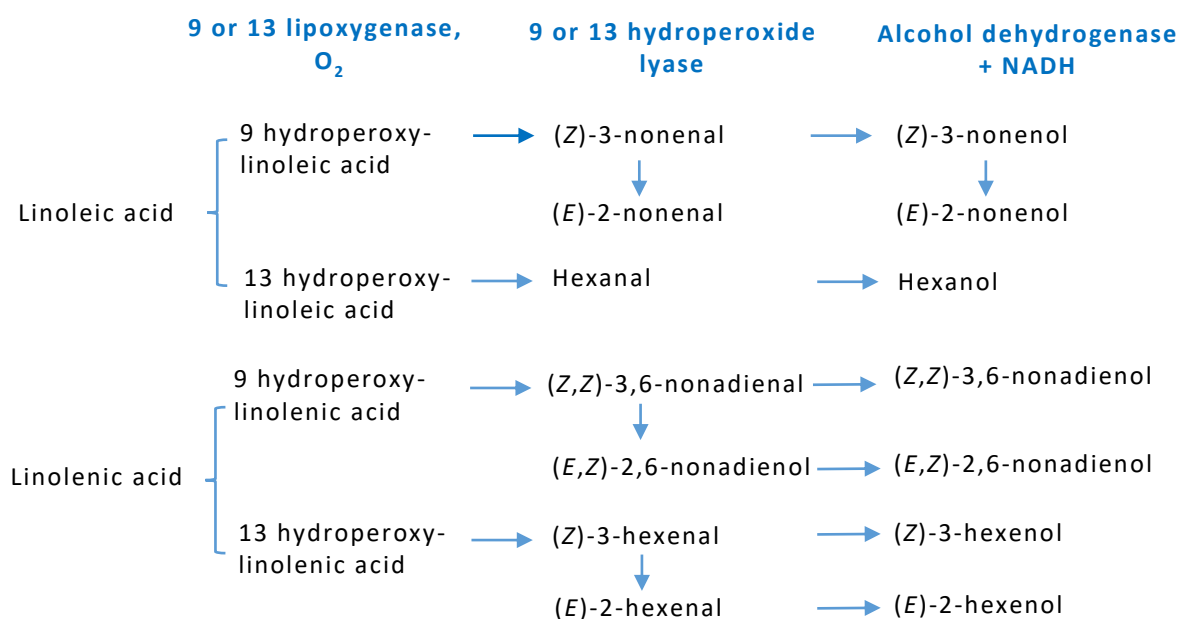


Figure 16. Major products, flavour precursors, and enzymes involved in the lipoxygenase pathway (Akacha and Gargouri, 2015).

C<sub>6</sub> aldehydes and alcohols are among the most abundant volatiles in tomato, imparting “green or grassy” notes. Their biosynthesis starts with linoleic (C<sub>18:2</sub>) or linolenic (C<sub>18:3</sub>) acid, which are acted upon by tomato LOX (TomloxC) to produce 13-hydroperoxides (Figure 17). In tomato, TomloxC is a chloroplast-targeted lipoxygenase isoform (Chen et al., 2004). The 13-hydroperoxides are then cleaved by 13-hydroperoxide lyase (13-HPL), releasing hexanal and (Z)-3-hexenal. (Z)-3-hexenal can be further converted to (E)-2-hexenal by enzymatic or non-enzymatic isomerisation. Finally, alcohol dehydrogenase2 (ADH2), the first enzyme convincingly linked to tomato C<sub>6</sub> volatile production, catalyses the reduction of C<sub>6</sub> aldehydes to corresponding alcohols (Baldwin et al., 2000; Wang et al., 2016).

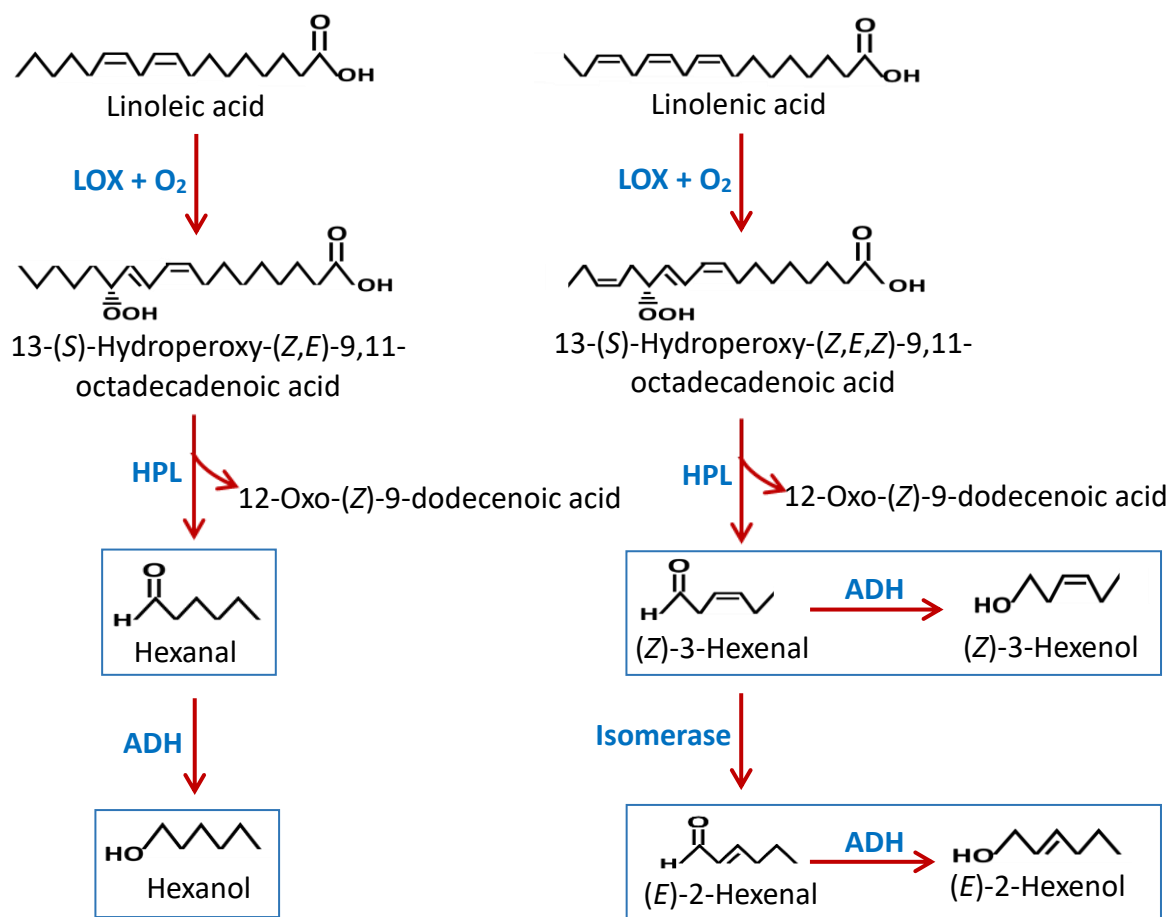


Figure 17. Enzymatic degradation of linoleic and linolenic acids via the lipoxygenase pathway to C<sub>6</sub> key aroma compounds in fruits and vegetables responsible for green notes (Christensen et al., 2007). Abbreviations: LOX, lipoxygenase; HPL, hydroperoxide lyase; ADH, alcohol dehydrogenase

### 3.3.3. Carotenoids

Carotenoids are responsible for fruit and vegetable pigments generally orange, red, and yellow. Beyond their functions as colorants and nutrients, carotenoids are crucial flavour precursors of fruits and vegetables (Vogel et al., 2010). Indeed, carotenoids can be broken down into smaller molecules known as apocarotenoids via the oxidative cleavage. The apocarotenoids are produced by enzymatic and non-enzymatic pathways. Non-enzymatic oxidative cleavage includes photo-oxygenation, (auto) oxidation, and thermal degradation processes. Conversely, the enzymatic pathway, also called carotenoid cleavage dioxygenases (CCDs), catalyses the selective cleavage of carotenoids. CCDs are a large family of non-heme iron-dependent enzymes. Depending on the position of the backbone (C<sub>40</sub>) cleavage, the CCDs can result in volatile aldehydes or ketones (Harrison and Bugg, 2014). A three-step mechanism is generally

required for the formation of the aroma compounds in food (Figure 18). The first step, also called oxidative cleavage is CCDs of the parent carotenoid giving rise to a primary cleavage product. The second is enzymatic transformation (e.g., reduction and glycosylation) in plant tissues. The final step is acid-catalysed conversion during processing of food (Winterhalter, 1996).

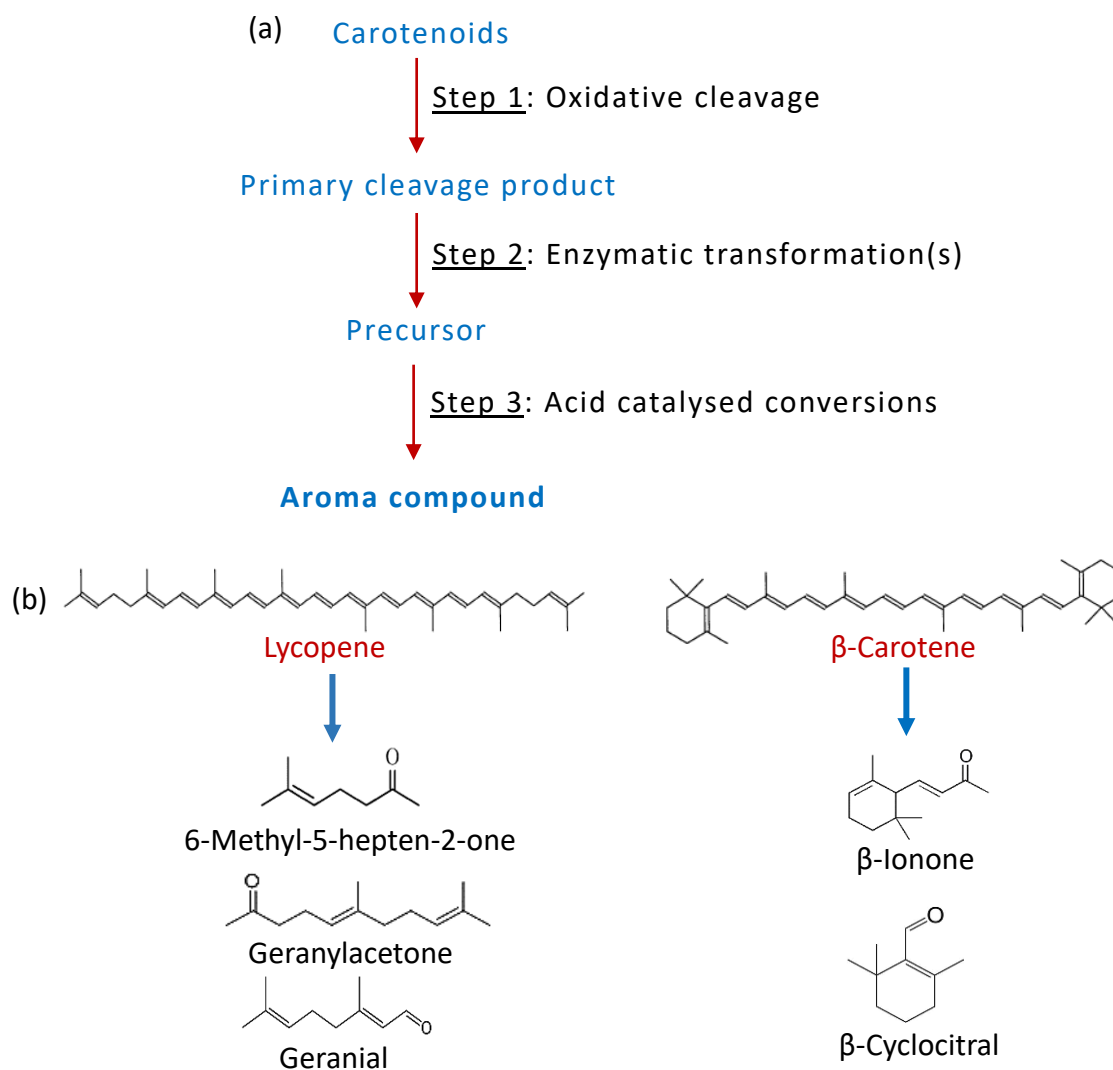


Figure 18. (a) General steps for the conversion of carotenoids into aroma compounds; (b) Aroma compounds released from carotenoids (Winterhalter, 1996).

Tomato fruits contain high levels of lycopene (linear carotenoid) and  $\beta$ -carotene (cyclic carotenoid) that can be degraded into apocarotenoids including  $\beta$ -cyclocitral, geranial,  $\beta$ -ionone,  $\beta$ - and  $\alpha$ -damascenone, geranylacetone, and 6-methyl-5-hepten-2-one (MHO). These aroma compounds accumulate at high level during fruit ripening and processing. Apocarotenoids are generally described as having fruity and/floral attributes. Although the apocarotenoids in tomato contain a very low concentration, they have extremely low odour

thresholds and humans can be quite sensitive to them. The apocarotenoids can be divided into two separate structural and perceptual (odour thresholds) classes. Linear apocarotenoids include MHO and geranylacetone with odour thresholds of 50 nL/L and 60 nL/L, respectively. Cyclic apocarotenoids are  $\beta$ -ionone,  $\beta$ -damascenone, and  $\beta$ -cyclocitral with odour thresholds of 0.007 nL/L, 0.002 nL/L, and 5 nL/L, respectively (Vogel et al., 2010).

### 3.3.4. Glycosides

A glycoside is a molecule in which a carbohydrate (glycone) is bound to another functional group (aglycone) via a glycosidic bond (Figure 19). A significant portion of aroma

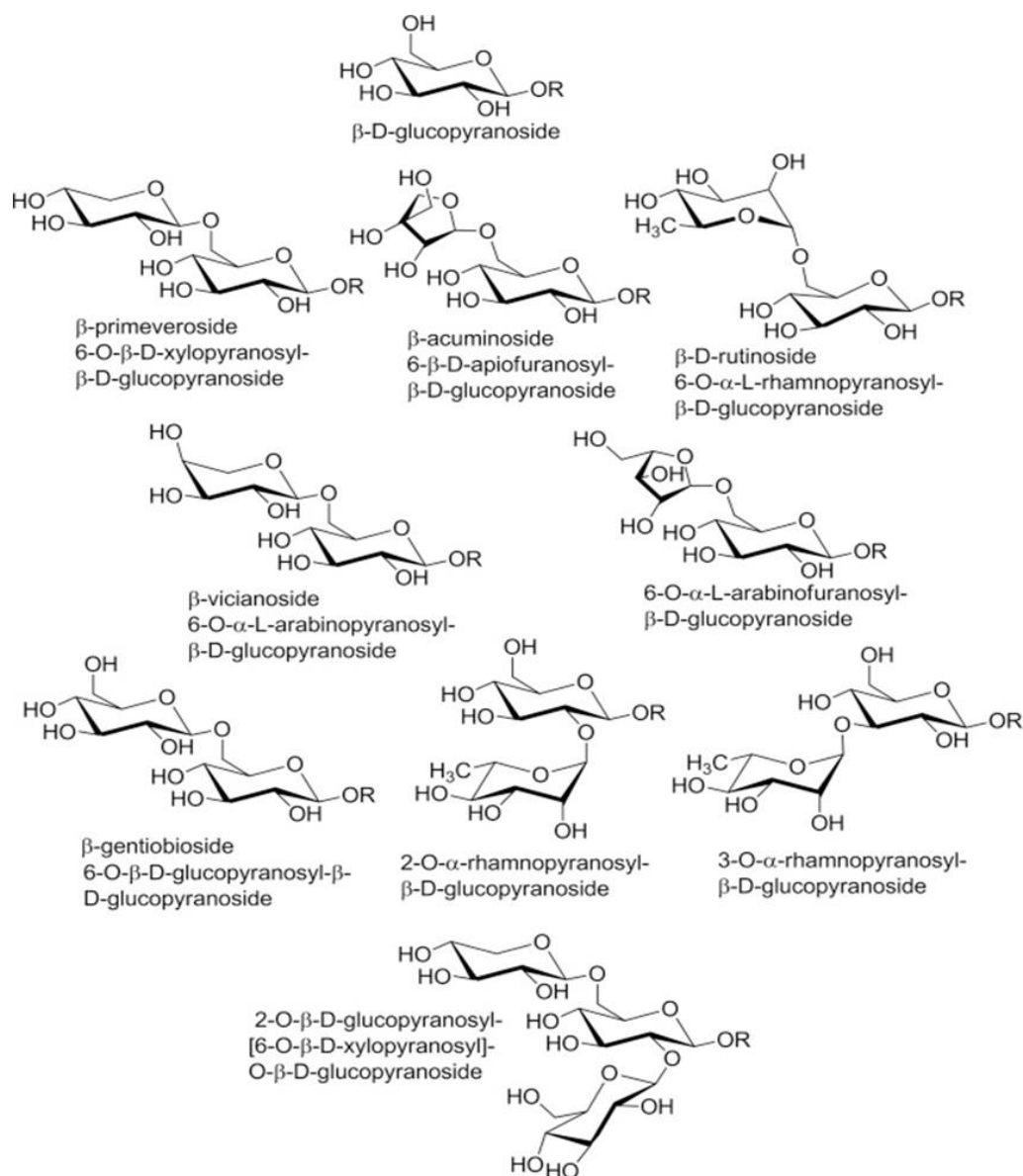


Figure 19. Structure of glycosidic aroma precursors from plant. R denotes aglycones (Song et al., 2018).

compounds accumulates in as non-volatile and odourless glycosides, also known as glycosidic aroma precursors. Glycosidic aroma compounds in fruits and vegetables are mainly *O*- $\beta$ -D-glucosides or *O*- $\beta$ -D-diglycosides. In few cases, triglycosides are observed (Winterhalter and Skouroumounis, 1997). The glycone part is represented by glucose or disaccharides (rhamnose-glucose, arabinose-glucose, and apiose-glucose) or trisaccharides. The aglycone moiety of glycosides includes monoterpenes,  $C_{13}$ -norisoprenoids, benzene derivatives, and aliphatic alcohols (Cabaroğlu et al., 2003). The aglycone can be released from glycosides by enzymes, acids or heat during maturation, storage or processing (e.g. fermentation) as shown in Figure 20 (Garcia et al., 2013; Reineccius, 2005). Some liberated aglycones can be odorous, in particular linalool, geraniol, and nerol. In contrast, some are able to give rise to potent flavour compounds, such as  $\beta$ -damascenone, vitispirane, and theaspirane by further enzymatic and/or chemical transformations during fruit juice or leaf products processing (Sarry and Günata, 2004).

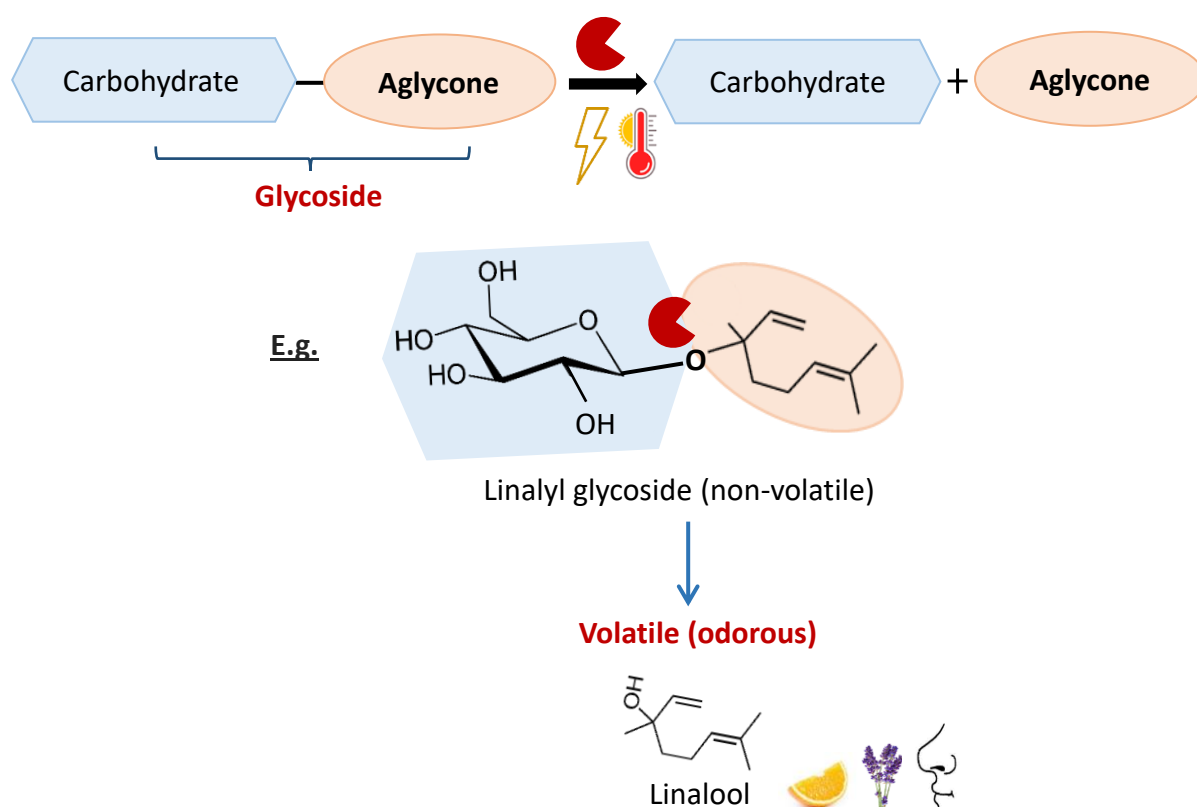


Figure 20. Hydrolysis of glycosides by  $\beta$ -glucosidase, acid or heat

Glycosides are found in tomatoes as glucosides and glucosyl esters of *p*-coumaric acid, caffeic acid, ferulic acid, and sinapic acid. Volatile compounds occur in tomato not only in free forms, but also in glycosidically bound form. Volatile glycosidically bound compounds are

released by acid or  $\beta$ -glucosidase hydrolysis. In fact, the volatile compounds are naturally released during the fruit ripening by endogenous enzymes,  $\beta$ -glucosidases. However, the natural process by endogenous  $\beta$ -glucosidases shows low activity, takes a long time, and cannot release the whole aromatic potential; therefore, supplementation with efficient exogenous  $\beta$ -glucosidases can enhance aroma compounds in wine and foods (Krisch et al., 2010; Sestelo et al., 2004). Few studies have investigated the release of volatile compounds from glycosidic precursors in tomatoes by using acid or exogenous  $\beta$ -glucosidases. The volatile compounds released were geraniol, linalool, (*E*)- and (*Z*)-linalool oxides, hotrienol,  $\alpha$ -terpineol,  $\beta$ -citronellol, 4-vinylguaiacol, 4-vinylphenol, 2-phenylethanol, benzyl alcohol, benzoic acid, phenylacetaldehyde, benzaldehyde, (*E*)-2-heptenal, 6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-ol, and other C<sub>13</sub>-norisoprenoids (Buttery et al., 1990; Marlatt et al., 1992; Ortiz-Serrano and Gil, 2010). C<sub>13</sub>-norisoprenoids such as  $\beta$ -damascenone (0.002 nL/L) and  $\beta$ -ionone (0.007 nL/L) were considered to be major contributors to tomato flavour due to their extremely low odour thresholds (Buttery et al., 1990).

#### 4. Aroma compounds analysis

Aroma compounds or odorants are organic molecules of low molecular weight (<400 Da) and volatile at atmospheric pressure and room temperature which causes an olfactory stimulation when they encounter the olfactory mucosa (Seuvre and Voilley, 2017). The odorants are one of the main attributes of consumer acceptability of food. They are formed because of physiological processes in fruits and vegetables, are produced by microorganisms in fermented processes and because of the processing (thermal treatment, cutting, etc.) of food products or raw materials (Jeleń, 2014). From more than 7000 volatile compounds identified in food, only 5% have an impact on food aroma (de Oliveira Felipe et al., 2020). Their impact on food aroma depends on their concentrations as low as ng/L, and on their odour thresholds (Jeleń, 2014). The odour thresholds (OT) are the lowest concentration of a certain aroma compound that is perceivable by the human sense. The OT are determined by placing an aroma compound in a background similar to a food medium and testing to determine the level at which it can be detected by smell (Baldwin, 2002).

Aroma compounds or odorants in food represent various chemical classes including terpene (hydrocarbons, alcohols, aldehydes, and ketones), C<sub>13</sub>-norisoprenoids, esters, lactone, carbonyl compounds, phenols, sulfur compounds, and heterocyclic compounds (Seuvre and Voilley, 2017). These compounds possess different volatility, polarity, chemical character, stability, and reactivity (Jeleń, 2014). Therefore, several factors have to be considered for a successful qualitative and quantitative analysis of aroma compounds in foods. They include:

- Complex nature and diversity of food aroma compounds to be generally determined in a single run
- Different concentrations of aroma compounds and other volatiles,
- Complexity of the matrix and effect on the release of the aroma compound,
- Extraction specificity guaranteeing maximum recovery of compounds and protecting analytes from decomposition,
- Stability of the matrix and of the aroma compound,
- Method performance-competitive to the human nose in terms of limits of detection (LOD) and quantification (LOQ) (Jeleń, 2014).

Despite the challenge of aroma analysis, three important approaches are commonly used to analyse the aroma compounds in food, including (Jeleń, 2014):

- Target analysis of specific odorants, is generally used for identification of known odoriferous compounds related to specific odour note of particular food, for example, off-flavors.
- Sensory guided analysis of key odorants is the approach that focuses on identifying the compounds responsible for the aroma of a given product. Beginning with the sensory profile analysis to obtain the main odour notes, then chromatographic methods are used to find key odorants.
- Profiling of aroma compounds is frequently used for the comparison of products, treatments, processes, and storage conditions (Jeleń, 2014).

Foods are complex products containing volatile and non-volatile compounds (carbohydrates, proteins, lipids, salt). The separation process of aroma compounds from raw material or food are called extraction. Extraction methods used for analysis of odour active compounds can be roughly divided into exhaustive and non-exhaustive extractions (Figure 21). Exhaustive extraction allows total transfer of aroma compounds from matrix into the extracting solvent, for example liquid/liquid extraction. Non-exhaustive extraction is based on partition of the analyte between matrix and the headspace (Jeleń, 2014). Furthermore, three main methods have been proposed for analysing aromas including analytical distillation, direct extraction, and headspace (HS) extraction (Augusto et al., 2003; Poole et al., 2016). Analytical distillation techniques consist in vapour distillation (steam-assisted distillation and hydro-distillation) and simultaneous distillation-extraction (also called Lickens-Nickerson method). Direct extraction involves application of liquid/liquid extraction (LLE), solid phase extraction (SPE), and supercritical fluid extraction (SFE) directly to the samples to isolate odorants. Finally yet importantly, HS extraction is based on the manipulation of the HS in contact with odorous materials, where a certain volume of HS is transferred from vial, which was equilibrated (at certain temperature and time) into injection port of gas chromatograph using different transfer method (using syringe or a loop transfer). Since the odorants are volatile, HS analysis or by a collection of the volatile compounds in HS using sorbent devices or cold traps are widely used. The common HS analysis techniques are static headspace analysis (SHS), dynamic headspace analysis (DHS), and headspace solid phase microextraction (HS-SPME) (Augusto et al., 2003). HS-SPME are most often applied to fruits/vegetables, wine, meat products, dairy, and beverages (Merkle et al., 2015).



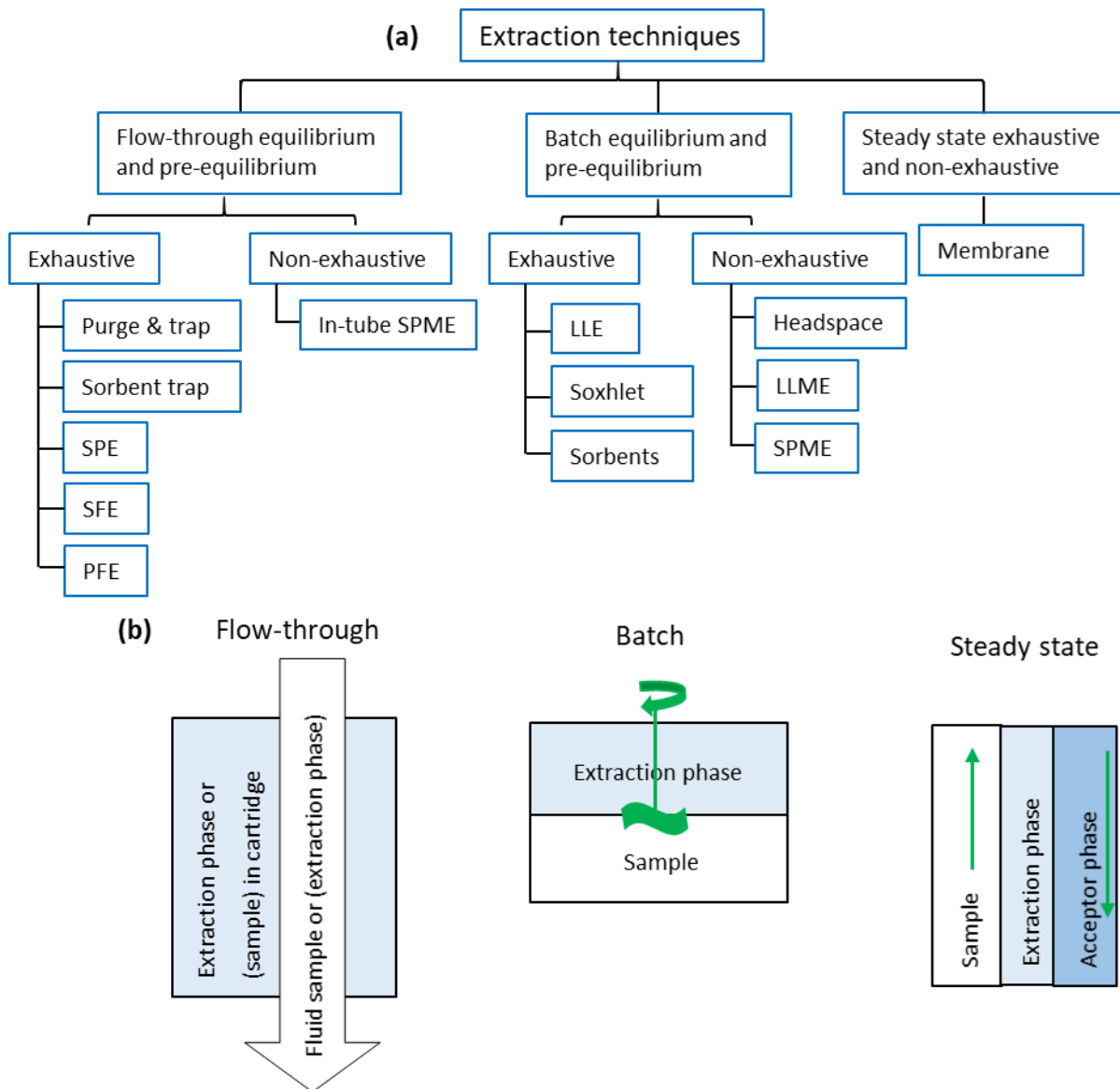


Figure 21. (a) General classification of extraction techniques; (b) Schematic showing the principles of the extraction process behind classification. Abbreviations: solid phase extraction (SPE), supercritical fluid extraction (SFE), pressurised fluid extraction (PFE), liquid-liquid extraction (LLE), liquid/liquid micro-extraction (LLME), solid phase micro-extraction (SPME) (Poole et al., 2016)

#### 4.1. SPME generalities

SPME is a solvent-free extraction method that uses a fused silica fibre coated with a thin film of sorbent to extract volatile analytes from a sample matrix. It was invented by Pawliszyn and Arthur in 1989. SPME integrates sampling, extraction, concentration and sample introduction into a single step. The method saves preparation time and disposal costs, and can improve detection limits. SPME has been commonly used in combination with gas

chromatography (GC) and GC/mass spectrometry (GC/MS). It has been successfully applied to a wide variety of compounds, in particular for the extraction of volatile and semi-volatile organic compounds from food, biological, and environmental samples (Vas and Vékey, 2004). The SPME apparatus is a very simple device. As shown in Figure 22, the fibre is housed within a syringe needle that protects the fibre and allows for easy penetration of sample and GC vial septa. Two important elements of SPME apparatus include SPME manual holder/autosampler and fibre assembly. The fibre assembly contains three parts, including a septum-piercing needle, a fibre attachment tubing, and a fused-silica fibre (1-2 cm long).

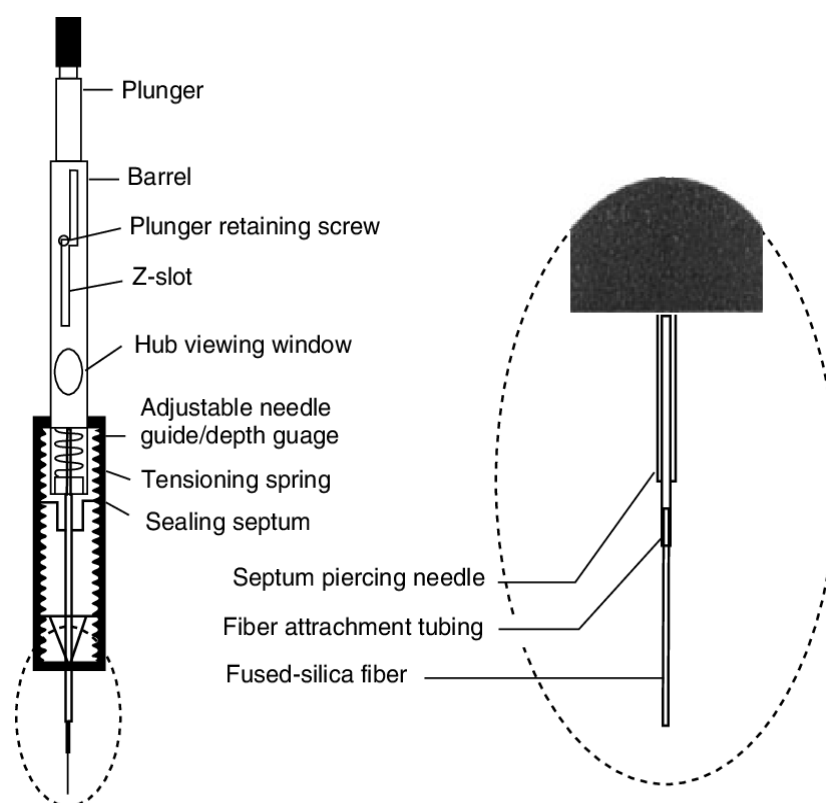


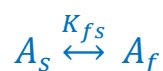
Figure 22. Schematic diagram of a commercial SPME device (Vas and Vékey, 2004)

#### 4.2. Technical aspects and analytical performance

The underlying principle of the SPME methodology is based on the partition of analytes between a coated fibre and a sample (Merkle et al., 2015). Mass transfer begins after exposure of fibre to the vapour phase above a solution (HS-SPME) or direct immersion in the solution (DI-SPME). This process is governed by the second law of thermodynamics. The amount of analyte extracted by the polymer coating in DI and HS mode could be determined by equations (1) and (2), respectively (Burgot and Pellerin, 2003; Pawliszyn, 1999). The choice of sampling mode and gas chromatography depends primarily on the polarity and volatility of the analyte.

Volatile analytes are most conveniently studied by HS-SPME followed by GC or GC/MS, whereas polar and non-volatile analytes are most often studied by DI-SPME, followed by GC. In addition, HS-SPME can reduce matrix effects and interference present in the liquid sample. DI-SPME is typically used with an aqueous sample free of carbohydrates, proteins, or fat and to quantify a target analyte (Roberts et al., 2000). HS-SPME takes less time to reach the equilibrium than DI-SPME because there is no liquid to prevent diffusion of the analyte onto the coating (Ho et al., 2006).

The equilibrium of analyte ( $A$ ) in DI mode with two phases (the fibre ( $f$ ) and the sample ( $s$ )):

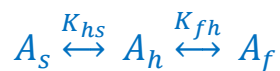


The number of moles of analyte absorbed/adsorbed by the polymeric coating ( $n_f$ ) is related to the overall equilibrium of the analyte in a two-phase system.

$$n_f = \frac{C_0 V_s V_f K_{fs}}{K_{fs} V_f + V_s} ; K_{fs} = \frac{C_f}{C_s} \quad (1)$$

Where  $C_0$  is the initial concentration of the analyte in the sample.  $C_f$  and  $C_s$  are the equilibrium concentrations of the analyte in the fibre and the sample, respectively.  $V_f$  and  $V_s$  are volume of coating and sample, respectively.  $K_{fs}$  is distribution constant (or partition coefficient) of the fibre/sample (Burgot and Pellerin, 2003; Pawliszyn, 1999).

On the other hand, extraction in HS mode introduces an additional equilibrium compared to DI mode. The equilibria of analyte ( $A$ ) in HS mode with three phases (the fibre ( $f$ ), the HS ( $h$ ), and the sample ( $s$ )):



The number of moles of analyte absorbed/adsorbed by the polymeric coating is related to the overall equilibrium of the analyte in the three-phase system.

$$n_f = \frac{C_0 V_f V_l K_{fh} K_{fs}}{K_{fh} K_{hs} V_f + K_{hs} V_h + V_s}; K_{hs} = \frac{C_h}{C_s}; K_{fh} = \frac{C_f}{C_s}; K_{fs} = K_{hs} K_{fh}$$

$$= \frac{C_f}{C_s} \quad (2)$$

Where  $C_0$  is the initial concentration of the analyte in the sample.  $C_f$ ,  $C_h$ , and  $C_s$  are the equilibrium concentrations of the analyte in fibre, HS, and sample, respectively.  $V_f$ ,  $V_h$ , and  $V_s$  are volume of coating, HS, and sample, respectively.  $K_{hs}$ ,  $K_{fh}$ , and  $K_{fs}$  are distribution constant of the HS/sample, fibre/HS, and fibre/sample, respectively (Burgot and Pellerin, 2003; Pawliszyn, 1999).

A few parameters such as the volatility and the concentration of the analyte and the ionic strength of the sample, which are related to the analyte and the fibre, control the extent of absorption/adsorption onto SPME fibre. The choice of the appropriate polarity and thickness of the coating on the fibre is also crucial. The agitation and heating can reduce the equilibration time of less volatile compounds (Ho et al., 2006). Some physical properties (polarity, volatility, and molecular weight) of organic compounds are shown in Table 2.

Table 2. Physical properties of organic compounds

		← volatility		
↑ polarity	polar	volatile alcohols ketones carboxylic acids	semi-volatile alcohols ketones carboxylic acids phenols	non-volatile high molecular electrolytes carbohydrates
	semi-polar	ethers esters aldehydes	ethers esters aldehydes epoxides heterocyclic compounds	protein carbohydrates
	non-polar	aliphatic compounds aromatic compounds  small	aliphatic compounds aromatic compounds alicyclic compounds medium	non-ionic polymers lignin large
		→ molecular weight		

### 4.2.1. Fibre coating type

The SPME fibre is coated with a thin polymer (stationary phase), which can be a liquid polymer, a solid sorbent, or a combination of both (Merkle et al., 2015). The coating is able to concentrate the organic analytes (or inorganic volatiles) during absorption/adsorption from the sample. The choice of polymer coating depends on the nature of the analyte, its polarity, and its volatility (Burgot and Pellerin, 2003). Several types of coating fibres are commercially available for the analysis of aroma compounds as shown in Table 3. SPME coatings can be broadly classified into four categories including type of coating, coating thickness, polarity, and absorbent/adsorbent mechanism. There are polydimethylsiloxane (PDMS), divinylbenzene (DVB), carbowax (CW; polyethylene glycol), carboxen (CAR), and polyacrylate (PA). These fibres exist in various coating combinations, blends or copolymers as well (Merkle et al., 2015). PDMS and PA are the most popular examples of absorption or liquid fibre coating. PDMS is the most useful coating because it has very good thermal stability up to 300 °C. PDMS fibre is better suited for analysing non-polar analytes, while PA fibre is more useful for extracting polar analytes. On the other hand, the primary extraction phase of mixed coatings is a porous solid. For instance, CAR/PDMS and DVB/CAR/PDMS fibres provide the best extraction efficiency for a wide range of analytes with different polarities and molecular weights (Merkle et al., 2015). Another porous polar solid coating is DVB, which is efficient in extracting polar compounds. Mixed coatings of PDMS (non-polar) and DVB (polar) often more efficiently extract bipolar compounds such as alcohols, aldehydes, ketones, ethers, and carboxylic acids (Merkle et al., 2015).

Table 3. Types of commercially available SPME coatings

Type of coating	Extraction mechanism	Polarity
7, 30, 100 µm Polydimethylsiloxane (PDMS)	Absorbent	Non-polar
85 µm Polyacrylate (PA)	Absorbent	Polar
60 µm Carbowax (CW)	Absorbent	Polar
Carboxen (CAR)/PDMS	Adsorbent	Bipolar
PDMS/ Divinylbenzene (DVB)	Adsorbent	Bipolar
DVB/CAR/PDMS	Adsorbent	Bipolar

### 4.2.2. Ionic strength, agitation, and heating

Salt concentration and salt type have a significant effect on HS-SPME extraction efficiency. The addition of salting-out reagents, usually NaCl,  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{Na}_2\text{SO}_4$ , or  $\text{K}_2\text{CO}_3$ , increase the ionic strength of solution, which can decrease the solubility of analytes (Burgot and Pellerin, 2003). Therefore, the amount of analyte absorbed/adsorbed on the fibre is increased. This effect depends on the particular analyte and salt concentration in the sample (Merkle et al., 2015). The ionic strength could be part of the extraction optimisation. Another important factor is agitation (stirring), which greatly improves the extraction yield and reduces time to reach equilibrium. Agitation increases precision especially for analytes with high distribution constants as well. Finally, temperature (heating) is crucial in HS-SPME extraction because it promotes the vaporisation of the analytes and their passage from the matrix to the HS (Burgot and Pellerin, 2003). Nevertheless, temperature is an adequate factor. If the temperature is too high (over 60 °C), it can create the effect of catalysis such as cooking, etc.

### 4.3. Identification and quantification

SPME technique is primarily used for qualitative or semi-quantitative (screening) studies. The traditional sampling methods attempt to remove completely the analytes of interest from the sample. In contrast, with SPME, the amount of analytes removed by the fibre is proportional to their concentration in the sample. Similar to traditional sampling methods, the quantification of SPME requires external calibration (e.g. drinking water), standard addition or internal standards. HS-SPME involves multi-phase equilibrium processes, internal standards are always recommended. Therefore, the physicochemical properties of the internal standards are essential considerations. Qualitative optimisation of the SPME parameters should be applied to determine the best coating and sampling conditions to use before selecting a quantitation approach and calibrating the instrument. The reproducibility and precision can be improved with fibre SPME through careful control and monitoring time and temperature (precisely constant) during sample extraction. The extraction time is a critical parameter in the SPME sampling process. [Figure 23](#) illustrates the typical relationship between extraction time and analyte absorbed/adsorbed on the fibre. Before equilibrium of fibre and sample, time is very critical. However, once equilibrium is reached, a small change in the extraction time does not have a critical influence on the quantitative results.

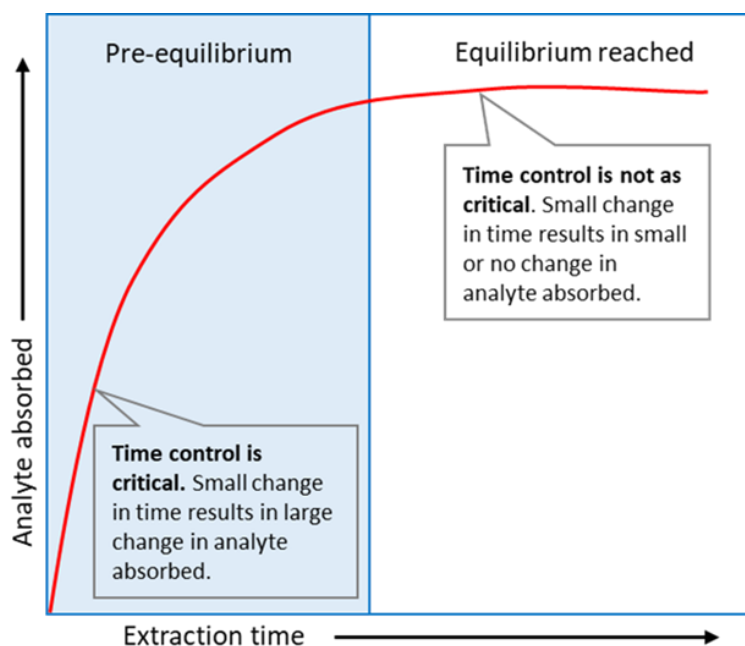


Figure 23. Time effect for SPME extraction (Vas and Vékey, 2004)

SPME is frequently used with gas chromatography-mass spectrometry (GC-MS) and gas chromatography olfactometry (GC-O) (Guillot et al., 2006). The simplified apparatus shows in Figure 24. GC-MS is based on their electron ionisation (EI) spectra and comparison with commercially available libraries (e.g. NIST or Wiley) (Jeleń, 2014). GC-O refers to the sniffing of GC effluent to determine which components possess odour. Many of the peaks detected by the GC actually contribute to our perception of flavours or fragrances.

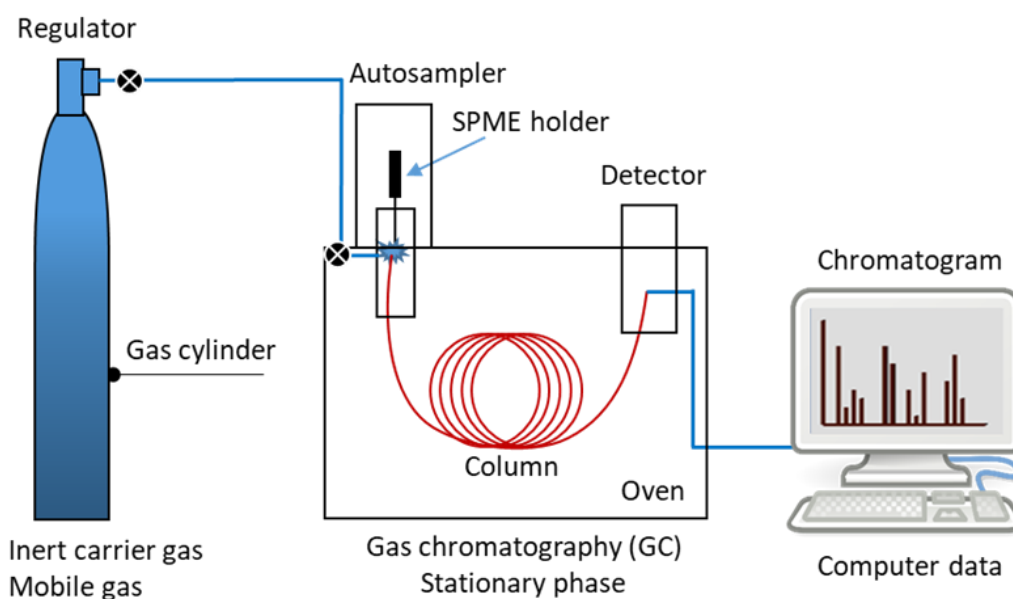


Figure 24. Simplified diagram of a gas chromatograph (Turner, 2020)

#### 4.4. SPME procedure

SPME is typically used for sampling gases (HS) or sampling solutions (direct extraction). The SPME needle is inserted into the appropriate position (e.g. through a septum into the HS), the needle protecting the fibre is retracted and the fibre is exposed to the environment (see in Figure 25). The polymer coating acts like a sponge, concentrating the analytes by absorption/adsorption processes. The kinetic of the SPME extraction process depends on a number of parameters, as described in section 4.2. Once equilibrium is reached or after a defined time, the fibre is retracted into the metal needle (for mechanical protection), and is transferred to the GC injector port. Finally, the fibre is pushed outside the metal needle and thermally desorbed the analyte from the fibre into the GC column. As there is no solvent, usually splitless injection is used. The sampling fibres can be used multiple times, hundreds of analyses in the case of HS analysis and dozens of times in the case of DI analysis.

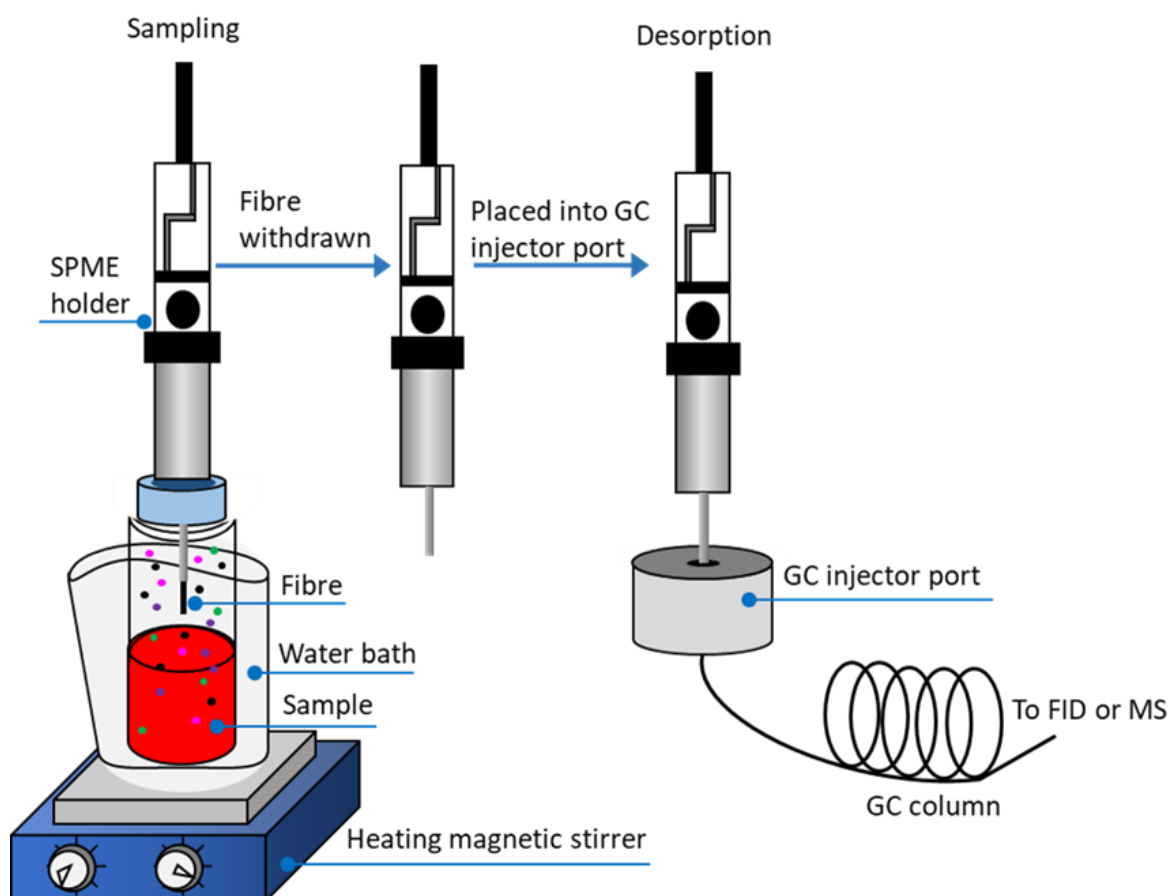


Figure 25. Diagram of headspace solid phase microextraction with gas chromatography flame ionisation detector or mass spectrophotometry (HS-SPME/GC-FID or-MS) (Schmidt and Podmore, 2015)



## 4.5. SPME optimisation

A few parameters such as fibre coating, heating temperature, time, and ionic strength are generally optimised in the development of the HS-SPME method prior to aroma analysis. Even though the properties of the fibre coating are well established as mentioned in section 4.3.1., choosing the appropriate fibre coating for individual matrix remains a challenge. Indeed, each fibre coating contains different affinity for target analytes. Thus, in practice, various fibre coatings are tested in order to obtain the most efficient extraction and representative aroma compounds in the sample. As shown in [Table 4](#), some researches have investigated on multiple fibre coatings (up to 5). Various heating temperature, extraction time, and ionic strength have also been explored (Feng et al., 2017; Ho et al., 2006; Sánchez-Palomo et al., 2005; Zhang et al., 2008, 2006). However, optimising SPME process takes time, therefore certain studies have reduced those parameters. Some studies have selected a fibre coating, heating temperature and extraction time (Bai et al., 2011; Di Cagno et al., 2009b; Serrano et al., 2009; Servili et al., 2000; Severo et al., 2016; Viljanen et al., 2011) ([Table 4](#)). For our study, we followed the extraction temperature and time from the literature review (Bai et al., 2011; Di Cagno et al., 2009b; Guillot et al., 2006; Junior et al., 2011; Severo et al., 2016). Nevertheless, two common approaches have been employed to optimise the HS-SPME method. The first approach is to set one parameter and vary the others. For example, to select the fibre coating, the extraction temperature and time have to be set and vice versa. The second technique is response surface methodology (Ma et al., 2013).

In practice, it is important to verify the fibre efficiency (fibre shelf life) and the fibre saturation. Fibre efficiency can be checked a few times with a standard solution or a sample throughout the experiment. The standard solution could be a mixture of 1 to 5 compounds of interest in water and keep in the freezer. The sample with internal standards can also be used, but it should be a consistent matrix in the freezer. If the results are repetitive, or decrease/increase steadily, therefore, the fibre is reusable, but the percent standard deviation should be included in the calculation. Fibre saturation is also crucial for efficient extraction. It can be verified by changing the volume of the sample, or by dilute the sample, and observing the change in analyte concentrations. It is also important to have at least one spare fibre in stock in case the working fibre is broken in the middle of the experiment.

Table 4. HS-SPME of volatile analytes in tomato, tomato product and fruits (\*selected fibre)

Matrix	Coating fibre	Condition equilibrium		Extraction conditions		Salt addition	Detection	Analytes	Reference
		T [°C]	Time [min]	T [°C]	Time [min]				
Tomato (3 mL)	50/30 µm DVB/CAR/PDMS	40	30	40	60	-	GC-MS	C <sub>6</sub> aldehydes and alcohols	Bai et al., (2011)
Tomato juice (5 g)	65 µm CAR/DVB	29	15	29	27	-	GC-MS	190 volatile compounds	Servili et al., (2000)
Tomato (0.1 g)	CAR/PDMS	70	10	70	50	-	GC-MS	20 volatile compounds	Serrano et al., (2009)
Tomato (3 g)	85 µm CW/PDMS 85 µm CAR/PDMS* 65 µm PDMS/DVB	35	30	35	60	-	GC-MS	21 volatile compounds	Beltran et al., (2006)
Fermented tomato juice (2 mL)	100 µm PDMS	40	30	40	30	-	GC-MS	196 volatile compounds	Di Cagno et al., (2009)
Cherry tomato (2 g)	75 µm CAR/PDMS	35	30	35	60	-	GC-MS	36 volatile compounds	Viljanen et al., (2011)
Tomato and strawberry	100 µm PDMS 65 µm PDMS/DVB 65 µm CW/DVB	23	60	23	12	-	GC-MS	8 volatile compounds	Song et al., (1998)
Tomato (5 g)	65 µm PDMS/DVB	-	-	25	60	20 mL of NaCl solution (20 g/L)	GC-MS	C <sub>6</sub> aldehydes and alcohols	Zhang et al., (2008)
Mango (5 g)	100 µm PDMS 65 µm CAR/PDMS* 65 µm CW/DVB 85 µm PA 75 µm PDMS/DVB	-	-	25	60	30 mL of NaCl solution (20 g/L)	GC-MS	24 volatile compounds (alcohols, aldehydes, ketones, aromatic compounds, etc.)	Zhang et al., (2006)
Chili peppers (1 g)	65 µm PDMS 65 µm DVB/PDMS 70 µm CW/DVB 75 µm CAR/PDMS 50/30 µm DVB/CAR/PDMS*	40	15	40	30	-	GC-FID; GC-MS	83 volatile compounds	Junior et al., (2011)
Strawberry (2 g)	75 µm CAR/PDMS	-	-	40	30	-	GC-MS	-	Severo et al., (2016)
Blackcurrant berries (1 g)	100 µm PDMS 65 µm PDMS/DVB 85 µm PA	-	-	55	20	-	GC	22 terpenes	Castillo and Dobson, (2002)
Brazilian fruits: cupuassu, caja, siriguela, graviola (1 g)	100 µm PDMS 85 µm PA 65 µm CW/DVB 75 µm CAR/PDMS	60	15	60	30	1 mL of saturated NaCl solution	GC-MS	Alcohols, esters, terpenoids, carbonyl compounds	Augusto et al., (2000)
Grape (skin 4 g and pulp 14 g)	65 µm PDMS/DVB 70 µm CW/DVB 50/30 µm DVB/CAR/PDMS	-	-	70	20	-	GC-MS	16 volatile compounds	Sánchez-Palomo et al., (2005)
Apricot purée (7 mL) or apricot pulp (50 g)	100 µm PDMS 65 µm PDMS/DVB 75 µm CAR/PDMS*	40	40	40	20	-	GC-FID; GC-MS; GC-O	26 volatile compounds	Guillot et al., (2006)

### III. BIBLIOGRAPHIC CONCLUSION AND OBJECTIVES

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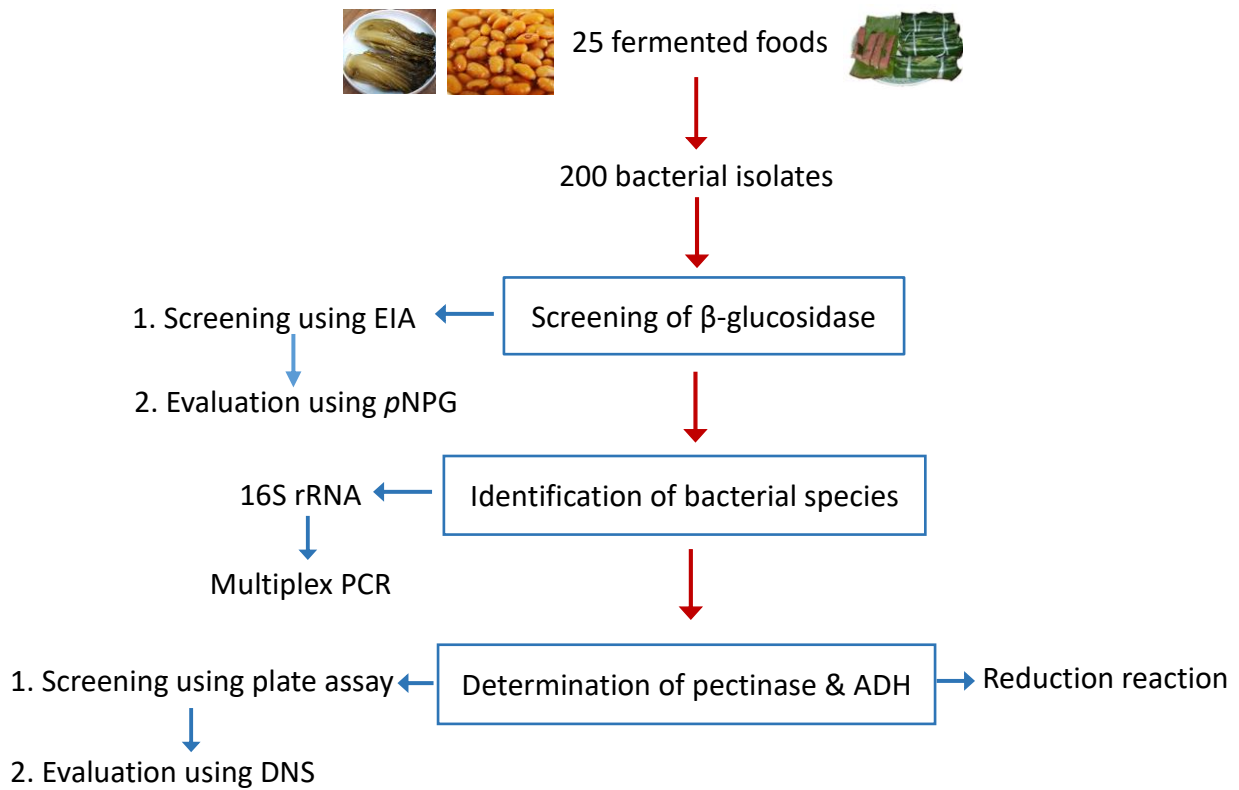
LAB have good growth capacities on various food matrices and produce very diverse enzymatic activities which are notably capable of positively modifying the organoleptic properties of fermented foods. Regarding fruits and vegetables, the bibliographic review highlights various traditional fermented foods for which lactic acid fermentation is spontaneous (or by backslopping). It is therefore realistic to imagine the development of new precision lactic acid fermentation (directed fermentation) for vegetables/fruits in order to direct the aromatic typicality of the product and the stability of this typicality. For this purpose, the bibliographic study make it possible to orient the selection of LAB starters towards the search for  $\beta$ -glucosidase and ADH activities which may have an impact on the aroma and pectinase on the texture and on the development capacity of strains in plant matrices. Because tomato is a highly consumed fruit/vegetable in the world and also very wasted, and the aroma and precursor contents are numerous, the tomato is a good matrix for exploring the aroma-release properties of selected starters.

Therefore, the main objective of this study is to obtain a diversity of aroma profiles of fermented tomatoes by using biotechnological approaches. In order to attain this, three important objectives are the followings:

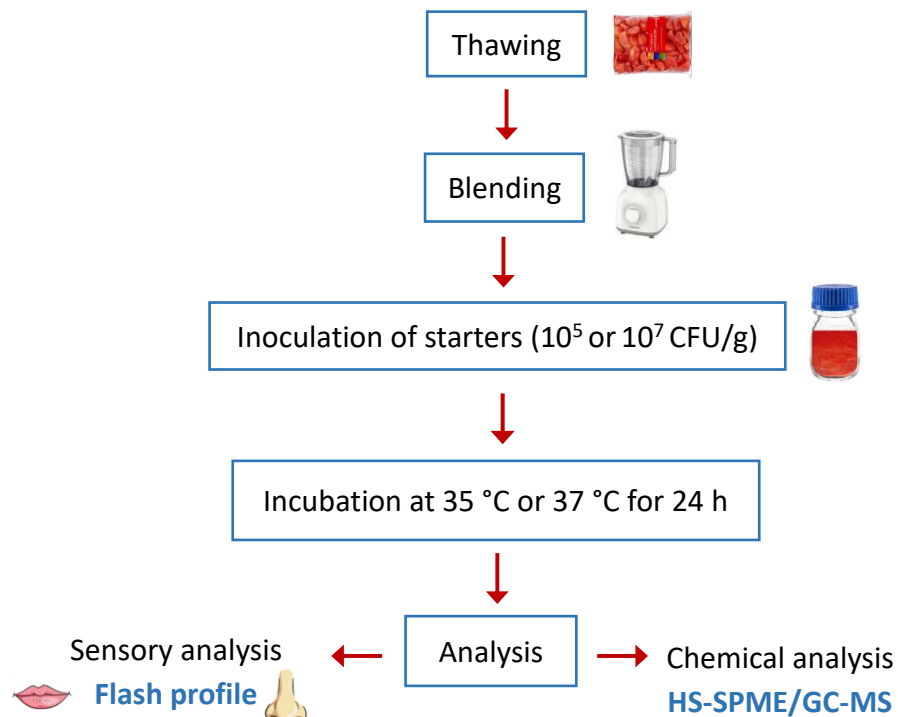
- (1) To evaluate the impact of starter diversity on the sensorial properties of fermented tomatoes
- (2) To identify and characterise the specific enzymatic activities, particularly  $\beta$ -glucosidases, alcohol dehydrogenases, and pectinases of LAB strains.
- (3) To characterise the role of LAB strains in the release of volatile aroma compounds in function of their enzymatic activities, using tomato as the fermentation matrix.

## IV. MATERIALS AND METHODS

### Experimental strategy for this study



### Fermentation process



## 1. Strains and microbiological techniques

### 1.1. Fermented foods and bacterial strains

Twenty-five samples were collected from different fermented foods from Cambodia or Vietnam as shown in Table 5. Each sample (20 g) was suspended in 80 mL of sterile tryptone salt (8.5 g/L NaCl and 1 g/L tryptone) and mixed with a Stomacher for 1 min. Serial dilutions were plated on MRS agar (VWR) and incubated for 48 h at 37 °C. Bacterial isolates of different morphology were selected and grown in MRS (pH 5.0) at 37 °C, except otherwise specified. The stationary phase of growth was reached after 20 h culture. *L. plantarum* B33, previously isolated from raw Vietnamese fermented pork (*nem chua*), was used for comparison purpose as a strain which has been selected as a starter for several applications (Cao-Hoang et al., 2013). A defined cocktail, called “cocktail D” was composed of 11 LAB strains isolated from legumes, wine and dairy products (Table 6).

Table 5. Fermented foods collected for this study and their origin

Matrix	Sample	Origin
Fermented vegetables	Fermented gherkins (2)	Phsar Pochentong, Phnom Penh, Cambodia
	Fermented cucumber pieces (1)	Phsar Depo, Phnom Penh, Cambodia
	Fermented young melons (1)	Phsar Pochentong, Phnom Penh, Cambodia
	Fermented mustard cabbages (5)	Phsar Pochentong and Phsar Depo, Phnom Penh, Cambodia
	Fermented shredded wild mustard leaves (2)	Phsar Depo, Phnom Penh, Cambodia Chợ Chính Kinh, Hanoi, Vietnam
	Fermented shredded white cabbages and carrots (1)	Chợ Châu Long, Hanoi, Vietnam
	Fermented Thai small round white aubergines (1)	Chợ Chính Kinh, Hanoi, Vietnam
	Fermented Thai big round white aubergines (1)	Chợ Chính Kinh, Hanoi, Vietnam
	Fermented shallot roots (2)	Phsar Depo, Phnom Penh, Cambodia Chợ Chính Kinh, Hanoi, Vietnam
	Fermented chili pepper puree (1)	Chợ Chính Kinh, Hanoi, Vietnam
Fermented legumes	Fermented soybeans (2)	Phsar Pochentong and Phsar Depo, Phnom Penh, Cambodia

Fermented meats	Raw fermented fish (1) Raw fermented pork (4)	Phsar Depo, Phnom Penh, Cambodia Chợ Hôm, Hanoi, Vietnam Dũng Nem - Đặc Sản Ước Lễ Gia Truyền, Hanoi, Vietnam
Raw material	Fresh banana leaf (1)	Dũng Nem - Đặc Sản Ước Lễ Gia Truyền, Hanoi, Vietnam

Table 6. Defined cocktail (D) compose of 11 LAB strains and their origin

Defined cocktail (called “cocktail D”)	Origin
<i>Pediococcus pentosaceus</i>	Wine of Burgundy
<i>Leuconostoc mesenteroides</i> ssp. <i>dextranicum</i> DSM 20484	Dairy product
<i>Leuconostoc mesenteroides</i> 19 D	Dairy product
<i>Leuconostoc</i> ssp. <i>cremoris</i> DSM 20200	-
<i>Streptococcus thermophilus</i> CIP6756	Gruyère cheese
<i>Lactobacillus helveticus</i> CNRZ303	Comté cheese
<i>Lactobacillus (Lactiplantibacillus) plantarum</i> ssp. <i>plantarum</i> CIP103151	Frozen peas
<i>Lactobacillus sakei</i> DSM20198	-
<i>Lactococcus lactis</i> ssp. <i>lactis</i> SL03	Dairy product
<i>Lactococcus lactis</i> ssp. <i>lactis</i> bv. <i>diacetylactis</i> SD17	Dairy product
<i>Lactococcus lactis</i> ssp. <i>cremoris</i> SC09	Dairy product

## 1.2. Genotypic identification of LAB

### a. DNA extraction and molecular identification by 16S rRNA gene sequencing

Genomic DNA was extracted using the InstaGene™ Matrix (Bio-Rad) with modifications. Each isolate (1 mL) grown 20 h at 37 °C was pelleted by centrifugation, washed twice with ultra-pure water, resuspended in 200 µL of InstaGene™ Matrix and incubated at 56 °C for 30 min. After vortex mixing at high speed for 10 s, the tubes were placed in a boiling water bath for 10 min. The supernatant containing the genomic DNA was recovered by centrifugation at 10,000 g for 3 min and stored at -20 °C until use. DNA were quantified by NanoDrop™. The V1-V9 regions of 16S rRNA gene were amplified using primers 16S-27 (5'-AGAGTTTGATCMTGGCTCAG-3') and 16S-1492R (5'-TACGGYTACCTTGTTACGACTT-3'). DNA sequencing reactions were performed by

Eurofins Genomics (Germany) using both forward and reverse primers (16S-SeqF, 5'-AGTAGGGAATCTTCCACA-3' and 16S-SeqR, 5'-CTTGCCACCTACGTATTA-3'). Each sample mixture (50 µL) contained 1 µM of both primers, 1 U of Phusion™ High-Fidelity DNA Polymerase (Thermo Fisher Scientific), 10 µL of PCR buffer 5X, 200 µM of each 2'-deoxynucleoside 5'-triphosphate, and 2 µL of DNA extract. The PCR was performed in a thermocycler (Bio-Rad) with an initial denaturation at 98 °C for 30 s, 35 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s and elongation at 72 °C for 30 s, and final extension at 72 °C for 5 min. DNA sequencing reactions were performed by Eurofins Genomics (Germany) using both forward and reverse primers (16S-SeqF, 5'-AGTAGGGAATCTTCCACA-3' and 16S-SeqR, 5'-CTTGCCACCTACGTATTA-3'). Taxonomic identification was determined by comparing the sequence of each isolate with those reported in the basic BLAST database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

### **b. Multiplex PCR assay**

Multiplex PCR assays were realised as described by Torriani et al. (2001). The PCR mixture was composed of four primers: 0.25 µM paraF (5'-GTCACAGGCATTACGAAAAC-3'), 0.25 µM pentF (5'-CAGTGGCGCGGTTGATATC-3'), 0.12 µM planF (5'-CCGTTTATGCGGAACACCTA-3'), and 0.25 µM pREV (5'-TCGGGATTACCAAACATCAC-3'). The difference in size of the amplicons allows to discriminate *L. plantarum*, *L. paraplantarum* and *L. pentosus* species (or *Lactiplantibacillus* sp.).

### **c. Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis**

Differential identification of the isolates was carried out through RAPD analysis. Four primers (Eurogentec) with arbitrarily chosen sequences (OPL5, 5'-ACGCAGGCAC-3'; P2, 5'-ATGTAACGCC-3'; P4, 5'-CCGCAGCGTT-3'; M13, 5'-GAGGGTGGCGGTTCT-3') were tested singly in four series of amplification (Corsetti et al., 2003; Di Cagno et al., 2009a; Lucena-Adrós et al., 2014). In the case of using the M13 primer, each 25-µL reaction mixture contained 1 µM of primer, 200 µM of each dNTP, 0.5 U of Phusion™ High-Fidelity DNA Polymerase (Thermo Fisher Scientific), 6 µL of DNA extract. The PCR program was an initial denaturation at 98 °C for 30 s, 35 cycles of 98 °C for 10 s, 46 °C for 60 s, 72 °C for 120 s, and a final extension at 72 °C for 5 min.

For OPL5, P2 or P4 primer, the 25- $\mu$ L PCR mixture contained 1.5 mM of MgCl<sub>2</sub>, 0.5  $\mu$ M of primer, 200  $\mu$ M of dNTP, 0.5 U of Taq polymerase (Thermo Fisher Scientific), and 6  $\mu$ L of DNA extract. The PCR program was: 94 °C for 3 min, 30 cycles of 94 °C for 30 s, 30 °C for 60 s, 72°C for 120 s, and 72 °C for 5 min.

The total PCR products were analysed by electrophoresis at 80 V for 4 h on 1.5% (w/v) agarose gel in 1X TAE buffer and the DNA was detected by UV transillumination after staining with ethidium bromide (0.5 mg/L). The molecular sizes of the amplified DNA fragments were estimated by comparison with Smartladder (Eurogentec). Randomly amplified polymorphic DNA-PCR (RAPD-PCR) analysis profiles were acquired by using the Gel Doc XR<sup>+</sup> system (Bio-Rad) and were compared using Image Lab software (Bio-Rad).

## 2. Enzymatic activities

### 2.1. Determination of $\beta$ -glucosidase activity

#### a. Screening for $\beta$ -glucosidase activity

$\beta$ -Glucosidase activity assays were conducted as described by Renchinkhand et al. (2015) with some modifications. Esculin or aesculin (coumarin glucoside) was used as a substrate to visualise the  $\beta$ -glucosidase activity.  $\beta$ -Glucosidases hydrolyse esculin to release  $\beta$ -D-glucose and esculetin. Esculetin reacts thereafter with iron salts, which forms a brown or black complex (Edberg et al., 1985). Therefore, a colony corresponding to an isolate positive for  $\beta$ -glucosidase activity turns brown or black. The esculin iron agar (EIA) was composed of esculin (1 g, Acros Organics), iron ammonium citrate (0.5 g), meat extract (3 g), peptone (5 g) and agar (16 g) in 1 litre of distilled water at pH 6.6. Bacterial isolates were grown for 24 h at 37 °C, plated on EIA, and incubated for 72 h at the same temperature.

#### b. Evaluation of $\beta$ -glucosidase activity

$\beta$ -Glucosidase activity was evaluated by the release of *p*-nitrophenol (*p*NP, Sigma-Aldrich) from *p*-nitrophenyl- $\beta$ -D-glucopyranoside (*p*NPG, Sigma-Aldrich N7006). The  $\beta$ -glucosidase activity was determined according to the method described by Grimaldi et al. (2005) with some adjustments. Bacterial cell suspension from 22 h culture at 25 °C was harvested by centrifugation (4,000 rpm, 7 min at 4 °C), washed twice with physiological water (0.9% NaCl, w/v), and normalised to a final optical density at 600 nm (OD<sub>600</sub>) of 0.8. A standardisation of the growth curves was previously made (number of CFU/OD over time).



Assays were prepared in a 96-well U-bottom microplate (ThermoFisher Scientific). Each well was filled with 40  $\mu\text{L}$  of 0.2 M McIlvane buffer at pH 5.0 (0.2 M of dipotassium hydrogen phosphate and 0.1 M of citric acid), 20  $\mu\text{L}$  of bacterial cell suspension and 20  $\mu\text{L}$  of *p*NPG solution (40 mM, pH 5.0). The microplate was incubated for 2 h at 37 °C. The reaction was stopped with 160  $\mu\text{L}$  of  $\text{Na}_2\text{CO}_3$  solution (0.5 M). The  $\text{Na}_2\text{CO}_3$  solution can change the pH above 10 (most enzymes are inactive) and increase the visibility intensity (*p*NP in anionic form). The microplate was then centrifuged (4,000 rpm, 15 min at 4 °C) and the supernatants (200  $\mu\text{L}$ ) were transferred to a new 96-well flat bottom microplate. The absorbance at 400 nm was measured by a multiplate spectrophotometer (FLUOstar Omega, BMG LABTECH). The blanks were prepared with physiological water instead of bacterial cells. Reactions were carried out in triplicate. The *p*NP calibration standards were between 0.013 and 0.302 mM. One unit of enzymatic activity (UA) was defined as the amount of *p*-nitrophenol (nmol) released per minute per milligram of cell dry weight.

## 2.2. Determination of alcohol dehydrogenase activity

The ADH activity of LAB resting cells was evaluated through the reduction of aldehydes or ketones into their corresponding alcohols. Substrates for the reduction reaction included hexanal (98%), (*E*)-2-hexenal (95%), (*E*)-2-heptenal (95%), (*E,E*)-2,4-decadienal (89%), 6-methyl-5-hepten-2-one (98%), and 6,10-dimethyl-5,9-undecadien-2-one (97%). They were purchased from Sigma-Aldrich. 4-Nonanol (96.5%) from Merck Millipore, was used as the internal standard. Stock solutions (1 g/L) were prepared in absolute ethanol. Working solutions (160 mg/L) were obtained by diluting the stock solutions with distilled water. The bacterial cell suspension from 20 h culture was obtained by centrifugation (4,000 rpm, 7 min at 4 °C), washed twice with physiological water and diluted to  $10^8$  CFU/mL according to a standardisation of the growth curves was previously made (number of CFU/OD over time). Each strain was grown in three independent cultures to determine the biological variation.

Bacterial ADH assays were carried out in 4 mL glass vials. Each vial contained 0.5 mL of substrate (20 mg/kg), 0.5 mL of glucose solution (3 g/kg), 0.5 mL of physiological water and 17  $\mu\text{L}$  of bacterial suspension to initiate the reduction reaction. The reduction reaction was conducted in triplicate at 37 °C for 1 h under agitation. Volatile compounds were quantified by gas chromatography coupled with flame ionisation detector (GC/FID, PerkinElmer, Clarus® 500) after extraction with diethyl ether according to Try et al. (2018) using 4-nonanol (35  $\mu\text{L}$ , 1.83 g/kg) as the internal standard. The oven temperature programme was modified to have an increase from 48 °C (hold for 2 min) to 110 °C at 2 °C/min, then from 110 °C to 120 °C at 10

°C/min and finally from 110 °C to 250 °C (hold for 2 min) at 30 °C/min. One unit of enzymatic activity (UA) was defined as the amount of a substrate (nmol) reduced into a product per minute per CFU.

### 2.3. Determination of pectinase activity

#### a. Screening for pectinase activity by plate assay

The pectinase activity of the isolates was screened by plate assay using three media. MRS medium with/without glucose was supplemented with 1% of citrus pectin (Sigma-Aldrich) and 0.5% of agarose in 1 litre of distilled water at pH 6.0 (Karam and Belarbi, 1995). Yeast extract pectin (YEP) contained citrus pectin (2.5 g), yeast extract (5 g), and agar (15 g) in one litre of distilled water at pH 7.2 (Karthik et al., 2011; Vidhyasagar et al., 2013). Mineral pectin (MP) consisted of citrus pectin (5 g), yeast extract (1 g), agar (15 g), Na<sub>2</sub>HPO<sub>4</sub> (6 g), KH<sub>2</sub>PO<sub>4</sub> (4 g), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (2 g) in one litre of distilled water and 1 mL of each element that composed FeSO<sub>4</sub> (0.1%), MgSO<sub>4</sub>·7H<sub>2</sub>O (0.1%), CaCl<sub>2</sub>·2H<sub>2</sub>O (0.1%), H<sub>3</sub>BO<sub>3</sub> (0.001%), MnSO<sub>4</sub>·H<sub>2</sub>O (0.001%), ZnSO<sub>4</sub>·H<sub>2</sub>O (0.007%), CuSO<sub>4</sub>·5H<sub>2</sub>O (0.001%) and MoO<sub>3</sub> (0.001%) (Bekhouche et al., 2006).

Each isolate was grown in a 10-mL MRS broth (pH 5.0) for 24 h at 30 °C and 10 µL of which was placed on pectin-containing plates for 3 to 4 days at 30 °C. After incubation, the MRS with/without glucose plates were stained with ruthenium red solution (0.02%, w/v). The excess dye was removed with distilled water. A dark-red halo surrounding the colony indicated polygalacturonases activity (Karam and Belarbi, 1995). Ruthenium red is able to bind to free carboxyl groups of pectin (Hou et al., 1999). On the other hand, the YEP and MP plates were stained with potassium iodide solution or Congo red (0.1%) to detect the clearance zone around the colony indicating the positive pectinase (Karthik et al., 2011; Vidhyasagar et al., 2013). The culture without a clear zone generally exhibited a ring of intense colouring around the colony. When pectin is used as the sole carbon source and the isolate is grown on this medium, the isolate would hydrolyse pectin if it possesses pectinase activity. As a result, iodine could not form a complex with pectin and that area would remain transparent while the area in which pectin is present would be a yellowish brown colour. Congo red interacts with (1-4)-β-D-glucan, (1-4)-β-D-xylan and (1-4)-α-D-polygalactopyranosyl uronic acid (Vidhyasagar et al., 2013; Woodward and Wiseman, 1982). All dyes solutions were freshly prepared each experiment.

## **b. Evaluation of pectinase activity by DNS**

The pectinase activity was measured by colorimetric method using 3,5-dinitrosalicylic acid (DNS). Each isolate was grown in a 10-mL MRS broth (pH 5.0) for 24 h at 30 °C. Cell free supernatants (CFS) were collected by centrifugation at 4,000 rpm for 10 min at 4 °C. The CFS (0.1 mL) was added to 0.5 mL of acetate buffer (25 mM, pH 5.0) with 0.5% pectin and incubated at 37 °C for 30 min. Blank was prepared the same manner by replacing the CFS with the heat-denatured enzyme (boiling for 5 min). After incubation, DNSA (1 mL) reagent was added and kept in boiling water bath for 5 min. After cooling in ice bath, the absorbance was read at 540 nm using spectrophotometer (Genesys TM 20, ThermoFisher Scientific). The concentration of reducing content was compared with standard D-galacturonic acid (Sigma-Aldrich). One enzyme unit of pectinase was defined by the enzyme activity which liberated one  $\mu\text{mol}$  of galacturonic acid per minute (Kashyap et al., 2000; Vidhyasagar et al., 2013).

## **3. Fermented tomato analysis**

### **3.1. Mashed tomato treatment**

Peeled frozen tomatoes (Picard Surgelés, France) were thawed and blended with a conventional blender (Philips HR2056/00, 350 W) for 1 min to obtain mashed tomatoes (MT). The MT (60 g) were fermented in a 100 mL Duran glass jar with a screw-cap. Stationary phase cultures were used to inoculate MT ( $10^5$  CFU/g of MT) into hermetic jars. MT were mixed and the jars were then kept in a static incubator for 24 h at 37 °C. The control “acid hydrolysis sample” was MT acidified to pH 3.7 with lactic acid. The “enzymatic hydrolysis sample” was MT treated with 1 U of almond  $\beta$ -glucosidases per g of tomato (Sigma-Aldrich, G0395-2.5KU) as control of the enzymatic activity. These conditions were noted LA and Glu, respectively. The control samples were also incubated for 24 h at 37 °C. Analyses were made extemporaneously. The mashed tomatoes (MT) were analysed immediately after blending as a reference without any treatment, and this condition was called “untreated tomatoes” (UT). The entire experiment was carried out in triplicate.

### **3.2. pH measurement and bacterial enumeration**

pH values were measured by an electronic pH meter (Jenco Electronics LTD Microcomputer). LAB enumeration was carried out by surface-inoculated plates of MRS agar. MT (20 g) was suspended in 80 mL of sterile NaCl solution (0.9%, w/v) and vortexed. Serial

dilutions were made and 10 µL of the appropriate decimal dilutions were plated on MRS agar. The colonies were visually counted after 48 h of incubation at 37 °C. The selected plates (3-30 colonies per plate) were expressed as colony forming units (CFU) per gram of the MT.

### 3.3. Sensory analysis by flash profile

Sensory analysis was conducted by flash profile method. Flash profile is a descriptive sensory analysis method, which gives freedom to the individual to decide on his/her own vocabulary to describe the sensory differences between products (Bredie et al., 2017). Seven adult panelists carried out a sensory evaluation of seven samples: tomatoes fermented with five different LAB starters, spontaneously fermented tomatoes and mashed tomatoes (without fermentation). Three LAB cocktails were isolated from traditional fermented foods brought from Cambodia and Vietnam. Cocktail P isolated from fermented gherkins, cocktail S from fermented salty soybean, and cocktail N from *nem chua* were chosen for this test due to their different origins such as vegetable, legume and meat respectively. The LAB cocktail was a mix of all LAB colonies grown on MRS plate. A defined cocktail (called cocktail D) was composed of 11 LAB strains isolated from legumes, wine and dairy products (Table 6). Mashed tomatoes were individually inoculated with the four cocktails or with *L. plantarum* B33 ( $10^7$  CFU/g), and incubated at 35 °C for 24 h.

### 3.4. Volatile compound extraction by HS-SPME

Volatile compounds were extracted by headspace-solid phase microextraction (HS-SPME). Three fibre coatings were tested on the MT to select the most suitable fibre coating for the tomato volatile compounds. One hundred µm polydimethylsiloxane (PDMS), 65 µm PDMS/divinylbenzene (PDMS/DVB) (Castillo and Dobson, 2002; Sánchez-Palomo et al., 2005; Song et al., 1998; Zhang et al., 2008) and 75 µm carboxen/PDMS (CAR/PDMS) (Beltran et al., 2006; Severo et al., 2016; Viljanen et al., 2011) were purchased from Sigma-Aldrich. The extraction was carried out in 20 mL headspace glass vial sealed tightly with crimp cap and PTFE/silicone septum. The MT (5 g) and 10 µL of 4-nonanol (70.77 mg/kg) as internal standard were placed in the vial. Before inserting the fibre, the sample vial was previously conditioned by stirring for 20 min at 40 °C. The fibre was then exposed to the headspace for 40 min at the same temperature (Di Cagno et al., 2009b). The SPME fibre analytes were desorbed by splitless injection at 250 °C for 5 min in the GC injector port.

### 3.5. Identification and quantification of volatile compounds

GC/FID was used to select the fibre coatings, while gas chromatography (PerkinElmer, Clarus 580) coupled with mass spectrometer (GC/MS, PerkinElmer, Clarus SQ 8S) was utilised to identify and quantify the volatile compounds. GC/FID was equipped with UBWax capillary column (30 m by 320  $\mu\text{m}$  by 0.25  $\mu\text{m}$ ) and GC/MS was equipped with Elite-5MS capillary column (30 m by 250  $\mu\text{m}$  by 0.25  $\mu\text{m}$ ). Nitrogen (GC/FID) and helium (GC/MS) were the carrier gas at a linear flow rate 4.3 mL/min and 1.0 mL/min, respectively. The oven temperature was programmed to increase from 48 °C (hold for 2 min) to 110 °C at 2 °C/min, then from 110 to 250 °C at 10 °C/min (hold for 5 min). Flame ionisation temperature was 300 °C and interface temperature was 240 °C. Mass spectra were obtained using an ionisation source with an electronic impact of 70 eV. Mass scans of the sample quadrupole type monitoring the range from 40-350 m/z. The identification of volatile compounds was mainly achieved by comparing their mass spectra with those of known compounds held in the NIST mass spectral database as well as laboratory databases.

### 4. Statistical data analysis

All experiments were carried out at least twice independently. The data were analysed using analysis of variance (one-way Anova) and Tukey's Honestly Significant Difference test (HSD) (the levels of significance 5%) by the XLSTAT statistical software (version 16.0). Principal component analysis (PCA) is a technique for identifying patterns in data, reducing the dimensions of dataset, and increasing interpretability but at the same time minimizing information loss (Jolliffe and Cadima, 2016). In this study, PCA was used to detect the correlation between variables, the volatile compounds in fermented tomatoes and their treatments.

## V. RESULTS AND DISCUSSION

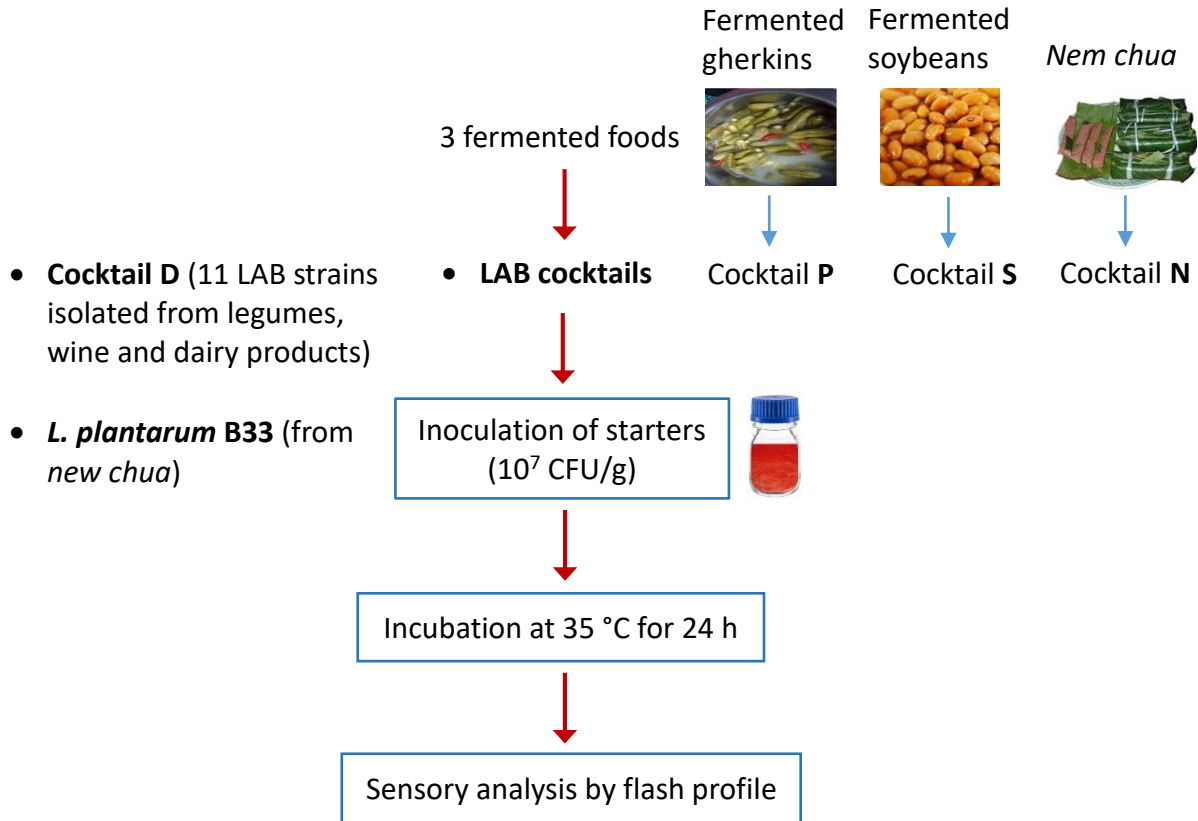
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### 1. Evaluation of the impact of starter diversity on flavour descriptors

Most of fermented foods in Cambodia and Vietnam are produced in small-scales, homemade and spontaneously fermented by the microflora (indigenous species) naturally present in the raw materials, the ingredients, and/or the production environment (La Anh, 2015). Both countries share some very common fermented foods including fermented fruits/vegetables (*chrouk* or *tram* (in Khmer) and *dua chua* (in Vietnamese)), shrimp, pork paste (*nem chua* (in Vietnamese)), and fish paste (*nem Trey* (in Khmer)). To our knowledge, little research has focused on the microflora of Vietnamese and Cambodian fermented foods. Nguyen et al. (2013) identified LAB in fermented mustard green and beet (*dua muoi* (in Vietnamese)) and fermented Thai aubergine (*ca muoi* (in Vietnamese)). *L. fermentum* (56.6%), *L. pentosus* (24.4%) and *L. plantarum* (17.1%) were the main LAB species. The least abundant species were *Pediococcus pentosaceus* (1.0%) and *L. brevis* (0.5%). LAB species (<0.1%) included *L. paracasei*, *L. pantheris* and *Pediococcus acidilactici* (Nguyen et al., 2013). These indigenous species exhibit a diversity of metabolic activities which give a unique character to each fermented product. The sensorial properties of these fermented foods are highly appreciated by consumers. It seems that the indigenous LAB play a key role in the sensorial quality of fermented foods. Since the microflora present in these fermented foods are numerous and diverse, it would take a long time to discover which strain provides the most interesting flavour. Therefore, it would be very interesting to use all the LAB microflora (called LAB cocktail) isolated from fermented foods as a starter for tomato fermentation. Tomatoes were chosen for this study due to the availability of this raw material as a by-product, the health benefit of this fruit/vegetable and the richness of aroma precursors.

The first aim of this study was to prove that LAB diversity can change aroma/flavour profiles of fermented tomatoes. (Ideally, it could have driven our selection of bacteria if one LAB cocktail have provided particularly interesting aroma and/or flavour compounds among the different LAB cocktail starters).

### Experimental strategy for this study



The pH of mashed tomatoes (MT) was around 4.3, while the pH of MT fermented with different starters significantly decreased (about 3.5) (Figure 26). The pH of MT with endogenous bacteria (spontaneously fermented tomatoes) barely decreased (4.1). This may be caused by the adventitious strains present in the matrix (tomato), equipment and/or production environment.

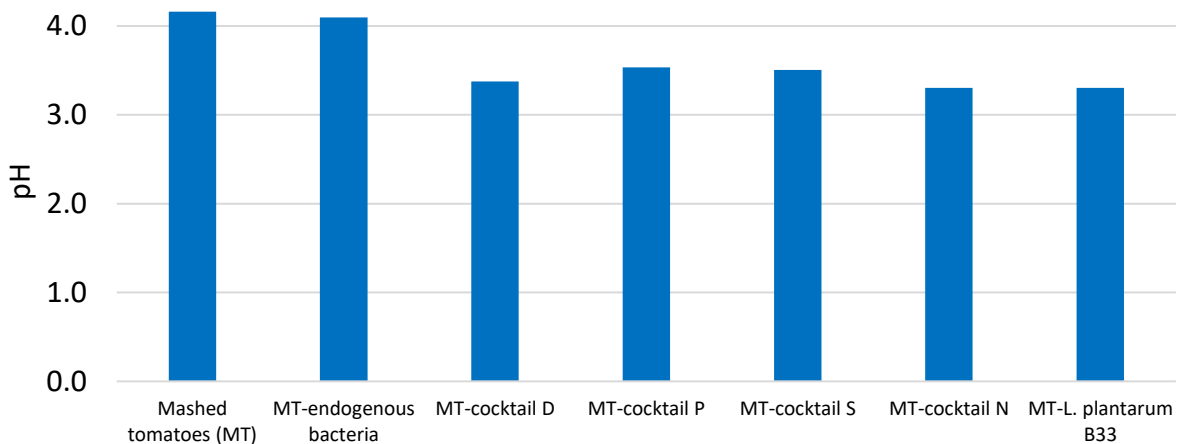


Figure 26. pH values of mashed tomatoes (MT) and MT fermented with different starters

The sensory evaluation mainly focused on the smell (aroma) and taste of the samples. Panelists described that tomatoes fermented with cocktail D (the defined cocktail in Table 6), MT and MT-endogenous bacteria had similar aroma note and taste such as green tomato and tomato-like taste (Table 7). Consequently, cocktail D had no impact on the flavour profile of fermented tomatoes. In contrast, tomatoes fermented with cocktail P, S and N showed the most intriguing results. The panelists described the aroma of these fermented tomatoes as grassy, fruity and floral notes (Table 7). Tomatoes fermented with these cocktails had a similar aroma profile to tomatoes fermented with *L. plantarum* B33. The LAB cocktails isolated from traditional fermented foods showed very promising aroma note compared to cocktail D. Furthermore, these fermented tomatoes also featured some interesting flavours such as umami, salty and bitter. However, the panelists did not appreciate the sourness of these fermented tomatoes. To lower acidity (sour taste), a few techniques such as reducing the amount of inoculum, incubation temperature and time could be possible. However, as the application of fermented tomatoes can be various (e.g. ketchup, tomato sauce), we decided to test another technique. Table sugar (2% w/w) was added after fermentation as an approach to remove the sourness perception. Consequently, panelists preferred fermented tomatoes with sugar over those without sugar. They also described that fermented tomatoes with sugar had a flavour profile similar to ketchup. Sugar was indeed an effective technique of eliminating the acidity taste of fermented tomatoes. Despite this, the panelists were not able to distinguish the typical aroma note of these samples. A disadvantage of this approach was that the added sugar might mask the typical and original aroma and flavour notes of the fermented tomatoes. Therefore, for future experiments, it should be better to avoid adding sugar and to modify fermentation conditions such as the amount of inoculums and the incubation time.

Table 7. Sensory evaluation of fermented tomatoes

Treatment	Description	
	Aroma	Flavor (taste)
Mashed tomatoes (MT)	Green tomato	Tomato-like
MT-endogenous bacteria	Green tomato	Tomato-like, sour, salty, bitter
MT-cocktail D	Green tomato, ethanol, plastic	Tomato-like, sour, umami
MT-cocktail P	Grassy, fruity, floral	Tomato-like, salty, vinegar, sour



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MT-cocktail S	Grassy, ethanol, fruity, floral, acid	umami, bitter, sour, sparking, sauerkraut
MT-cocktail N	Fruity, floral, acid	Salty, bitter, sour, sauerkraut
MT- <i>L. plantarum</i> B33	Grassy, cereal, fruity, honey	Salty, sour

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Tomatoes fermented with cocktail P, S, and N and *L. plantarum* B33 revealed a few important aroma notes such as grassy (green or cut grass), fruity and floral. This sensory evaluation showed the impact of these LAB cocktails on fermented tomatoes. These aroma notes might be released from the enzymatic activities of LAB. Hence, it is very important to test different strains according to criteria defined in advance. The grassy note is typically due to aldehydes and alcohols derived from the LOX pathway and its intensity depends on the aldehydes/alcohols ratio. The ADH enzyme is responsible for the interconversion between two volatile families. Therefore, it is necessary to verify whether those bacterial isolates produce ADH activity. The fruity and floral notes were normally attributed to volatile ketones and terpenes. However, most ketones and terpenes found in fruits/vegetables are not in the free form (volatile), but as non-volatile and flavourless glycoconjugates (i.e. glycosides). Acid or  $\beta$ -glucosidase can hydrolyse glycoconjugates and release these volatile ketones and terpenes. This may explain the fruity and floral aromas presented in the fermented tomatoes. Thus, it is important to demonstrate the  $\beta$ -glucosidase activity of the bacterial isolates and possibly to differentiate between acid hydrolysis and enzymatic activities.

In conclusion, cocktail P, S and N considerably modified the aroma and flavour profiles of fermented tomatoes, which is probably due to ADH and  $\beta$ -glucosidase activities. Therefore, demonstrating the enzymatic activities is a key step for this work. Pectinases are another enzymatic group that would be also determined on the bacterial isolates. Little work has focused on LAB pectinases, yet pectinases could play a key role in fermented fruits and vegetables. They can make the plant tissues less rigid and release nutrients for bacterial growth.

## 2. Selection of LAB strains for enzymatic activities interesting for food fermentation

Besides its great potential to increase in the shelf life of perishable raw materials, innovation, detoxification, nutritional enrichment, and reduction of fuel consumption, lacto-fermentation can also have an impact on the sensorial properties of a product (Beena Divya et al., 2012; Leroy and De Vuyst, 2004; Steinkraus, 1995). Although traditionally spontaneous fermentation usually brings some suitable properties, the selection of starters based on specific enzymatic activities can bring a step forward in precision fermentation, i.e. the process that allows us to program microorganisms to produce almost any complex organic molecule (Tubb and Seba, 2019), leading to food exhibiting specific sensorial properties. The present work follows this approach focusing on some activities able to bring important sensorial notes to tomatoes.

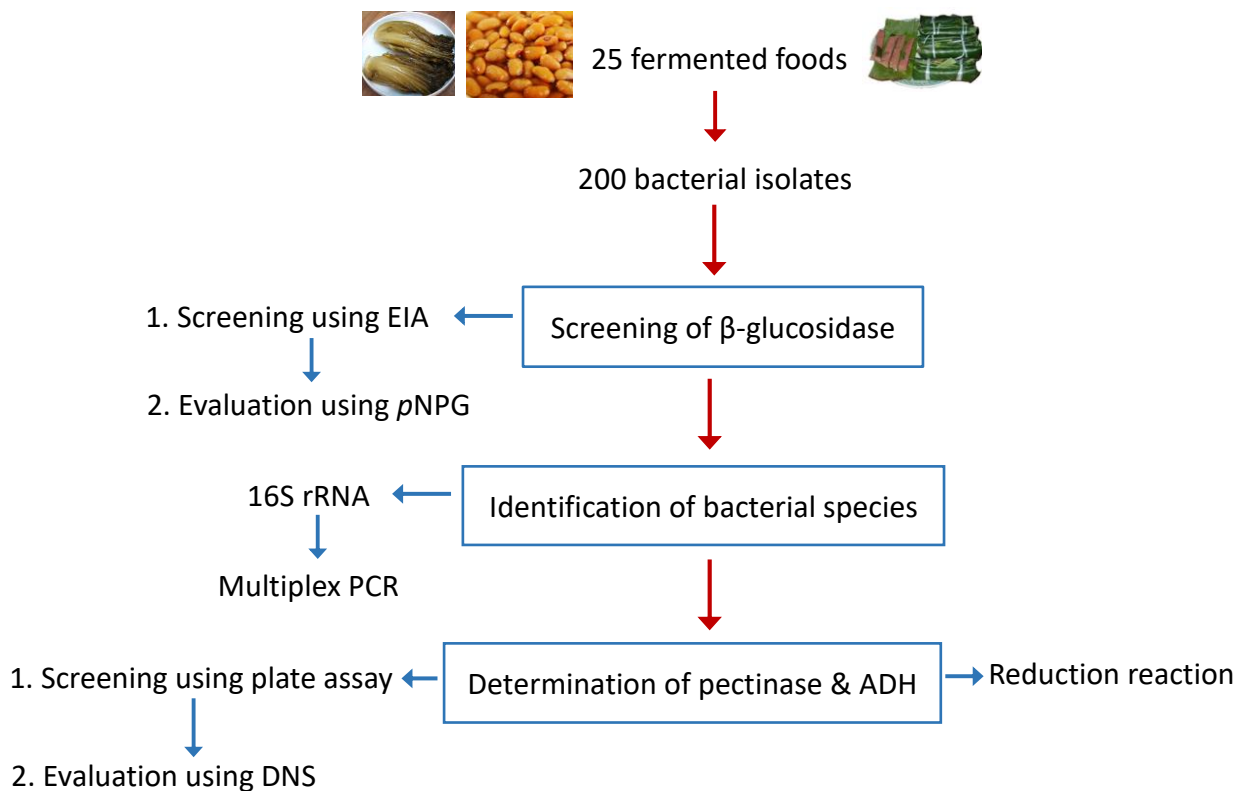
Numerous reviews have shown the essential use of  $\beta$ -glucosidases, alcohol dehydrogenases, and pectinases in various applications. In fruit and vegetable processing,  $\beta$ -glucosidases and ADH have been significantly involved in the release of volatile aroma compounds (Fan et al., 2011; Gargouri et al., 2004; Hu et al., 2019; Krisch et al., 2010; Sestelo et al., 2004; Su et al., 2010). Some LAB are capable of producing enzymes such as  $\beta$ -glucosidase (Michlmayr and Kneifel, 2014), alcohol dehydrogenase (Haberland et al., 2002; Hu et al., 2019), and pectinase (Vidhyasagar et al. 2013). Many studies have shown the ability and use of fungal  $\beta$ -glucosidases to improve the sensory quality of wines and foods (Ahmed et al., 2017). However, a few studies have focused on the ability of LAB to produce  $\beta$ -glucosidases. *Leuconostoc mesenteroides* (Gueguen et al., 1997a), *L. plantarum* USC1 (Sestelo et al., 2004), *Lactobacillus* spp. and *Pediococcus* spp. (Grimaldi et al., 2005a), and *L. brevis* and *L. casei* (Hernandez-Orte et al., 2009) isolated from wines and fermented foods exhibited  $\beta$ -glucosidase activities.

ADH is an important enzyme since it can contribute to the flavour development by interconverting aldehydes or ketones into alcohols. Despite the fact that their encoding genes are generally present in LAB genomes, ADH activities have only rarely been reported in LAB. *L. kefir* DSM 20587 was able to reduce 2,5-hexanedione into (2*R*,5*R*)-hexanediol (Haberland et al., 2002) in specific resting whole cells conditions. Few studies characterised purified ADHs from LAB. In cheese, Hu et al. (2019) found five ADHs from *L. reuteri* DSM20016 which were able to reduce aldehydes into their corresponding alcohols, for instance, 3-methylbutanal to 3-methylbutanol, butyraldehyde to 2-butanol, hexanal to hexanol and phenylacetaldehyde to phenylethanol, etc.

Pectinases are an enzymatic group that catalyse the degradation of pectic substances (Pedrolli et al., 2009; Singh et al., 2019). Little work has focused on the pectinolytic enzymes of LAB, yet these enzymes could play an important role in fermented fruits and vegetables. Indeed, pectinases can make the plant tissues less rigid and release nutrients for the growth of bacteria (Juven et al., 1985; Vidhyasagar et al., 2013). This mechanism might also allow  $\beta$ -glucosidases to access glycosides in fruit and vegetable matrices. *Leuconostoc mesenteroides* isolated from tomato juice (Juven et al., 1985), *Pediococcus pentosaceus* (VJ13, VJ41, and VJ56) and *Leuconostoc lactis* VJ52 from *idli batter* (Indian fermented rice cake) (Vidhyasagar et al., 2013), *L. pentosus* SJ65 and *L. plantarum* ssp. *argenteratensis* (SJ6, SJ33, SJ37, and SJ40) from *uttapam batter* (Indian fermented rice cake) produced pectinases. *Leuconostoc* sp. from black olives, and *Lactobacillus* spp., *Leuconostoc* sp., and *Pediococcus* spp. (green olives) synthesised polygalacturonases (Bekhouche et al., 2006).

Therefore, the second purpose of this study was to identify and characterise the specific enzymatic activities, particularly  $\beta$ -glucosidases, alcohol dehydrogenases and pectinases of LAB from traditional fermented foods in Cambodia and Vietnam.

#### Experimental strategy for this study



## 2.1. $\beta$ -Glucosidase activity

Two hundred isolates were selected from 25 fermented foods and screened on EIA for  $\beta$ -glucosidase activity. Eighty isolates were positive for  $\beta$ -glucosidase according to the brown or black colour of their colony (Figure 27). The  $\beta$ -glucosidase activity of these 80 isolates was evaluated at 37 °C (from 22 h culture). Fourteen isolates had an activity greater than 10 UA

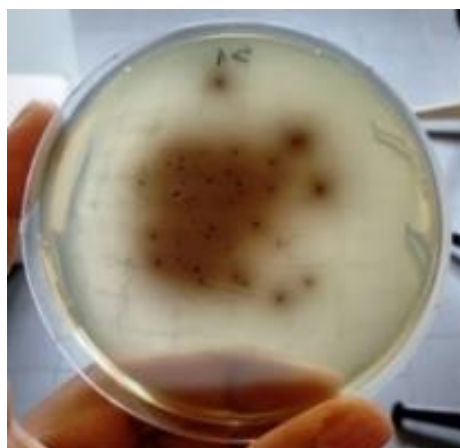


Figure 27.  $\beta$ -Glucosidase activity of bacterial isolates on Esculin Iron Agar plate. The brown/black colony is  $\beta$ -glucosidase positive.

(Figure 28), while the other 66 had a  $\beta$ -glucosidase activity lower than 10 UA (data not shown). The highest activity detected was 27 UA for isolates C022-2B and C022-3B. Other isolates exhibited about half this activity like V0023-4B2 (12 UA). For comparison, *L. plantarum* B33 presented a  $\beta$ -glucosidase activity of 17 UA. The quantification of activity was also checked for V013-1A, which was negative for  $\beta$ -glucosidase activity on EIA plate, and no activity was detected in the pNPG assay, confirming its phenotype.

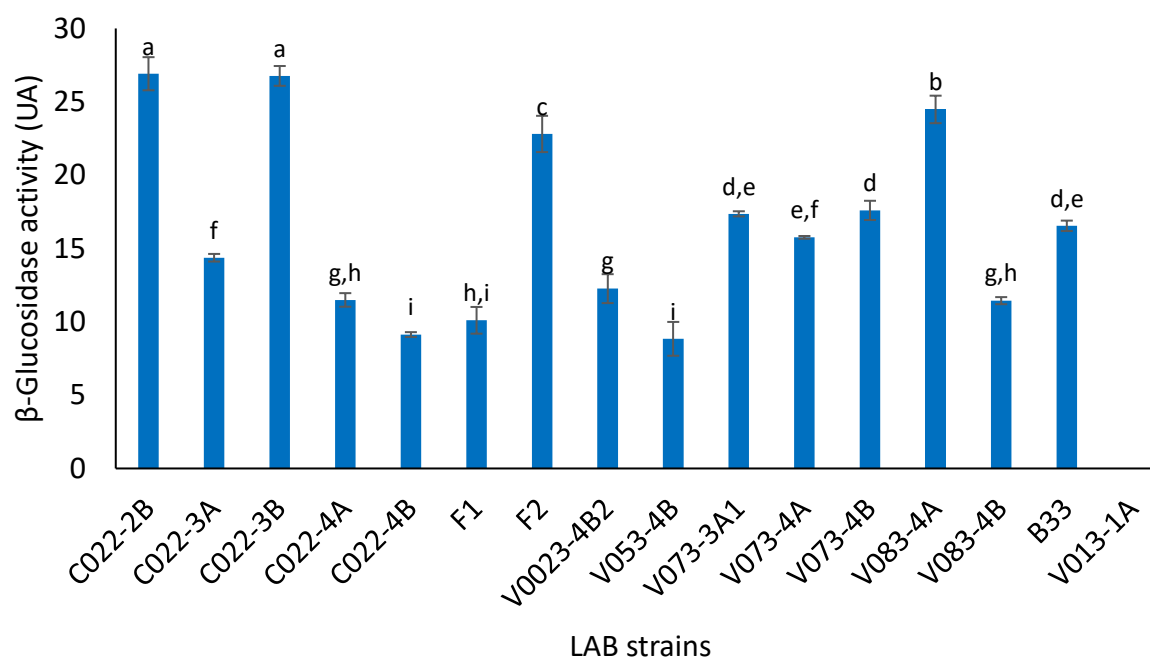


Figure 28.  $\beta$ -Glucosidase activity of LAB, 15 strains and *L. plantarum* B33 grown 22 h at 25 °C. Mean values are from three biological repeats. UA, nmol of *p*-nitrophenol per minute per milligram of cell dry weight at 37 °C. Different letters show significant differences ( $p < 0.05$ ) among the different strains.

LAB  $\beta$ -glucosidase can be localised as intracellular, extracellular, and cell-bound  $\beta$ -glucosidase (Table 8 and 9). This study focused only on whole cells (cell-bound  $\beta$ -glucosidase) due to its subsequent use as a starter in fermented vegetables. Our results showed that the  $\beta$ -glucosidase activities of LAB isolated from Asian fermented foods were much lower than those of previous research, except for *L. brevis* SK3 (10.6 UA) were similar to our results (Table 8). However, it is difficult to compare our results to the previous research since LAB  $\beta$ -glucosidase activities may vary from strain to strain. The biosynthesis of  $\beta$ -glucosidases depends considerably on the growth medium, oxygen, temperature, and growth phase. Kok et al. (2012) found that *L. rhamnosus* NRRL 442 produced substantially cell-bound  $\beta$ -glucosidases at pH 6.0, 40 °C, and anaerobic conditions. Gueguen et al. (1997a) found that *Leuconostoc mesenteroides* produced higher intracellular  $\beta$ -glucosidases in medium containing arbutin rather than glucose and salicin. Sestelo et al. (2004) showed that *L. plantarum* USC1 exhibited the highest extracellular  $\beta$ -glucosidases at the beginning of the stationary phase and that the optimal condition is the presence of oxygen and a low glucose concentration (0.2%). To explore the maximum  $\beta$ -glucosidase production from LAB, bacterial growth medium (pH and carbon sources), temperature, and growth phase are important to reconsider.

Table 8.  $\beta$ -Glucosidase activity of whole cells of lactic acid bacteria isolated from different origin

LAB strains	Origin	$\beta$ -glucosidase activity of whole cells (UA)	Conditions	Reference
<i>Lactobacillus</i> sp. (Lac19)	Commercial olive product	$0.27 \times 10^6$	Isolated after growth on modified MRS (pH 5.0) at 25 °C; Enzymatic assays at pH 5.0 and 37 °C ( <i>p</i> NPG substrate)	Grimaldi et al. (2005)
<i>L. plantarum</i> (Lac 26)	Experimental starter culture	$0.17 \times 10^6$		
<i>Pediococcus</i> sp. (Ped18)	Commercial olive product	$3.80 \times 10^6$		
<i>L. rhamnosus</i> NRRL 442	-	$0.63 \times 10^3$	Enzymatic assays at pH 5.0 and 37 °C ( <i>p</i> NPG )	Kok et al. (2012)
<i>L. brevis</i> SK3	Freeze-dried starter culture	10.6	Isolated after growth on modified MRS (pH 5.0) at 25 °C; Enzymatic assays at pH 5.5 and 37 °C ( <i>p</i> NPG )	Michlmayr et al. (2010)
<i>L. plantarum</i> KFRI 00144	Korean food research institute	$(79.17 \times 10^6)$	Isolated after growth on MRS at 37 °C; Enzymatic assays at pH 7.0 and 37 °C ( <i>p</i> NPG )	Pyo et al. (2005)
<i>L. bulgaricus</i> KFRI 00343		$(6.92 \times 10^6)$		
<i>L. acidophilus</i>		$(17.59 \times 10^6)$		
<i>L. delbrueckii</i>		$(50.58 \times 10^6)$		

UA, nmol of *p*-nitrophenol per minute per milligram of cell dry weight at 37 °C.

Table 9. Characteristic of  $\beta$ -glucosidases isolated from lactic acid bacteria (Michlmayr and Kneifel, 2014)

LAB strains	Location	Specific activity (UA)	pH optimum	Conditions (growth medium)	Reference
<i>Leuconostoc mesenteroides</i>	Intracellular	$73.5 \times 10^3$	5.5-6.0	arbutin	Gueguen et al. (1997)
<i>L. brevis</i> SK3	Intracellular	$71 \times 10^3$	5.5	glucose	Michlmayr et al. (2010)
<i>L. plantarum</i> USC1	Extracellular	$(4.9) \times 10^3$	5.0	glucose	Sestelo et al. (2004)
<i>L. casei</i> ATCC 393	Intracellular	11	6.3	cellobiose	Coulon et al. (1998)
<i>L. rhamnosus</i> CRL981	Intracellular	$23 \times 10^3$	6.4	glucose	Marazza et al. (2009)

UA, nmol of *p*-nitrophenol per minute per milligram of cell dry weight at 37 °C.

## 2.2. Duplicate isolates elimination

To discard duplicate isolates among the  $\beta$ -glucosidase-positive isolates, the RAPD-PCR profiles from 55 isolates (the 14 isolates with the highest activities and 41 isolates randomly selected) were analysed. As an example, the different banding patterns obtained with four isolates are shown in Figure 29. In the case of the two isolates C022-3B and V0023-4B2, the PCR-amplified DNA fragments with the primers P2, P4, and M13 were identical. On the contrary, the primers OPL5 generated two different profiles which enabled us to distinguish these isolates (Figure 29). The analysis of the amplification profiles of the 55 isolates with the primers P2, P4, OPL5, and M13 released 30, 33, 33, and 36 different RAPD-PCR profiles, respectively. Finally, the comparison of the combined RAPD-PCR fingerprinting profiles provided 40 different strains. It revealed redundancy among the 41 isolates with  $\beta$ -glucosidase activity lower than 10 UA but not among the 14 isolates with an activity greater than 10 UA (Figure 28).

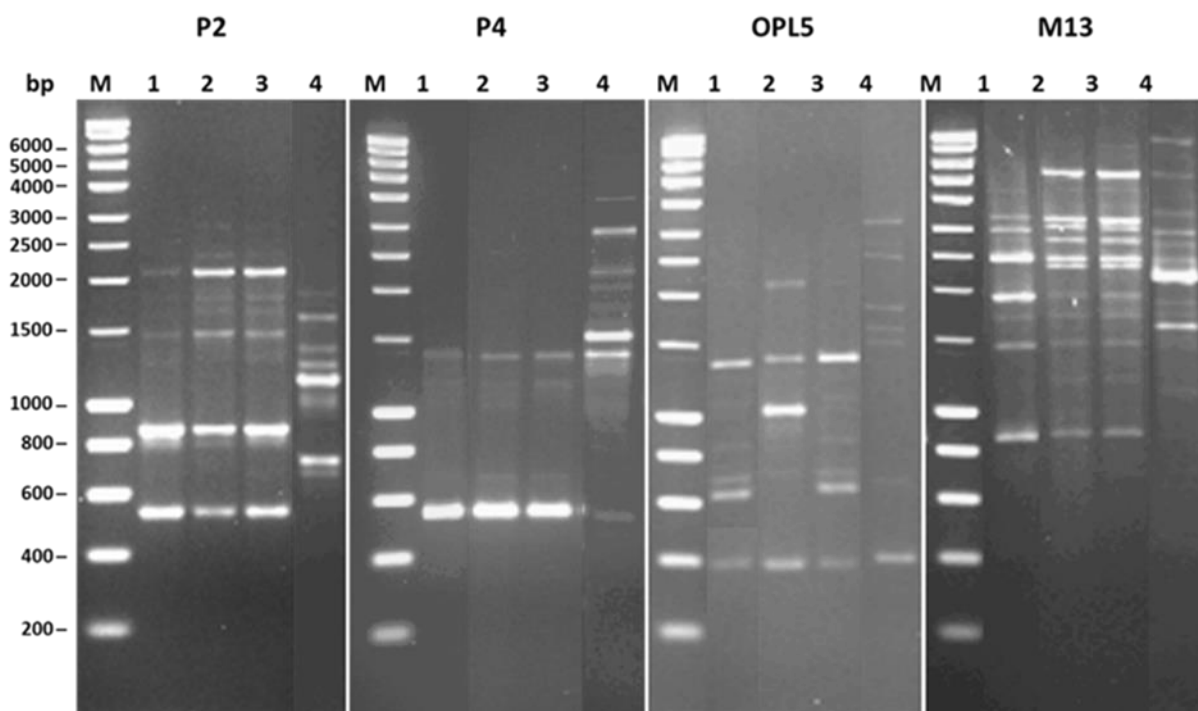


Figure 29. RAPD-PCR profiles of LAB isolates from fermented foods using primers P2, P4, OPL5, and M13. Lanes: **1**, C022-2B; **2**, C022-3B; **3**, V0023-4B2; **4**, F2; **M**, Molecular weight markers (SmartLadder; Eurogentec).

### 2.3. Bacterial species identification by 16S rRNA and multiplex PCR

All the 14  $\beta$ -glucosidase-positive strains, belonged to *Lactiplantibacillus* group (new taxonomic nomenclature (Zheng et al., 2020)) (*L. plantarum*, *L. paraplantarum* and *L. pentosus*) and the  $\beta$ -glucosidase-negative (V013-1A) was *Lactobacillus* (*Limosilactobacillus* in the new taxonomy (Zheng et al., 2020)) *fermentum* according to the 16S rRNA gene sequencing (Table 10). As 16S rRNA gene sequencing cannot discriminate between the three species, multiplex PCR assays were carried out on the 14  $\beta$ -glucosidase-positive strains. Thirteen strains had fragments of the same size of 318 bp corresponding to *L. plantarum*. Only strain F2 gave a fragment of 218 bp corresponding to *L. pentosus* (Figure 30).

Table 10. Lactic acid bacteria species identification by molecular approaches and their fermented foods origin

Strain code	Species	Fermented food	Country
C022-2B	<i>L. plantarum</i>	Fermented shallot roots	Cambodia
C022-3A	<i>L. plantarum</i>	Fermented shallot roots	Cambodia
C022-3B	<i>L. plantarum</i>	Fermented shallot roots	Cambodia
C022-4A	<i>L. plantarum</i>	Fermented shallot roots	Cambodia
C022-4B	<i>L. plantarum</i>	Fermented shallot roots	Cambodia
F1	<i>L. plantarum</i>	Raw fermented fish “ <i>nem trey</i> ”	Cambodia
F2	<i>L. pentosus</i>	Raw fermented fish “ <i>nem trey</i> ”	Cambodia
V0023-4B2	<i>L. plantarum</i>	Raw fermented pork “ <i>nem chua</i> ”	Vietnam
V053-4B	<i>L. plantarum</i>	Fermented small round aubergine	Vietnam
V073-3A1	<i>L. plantarum</i>	Fermented shallot roots	Vietnam
V073-4A	<i>L. plantarum</i>	Fermented shallot roots	Vietnam
V073-4B	<i>L. plantarum</i>	Fermented shallot roots	Vietnam
V083-4A	<i>L. plantarum</i>	Fresh banana leaf	Vietnam
V083-4B	<i>L. plantarum</i>	Fresh banana leaf	Vietnam
V013-1A	<i>L. fermentum</i>	Raw fermented pork “ <i>nem chua</i> ”	Vietnam
B33	<i>L. plantarum</i>	Raw fermented pork “ <i>nem chua</i> ”	Vietnam



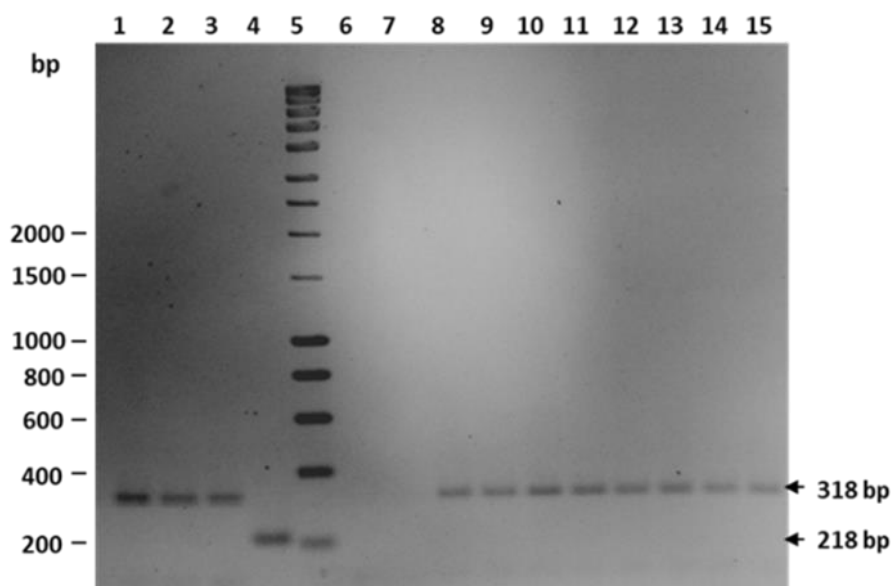


Figure 30. PCR multiplex analysis of the *Lactiplantibacillus* group. Lane: **1**, V0023-4B2; **2**, V053-4B; **3**, F1; **4**, F2; **5**, Molecular weight markers (SmartLadder; Eurogentec); **6**, H<sub>2</sub>O; **7**, V013-1A; **8**, C022-2B; **9**, C022-3A; **10**, C022-3B; **11**, C022-4A; **12**, C022-4B; **13**, V073-3A1; **14**, V073-4A; **15**, V073-4B. DNA molecular weight markers (bp) are shown on the left.

#### 2.4. Pectinase activity

Three solid media, MRS (with or without glucose), YEP, and MP and three different dyes (Congo red, ruthenium red, and potassium iodide) were used to visualise the pectinolytic activity of the LAB isolates. After staining with two dye solutions (potassium iodide solution or Congo red), the clearance zone around the colony would indicate positive pectinase. Although the clearance zone was likely to observe in [Figure 31B](#) and [C](#), it was not caused by pectinase activity but rather by the visible patch of LAB colonies. In contrast, after staining with ruthenium red, a dark-red halo surrounding the colony would indicate polygalacturonase activity. Even though two colonies in [Figure 31A](#) appeared to have a dark-red halo, this dark-red colour may be caused by the dryness surrounding the colony. Therefore, it is impossible to draw an absolute conclusion from this test as the results may be false positive and/or false negative. LAB isolates might exhibit extremely low pectinase activity and below the threshold of detection in plate assays.

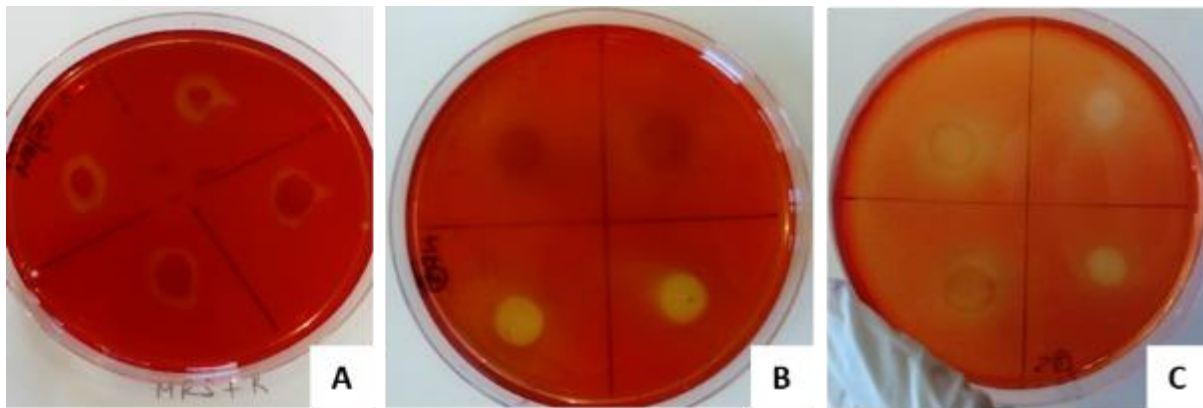


Figure 31. Pectin degradation Petri plate assay after staining with different dyes. **A**, MRS with ruthenium red; **B**, YEP with Congo red; **C**, MP with potassium iodide.

Another strategy to evaluate the pectinase activity of LAB isolates was DNSA method. However, the results of the absorbance of the test were often lower than the blank values showing that the DNSA method might not be able to detect the tiny amounts of pectinases of the LAB isolates. A viscometry approach might be necessary to detect low pectinolytic activities but it seems difficult to be developed in a screening goal. *Leuconostoc mesenteroides* and *L. plantarum* were evaluated successfully by this approach since pectinase activity could cause a rapid drop of pectic substance solution viscosity (Juven et al., 1985; Sakellaris et al., 1988). Furthermore, in order to develop a method to visualise the pectinolytic activity in Petri plates, a positive pectinase strain is worth to have. Another modification would be to leave the staining dye (0.05% ruthenium red) solution in Petri plates for a longer time (one hour) (Banu et al., 2010).

## 2.5. Alcohol dehydrogenase activity

Four strains selected for their diverse  $\beta$ -glucosidase activity were tested for their potential to reduce aldehydes into their corresponding alcohols: *L. plantarum* C022-2B and C022-3B, the two highest producer strains, *L. plantarum* V0023-4B2, an intermediate producer, and *L. fermentum* V013-1A, exhibiting no detectable  $\beta$ -glucosidase activity. Hexanal and (*E*)-2-hexenal were used as test substrates because they are short-chain saturated and unsaturated aldehydes present in mashed tomatoes and important contributors to the tomato aroma (Wang et al., 2016). *L. fermentum* V013-1A was able to reduce hexanal ( $3.85 \times 10^{-8}$  UA) and (*E*)-2-hexenal ( $2.47 \times 10^{-8}$  UA) into hexanol and (*E*)-2-hexenol, respectively (Table 11). No alcohol was detected in the *L. plantarum* C022-2B, C022-3B, and V0023-4B2 vials under the above conditions. Therefore, an adjustment was made by concentrating bacterial cells ten times.

Indeed, *L. plantarum* C022-2B, C022-3B, and V0023-4B2 could only reduce hexanal with a far lower reduction rate than *L. fermentum* V013-1A. For *L. plantarum* strains, no reduction of (*E*)-2-hexenal was observed.

Table 11. Reduction of aldehydes into alcohols by ADH activity of *L. plantarum* C022-2B, C022-3B and V0023-4B2 and *L. fermentum* V013-1A

Strain	Substrate disappearance rate ( $\times 10^{-8}$ UA)		Product appearance rate ( $\times 10^{-8}$ UA)	
	<i>L. plantarum</i> C022-2B	Hexanal	$0.44 \pm 0.05$	Hexanol
	( <i>E</i> )-2-hexenal	n.d.	( <i>E</i> )-2-hexenol	n.d.
<i>L. plantarum</i> C022-3B	Hexanal	$< 0.44$	Hexanol	$< 0.31$
	( <i>E</i> )-2-hexenal	n.d.	( <i>E</i> )-2-hexenol	n.d.
<i>L. plantarum</i> V0023-4B2	Hexanal	$< 0.44$	Hexanol	$< 0.31$
	( <i>E</i> )-2-hexenal	n.d.	( <i>E</i> )-2-hexenol	n.d.
<i>L. fermentum</i> V013-1A	Hexanal	$3.85 \pm 0.18$	Hexanol	$3.70 \pm 0.42$
	( <i>E</i> )-2-hexenal	$2.47 \pm 0.67$	( <i>E</i> )-2-hexenol	$2.58 \pm 0.26$

n.d. = not detectable

Values are expressed as means of triplicate experiments. One unit of enzymatic activity (UA) was defined as the amount of a substrate (nmol) reduced into a product per minute per CFU.

*L. fermentum* V013-1A seemed to have the greatest potential for aldehyde reduction ability. Consequently, more aldehyde and ketone substrates were tested. As shown in Table 12, *L. fermentum* V013-1A was capable of converting (*E*)-2-heptenal ( $2.46 \times 10^{-8}$  UA) and (*E,E*)-2,4-decadienal ( $3.69 \times 10^{-8}$  UA) to (*E*)-2-heptenol and (*E,E*)-2,4-decadienol, respectively. This strain could also convert 6,10-dimethyl-5,9-undecadien-2-one ( $1.26 \times 10^{-8}$  UA) to 6,10-dimethyl-5,9-undecadien-2-ol but not 6-methyl-5-hepten-2-one. Therefore, *L. fermentum* V013-1A was able to reduce aldehydes and ketones to their corresponding alcohols with a reduction ability greater for aldehydes than for ketones.

Table 12. Reduction of aldehydes and ketones into alcohols by ADH activity of *L. fermentum* V013-1A

Strain	Substrate disappearance rate ( $\times 10^{-8}$ UA)	Product appearance rate ( $\times 10^{-8}$ UA)		
<i>L. fermentum</i>	( <i>E</i> )-2-heptenal	2.46 $\pm$ 0.25	( <i>E</i> )-2-heptenol	3.13 $\pm$ 0.26
V013-1A	( <i>E,E</i> )-2,4-decadienal	3.69 $\pm$ 0.00	( <i>E,E</i> )-2,4-decadienol	3.22 $\pm$ 0.00
	6-Methyl-5-hepten-2-one	n.d.	6-Methyl-5-hepten-2-ol	n.d.
	6,10-Dimethyl-5,9-undecadien-2-one	1.26 $\pm$ 0.14	6,10-Dimethyl-5,9-undecadien-2-ol	0.81 $\pm$ 0.08

n.d. = not detectable

Values are expressed as means of triplicate experiments. One unit of enzymatic activity (UA) was defined as the amount of a substrate (nmol) reduced into a product per minute per CFU.

In conclusion, first of all, our study has demonstrated that  $\beta$ -glucosidase activity is widespread among LAB from Asian fermented foods since of the 200 bacterial isolates screened, 80 were positive for  $\beta$ -glucosidase. However, most of bacteria exhibited low activity. Only 14 strains showed  $\beta$ -glucosidase activity greater than 10 UA and the highest  $\beta$ -glucosidase activity was 27 UA. Among them, thirteen were *L. plantarum* and one was *L. pentosus*. According to this result, one could hypothesise that all *L. plantarum/paraplantarum/pentosus* strains found in fermented foods are positive for  $\beta$ -glucosidase activity. In fact, this is not obvious since *L. plantarum* is generally the dominant species in lacto-fermented foods. Identification of a representative panel of  $\beta$ -glucosidase-negative isolates should be necessary to rule on this assumption.

Second, our results have highlighted differences between bacterial isolates concerning ADH activity. The resting cells of *L. fermentum* V013-1A demonstrated the highest reduction ability for hexanal ( $3.85 \times 10^{-8}$  UA) and (*E*)-2-hexenal ( $2.47 \times 10^{-8}$  UA), whereas, the resting cells of *L. plantarum* C022-2B, C022-3B, and V0023-4B2 exhibited similar ADH reduction ability for only hexanal ( $0.44 \times 10^{-8}$  UA). The resting cells of *L. fermentum* V013-1A was also able to reduce (*E*)-2-heptenal ( $2.46 \times 10^{-8}$  UA), (*E,E*)-2,4-decadienal ( $3.69 \times 10^{-8}$  UA), and geranylacetone ( $1.26 \times 10^{-8}$  UA).

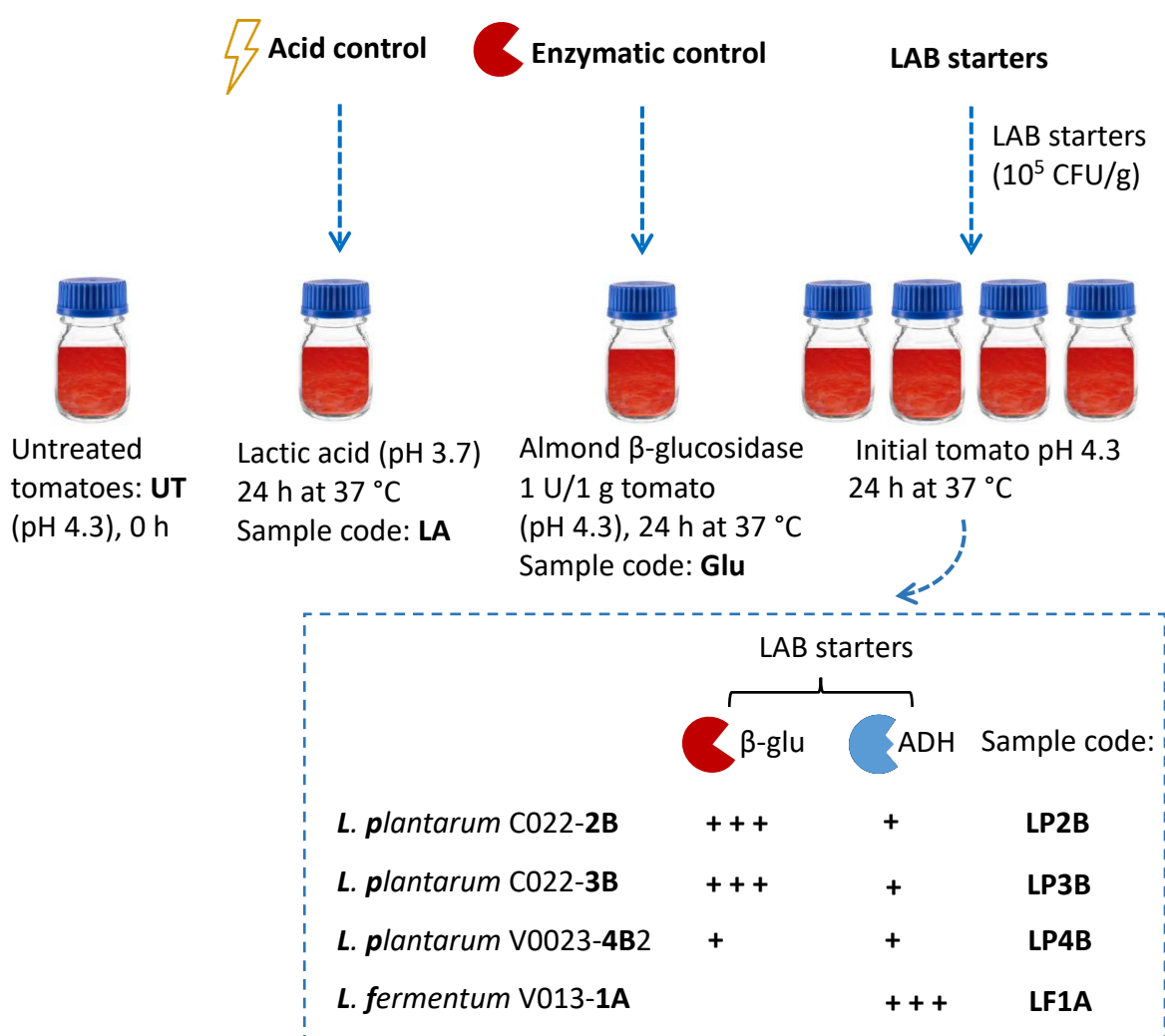
Unfortunately, the method of screening for pectinase activity of LAB strains was not validated because LAB strains might exhibit extremely low pectinase activity and below the threshold of detection in plate assays.

### 3. Application of aromatic starter cultures in fermented tomatoes

LAB have been known to enhance the flavour of fermented products thanks to their metabolism. Homofermentative LAB produce lactic acid as the main end-product of carbohydrate fermentation while heterofermentative LAB produce in addition other products such as acetic acid, carbon dioxide, ethanol, acetoin, and diacetyl (Gänzle, 2015; Leroy and De Vuyst, 2004). The volatiles generated by heterofermentation can provide special tastes and flavours to final fermented foods. The characteristic of many fermented foods depends therefore on LAB metabolism. Furthermore, the genesis of flavour compounds mainly rely on the specific ability of different bacterial strains to convert precursors derived from carbohydrates, proteins, fatty acids, carotenoids and glycosides (Bancalari et al., 2017). The newly generated volatile compounds are added to the pool of flavouring compounds that are already present in plant matrix though in plant, these latter are mostly encountered under a glycosylated form to decrease their toxicity (Song et al., 2018).  $\beta$ -Glucosidases are key enzymes for releasing aroma compounds (e.g. monoterpene, norisoprenoids, benzene derivatives and long-chain aliphatic alcohols) from glycosidic precursors present in fruits and vegetables (Krisch et al., 2010). They are utilised to enhance the flavour of wine (Sestelo et al., 2004), tea (Su et al., 2010), and fruit juice (Fan et al., 2011). ADH is another family of enzymatic activities which could be important for modifying aroma profiles in fruits and vegetables (Gargouri et al., 2004). ADH catalyses the transformation of aldehydes or ketones into their corresponding alcohols, acids or esters (Hu et al., 2019).

Four LAB strains exhibiting distinct  $\beta$ -glucosidase activities (high, medium or negative) and ADH activities were used as starters in mashed tomatoes. The strain ability to release volatile compounds from the tomato aroma precursors was analysed in regard to their  $\beta$ -glucosidase and ADH activities. Therefore, the third objective of this study was to characterise the role of LAB strains in the release of volatile aroma compounds in function of their enzymatic activities, using tomato as the fermentation matrix.

## Experimental strategy for this study



The four strains exhibiting various enzymatic properties were used individually as starters for tomato fermentation. The mashed tomatoes (MT) were analysed immediately after blending as a reference without any treatment, and this condition was called untreated tomatoes (UT). The MT were then treated with lactic acid at pH 3.70 as a control of acid hydrolysis and with  $\beta$ -glucosidase as a control of enzymatic activity. These conditions were noted LA and Glu, respectively.

### 3.1. pH and bacterial enumeration in fermented tomatoes

For the four strains, during fermentation of MT the pH value dropped to 3.5-3.8 and the total number of lactobacilli increased from  $1.0 \times 10^5$  CFU/g (initial inoculation) to  $1.2 \times 10^8$  -  $1.5 \times 10^9$  CFU/g (Table 13), demonstrating that the four strains were able to grow and ferment MT individually. For LA and Glu, lactobacilli counts were  $1.1 \times 10^7$  and  $5.4 \times 10^7$  CFU/g after

incubation, showing that a spontaneous fermentation took place reaching lower bacterial counts than with starters (Table 13).

Table 13. Total lactobacilli count on MRS agar plates (CFU/g) and pH of tomatoes fermented with the selected LAB strains, and controls

Treatment	pH	Total lactobacilli (CFU/g)
Untreated tomatoes (UT)	4.3	$8.3 \times 10^2$
Lactic-acid-treated tomatoes (LA)	3.7	$1.1 \times 10^7$
Glucosidase-treated tomatoes (Glu)	4.1	$5.4 \times 10^7$
Fermented tomatoes with <i>L. plantarum</i> C022-2B (LP2B)	3.7	$7.5 \times 10^8$
Fermented tomatoes with <i>L. plantarum</i> C022-3B (LP3B)	3.7	$7.0 \times 10^8$
Fermented tomatoes with <i>L. plantarum</i> V0023-4B2 (LP4B)	3.8	$1.5 \times 10^9$
Fermented tomatoes with <i>L. fermentum</i> V013-1A (LF1A)	3.5	$1.2 \times 10^8$

For all conditions, tomatoes were incubated for 24 h at 37 °C and analysed immediately, except for UT for which tomatoes were not incubated. Data are means of three independent experiments.

### 3.2. Fibre coating selection

Three fibre coatings (PDMS, PDMS/DVB, and CAR/PDMS) were tested on mashed tomatoes spiked with an internal standard (4-nonanol). The volatile compounds of the mashed tomatoes were analysed by GC/FID. The total peak area of volatile compounds of mashed tomatoes obtained from PDMS, PDMS/DVB, and CAR/PDMS was approximately 10 000, 84 000, and 96 000, respectively. PDMS showed the smallest amount of total peak area whereas PDMS/DVB and CAR/PDMS coatings displayed a similar amount of total peak area. PDMS/DVB and CAR/PDMS are mixed coating with adsorption extraction mechanism. However, PDMS/DVB and CAR/PDMS showed completely dissimilar peak volatile profiles (Figure 32A). As illustrated in Figure 32A, the CAR/PDMS chromatogram (blue) shows higher peak areas of low molecular weight compounds and compatible only with short chain molecules. This is probably due to its small micropores (Vas and Vékey, 2004). On the other hand, PDMS/DVB shows the highest sensitivity for compounds of medium and low volatility. This coating fibre give the most diverse volatile compounds profiles, in particular for aldehydes, alcohols, ketones, and terpenes. The PDMS chromatogram also seems to provide various volatile compounds profiles but the peaks of volatile compounds were much smaller than those

of PDMS/DVB (Figure 32B). Therefore, PDMS/DVB was the preferred coating material for extracting tomato aroma, and PDMS/DVB would be used for future experiments.

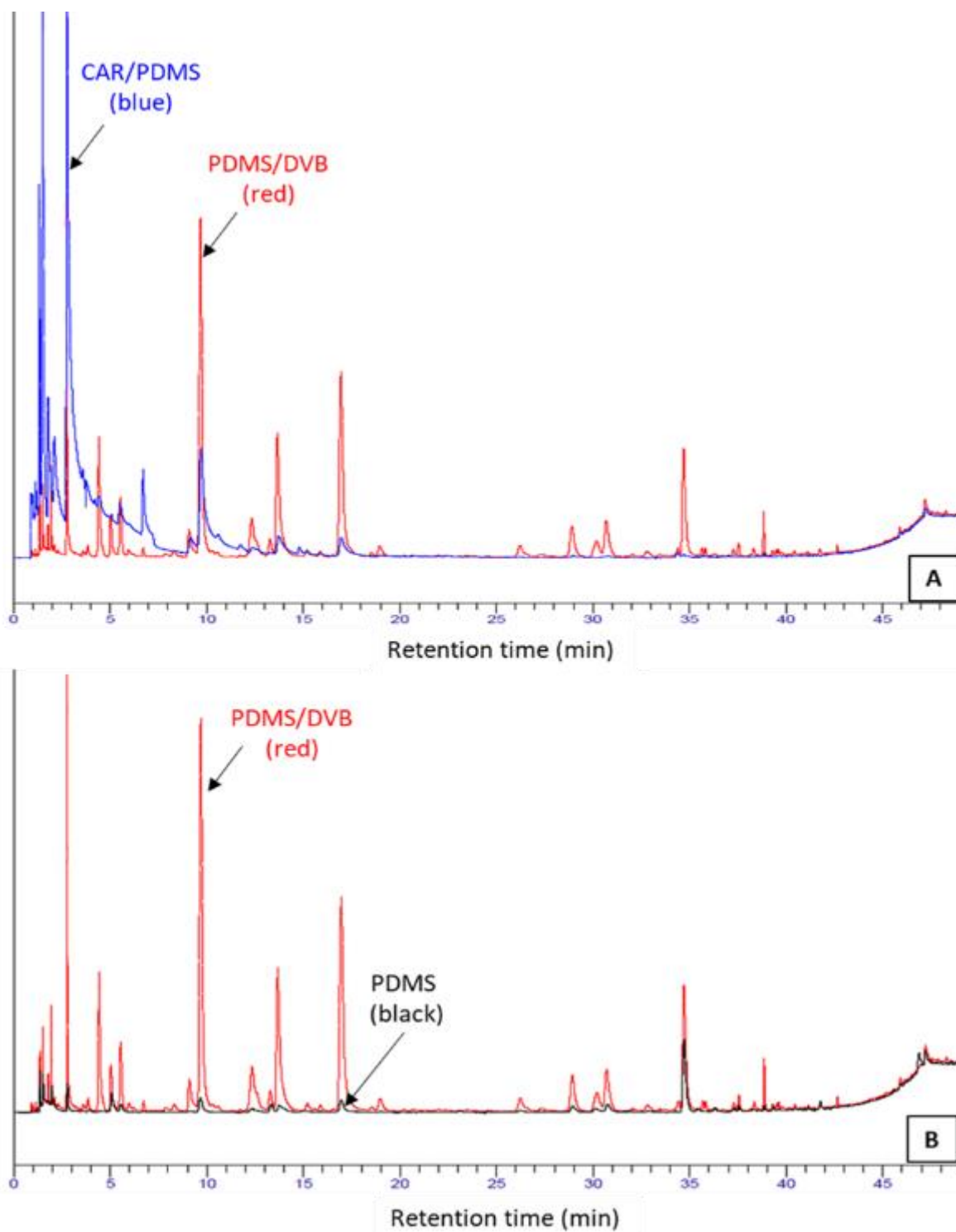


Figure 32. Chromatogram of volatile compounds in mashed tomatoes obtained by fibre coating PDMS (black), PDMS/DVB (red), and CAR/PDMS (blue) with headspace-solid phase microextraction/gas chromatography-flame ionisation detection (HS-SPME/GC-FID).



### 3.3. Volatile compounds in fermented tomatoes

The volatile compounds in UT, LA, Glu, and fermented tomatoes were extracted using PDMS/DVB coating. They were identified and quantified by GC/MS. Fifty-eight volatile compounds were grouped according to the chemical classes: aldehydes, alcohols, ketones, terpenes, organic acids, and miscellaneous (Table 14).

Table 14. Volatile compounds (ppb) found in tomato after treatment

Chemical class	Treatment						
	UT	MT-LA	MT-Glu	MT-LP2B	MT-LP3B	MT-LP4B	MT-LF1A
<b>Aldehydes</b>							
Hexanal	135.85 <sup>a</sup>	92.21 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
( <i>E</i> )-2-hexenal	18.10 <sup>a</sup>	17.07 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
Heptanal	6.41 <sup>a</sup>	1.36 <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
( <i>E</i> )-2-heptenal	15.65 <sup>a</sup>	14.81 <sup>a</sup>	nd <sup>a</sup>	7.77 <sup>a</sup>	tr <sup>a</sup>	3.56 <sup>a</sup>	nd <sup>a</sup>
( <i>E</i> )-2-octenal	60.19 <sup>a</sup>	58.46 <sup>a</sup>	1.78 <sup>b</sup>	49.63 <sup>ab</sup>	11.92 <sup>ab</sup>	13.60 <sup>ab</sup>	nd <sup>b</sup>
Nonanal	16.77 <sup>a</sup>	6.7 <sup>b</sup>	nd <sup>c</sup>	tr <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>
( <i>E</i> )-2-nonenal	9.38 <sup>a</sup>	9.70 <sup>a</sup>	nd <sup>b</sup>	3.29 <sup>ab</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
Decanal	9.97 <sup>a</sup>	5.99 <sup>ab</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
( <i>E,E</i> )-2,4-decadienal	31.68 <sup>ab</sup>	18.17 <sup>bc</sup>	nd <sup>c</sup>	42.98 <sup>a</sup>	32.97 <sup>ab</sup>	nd <sup>c</sup>	nd <sup>c</sup>
Benzaldehyde	nd <sup>b</sup>	nd <sup>b</sup>	13.39 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
β-Cyclocitral	4.76 <sup>a</sup>	5.85 <sup>a</sup>	3.54 <sup>a</sup>	3.74 <sup>a</sup>	4.44 <sup>a</sup>	4.06 <sup>a</sup>	4.09 <sup>a</sup>
β-Citral	11.62 <sup>a</sup>	9.51 <sup>a</sup>	10.44 <sup>a</sup>	8.11 <sup>a</sup>	5.95 <sup>a</sup>	6.79 <sup>a</sup>	1.88 <sup>a</sup>
α-Citral	31.12 <sup>a</sup>	15.26 <sup>b</sup>	16.25 <sup>b</sup>	14.36 <sup>b</sup>	8.02 <sup>b</sup>	8.48 <sup>b</sup>	4.28 <sup>b</sup>
4-Methyl-3-cyclohexene-1-carboxaldehyde	tr <sup>a</sup>	1.60 <sup>a</sup>	2.97 <sup>a</sup>	tr <sup>a</sup>	nd <sup>a</sup>	nd <sup>a</sup>	nd <sup>a</sup>
3-Methyl-3-(4-methyl-3-pentenyl)-oxiranecarboxaldehyde	6.48 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
<b>Total</b>	<b>358.80</b>	<b>256.69</b>	<b>48.37</b>	<b>131.48</b>	<b>64.10</b>	<b>36.49</b>	<b>10.25</b>
<b>Alcohols</b>							
2,3-Butanediol	nd <sup>b</sup>	nd <sup>b</sup>	2.46 <sup>b</sup>	12.27 <sup>a</sup>	tr <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
Hexanol	nd <sup>c</sup>	6.25 <sup>c</sup>	32.10 <sup>bc</sup>	27.93 <sup>bc</sup>	51.31 <sup>ab</sup>	50.45 <sup>ab</sup>	74.05 <sup>a</sup>
1-Heptanol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	3.86 <sup>a</sup>	nd <sup>b</sup>
2-Heptanol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	5.29 <sup>a</sup>
( <i>E</i> )-2-heptenol	nd <sup>b</sup>	nd <sup>b</sup>	7.98 <sup>a</sup>	7.37 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	6.06 <sup>a</sup>
1-Octanol	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	4.43 <sup>b</sup>	nd <sup>c</sup>	15.07 <sup>a</sup>	nd <sup>c</sup>
1-Octen-3-ol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	1.50 <sup>a</sup>
( <i>E</i> )-2-octenol	nd <sup>d</sup>	nd <sup>d</sup>	22.20 <sup>a</sup>	15.42 <sup>b</sup>	6.60 <sup>c</sup>	5.75 <sup>c</sup>	20.47 <sup>a</sup>
1-Nonanol	nd <sup>b</sup>	nd <sup>b</sup>	tr <sup>b</sup>	3.37 <sup>a</sup>	3.41 <sup>a</sup>	tr <sup>b</sup>	2.10 <sup>a</sup>
2-Nonanol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	2.70 <sup>a</sup>
( <i>Z</i> )-3-nonenol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	7.64 <sup>a</sup>
( <i>E</i> )-2-nonenol	nd <sup>b</sup>	nd <sup>b</sup>	1.34 <sup>b</sup>	6.39 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	5.65 <sup>a</sup>
( <i>E,E</i> )-2,4-decadienol	nd <sup>b</sup>	nd <sup>b</sup>	2.74 <sup>ab</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	4.96 <sup>a</sup>
6-Methyl-5-hepten-2-ol	nd <sup>d</sup>	nd <sup>d</sup>	9.84 <sup>d</sup>	33.70 <sup>b</sup>	35.38 <sup>b</sup>	21.74 <sup>c</sup>	163.19 <sup>a</sup>

6,10-Dimethyl-5,9-undecadien-2-ol	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	10.49 <sup>b</sup>	10.33 <sup>b</sup>	12.74 <sup>b</sup>	25.75 <sup>a</sup>
2-Methoxy phenol	5.86 <sup>a</sup>	5.97 <sup>a</sup>	5.21 <sup>a</sup>	3.55 <sup>a</sup>	10.39 <sup>a</sup>	5.41 <sup>a</sup>	5.93 <sup>a</sup>
2-Phenylethanol	nd <sup>d</sup>	1.74 <sup>d</sup>	21.17 <sup>a</sup>	9.70 <sup>bc</sup>	16.32 <sup>ab</sup>	12.28 <sup>bc</sup>	5.20 <sup>cd</sup>
4-Ethylphenol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	15.50 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>
<b>Total</b>	<b>5.86</b>	<b>13.96</b>	<b>105.44</b>	<b>134.62</b>	<b>150.05</b>	<b>128.09</b>	<b>330.47</b>
<b>Ketones</b>							
1-Octen-3-one	3.24 <sup>a</sup>	0.84 <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
6-Methyl-5-hepten-2-one	139.57 <sup>a</sup>	184.69 <sup>a</sup>	219.43 <sup>a</sup>	246.69 <sup>a</sup>	269.49 <sup>a</sup>	207.21 <sup>a</sup>	4.31 <sup>b</sup>
6,10-Dimethyl-5,9-undecadien-2-one	78.88 <sup>a</sup>	84.91 <sup>a</sup>	81.83 <sup>a</sup>	78.46 <sup>a</sup>	62.47 <sup>ab</sup>	51.51 <sup>ab</sup>	4.23 <sup>b</sup>
2-Undecanone	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	28.83 <sup>a</sup>	8.85 <sup>b</sup>
α-Ionone	nd <sup>b</sup>	2.10 <sup>a</sup>	tr <sup>ab</sup>	tr <sup>ab</sup>	tr <sup>ab</sup>	tr <sup>ab</sup>	nd <sup>b</sup>
1-(2-Methyl-cyclopenten-1-yl)-ethanone	tr <sup>b</sup>	3.20 <sup>ab</sup>	tr <sup>b</sup>	5.54 <sup>a</sup>	4.74 <sup>a</sup>	tr <sup>b</sup>	3.25 <sup>ab</sup>
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	12.23 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	tr <sup>b</sup>
5-Ethyl-2,4-dimethyl-4-hepten-3-one	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	6.05 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
<b>Total</b>	<b>222.49</b>	<b>275.75</b>	<b>302.86</b>	<b>349.78</b>	<b>337.49</b>	<b>289.15</b>	<b>21.43</b>
<b>Terpenes</b>							
(Z)-geraniol	nd <sup>b</sup>	nd <sup>b</sup>	1.73 <sup>b</sup>	9.86 <sup>a</sup>	6.06 <sup>a</sup>	6.94 <sup>a</sup>	7.73 <sup>a</sup>
6,7-Dihydrogeraniol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	tr <sup>b</sup>	3.12 <sup>a</sup>	4.21 <sup>a</sup>
Melonol	nd <sup>d</sup>	nd <sup>d</sup>	nd <sup>d</sup>	5.16 <sup>a</sup>	tr <sup>c</sup>	2.69 <sup>b</sup>	tr <sup>c</sup>
Linalool	nd <sup>b</sup>	1.52 <sup>ab</sup>	tr <sup>ab</sup>	5.35 <sup>a</sup>	3.84 <sup>ab</sup>	tr <sup>ab</sup>	2.58 <sup>ab</sup>
D-limonene	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	9.38 <sup>a</sup>	8.15 <sup>a</sup>	5.43 <sup>a</sup>
(Z)-2,6-dimethyl-2,6-octadiene	8.82 <sup>a</sup>	7.90 <sup>a</sup>	5.77 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
(3Z,5Z)-2,7-dimethyl-3,5-octadiene	6.98 <sup>a</sup>	1.75 <sup>b</sup>	4.23 <sup>b</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>
<b>Total</b>	<b>15.80</b>	<b>11.17</b>	<b>12.52</b>	<b>20.37</b>	<b>20.88</b>	<b>21.69</b>	<b>20.75</b>
<b>Organic acid and their derivatives</b>							
Acetic acid	nd <sup>c</sup>	nd <sup>c</sup>	26.69 <sup>c</sup>	93.56 <sup>b</sup>	tr <sup>c</sup>	7.36 <sup>c</sup>	148.58 <sup>a</sup>
Hexyl ester acetic acid	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	tr <sup>c</sup>	5.45 <sup>a</sup>	nd <sup>c</sup>	3.08 <sup>b</sup>
Octanoic acid	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	5.74 <sup>a</sup>	4.27 <sup>b</sup>
Methyl ester octanoic acid	5.94 <sup>a</sup>	3.25 <sup>b</sup>	tr <sup>b</sup>	3.13 <sup>b</sup>	tr <sup>b</sup>	tr <sup>b</sup>	2.36 <sup>b</sup>
<b>Total</b>	<b>5.94</b>	<b>3.25</b>	<b>27.49</b>	<b>97.49</b>	<b>7.05</b>	<b>13.90</b>	<b>158.28</b>
<b>Miscellaneous</b>							
Methoxy-phenyl-oxime	1.89 <sup>a</sup>	2.82 <sup>a</sup>	2.18 <sup>a</sup>	nd <sup>a</sup>	tr <sup>a</sup>	3.90 <sup>a</sup>	6.56 <sup>a</sup>
2,7-Dimethyl-oxepine	nd <sup>a</sup>	7.29 <sup>a</sup>	nd <sup>a</sup>	nd <sup>a</sup>	8.73 <sup>a</sup>	8.65 <sup>a</sup>	8.26 <sup>a</sup>
2-Isobutylthiazole	14.89 <sup>a</sup>	tr <sup>c</sup>	nd <sup>c</sup>	18.26 <sup>a</sup>	12.37 <sup>ab</sup>	13.66 <sup>ab</sup>	4.52 <sup>bc</sup>

3-(4-Methyl-3-pentenyl)-furan	7.79 <sup>ab</sup>	7.30 <sup>b</sup>	8.32 <sup>ab</sup>	14.01 <sup>ab</sup>	14.46 <sup>ab</sup>	13.93 <sup>ab</sup>	16.88 <sup>a</sup>
1-Isocyano-3-methylbenzene	nd <sup>d</sup>	nd <sup>d</sup>	nd <sup>d</sup>	6.17 <sup>a</sup>	tr <sup>c</sup>	3.39 <sup>b</sup>	3.66 <sup>b</sup>
Methyl salicylate	11.66 <sup>a</sup>	9.91 <sup>a</sup>	nd <sup>a</sup>	5.74 <sup>a</sup>	7.22 <sup>a</sup>	nd <sup>a</sup>	nd <sup>a</sup>
<b>Total</b>	<b>36.23</b>	<b>28.12</b>	<b>10.50</b>	<b>44.18</b>	<b>44.54</b>	<b>43.54</b>	<b>158.28</b>

Samples were tomatoes fermented with *L. plantarum* C022-2B (sample LP2B), C022-3B (LP3B), and V0023-4B2 (LP4B) and *L. fermentum* V013-1A (LF1A). Abbreviations: UT, untreated tomatoes; LA, lactic-acid-treated tomatoes; Glu, glucosidase-treated tomatoes; using PDMS/DVB coating with headspace-solid phase microextraction/gas chromatography-mass spectrometry (HS-SPME/GC-MS).

“a, b, c, d” Values in the same line followed by different letters are significantly different ( $p < 0.05$ ). tr, less than 0.80 ppb; nd, not detected.

As shown in Figure 33, most of aldehydes found in untreated tomato (UT) had their concentration decreasing, at least three times, and sometimes even reached zero during fermentation and  $\beta$ -glucosidase treatment. They also decreased, but slightly (about 1.4 folds), during the lactic acid treatment (LA). Among fermented tomatoes, LF1A (tomatoes fermented with *L. fermentum* V013-1A) contained the lowest amount of aldehydes with a 36-fold decrease compared to UT. Only  $\beta$ -cyclocitral,  $\beta$ -citral, and  $\alpha$ -citral remained in all fermented tomatoes (Table 14). The levels of aldehydes derived from the LOX pathway such as hexanal (C=6), 2-hexenal (C=6), 2-octenal (C=8), and 2-nonenal (C=9) were similar in UT and LA whereas they were not detectable or very low in the other treatments (Figure 34).

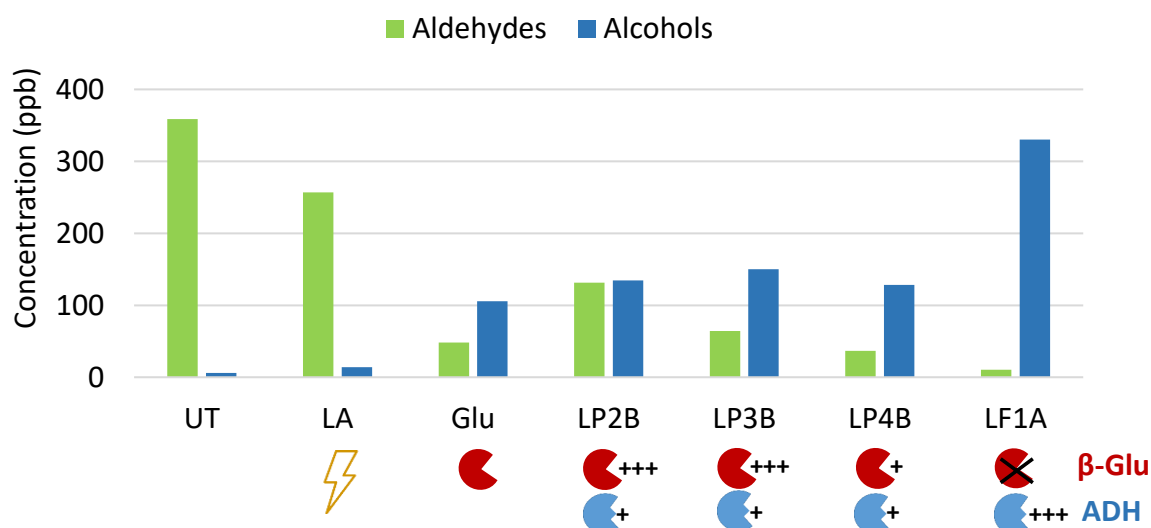


Figure 33. Total concentration of aldehydes and alcohols in treated and fermented tomatoes. Four strains exhibiting various enzymatic properties ( $\beta$ -glucosidase ( $\beta$ -glu) and alcohol dehydrogenase (ADH); high (+++) and low (+)) were used individually as starters for tomato fermentation. Abbreviations: LP2B, tomatoes fermented with *L. plantarum* C022-2B; LP3B,

with *L. plantarum* C022-3B; LP4B, with *L. plantarum* V0023-4B2; LF1A, with *L. fermentum* V013-1A; UT, untreated tomatoes; LA, lactic-acid-treated tomatoes; Glu, glucosidase-treated tomatoes

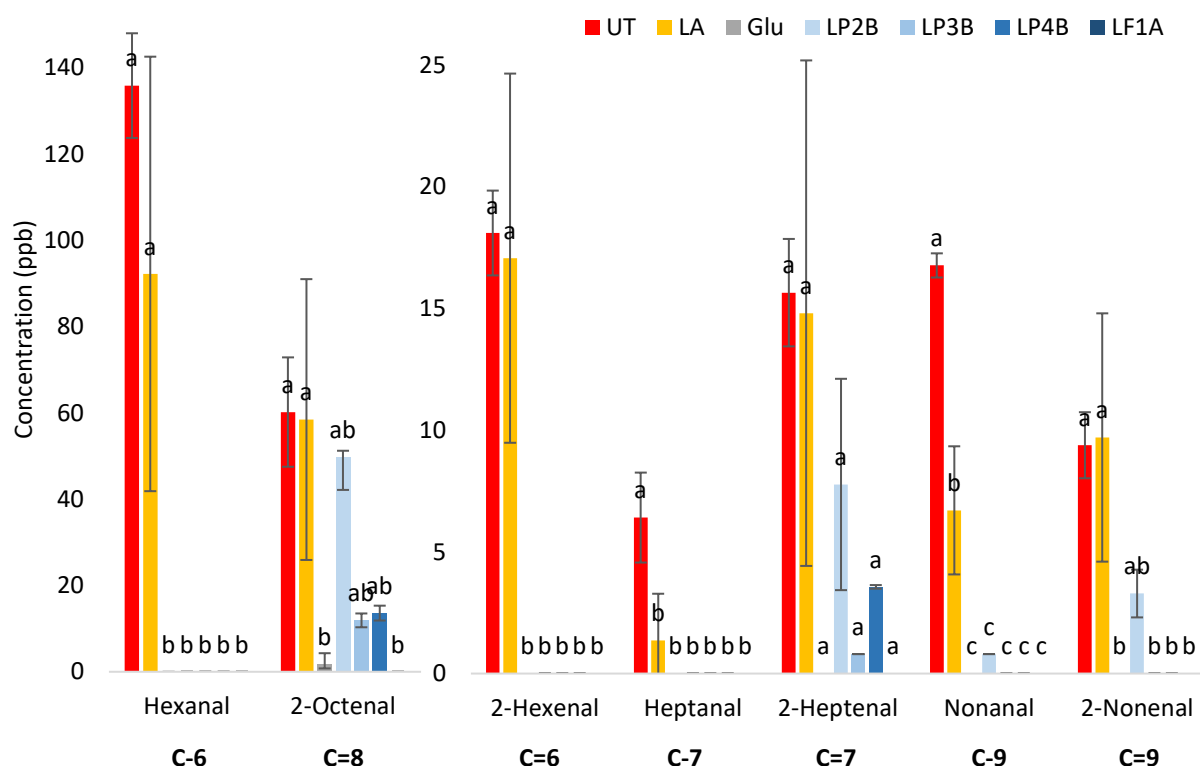


Figure 34. LOX-pathway-derived aldehydes from fatty acids in treated and fermented tomatoes. Abbreviations: LP2B, tomatoes fermented with *L. plantarum* C022-2B; LP3B, with *L. plantarum* C022-3B; LP4B, with *L. plantarum* V0023-4B2; LF1A, with *L. fermentum* V013-1A; UT, untreated tomatoes; LA, lactic-acid-treated tomatoes; Glu, glucosidase-treated tomatoes. C– and C= are saturated and unsaturated aldehydes, respectively. Means with different letters (a, b, c, d) are significantly different ( $p < 0.05$ ).

Unlike aldehydes, most alcohols detected in our study were not found in UT and LA (Figure 33). Inversely correlated with the degradation of aldehydes, the concentration of alcohols significantly increased during fermentation and  $\beta$ -glucosidase treatment. The level of alcohols was the highest in LF1A, where it was about twice as high as in other fermented tomatoes. The alcohols present in high concentrations in all fermented tomatoes were hexanol (C–6) and 2-octenol (C=8) (the two volatiles derived from the LOX pathway) in Figure 35, and 6-methyl-5-hepten-2-ol (C=8) and 6,10-dimethyl-5,9-undecadien-2-ol (C=13) in Table 14. Low concentrations of phenolic volatiles, including 2-phenylethanol and 4-ethylphenol, appeared during fermentation and treatment (Table 14). 2,3-Butanediol only increased significantly in LP2B (tomatoes fermented with *L. plantarum* C022-2B) (Table 14).

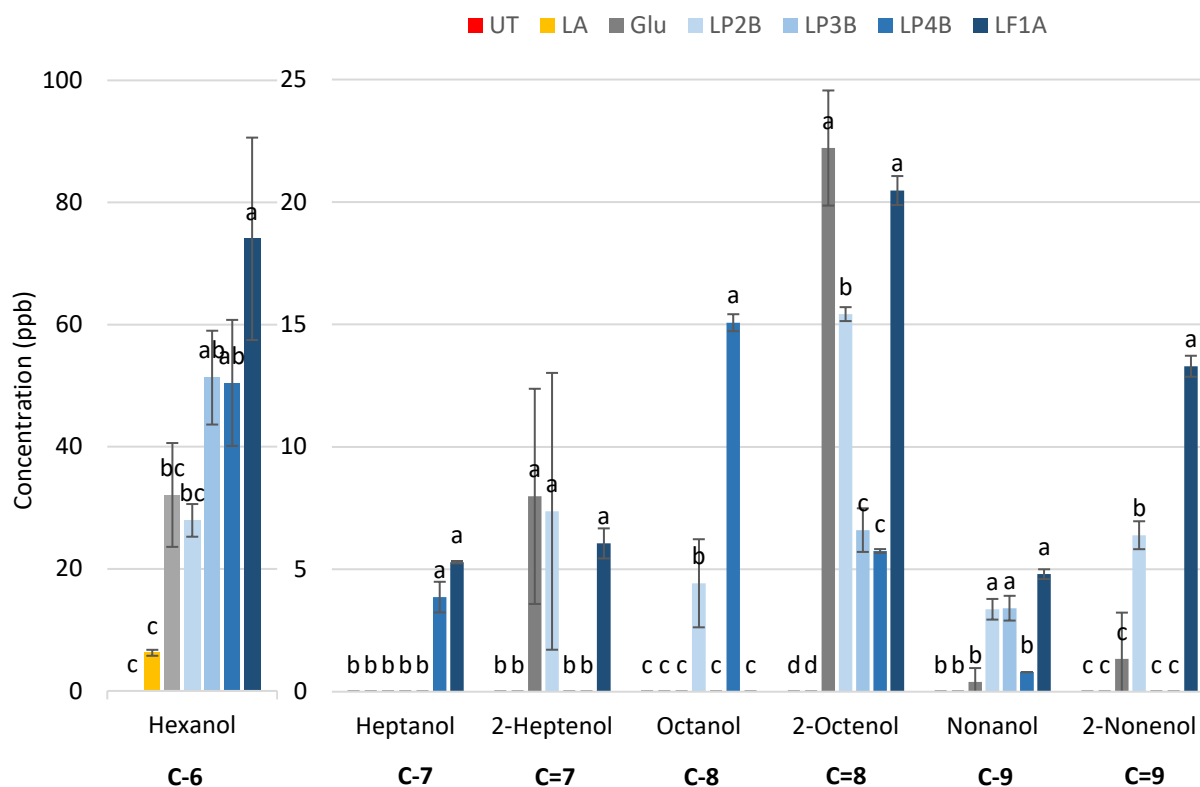


Figure 35. Alcohol volatiles derived from fatty acids by alcohol dehydrogenase activity. Abbreviations: LP2B, tomatoes fermented with *L. plantarum* C022-2B; LP3B, with *L. plantarum* C022-3B; LP4B, with *L. plantarum* V0023-4B2; LF1A, with *L. fermentum* V013-1A; UT, untreated tomatoes; LA, lactic-acid-treated tomatoes; Glu, glucosidase-treated tomatoes; C– and C= are saturated and unsaturated alcohols, respectively. Means with different letters (a, b, c, d) are significantly different ( $p < 0.05$ ).

The concentration of ketones increased moderately in all fermented tomatoes and in Glu and LA, except in LF1A where the concentration decreased drastically (Figure 36). Ketones levels were similar in LP2B and LP3B (tomatoes fermented with *L. plantarum* C022-2B and C022-3B, respectively). These levels were both higher than those in LP4B (tomatoes fermented with *L. plantarum* V0023-4B2), Glu, and LA. 6-Methyl-5-hepten-2-one (MHO) and geranylacetone were the most abundant ketones in UT, Glu, LA, and fermented tomatoes, but they decreased significantly in LF1A (Table 14).

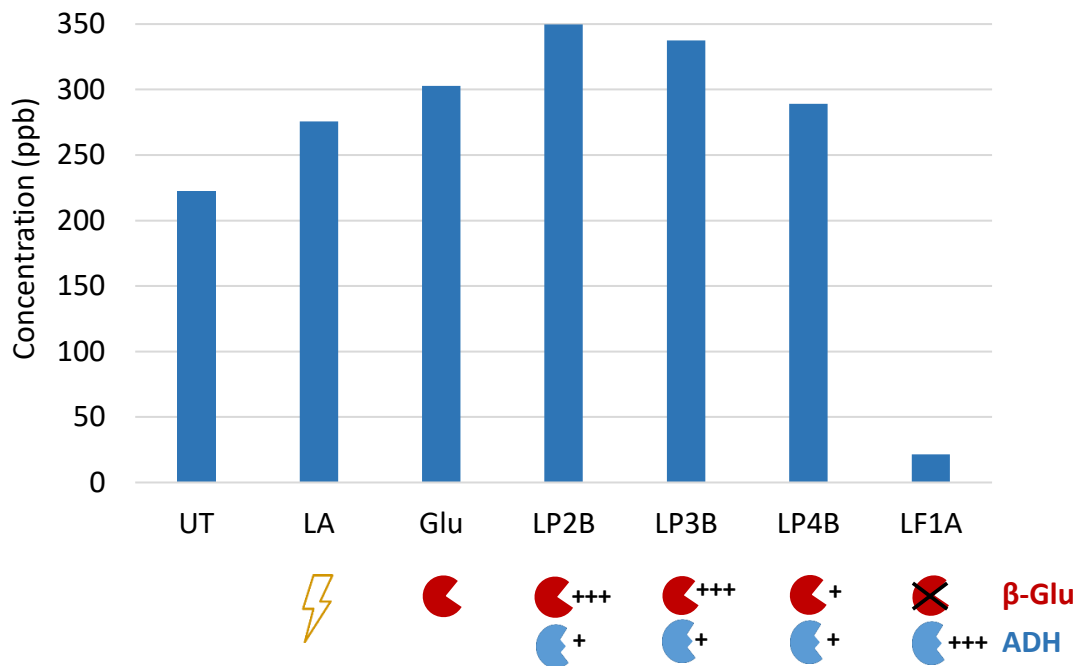


Figure 36. Total concentration of ketones. Four strains exhibiting various enzymatic properties ( $\beta$ -glucosidase ( $\beta$ -glu) and alcohol dehydrogenase (ADH); high (+++) and low (+) were used individually as starters for tomato fermentation. Abbreviations: LP2B, tomatoes fermented with *L. plantarum* C022-2B; LP3B, with *L. plantarum* C022-3B; LP4B, with *L. plantarum* V0023-4B2; LF1A, with *L. fermentum* V013-1A; UT, untreated tomatoes; LA, lactic-acid-treated tomatoes; Glu, glucosidase-treated tomatoes

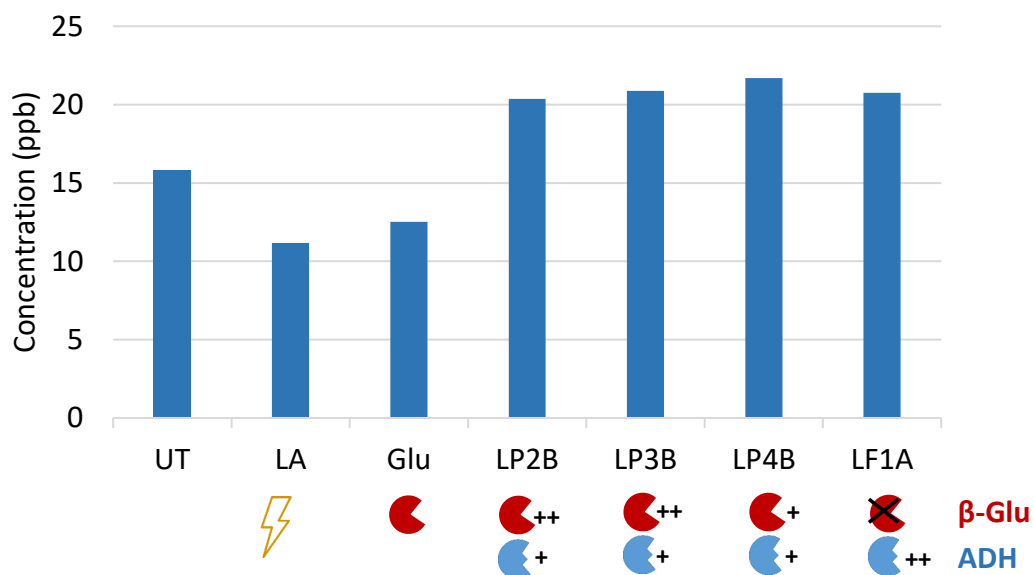


Figure 37. Total concentration of terpenes. Four strains exhibiting various enzymatic properties ( $\beta$ -glucosidase ( $\beta$ -glu) and alcohol dehydrogenase (ADH); high (+++) and low (+)) were used individually as starters for tomato fermentation. Abbreviations: LP2B, tomatoes fermented with *L. plantarum* C022-2B; LP3B, with *L. plantarum* C022-3B; LP4B, with *L. plantarum* V0023-

4B2; LF1A, with *L. fermentum* V013-1A; UT, untreated tomatoes; LA, lactic-acid-treated tomatoes; Glu, glucosidase-treated tomatoes

Although the total concentration of terpenes did not change considerably during fermentation, several terpenes important for aroma profiles appeared, including (*Z*)-geraniol, 6,7-dihydrogeraniol, melonol, linalool, and D-limonene (Figure 37 and 38). Acetic acid was undetected in UT but it was found in LP2B and LF1A (Table 14).

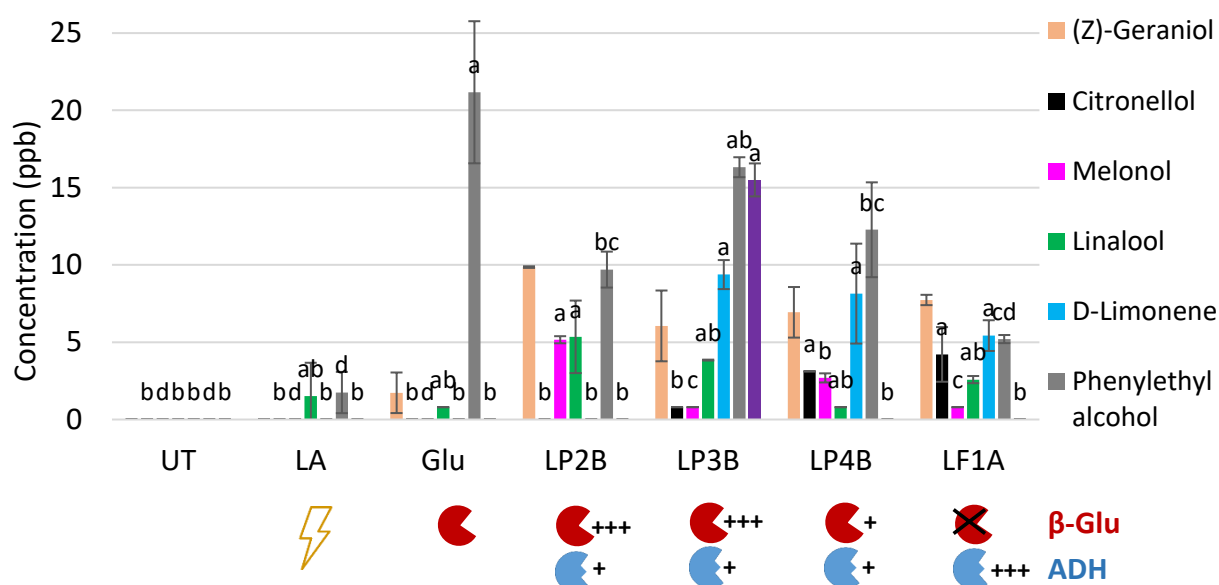


Figure 38. Glycosidically bound volatile compounds. Four strains exhibiting various enzymatic properties ( $\beta$ -glucosidase ( $\beta$ -glu) and alcohol dehydrogenase (ADH); high (+++) and low (+)) were used individually as starters for tomato fermentation. Abbreviations: LP2B, tomatoes fermented with *L. plantarum* C022-2B; LP3B, with *L. plantarum* C022-3B; LP4B, with *L. plantarum* V0023-4B2; LF1A, with *L. fermentum* V013-1A; UT, untreated tomatoes; LA, lactic-acid-treated tomatoes; Glu, glucosidase-treated tomatoes. Means with different letters (a, b, c, d) are significantly different ( $p < 0.05$ ).

Principal component analysis (PCA) was used to identify patterns and detect correlations between volatile compounds and tomato treatments. The first and second components represented 67.90% and 24.67% of the total variance, respectively (Figure 39). The correlation circle (left) exposed that all the variables were far from the centre. LA was close to UT, which indicates that they were significantly positively correlated. Acid hydrolysis therefore was unlikely to modify the aroma profiles of tomatoes. In contrast, LP2B, LP3B, LP4B, and Glu were almost orthogonal with UT, which signifies that they were not correlated with UT. LF1A was nearly on the opposite side of UT, which suggests that they were significantly negatively correlated. Consequently,  $\beta$ -glucosidase activity and LAB had a considerable effect on the

volatiles of tomatoes. The observation chart (right) clearly shows the effects of the treatments on volatiles of tomatoes. UT and LA were rich in aldehydes. LP2B, LP3B, LP4B, and Glu were likely abundant in ketones. LF1A differed considerably from other fermented tomatoes with the abundant presence of alcohols and acetic acid. These results highlight the effect of LAB on volatiles of fermented tomatoes.

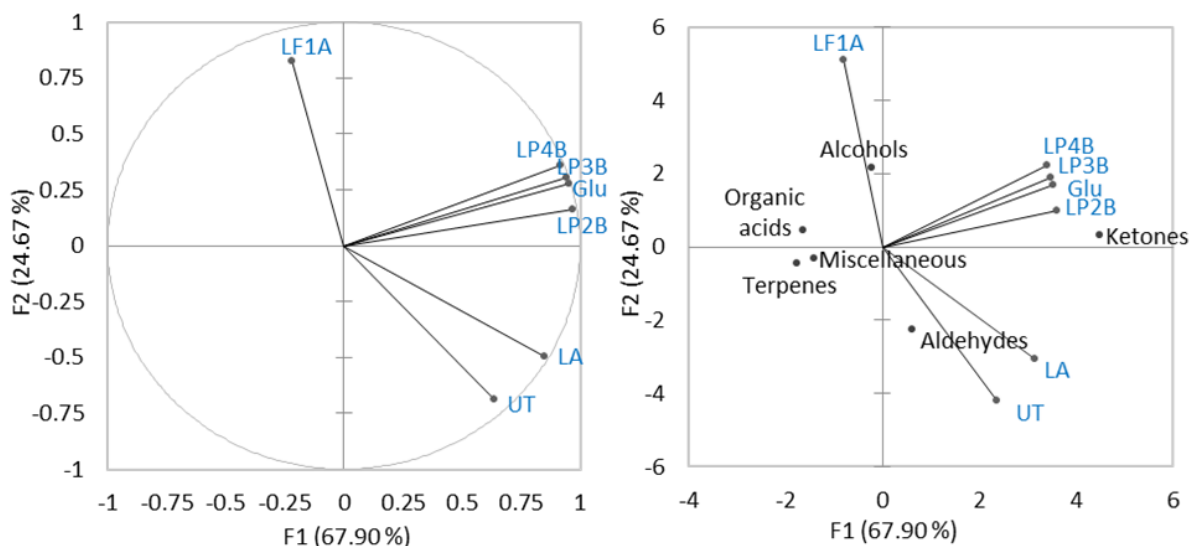


Figure 39. Score plot of the first and second principal component (PC) after PC analysis based on volatile compounds concentration found in tomato after treatments (Table 14). Abbreviations: LP2B, tomatoes fermented with *L. plantarum* C022-2B; LP3B, with *L. plantarum* C022-3B; LP4B, with *L. plantarum* V0023-4B2; LF1A, with *L. fermentum* V013-1A; UT, untreated tomatoes; LA, lactic-acid-treated tomatoes; Glu, glucosidase-treated tomatoes

The first target of our work was the volatiles produced during plant metabolism. These volatiles in plant can exhibit toxicity or have a communication role and therefore, the plant itself inactivates them through glycosylation to decrease toxicity or to control communication between plant cells (Song et al., 2018). Most of the volatiles accumulate in fruits/vegetables as non-volatile and odourless glycosides. These flavouring volatiles can be activated sensorially again by hydrolysing the sugar bond and releasing the volatile aglycone. This reaction can be catalysed by glucosidase enzymes (Sarry and Günata, 2004; Song et al., 2018).  $\beta$ -Glucosidase genes are widespread in LAB genomes.  $\beta$ -Glucosidase activities of LAB have been reported in many plant food matrices such as olives, soybeans, grapes, and cassava. They can hydrolyse a broad range of substrates (Michlmayr and Kneifel, 2014) but their substrate (aglycone) specificity is difficult to predict based on gene sequence. Thus, the first step in screening for specific  $\beta$ -glucosidases must be experimental. Aglycone specificity seems to have evolved



during bacterial adaptation to the ecological niche as well as the environmental conditions for the induction of the enzymatic activity (Michlmayr and Kneifel, 2014). These assumptions suggest that the selection of LAB from various type of fermented foods would permit to get various  $\beta$ -glucosidase activities and therefore various aroma profiles.

The selected strains exhibiting  $\beta$ -glucosidase activity have been evaluated for their capability to release volatile compounds in comparison with the effect of lactic acid to stimulate hydrolysis and with the addition of a  $\beta$ -glucosidase enzyme from almonds. In our study, terpenoids were the main volatiles released after  $\beta$ -glucosidase treatment. A slight increase in the concentration of terpene ketones was observed not only in Glu but also in LA (Figure 36), confirming that, besides  $\beta$ -glucosidase activity, acid hydrolysis had also an impact on ketones generation. For the glucosidase-exhibiting strains, the result of volatiles generation correlates with the  $\beta$ -glucosidase activity values with ketones levels higher in LP2B and LP3B than in LP4B ( $\beta$ -glucosidase activity: 27 UA for *L. plantarum* C022-2B and C022-3B and 12 UA for *L. plantarum* V0023-4B2). MHO and geranylacetone were the most abundant ketones in all treatments except in LF1A (Table 14). These compounds give fruity or floral notes in tomato fruits (Wang et al., 2016) and they are initially derived from lycopene (Vogel et al., 2010).

Other important terpenes such as (*Z*)-geraniol, 6,7-dihydrogeraniol, melonol, linalool, and D-limonene were also detected in fermented tomatoes (Figure 38). They can theoretically be released from fruit glycosides by  $\beta$ -glucosidase or acid hydrolysis (Buttery et al., 1990; Fenoll et al., 2009; Hellín et al., 2010; Marlatt et al., 1992; Ortiz-Serrano and Gil, 2010). However, the results in LA and Glu were not significantly different from the ones in UT, whereas several important increases in concentration were observed after fermentation although this was strain- and compound-specific. For instance, geraniol appeared in all fermentations while limonene increased significantly in three media (LP3B, LP4B, and LF1A), but not in LP2B (Figure 38). This latter medium was the one containing the highest concentration of melonol and linalool. All these results suggest that the release of terpenoids could either come from glycosides after the action of specific glucosidases or from other metabolic activities present differently in the lactic acid bacteria. Our results show that the production of volatile terpenes is a characteristic showing differences at the strain level in lactic acid bacteria.

A family of flavouring compounds exhibiting a huge importance in many plant products is the green note family. These compounds, volatile aldehydes and alcohols, are generated from the LOX pathway and are responsible for the fresh and green sensorial notes of fruits and vegetables. The LOX pathway is a multi-enzymatic system in which polyunsaturated fatty acids are converted into aldehydes and alcohols by the sequential action of lipoxygenase,

hydroperoxide lyase (HPL), and alcohol dehydrogenase (ADH) (Akacha and Gargouri, 2015; Christensen et al., 2007). This pathway generally takes place in fruits during ripening process or upon tissue disruption (e.g. maceration or blending). Therefore, most of the aldehydes detected in UT and LA were likely released from fatty acids via this pathway. The LOX-pathway-derived aldehydes include hexanal, (*E*)-2-hexenal, heptanal, (*Z*)-2-heptenal, (*E*)-2-octenal, nonanal, and (*E*)-2-nonanal (Figure 34). The LOX-alcohols homologues of aldehydes were not detected in UT, showing that the final reduction of aldehydes by ADH was not active (Figure 35). However, this reduction of green notes is important for the sensorial properties as alcohols exhibit either the same sensorial properties as aldehydes but with higher detection thresholds or exhibit notes less green and fruitier (Hu et al., 2019; Wang et al., 2016). Green notes are typical of tomato flavour but their transformation to fruity notes can bring interesting sensorial properties to the fermented products. When we investigated the ADH activity of the various strains in resting cells, we noticed that *L. fermentum* exhibited considerably higher activities than *L. plantarum* strains on most aldehydes and ketones present in the medium (Table 11 and 12). Our test with resting cells presented a slight difference of ketone reduction compared to what occurred in tomatoes. For instance, 6-methyl-5-hepten-2-one (MHO) was not reduced with resting cells (Table 12) but its corresponding alcohol 6-methyl-5-hepten-2-ol was found in LF1A (Table 14).

In contrast to this wide-range activity of *L. fermentum* ADH, ADHs of *L. plantarum* strains were substrate selective. *L. plantarum* C022-3B and V0023-4B2 were active only on saturated aldehydes, while C022-2B was active on unsaturated aldehydes (Figure 40). ADHs of *L. plantarum* strains were also active on unsaturated ketones (Table 14). LAB genomes possess several *adh* family genes which code various functional domains (Haberland et al., 2002; Hu et al., 2019). For instance, *L. plantarum* WCFS1 possessed nine *adh* genes and *L. fermentum* IFO 3956 ten *adh* genes among which only four are *L. plantarum* homologs (Bioinformatic analysis using Biocyc database) (Karp et al., 2017). The differences of substrate specificity observed between ADH activities of the two species could be due to the presence of specific ADH enzymes. This point of characterisation of the diversity of *adh* genes in relation with the activity will be our interest for the next study.

Eventually, besides the strain potential to release terpenoids or to transform aldehydes and ketones into alcohols, we also found a sign of heterolactic fermentation in fermented products. Indeed, acetic acid was detected in fermented matrices but not in UT (Table 14). Unsurprisingly, the medium containing the highest concentration was LF1A. This is consistent with the fact that *L. fermentum* V013-1A is obligately heterofermentative (Ibrahim, 2016).

Acetic acid together with 2,3-butanediol were also identified in LP2B (Table 14), showing that *L. plantarum* C022-2B was probably heterofermentative during tomato fermentation (Ibrahim, 2016; Renchinkhand et al., 2015).

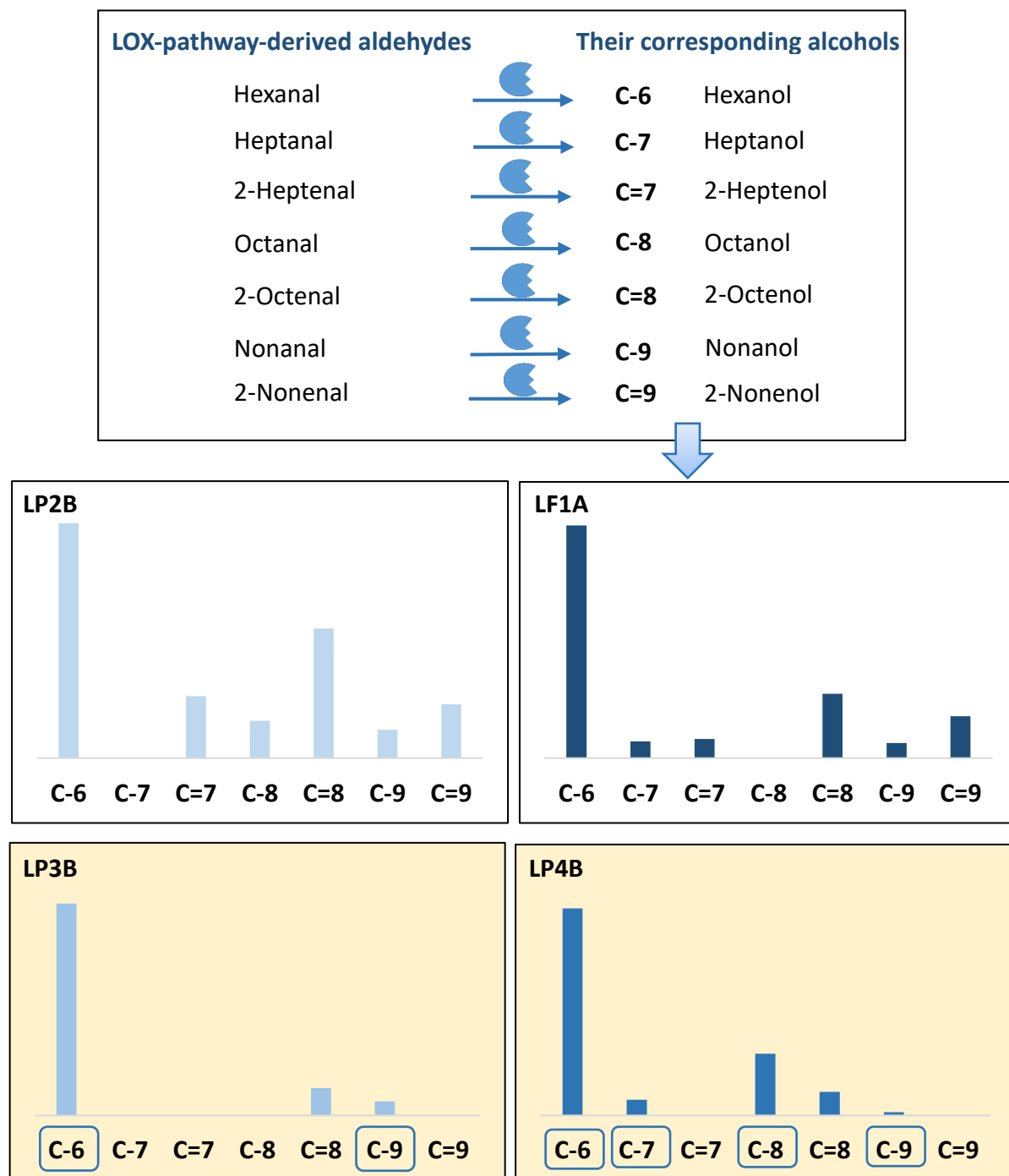


Figure 40. LOX-alcohols homologues of aldehydes from fatty acids by bacterial alcohol dehydrogenase (ADH) activity. Abbreviations: LP2B, tomatoes fermented with *L. plantarum* C022-2B; LP3B, with *L. plantarum* C022-3B; LP4B, with *L. plantarum* V0023-4B2; LF1A, with *L. fermentum* V013-1A; UT, untreated tomatoes; LA, lactic-acid-treated tomatoes; Glu, glucosidase-treated tomatoes; C- and C= are saturated and unsaturated alcohols, respectively.

Letter C in rectangular (e.g. **C-6**) represents the corresponding alcohols (e.g. C-6: hexanol) which were reduced from aldehydes by bacterial ADH.

Similar research by Di Cagno et al. (2009b) on tomato juices fermented with allochthonous *L. plantarum* LP54 was discriminated from autochthonous strains, especially by high levels of esters, alcohols and sulphur compounds, and a few furans.

In conclusion, the use of the selected LAB isolates as starters demonstrated that these strains caused a broad change in the aroma profiles of the fermented tomatoes. Tomatoes fermented with *L. plantarum* C022-2B, C022-3B, and V0023-4B2 were rich in ketones, whereas tomatoes fermented with *L. fermentum* V013-1A were abundant in alcohols. A majority of alcohols in fermented tomatoes were obtained from bacterial ADH activity. In contrast, untreated tomatoes were rich in aldehydes. Overall, *L. plantarum* strains producing  $\beta$ -glucosidase had no notable effect on carotenoids derivative compounds and on glycosidically bound aroma compounds. Nevertheless, fermentation increased significantly glycosidically bound aroma compounds such as (*Z*)-geraniol, 6,7-dihydrogeraniol, melonol, linalool, D-limonene, phenylethyl alcohol, and 4-ethylphenol. These aroma compounds are very important for fermented tomatoes because they bring the floral and fruity notes. ADH activity of the selected strains had a significant effect on the aldehydes/alcohols and ketones/alcohols ratios. The three *L. plantarum* strains were substrate selective. *L. plantarum* C022-3B and V0023-4B2 could reduce only saturated aldehydes (C<sub>6</sub>-C<sub>9</sub>) while *L. plantarum* C022-2B could reduce saturated and unsaturated aldehydes (C<sub>6</sub>-C<sub>9</sub>) in fermented tomatoes, but *L. plantarum* strains had similar abilities to reduce unsaturated ketones (C<sub>13</sub>). *L. fermentum* V013-1A was capable of reducing a wide variety of volatile compounds, saturated and unsaturated aldehydes (C<sub>6</sub>-C<sub>10</sub>) and unsaturated ketones (C<sub>8</sub> and C<sub>13</sub>). Among the four strains, *L. fermentum* V013-1A showed the highest ADH activity in resting cells as well as in fermented tomatoes. The fermented tomatoes seem to favour the ADH activity of *L. fermentum* V013-1A.

Since *L. plantarum* C022-2B, C022-3B, and V0023-4B2 were able to produce both ADH and  $\beta$ -glucosidase activities, these strains can be potential starters to decrease the off-flavour and anti-nutrients. Aldehydes and alcohols are responsible for off-flavour, but alcohols have generally higher odour threshold than aldehydes. *L. plantarum* strains with ADH could reduce aldehydes to alcohols; therefore, they can potentially decrease off-flavours. Shi, (2020) found *L. plantarum* was able to eliminate off-flavour of fermented pea protein isolates. Around 42% aldehydes and 64% ketones content were removed, and a small amount of alcohols were produced. Li et al. (2014) showed that *L. plantarum* 70810 decreased off-flavour, particularly

hexanal of fermented soymilk. Furthermore, *L. plantarum* strains with  $\beta$ -glucosidase probably remove the anti-nutritional compounds that are frequently in the form of glycosides (e.g.  $\alpha$ -galactosides and pyrimidine glycosides in faba bean) (Rizzello et al., 2017). Other benefits of  $\beta$ -glucosidase activity over lacto-fermented foods have been previously described in section 1.4.2.3. Therefore, the combination of ADH and  $\beta$ -glucosidase activities of *L. plantarum* strains may have many positive effects on antinutrient reduction and on sensory quality of fermented legume-based foods. Despite the fact that *L. fermentum* V013-1A did not produce  $\beta$ -glucosidase, it showed the highest ADH activity. Therefore, mixing *L. fermentum* V013-1A and *L. plantarum* C022-2B, C022-3B or V0023-4B2 could eliminate the off-flavour. In addition, since these strains altered tremendously the aroma profiles of fermented tomatoes, it is worth testing these strains on fruit or vegetable by-products like tomato pomace, grape marc, etc. This approach can revalue those by-products as well as decrease the environmental pollution.

## VI. CONCLUSION AND PERSPECTIVES

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### Conclusion

First, our study has demonstrated that  $\beta$ -glucosidase activity is widespread among LAB from Asian fermented foods since of the 200 bacterial isolates screened, 80 were positive for  $\beta$ -glucosidase. However, most of bacteria exhibited low activity. Only 14 strains showed  $\beta$ -glucosidase activity greater than 10 UA and the highest  $\beta$ -glucosidase activity was 27 UA. Among them, thirteen were *L. plantarum* and one was *L. pentosus*. According to this result, one could hypothesise that all *L. plantarum/paraplantarum/pentosus* strains found in fermented foods are positive for  $\beta$ -glucosidase activity. In fact, this is not obvious since *L. plantarum* is generally the dominant species in lacto-fermented foods. Identification of a representative panel of  $\beta$ -glucosidase-negative isolates should be necessary to rule on this assumption.

Second, our results have highlighted differences between bacterial isolates concerning ADH activity that would be important for the aroma profiles of fermented vegetables. The resting cells of *L. fermentum* V013-1A demonstrated the highest reduction ability for hexanal ( $3.85 \times 10^{-8}$  UA) and (*E*)-2-hexenal ( $2.47 \times 10^{-8}$  UA), whereas the resting cells of *L. plantarum* C022-2B, C022-3B, and V0023-4B2 exhibited similar ADH reduction ability for only hexanal ( $0.44 \times 10^{-8}$  UA). The resting cells of *L. fermentum* V013-1A was also able to reduce (*E*)-2-heptenal ( $2.46 \times 10^{-8}$  UA), (*E,E*)-2,4-decadienal ( $3.69 \times 10^{-8}$  UA), and geranylacetone ( $1.26 \times 10^{-8}$  UA).

Third, the use of the selected LAB isolates as starters demonstrated that these strains caused a broad change in the aroma profiles of the fermented tomatoes. Tomatoes fermented with *L. plantarum* C022-2B, C022-3B, and V0023-4B2 were rich in ketones, whereas tomatoes fermented with *L. fermentum* V013-1A were abundant in alcohols. A majority of alcohols in fermented tomatoes were obtained from bacterial ADH activity. In contrast, untreated tomatoes were rich in aldehydes. Overall, *L. plantarum* strains producing  $\beta$ -glucosidase had no notable effect on carotenoids derivative compounds and on glycosidically bound aroma compounds. Nevertheless, fermentation increased significantly glycosidically bound aroma compounds such as (*Z*)-geraniol, 6,7-dihydrogeraniol, melonol, linalool, D-limonene, phenylethyl alcohol, and 4-ethylphenol. These aroma compounds are very important for fermented tomatoes because they bring the floral and fruity notes. ADH activity of the selected strains had a significant effect on the aldehydes/alcohols and ketones/alcohols ratios. The three *L. plantarum* strains were substrate selective. *L. plantarum* C022-3B and V0023-4B2 could reduce only saturated aldehydes (C<sub>6</sub>-C<sub>9</sub>) while *L. plantarum* C022-2B could reduce saturated and unsaturated

aldehydes (C<sub>6</sub>-C<sub>9</sub>) in fermented tomatoes, but *L. plantarum* strains had similar abilities to reduce unsaturated ketones (C<sub>13</sub>). *L. fermentum* V013-1A was capable of reducing a wide variety of volatile compounds, saturated and unsaturated aldehydes (C<sub>6</sub>-C<sub>10</sub>) and unsaturated ketones (C<sub>8</sub> and C<sub>13</sub>). Among the four strains, *L. fermentum* V013-1A showed the highest ADH activity in resting cells as well as in fermented tomatoes. The fermented tomatoes seem to favour the ADH activity of *L. fermentum* V013-1A.

### Perspectives

This work has raised several points that merit further studies.

Many aroma compounds in foods are produced in trace amounts, which are below the thresholds of most analytical instruments, but can be detected by human olfaction (El Hadi et al., 2013). Each starter considerably altered the flavour compounds in fermented tomatoes. Most aldehydes disappeared, alcohols increased significantly, and terpenes and ketones increased slightly in fermented tomatoes. The pH values of fermented tomatoes dropped significantly. These changes have an effect on the aroma and taste of the final products. Consequently, it is important to perform sensory evaluation of fermented tomatoes to confirm their acceptability and further application.

LAB isolated from Cambodian and Vietnamese fermented foods show promising enzymatic activities especially alcohol dehydrogenases and  $\beta$ -glucosidases. The ADH activity of the four strains tested on fermented tomatoes reduced various aldehydes and ketones. These strains are worth testing with more substrates. They can be a catalyst for the enantioselective transformations of ketones into corresponding alcohols. Chiral alcohols are high value products for pharmaceuticals and fine chemicals (Hu et al., 2019; Zheng et al., 2017). *L. kefir* DSM 20587 were able to reduce (2,5)-hexanedione to (2*R*,5*R*)-hexanediol (Haberland et al., 2002) and acetophenone to R(+)-phenylethanol (Hummel, 1990). The ADH of *L. brevis* is a versatile catalyst for enantioselective reduction of ketones (Leuchs and Greiner, 2011). *L. fermentum* V013-1A could be the first strain to experiment because of the higher ADH activity.

ADH activity depends on the cofactor and effectors in the medium (Gargouri et al., 2004; Hu et al., 2019). ADH interconverts aldehydes to alcohols when there is NAD<sup>+</sup>/NADH, and ketones to alcohols when there is NADP<sup>+</sup>/NADPH. The effectors are pH, metal ions (ZnSO<sub>4</sub>, MgCl<sub>2</sub>, CaCl<sub>2</sub>, CuCl<sub>2</sub>, FeCl<sub>2</sub>, MnCl<sub>2</sub>, etc.), and metal ion concentration. This study focused on whole LAB cells. It would be very interesting to explore the kinetic parameter ( $K_m$  and  $V_{max}$ ) of the purified ADH activity of these strains and the effectors in the medium, which allows to study substrate selectivity and explain why some strains transform saturates, and others

unsaturated aldehydes and ketones. Other parameters such as growth temperature, thermal stability, pH stability, and substrate specificity also worth investigating. Nine purified ADH were identified in *L. reuteri* DSM20016 (Chen et al., 2016; Hu et al., 2019).

A significant amount of aroma compounds is accumulated in fruits and vegetables in the form of glycosides. Glycosides are found in various forms, mainly *O*- $\beta$ -D-glucosides, *O*- $\beta$ -D-diglycosides, and triglycosides (a few cases). To confirm the  $\beta$ -glucosidase activity of LAB in this study, a glycosidic substrate (*p*-nitrophenyl- $\beta$ -D-glucopyranoside) was used. Due to the complex and unknown structure of glycosides in fruits and vegetables, many researchers have tested glycosidases on several commercially available glycosides prior to the plant matrix experiment. Common commercial glycosides are *p*-nitrophenyl (*p*NP)- $\beta$ -D-glucopyranoside, *p*NP- $\alpha$ -D-glucopyranoside, *p*NP- $\beta$ -D-xylopyranoside, *p*NP- $\alpha$ -L-rhamnopyranoside, *p*NP- $\alpha$ -L-arabinofuranoside, and *p*NP- $\alpha$ -L-arabinopyranoside (Grimaldi et al., 2005b; Michlmayr et al., 2010; Pérez-Martín et al., 2012). *L. plantarum* strains producing  $\beta$ -glucosidases are worth testing with various glycosides as part of the screening strategy, which may increase the possibility of glycoside hydrolysis in the plant matrix.

Some parameters including pH, temperature, glucose/fructose, and substrate specificity (i.e. glycosides) influence the enzymatic assays of intracellular, extracellular, and cell-bound  $\beta$ -glucosidases. Enzymatic inhibitors include  $Hg^{2+}$ ,  $Ag^{2+}$ , and  $Zn^{2+}$ , while enzymatic activators are methanol, ethanol, propanol, and butanol (Grimaldi et al., 2005a, 2005b; Gueguen et al., 1997a; Michlmayr et al., 2010; Pérez-Martín et al., 2012; Sestelo et al., 2004). Glucose, fructose, galactose or mannose was likely to repress LAB  $\beta$ -glucosidase activities.  $\beta$ -Glucosidases were induced by cellobiose, lactose, salicin, ribose, glucose or arbutin (Michlmayr and Kneifel, 2014). However, each LAB strain has its optimal growth conditions and its own specificity for  $\beta$ -glucosidase activities. Therefore, to deepen understanding of the  $\beta$ -glucosidase activities of LAB strains, these parameters have to be investigated.



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# **Thèse résumé**

**Criblage des bactéries lactiques pour leur utilisation comme ferments aromatiques lors de la fermentation des légumes**

**Da LORN**

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## I. INTRODUCTION

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Pour réussir la transition vers un système alimentaire durable, abordable, fiable et de haute qualité au cours de la prochaine décennie, un rapport de l'OMS/FAO recommande une consommation d'au moins 400 g de fruits et légumes par jour (à l'exclusion des pommes de terre et autres tubercules féculents) (OMS, 2003). La fermentation lactique est un procédé traditionnel qui pourrait être un moyen écologique de diversifier l'offre de fruits et légumes et donc d'augmenter leur consommation quotidienne. Généralement, cinq caractéristiques intéressantes sont avancées pour expliquer l'utilisation des LAB dans les aliments fermentés: augmentation de la durée de conservation des matières premières périssables, innovation, détoxification, enrichissement nutritionnel et réduction de la consommation de carburant (Steinkraus, 1995). Alors que la fermentation lactique est à peu près anecdotique pour les fruits et légumes dans les pays occidentaux (mais avec quelques exemples populaires comme la choucroute, les olives, les câpres, le navet et les cornichons) (Di Cagno et al., 2013; Tamang et al., 2016b), il est populaire en Asie. En effet, en Asie, les fruits ou légumes lacto-fermentés sont servis en entrée, en accompagnement ou en ingrédient pour préparer le plat principal. Les matières premières sont très diverses, ce qui donne des produits comme *sunki* (feuilles de betterave rouge fermentées japonaises) (Endo et al., 2008), *kimchi* (chou nappa avec d'autres légumes fermenté coréen) (Patra et al., 2016), *sinki* (radis fermenté indien) (Tamang et al., 2005), *dua muoi* (feuilles de moutarde, betteraves et aubergines fermentées vietnamiennes) (Nguyen et al., 2013) et *chrourk* ou *tram* (fruits ou légumes fermentés cambodgiens).

La fermentation lactique est considérée comme une biotechnologie simple et précieuse pour préserver et/ou améliorer les propriétés sanitaires, nutritionnelles et de durée de conservation des fruits et légumes (Fan et Hansen, 2012; Tamang et al., 2016a). Elle est également très importante pour les propriétés sensorielles. Les bactéries lactiques (LAB) sont connues pour améliorer la saveur des produits fermentés grâce à leur métabolisme. Les LAB homofermentaires produisent de l'acide lactique comme principal produit de la fermentation des glucides, tandis que les LAB hétérofermentaires produisent en outre d'autres produits tels que l'acide acétique, le dioxyde de carbone, l'éthanol, l'acétoïne et le diacétyl (Gänzle, 2015; Leroy et De Vuyst, 2004). Les composés volatils générés par l'hétérofermentation peuvent donner des goûts et des saveurs particulières aux aliments fermentés. La caractéristique de nombreux aliments fermentés dépend donc du métabolisme du LAB.

De plus, la genèse des composés d'arômes repose principalement sur la capacité spécifique de différentes souches bactériennes à convertir les précurseurs dérivés des glucides,

des protéines, des acides gras, des caroténoïdes et des glycosides (Bancalari et al., 2017). Les composés volatils nouvellement générés sont ajoutés au pool de composés d'arômes déjà présents dans la matrice végétale bien que dans la plante, ces derniers se rencontrent principalement sous forme glycosylés pour diminuer leur toxicité (Song et al., 2018). Les  $\beta$ -glucosidases sont des enzymes clés pour libérer des composés d'arômes à partir des précurseurs glucosidiques présents dans les fruits (Krisch et al., 2010). Ils sont utilisés pour améliorer les arômes du vin (Sestelo et al., 2004), du thé (Su et al., 2010) et des jus de fruits (Fan et al., 2011). Certaines LAB tels que *Leuconostoc mesenteroides* (Gueguen et al., 1997), *Lactobacillus* (*Lactiplantibacillus* dans la nouvelle taxonomie (Zheng et al., 2020)) *plantarum* USC1 (Sestelo et al., 2004), *Lactobacillus* ssp. et *Pediococcus* ssp. (Grimaldi et al., 2005) isolés à partir de vins et d'aliments fermentés sont capables de présenter une activité  $\beta$ -glucosidase (Michlmayr et Kneifel, 2014). Ce phénotype est spécifique à la souche, comme rapporté par Renchinkhand et al. (2015) qui n'ont trouvé que six souches positives pour la  $\beta$ -glucosidase parmi 28 souches LAB isolées à partir de *kimchi*. L'alcool déshydrogénase (ADH) est une autre famille d'activités enzymatiques qui pourraient être importantes pour modifier les profils aromatiques des fruits et légumes. L'ADH catalyse la transformation d'aldéhydes ou de cétones en leurs alcools, acides ou esters correspondants (Gargouri et al., 2004; Hu et al., 2019). Malgré le fait que leurs gènes codants soient généralement présents dans les génomes LAB, les activités ADH n'ont été que rarement rapportées dans LAB. *Lactobacillus* (*Lentilactobacillus* dans la nouvelle taxonomie (Zheng et al., 2020)) *kéfir* DSM 20587 a pu réduire le 2,5-hexanedione en (2*R*,5*R*)-hexanediol (Haberland et al., 2002) dans des conditions spécifiques de cellules non proliférantes (resting whole cells). Peu d'études ont caractérisé les ADH purifiés de LAB. Dans le fromage, Hu et al. (2019) ont trouvé cinq ADH de *Lactobacillus* (*Limosilactobacillus* dans la nouvelle taxonomie (Zheng et al., 2020)) *reuteri* DSM20016 qui pourraient réduire les aldéhydes en leurs alcools correspondants, par exemple, le 3-méthylbutanal en 3-méthylbutanol, le butyraldéhyde en 2-butanol, hexanal en hexanol, phénylacétaldéhyde en phényléthanol, etc.

Pour améliorer les profils aromatiques par fermentation, les fruits ou légumes doivent être fermentés par des starters LAB possédant des activités métaboliques et enzymatiques adéquates envers la matrice végétale. Cela nécessite de sélectionner les ferments (starters) en fonction de leurs activités enzymatiques dans la matrice, qui ne peuvent pas être totalement prédites en conditions de laboratoire.

## II. OBJECTIFS

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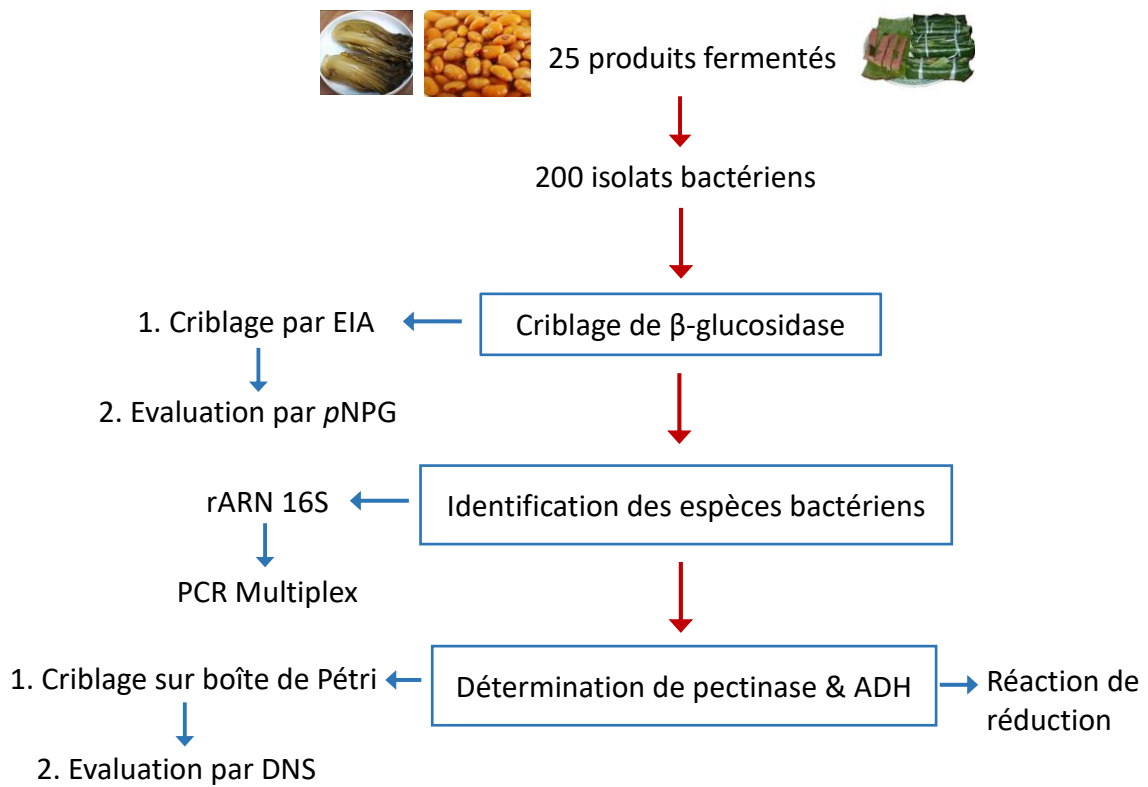
Les LAB ont de bonnes capacités de croissance sur différentes matrices alimentaires et produisent des activités enzymatiques très diverses qui sont notamment capables de modifier positivement les propriétés organoleptiques des aliments fermentés. Concernant les fruits et légumes, la revue bibliographique met en évidence divers aliments fermentés traditionnels pour lesquels la fermentation lactique est spontanée (ou par backslopping). Il est donc réaliste d'imaginer le développement d'une nouvelle fermentation lactique de précision (fermentation dirigée) pour les légumes/fruits afin d'orienter la typicité aromatique du produit et la stabilité de cette typicité. A cet effet, l'étude bibliographique permet d'orienter la sélection des starters LAB vers la recherche d'activités  $\beta$ -glucosidases et ADH pouvant avoir un impact sur l'arôme et la pectinase sur la texture et sur la capacité de développement des souches dans les matrices végétales. Etant donné que la tomate est un fruit/légume très consommé dans le monde et également très gaspillé, et que les teneurs en arômes et précurseurs sont nombreuses, la tomate est une bonne matrice pour explorer les propriétés de libération d'arôme de starters sélectionnés.

Par conséquent, l'objectif principal de cette étude est d'obtenir une diversité de profils aromatiques des tomates fermentées en utilisant des approches biotechnologiques. Pour y parvenir, trois objectifs importants sont les suivants :

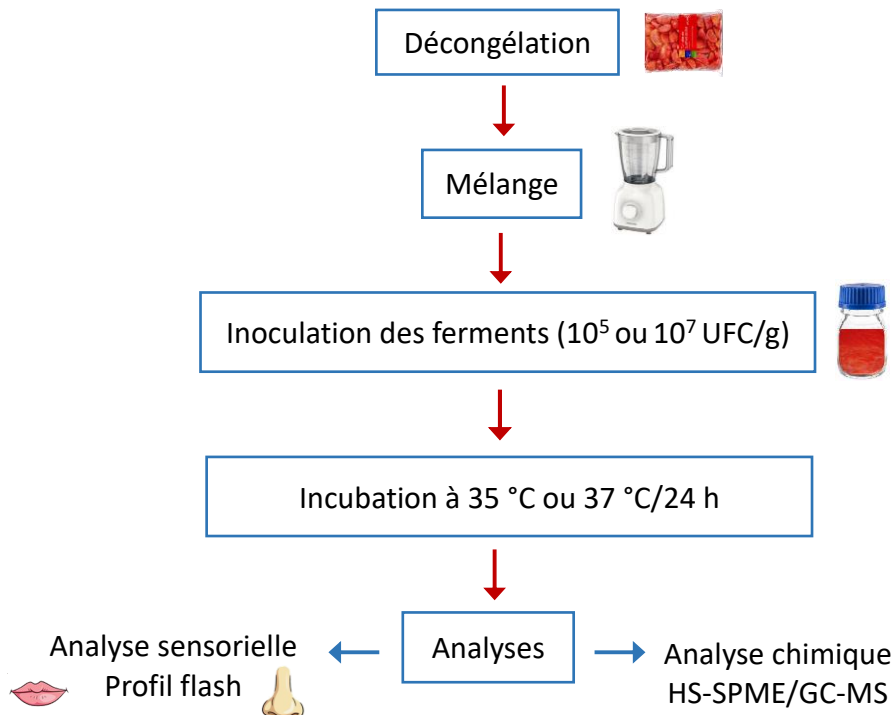
- (1) Évaluer l'impact de la diversité des ferments (starters) sur les propriétés sensorielles des tomates fermentées
- (2) Identifier et caractériser les activités enzymatiques spécifiques, en particulier les  $\beta$ -glucosidases, les alcool déshydrogénases et les pectinases des souches LAB.
- (3) Caractériser le rôle des souches LAB dans la libération de composés d'arômes en fonction de leurs activités enzymatiques, en utilisant la tomate comme matrice de fermentation.



## Stratégie expérimentale pour cette étude



## Processus de fermentation

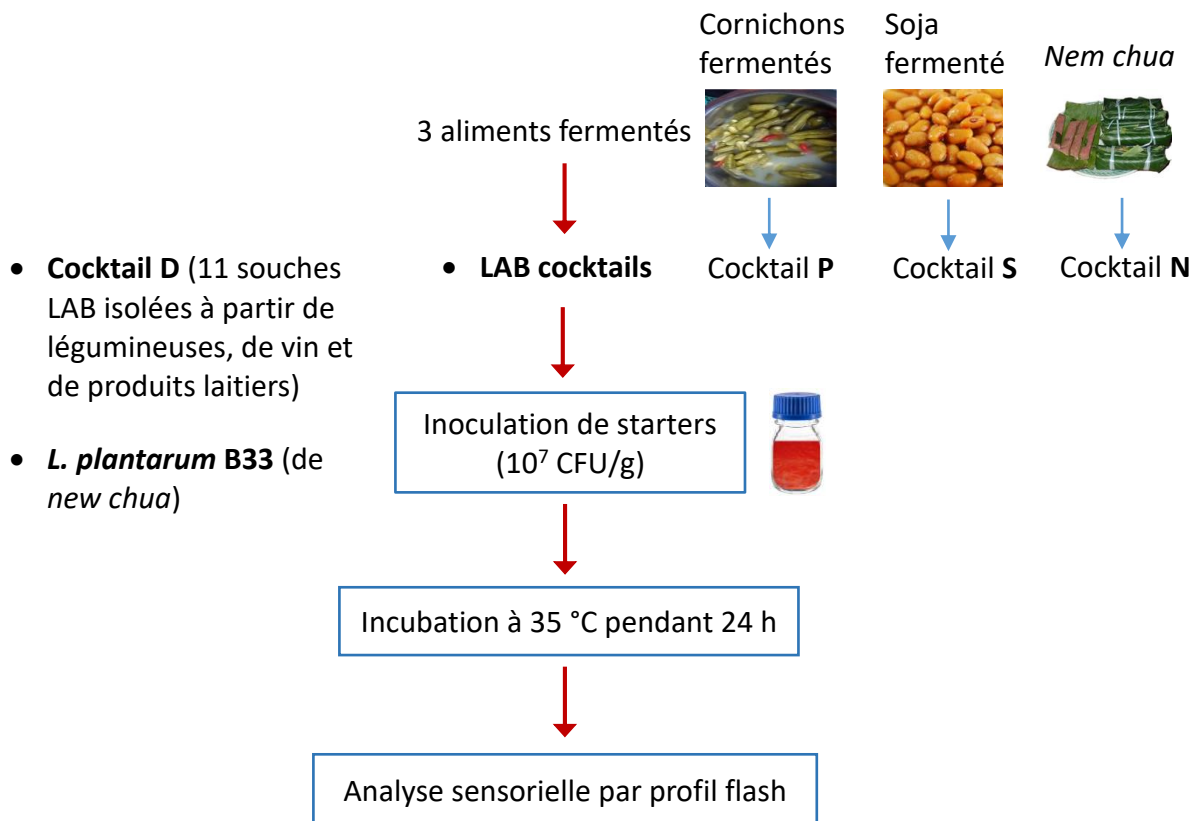


### III. RESULTATS ET DISCUSSION

#### 1. Évaluation de l'impact de la diversité des starters sur les descripteurs de flaveur

Le principal objectif de cette étude était d'obtenir une diversité de profils aromatiques des tomates fermentées en utilisant des approches biotechnologiques. Pour y parvenir, dans un premier temps, des cocktails de bactéries lactiques (LAB) isolés à partir d'aliments fermentés cambodgiens et vietnamiens ont été utilisés comme starters dans les tomates fermentées. L'objectif de cet essai était de prouver que la diversité des LAB peut modifier les profils d'arôme/flaveur des tomates fermentées. (Idéalement, cela aurait pu conduire notre sélection de bactéries si un cocktail LAB avait fourni des composés d'arômes et/ou de saveurs particulièrement intéressants parmi les différents starters de cocktail LAB).

#### Stratégie expérimentale pour cette étude



Le pH de la purée de tomates (MT : mashed tomatoes) était d'environ 4,3, tandis que le pH de la MT fermentée avec différents starters a considérablement diminué (environ 3,5) (Figure 1). Le pH des MT avec des bactéries endogènes (tomates fermentées spontanément) a à peine diminué (4,1). Cela peut être causé par les souches adventives présentes dans la matrice (tomate), l'équipement et/ou l'environnement de production.

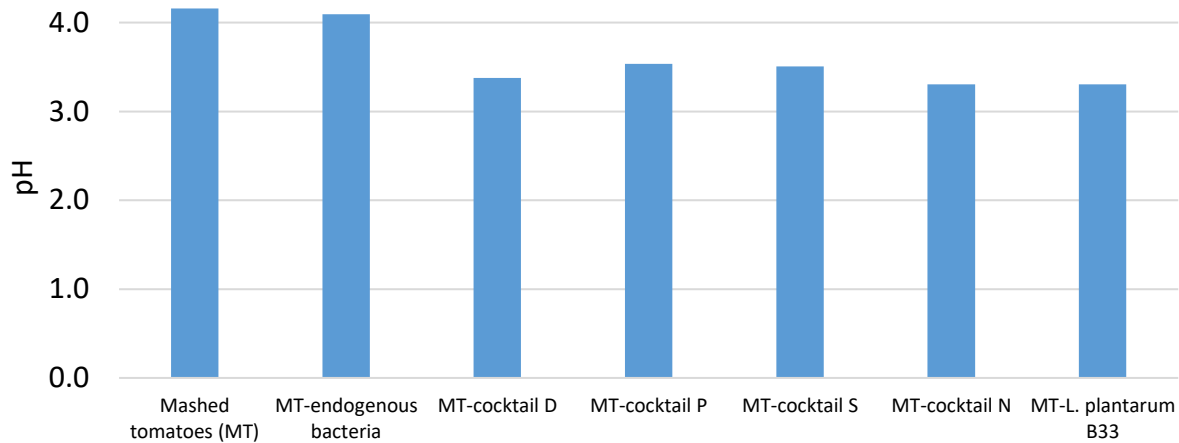


Figure 1. Valeurs de pH de la purée de tomates (MT : mashed tomatoes) et de la MT fermentées avec des différents starters

L'analyse sensorielle a été réalisée par la méthode du profil flash et s'est principalement concentrée sur l'odeur (arôme) et le saveur (goût) des échantillons. Les panélistes ont décrit que les tomates fermentées avec le cocktail D (le « cocktail défini » composé de 11 souches LAB), les purées de tomates (MT) et les tomates fermentées avec les bactéries endogènes avaient une note aromatique et un goût similaires, tels que la tomate verte et le goût de tomate (Tableau 1). Par conséquent, le cocktail D n'a eu aucun impact sur le profil de flaveur des tomates fermentées. En revanche, les tomates fermentées avec le cocktail P (isolé de cornichons fermentés), le cocktail S (isolé de soja fermenté) et le cocktail N (isolé de *nem chua*) ont montré les résultats les plus intrigants. Les panélistes ont décrit l'arôme de ces tomates fermentées comme des notes vertes, fruitées et florales (Tableau 1). Les tomates fermentées avec ces cocktails avaient un profil aromatique similaire à celui des tomates fermentées avec *L. plantarum* B33 (isolée de *nem chua*). Les cocktails LAB isolés à partir d'aliments fermentés traditionnels ont montré une note aromatique très prometteuse par rapport au cocktail D. De plus, ces tomates fermentées présentaient également des saveurs intéressantes telles que l'umami, le salé et l'amer. Cependant, les panélistes n'ont pas apprécié l'acidité de ces tomates fermentées. Pour diminuer l'acidité (goût aigre), quelques techniques telles que la réduction de la quantité d'inoculum, de la température et de la durée d'incubation pourraient être possibles. Cependant, comme l'application des tomates fermentées peut être diverse (par exemple, ketchup, sauce tomate), nous avons décidé de tester une autre technique. Du sucre de table (2% p/p) a été ajouté après la fermentation afin d'enlever la perception d'aigreur. Par conséquent, les panélistes ont préféré les tomates fermentées avec du sucre à celles sans sucre. Ils ont

également décrit que les tomates fermentées avec du sucre avaient un profil de goût similaire à celui du ketchup. Le sucre était en effet une technique efficace pour éliminer le goût d'acidité des tomates fermentées. Malgré cela, les panélistes n'ont pas pu distinguer la note aromatique typique de ces échantillons. L'inconvénient de cette approche est que le sucre ajouté peut masquer les notes d'arôme et de saveur typiques et originales des tomates fermentées. Par conséquent, pour les expériences futures, il serait préférable d'éviter d'ajouter du sucre et de modifier les conditions de fermentation telles que la quantité d'inoculum et le temps d'incubation.

Tableau 1. Évaluation sensorielle des tomates fermentées

Traitement	Description	
	Odeur (arôme)	Saveur (goût)
Purée de tomates (MT)	Tomate verte	Tomate
MT- bactéries endogènes	Tomate verte	Tomate, aigre, salé, amer
MT- cocktail D	Tomate verte, éthanol, plastique	Tomate, aigre, <b>umami</b>
MT- cocktail P	<b>Note verte, herbe coupée, fruité, floral</b>	Tomate, <b>salé</b> , vinaigre, aigre
MT- cocktail S	<b>Note verte, herbe coupée,</b> éthanol, <b>fruité, floral</b> , acide	<b>Umami</b> , amer, aigre, pétillant, choucroute
MT- cocktail N	<b>Fruité, floral</b> , acide	<b>Salé</b> , amer, aigre, choucroute
MT- <i>L. plantarum</i> B33	<b>Note verte, herbe coupée,</b> céréales, <b>fruité, miel</b>	<b>Salé</b> , aigre

Les tomates fermentées avec le cocktail P, S et N et *L. plantarum* B33 ont révélé quelques notes aromatiques importantes telles que l'herbe (verte ou coupée), fruitée et florale. Cette évaluation sensorielle a montré l'impact de ces cocktails LAB sur les tomates fermentées. Ces notes aromatiques pourraient être libérées par les activités enzymatiques de LAB. Il est donc très important de tester différentes souches selon des critères définis au préalable. La note herbacée est généralement due aux aldéhydes et aux alcools dérivés de la voie lipoxygénase (LOX) et son intensité dépend du rapport aldéhydes/alcools. L'enzyme ADH est responsable de l'interconversion entre deux familles volatiles. Par conséquent, il est nécessaire de vérifier si ces isolats bactériens produisent une activité ADH. Les notes fruitées et florales ont été

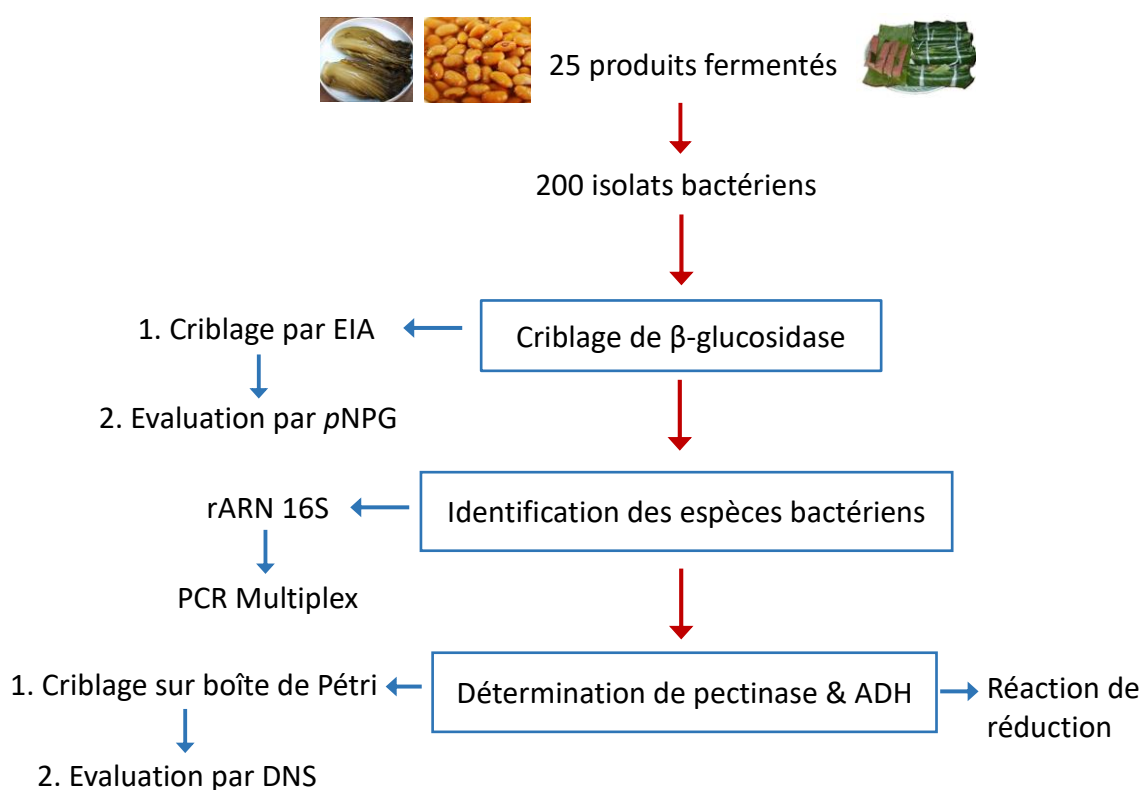
normalement attribuées aux cétones et terpènes volatiles. Cependant, la plupart des cétones et des terpènes présents dans les fruits et légumes ne sont pas sous forme libre (volatile), mais sous forme de glycoconjugués (c'est-à-dire des glycosides : non volatils). L'acide ou  $\beta$ -glucosidase peut hydrolyser les glycoconjugués et libérer ces cétones et terpènes volatiles. Cela peut expliquer les arômes fruités et floraux présentés dans les tomates fermentées. Il est donc important de démontrer l'activité  $\beta$ -glucosidase des isolats bactériens et éventuellement de différencier l'hydrolyse acide des activités enzymatiques.

En conclusion, les cocktails P, S et N ont considérablement modifié les profils d'arômes et de saveurs des tomates fermentées, ce qui est probablement dû aux activités ADH et  $\beta$ -glucosidase. Par conséquent, la démonstration des activités enzymatiques est une étape clé de ce travail.

## 2. Sélection de souches de bactéries lactiques pour des activités enzymatiques intéressantes pour la fermentation alimentaire

Grâce à l'analyse sensorielle et à l'étude bibliographique, nous sommes en mesure d'orienter la sélection des starters LAB vers la recherche des activités  $\beta$ -glucosidase et ADH pouvant avoir un impact sur l'arôme. Cela nous a conduit à poursuivre le second objectif qui était d'identifier et de caractériser des activités enzymatiques spécifiques, en particulier la  $\beta$ -glucosidase et l'ADH de LAB provenant d'aliments fermentés traditionnels au Cambodge et au Vietnam.

### Stratégie expérimentale pour cette étude



### 2.1. Activité de la $\beta$ -glucosidase

Deux cents isolats ont été sélectionnés parmi 25 aliments fermentés et criblés sur gélose au fer esculine (esculin iron agar (EIA)) pour l'activité  $\beta$ -glucosidase. Quarante-vingt isolats étaient positifs pour la  $\beta$ -glucosidase selon la couleur brune ou noire de leur colonie. L'activité  $\beta$ -glucosidase a été évaluée par la libération de *p*-nitrophénol à partir du *p*-nitrophényl- $\beta$ -D-glucopyranoside (*p*NPG) à 37 °C. Quatorze isolats avaient une activité supérieure à 10 UA (Figure 2), tandis que les 66 autres avaient une activité de  $\beta$ -glucosidase inférieure à 10 UA

(données non présentées). L'activité la plus élevée détectée était de 27 UA pour les isolats C022-2B et C022-3B. D'autres isolats ont présenté environ la moitié de cette activité comme V0023-4B2 (12 UA). A titre de comparaison, *L. plantarum* B33 a présenté une activité  $\beta$ -glucosidase de 17 UA. La quantification de l'activité a également été vérifiée pour V013-1A, qui était négative pour l'activité  $\beta$ -glucosidase sur gélose d'EIA, et aucune activité n'a été détectée dans le test *p*NPG, confirmant son phénotype.

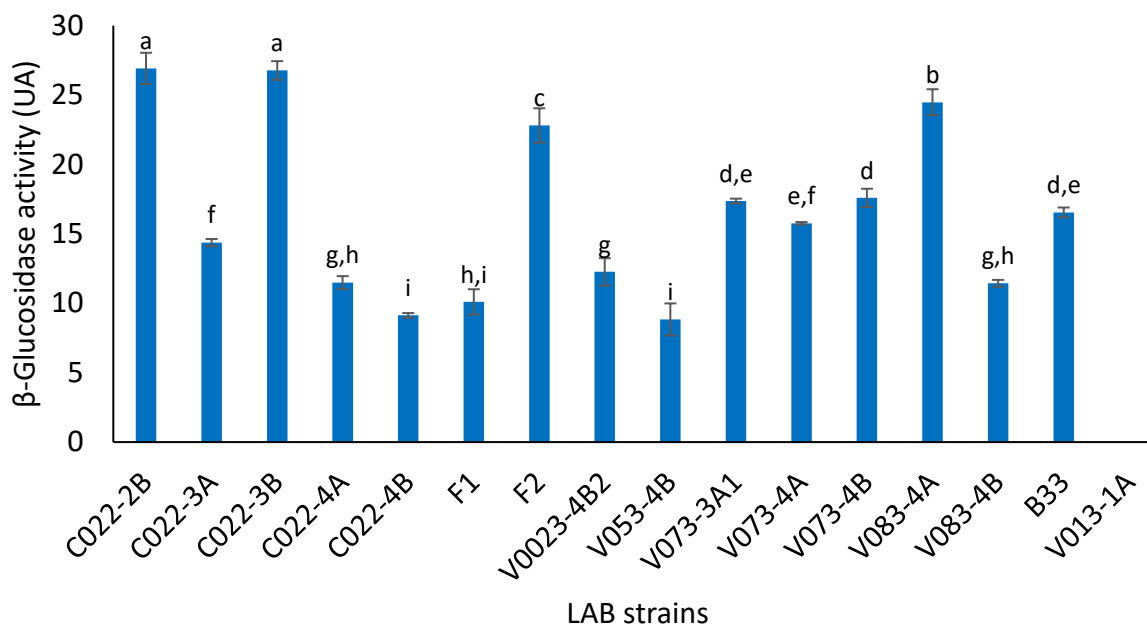


Figure 2. Activité  $\beta$ -glucosidase de bactéries lactiques, 15 souches et *L. plantarum* B33 cultivées 22 h à 25 °C. Les valeurs moyennes proviennent de trois répétitions biologiques. UA, nmol de *p*-nitrophénol par minute par milligramme de poids sec de cellule à 37 °C. Différentes lettres montrent des différences significatives ( $p < 0,05$ ) entre les différentes souches.

## 2.2. Élimination des isolats en double

Pour éliminer les isolats en double parmi les isolats positifs pour la  $\beta$ -glucosidase, les profils RAPD-PCR de 55 isolats (les 14 isolats avec les activités les plus élevées et 41 isolats sélectionnés au hasard) ont été analysés. A titre d'exemple, les différents motifs de bandes obtenus avec quatre isolats sont présentés sur la Figure 3. Dans le cas des deux isolats C022-3B et V0023-4B2, les fragments d'ADN amplifiés par PCR avec les amorces P2, P4 et M13 étaient identiques. Au contraire, les amorces OPL5 ont généré deux profils différents qui nous ont permis de distinguer ces isolats (Figure 3). L'analyse des profils d'amplification des 55 isolats avec les amorces P2, P4, OPL5 et M13 a libéré respectivement 30, 33, 33 et 36 profils RAPD-PCR différents. Enfin, la comparaison des profils combinés d'empreintes génétique

RAPD-PCR a fourni 40 souches différentes. Il a révélé une redondance parmi les 41 isolats avec une activité de  $\beta$ -glucosidase inférieure à 10 UA mais pas parmi les 14 isolats avec une activité supérieure à 10 UA (Figure 2).

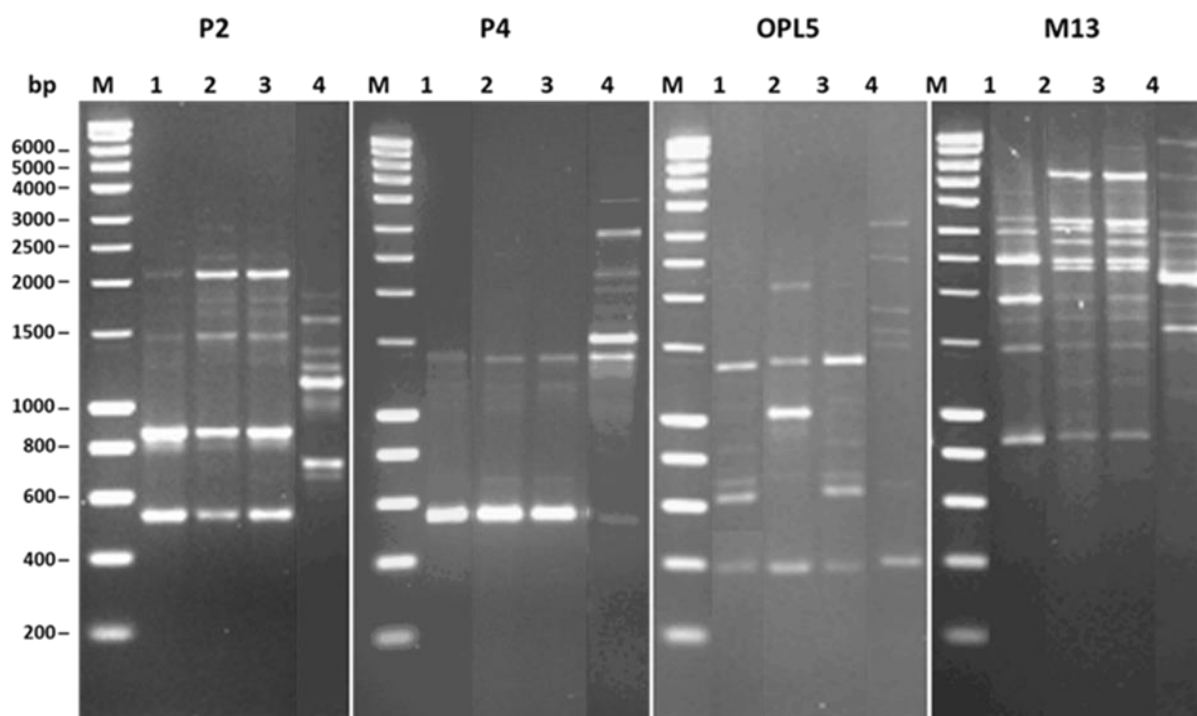


Figure 3. Profils RAPD-PCR d'isolats de bactéries lactiques provenant d'aliments fermentés en utilisant les amorces P2, P4, OPL5 et M13. Pistes : 1, C022-2B; 2, C022-3B; 3, V0023-4B2; 4, F2; M, marqueurs de poids moléculaire (SmartLadder; Eurogentec).

### 2.3. Identification des espèces bactériennes par ARNr 16S et PCR multiplex

Toutes les 14 souches positives pour la  $\beta$ -glucosidase appartenait au groupe *Lactiplantibacillus* (nouvelle nomenclature taxonomique (Zheng et al., 2020)) (*L. plantarum*, *L. paraplantarum* et *L. pentosus*) et la  $\beta$ -glucosidase-négative (V013-1A) était *Lactobacillus* (*Limosilactobacillus* dans la nouvelle taxonomie (Zheng et al., 2020)) *fermentum* selon le séquençage du gène de l'ARNr 16S (Tableau 2). Comme le séquençage du gène de l'ARNr 16S ne peut pas faire la distinction entre les trois espèces, des tests de PCR multiplex ont été réalisés sur les souches de 14  $\beta$ -glucosidases positives. Treize souches avaient des fragments de la même taille de 318 pb correspondant à *L. plantarum*. Seule la souche F2 a donné un fragment de 218 pb correspondant à *L. pentosus* (Figure 4).



Tableau 2. Identification des espèces de bactéries lactiques par approches moléculaires et origine de leurs aliments fermentés

Code de souche	Espèce	Aliment fermenté	Pays
C022-2B	<i>L. plantarum</i>	Racines d'échalote fermentées	Cambodge
C022-3A	<i>L. plantarum</i>	Racines d'échalote fermentées	Cambodge
C022-3B	<i>L. plantarum</i>	Racines d'échalote fermentées	Cambodge
C022-4A	<i>L. plantarum</i>	Racines d'échalote fermentées	Cambodge
C022-4B	<i>L. plantarum</i>	Racines d'échalote fermentées	Cambodge
F1	<i>L. plantarum</i>	Poisson cru fermenté "nem trey"	Cambodge
F2	<i>L. pentosus</i>	Poisson cru fermenté "nem trey"	Cambodge
V0023-4B2	<i>L. plantarum</i>	Porc fermenté cru "nem chua"	Vietnam
V053-4B	<i>L. plantarum</i>	Petite aubergine ronde fermentée	Vietnam
V073-3A1	<i>L. plantarum</i>	Racines d'échalote fermentées	Vietnam
V073-4A	<i>L. plantarum</i>	Racines d'échalote fermentées	Vietnam
V073-4B	<i>L. plantarum</i>	Racines d'échalote fermentées	Vietnam
V083-4A	<i>L. plantarum</i>	Feuille de bananier fraîche	Vietnam
V083-4B	<i>L. plantarum</i>	Feuille de bananier fraîche	Vietnam
V013-1A	<i>L. fermentum</i>	Porc fermenté cru "nem chua"	Vietnam
B33	<i>L. plantarum</i>	Porc fermenté cru "nem chua"	Vietnam

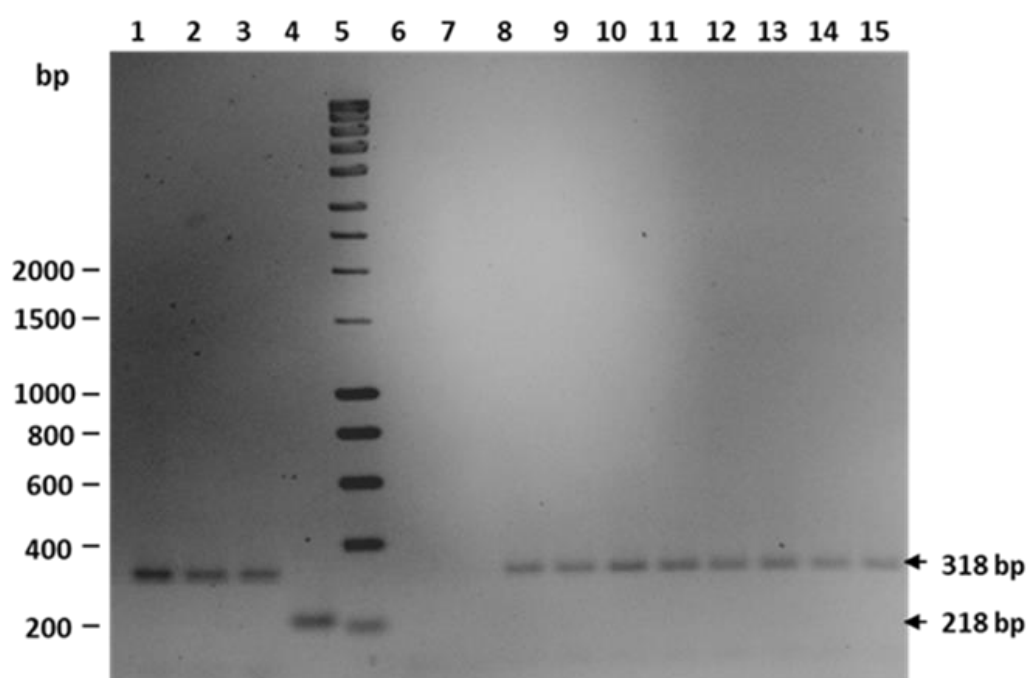


Figure 4. Analyse par PCR multiplex du groupe *Lactiplantibacillus*. Piste : **1**, V0023-4B2; **2**, V053-4B; **3**, F1; **4**, F2; **5**, marqueurs de poids moléculaire (SmartLadder; Eurogentec); **6**, H<sub>2</sub>O; **7**, V013-1A; **8**, C022-2B; **9**, C022-3A; **10**, C022-3B; **11**, C022-4A; **12**, C022-4B; **13**, V073-

3A1; 14, V073-4A; 15, V073-4B. Les marqueurs de poids moléculaire de l'ADN (pb) sont indiqués sur la gauche.

## 2.4. Activité de l'alcool déshydrogénase

Quatre souches sélectionnées pour leur activité  $\beta$ -glucosidase diversifiée ont été testées pour leur potentiel à réduire les aldéhydes en alcools correspondants : *L. plantarum* C022-2B et C022-3B, les deux souches les plus productrices, *L. plantarum* V0023-4B2, producteur intermédiaire et *L. fermentum* V013-1A, ne montrant aucune activité de  $\beta$ -glucosidase détectable. L'hexanal et le (*E*)-2-hexenal ont été utilisés comme substrats d'essai car ce sont des aldéhydes saturés et insaturés à chaîne courte présents dans la purée de tomates et contributeurs importants à l'arôme de la tomate (Wang et al., 2016). *L. fermentum* V013-1A a pu réduire l'hexanal ( $3,85 \times 10^{-8}$  UA) et le (*E*)-2-hexenal ( $2,47 \times 10^{-8}$  UA) en hexanol et (*E*)-2-hexénol, respectivement (Tableau 3). Aucun alcool n'a été détecté dans les flacon vials de *L. plantarum* C022-2B, C022-3B et V0023-4B2 dans les conditions ci-dessus. Par conséquent, un ajustement a été effectué en concentrant dix fois les cellules bactériennes. En effet, *L. plantarum* C022-2B, C022-3B et V0023-4B2 ne pouvaient réduire que l'hexanal avec un taux de réduction beaucoup plus faible que *L. fermentum* V013-1A. Pour les souches de *L. plantarum*, aucune réduction du (*E*)-2-hexenal n'a été observée.

Tableau 3. Réduction des aldéhydes en alcools par l'activité ADH de *L. plantarum* C022-2B, C022-3B et V0023-4B2 et *L. fermentum* V013-1A

Strain	Substrate disappearance rate ( $\times 10^{-8}$ UA)		Product appearance rate ( $\times 10^{-8}$ UA)	
	Substrate	Rate	Product	Rate
<i>L. plantarum</i> C022-2B	Hexanal	$0.44 \pm 0.05$	Hexanol	$0.31 \pm 0.11$
	( <i>E</i> )-2-hexenal	n.d.	( <i>E</i> )-2-hexenol	n.d.
<i>L. plantarum</i> C022-3B	Hexanal	< 0.44	Hexanol	< 0.31
	( <i>E</i> )-2-hexenal	n.d.	( <i>E</i> )-2-hexenol	n.d.
<i>L. plantarum</i> V0023-4B2	Hexanal	< 0.44	Hexanol	< 0.31
	( <i>E</i> )-2-hexenal	n.d.	( <i>E</i> )-2-hexenol	n.d.
<i>L. fermentum</i> V013-1A	Hexanal	$3.85 \pm 0.18$	Hexanol	$3.70 \pm 0.42$
	( <i>E</i> )-2-hexenal	$2.47 \pm 0.67$	( <i>E</i> )-2-hexenol	$2.58 \pm 0.26$

n.d. = non détectable

Les valeurs sont exprimées sous forme d'expériences en triple. Une unité d'activité enzymatique (UA) a été définie comme la quantité d'un substrat (nmol) réduite en un produit par minute et par UFC.

*L. fermentum* V013-1A semble avoir le plus grand potentiel de capacité de réduction des aldéhydes. Par conséquent, plus de substrats d'aldéhyde et de cétone ont été testés. *L. fermentum* V013-1A était capable de réduire (*E*)-2-hepténal ( $2,46 \times 10^{-8}$  UA) et (*E,E*)-2,4-décadiénal ( $3,69 \times 10^{-8}$  UA) en (*E*)-2-hepténol et (*E,E*)-2,4-décadiénol, respectivement (Tableau 4). Cette souche pourrait également convertir le 6,10-diméthyl-5,9-undécadién-2-one ( $1,26 \times 10^{-8}$  UA) en 6,10-diméthyl-5,9-undécadién-2-ol mais pas en 6-méthyl-5-heptén-2-one. Par conséquent, *L. fermentum* V013-1A était capable de réduire les aldéhydes et les cétones en leurs alcools correspondants avec une capacité de réduction plus grande pour les aldéhydes que pour les cétones.

Tableau 4. Réduction des aldéhydes et cétones en alcools par activité ADH de *L. fermentum* V013-1A

Strain	Substrate disappearance rate ( $\times 10^{-8}$ UA)	Product appearance rate ( $\times 10^{-8}$ UA)		
<i>L. fermentum</i> V013-1A	( <i>E</i> )-2-heptenal	$2.46 \pm 0.25$	( <i>E</i> )-2-heptenol	$3.13 \pm 0.26$
	( <i>E,E</i> )-2,4-decadienal	$3.69 \pm 0.00$	( <i>E,E</i> )-2,4-decadienol	$3.22 \pm 0.00$
	6-Methyl-5-hepten-2-one	n.d.	6-Methyl-5-hepten-2-ol	n.d.
	6,10-Dimethyl-5,9-undecadien-2-one	$1.26 \pm 0.14$	6,10-Dimethyl-5,9-undecadien-2-ol	$0.81 \pm 0.08$

n.d. = non détectable

Les valeurs sont exprimées sous forme d'expériences en triple. Une unité d'activité enzymatique (UA) a été définie comme la quantité d'un substrat (nmol) réduite en un produit par minute et par UFC.

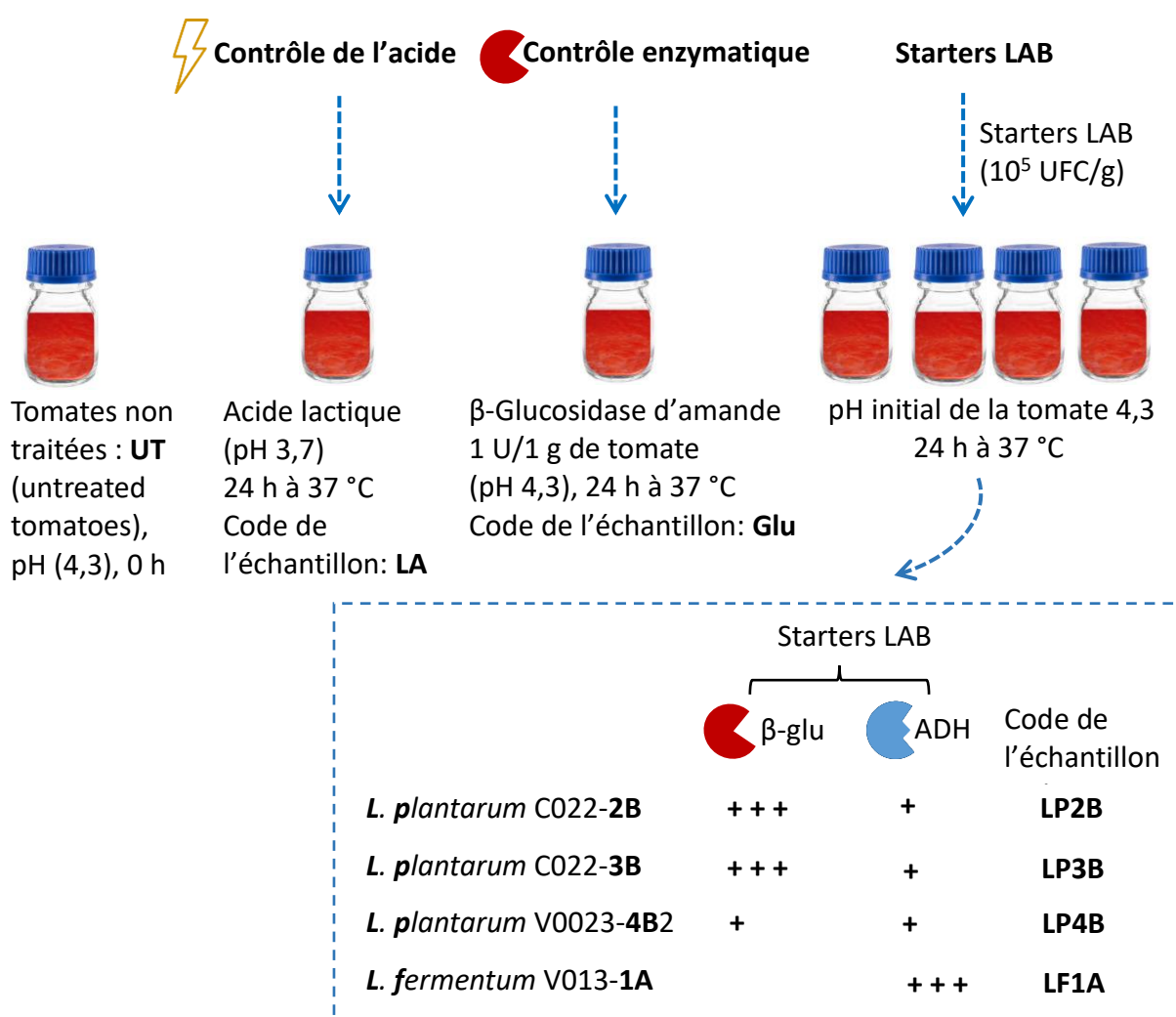
En conclusion, tout d'abord, notre étude a démontré que l'activité  $\beta$ -glucosidase est répandue parmi les LAB issus d'aliments fermentés asiatiques puisque sur les 200 isolats bactériens criblés, 80 étaient positifs pour la  $\beta$ -glucosidase. Cependant, la plupart des bactéries montraient une faible activité. Seules 14 souches ont montré une activité  $\beta$ -glucosidase supérieure à 10 UA et l'activité  $\beta$ -glucosidase la plus élevée était de 27 UA. Parmi eux, treize étaient *L. plantarum* et un était *L. pentosus*. D'après ce résultat, on pourrait émettre l'hypothèse que toutes les souches de *L. plantarum/paraplantarum/pentosus* trouvées dans les aliments fermentés sont positives pour l'activité  $\beta$ -glucosidase. En fait, cela n'est pas évident puisque *L. plantarum* est généralement l'espèce dominante dans les aliments lacto-fermentés. L'identification d'un panel représentatif d'isolats  $\beta$ -glucosidase négatifs devrait être nécessaire pour statuer sur cette hypothèse.

Deuxièmement, nos résultats ont mis en évidence des différences entre les isolats bactériens concernant l'activité ADH. Les cellules non proliférantes de *L. fermentum* V013-1A ont démontré la capacité de réduction la plus élevée pour l'hexanal ( $3,85 \times 10^{-8}$  UA) et (*E*)-2-hexéanal ( $2,47 \times 10^{-8}$  UA), tandis que les cellules non proliférantes de *L. plantarum* C022-2B, C022-3B et V0023-4B2 ont présenté une capacité similaire de réduction de l'ADH pour seulement l'hexanal ( $0,44 \times 10^{-8}$  UA). Les cellules non proliférantes de *L. fermentum* V013-1A ont également été capables de réduire (*E*)-2-heptéanal ( $2,46 \times 10^{-8}$  UA), (*E, E*)-2,4-décadiéanal ( $3,69 \times 10^{-8}$  UA) et géranylacétone ( $1,26 \times 10^{-8}$  UA).

### 3. Application de cultures de starter aromatiques dans les tomates fermentées

Quatre souches de LAB présentant des activités  $\beta$ -glucosidases distinctes (élevées, moyennes ou négatives) et des activités ADH ont été utilisées comme starters dans la purée de tomates. La capacité de la souche à libérer des composés volatils à partir des précurseurs d'arômes de tomate a été analysée concernant leurs activités  $\beta$ -glucosidase et ADH. En conséquence, l'objectif de cette étude était de caractériser le rôle des souches LAB dans la libération de composés d'arômes en fonction de leurs activités enzymatiques, en utilisant la tomate comme matrice de fermentation.

#### Stratégie expérimentale pour cette étude



Les quatre souches présentant diverses propriétés enzymatiques ont été utilisées individuellement comme starters pour la fermentation de la tomate. La purée de tomates (MT : mashed tomatoes) a été analysée immédiatement après le mélange comme référence sans aucun

traitement, et cette condition a été appelée tomates non traitées (UT : untreated tomatoes). Ensuite, les MT ont été traités avec de l'acide lactique à pH 3,70 comme contrôle de l'hydrolyse acide et avec de la  $\beta$ -glucosidase comme contrôle de l'activité enzymatique. Ces conditions ont été notées LA (lactic-acid-treated tomatoes) et Glu (glucosidase-treated tomatoes), respectivement.

### 3.1. pH et dénombrement bactérien dans les tomates fermentées

Pour les quatre souches, pendant la fermentation de la MT, le pH a baissé à 3,5-3,8 et le nombre total de lactobacilles est passé de  $1,0 \times 10^5$  UFC/g (inoculation initiale) à  $1,2 \times 10^8$  -  $1,5 \times 10^9$  UFC/g (Tableau 5), démontrant que les quatre souches étaient capables de cultiver et de fermenter la MT individuellement. Pour LA et Glu, le nombre de lactobacilles était de  $1,1 \times 10^7$  et  $5,4 \times 10^7$  UFC/g après incubation, ce qui montre qu'une fermentation spontanée a eu lieu, atteignant un nombre de bactéries inférieur à celui des starters (Tableau 5).

Tableau 5. Dénombrement total des lactobacilles sur boîtes de gélose MRS (UFC/g) et pH des tomates fermentées avec les souches de bactéries lactiques sélectionnées et les témoins

Treatment	pH	Total lactobacilli (CFU/g)
Untreated tomatoes (UT)	4,3	$8,3 \times 10^2$
Lactic-acid-treated tomatoes (LA)	3,7	$1,1 \times 10^7$
Glucosidase-treated tomatoes (Glu)	4,1	$5,4 \times 10^7$
Fermented tomatoes with <i>L. plantarum</i> C022-2B (LP2B)	3,7	$7,5 \times 10^8$
Fermented tomatoes with <i>L. plantarum</i> C022-3B (LP3B)	3,7	$7,0 \times 10^8$
Fermented tomatoes with <i>L. plantarum</i> V0023-4B2 (LP4B)	3,8	$1,5 \times 10^9$
Fermented tomatoes with <i>L. fermentum</i> V013-1A (LF1A)	3,5	$1,2 \times 10^8$

Dans toutes les conditions, les tomates ont été incubées pendant 24 h à 37 °C et analysées immédiatement, à l'exception de l'UT pour laquelle les tomates n'ont pas été incubées. Les données sont des moyennes de trois expériences indépendantes.

### 3.2. Composés volatils dans les tomates fermentées

Les composés volatils dans UT, LA, Glu et les tomates fermentées ont été extraits par HS-SPME. Parmi les trois revêtements de fibres testés, le PDMS/DVB était le plus convenable car il donnait des résultats avec toutes les familles de composés. Les composés volatils ont été identifiés et quantifiés par GC/MS. Cinquante-huit composés volatils ont été regroupés selon

les classes chimiques : aldéhydes, alcools, cétones, terpènes, acides organiques et divers (Tableau 6).

Tableau 6. Composés volatils (ppb) trouvés dans la tomate après traitement

Chemical class	Treatment						
	UT	MT-LA	MT-Glu	MT-LP2B	MT-LP3B	MT-LP4B	MT-LF1A
<b>Aldehydes</b>							
Hexanal	135.85 <sup>a</sup>	92.21 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
(E)-2-hexenal	18.10 <sup>a</sup>	17.07 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
Heptanal	6.41 <sup>a</sup>	1.36 <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
(E)-2-heptenal	15.65 <sup>a</sup>	14.81 <sup>a</sup>	nd <sup>a</sup>	7.77 <sup>a</sup>	tr <sup>a</sup>	3.56 <sup>a</sup>	nd <sup>a</sup>
(E)-2-octenal	60.19 <sup>a</sup>	58.46 <sup>a</sup>	1.78 <sup>b</sup>	49.63 <sup>ab</sup>	11.92 <sup>ab</sup>	13.60 <sup>ab</sup>	nd <sup>b</sup>
Nonanal	16.77 <sup>a</sup>	6.7 <sup>b</sup>	nd <sup>c</sup>	tr <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>
(E)-2-nonenal	9.38 <sup>a</sup>	9.70 <sup>a</sup>	nd <sup>b</sup>	3.29 <sup>ab</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
Decanal	9.97 <sup>a</sup>	5.99 <sup>ab</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
(E,E)-2,4-decadienal	31.68 <sup>ab</sup>	18.17 <sup>bc</sup>	nd <sup>c</sup>	42.98 <sup>a</sup>	32.97 <sup>ab</sup>	nd <sup>c</sup>	nd <sup>c</sup>
Benzaldehyde	nd <sup>b</sup>	nd <sup>b</sup>	13.39 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
β-Cyclocitral	4.76 <sup>a</sup>	5.85 <sup>a</sup>	3.54 <sup>a</sup>	3.74 <sup>a</sup>	4.44 <sup>a</sup>	4.06 <sup>a</sup>	4.09 <sup>a</sup>
β-Citral	11.62 <sup>a</sup>	9.51 <sup>a</sup>	10.44 <sup>a</sup>	8.11 <sup>a</sup>	5.95 <sup>a</sup>	6.79 <sup>a</sup>	1.88 <sup>a</sup>
α-Citral	31.12 <sup>a</sup>	15.26 <sup>b</sup>	16.25 <sup>b</sup>	14.36 <sup>b</sup>	8.02 <sup>b</sup>	8.48 <sup>b</sup>	4.28 <sup>b</sup>
4-Methyl-3-cyclohexene-1-carboxaldehyde	tr <sup>a</sup>	1.60 <sup>a</sup>	2.97 <sup>a</sup>	tr <sup>a</sup>	nd <sup>a</sup>	nd <sup>a</sup>	nd <sup>a</sup>
3-Methyl-3-(4-methyl-3-pentenyl)-oxiranecarboxaldehyde	6.48 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
<b>Total</b>	<b>358.80</b>	<b>256.69</b>	<b>48.37</b>	<b>131.48</b>	<b>64.10</b>	<b>36.49</b>	<b>10.25</b>
<b>Alcohols</b>							
2,3-Butanediol	nd <sup>b</sup>	nd <sup>b</sup>	2.46 <sup>b</sup>	12.27 <sup>a</sup>	tr <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
1-Hexanol	nd <sup>c</sup>	6.25 <sup>c</sup>	32.10 <sup>bc</sup>	27.93 <sup>bc</sup>	51.31 <sup>ab</sup>	50.45 <sup>ab</sup>	74.05 <sup>a</sup>
1-Heptanol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	3.86 <sup>a</sup>	nd <sup>b</sup>
2-Heptanol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	5.29 <sup>a</sup>
(E)-2-hepten-1-ol	nd <sup>b</sup>	nd <sup>b</sup>	7.98 <sup>a</sup>	7.37 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	6.06 <sup>a</sup>
1-Octanol	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	4.43 <sup>b</sup>	nd <sup>c</sup>	15.07 <sup>a</sup>	nd <sup>c</sup>
1-Octen-3-ol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	1.50 <sup>a</sup>
(E)-2-octen-1-ol	nd <sup>d</sup>	nd <sup>d</sup>	22.20 <sup>a</sup>	15.42 <sup>b</sup>	6.60 <sup>c</sup>	5.75 <sup>c</sup>	20.47 <sup>a</sup>
1-Nonanol	nd <sup>b</sup>	nd <sup>b</sup>	tr <sup>b</sup>	3.37 <sup>a</sup>	3.41 <sup>a</sup>	tr <sup>b</sup>	2.10 <sup>a</sup>
2-Nonanol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	2.70 <sup>a</sup>
(Z)-3-nonen-1-ol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	7.64 <sup>a</sup>
(E)-2-nonen-1-ol	nd <sup>b</sup>	nd <sup>b</sup>	1.34 <sup>b</sup>	6.39 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	5.65 <sup>a</sup>
(E,E)-2,4-decadien-1-ol	nd <sup>b</sup>	nd <sup>b</sup>	2.74 <sup>ab</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	4.96 <sup>a</sup>
6-Methyl-5-hepten-2-ol	nd <sup>d</sup>	nd <sup>d</sup>	9.84 <sup>d</sup>	33.70 <sup>b</sup>	35.38 <sup>b</sup>	21.74 <sup>c</sup>	163.19 <sup>a</sup>
6,10-Dimethyl-5,9-undecadien-2-ol	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	10.49 <sup>b</sup>	10.33 <sup>b</sup>	12.74 <sup>b</sup>	25.75 <sup>a</sup>
2-Methoxy phenol	5.86 <sup>a</sup>	5.97 <sup>a</sup>	5.21 <sup>a</sup>	3.55 <sup>a</sup>	10.39 <sup>a</sup>	5.41 <sup>a</sup>	5.93 <sup>a</sup>
2-Phenylethanol	nd <sup>d</sup>	1.74 <sup>d</sup>	21.17 <sup>a</sup>	9.70 <sup>bc</sup>	16.32 <sup>ab</sup>	12.28 <sup>bc</sup>	5.20 <sup>cd</sup>
4-Ethylphenol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	15.50 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>

<b>Total</b>	<b>5.86</b>	<b>13.96</b>	<b>105.44</b>	<b>134.62</b>	<b>150.05</b>	<b>128.09</b>	<b>330.47</b>
<b>Ketones</b>							
1-Octen-3-one	3.24 <sup>a</sup>	0.84 <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
6-Methyl-5-hepten-2-one	139.57 <sup>a</sup>	184.69 <sup>a</sup>	219.43 <sup>a</sup>	246.69 <sup>a</sup>	269.49 <sup>a</sup>	207.21 <sup>a</sup>	4.31 <sup>b</sup>
6,10-Dimethyl-5,9-undecadien-2-one	78.88 <sup>a</sup>	84.91 <sup>a</sup>	81.83 <sup>a</sup>	78.46 <sup>a</sup>	62.47 <sup>ab</sup>	51.51 <sup>ab</sup>	4.23 <sup>b</sup>
2-Undecanone	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	28.83 <sup>a</sup>	8.85 <sup>b</sup>
α-Ionone	nd <sup>b</sup>	2.10 <sup>a</sup>	tr <sup>ab</sup>	tr <sup>ab</sup>	tr <sup>ab</sup>	tr <sup>ab</sup>	nd <sup>b</sup>
1-(2-Methyl-cyclopenten-1-yl)-ethanone	tr <sup>b</sup>	3.20 <sup>ab</sup>	tr <sup>b</sup>	5.54 <sup>a</sup>	4.74 <sup>a</sup>	tr <sup>b</sup>	3.25 <sup>ab</sup>
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	12.23 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	tr <sup>b</sup>
5-Ethyl-2,4-dimethyl-4-hepten-3-one	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	6.05 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
<b>Total</b>	<b>222.49</b>	<b>275.75</b>	<b>302.86</b>	<b>349.78</b>	<b>337.49</b>	<b>289.15</b>	<b>21.43</b>
<b>Terpenes</b>							
(Z)-geraniol	nd <sup>b</sup>	nd <sup>b</sup>	1.73 <sup>b</sup>	9.86 <sup>a</sup>	6.06 <sup>a</sup>	6.94 <sup>a</sup>	7.73 <sup>a</sup>
6,7-Dihydrogeraniol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	tr <sup>b</sup>	3.12 <sup>a</sup>	4.21 <sup>a</sup>
Melonol	nd <sup>d</sup>	nd <sup>d</sup>	nd <sup>d</sup>	5.16 <sup>a</sup>	tr <sup>c</sup>	2.69 <sup>b</sup>	tr <sup>c</sup>
Linalool	nd <sup>b</sup>	1.52 <sup>ab</sup>	tr <sup>ab</sup>	5.35 <sup>a</sup>	3.84 <sup>ab</sup>	tr <sup>ab</sup>	2.58 <sup>ab</sup>
D-limonene	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	9.38 <sup>a</sup>	8.15 <sup>a</sup>	5.43 <sup>a</sup>
(Z)-2,6-dimethyl-2,6-octadiene	8.82 <sup>a</sup>	7.90 <sup>a</sup>	5.77 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
(3Z,5Z)-2,7-dimethyl-3,5-octadiene	6.98 <sup>a</sup>	1.75 <sup>b</sup>	4.23 <sup>b</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>
<b>Total</b>	<b>15.80</b>	<b>11.17</b>	<b>12.52</b>	<b>20.37</b>	<b>20.88</b>	<b>21.69</b>	<b>20.75</b>
<b>Organic acid and their derivatives</b>							
Acetic acid	nd <sup>c</sup>	nd <sup>c</sup>	26.69 <sup>c</sup>	93.56 <sup>b</sup>	tr <sup>c</sup>	7.36 <sup>c</sup>	148.58 <sup>a</sup>
Hexyl ester acetic acid	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	tr <sup>c</sup>	5.45 <sup>a</sup>	nd <sup>c</sup>	3.08 <sup>b</sup>
Octanoic acid	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	5.74 <sup>a</sup>	4.27 <sup>b</sup>
Methyl ester octanoic acid	5.94 <sup>a</sup>	3.25 <sup>b</sup>	tr <sup>b</sup>	3.13 <sup>b</sup>	tr <sup>b</sup>	tr <sup>b</sup>	2.36 <sup>b</sup>
<b>Total</b>	<b>5.94</b>	<b>3.25</b>	<b>27.49</b>	<b>97.49</b>	<b>7.05</b>	<b>13.90</b>	<b>158.28</b>
<b>Miscellaneous</b>							
Methoxy-phenyl-oxime	1.89 <sup>a</sup>	2.82 <sup>a</sup>	2.18 <sup>a</sup>	nd <sup>a</sup>	tr <sup>a</sup>	3.90 <sup>a</sup>	6.56 <sup>a</sup>
2,7-Dimethyl-oxepine	nd <sup>a</sup>	7.29 <sup>a</sup>	nd <sup>a</sup>	nd <sup>a</sup>	8.73 <sup>a</sup>	8.65 <sup>a</sup>	8.26 <sup>a</sup>
2-Isobutylthiazole	14.89 <sup>a</sup>	tr <sup>c</sup>	nd <sup>c</sup>	18.26 <sup>a</sup>	12.37 <sup>ab</sup>	13.66 <sup>ab</sup>	4.52 <sup>bc</sup>
3-(4-Methyl-3-pentenyl)-furan	7.79 <sup>ab</sup>	7.30 <sup>b</sup>	8.32 <sup>ab</sup>	14.01 <sup>ab</sup>	14.46 <sup>ab</sup>	13.93 <sup>ab</sup>	16.88 <sup>a</sup>
1-Isocyano-3-methylbenzene	nd <sup>d</sup>	nd <sup>d</sup>	nd <sup>d</sup>	6.17 <sup>a</sup>	tr <sup>c</sup>	3.39 <sup>b</sup>	3.66 <sup>b</sup>
Methyl salicylate	11.66 <sup>a</sup>	9.91 <sup>a</sup>	nd <sup>a</sup>	5.74 <sup>a</sup>	7.22 <sup>a</sup>	nd <sup>a</sup>	nd <sup>a</sup>



<b>Total</b>	<b>36.23</b>	<b>28.12</b>	<b>10.50</b>	<b>44.18</b>	<b>44.54</b>	<b>43.54</b>	<b>158.28</b>
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Les échantillons étaient des tomates fermentées avec *L. plantarum* C022-2B (échantillon LP2B), C022-3B (LP3B) et V0023-4B2 (LP4B) et *L. fermentum* V013-1A (LF1A). Abréviations : UT, tomates non traitées; LA, tomates traitées à l'acide lactique; Glu, tomates traitées à la glucosidase; utilisant un revêtement PDMS/DVB avec microextraction en phase solide d'espace de tête/chromatographie en phase gazeuse-spectrométrie de masse (HS-SPME/GC-MS).

« a, b, c, d » Les valeurs de la même ligne suivies de lettres différentes sont significativement différentes ( $p < 0,05$ ). tr, inférieur à 0,80 ppb; nd, non détecté

Comme le montre la [Figure 5](#), la plupart des aldéhydes trouvés dans la tomate non traitée (UT : untreated tomatoes) avaient leur concentration décroissante, au moins trois fois, et parfois même atteint zéro lors de la fermentation et du traitement avec la  $\beta$ -glucosidase. Ils ont également diminué, mais légèrement (environ 1,4 fois), lors du traitement à l'acide lactique (LA). Parmi les tomates fermentées, la LF1A (tomates fermentées avec *L. fermentum* V013-1A) contenait la plus faible quantité d'aldéhydes avec une diminution de 36 fois par rapport à l'UT. Seuls le  $\beta$ -cyclocitral, le  $\beta$ -citral et le  $\alpha$ -citral sont restés dans toutes les tomates fermentées ([Tableau 6](#)). Les niveaux d'aldéhydes dérivés de la voie LOX tels que l'hexanal (C=6), le 2-hexéanal (C=6), le 2-octéanal (C=8) et le 2-nonéanal (C=9) étaient similaires dans UT et LA alors qu'ils n'étaient pas détectables ou très faibles dans les autres traitements ([Figure 6](#)).

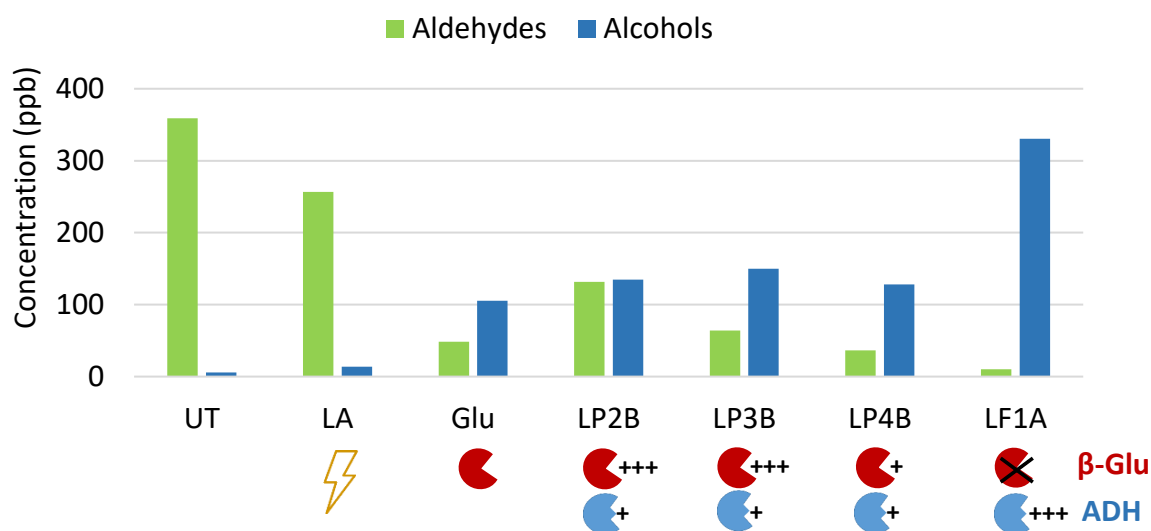


Figure 5. Concentration totale d'aldéhydes et d'alcools dans les tomates traitées et fermentées. Quatre souches présentant diverses propriétés enzymatiques ( $\beta$ -glucosidase ( $\beta$ -glu) et alcool déshydrogénase (ADH); haute (+++) et faible (+)) ont été utilisées individuellement comme starters pour la fermentation de la tomate. Abréviations : LP2B, tomates fermentées avec *L. plantarum* C022-2B; LP3B, avec *L. plantarum* C022-3B; LP4B, avec *L. plantarum* V0023-

4B2; LF1A, avec *L. fermentum* V013-1A; UT, tomates non traitées; LA, tomates traitées à l'acide lactique; Glu, tomates traitées à la glucosidase

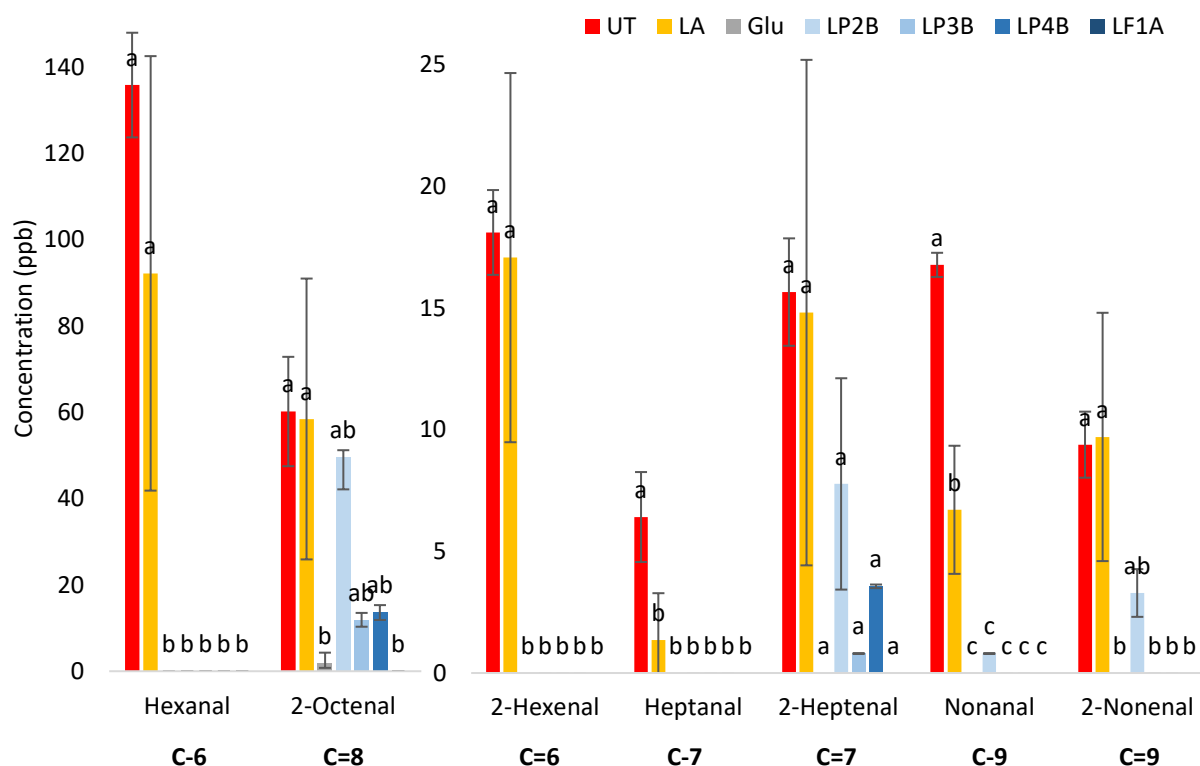


Figure 6. Aldéhydes dérivés de la voie lipoxygénase d'acides gras dans les tomates traitées et fermentées. Abréviations : LP2B, tomates fermentées avec *L. plantarum* C022-2B; LP3B, avec *L. plantarum* C022-3B; LP4B, avec *L. plantarum* V0023-4B2; LF1A, avec *L. fermentum* V013-1A; UT, tomates non traitées ; LA, tomates traitées à l'acide lactique; Glu, tomates traitées à la glucosidase. C– et C= sont des aldéhydes saturés et insaturés, respectivement. Les moyennes avec des lettres différentes (a, b, c, d) sont significativement différentes ( $p < 0,05$ ).

Contrairement aux aldéhydes, la plupart des alcools détectés dans notre étude n'ont pas été retrouvés dans UT et LA (Figure 5). Inversement corrélée à la dégradation des aldéhydes, la concentration en alcools a augmenté significativement lors de la fermentation et du traitement  $\beta$ -glucosidase. Le niveau d'alcools était le plus élevé dans LF1A, où il était environ deux fois plus élevé que dans les autres tomates fermentées. Les alcools présents en concentrations élevées dans toutes les tomates fermentées étaient le hexanol (C–6) et le 2-octénol (C=8) (les deux volatils dérivés de la voie LOX) dans la Figure 7, et le 6-méthyl-5-heptén-2-ol (C=8) et le 6,10-diméthyl-5,9-undécadién-2-ol (C=13) dans le Tableau 6. De faibles concentrations de volatils phénoliques, y compris le 2-phényléthanol et le 4-éthylphénol, sont apparues pendant la fermentation et traitement (Tableau 6). Le 2,3-butanediol n'a augmenté de manière significative que dans le LP2B (tomates fermentées avec *L. plantarum* C022-2B) (Tableau 6).

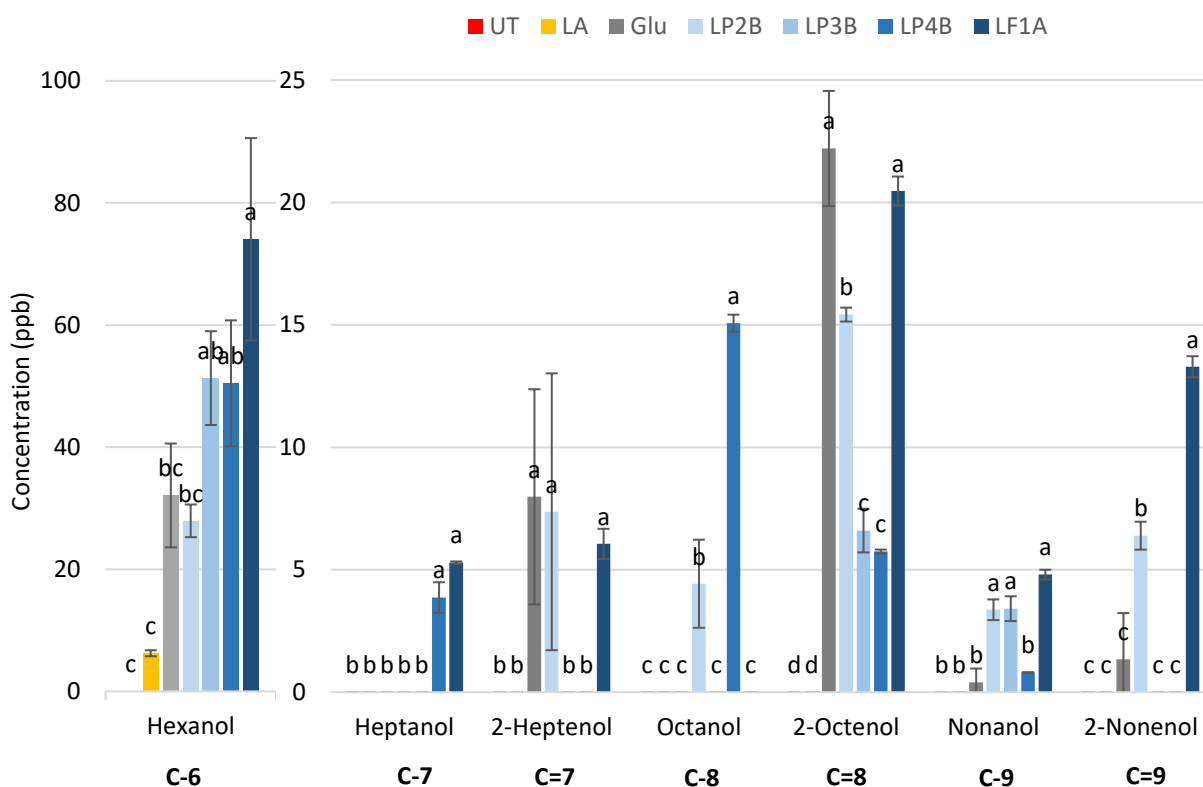


Figure 7. Alcools volatils dérivés d'acides gras par activité de l'alcool déshydrogénase. Abréviations : LP2B, tomates fermentées avec *L. plantarum* C022-2B; LP3B, avec *L. plantarum* C022-3B; LP4B, avec *L. plantarum* V0023-4B2; LF1A, avec *L. fermentum* V013-1A; UT, tomates non traitées; LA, tomates traitées à l'acide lactique; Glu, tomates traitées à la glucosidase. C– et C= sont des alcools saturés et insaturés, respectivement. Les moyennes avec des lettres différentes (a, b, c, d) sont significativement différentes ( $p < 0,05$ ).

La concentration de cétones a augmenté modérément dans toutes les tomates fermentées et dans Glu et LA, sauf dans LF1A où la concentration a considérablement diminué (Figure 8). Les niveaux de cétones étaient similaires dans LP2B et LP3B (tomates fermentées avec *L. plantarum* C022-2B et C022-3B, respectivement). Ces niveaux étaient tous deux plus élevés que ceux de LP4B (tomates fermentées avec *L. plantarum* V0023-4B2), Glu et LA. La 6-méthyl-5-heptén-2-one (MHO) et la géranylacétone étaient les cétones les plus abondantes dans UT, Glu, LA et tomates fermentées, mais elles ont diminué de manière significative dans LF1A (Tableau 6).

Bien que la concentration totale de terpènes n'ait pas changé considérablement pendant la fermentation, plusieurs terpènes importants pour les profils aromatiques sont apparus, notamment le (Z)-géraniol, le 6,7-dihydrogéraniol, le melonol, le linalol et le D-limonène (Figures 9 et 10). L'acide acétique n'a pas été détecté dans UT, mais il a été trouvé dans LP2B et LF1A (Tableau 6).

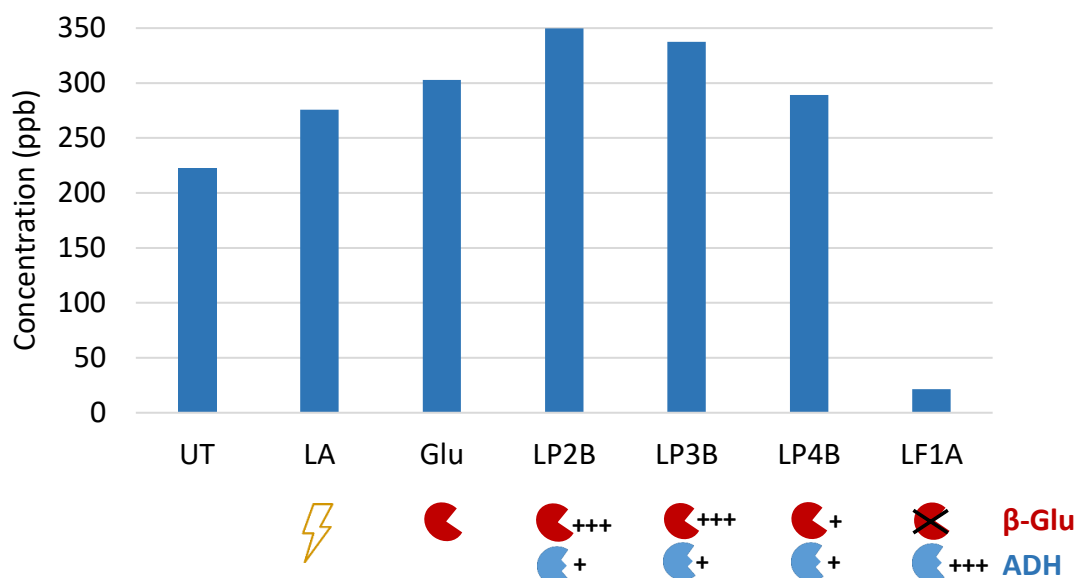


Figure 8. Concentration totale de cétones. Quatre souches présentant diverses propriétés enzymatiques ( $\beta$ -glucosidase ( $\beta$ -glu) et alcool déshydrogénase (ADH); haute (+++) et faible (+)) ont été utilisées individuellement comme starters pour la fermentation de la tomate. Abréviations : LP2B, tomates fermentées avec *L. plantarum* C022-2B; LP3B, avec *L. plantarum* C022-3B; LP4B, avec *L. plantarum* V0023-4B2; LF1A, avec *L. fermentum* V013-1A; UT, tomates non traitées; LA, tomates traitées à l'acide lactique; Glu, tomates traitées à la glucosidase

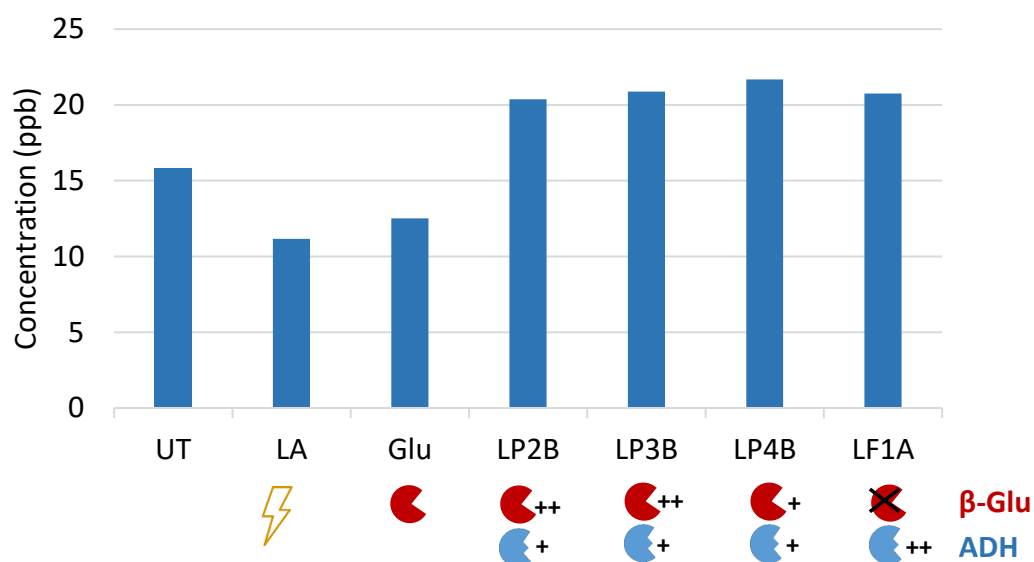


Figure 9. Concentration totale de terpènes. Quatre souches présentant diverses propriétés enzymatiques ( $\beta$ -glucosidase ( $\beta$ -glu) et alcool déshydrogénase (ADH); haute (+++) et faible (+)) ont été utilisées individuellement comme starters pour la fermentation de la tomate. Abréviations : LP2B, tomates fermentées avec *L. plantarum* C022-2B; LP3B, avec *L. plantarum* C022-3B; LP4B, avec *L. plantarum* V0023-4B2; LF1A, avec *L. fermentum* V013-

1A; UT, tomates non traitées; LA, tomates traitées à l'acide lactique; Glu, tomates traitées à la glucosidase

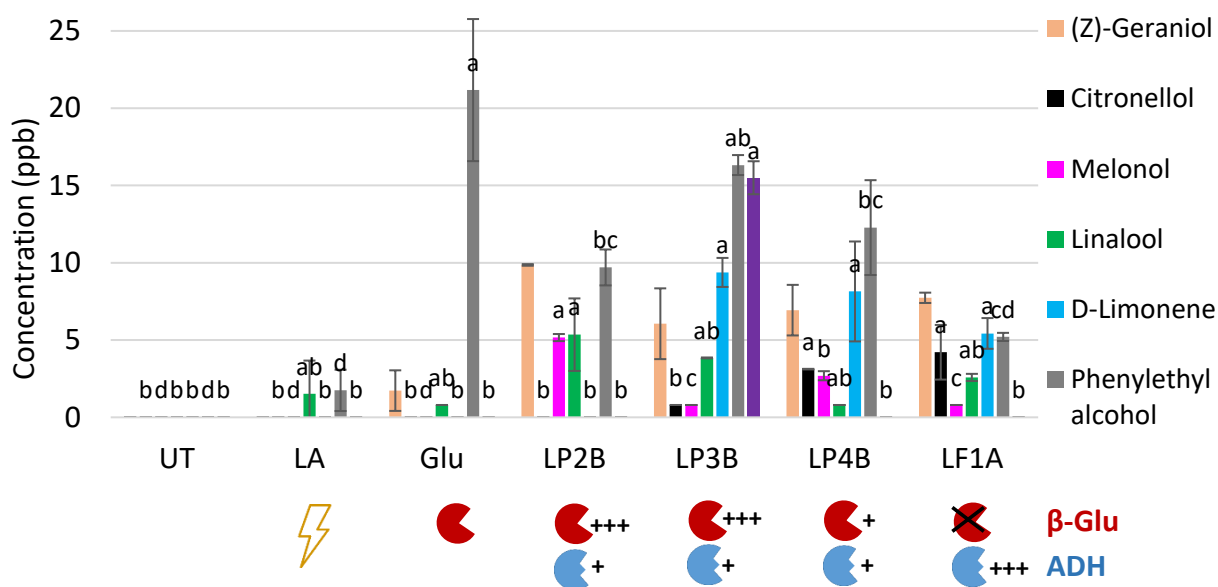


Figure 10. Composés volatils glycosylés. Quatre souches présentant diverses propriétés enzymatiques ( $\beta$ -glucosidase ( $\beta$ -glu) et alcool déshydrogénase (ADH); haute (++++) et faible (+)) ont été utilisées individuellement comme starters pour la fermentation de la tomate. Abréviations : LP2B, tomates fermentées avec *L. plantarum* C022-2B; LP3B, avec *L. plantarum* C022-3B; LP4B, avec *L. plantarum* V0023-4B2; LF1A, avec *L. fermentum* V013-1A; UT, tomates non traitées; LA, tomates traitées à l'acide lactique; Glu, tomates traitées à la glucosidase. Les moyennes avec des lettres différentes (a, b, c, d) sont significativement différentes ( $p < 0,05$ ).

L'analyse en composantes principales (ACP) a été utilisée pour identifier les modèles et détecter les corrélations entre les composés volatils et les traitements à la tomate. Les première et deuxième composantes représentaient 67,90% et 24,67% de la variance totale, respectivement (Figure 11). Le cercle de corrélation (à gauche) a révélé que toutes les variables étaient éloignées du centre. LA était proche d'UT, ce qui indique qu'elles étaient significativement corrélées positivement. Il est donc peu probable que l'hydrolyse acide modifie les profils aromatiques des tomates. En revanche, LP2B, LP3B, LP4B et Glu étaient presque orthogonaux avec UT, ce qui signifie qu'ils n'étaient pas corrélés avec UT. LF1A était presque du côté opposé d'UT, ce qui suggère qu'elles étaient significativement corrélées négativement. Par conséquent, l'activité  $\beta$ -glucosidase et les LAB ont eu un impact considérable sur les composés volatils des tomates. La carte d'observation (à droite) montre clairement les effets des traitements sur les composés volatils des tomates. UT et LA étaient riches en aldéhydes. LP2B, LP3B, LP4B et Glu étaient probablement abondants dans les cétones. LF1A différait

considérablement des autres tomates fermentées par la présence abondante d'alcools et d'acide acétique. Ces résultats mettent en évidence l'effet des LAB sur les composés volatils des tomates fermentées.

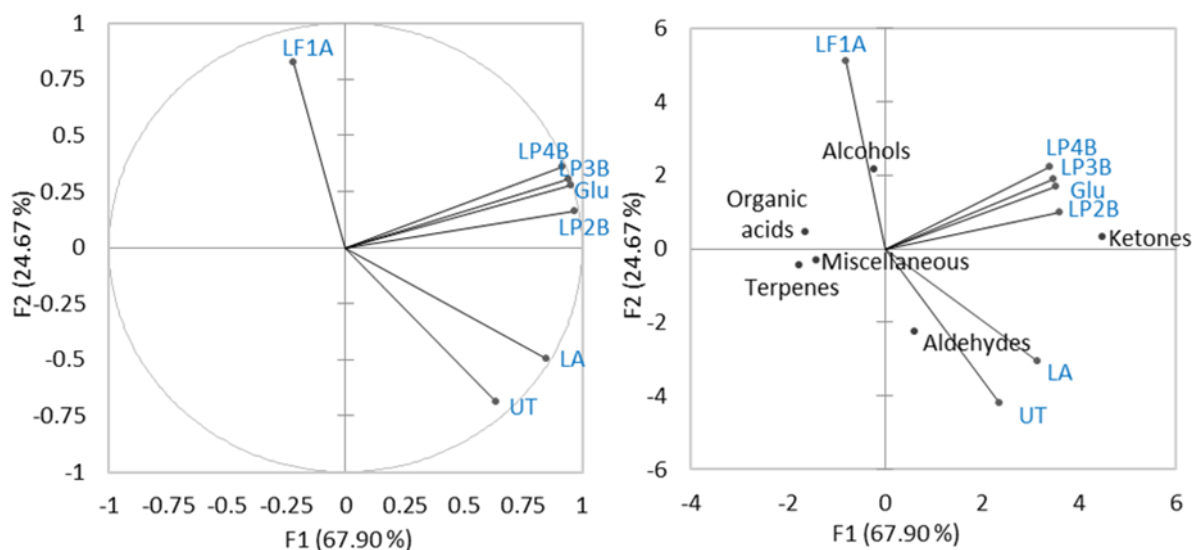


Figure 11. Graphique des scores de la première et de la deuxième composante principale (CP) après analyse CP sur la base de la concentration de composés volatils trouvée dans la tomate après les traitements (Tableau 6). Abréviations : LP2B, tomates fermentées avec *L. plantarum* C022-2B; LP3B, avec *L. plantarum* C022-3B; LP4B, avec *L. plantarum* V0023-4B2; LF1A, avec *L. fermentum* V013-1A; UT, tomates non traitées; LA, tomates traitées à l'acide lactique; Glu, tomates traitées à la glucosidase

Le premier objectif de notre travail était les composés volatils produits lors du métabolisme des plantes. Ces composés volatils dans la plante peuvent présenter une toxicité ou avoir un rôle de communication et par conséquent, la plante elle-même les inactive par glycosylation pour diminuer la toxicité ou pour contrôler la communication entre les cellules végétales (Song et al., 2018). La plupart des composés volatils s'accumulent dans les fruits/légumes sous forme de glycosides non volatils et inodores. Ces composés volatils d'arômes peuvent être à nouveau activés sensoriellement en hydrolysant la liaison glycosique et en libérant l'aglycone volatile. Cette réaction peut être catalysée par des enzymes  $\beta$ -glucosidases (Sarry et Günata, 2004; Song et al., 2018). Les gènes de  $\beta$ -glucosidase sont répandus dans les génomes LAB. Des activités de  $\beta$ -glucosidase des LAB ont été signalées dans de nombreuses matrices alimentaires végétales telles que l'olive, le soja, le raisin et le manioc. Ils peuvent hydrolyser une large gamme de substrats (Michlmayr et Kneifel, 2014) mais leur spécificité de substrat (aglycone) est difficile à prédire en fonction de la séquence du gène. Ainsi, la première étape du criblage de  $\beta$ -glucosidases spécifiques doit être expérimentale. La

spécificité des aglycones semble avoir évolué au cours de l'adaptation bactérienne à la niche écologique ainsi que des conditions environnementales d'induction de l'activité enzymatique (Michlmayr et Kneifel, 2014). Ces hypothèses suggèrent que la sélection de LAB à partir de divers types d'aliments fermentés permettrait d'obtenir diverses activités de  $\beta$ -glucosidases et donc divers profils d'arômes.

Les souches sélectionnées présentant une activité  $\beta$ -glucosidase ont été évaluées pour leur capacité à libérer des composés volatils en comparaison avec l'effet de l'acide lactique pour stimuler l'hydrolyse et avec l'ajout d'une enzyme  $\beta$ -glucosidase d'amandes. Dans notre étude, les terpénoïdes étaient les principaux volatils libérés après un traitement à la  $\beta$ -glucosidase. Une légère augmentation de la concentration de cétones terpéniques a été observée non seulement dans Glu mais aussi dans LA (Figure 8), confirmant qu'outre l'activité  $\beta$ -glucosidase, l'hydrolyse acide avait également un impact sur la génération de cétones. Pour les souches présentant de la glucosidase, le résultat de la génération de composés volatils est en corrélation avec les valeurs d'activité de la  $\beta$ -glucosidase avec des niveaux de cétone plus élevés dans LP2B et LP3B que dans LP4B (activité  $\beta$ -glucosidase : 27 UA pour *L. plantarum* C022-2B et C022-3B et 12 UA pour *L. plantarum* V0023-4B2). La MHO et la géranylacétone étaient les cétones les plus abondantes dans tous les traitements, sauf dans LF1A (Table 6). Ces composés donnent des notes fruitées ou florales à la tomate (Wang et al., 2016) et ils sont initialement dérivés du lycopène (Vogel et al., 2010).

D'autres terpènes importants tels que le (*Z*)-géraniol, le 6,7-dihydrogéraniol, le melonol, le linalol et le D-limonène ont également été détectés dans les tomates fermentées (Figure 10). Ils peuvent théoriquement être libérés des glycosides de fruits par la  $\beta$ -glucosidase ou l'hydrolyse acide (Buttery et al., 1990; Fenoll et al., 2009; Hellín et al., 2010; Marlatt et al., 1992; Ortiz-Serrano et Gil, 2010). Cependant, les résultats dans LA et Glu n'étaient pas significativement différents de ceux dans UT alors que plusieurs augmentations importantes de concentration ont été observées après la fermentation bien que cela soit spécifique à la souche et au composé. Par exemple, le géraniol est apparu dans toutes les fermentations tandis que le limonène a augmenté de manière significative dans trois milieux (LP3B, LP4B et LF1A) mais pas dans LP2B (Figure 10). Ce dernier milieu était celui contenant la plus forte concentration de melonol et de linalol. Tous ces résultats suggèrent que la libération de terpénoïdes pourrait provenir soit des glycosides après l'action de glucosidases spécifiques, soit d'autres activités métaboliques présentes différemment chez les bactéries lactiques. Nos résultats montrent que la production de terpènes volatils est une caractéristique présentant des différences au niveau de la souche en LAB.

Une famille de composés d'arômes présentant une importance énorme dans de nombreux produits végétaux est la famille des notes vertes. Ces composés, aldéhydes et alcools volatils, sont issus de la voie lipoxigénase (LOX) et sont responsables des notes sensorielles fraîches et vertes des fruits et légumes. La voie LOX est un système multi-enzymes dans lequel les acides gras polyinsaturés sont convertis en aldéhydes et alcools par l'action séquentielle de la lipoxigénase, de l'hydroperoxydase (HPL) et de l'alcool déshydrogénase (ADH) (Akacha et Gargouri, 2015; Christensen et al., 2007). Cette voie se produit généralement dans les fruits lors de la maturation ou la rupture tissulaire (macération ou mélange). Par conséquent, la plupart des aldéhydes détectés dans UT et LA étaient probablement libérés d'acides gras via la voie LOX. Les aldéhydes dérivés de la voie LOX comprennent l'hexanal, le (*E*)-2-hexenal, l'heptanal, le (*Z*)-2-heptenal, le (*E*)-2-octenal, le nonanal et le (*E*)-2-nonanal (Figure 6). Les homologues LOX-alcools des aldéhydes n'ont pas été détectés dans UT, montrant que la réduction finale des aldéhydes par l'ADH n'était pas active (Figure 7). Cependant, cette réduction des notes vertes est importante pour les propriétés sensorielles car les alcools présentent soit les mêmes propriétés sensorielles que les aldéhydes mais avec des seuils de détection plus élevés, soit des notes moins vertes et plus fruitées (Hu et al., 2019; Wang et al., 2016). Les notes vertes sont typiques de l'odeur de tomate mais leur transformation en notes fruitées peut apporter des propriétés sensorielles intéressantes au produit fermenté. Lorsque nous avons étudié l'activité ADH des différentes souches dans les cellules non proliférantes, nous avons remarqué que *L. fermentum* présentait des activités considérablement plus élevées que les souches de *L. plantarum* sur la plupart des aldéhydes et cétones présents dans le milieu (Tableaux 3 et 4). Notre test avec des cellules non proliférantes a présenté une légère différence de réduction des cétones par rapport à ce qui s'est produit dans les tomates. Par exemple, la 6-méthyl-5-heptén-2-one (MHO) n'a pas été réduite avec les cellules non proliférantes (Tableau 4) mais son alcool correspondant, le 6-méthyl-5-heptén-2-ol a été trouvé dans LF1A (Tableau 6).

Contrairement à cette large gamme d'activité ADH de *L. fermentum*, les ADH des souches de *L. plantarum* étaient sélectives au substrat. *L. plantarum* C022-3B et V0023-4B2 étaient actives uniquement sur les aldéhydes saturés, tandis que *L. plantarum* C022-2B était active sur les aldéhydes insaturés (Figure 12). Les ADH des souches de *L. plantarum* étaient également



Aldéhydes dérivés de la voie LOX		Leurs alcools correspondants
Hexanal		C-6 Hexanol
Heptanal		C-7 Heptanol
2-Heptenal		C=7 2-Heptenol
Octanal		C-8 Octanol
2-Octenal		C=8 2-Octenol
Nonanal		C-9 Nonanol
2-Nonenal		C=9 2-Nonenol

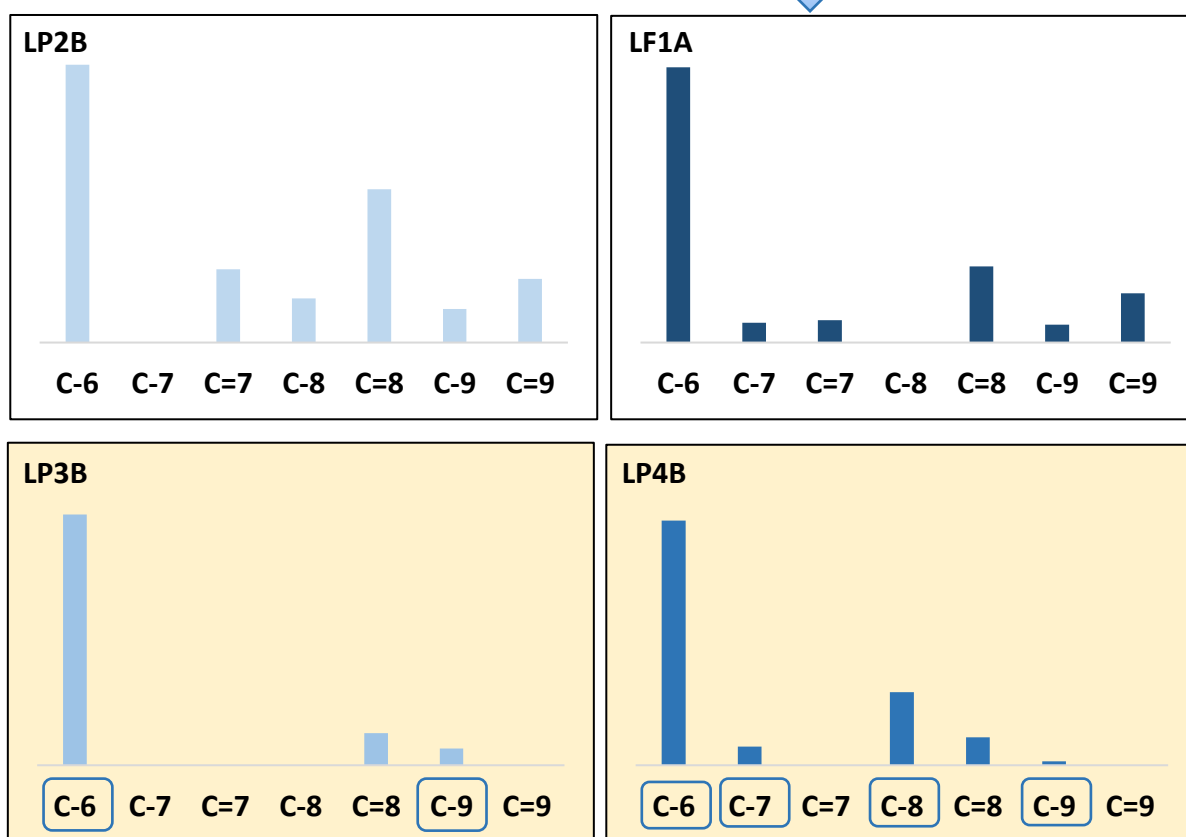


Figure 12. LOX-alcools homologues d'aldéhydes d'acides gras par activité d'alcool déshydrogénase bactérienne (ADH). Abréviations : LP2B, tomates fermentées avec *L. plantarum* C022-2B; LP3B, avec *L. plantarum* C022-3B; LP4B, avec *L. plantarum* V0023-4B2; LF1A, avec *L. fermentum* V013-1A; UT, tomates non traitées; LA, tomates traitées à l'acide lactique; Glu, tomates traitées à la glucosidase; C- et C= sont des alcools saturés et insaturés, respectivement. La lettre C en forme rectangulaire (e.g. **C-6**) représente les alcools correspondants (e.g. C-6 : hexanol) qui ont été réduits d'aldéhydes par l'ADH bactérienne.

actives pour les cétones insaturées (Tableau 6). Les génomes LAB possèdent plusieurs gènes de la famille *adh* qui codent divers domaines fonctionnels. Par exemple, *L. plantarum* WCFS1 possédait neuf gènes *adh* et *L. fermentum* IFO 3956 dix gènes *adh* dont quatre seulement sont des homologues de *L. plantarum* (Analyse bio-informatique à l'aide de la base de données Biocyc) (Karp et al., 2017). Les différences de spécificité de substrat observées entre les activités ADH des deux espèces pourraient être dues à la présence d'enzymes ADH spécifiques. Ce point de caractérisation de la diversité des gènes *adh* en relation avec l'activité sera notre intérêt pour la prochaine étude.

Finalement, outre le potentiel de la souche à libérer des terpénoïdes ou à transformer des aldéhydes et des cétones en alcools, nous avons également trouvé un signe de fermentation hétérolactique dans les produits fermentés. En effet, l'acide acétique a été détecté dans les matrices fermentées mais pas dans UT (Tableau 6). Sans surprise, le milieu contenant la concentration la plus élevée était LF1A. Ceci est cohérent avec le fait que *L. fermentum* V013-1A est obligatoirement hétérofermentaire (Ibrahim, 2016). L'acide acétique et le 2,3-butanediol ont également été identifiés dans LP2B (Tableau 6), montrant que *L. plantarum* C022-2B était probablement hétérofermentaire pendant la fermentation de la tomate (Ibrahim, 2016; Renchinkhand et al., 2015).

Une recherche similaire de Di Cagno et al. (2009b) sur les jus de tomates fermentés avec *L. plantarum* LP54 allochtone a été distingué des souches autochtones, en particulier par des niveaux élevés d'esters, d'alcools et de composés soufrés et de quelques furanes.

En conclusion, l'utilisation des isolats sélectionnés comme starters a démontré que ces souches provoquaient un changement important dans les profils aromatiques des tomates fermentées. Les tomates fermentées avec *L. plantarum* C022-2B, C022-3B et V0023-4B2 étaient riches en cétones tandis que les tomates fermentées avec *L. fermentum* V013-1A étaient abondantes en alcools. Une majorité d'alcools dans les tomates fermentées a été obtenue à partir de l'activité ADH bactérienne. En revanche, les tomates non traitées étaient riches en aldéhydes. Dans l'ensemble, les souches de *L. plantarum* produisant de la  $\beta$ -glucosidase n'ont eu aucun effet notable sur les dérivés de caroténoïdes et sur les composés d'arômes glycosylés. Néanmoins, la fermentation a augmenté de manière significative les composés d'arômes glycosylés tels que le (*Z*)-géraniol, le 6,7-dihydrogéraniol, le melonol, le linalol, le D-limonène, l'alcool phényléthylique et le 4-éthylphénol. Ces composés d'arômes sont très importants pour les tomates fermentées car ils apportent des notes florales et fruitées. L'activité ADH des souches sélectionnées avait un effet significatif sur les rapports aldéhydes/alcools et cétones/alcools. Les trois souches de *L. plantarum* étaient sélectives au substrat. *L. plantarum*

C022-3B et V0023-4B2 pourraient réduire uniquement les aldéhydes saturés (C<sub>6</sub>-C<sub>9</sub>) tandis que *L. plantarum* C022-2B pourrait réduire les aldéhydes saturés et insaturés (C<sub>6</sub>-C<sub>9</sub>) dans les tomates fermentées, mais les souches de *L. plantarum* avaient des capacités similaires pour réduire les cétones insaturées (C<sub>13</sub>). *L. fermentum* V013-1A était capable de réduire une grande variété de composés volatils, d'aldéhydes saturés et insaturés (C<sub>6</sub>-C<sub>10</sub>) et de cétones insaturées (C<sub>8</sub> et C<sub>13</sub>). Parmi les quatre souches, *L. fermentum* V013-1A a montré l'activité ADH la plus élevée dans les cellules non proliférantes ainsi que dans les tomates fermentées. Les tomates fermentées semblent favoriser l'activité ADH de *L. fermentum* V013-1A.

Puisque *L. plantarum* C022-2B, C022-3B et V0023-4B2 étaient capables de produire à la fois des activités ADH et  $\beta$ -glucosidase, ces souches peuvent être des ferments (starters) potentiels pour diminuer le mauvais goût et les anti-nutriments. Les aldéhydes et les alcools sont responsables du mauvais goût, mais les alcools ont généralement un seuil de perception d'odeur plus élevé que les aldéhydes. Les souches de *L. plantarum* avec ADH pourraient réduire les aldéhydes en alcools; par conséquent, ils peuvent potentiellement diminuer les odeurs indésirables. Shi, (2020) a découvert que *L. plantarum* était capable d'éliminer le mauvais goût des isolats de protéines de pois fermentés. Environ 42% d'aldéhydes et 64% de cétones ont été éliminés et une petite quantité d'alcools a été produite. Li et al. (2014) ont montré que *L. plantarum* 70810 diminuait le mauvais goût, en particulier l'hexanal du lait de soja fermenté. De plus, les souches de *L. plantarum* avec  $\beta$ -glucosidase éliminent probablement les composés antinutritionnels qui se présentent fréquemment sous forme de glycosides (par exemple, les  $\alpha$ -galactosides et les pyrimidines glycosides dans la fève) (Rizzello et al., 2017). D'autres avantages de l'activité  $\beta$ -glucosidase par rapport aux aliments lacto-fermentés ont été précédemment décrits dans l'étude bibliographique de la thèse. Par conséquent, la combinaison des activités ADH et  $\beta$ -glucosidase des souches de *L. plantarum* peut avoir de nombreux effets positifs sur la réduction des antinutriments et sur la qualité sensorielle des aliments à base de légumineuses fermentées. Malgré le fait que *L. fermentum* V013-1A ne produisait pas de  $\beta$ -glucosidase, il présentait l'activité ADH la plus élevée. Par conséquent, le mélange de *L. fermentum* V013-1A et *L. plantarum* C022-2B, C022-3B ou V0023-4B2 pourrait éliminer le mauvais goût. De plus, comme ces souches ont considérablement modifié les profils aromatiques des tomates fermentées, il vaut la peine de tester ces souches sur des sous-produits de fruits ou de légumes comme le marc de tomate, le marc de raisin, etc. Cette approche peut revaloriser ces sous-produits ainsi que diminuer la pollution environnementale.

## IV. CONCLUSION ET PERSPECTIVES

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### Conclusion

Premièrement, notre étude a démontré que l'activité  $\beta$ -glucosidase est répandue parmi les LAB issus d'aliments fermentés asiatiques puisque sur les 200 isolats bactériens criblés, 80 étaient positifs pour la  $\beta$ -glucosidase. Cependant, la plupart des bactéries montraient une faible activité. Seules 14 souches ont montré une activité  $\beta$ -glucosidase supérieure à 10 UA et l'activité  $\beta$ -glucosidase la plus élevée était de 27 UA. Parmi eux, treize étaient *L. plantarum* et un était *L. pentosus*. D'après ce résultat, on pourrait émettre l'hypothèse que toutes les souches de *L. plantarum/paraplantarum/pentosus* trouvées dans les aliments fermentés sont positives pour l'activité  $\beta$ -glucosidase. En fait, cela n'est pas évident puisque *L. plantarum* est généralement l'espèce dominante dans les aliments lacto-fermentés. L'identification d'un panel représentatif d'isolats  $\beta$ -glucosidase négatifs devrait être nécessaire pour statuer sur cette hypothèse.

Deuxièmement, nos résultats ont mis en évidence des différences entre les isolats bactériens concernant l'activité ADH. Les cellules non proliférantes de *L. fermentum* V013-1A ont démontré la capacité de réduction la plus élevée pour l'hexanal ( $3,85 \times 10^{-8}$  UA) et (*E*)-2-hexéanal ( $2,47 \times 10^{-8}$  UA), tandis que les cellules non proliférantes de *L. plantarum* C022-2B, C022-3B et V0023-4B2 ont présenté une capacité similaire de réduction de l'ADH pour seulement l'hexanal ( $0,44 \times 10^{-8}$  UA). Les cellules non proliférantes de *L. fermentum* V013-1A ont également été capables de réduire (*E*)-2-heptéanal ( $2,46 \times 10^{-8}$  UA), (*E, E*)-2,4-décadiéanal ( $3,69 \times 10^{-8}$  UA) et géranylacétone ( $1,26 \times 10^{-8}$  UA).

Troisièmement, l'utilisation des isolats sélectionnés comme starters a démontré que ces souches provoquaient un changement important dans les profils aromatiques des tomates fermentées. Les tomates fermentées avec *L. plantarum* C022-2B, C022-3B et V0023-4B2 étaient riches en cétones tandis que les tomates fermentées avec *L. fermentum* V013-1A étaient abondantes en alcools. Une majorité d'alcools dans les tomates fermentées a été obtenue à partir de l'activité ADH bactérienne. En revanche, les tomates non traitées étaient riches en aldéhydes. Dans l'ensemble, les souches de *L. plantarum* produisant de la  $\beta$ -glucosidase n'ont eu aucun effet notable sur les dérivés de caroténoïdes et sur les composés d'arômes glycosylés. Néanmoins, la fermentation a augmenté de manière significative les composés d'arômes glycosylés tels que le (*Z*)-géraniol, le 6,7-dihydrogéraniol, le melonol, le linalol, le D-limonène, l'alcool phényléthylrique et le 4-éthylphénol. Ces composés d'arômes sont très importants pour les tomates fermentées car ils apportent des notes florales et fruitées. L'activité ADH des souches sélectionnées avait un effet significatif sur les rapports aldéhydes/alcools et

cétones/alcools. Les trois souches de *L. plantarum* étaient sélectives au substrat. *L. plantarum* C022-3B et V0023-4B2 pourraient réduire uniquement les aldéhydes saturés (C<sub>6</sub>-C<sub>9</sub>) tandis que *L. plantarum* C022-2B pourrait réduire les aldéhydes saturés et insaturés (C<sub>6</sub>-C<sub>9</sub>) dans les tomates fermentées, mais les souches de *L. plantarum* avaient des capacités similaires pour réduire les cétones insaturées (C<sub>13</sub>). *L. fermentum* V013-1A était capable de réduire une grande variété de composés volatils, d'aldéhydes saturés et insaturés (C<sub>6</sub>-C<sub>10</sub>) et de cétones insaturées (C<sub>8</sub> et C<sub>13</sub>). Parmi les quatre souches, *L. fermentum* V013-1A a montré l'activité ADH la plus élevée dans les cellules non proliférantes ainsi que dans les tomates fermentées. Les tomates fermentées semblent favoriser l'activité ADH de *L. fermentum* V013-1A.

### Perspectives

Ces travaux ont soulevé plusieurs points qui méritent d'être approfondis.

De nombreux composés d'arômes dans les aliments sont produits à l'état de traces, qui sont inférieurs aux seuils de la plupart des instruments d'analyse, mais peuvent être détectés par olfaction humaine (El Hadi et al., 2013). Chaque stater a considérablement modifié les composés d'arômes des tomates fermentées. La plupart des aldéhydes ont disparu, les alcools ont augmenté de manière significative et les terpènes et les cétones ont légèrement augmenté dans les tomates fermentées. Les valeurs de pH des tomates fermentées ont considérablement baissé. Ces changements ont un effet sur l'arôme et le goût des produits finis. Par conséquent, il est important d'effectuer une évaluation sensorielle des tomates fermentées pour confirmer leur acceptabilité et leur application ultérieure.

Les LAB isolées d'aliments fermentés cambodgiens et vietnamiens montrent des activités enzymatiques prometteuses, en particulier les alcool déshydrogénases et les β-glucosidases. L'activité ADH des quatre souches testées sur des tomates fermentées a réduit divers aldéhydes et cétones. Ces souches valent la peine d'être testées avec plus de substrats. Ils peuvent être un catalyseur pour les transformations énantiosélective des cétones en alcools correspondants. Les alcools chiraux sont des produits de grande valeur pour les produits pharmaceutiques et chimiques fins (Hu et al., 2019; Zheng et al., 2017). *L. kefir* DSM 20587 a pu réduire la (2,5)-hexanedione en (2R,5R)-hexanediol (Haberland et al., 2002) et l'acétophénone en R(+)-phényléthanol (Hummel, 1990). L'ADH de *L. brevis* est un catalyseur polyvalent pour la réduction énantiosélective des cétones (Leuchs et Greiner, 2011). *L. fermentum* V013-1A pourrait être la première souche à expérimenter en raison de l'activité ADH plus élevée.

L'activité ADH dépend du cofacteur et des effecteurs dans le milieu (Gargouri et al., 2004; Hu et al., 2019). L'ADH convertit les aldéhydes en alcools lorsqu'il y a du NAD<sup>+</sup>/NADH et les

cétones en alcools lorsqu'il y a du NADP<sup>+</sup>/NADPH. Les effecteurs sont le pH, les ions métalliques (ZnSO<sub>4</sub>, MgCl<sub>2</sub>, CaCl<sub>2</sub>, CuCl<sub>2</sub>, FeCl<sub>2</sub>, MnCl<sub>2</sub>, etc.) et la concentration en ions métalliques. Cette étude s'est concentrée sur des cellules LAB entières. Il serait très intéressant d'explorer le paramètre cinétique ( $K_m$  et  $V_{max}$ ) de l'activité ADH purifiée de ces souches, et les effecteurs dans le milieu, ce qui permet d'étudier la sélectivité des substrats et d'expliquer pourquoi certaines souches transforment les saturés, et d'autres les aldéhydes et cétones insaturés. D'autres paramètres tels que la température de croissance, la stabilité thermique, la stabilité du pH et la spécificité du substrat méritent également d'être étudiés. Neuf ADH purifiés ont été identifiés dans *L. reuteri* DSM20016 (Chen et al., 2016; Hu et al., 2019).

Une quantité importante de composés d'arômes s'accumule dans les fruits et légumes sous forme de glycosides. Les glycosides se trouvent sous diverses formes, principalement les *O*- $\beta$ -D-glucosides, les *O*- $\beta$ -D-diglycosides et les triglycosides (quelques cas). Pour confirmer l'activité  $\beta$ -glucosidase des LAB dans cette étude, un substrat glycosidique (*p*-nitrophényl- $\beta$ -D-glucopyranoside) a été utilisé. En raison de la structure complexe et inconnue des glycosides dans les fruits et légumes, de nombreux chercheurs ont testé des glycosidases sur plusieurs glycosides disponibles dans le commerce avant l'expérience de la matrice végétale. Les glycosides commerciaux courants sont le *p*-nitrophényl (*p*NP)- $\beta$ -D-glucopyranoside, le *p*NP- $\alpha$ -D-glucopyranoside, le *p*NP- $\beta$ -D-xylopyranoside, le *p*NP- $\alpha$ -L-rhamnopyranoside, le *p*NP- $\alpha$ -L-arabinofuranoside et *p*NP- $\alpha$ -L-arabinopyranoside (Grimaldi et al., 2005b; Michlmayr et al., 2010; Pérez-Martín et al., 2012). Les souches de *L. plantarum* produisant des  $\beta$ -glucosidases méritent d'être testées avec divers glycosides dans le cadre de la stratégie de criblage, ce qui peut augmenter la possibilité d'hydrolyse des glycosides dans la matrice végétale.

Certains paramètres tels que le pH, la température, le glucose/fructose et la spécificité du substrat (c'est-à-dire les glycosides) influencent les dosages enzymatiques des  $\beta$ -glucosidases intracellulaires, extracellulaires et liées à la membrane cellulaire. Les inhibiteurs enzymatiques comprennent Hg<sup>2+</sup>, Ag<sup>2+</sup> et Zn<sup>2+</sup>, tandis que les activateurs enzymatiques sont le méthanol, l'éthanol, le propanol et le butanol (Grimaldi et al., 2005a, 2005b; Gueguen et al., 1997; Michlmayr et al., 2010; Pérez-Martín et al., 2010; Pérez-Martín et al., 2012; Sestelo et al., 2004). Le glucose, le fructose, le galactose ou le mannose était susceptible de réprimer les activités  $\beta$ -glucosidases de LAB. Les  $\beta$ -glucosidases ont été induites par le cellobiose, le lactose, la salicine, le ribose, le glucose ou l'arbutine (Michlmayr et Kneifel, 2014). Cependant, chaque souche LAB a ses conditions de croissance optimales et sa propre spécificité pour les activités  $\beta$ -glucosidases. Par conséquent, pour approfondir la compréhension des activités  $\beta$ -glucosidases des souches de LAB, ces paramètres doivent être étudiés.

