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Rhéologie de suspensions hétérogènes concentrées : applications aux bols alimentaires et aux jus gastriques d'aliments solides.

Jeremy Patarin

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THÈSE

Pour obtenir le grade de

DOCTEUR DE L'UNIVERSITÉ DE GRENOBLE

Spécialité : **Mécanique des fluides, Procédés, Energétique**

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Présentée par

Jérémy PATARIN

Thèse dirigée par **Albert MAGNIN**

préparée au sein du **Laboratoire Rhéologie et Procédés**
dans l'École Doctorale **IMEP2**

Rhéologie de suspensions hétérogènes concentrées : applications aux bols alimentaires et aux jus gastriques issus d'aliments solides

Thèse soutenue publiquement le **12 décembre 2014**,
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A ma mère

Avant-Propos

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Introduction générale

Le corps humain est le lieu de multiples écoulements de fluides, majoritairement non-newtoniens. À ce titre, nous pouvons citer les écoulements du sang dans les artères, des fluides lacrymaux à la surface des yeux, du liquide synovial sollicité dans les articulations, mais aussi des aliments dont la nature physique change tout au long du tube digestif : les bols alimentaires en bouche, les jus gastriques dans l'estomac et l'intestin grêle, et enfin les selles dans le gros intestin. Les travaux présentés dans cette thèse s'intéressent particulièrement aux aliments « solides » au cours des processus mécaniques de mastication, de déglutition et de transport jusqu'à la sortie de l'estomac, dont la nature se rapproche d'une suspension d'objets déformables dans une matrice suspendante elle-même non newtonienne. La caractérisation rhéologique de ces suspensions est indispensable pour comprendre leur comportement au cours des différents processus. À ce jour, peu de travaux expérimentaux et de théories sont consacrés aux mesures des propriétés rhéologiques des suspensions de particules dans un fluide à seuil, et encore moins appliqués à l'alimentaire. En effet, le comportement des suspensions est complexe et encore loin d'être totalement modélisé. Cette complexité provient notamment de la diversité des natures des interactions entre particules (colloïdales, hydrodynamiques, frictionnelles, de collision) et des propriétés physiques des particules (forme, flottabilité, déformation, sensibilité à l'agitation thermique) présentes dans la suspension étudiée.

Ainsi, le challenge de cette rhéométrie est triple, en travaillant sur : des suspensions hétérogènes fortement concentrées, entre 30 et 60% v/v ; des volumes faibles de 5 à 100 ml au regard de la granulométrie allant de 1 à 5mm ; des durées de vie *in vivo* et a fortiori *ex vivo* courtes, de l'ordre de la minute. Ainsi primo la rhéométrie ne peut être faite qu'*ex vivo* avec une méthode macroscopique. Secundo, la rhéométrie sur ces suspensions nous amène aux frontières de la mécanique des milieux continus avec des rapports de longueur parfois proche de 3. Tertio, les temps de manipulation courts, inférieurs à la minute, nécessitent des méthodes et de protocoles dédiés. La rhéométrie mise en œuvre doit être une rhéométrie du compromis.

Au-delà de la production de nouveaux savoirs, les enjeux de ces travaux sont sociétaux et industriels. Le surpoids et l'obésité augmentent à un rythme alarmant parmi les citoyens de

l'UE et les conséquences sur la qualité de vie sont importantes (mauvaise santé, exclusion sociale, etc.) Ils sont à l'origine de graves problèmes de santé, comme les maladies cardiovasculaires et le diabète de type 2. Dans un livre vert, la Commission européenne a dressé une liste des origines de cette tendance, avec notamment l'importance des habitudes alimentaires chez l'être humain. Au travers d'une nouvelle politique de santé publique (Plan National Nutritionnel Santé), les recommandations ont pour objet la réduction de la teneur en matière grasse, en sel et en sucres simples des produits.

Dans ce contexte, l'ensemble de l'industrie agroalimentaire doit développer de nouvelles stratégies adaptées à ces recommandations. La modification des formulations et des ingrédients majeurs perturbe notamment la disponibilité et la libération des composés aromatiques. Le risque est de perdre les qualités organoleptiques des produits, indispensables en vue de l'acceptabilité sensorielle des consommateurs, pour qui manger doit rester un plaisir. Afin d'éviter des étapes de reformulation fastidieuses et coûteuses, car souvent empiriques, il est nécessaire de comprendre les mécanismes qui déterminent la qualité sensorielle d'un produit et de proposer ainsi des outils de formulation raisonnée des aliments. Ainsi, **comment la texture des aliments influe la rhéologie et la structure de bols alimentaires produits en bouche, qui a son tour participe à la libération aromatique ?** La réponse à cette question est un des objectifs du programme ANR Sens In Mouth, basé sur l'étude du système gras/eau incorporant une base aromatique : les fromages.

L'industrie peut être aussi amenée à concevoir de nouveaux produits alimentaires liés à des populations spécifiques, notamment diabétiques. Afin de contrer les troubles de la glycémie, une voie possible est la régulation du transit intestinal en tentant de modifier la rhéologie des jus gastriques. Ainsi, **comment les propriétés physiques des aliments influent sur la rhéologie et la structure des jus gastriques, et la rhéologie de jus gastriques a-t-elle un effet sur les cinétiques de vidange gastrique ?** Le programme ANR NOMAC tente d'apporter des réponses à ces questions, avec une étude centrée sur un aliment du quotidien, le pain. Il est constitué majoritairement de farines de céréales, dont la composition en fibres peut varier et influencer la rhéologie des jus gastriques après consommation.

Dans ce large contexte industriel, la contribution de cette thèse est de montrer dans une première partie l'influence de la rhéologie et de la structure des bols alimentaires sur la

libération aromatique en bouche au cours des processus de mastication et de déglutition, et dans une seconde partie le rôle des propriétés rhéologiques des jus gastriques au cours du temps lors du processus de vidange gastrique. Nous avons adopté pour cette thèse l'organisation suivante.

Partie 1 : Rhéologie des fromages, des bols alimentaires associés, et rôle de la rhéologie dans la libération aromatique

Les bols alimentaires sont issus de la mastication d'aliments solides. Ils peuvent être considérés comme des suspensions de particules déformables issues de l'aliment de base, suspendues dans une matrice composée majoritairement de la salive, au comportement non-newtonien (Schipper et al., 2007). Connaître la structure et quantifier les propriétés rhéologiques des bols alimentaires est primordial afin de comprendre les paramètres qui gouvernent la libération aromatique à l'origine de la perception sensorielle. Nous proposons un plan d'analyse en trois points :

- effectuer la rhéométrie des bases fromagères ;
- caractériser la structure et les propriétés rhéologiques des bols alimentaires ;
- analyser l'influence des panelistes sur les propriétés rhéologiques et dégager des corrélations avec les libérations aromatiques.

Ces problématiques ont été traitées dans le cadre du programme ANR Sens In Mouth, incluant le Laboratoire Rhéologie et Procédés, les laboratoires de l'INRA CSGA FLAVI, GMPA, le laboratoire DIDO de l'Université de Clermont-Ferrand et deux industriels : les Fromageries Bel et la SOREDAB.

Chapitre 1.1

La connaissance de la rhéologie de l'aliment de base est indispensable pour mener une analyse cohérente sur les bols alimentaires générés à partir de celui-ci. Un premier article est donc dédié à la rhéométrie de fromages ayant différentes textures, contrôlées par le ratio gras/maigre et la teneur en eau. Deux techniques de rhéométrie sont utilisées et les résultats respectifs sont comparés afin de se soustraire de différents artefacts de mesures : les effets de la force normale sur les propriétés en cisaillement et le glissement aux interfaces. Les propriétés rhéologiques sont enfin corrélées avec des données sensorielles issues d'un panel de testeurs entraînés au protocole d'analyse de texture.

Chapitre 1.2

La nature de suspensions hétérogènes concentrées, les faibles volumes et la courte durée de vie des bols alimentaires ont conduit au développement d'une méthode basée sur la compression. Cette méthode permet en moins d'une minute de caractériser en volume les bols alimentaires à différents taux de cisaillement, celui-ci augmentant à mesure que l'entrefer décroît à une vitesse constante. La méthodologie a nécessité le développement d'un instrument de mesure au sein du Laboratoire Rhéologie et Procédés, incluant une interface de contrôle et un code de calcul pour le traitement. Les propriétés rhéologiques de volume et des longueurs caractéristiques sont extraites, et sont corrélées à des propriétés analogues données par des tests connexes afin de valider les résultats issus de la première méthode d'extraction. De plus la méthode est validée sur des suspensions modèles dans des conditions équivalentes à celles des bols alimentaires réels. L'appareil a été installé au sein du laboratoire CSGA FLAVI, et une équipe a été formée pour travailler sur l'instrument. L'équipe spécialisée a ensuite réalisé l'étude clinique sur l'ensemble des panelistes et pour six fromages types. Les données ont été ensuite analysées au sein du Laboratoire Rhéologie et Procédés. Les résultats issus du panel démontrent la forte influence des individus sur les propriétés rhéologiques, avec une variabilité intra-individuelle raisonnable. Une modélisation sous forme de suspensions des bols est proposée afin d'extraire les propriétés rhéologiques de la matrice suspendante.

Chapitre 1.3

Le dernier chapitre de cette première partie met en évidence un lien entre les propriétés rhéologiques du produit initial, celles du bol alimentaire, et la libération aromatique. Deux étapes physiologiques clefs expliquent la libération des stimuli. Lors de la phase buccale, la déstructuration de l'aliment par la mastication gouverne la libération des stimuli. Lors de la phase pharyngée, la biomécanique de la déglutition régit l'enduction des muqueuses pharyngées par le produit et ainsi la libération des composés d'arôme présents dans cette couche résiduelle.

La compréhension des mécanismes gouvernant la libération des stimuli orosensoriels permettrait leur modélisation. À partir de la composition et des propriétés physiques du produit, ainsi que de paramètres physiologiques de l'individu, il serait ainsi possible de

simuler les phénomènes qui vont gouverner la libération de stimuli en bouche. L'influence des modifications de propriétés du produit ou de « consommation » de l'aliment sur les dynamiques de libération pourrait être quantifiée. Ainsi, une démarche de conception des produits selon des critères prédéfinis serait envisageable, notamment basée sur des modèles comportementaux.

Partie 2 : Rhéologie des jus gastriques

Le pain, et plus particulièrement la baguette française possède un index glycémique élevé. Il est donc susceptible de participer à la création d'une insulino-résistance du fait de l'excursion insulino-glycémique postprandiale importante (Kaplan et al., 2001). Une des options pour réduire cette excursion glycémique est de manipuler la vidange de l'estomac (Jebb, 2005). En effet, la vidange gastrique module la libération des nutriments au niveau de l'intestin grêle. C'est pourquoi l'index glycémique des nutriments contenant des glucides est d'abord fonction de la forme de la vidange de l'estomac (Edelbroek et al., 1993; Hebbard et al., 1996, 1995; Horowitz et al., 1993). De plus, l'index glycémique peut être manipulé par différentes stratégies qui tendent à inhiber la vidange de l'estomac. C'est pourquoi une des solutions pour réduire l'excursion glycémique postprandiale consiste à augmenter la viscosité du chyme gastrique par la modification des taux de fibres alimentaires (Guerin et al., 2001), effet vérifié pour des aliments fluides. Dans ce but, l'ANR Nomac visait à sélectionner différentes variétés de blés afin de créer des farines avec des ratios amylose/amylopectine différents. Devant les difficultés rencontrées par les partenaires pour obtenir une gamme de ratios significativement différents, le choix a été fait de modifier ce ratio par adjonction d'amylose ou d'amylopectine à une farine standard.

L'objectif de l'étude porté au sein du Laboratoire Rhéologie et Procédés est d'évaluer si l'adjonction d'amylose à la farine servant à la panification est à elle seule suffisante pour modifier les caractéristiques de gélification dans l'estomac et donc la vitesse d'évacuation du repas par l'estomac. Avec des farines aux taux de fibres très différents, des pains sont créés afin de mener en parallèle une campagne de rhéométrie sur les jus gastriques et de visualisation de la vidange gastrique. Le modèle porcin a été choisi pour sa similitude avec l'humain, et les porcs ont été adaptés aux études par le laboratoire ADNC Senah. L'objectif final est, au travers de cette modification de l'évacuation de l'estomac, de réduire l'excursion

glycémique et donc l'index glycémique du pain. Nous proposons donc un plan d'analyse en deux points :

- caractériser la structure et les propriétés rhéologiques des jus gastriques pour différents taux de fibres et pour différents temps de digestion ;
- analyser l'effet des propriétés rhéologiques sur les cinétiques de vidange gastrique.

Les études ont été menées dans le cadre du programme ANR NOMAC, incluant le Laboratoire Rhéologie et Procédés, les Laboratoire de l'INRA BIA, ADNC Senah, IATE, GDEC, le CIC du CHU de Rennes et un industriel, LIMAGRAIN.

Chapitre 2.1

L'objectif de ce premier chapitre est de mettre en place une méthodologie de mesure adéquate pour caractériser les jus gastriques issus de porcs, choisis comme représentatifs du modèle humain. Les porcs ont été au préalable opérés par l'équipe du laboratoire ADNC Senah afin de leur implanter une canule gastrique, indispensable pour effectuer les prélèvements *in vivo*. L'instrumentation supportant ce protocole a été réalisée au sein du Laboratoire Rhéologie et Procédés, de manière à rendre la mesure la plus ergonomique possible et à enregistrer automatiquement le maximum d'informations (vitesse de cisaillement, couple, vidéo des champs de déformation, photographies des jus gastriques). Les personnels en charge du soin des porcs ont été formés à l'utilisation de l'instrument afin que les campagnes de mesures soient menées. Après ingestion de pain par les porcs, les jus ont été récoltés au travers de la canule gastrique à différents temps de digestion. Les jus ont été testés par scissométrie, et les résultats ont été analysés au sein du Laboratoire Rhéologie et Procédés. Les premiers enjeux ont été de corrélérer la rhéologie du fluide avec le temps de vidange gastrique, et de dégager des hypothèses sur la structure des jus durant leur séjour gastrique. Avec le temps, les jus perdent en consistance pour passer d'une nature de fluide viscoplastique à un fluide presque newtonien, quel que soit le taux de fibres contenu dans les repas d'épreuve. Le second point est, au regard de la structure, de suivre l'évolution de la viscosité à temps de séjour et à taux de cisaillement constants en fonction du taux de fibres. Le rapport de concentration en amylopectine sur l'amylose a un effet significatif pour un temps de digestion long (120 min). Aux temps courts (30 et 60 minutes), l'influence du taux de fibres est noyée dans les variabilités de structures gouvernées par l'activité masticatoire en amont.

Chapitre 2.2

En parallèle des essais de rhéométrie, le suivi des cinétiques de vidanges sur un autre groupe de porcs modèles a été réalisé au sein du laboratoire ADNC Senah. L'enjeu est de connaître l'effet de la rhéologie sur la vidange gastrique. Le chapitre précédent montre un effet du taux de fibres sur la rhéologie des jus gastriques, notamment au temps long. Cependant, le même taux de fibres ne semble pas avoir d'effets significatifs sur les cinétiques de vidanges du panel de porcs. Ces résultats démontrent qu'avec des propriétés rhéologiques variées, les cinétiques de vidanges ne varient pas de manière significative. Les repas d'épreuve ayant pour point commun d'être isocaloriques, la charge énergétique pourrait donc être le facteur déterminant pour le contrôle de la vidange gastrique et donc de l'excursion glycémique.

Une conclusion générale se trouve à la fin de cette thèse. Elle synthétise les différents résultats et met en relief l'utilité des méthodes présentées. Elle propose des recommandations et des perspectives vis-à-vis de travaux futurs.

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PARTIE I :

Rhéologie des fromages, des bols alimentaires associés, et rôle de la rhéologie dans la libération aromatique

Chapitre 1.1

La rhéométrie des fromages

Avant d'étudier les bols alimentaires, il est impératif de caractériser les produits solides qui en sont à l'origine, dans le cas présent des fromages. Les propriétés rhéologiques mesurées peuvent être reliées à la fois à la perception sensorielle, et aux mécanismes de fragmentation au cours de la mastication. Ce premier article présente une méthodologie fiable afin d'effectuer la rhéométrie de fromage au travers de la technique dit de « scissomètre », notamment sous oscillations. Il compare les résultats de cette technique à ceux de la rhéométrie plan-plan, soit deux techniques en cisaillement, qui diffèrent ainsi de la compression. Les avantages de la scissométrie dans le contexte sont notamment une facilité de mise en place, une dessiccation moindre, et un affranchissement des problèmes de glissement aux interfaces et d'efforts normaux. Deux types de sollicitations sont appliqués : des grandes déformations allant jusqu'à la fracturation du produit, et des oscillations à déformations imposées pour caractériser les propriétés viscoélastiques. L'étude met en perspective les problèmes d'interface qui peuvent être rencontrés avec des essais en rhéométrie plan-plan, ainsi que les conséquences des efforts normaux sur les mesures en cisaillement sur les fromages. Les propriétés rhéologiques mesurées des produits sont :

- le seuil d'écoulement ou de rupture
- la déformation critique à la rupture ou à la mise en écoulement
- le module élastique et la déformation qui correspond à la sortie du domaine élastique.

De plus, des visualisations du champ de déformation permettront de montrer les modes de rupture des différentes bases de fromage et de valider les essais. La partie discussion de l'article à venir traite des avantages et des inconvénients des deux méthodes aux vues des résultats obtenus. Les relations entre rhéologie et propriétés sensorielles (texture) sont aussi mises en avant dans les résultats de ce premier chapitre.

Vane and plate-plate rheometry of cheeses under oscillations and large strains: a comparative study and experimental conditions analysis

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Abstract:

The rheological behaviour of cheeses of different textures was analysed using two techniques: vane tests and plate-plate rheometry. The yield or failure stress and failure strain were measured by means of large-strain shear tests (LSS). The elastic and viscous moduli and the linear strain region (LVR) were measured by means of small amplitude oscillation shear tests (SAOS). This study underlines the importance of the experimental conditions to guarantee the quality of the results and, in particular, controlling the interface adherence conditions and loading effects in the case of plate-plate rheometry. Comparison of these two techniques shows that vane tests are more efficient, especially with respect to the set-up, and provide greater control of the measurements. The rheological properties measured by the tests are correlated with data on the composition of the cheeses and the results of sensory analysis.

1. Introduction

The knowledge of rheological properties of cheese may be useful to control quality and to understand their behaviour during consumption. A recurrent question is the right choice of measurement technique, considering the nature of the cheese. This choice depends upon the applications context and the control of environmental conditions. Various types of rheometric tests may be applied to cheeses: compression, uniaxial elongation, cutting tests, plate-plate rheometry, vane tests, etc. The review produced by Gunasekaran and Ak (2003) describes these various types of tests applied to the rheology of cheeses. Of these, plate-plate rheometry, vane tests and compression are very commonly used to determine rheological characteristics under shear conditions. This article focuses on plate-plate rheometry and vane tests. These two techniques produce a comparable rheological characterisation. Two types of stimulation are applied with each technique: large-strain shear tests (LSS) and small amplitude oscillation shear tests (SAOS). Each type of stimulation provides linear and non-linear properties of the material under shear strains.

LSS plate-plate rheometry is mainly used to link rheological properties to sensory characteristics (Foegeding & Drake, 2007). SAOS plate-plate rheometry is used on different types of cheeses in particular to characterise their structure (Everett & Auty, 2008; Graiver, Zaritzky, & Califano, 2006; Ma, Drake, Barbosa-Canovas, & Swanson, 1996; Messens, Van de Walle, Arevalo, Dewettinck, & Huyghebaert, 2000; Muliawan & Hatzikiriakos, 2007; Subramanian & Gunasekaran, 1997; Tunick et al., 1990; Venugopal & Muthukumarappan, 2003).

Vane testing was initially used to determine the yield point or failure stress of various materials (Baravian, Lalante, & Parker, 2002; Barnes & Nguyen, 2001; Keentok, Milthorpe, & O'Donovan, 1985; Sherwood & Meeten, 1991; Sherwood, 2008). The work of Martin et al. (2005) involved LSS vane tests applied to different foodstuffs. But no study of cheeses has used SAOS vane tests. Yet the dynamic stimulations created by this technique can also be used to study the viscoelastic properties of the materials. One of the first articles to study elasticity below the yield point using small strain vane tests is that of Alderman et al. (1991) concerning clay gels, but oscillations aren't used. Zhang et al. (1998) used oscillations with a vane geometry to determine the viscoelastic properties of foams. In the food industry, the

studies carried out by Junus and Briggs (2001) and Guggisberg et al. (2009) used SOAS vane test to characterise yoghurts.

This study investigates the experimental conditions of plate-plate rheometry and vane tests, and compares the results produced by these two techniques with SAOS and LSS tests. This work is based on a study of four model cheeses with different textures.

The first aim was to show the effect of the experimental conditions on the results, such as adherence to the measuring interfaces and normal loading. The strain fields were visualised for this purpose. The second aim was to identify the relative advantages of the two techniques, and describe limitations to help potential users choose one method over another. Examples of correlations data were provided between the rheological properties, the composition of the cheeses and sensory analysis, to illustrate the use of proper rheological measurements.

2. Material and methods

2.1. Materials

Four cheddar-based melted cheeses were developed by Fromagerie Bel (Paris, France) and Soredab (La Boissière-Ecole, France). The cheeses were created especially for the study and do not include any texturing agent. Cheeses were produced using different fat levels, water content and processes to obtain four products that differed in composition and texture (Table 1). The ratios of fat to dry matter in these cheeses varied, from 28 for the low-fat (l) cheeses to 50 for the high-fat (h) cheeses. Within each ratio of fat to dry matter, variations in water content led to two levels of firmness (F = firm, S = soft). Three replicates of each type of cheese were manufactured to be tested 15 days after production. The products were stored in their wrapped trays in a refrigerator at 4°C. The day before the measurements, the products still in their trays were placed in a room at a temperature of 20°C.

Plastic rings were placed in some of the trays when the cheeses were being moulded within. In this way the vane tests could be carried out with a known gap, enabling the strain applied to the sample to be calculated. The calculations are explained in the following section.

2.2. Methods

The following rheological properties were measured by vane tests and plate-plate rheometry. Firstly, the yield or failure stress and the associated critical failure strain were measured by means of large-strain (LSS) tests. Secondly, the elastic and viscous moduli, phase shift and linear strain region (LVR) strain were measured by means of small amplitude oscillation shear (SAOS) tests. The strain field was visualised to monitor the strain and failure modes of the different cheese bases. Visualisation was important in validating the tests.

2.2.1. Plate-plate rheometry

The tests were carried out using an Ares controlled-strain rheometer (TA Instruments, New Castle, DE, USA) with plate geometries 25 mm in diameter. Immediately before the experiment, a 3 mm slice was cut off with a wire from the unwrapped tray of the tested cheese. After the sampling, the tray was re-wrapped to prevent the cheese from drying out. A cylindrical sample was then cut with a die cutter from the slice of cheese. Three samples per tray were tested.

To analyse the effect of surface conditions between the geometries and the cheeses, three different types of interfaces were used: smooth aluminium surfaces with a roughness less than 1 μm ; two rough surfaces (sand paper) with respective roughness values of 260 μm and 130 μm ; and surfaces glued with cyanoacrylate glue and brought into contact with the sample during the installation phase. Roughness of the plates and the sand papers was used as stated by the manufacturers, and also measured using a profilometer (Mitutoyo, Kawasaki, Japan).

In the case where glue was not used, each cylindrical sample was centered and then squeezed at a constant normal load (N) of 10g, aiming to ensure adhesion by static friction. When the samples were stuck to the surface of the plates, the lower plate was first coated with glue and the sample was placed in the centre. The upper plate, previously coated with glue, was then brought into contact with the sample under a normal load of 50 g. The glue polymerised in a few seconds and the load placed on the sample was then set to any chosen value. A load of 10 g was used for comparison tests with other types of surface. A load 0 g

was used for comparison tests with the vane test. Other specific tests used various values of N to investigate their effects on rheological properties using plate-plate rheometry.

The temperature during the tests was regulated ($\pm 1^\circ\text{C}$) by the Peltier effect. The cutting operation and the sample setting took less than 5 minutes. Rheometric tests took a maximum of 3 minutes to be carried out to avoid drying.

The rheometer gives a stress measurement for an imposed angular displacement. It is possible to obtain the imposed strain (γ) and the stress (τ) measured on the outer diameter of the measuring plate (Macosko, 1994).

$$\gamma = \theta R/h \quad (1)$$

$$\tau = 2M / (\pi R^3) \quad (2)$$

where R is the radius of the geometry, h is the gap, θ is the angular displacement and M is the torque.

In the large strain tests, the sample was subjected to a low speed rotation of 0.01 rad.s^{-1} until failure occurred. The maximum stress (τ_{max}) reached during the test was noted. The maximum stress corresponds to the failure stress when the cheese does not slip at the interfaces. With no slip, the critical failure strain (γ_c) was associated with the failure stress. An example curve is shown in Fig.1. The plate-plate rheometry test is illustrated by photographs a) to f) on Fig.2. Rupture was seen on photograph d).

In the case of the oscillation tests, the rheometer measured the change in the material's stress response as a function of the strain imposed, as well as the phase shift between the two signals. The complex modulus (G^*) was thus defined. The two components of G^* are the elastic modulus (G') and the viscous modulus (G''). G' and G'' are a function of the amplitude of the imposed strain (γ_0), the amplitude of the measured stress (τ_0) and the phase shift between the strain and the stress (δ). The value of $\tan \delta$ corresponds to the ratio of G'' to G' , and was used to evaluate viscous effects in relation to elastic effects.

$$G^* = G' + iG'' \quad (3)$$

$$G' = \tau_0 / \gamma_0 \cdot \cos \delta \quad (4)$$

$$G'' = \tau_0 / \gamma_0 \cdot \sin \delta \quad (5)$$

The samples were subjected to successive increments of strain from 0.001% to 5% at a frequency of 1Hz. The frequency chosen corresponds to the order of magnitude of the characteristic times of the stresses that the cheeses undergo during chewing. The curves typically displayed a plateau at small strains, the linear viscoelastic region (LVR), and then quickly decreased. The respective values of the plateaus of G' and G'' characterise the LVR of the cheeses. The LVR limit strain (γ_{lin}) marks the end of the LVR, where G' and G'' begin to decrease. Example curves are given in Fig.3 for cheese Fl.

2.2.2. Vane tests

The vane tests were carried out with an ARG2 controlled-stress rheometer (TA Instruments), using a four-blade geometry 10 mm in diameter, with a blade thickness of 1 mm. The edges of the vane were sharpened to avoid any fracture during penetration. The vane was inserted to a depth of 10 mm at a speed of 0.5 mm.s^{-1} .

A four-blade vane was chosen for this study to avoid creating cracks and damaging the product when it was being inserted. The number of blades is a compromise between the accuracy of measurement and the firmness of the product being analysed (Zhang et al., 1998).

In the LSS tests, the sample was subjected to a low speed rotation of 0.01 rad.s^{-1} until failure occurred. The measured torque (M) gives a direct indication of the shear stress, τ (Daubert & Foegeding, 2010; Macosko, 1994; Truong & Daubert, 2001; Yoshimura, Prud'homme, Princen, & Kiss, 1987), where the vane is partly inserted.

$$\tau = M / (\pi D^3 / 2 \cdot (H/D + 1/6)) \quad (6)$$

where M is the torque, H is the depth to which the vane was inserted and D is the vane diameter.

Fig.4 illustrates different the stages during a LSS vane test. From a) to c) the sample underwent elastic strain; failure occurred at d) and continued to f).

Measuring strain under wide gap conditions, with a ratio of inner radius to outer radius less than 0.5, is not trivial. The methods of Truong et al. (2002) based on observations by Keentok et al. (1985) were applied in this study to obtain an order of magnitude of the strain. Those methods involved the use of an approximate size for the deformed area of fluid around

the vane, of diameter (D). The diameter of this area (D_f) was measured by placing several marks on the surface of the cheese (Fig.4). It was then possible to determine the strain using the equation:

$$\gamma = \theta / (D_f/D - 1) \quad (7)$$

The values obtained for the ratio D_f/D were 2.1 for cheese Fh, 1.8 for Sh, 2.9 for Fl and 2.7 for Sl. The ratio is therefore not always 2, which is the value proposed by Keentok et al. (1985) for a gelatinous gel. Measuring the diameter of the sheared area of each cheese is mandatory to obtain an accurate value. Truong's approximation (2002), which assumes that the ratio is close to 2 for all cheeses, may provide relatively good results.

For the purpose of this study, the use of a large gap that enables a Couette flow analogy was preferred to calculate the strain γ :

$$\gamma = 2\theta / (1 - (R_i/R_e)^2) \quad (8)$$

where γ is the angular strain in radians, R_i is the radius of the vane and R_e is the radius of the ring.

This method required that the cheeses were formed with rings inside the trays. The rings chosen had an inner radius of 15 mm, thus the cheese disk inside each ring had an outer diameter $R_e = 15$ mm. A ring with cheese inside was removed from the trays and fixed to the rheometer. The cheese sample stayed stuck to the inner surface of the ring during the experiment. The change in shear stress versus strain was plotted using the previous calculations (Fig.1).

In the SOAS tests, the samples were subjected to successive strain increments from 0.001% to 5% at a frequency of 1Hz. The plateau values of G' and G'' were thus deduced, as well as the strain at the end of the linear domain (γ_{lin}). An example of a curve is displayed on Fig.3 for cheese Fl.

2.2.3. Sensory analysis

A panel of experts (12 people) was formed and trained in the sensory evaluation of different types of cheese. This panel worked for 8 months exclusively for the study. The vocabulary used was defined on the basis of the first formulation tests. For this paper we

chose to focus on three attributes. Firstly, “Firm” is considered to be the resistance of the product to compression between the tongue and palate. Firm should be understood as opposed to soft (water is taken as an extreme example). Secondly, “Elastic” is considered to be the ability of a product to recover its shape and dimensions after compression (the gelatine sweets are taken as an extreme example). Thirdly, “Pasty” is considered to be the ability of the product to occupy the volume of the mouth with saliva intake, and therefore not easily liquefiable.

The panel members were trained to score on a 0 to 10 linear scale with no graduations. The products were cut with a die cutter and tasted at a temperature of $17\pm 1^\circ\text{C}$. The products were anonymous and presented in monadic sequence using a Latin square. No more than six products were presented in any session. The results were processed using Duncan’s test to highlight differences between the cheeses. The various letters illustrate significant differences at a 5% threshold.

3. Results

3.1. Influence of experimental conditions for plate-plate rheometry tests

3.1.1. Interface adherence condition

The first aim was to analyse the effect of surface conditions between the measuring plates and the cheeses. Three different types of interface were used: smooth aluminium surfaces; two rough surfaces (sand paper) with respective roughness values of $260\ \mu\text{m}$ and $130\ \mu\text{m}$; and surfaces glued with cyanoacrylate glue. Series of LSS plate-plate rheometry tests were carried out by varying the surface condition. The samples were subjected to a shear rate of $0.01\ \text{rad}\cdot\text{s}^{-1}$.

Before carrying out the test, carbon black powder was used as a marker, making a line across the upper plate, the cheese and lower plate. The distortion of these marks was filmed with a video camera to check whether the strain field was homogeneous (Fig.2). The sequence of images from a) to f) shows a test in which cheese F1 failed without any slip at the wall, where the surfaces were glued. From images a) to c), the sample can be seen to be deformed linearly. The mark remains straight and there is no shift at the interface between the cheese and the plate. The sample breaks in image c) and the failure continues as far as image f).

Photographs g) to i) show an example of slip at the interface with the upper plate, where the surface is rough (130 μm). A shift appears between the free surface of the cheese and the upper plate in image h). The strain in the cheese no longer changes in image i).

Fig.5 shows a typical response for cheese F1, which was subjected to increments in shear rate with glued, rough and smooth surfaces. The stress response to small strain up to 0.3 is identical. Beyond this strain, the adherence condition only occurs for the glued surfaces. The rough surfaces do not guarantee adherence. Indeed, surface roughness damages the sample surface during initial loading, and creates micro-cracks at the interface. Loading is nevertheless necessary to maintain adherence through friction. During the test, the micro-cracks spread and a layer of debris formed at the interface. This layer lubricated the contact and led to premature slip, even in comparison with the smooth surface sample.

These observations can be seen in the photographs of Fig.2. Images a) to f) show the experiment for cheese F1 glued to the measuring plates. The sample appeared to break in image c) and the failure continued up to image f). The images from g) to i) show the experiment with a rough surface (260 μm). Slip occurred at h) and continued. The mark on the upper plate no longer corresponded with the mark on the cheese. The peak stress observed did not correspond to failure stress, but to the stress at which slip at the surface begins. This stress is not characteristic of the bulk properties of the cheese.

Although it is common to use smooth and rough surfaces in rheometry, it is rarer to use glue, as this is more restrictive. Only Truong and Daubert (2001) and Tunick et al. (1990) discuss tests with samples glued to ensure contact up to failure under large strain. Our observations confirm the importance of setting up a system for visualising the strain field to check the domain of validity of plate-plate rheometry experiments.

3.1.2. Influence of normal force N

SAOS tests were carried out with glued surfaces, where variations of N were applied at a constant frequency and strain. The viscoelastic properties of the cheese can thus be measured as a function of N. The results for cheese F1 show that any increase in N leads to a proportional increase in G' and G'' , as shown on Fig.6. Similarly, if tension is applied to the samples, the moduli decrease until the sample becomes detached. A 1500g variation in loading produces an increase of more than 10% in the values of G' and G'' . Loading is

necessary when rough or smooth surfaces are used to guarantee adherence through friction. Uncontrolled loading leads to incorrect results for a given cheese. Furthermore, the effect of loading on the results depends upon the cheese. A no-load condition during a test may be important to reduce the uncertainties involved. In the rest of this study, the values of the elastic and viscous moduli and the failure stresses and strains correspond to a zero load following gluing. A possible explanation of the effects of N on the viscoelastic properties of the cheese is given in the discussion section.

3.2. Rheometry, formulation and sensory analysis: correlations.

3.2.1. Accuracy and standard deviations

An acceptable level of reproducibility is observed for each class of rheometric test. This is illustrated by the standard deviation expressed as a percentage in Table 2. Some of these variations may be imputed to variations in temperature, which have a significant influence on the mechanical properties of these fat-based products. The temperature is regulated in the area immediately around the sample by a Peltier effect. The potential variation is therefore kept to 1°C from ambient room temperature at 20°C.

Some of the variations are also due to the sampling process. The cheese is relatively non-homogeneous, which means that there are local variations in its mechanical properties. As vane testing uses a larger quantity of the product, it averages out the effect of these irregularities to a greater extent. The variance values are indeed higher in plate-plate rheometry than in vane tests (Table 2).

3.2.2. Comparison between vane tests and plate-plate rheometry

The results obtained from the rheometry tests and the sensory analysis are compiled in Table 2. The LSS and SAOS tests were treated separately to explore the differences between the two measurement techniques.

In the case of the SAOS tests, there is a good correlation between the phase shifts (δ) resulting from both measurement techniques. The deviation is 5%, which is of the order of magnitude of the measurement errors. The correlation between the LVR strains (γ_{lin}) depends upon the type of cheese: the deviation for high fat cheeses is 10%, comparable to the uncertainties; the deviation is 15% for low fat cheeses, also comparable to the uncertainties.

The deviations for the elastic modulus (G') and viscous modulus (G'') vary from one cheese to another, 6% for Fh, 14% for Sh, 14% for Fl and 21% for Sl. The deviations for low fat cheeses are slightly greater than the cumulative uncertainties of the two techniques. The higher values from the plate-plate rheometry measurements compared to the vane tests are probably due to the initial assumptions concerning calculations of strains, explained in the next paragraph.

For LSS tests, the failure stress values (τ_{\max}) obtained with plate-plate rheometry and vane tests had the same order of magnitude, with the deviation between 5% and 10%. Variable deviations for τ_{\max} values have been reported (Junus & Briggs, 2001; Truong & Daubert, 2001). The failure strain values (γ_c) are to be examined with care. Theoretically speaking, LSS plate-plate rheometry and LSS vane tests with a large gap require correcting the strain calculations. This correction depends upon the rheological behaviour of the product. Indeed, strain is not uniform throughout the sample. The corrections described in the work by Macosko (1994) must be applied on the basis of flow tests under steady conditions, but it is not possible to obtain such flows in the case of the cheeses studied here and so this correction cannot be applied. However, we ensured that strain was applied through the gap when using both techniques by choosing appropriate dimensions. Still, the tests carried out with both techniques produced comparable results for strain.

3.2.3. Correlations between rheometry, sensory analysis and cheese composition

τ_{\max} follow the cheese firmness scale, regardless of the fat content. γ_c values are higher in the case of low fat cheeses compared to high fat cheeses. The link between δ and γ_c ($R^2=0.87$) characterises a cheese's ductility. Those observations confirm the ductile nature of low fat cheeses and the brittle nature of high fat cheeses.

A panel of experts provided a sensory analysis using descriptors for the various products. This analysis can provide preliminary support for the formulation work and in particular enabled a significantly different range of firmness to be obtained, as confirmed by the Duncan test. From these different descriptors, the aspects of firmness, elasticity and pastiness were examined. The results of these descriptors and the Duncan test are presented in Table 2. The sensory data at 17°C are comparable to the rheological data measured at 20°C.

Correlations between different parameters derived from the composition of the cheeses, the vane tests and the sensory analysis were calculated. There is a strong link ($R^2=0.995$) between the δ and the fat content of the cheeses. This dependence of δ is emphasised in the literature (Everett & Auty, 2008; Gunasekaran & Ak, 2003; Messens et al., 2000). This result is independent of the firmness scale. The greater the $\tan \delta$ value, the more important the viscous response is in comparison to the elastic response. Furthermore, δ may be linked ($R^2=0.97$) to pastiness, identified in the sensory analysis. The fat content may be the dominant factor causing this sensation, and thus can be characterised rheologically by δ values.

There is a weak correlation ($R^2=0.72$) between the firmness identified in the sensory analysis and the dry matter of the products. The quantity of water in cheese has an effect on the firmness, but doesn't seem to be the only causative parameter. No rheological parameters matched with the descriptor of firmness.

The failure stress can be related to the dry matter ($R^2=0.80$), the fat content ($R^2=0.87$) and the moisture in non-fat substance ($R^2=0.86$). In addition, G' is correlated with the dry matter ($R^2=0.78$), the fat content ($R^2=0.87$) and the moisture in non-fat substance ($R^2=0.85$). These correlations confirm the effects of different formulations on the mechanical properties of the cheese.

There is weak link between the “elasticity” of the cheese identified in the sensory analysis and G' ($R^2=0.71$). In contrast, there is a stronger correlation between this descriptor and γ_{lin} ($R^2=0.86$), γ_c ($R^2=0.88$) and δ ($R^2=0.89$). This observation may be explained by the definition of “elasticity”, which is the ability of the product to recover its shape after the application of stress. Moreover, a strong link between “elasticity” and the moisture in non-fat substance should be noted ($R^2=0.93$).

4. Discussion

The test most commonly used in the food industry for analysing texture is compression, in particular, the texture profile analysis (TPA). However, it has its limits. For example, the interface conditions of perfect slip or perfect adherence are sometimes difficult to obtain or control, which leads to uncertain results. Oscillations are rarely used during compression, as the product's rheological parameters cannot precisely be determined in

dynamic tests (Mert & Campanella, 2008). Rotational rheometry is easier to use in this respect.

Oscillatory rotational rheometry has been used in many studies, as mentioned in the introduction. Problems previously highlighted show the complexity of making and installing samples in the case of plate-plate rheometry. Sample loading is an important aspect that is rarely described in the literature. Correct and appropriate normal load (N) is required as it guarantees adherence according to Coulomb friction (the tangential friction force is proportional to the loading). As the load is increased, the friction increases and slip is repelled. Most oscillatory studies use rough or scored surfaces to prevent slip, however, this is liable to cause damage near the surfaces. In addition, loading has a direct effect on the properties of the cheeses. Indeed, studies at the microscopic and mesoscopic levels (Gunasekaran & Ak, 2003) have shown that cheeses are not really homogeneous. In reality, they consist of aggregates with several length scales, ranging from a hundred microns to several centimetres. These aggregates, which are formed by a porous protein network, interact mechanically with one another. During a shear test under a given load, more elements are brought into contact and interact. This increases the values of G' and G'' in proportion to N (Fig.6). Similarly, if tension is applied to the sample, the moduli decrease as there is less contact between the elements. The work of Zhang et al. (1998) also studies the dependence of G' and G'' in relation to normal loading on polyurethane foams. In this study, the values of G' and G'' reach a plateau above a certain loading. This plateau represents the state where all the material is fully stimulated (all micro-structures are bonded). In the case of cheese, the sample is damaged before the plateau is reached. This is why we chose to measure the values of G' and G'' with no loading, and comparing these values with the values measured in the vane tests where the sample is not subjected to any normal forces. All of these observations show that it is less restricting to use LSS or SAOS vane tests for cheeses than plate-plate rheometry, for equivalent results.

5. Conclusions

This study provides essential methodological details for carrying out properly controlled tests, especially in the context of oscillating rheometry. The plate-plate rheometer and vane tests produced comparable results, however, a plate-plate rheometer requires more caution. Adherence conditions and the effects of the normal force must be controlled to

perform valid tests. Moreover it is mandatory to visualise the strain fields. Vane test are recommended for SAOS and LSS tests as they are easier to carry out than tests involving plate-plate rheometry to characterise cheese. Rheological characterisation, by means of vane tests, provides parameters that can be correlated with the sensory properties of the cheeses and their mechanical properties defined at the time of formulation.

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6. Figures

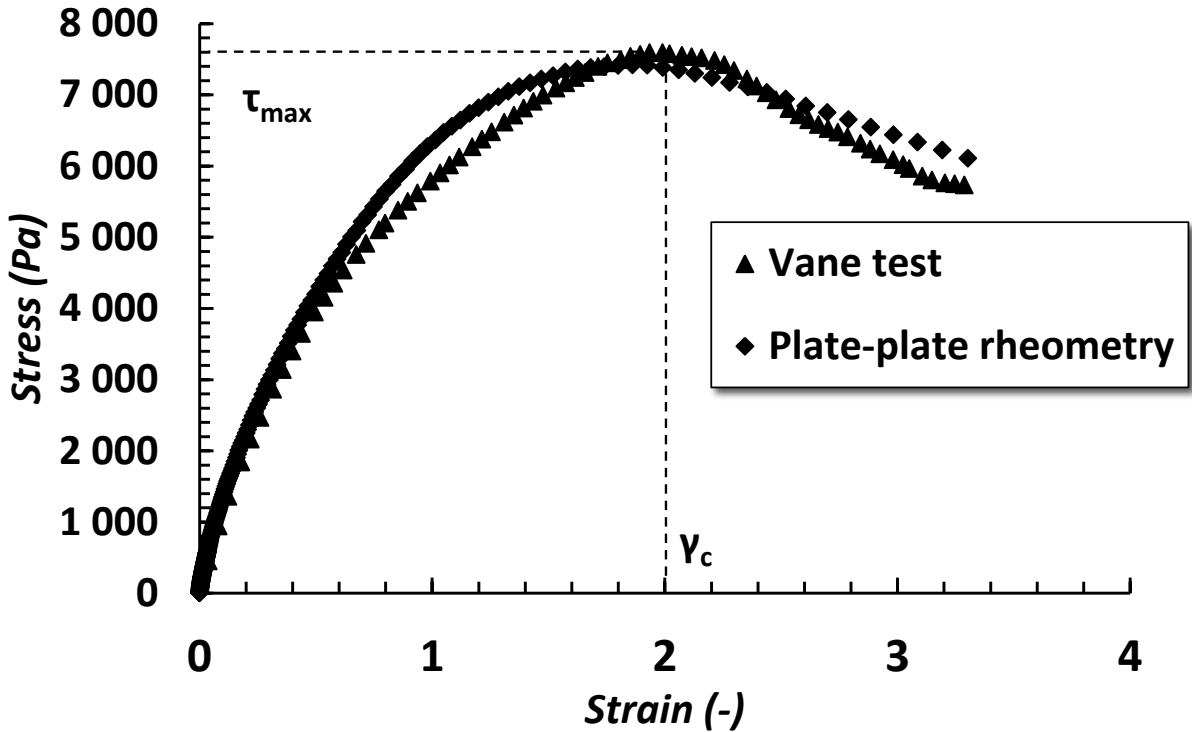


Fig.1. Example of large-strain shear (LSS) tests on cheese FI for plate-plate rheometry (◆) and vane test (▲). Shear stress is plotted against strain. The critical failure strain γ_c and the failure stress τ_{max} are respectively the values of strain and stress at the peak point.

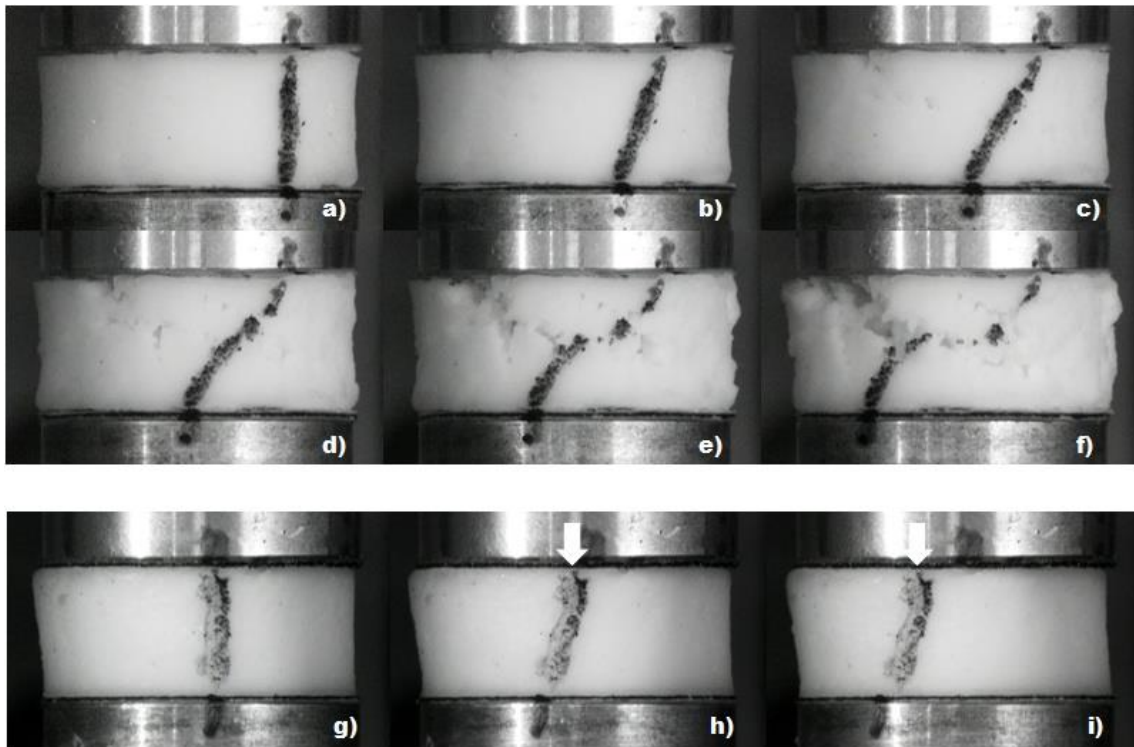


Fig.2. Visualisation of the strain field during a plate-plate rheometry test with a strain rate of 0.01 rad/s, for cheese Fl. Marks in line were placed across the cheese and on the measuring plates. The thickness of the cheese in these photos (10mm) is deliberately greater than that of the samples used in the study (3mm) so that they are easier to see. Glued surfaces: (a) initial state, (b) failure strain, (c) start of failure, (d) failure, (e) (f) extensive failure. Rough surfaces: (g) Initial state, (h) start of slip, (i) extensive slip.

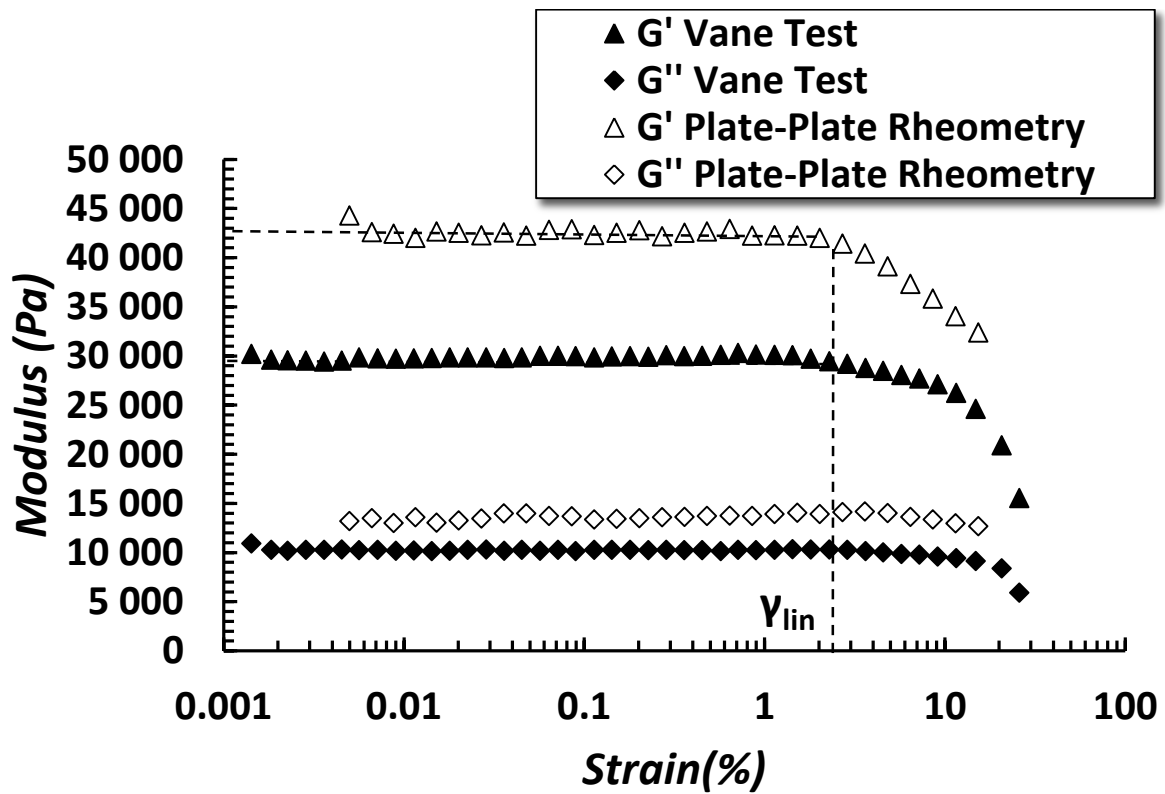


Fig.3. Example of curves obtained with small amplitude oscillation shear (SAOS) tests for cheese FI, with a strain sweep. The elastic modulus (G') and the viscous modulus (G'') are reported. The end of the linear viscoelastic region (LVR) is spotted at the limit strain γ_{lin} .

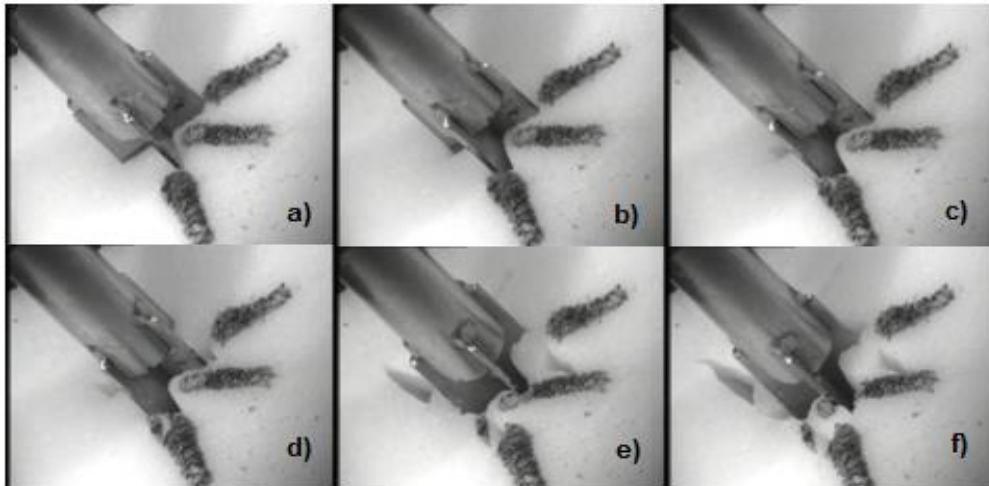


Fig.4. Visualisation of the strain field during a vane test with a strain rate of 0.01 rad/s, using cheese FL. (a) Initial state, (b) Elastic strain, (c) Failure strain, (d) (e) (f) Failure. The marks are used to determine the diameter of the sheared area in the case where the gap is considered to be infinite.

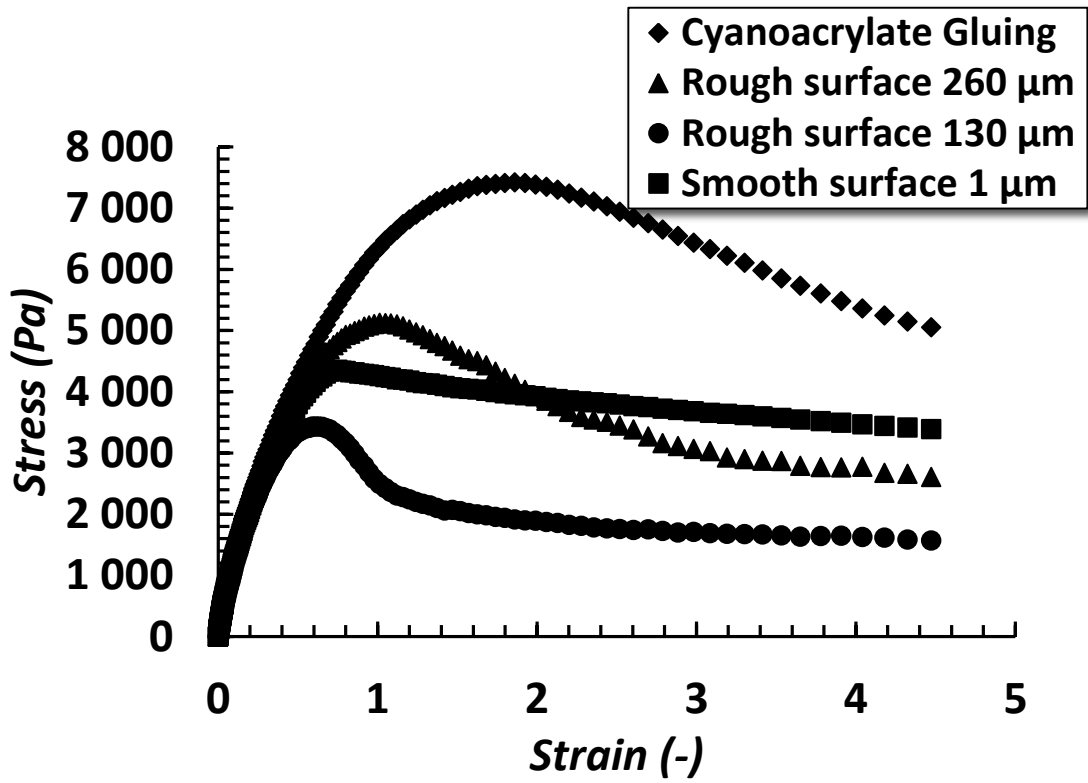


Fig.5. Large-strain shear (LSS) plate-plate rheometry tests using cheese F1 and different types of surfaces, at low rotational velocity (0.01 rad.s^{-1}). Premature slip occurs before failure with both rough and smooth surfaces.

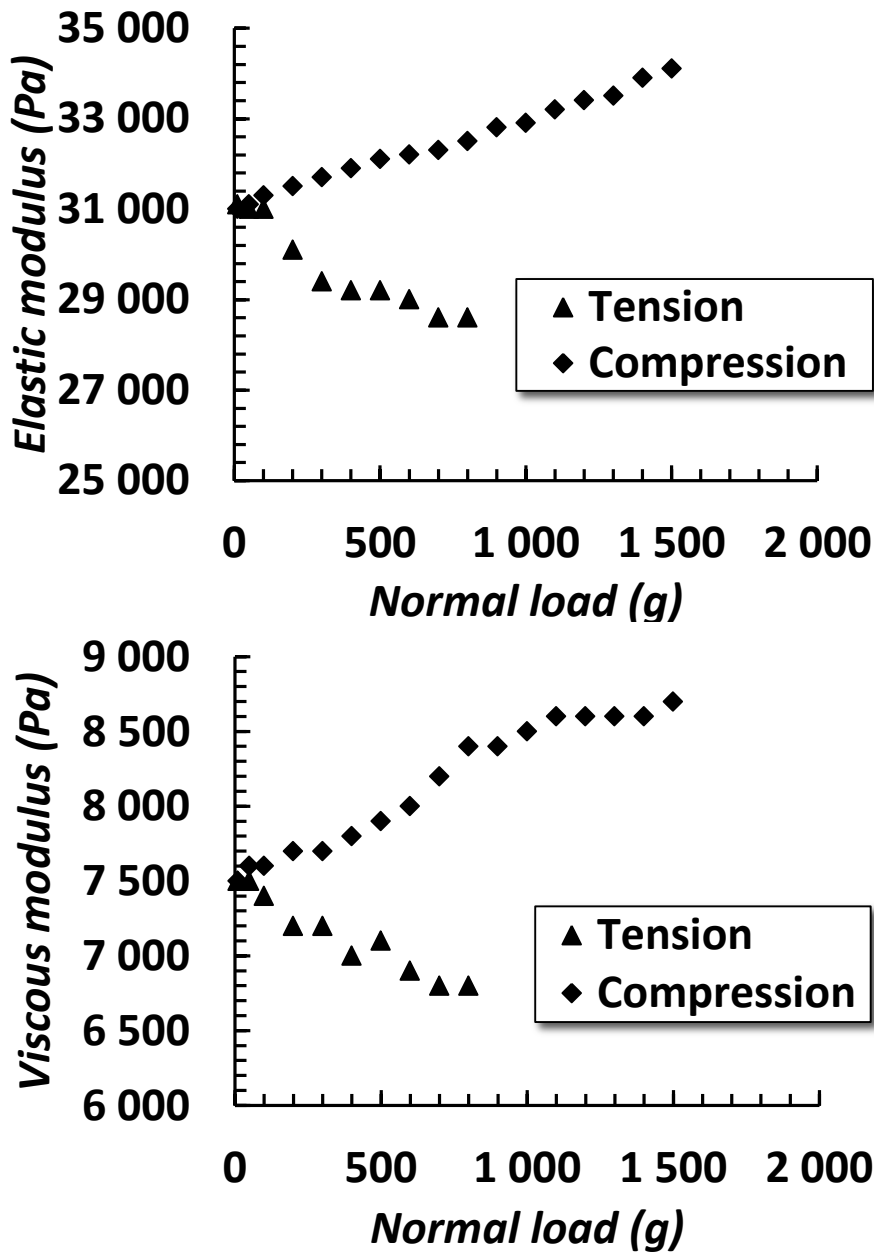


Fig.6. Change in elastic modulus (G') and viscous modulus (G'') according to normal load (N) applied to a cylindrical sample of cheese S1 (under compression (a) or tension (b)). The SOAS test was carried out at a constant frequency of 1Hz and a constant strain of 1%.

Table 1

Designation and composition of cheeses.

Code	Description	Dry extract (% w/w)	Fat/Dry content (% w/w)	Moisture/Non-fat (calculated) (% w/w)
Fh	Firm, High fat	51.5 ± 0.7	48.3 ± 0.6	12.9
Fl	Firm, Low fat	45.6 ± 0.4	25.0 ± 0.1	18.6
Sh	Soft, High fat	45.5 ± 0.5	48.4 ± 0.5	12.8
Sl	Soft, Low fat	41.7 ± 0.3	25.0 ± 0.1	18.2

Cheeses were replicated over three different productions

Table 2

Results for vane and plate-plate rheometry with small amplitude oscillation shear (SOAS) and large-strain shear (LSS) tests, as well as for sensory analysis.

Cheese	Sensory Analysis			Plate-Plate Rheometry						Vane Test					
				SOAS			LSS			SOAS			LS		
	Firmness	Elasticity	Pasty	G'	G''	δ	γ_{lin}	τ_{max}	γ_c	G'	G''	δ	γ_{lin}	τ_{max}	γ_c
			(kPa)	(kPa)	(°)	(%)	(kPa)	(-)	(kPa)	(kPa)	(°)	(%)	(kPa)	(-)	
Fh	5.07	0.87	4.86	263	55.0	11.8	0.3	23.1	0.7	256	52.0	11.5	0.3	21.3	0.6
<i>Duncan/SD%</i>	<i>A</i>	<i>C</i>	<i>A</i>	<i>4.8</i>	<i>8.4</i>	<i>5.1</i>	<i>14</i>	<i>12</i>	<i>9.7</i>	<i>10</i>	<i>11</i>	<i>0.5</i>	<i>6.6</i>	<i>7.3</i>	<i>9.5</i>
Fl	4.37	5.36	1.19	44.5	14.6	18.2	2.0	7.23	1.2	38.1	12.5	18.9	1.7	6.87	2.0
<i>Duncan/SD%</i>	<i>B</i>	<i>A</i>	<i>B</i>	<i>7.9</i>	<i>8.3</i>	<i>1.3</i>	<i>10</i>	<i>2.2</i>	<i>9.0</i>	<i>5.8</i>	<i>6.0</i>	<i>0.5</i>	<i>14</i>	<i>3.8</i>	<i>1.8</i>
Sh	3.09	0.41	4.6	183	33.6	10.5	0.5	16.1	0.8	160	29.0	10.8	0.5	15.2	0.6
<i>Duncan/SD%</i>	<i>C</i>	<i>C</i>	<i>A</i>	<i>8.6</i>	<i>8.2</i>	<i>6.0</i>	<i>7.8</i>	<i>3.5</i>	<i>8.6</i>	<i>7.4</i>	<i>7.9</i>	<i>0.8</i>	<i>3.7</i>	<i>3.7</i>	<i>6.3</i>
Sl	3.06	3.67	1.69	30.0	9.11	18.1	2.0	4.51	1.3	23.7	7.22	19.3	1.8	4.24	2.1
<i>Duncan/SD%</i>	<i>C</i>	<i>B</i>	<i>B</i>	<i>9.0</i>	<i>6.0</i>	<i>2.3</i>	<i>11</i>	<i>6.7</i>	<i>5.4</i>	<i>5.0</i>	<i>5.0</i>	<i>0.5</i>	<i>13</i>	<i>14</i>	<i>5.1</i>

The values are averaged over three tests in the case of the rheological measurements.

Standard deviation (SD%) are expressed as a percentage.

Duncan test: different capital letter indicate significant differences at 95% confidence level of the descriptors.

Fh, firm, high fat; Fl: firm, low fat; Sh, soft, high fat; Sl, soft, low fat; G', elastic modulus; G'', viscous modulus;

δ , the phase shift; γ_{lin} , linear viscoelastic region (LVR) limit strain, which marks the point at which G' and G'' begin to decrease; τ_{max} , failure stress; γ_c , critical failure strain.

Chapitre 1.2

Rhéologie et modélisation des bols alimentaires issus de fromages

Le rôle principal de la mastication est de transformer une bouchée de nourriture en un bol prêt à être avalé. Ce procédé peut donner des aspects relativement différents au bol, allant du liquide peu visqueux à un ensemble de solides mis en suspension, en passant par des fluides viscoplastiques. La taille des particules est un facteur important déclenchant la déglutition, expliquant pourquoi la structure du bol (et la taille des particules) est généralement similaire entre les individus, quels que soient le temps et l'efficacité de la mastication (Peyron et al., 2004). Dans le bol alimentaire, la salive a de nombreuses fonctions biologiques et joue donc un rôle important dans le cadre de la perception en bouche des produits alimentaires (Schiffer 2007).

Nous allons nous pencher sur le cas des bols issus de la consommation de fromages, dont deux familles se dessinent : les bols homogènes sans particule et les bols à morceaux déformables de taille millimétrique. La première famille est issue de fromages dits « mous », qui nécessitent peu de mastication. Ils sont plutôt mélangés avec la salive par des écoulements de compression et de cisaillement principalement entre le palais et la langue. Il en résulte un bol homogène composé en grande partie du produit de base, mélangé avec de la salive. Même à des temps courts, de l'ordre de la dizaine de secondes, l'émulsion eau/gras qui compose le fromage est déstabilisée par la salive. Au final, le fromage dilué et les macromolécules contenues dans la salive font apparaître un comportement viscoplastique.



Fig.1 : Bol alimentaire à morceaux issu d'un fromage ferme, avec des morceaux de fromages de 0.1 à 4 mm.

La deuxième famille de bols alimentaires est issue de fromages avec des seuils de contrainte grands (>5000 Pa). Les produits sont mastiqués par les dents, dont résulte un bol cohésif composé de morceaux, de tailles comprises entre 0,1 et 4 mm (Fig.1). Ce bol est composé de morceaux de fromage encore non travaillés, qui sont petit à petit attaqués en surface par la salive. Le caractère non continu des bols, avec un rapport de la taille des particules sur la taille du système compris entre 3 et 10, permet de mesurer des propriétés rhéologiques de volume à la limite de la mécanique des milieux continus. La phase « fluide » suspendante est composée de salive et de fromage dissout. Les propriétés viscoplastiques de la matrice liquide participent grandement à la cohésion du bol, ce qui implique qu'elle reste difficilement séparable des morceaux.

Le challenge de cette partie est la caractérisation rhéologique de bols issus d'aliments formant des morceaux en bouche. La méthode d'analyse d'essais en compression est la principale originalité de cette étude, qui permet de générer des paramètres quantitatifs en lien avec la perception sensorielle. La plupart des études précédentes dans ce domaine ont été effectuées sur les liquides et produits semi-solides. Les modèles n'ont pas pris en compte la dilution par la salive. L'enjeu est de développer une méthodologie de mesure des caractéristiques rhéologiques, adaptée aux suspensions hétérogènes concentrées. De par l'originalité de la méthode développée, une étape de validation est apparue incontournable pour sélectionner des variables fiables qui pourront être utilisées dans les modèles. Cette méthode a nécessité le développement d'un outil de compression (Fig.2), testé sur des suspensions modèles. Une autre contrainte est son ergonomie et sa portabilité, car

l'instrument devait être installé au sein de l'équipe FLAVI du CSGA pour les études cliniques, et être opéré par des personnes spécialistes, non-experts en rhéologie. Ces conditions ont nécessité le développement d'une interface de contrôle simplifié, programmé sur Labview®. Outre la mesure des paramètres nécessaires aux calculs des propriétés rhéologiques, des prises de vue et de température ont permis de valider l'exactitude des données récoltées. Le dispositif d'actionneur permet d'appliquer une vitesse de descente constante, contrôlée par un capteur de position LVDT indépendant du moteur. A partir d'une force de 50N, le plateau supérieur est libéré grâce à une liaison pivot semi-glissante, dans le but de protéger les capteurs de force d'une éventuelle surcharge. Deux capteurs en série sont utilisés pour couvrir deux gammes de mesure. Suite à trois campagnes d'essais, les données liées à la rhéologie ont été extraites grâce à un programme développé sur Matlab® (Fig.3), sur près de 900 courbes de compression.

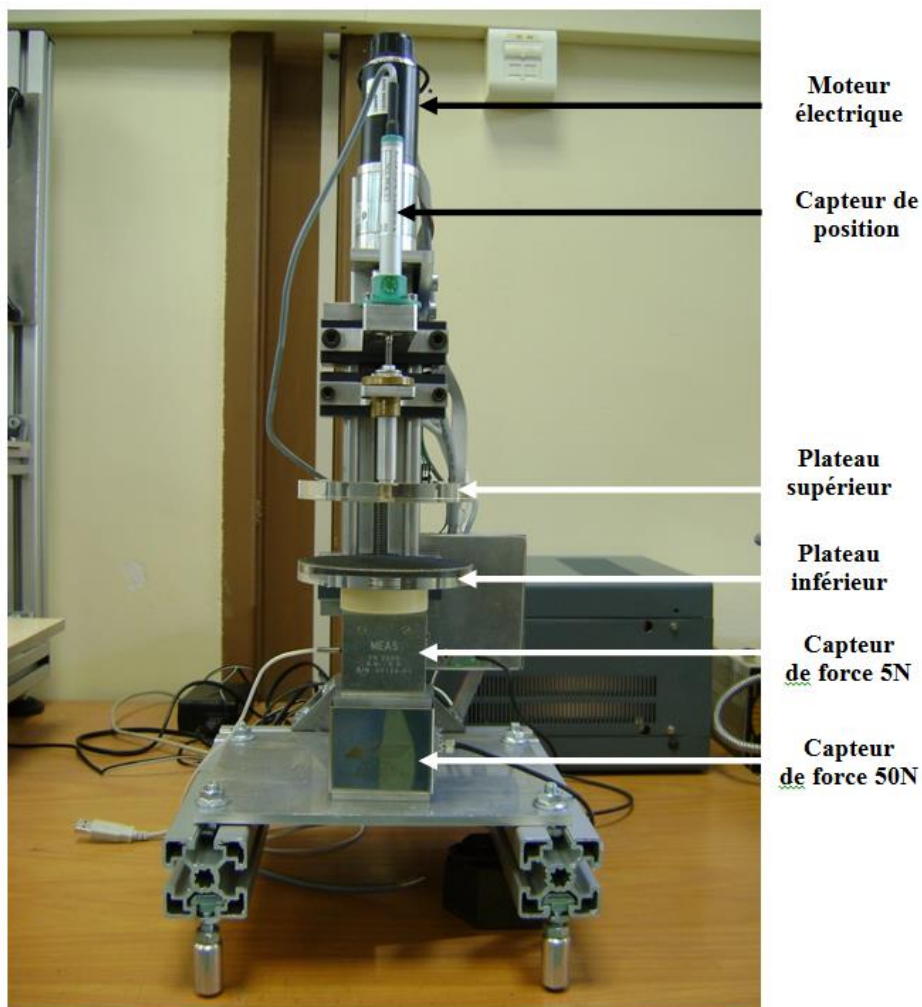


Fig.2 : Prise de vue du rhéomètre de compression.

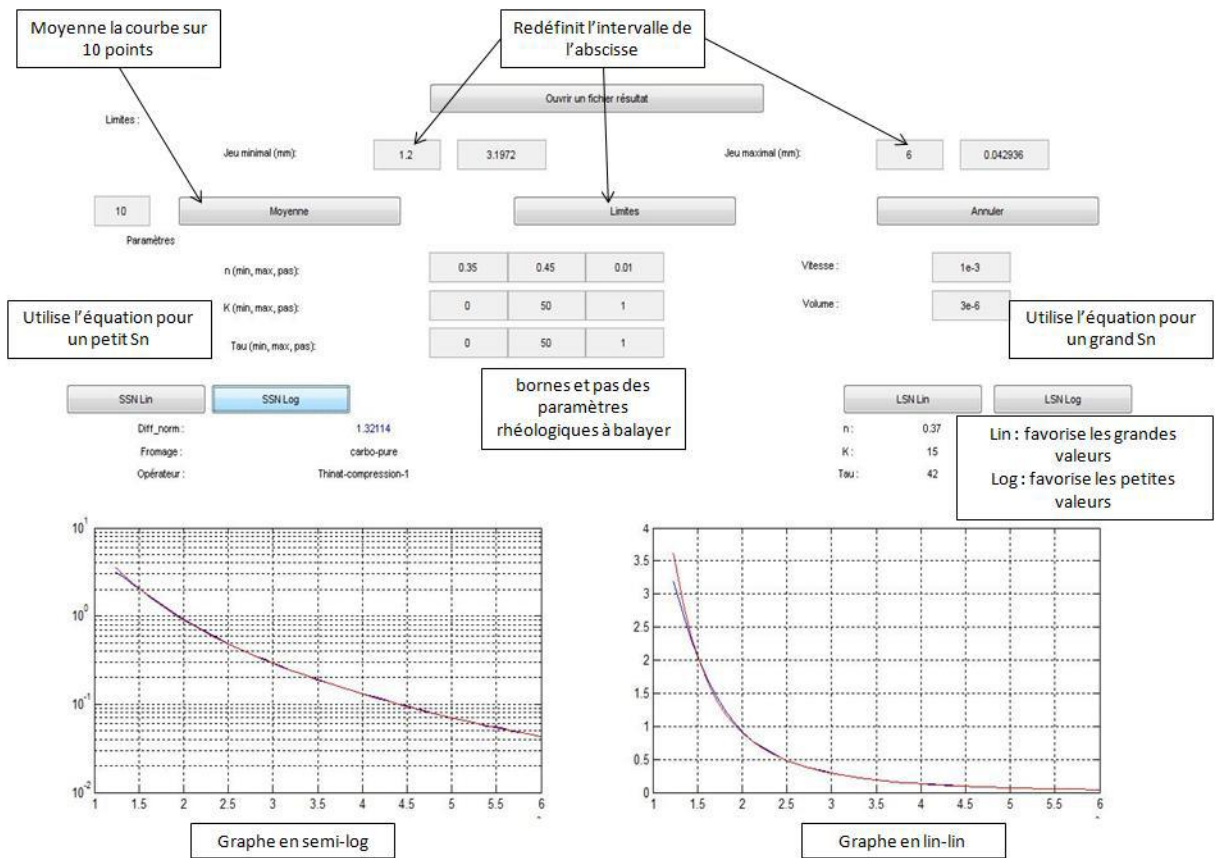


Fig.3 : Vue de l'écran de contrôle du logiciel de traitement des données issues des courbes de compression.

Afin de valider la méthodologie de mesure des propriétés rhéologiques à partir de courbes de compression, des suspensions modèles ont été mises au point. Les premiers modèles ont été fabriqués avec des matrices fluides constituées d'huile de silicone (au comportement newtonien) ou de gel Carbopol de consistance. Trois types de particules sphériques ont été ajoutés à ces fluides : des billes en caoutchouc (EPDM) au comportement élastique, des billes de polystyrène au comportement élastoplastique, et des billes de Plastiline au comportement plastique (Fig.4). Les résultats concernant les suspensions à base d'huile de silicone ont été vite écartés, car les particules ont tendance à sédimenter rapidement avant même qu'un essai de compression puisse être mené. L'utilisation du gel de Carbopol, fluide à seuil, a été plus fructueuse. Toutefois les billes en EPDM sont à l'origine de structurations au cours de la compression, créées par blocage des particules fortement élastiques les unes contre les autres. La structuration suivie de la rupture donne ainsi naissance à des pics de force

(Fig.5), non représentatifs des profils de compression obtenus sur des bols alimentaires réels. Ainsi la validation du dispositif instrumental a été réalisée avec des modèles de suspensions avec des particules purement plastiques, plus représentatifs de la réalité des bols alimentaires.



Fig.4 : Suspensions modèles à base de Carbopol et de billes d'EPDM (a), de polystyrène expansé (b) et de Plastiline (c), avant compression.

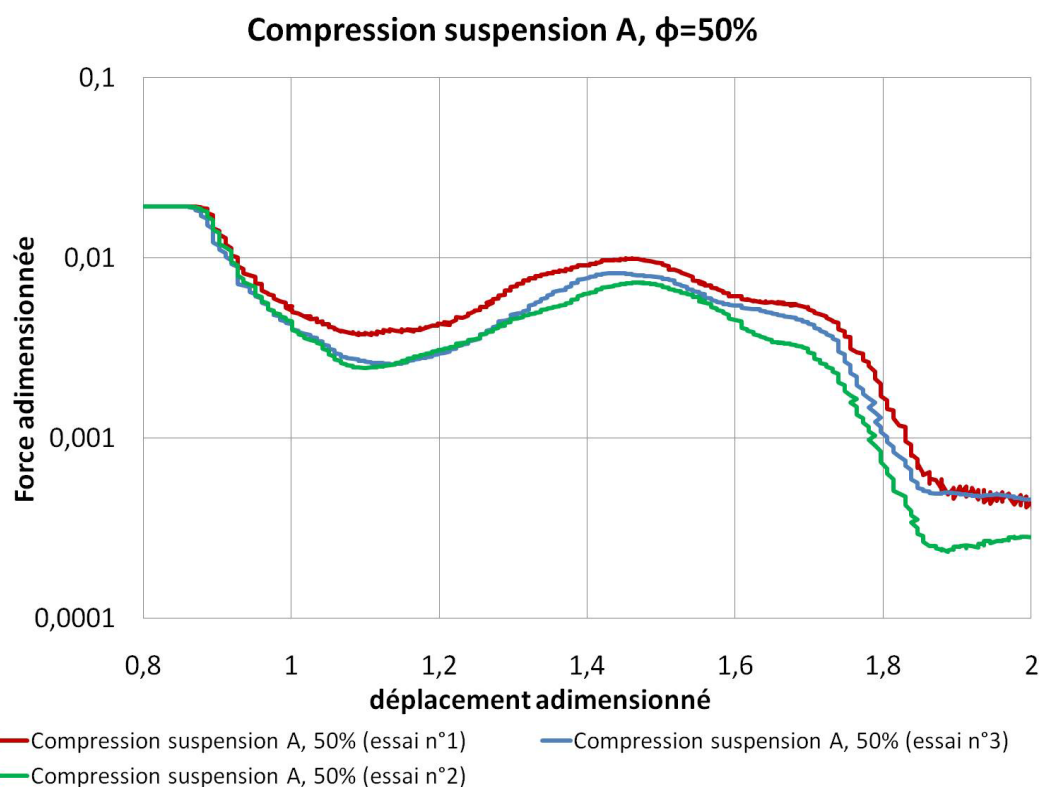


Fig.5 : Courbes de compression d'une suspension Carbopol/EPDMS pour trois essais. Le pic de force au centre des courbes est dû aux coincements/décoinçements des particules. La force a été adimensionnée par la réponse des particules seules à la compression, et le déplacement par le diamètre des particules (3mm).

Dans l'article à venir, le gel de Carbopol est conservé comme matrice fluide avec un seuil de contrainte autour de 40 Pa, couplé avec des billes de Plastiline de 2mm et 3mm de diamètre, de seuils intrinsèques 100, 300 et 750 fois supérieurs à celui de la matrice fluide. Ce fluide est utilisé pour valider l'analyse des courbes de compression, réalisée par le calage d'un modèle d'Herschel-Bulkley sur les différentes phases constitutives. La compression de ces suspensions modèles est réalisée à l'aide du rhéomètre d'écrasement, développé également pour l'étude de bols alimentaires de fromage. L'article déroule les résultats obtenus, et propose d'appliquer un modèle de suspension aux bols alimentaires réels, afin d'évaluer les propriétés rhéologiques de la phase suspendante au sein des bols.

Rheological characterizations of concentrated heterogeneous suspensions by compression test: application to human bolus from cheese

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Abstract:

Human boluses from cheese are heterogeneous suspensions of cheese particles and saliva. The main scope of this study is to determine the rheological properties of those boluses. A rheometrical device has been designed that makes allowance for the nature, small quantity and short lifetime of boluses. The boluses were considered as Herschel-Bulkley fluids. Model suspensions of spheres made in viscoplastic material (Plastiline) into a yield stress fluid (Carbopol gel) were created to mimic the bolus and to build the methodology. The aim of the model is to validate all the parameters obtained from the Herschel-Bulkley analysis, thanks to another rheometrical assessment method: consistometry. A suspension model was proposed to link the properties of the whole suspension to those of the suspending matrix. Thus, several characteristics of model suspensions and boluses were estimated: viscosity, yield stress, consistency, flow index, particle size distribution and volume fraction.

1. Introduction

Food boluses and the mechanical transformation of food in the mouth have been examined in numerous studies in recent years. It has been shown that food rheology is an essential factor in the stages of mastication (Lucas et al., 2002) and in the release of flavor compounds (Malone et al., 2003; Salles et al., 2010). Some authors (de Wijk et al., 2003a, 2003b) have concentrated on the sensations of texture in the mouth, by performing mechanical and sensory studies using different panels. However in-mouth processes depend upon panelists (Gierczynski et al., 2008; Mishellany-Dutour et al., 2012, 2012).

The release of aromas from dairy matrices is connected with the masticatory process (Feron et al., 2014), in particular with the way in which products spread around the mouth and hence with the mechanical capability of the boluses to create areas of exchange with the ambient medium (Doyennette et al., 2014; Trelea et al., 2008). Semi-solid food products tend to create a cohesive concentrated suspension while they are chewed (Chen, 2009; Peyron et al., 2004; Prinz and Lucas, 1997). Inside the bolus, the liquid fraction between the food particles can be a great source of aroma release, as it can coat the mouth during the mastication phase and stick on mucosa after swallowing (Repoux et al., 2012a). The liquid fraction is mainly composed of saliva, which has a non-Newtonian behavior (Schipper et al., 2007).

Chen and Lolivret (2011) investigated bolus rheology from different foods, and the boluses generated were semi-fluids without particles inside. The present paper aims to characterize boluses as semi-fluids which contain millimeter-size particles. The rheological characterization of these food boluses involves working with small quantities and over short time periods, because of the rapid changes in the physicochemical properties and thermal kinetics of the boluses just after sampling. The original challenge to be fulfilled by this study is to determine the bulk properties, i.e. rheological properties, particle distribution and volume fraction, of bolus from semi-solid food taking into account the constraints indicated above. An additional challenge is to evaluate the rheological properties of the fluid matrix inside the bolus, using a suspension model with specific hypotheses. The fluid matrix can, in effect, play a major role in the bolus properties (Drago et al., 2011), aroma release (Trelea et al., 2008) and mouth coating. Both challenges involve the creation of a new methodology, using the compression technique with a specially designed, dedicated device.

The first step is to perform a rheological analysis of the suspension model to mimic food bolus behavior, with deformable particles immersed in a yield stress fluid. Several parameters will be derived from the rheological analysis, in order to quantify and validate the mechanical properties of the suspension. In a second step, real boluses from 28 panelists will be tested and the same analysis will be performed. The effects of each individual will be monitored. Finally a suspension modeling procedure will be proposed to calculate the properties of the suspending matrix.

2. Materials

2.1. Cheese base

A cheddar-based melted cheese was developed by FROMAGERIE BEL (Paris, France). The cheese was produced for the study and did not include any texturing agent. The dry extract DM_{cheese} was measured at 45.6%, and the fat in dry matter ratio was targeted at 25%. The cheese was subjected to rheological characterization in order to determine its yield stress, τ_{max} was 7500 Pa, following a vane test protocol (Patarin et al., 2014).

2.2. Subjects

Twenty-eight panelists (males and females from 20 to 60 years old) were recruited. They were selected from a group of 100 volunteers based on their good dental status (no missing teeth – except third molar, no occlusion disorder) and on the repeatability of measured physiological parameters (salivary flow rate at rest and under stimulated conditions, respiratory flux and salivary composition) and aroma release profile (Repoux et al., 2012b).

All the subjects gave their informed consent. The protocol of the study was submitted to the local ethical committee and approved on 17th April 2008 by the *Comité de Protection des Personnes Est-1* (N°2008/15) and on 8th August 2008 by the *Direction Générale de la Santé - France* (N° DGS2008-0196).

2.3. Description of food boluses

From the visual point of view, cheese boluses are suspensions of particles of varying numbers and sizes, typically millimetric. These are suspended in and lubricated by a liquid phase. Prinz

and Lucas (1997) proposed a cohesion model for a heterogeneous bolus with particles that are dissolved by saliva. This opposes viscous effects due to the suspending phase, which ensures the cohesion of the particles, and the effects of surface tension. But the model does not take into account the plastic properties of the suspending matrix. The cheese particles are degraded on the surface by saliva, which softens the peripheral area of each particle. Part of the cheese is also dissolved in saliva creating the suspending matrix around the particles that thus corresponds to an o/w emulsion consisting of saliva and dissolved cheese induced by the mastication process. This phase enables the bolus to retain remarkable cohesive properties in spite of its heterogeneous nature. This explains why a viscoplastic fluid was chosen as a model suspending matrix, and another viscoplastic fluid as deformable particles.

2.4. Model fluids

The use of model fluids is proposed in order to validate the use of a compression device for the rheological characterization of concentrated suspension. A yield stress fluid was chosen as the suspending matrix, to simulate the behavior of the saliva/dissolved cheese mix. A transparent aqueous gel of Carbopol 940 (Lubrizol, Wickliffe, OH, USA) was formulated. Carbopol gels are well-known yield stress fluids (Piau, 2007). Rheometrical tests were performed on an AR-G2 (TA Instruments, New Castle, DE, USA) with cone-plate geometry with rough surface to avoid slippage at the wall (Magnin and Piau, 1987). The flow curve can be fitted by a Herschel-Bulkley model (Eq.2) with the following parameters (Piau, 2007): a consistency $K_0=15.4 \text{ Pa}\cdot\text{s}^{0.32}$, a yield stress $s_0=40.0\text{Pa}$ and a shear thinning index $n=0.32$.

Particles have been chosen to mimic the behavior of the cheese particles. Small balls of Plastiline (Bumpodo, Italy) were created. Plastiline is a pasty material which has a viscoplastic behavior. Plastiline is a mix of sulfur, kaolin and mineral oil, and has the advantage of not dissolving in a water base matrix, like the Carbopol gel. The yield stress of the Plastiline was adjusted by using various concentrations of oil, aiming to create three levels of yield stress. A vane test technique was used to characterize the yield stress of the Plastiline (Barnes, 1989; Barnes and Nguyen, 2001), with a 4-blade vane set on a AR-G2 rheometer. The formulations had a yield stress of respectively 4140, 12600 and 29800 Pa, knowing that the cheese has a yield stress of 16 000 Pa. Spherical balls were created by hand, to reach a diameter d_{part} of 2 and 3mm. The polydispersity of the balls is $\pm 4.6\%$ around the average

diameter. The density of Plastiline is $\rho=1395 \text{ kg/m}^3$. Balls were added to the Carbopol matrix and the volume fraction Φ_v was fixed at 20, 30, 40 and 50% for the experiments, with a precision of 3%.

3. Methods

3.1. Instrumentation

Numerous constraints have to be taken into account in order to perform rheological characterization of the boluses. An individual produces a bolus of limited volume, between 4 and 10 cm^3 . The particle size is large, typically a few millimeters. The food boluses studied are therefore at the limit of the conditions required for them to be considered as continuous media. Furthermore, the rapid physical, chemical and thermal changes taking place in the bolus mean that a fast, simple method has to be used. These constraints finally led to the adoption of the compression technique for the rheological test, along with suitable measurement protocols and analysis.

The compression device was designed and dedicated to the fluids being studied. It consists of a mobile upper plate and fixed lower plate. Compression is applied at a constant velocity $V=1\text{mm}\cdot\text{s}^{-1}$ over a maximum distance of 50mm, obtained with a speed-controlled motor. Displacement of the upper plate was monitored using a LVD sensor with an accuracy of 0.05 mm. The fluid was subjected to a force up to $F=50\text{N}$ with an accuracy of 0.01N. The test stopped when the maximum force was reached. The orders of magnitude of velocity, forces and stresses applied correspond to those found during a mastication process in the mouth (Mioche and Peyron, 1995; Ono et al., 2004). To impose an adherence condition at the fluid matrix interfaces, the surfaces of the plates were roughened (Magnin and Piau, 1987). Of course this ensures only the adherence of the suspending fluid, not the particles. Still it will be demonstrated that this fluid governs the system flow during the most interesting part of the test.

3.2. Experimental protocol

The panelists were given 6 g of cheese to chew and spat out the bolus freely when they felt ready to swallow. The bolus samples, with a volume between 4 and 10 cm^3 , were spat into a

truncated syringe. The panelists were told not to add air in the syringe as it affects the compression results. Moreover, mastication is a process where the air is for the most part separated from the bolus before swallowing (Buettner et al., 2001).

A constant volume of $\Lambda = 3 \text{ cm}^3$ of bolus was then loaded with the syringe in the center of the lower plate for rheological characterization by compression. Although the bolus was split in two, the tested part of the bolus was considered representative of the whole bolus in terms of bulk properties, as it was previously well mixed in the mouth.

Less than 30 seconds elapsed between the bolus being spat out and the start of the experiment. The temperature of the samples was measured systematically before compression. A picture was taken of the bolus placed on the lower plate before each experiment began. This picture (Fig.1) was used to evaluate the initial height of the sample. As detailed below, this can be used to evaluate the yield stress by consistometry. The picture was also used to check the volume of the sample Λ . The part of the food bolus not used for the compression test was carefully spread and photographed for approximate evaluation of the particle size distribution (Fig.1d).

3.3. Evaluation of the yield stress and particle size distribution of the boluses

Two types of pictures were taken in addition to performing the compression tests. An initial picture of the food bolus sample prior to compression (Fig.1a & b) provides an order of magnitude of the yield stress by using consistometry or slump test (Pashias et al., 1996; Piau, 2005). The yield stress s_v can be estimated from the final height h_v of uncollapsed product after setting the bolus on the lower plate:

$$s_v \approx \frac{1}{2} \rho \cdot h_v \cdot g \quad (1)$$

where ρ is the density of the bolus and g is the acceleration of gravity.

Formula (1) represents the balance of forces due to the yield stress and forces of gravity. Its validity is subject to two conditions: (i) the bolus must have collapsed after the setting, since, if this were not the case, the value s_v would only be a lower bound for the real yield stress; (ii) stresses caused by gravity must predominate. With small volumes, this is an approximate

approach as surface tension generates stresses that certainly play a significant role. These stresses values will be compared with those given by the Herschel-Bulkley model in order to validate the parameters derived from the analysis.

The second picture (Fig.1d & e) shows the part of the bolus not used for the compression tests, which is delicately spread. This procedure gives a visual order of magnitude of the maximum average size of the particles d_{part} and thus an approximation of the particle size distribution of the bolus.

3.4. Rheological analysis of the boluses

The boluses are assumed to be non-thixotropic yield-stress fluids. They will be considered to be homogeneous, modeled by a Herschel-Bulkley fluid with a constitutive law given by the expression:

$$\begin{cases} \underline{\underline{\tau}} = 2 \left(K \dot{\underline{\underline{\gamma}}}^{n-1} + \frac{s}{\dot{\underline{\underline{\gamma}}}} \right) \dot{\underline{\underline{\gamma}}} & \text{if } \tau > s \\ \dot{\underline{\underline{\gamma}}} = 0 & \text{if } \tau < s \end{cases} , \quad (2)$$

$$\dot{\underline{\underline{\gamma}}} = \left(\frac{1}{2} \text{tr} \left(\underline{\underline{\dot{\gamma}}}^2 \right) \right)^{1/2} , \quad (3)$$

$$\tau = \left(\frac{1}{2} \text{tr} \left(\underline{\underline{\tau}}^2 \right) \right)^{1/2} , \quad (4)$$

where n is the power-law index, K the consistency, s the yield stress, $\underline{\underline{\dot{\gamma}}}$ the strain rate tensor, $\underline{\underline{\tau}}$ the stress tensor, $\dot{\gamma}$ the shear rate and τ the second invariant of the stress tensor.

Analytical solutions have been proposed for the change in compression force. For Herschel-Bulkley fluids, at constant volume and with wall adherence conditions, Covey and Stanmore (1981) proposed formulas depending upon the characteristics of the bolus. The criterion S_N (Eq.5) is used to choose the appropriate equation to model the behavior: Eq.6 if $S_N < 1$ and Eq.7 if $S_N > 1$. S_N was calculated by iteration: a first fit was performed assuming $S_N > 1$ to get the rheological properties. Those properties were then used to calculate a new S_N value and a new fit was performed if $S_N < 1$. Equations are:

$$S_N = \frac{\Lambda^{1/2} V K^{1/n}}{h^{5/2} \pi^{1/2} s^{1/n}} \quad (5)$$

$$F = \frac{2s\Lambda^{3/2}}{3h^{5/2}\pi^{1/2}} + \frac{2\pi K^{1/(n+1)} s^{n/(n+1)} V^{n/(n+1)}}{h^{(3n+1)/(n+1)}} \left(\frac{n+1}{n}\right)^{n/(n+1)} \left(\frac{n+1}{4n+3}\right) \left(\frac{\Lambda}{h\pi}\right)^{\frac{4n+1}{2n+2}} \quad (6)$$

$$F = \frac{s\Lambda^{3/2}}{h^{5/2}\pi^{1/2}} + \frac{2\pi K V^n}{h^{2n+1}} \left(\frac{2n+1}{n}\right)^n \left(\frac{1}{n+3}\right) \left(\frac{\Lambda}{h\pi}\right)^{\frac{n+3}{2}} \quad (7)$$

where F is the compression force, h the height between the plates, V the compression velocity, Λ the fluid volume.

This approach was described in Engmann's literature review (2005) concerning compression, for the study of a shear-thinning yield-stress fluid. Mossaz et al. (2010) used this approach to characterize dairy products. The modeling procedure is one method of analysis among others. The advantage of the present analysis method in relation to this technique is that it identifies rheological parameters that can be related to the rheology of the cheeses, bucco-pharyngeal mechanisms and other physiological parameters.

3.5. Evaluation of the volume fraction in boluses

In addition to the rheological characterization, which is the first challenge of this study, an attempt was made to evaluate the volume fraction of the bolus, and finally to propose a suspension model. The volume fraction of the boluses was determined by measuring the dry matter of the bolus DM_{bolus} and knowing the dry matter of the cheese DM_{cheese} . After being tested, the boluses were weighed and then desiccated. The ratio of the dry weight/initial weight provided the dry matter of the bolus. Its volume fraction Φ_v was then estimated by mass fraction Φ_{mass} by the equation:

$$\phi_v \approx \phi_{mass} = \frac{DM_{bolus}}{DM_{cheese}} \quad (8)$$

This approach is only an approximation since part of the cheese dry matter is dissolved in the suspending matrix. Thus, bolus volume fraction Φ_v is overestimated. The cheese tested was checked to determine that less than 10% of cheese dry matter went into the suspending matrix, by measuring the weight of the particle inside the bolus in comparison to the weight of

the initial cheese sample. Those values of volume fraction were used to propose a suspension model for the boluses.

3.6. Bolus modeling: fluid matrix properties

The additional challenge of this study is to determine the rheological properties of the fluid matrix inside the bolus, based on the rheological characterization. For this purpose, a concentrated suspensions theory was used to model human cheese boluses. This theory gives the relationship between rheological properties of the suspension and particle concentration in the model case (Chateau et al., 2008; Eilers, 1941; Krieger and Dougherty, 1959; Merkak, 2007; Mooney, 1951). The empirical model of Krieger- Dougherty is the most commonly used. By integrating the limits of Einstein's model at low concentration and based on the assumption of infinite viscosity when the volume fraction tends to the maximum volume fraction, Krieger and Dougherty derived the equation:

$$\mu = \mu_0 \left(1 - \frac{\phi}{\phi_m}\right)^{-[\eta]\phi_m} \quad (9)$$

The intrinsic viscosity $[\eta]$ and the maximum packing volume fraction Φ_m drastically depend upon the shape of the particles and their size distribution (Barnes, 1989). In the case of a random arrangement of monodisperse spherical particles, $[\eta]$ and Φ_m are respectively equal to 2.5 and 63.5%, based on Einstein's equation for low volume fraction suspensions. However, this model is only valid for a Newtonian suspending fluid. Φ_m is representative of the ability of the suspension to create a compact structure, and $[\eta]$ is representative of particle shape.

More recently, studies on suspensions using Bingham fluids or Herschel-Bulkley fluids were performed with a suspending matrix (Luu, 2009; Merkak, 2007), aiming to link the rheological parameters of the suspensions, such as yield stress, consistency or flow index, to the parameters of the suspending matrix. Chateau *et al.* (2008) proposed relationships between suspension parameters (s ; K ; n) and suspending matrix parameters (s_0 ; K_0 ; n_0), with Eq.10 or Eq.11:

$$s = s_0 \sqrt{(1 - \phi) \left(1 - \frac{\phi}{\phi_m}\right)^{-[\eta]\phi_m}} \quad (10)$$

$$K = K_0 \sqrt{(1 - \phi)^{1-n} \left(1 - \frac{\phi}{\phi_m}\right)^{-[\eta](n+1)\phi_m}} \quad (11)$$

Based on this model, n was assumed independent of the volume fraction, i.e. $n=n_0$. This hypothesis was used to get more accurate results of s and K using the model of Covey and Stanmore on model suspensions. For analysis purposes, dimensionless yield stress $[s]$ and consistency $[K]$ were used, calculated from the ratios of Herschel-Bulkley parameters of the whole suspension in the flow phase, to those from the suspending matrix: $[s] = s / s_0$ and $[K] = K / K_0$.

3.7. Statistical analyses

ANOVA tests with Prism (GraphPad Software, USA) were performed on rheological parameters for each panelist. For correlation analyses, Pearson tests were performed on a different set of parameters, including the uncertainties and variations due to repetitions.

4. Results

4.1. Results for model suspensions

4.1.1. Raw curves and analysis

Examination of the compression curves reveals three major phases (Fig.2). To better understand the different squeeze phases, Fig.3 shows the behavior of the particles inside the suspension according to the dimensionless height $[h]$, the ratio of the height h to the diameter of the particle.

During the first phase, called “flow phase”, the suspension flows in bulk and the particles are reorganized upon a squeeze flow. For the flow phase, a model based on Eq.6 or Eq.7 is fit on the curves, depending upon the S_N value. The model fit also enables the limits of the flow phase to be positioned, and thus indicates transition heights between the phases. The height at which the flow phase begins is noted h_{flow} . The Herschel-Bulkley parameters determined are the shear-thinning index $n_{\text{flow}}=n_0$, the yield stress s_{flow} and the consistency K_{flow} . In the case of boluses that have already collapsed due to gravity after setting, a consistometry test was then performed to validate the yield stress as described above Eq.1. The yield stress values s_{flow} can

be correlated to the values of s_v on Fig.4 for model suspensions which collapsed prior to compression due to gravity effect. The correlation cannot be made for higher yield stressed suspension ($\Phi_v > 50\%$) as the initial heights $h_0 > h_{flow}$ was too low to initiate gravity flow. In order to make further analyses on model suspensions, dimensionless yield stress $[s] = s_{flow} / s_0$ and consistency $[K] = K_{flow} / K_0$ will be used.

The second zone occurs for a dimensionless height $h_{trans}/d_{part} = 2.02 \pm 0.25$. Within this zone, particles are rearranged in a unique slice. The shape of this area is variable depending upon the experiments: some balls will be able to spread while others are blocked by those located nearby. This phase is therefore governed by lubrication effects and the continuous media theory can no longer be applied. Even if no rheological parameters can be determined, this phase is representative of lubrication phenomena between particles, as they occurred in the mouth during the mastication process. Only a discrete model of mechanics would be able to propose suitable quantitative parameters, which is not within the scope of this study.

The last phase is called the "particles phase" and begins at a height h_{part} . The compression profile of the exact number of particles without suspending matrix fits the compression profile of the suspension (Fig.2). In this phase, particles govern the mechanical behaviour, and the viscous component becomes negligible in comparison to the yield stress component s_{part} , which can be linked to the yield stress of the particles with a precision of 5%. It proves that the water-based Carbopol and the oil-based Pastiline do not interact during the compression test. Moreover, h_{part} values happen to be proportional to d_{part} , with a ratio $h_{part}/d_{part} = 0.81 \pm 0.07$. This relationship in the case of model suspensions can be explained by the conservation of the particle volume. Assuming a slice full of spherical particles connected one to another by their edge, the volume occupied by the suspending matrix will be expelled during the compression due to the yield stress difference. Then, the volume $V_{part} = \frac{4}{3}\pi \frac{d_{part}^3}{8}$ of the particles will occupy a cubic volume $V_{tot} = h_{part}^3$ as it can no longer expand. Thus, $h_{part} = \sqrt[3]{\frac{\pi}{6}} d_{part} \approx 0.8d_{part}$. The relationship is valid for particles of 2mm and 3mm diameter.

In these experiments, squeezing is continued until the force reaches 50N at a height h_{end} . A corresponding spreading surface $S_{end} = \Lambda/h_{end}$ is defined to evaluate the ability of the bolus to

create a surface under a load of 50N. S_{end} can be used to discriminate chewing strategy through a panel of individuals (Yven et al., 2012).

4.1.2. Effects of the volume fraction

Dimensionless Herschel-Bulkley parameters $[s]$ and $[K]$ from the flow phase are plotted on Fig.5 according to the volume fraction Φ_v . Firstly, a good reproducibility of compression test results was observed. Variations are mainly due to sample preparation and more specifically to the particle creation process. But as the volume fraction increases, the variability of $[s]$ and $[K]$ tends to be greater, because of the limit of the continuous medium conditions, as we are working on the edge of the model. However, $[s]$ and $[K]$ tend to increase as Φ_v rises, in accordance with suspension theories. A proposed modeling based on the Chateau model will be debated in the discussion section.

4.1.3. Effects of particle size distribution and particle yield stress

In the case of real human bolus, the particle size distribution is polydisperse. To evaluate the potential effect of particle size, particles with different diameters (2mm and 3mm) were used in model suspensions. Suspensions were made using different proportions in volume of 2mm and 3mm particles (0/100; 25/75; 50/50; 75/25; 100/0) for a constant volume fraction $\Phi_v = 40\%$. The effect of size distribution on dimensionless Herschel-Bulkley parameters $[s]$ and $[K]$ was investigated.

In the case of monodisperse suspensions, $[s]$ and $[K]$ do not significantly depend upon particle size (Fig.6), since the standard deviations overlap. This observation validates the hypothesis of continuum mechanics used for these monodisperse suspensions. For polydisperse suspensions, $[s]$ and $[K]$ increase until the distribution contains 50/50 in volume of the two diameters (Fig.6). This phenomenon may be caused by the smaller particles that are interposed between the larger ones, forming an even more compact structure.

Suspensions at a constant $\Phi_v = 40\%$ were prepared with 3mm particles of several yield stresses (4000, 12000 and 30000 Pa). The resulting values of $[s]$ and $[K]$ are independent of particle yield stress as can be seen on Fig.7. This observation validates the hypothesis that particles do not interact and are not subject to direct stress during the flow phase.

4.2. Results for human cheese bolus

4.2.1. Raw curves and analysis

Fig.8 presents a typical compression curve of a bolus obtained from an individual consuming the cheeses studied. Examination of the compression curves reveals only two phases compared to the three phases identified in the model suspension: the transition phase between the flow phase and the particles phase no longer exists. Boluses are much more polydisperse than the suspensions tested in the model, and thus the rearrangement of particles between bulk flow and particle compression is much smoother. Like the model suspension, human boluses were in two configurations prior to compression. Either they were collapsed due to the gravity effect, or they stood on the lower plate as their yield stress was higher than the stress due to gravity. In the first case, a consistometry test was performed to obtain yield stresses s_v using Eq.1, in the same manner as with the model suspension. In the second case, calculations shown in the next section were performed to obtain an order of magnitude of the yield stress.

The parameters obtained from compression curves are displayed in Table 1. Temperature measured before compression test was in average at $32\pm 2^\circ\text{C}$. With regard to the individuals, rheological properties of boluses may vary from day to day. The experiments were therefore repeated twice with the same individual on a different day. Each panelist tends to create similar boluses, i.e. with the same rheological properties ($p < 0.05$). Among the panelists, p was found to be greater than 0.05 in an overall approach (every parameter), which means that the parameters are dependent on the panelists. This confirmed that each panelist has his/her own strategy for creating a bolus from the same cheese matrix. However, deviations for h_{part} and S_{end} are relatively small taking into account the uncertainties. In particular, S_{end} , the surface created by the bolus at the end of compression, was found to be an accurate parameter that can discriminate between the mastication strategies of panelists during chewing (Yven et al., 2012).

4.2.2. Validation of parameters during the flow phase

In the case of collapsed boluses prior to compression, the yield stress values s_{flow} can be correlated to the values of s_v with a good correlation (Fig.9). Like the model suspension, the correlation is not valid for non-collapsed boluses. To validate the yield stress inside the flow

phase for these kinds of boluses, it was proposed to calculate the mean stress in the sample at the beginning of the flow phase. Starting from Eq.13, the shear stress τ_{rz} within the sample is given by:

$$\tau_{rz} = \left(\frac{h}{2} - z\right) \frac{\partial P}{\partial r} \quad (13)$$

P is the pressure. An order of magnitude of the stress applied to the sample at a given height, here h_{flow} , can thus be calculated:

$$\tau_{\text{flow}} \sim h_{\text{flow}} \frac{1}{r_{\text{flow}}} \frac{F_{\text{flow}}}{S_{\text{flow}}} \quad (14)$$

$$\tau_{\text{flow}} \sim h_{\text{flow}} F_{\text{flow}} \pi^{1/2} \left(\frac{h_{\text{flow}}}{\Lambda}\right)^{3/2} \quad (15)$$

F_{flow} is the compression force at the beginning of the flow phase, S_{flow} the contact area between the bolus and the plates at h_{flow} , r_{flow} the radius of the associated cylindrical sample and Λ the sample volume. The stress τ_{flow} , evaluated at h_{flow} , is then considered as an evaluation of the yield stress obtained through the analysis performed for non-collapsed boluses, marking the beginning of the flow phase. s_{flow} , derived from the model fit is correlated to τ_{flow} (Fig.10). Both correlations between s_v and s_{flow} for collapsed boluses and between τ_{flow} and s_{flow} for non-collapsed boluses validate the results given by the Herschel-Bulkley fits on compression curves from real human boluses.

4.2.3. Validation of parameters during the particle phase

The particle size distribution study was based on an analysis of the bolus photographs (Fig.1d), whereby it was possible to estimate the characteristic maximum size of the particles contained in the bolus, denoted d_{part} . The transition height h_{part} also provides information on this characteristic, where fluid behavior gives way to solid behavior. The heights h_{part} have been correlated with the characteristic sizes d_{part} (Fig.11). The parameter h_{part} can subsequently be used to quantify the size of the particles in the case of human bolus from a semi-solid meal.

5. Discussion

5.1. Application of the Chateau theory to model suspensions

An attempt was made to model the relationship between the yield stress and the consistency with the volume fraction, using a model proposed by Chateau (2008). As shown in the method section, the model depends upon two parameters, the intrinsic viscosity $[\eta]$ and the maximum packing volume fraction Φ_m . The hypotheses of the model are suspensions of non-colloidal, monodisperse, hard, and spherical particles. In our suspension, particles can be considered as spherical, but can stick one to another if they are in contact. Moreover, the degree of yield stress is a variable in our system. The results obtained from the Chateau model are plotted on Fig.5 retaining the value of $[\eta] = 2.5$, as derived from Einstein's equation (Einstein, 1911, 1906), and $n = n_0$, with a shear-thinning index equal to that of the suspending matrix. To obtain the best fit with the experimental curves of $[K]$ and $[s]$, a value of $\Phi_m = 55\%$ was chosen. The value is smaller than the classical maximum packing fraction from a random stack of spherical particle ($\Phi_m = 63,5\%$) or from a perfect Apollonian stack ($\Phi_m = 74\%$), but this is logical since the particles in this study are not perfect. As the model tends to suit experimental values of $[s]$ on Fig.5b, the values of $[K]$ are higher, mostly at $\Phi_v = 50\%$. This discrepancy may be due to sticky interactions between the particles due to a jamming effect, which can cause additional viscous loss.

Fig.6 and Fig.7 show a plot of the results of a Chateau model for $\Phi_v = 40\%$, $\Phi_m = 55\%$. $[\eta] = 2.5$. Experimental values of $[K]$ and $[s]$ are closer from the model in the case of a monodisperse suspension (Fig.6), which is in line with the model hypothesis of monodisperse suspensions. Particle yield stress τ_{max} has hardly any significant effect on $[K]$ and $[s]$, but as τ_{max} increases, the uncertainties decrease and the values seem to tend to the theoretical values given by the model. This trend is logical given the model hypothesis of hard particle: as particles become increasingly harder, the model better fits the experiment.

5.2. Application of the Chateau theory to human bolus

In the context of semi-solid food boluses, rheological properties can play an important role in flavor release (Trelea et al., 2008). More specifically, the liquid phase is a vector of aroma since it coats the mouth and the throat during mastication and swallowing (Marie Repoux, et

al., 2012). Once the bolus has been swallowed, a fraction of the liquid remains (Buettner et al., 2001) and extensively participates in the aroma release. Knowledge of its rheological properties is therefore mandatory to validate any mechanical model of aroma release. As the liquid matrix cannot be extracted from the bolus, the previous model of suspension on human food boluses is applied.

Applying the Chateau model to the human bolus, similarly to model suspensions, has to be done with care. Firstly, particles are soft and very polydisperse, and hardness and polydispersity have an effect on the results as shown previously. Secondly, an unknown fraction of cheese has been dissolved in the suspending matrix. The evaluation of the volume fraction, based on the saliva added during mastication, tends therefore to be overestimated. In the case of the cheese tested, it was found that less than 10% of the mass of the particle dissolves in the liquid media.

Herschel-Bulkley parameters s_{flow} , and K_{flow} , obtained from the flow phase of compression curves of boluses, have been plotted as a function of the volume fraction Φ_v (Fig.12). s_{flow} , and K_{flow} increase with the volume fraction, which confirms the suspension behaviour. An attempt was therefore made to apply a Chateau model with $[\eta] = 2.5$ and $\Phi_m = 71\%$ for each bolus. It is proposed to use the model with higher maximum packing fraction, keeping the intrinsic viscosity from a suspension of spherical hard spheres, quite different from the particle shape inside the bolus. This is because, in some cases, a high bolus compactness was observed due to the shape of particles, stuck with a little liquid matrix. To fit a Chateau model for each bolus, the set of parameters of bolus properties (n_{flow} ; s_{flow} ; K_{flow}) is used to get the set of parameters of the related suspending matrix (n_0 ; s_0 ; K_0), with the hypothesis $n_0 = n_{\text{flow}}$.

The properties of the suspending matrix are shown in Table 1. Standard deviations of s_0 and K_0 are high with respect to the panelists, which confirms that each panelist creates different boluses in terms of rheology. The choice of $[\eta] = 2.5$ and $\Phi_m = 71\%$ can be discussed, since the maximum packing fraction can vary with panelist, as well as the intrinsic viscosity which depends upon the shape of the particle.

6. Conclusions

The main objective of this work was to present a methodology using compression flow, to assess the rheological properties of cheese-based food boluses. The results can be used to characterize the boluses quantitatively and thus help identify the pertinent mechanical parameters which rule the mastication and swallowing process, but also the aroma release pattern. The intrinsic correlations between Herschel-Bulkley parameters and consistometry results support the validity of the findings in the case of model suspension and human boluses from a semi-solid meal, even if the system studied is at the edge limit for continuum mechanics.

The second objective was to propose a suspension model, with a view to estimating the rheological properties of the suspending matrix. Knowledge of the suspending fluid properties is mandatory for building a behaviour model of the boluses *in vivo*. Those properties may be causative to the coating process during mastication and swallowing, and by using them in a proper mechanistic model it may be possible to predict aroma release as a function of characteristics of the individuals and also of the products consumed. Those relationships will be published in a forthcoming study.

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7. Figures

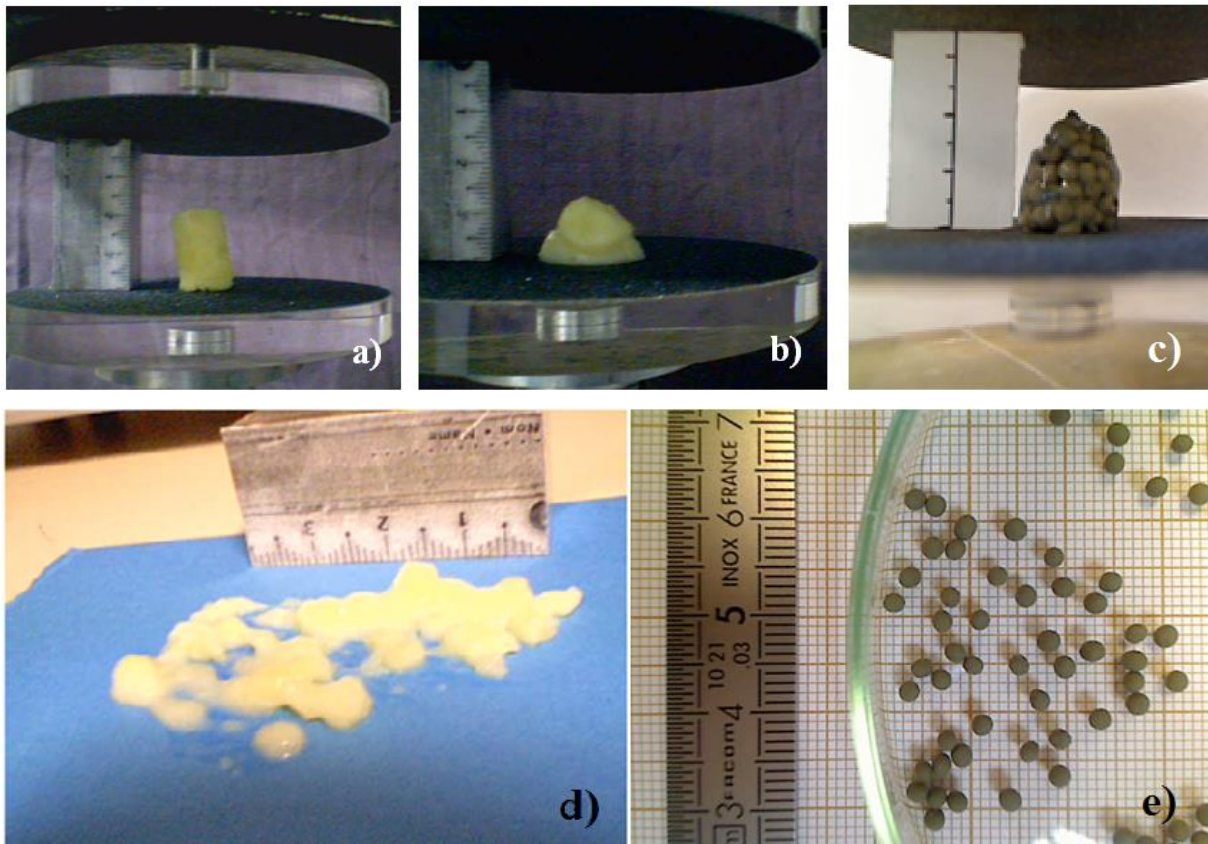


Fig.1: Photographs of boluses before the start of the compression test. Different configurations may be distinguished: (a) The sample has not collapsed, gravity is not sufficient to cause the bolus to flow; (b) The sample has collapsed to different degrees under its own weight; (c) a model suspension prior compression. Photograph (d) and (e) are representative of the particles size in the bolus and in the model suspension.

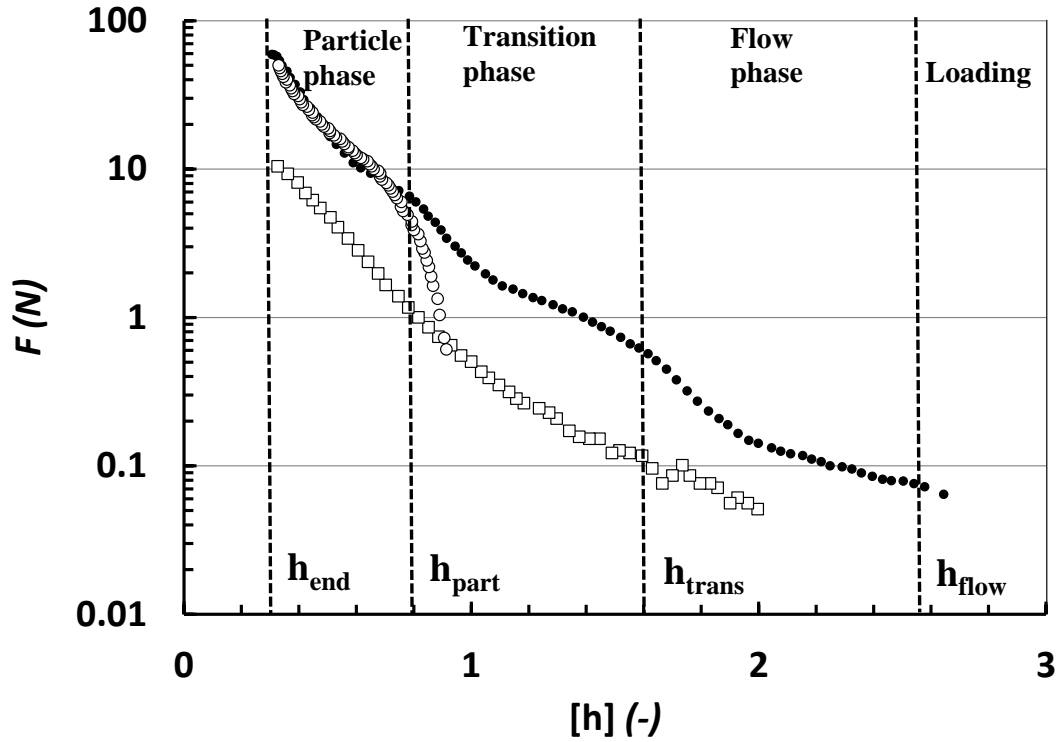


Fig.2: Description of the succession of phases and delimitation of the three behaviors. The compression curve (●) is from a model suspension at $\Phi_v = 30\%$ and $d_{\text{part}} = 3\text{mm}$, with a gel of Carbopol as suspending matrix. The distinction of the three major phases is highlighted by the dotted lines. The loading area corresponds to the beginning of the squeeze, where the top surface of the sample is not yet fully in contact with upper plate. A compression curve (□) of the same volume ($\Lambda = 3 \text{ cm}^3$) of Carbopol gel is also plotted. The order of magnitude in term of force is 5 times lower for the Carbopol alone. The compression curve of exact same number of particles set in pack, without suspending matrix, is plotted with (○). Once the particle phase is reached on the compression curve of suspensions, it overlaps the compression curve of particles alone.

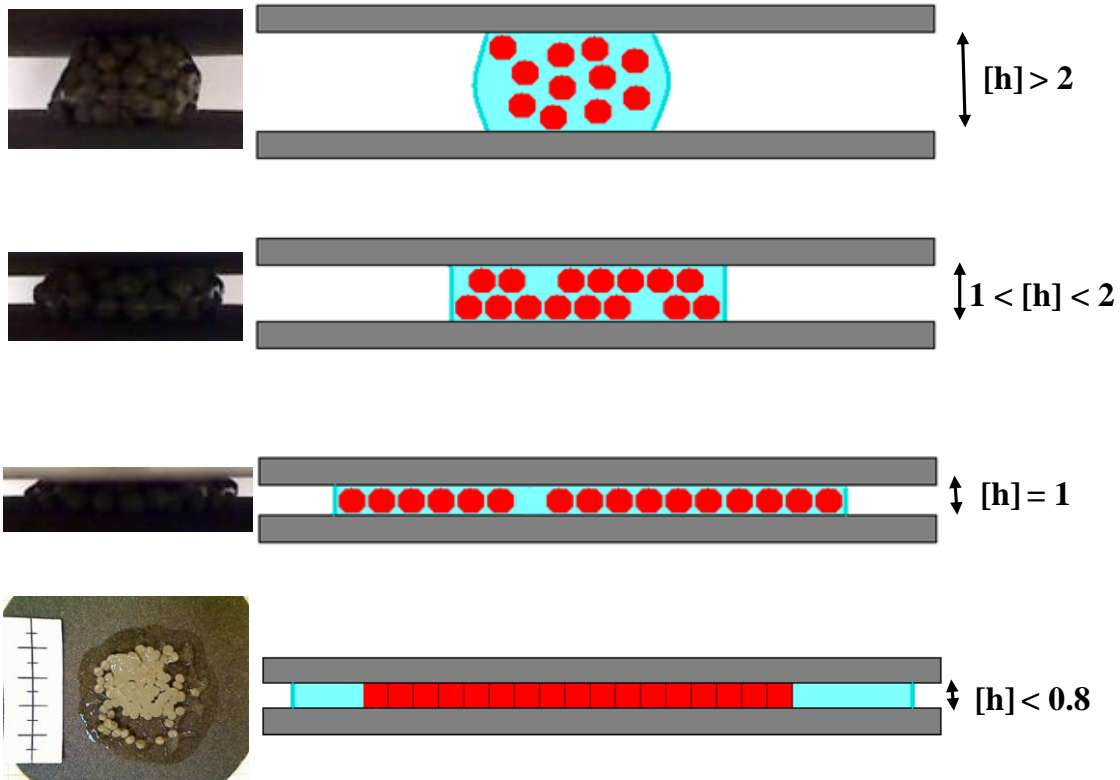


Fig.3: Particles behaviour during the squeeze flow. For $[h] > 2$, there is only weak interaction between particles and the viscous behaviour is predominant. For $1 < [h] < 2$, particles begin to contact each other and the particle behaviour is more and more predominant. For $[h] < 0.8$, particles are squeezed and the suspending matrix is expelled.

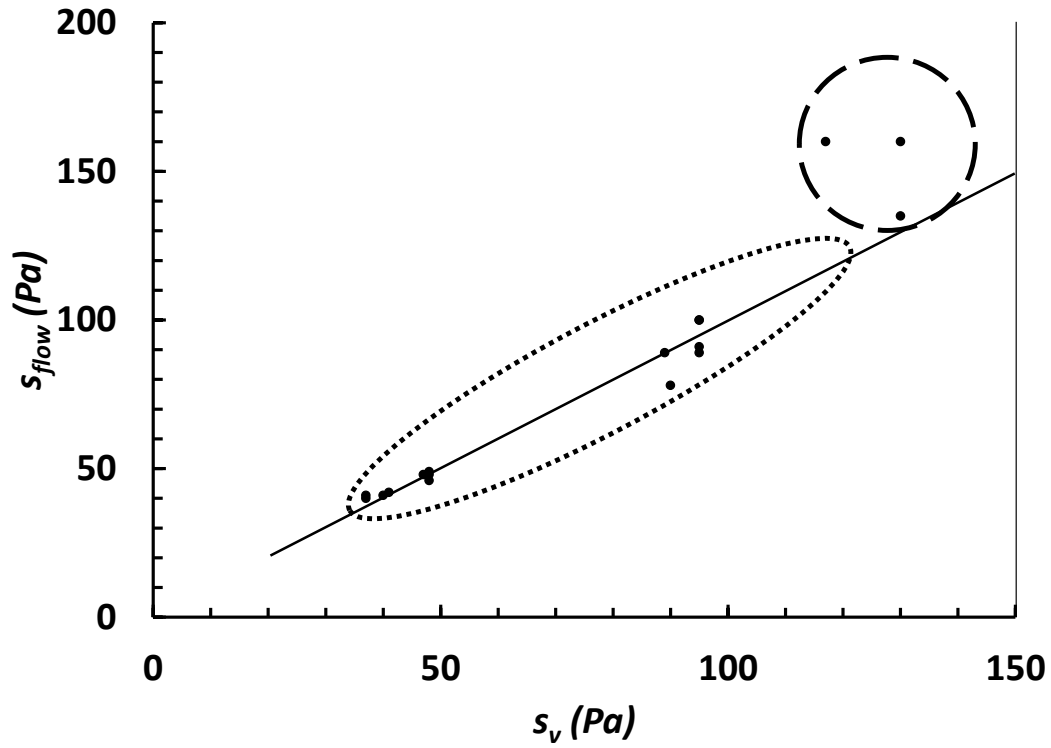


Fig.4: Correlation between yield stress s_v calculated with consistometry, and yield stress s_{flow} of model suspensions from the Herschel-Bulkley fit. The correlation is only valid for collapsed model suspensions (zone - - -), with $R^2=0.98$. The correlation is no longer valid for uncollapsed suspensions (zone — — —), where $\Phi_v > 50\%$ in the case of model suspensions.

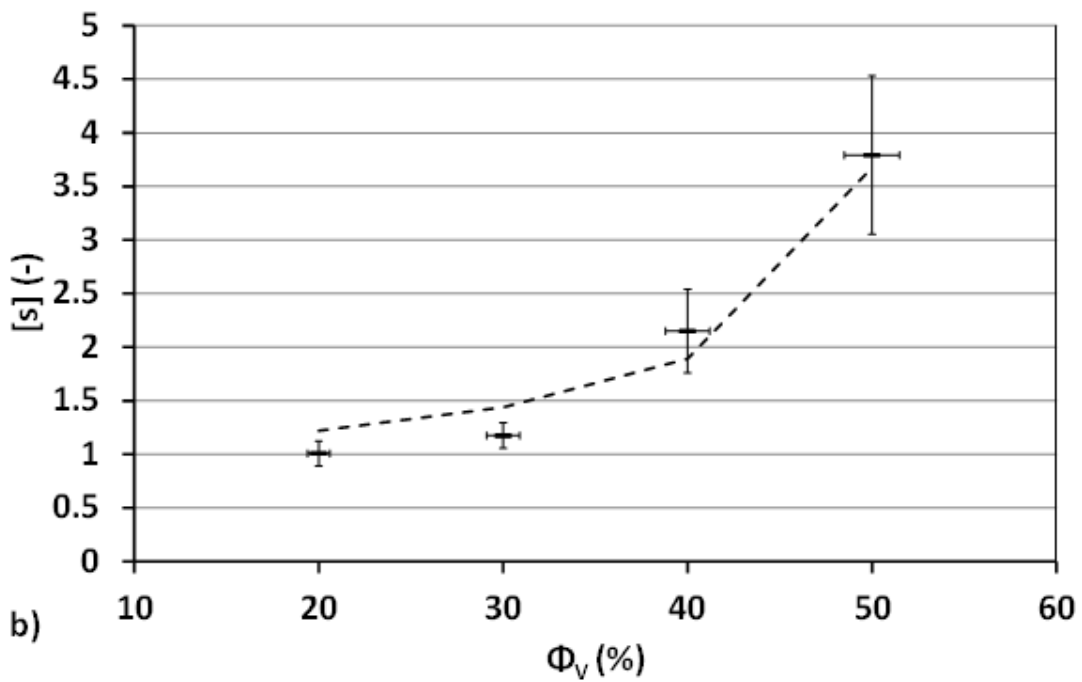
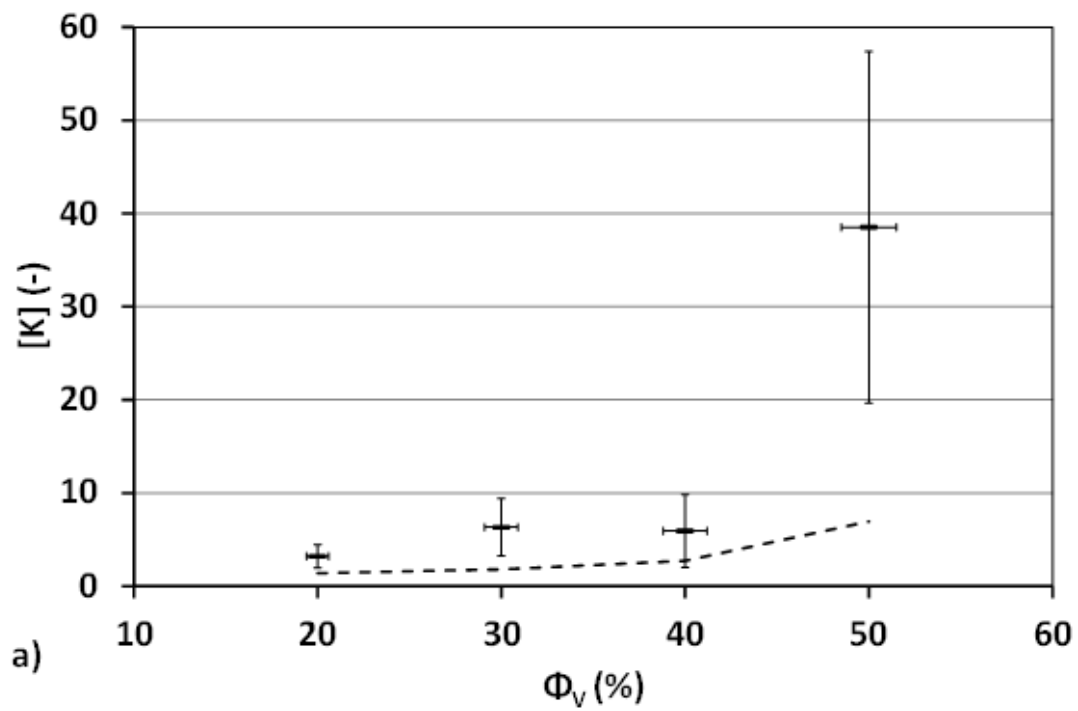


Fig.5: For model suspensions, dimensionless consistency [K] and yield stress [s] are plotted according to Φ_V . A fit of the Chateau model is presented with the intrinsic viscosity $[\eta] = 2.5$ and the maximum packing volume fraction $\Phi_m = 55\%$.

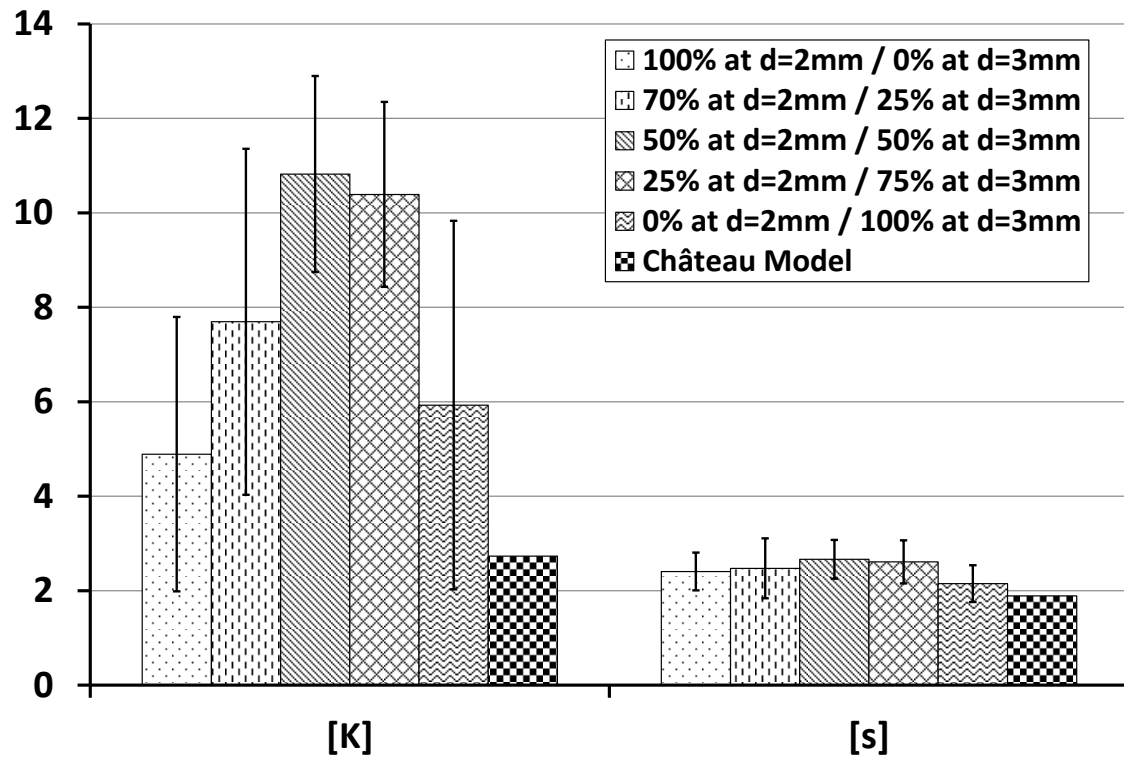


Fig.6: Effects of polydispersity on dimensionless consistency [K] and yield stress [s] for model suspension at a constant $\Phi_v = 40\%$, with different proportion of particles of 2 and 3 mm diameters. Comparison is made with a Chateau model ($[\eta] = 2.5$; $\Phi_m = 55\%$).

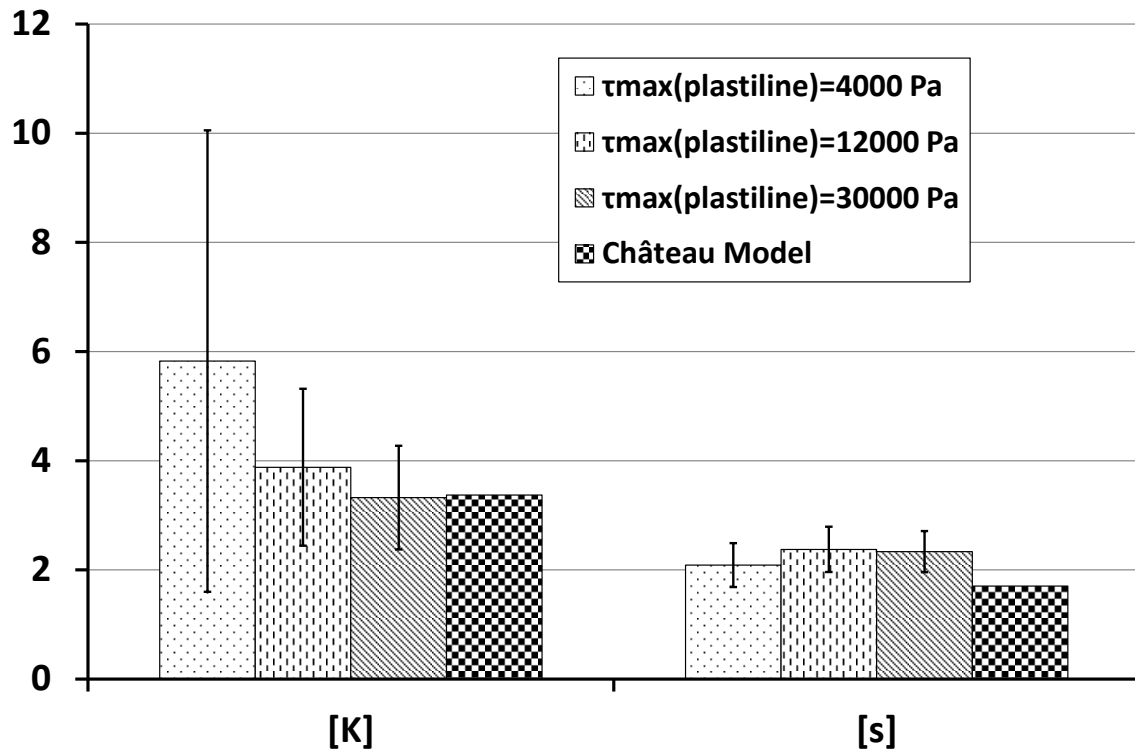


Fig.7: Effects of particles yield stress on dimensionless consistency [K] and yield stress [s] for model suspension at a constant $\Phi_v = 40\%$. Comparison is made with a Chateau model ($[\eta] = 2.5$; $\Phi_m = 55\%$).

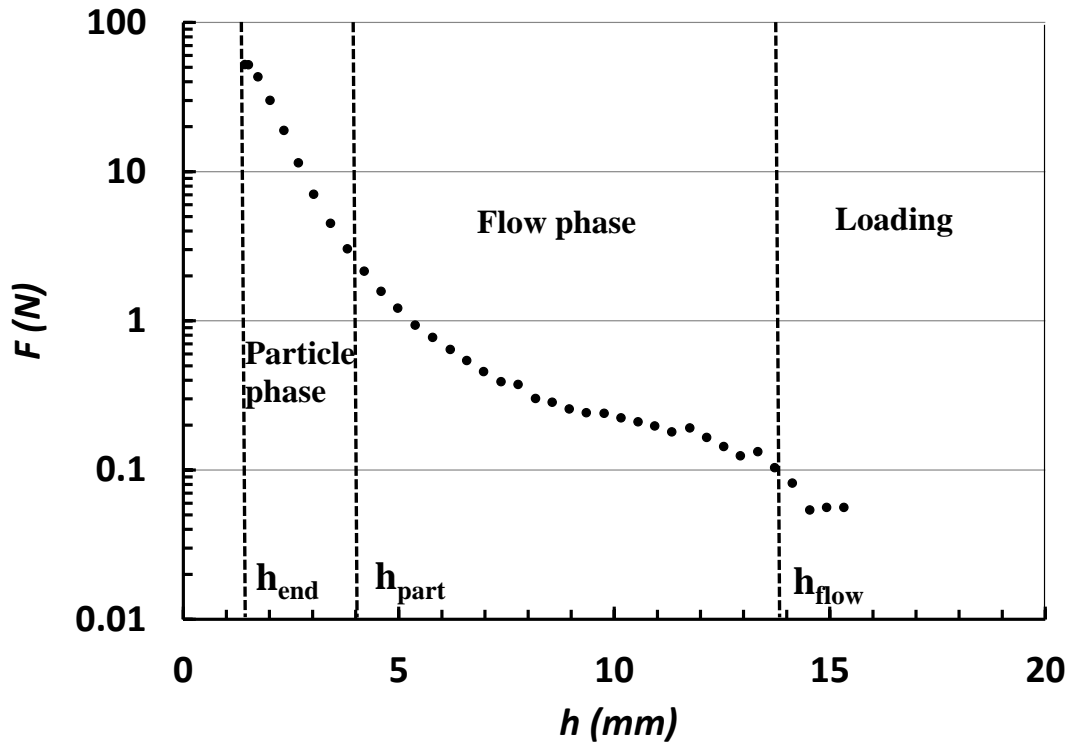


Fig.8: Description of the succession of phases, delimited by dotted line, for a human bolus from cheese. The compression curve (\bullet) is from a panelist after eating a cheese sample. The loading area corresponds to the beginning of the squeeze, where the top surface of the sample is not yet fully in contact with upper plate.

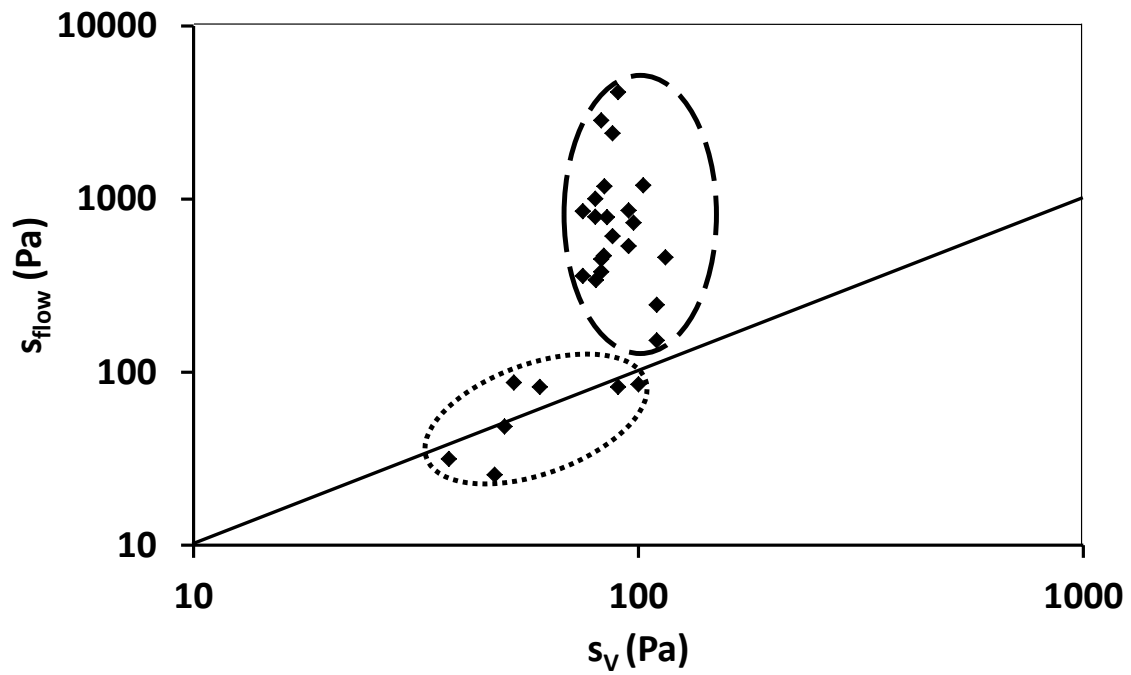


Fig.9: Correlation between yield stress s_v calculated with consistometry, and yield stress s_{flow} of human boluses from the Herschel-Bulkley fit. The correlation is only valid for collapsed model suspensions (zone - - -), with $R^2=0.88$. The correlation is no longer valid for uncollapsed suspensions (zone . . .).

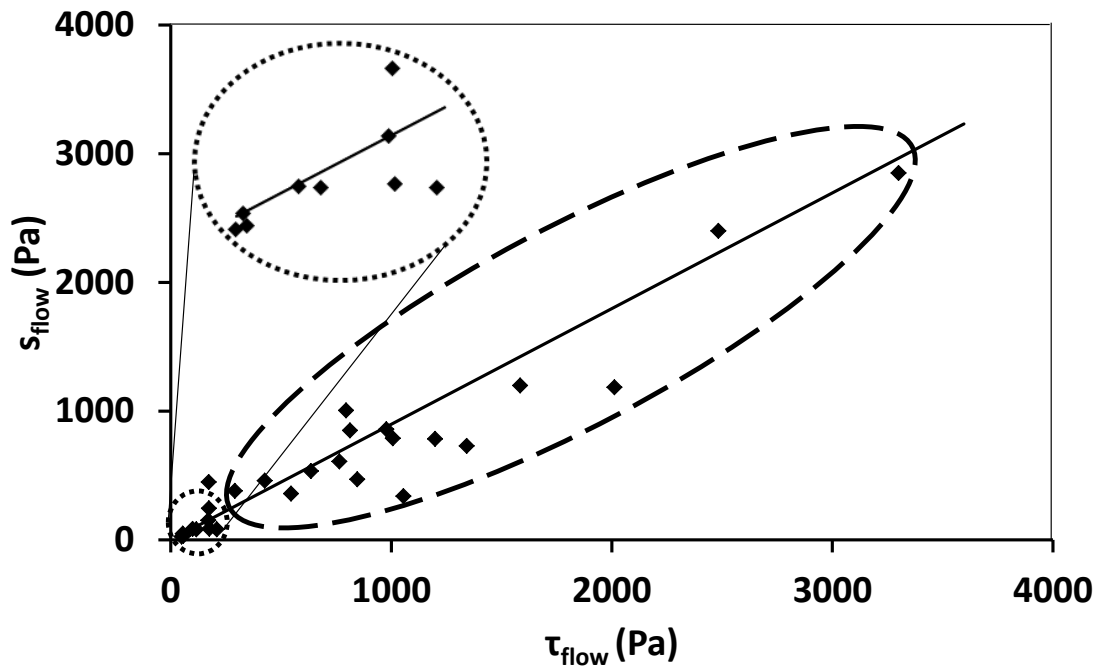


Fig.10: Correlation between τ_{flow} , measured stress at the beginning the flow phase, and the yield stress s_{flow} of human boluses extracted from the Herschel-Bulkley fit. The correlation is only valid for collapsed boluses (zone - - -) and uncollapsed boluses (zone — — —), with $R^2=0.90$.

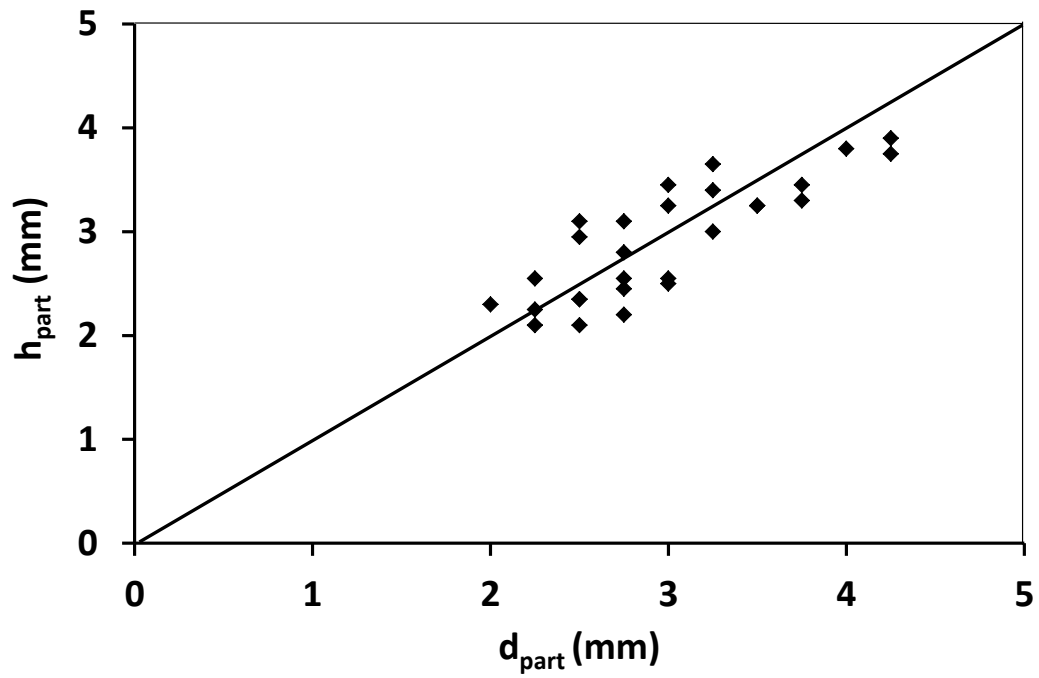


Fig.11: Correlation between d_{part} , the maximum particle diameter observed on boluses picture, and h_{part} , the height at the beginning of the particle phase extracted from the Herschel-Bulkley fit. The correlation has a regression coefficient of $R^2=0.72$.

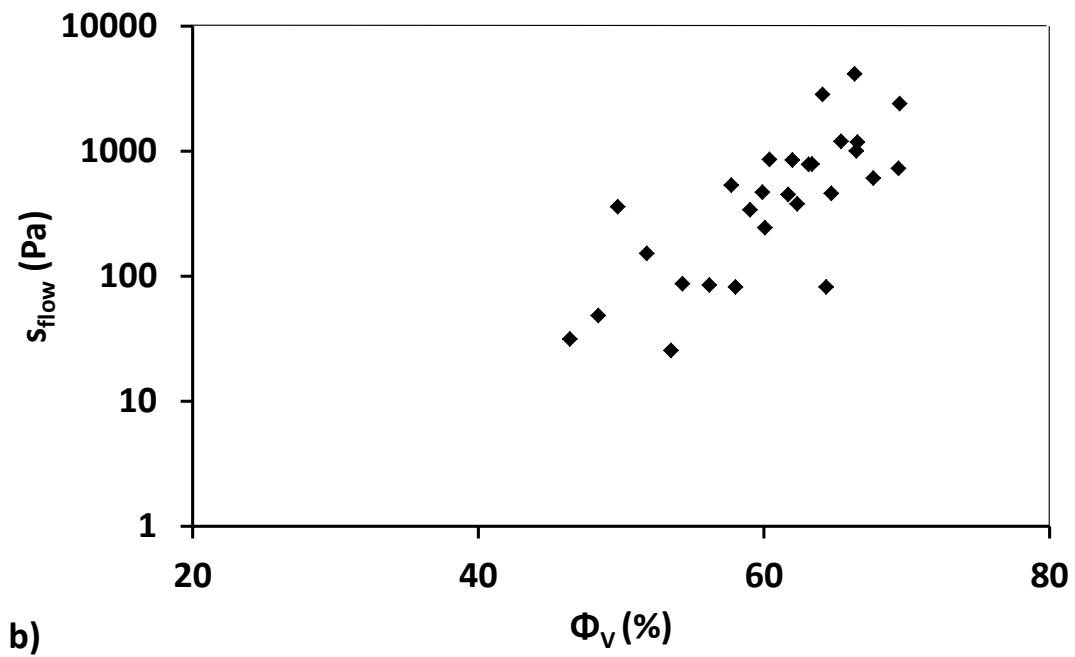
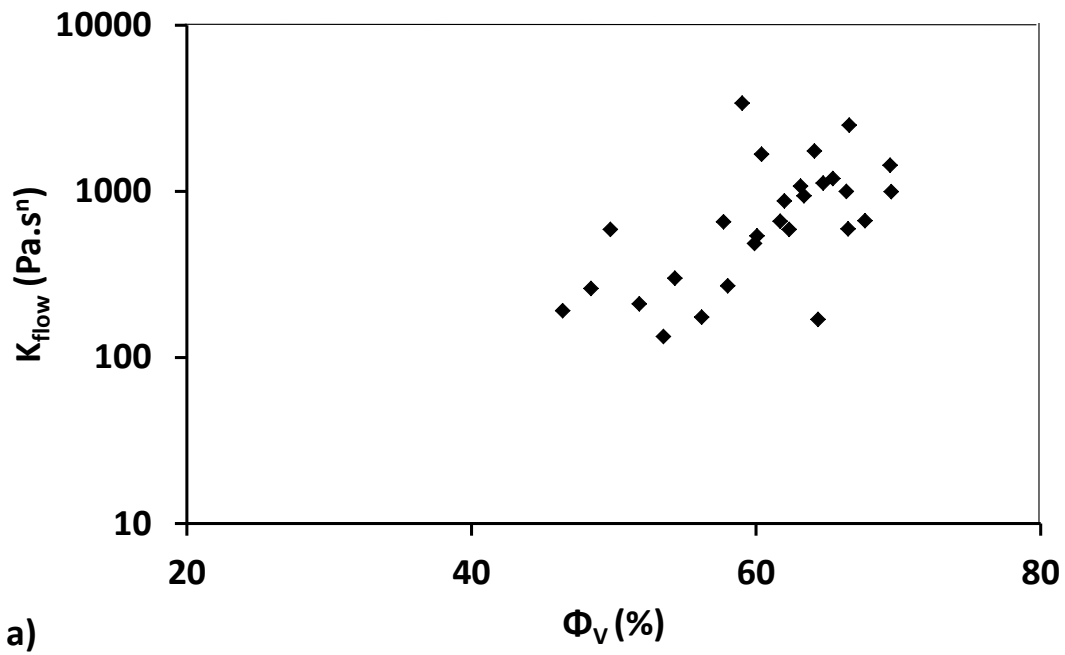


Fig.12: Consistency K_{flow} and yield stress s_{flow} are displayed according to Φ_V from human bolus. After fitting an Herchel-Bulkley model, values are the mean of 2 repetitions over 28 panelists.

Table 1

Herschel-Bulkley parameters of human boluses and related data, extracted from compression curves, as well as Herschel-Bulkley parameters of the suspending matrix calculated with the suspension model proposed. Standard deviations are calculated over a panel of 28 individuals.

Parameters	Suspension Rheological properties						Other suspension properties			Suspending matrix rheological properties	
	n_{flow}	S_{flow}	K_{flow}	h_{flow}	s_{part}	h_{part}	d_{part}	S_{end}	ϕ_v	s_0	K_0
Unit	(-)	(Pa)	(Pa.s ^{n_{flow}})	(mm)	(Pa)	(mm)	(mm)	(mm ²)	(%)	(Pa)	(Pa.s ^{n_{flow}})
Moyenne	0.74	759	873	9.8	3242.5	3	3	2004	60.4	137	57
SD (%)	15	80	61	17	45	17	16	25	8.3	65	88

Chapitre 1.3

Rôle de la rhéologie des bols alimentaires issus de fromages dans la libération aromatique

Le processus de consommation d'un aliment facilite l'ingestion de nourriture et contribue également à la libération des stimuli responsables de la perception. D'un point de vue mécanique, le processus se déroule en trois phases distinctes (Fig.1 a-b) :

- la mastication, où les aliments sont mâchés et mélangés à la salive. Elle a pour but de détruire les macrostructures, déstructuration renforcée par l'action d'enzymes, d'augmenter la température et de diminuer la consistance du bol alimentaire. Suite à la mastication, le mouvement de déglutition est initié par la pression de la langue sur le palais osseux.

- la phase pharyngée, qui commence au moment du déclenchement du réflexe de déglutition, dure environ 1 seconde, et finit par la fermeture du sphincter œsophagien supérieur. Au cours de cet écoulement, une partie du bol alimentaire est déposé sur les muqueuses pharyngées.

- le transport du bol alimentaire par le péristaltisme primaire et secondaire, avec des contractions et des relâchements successifs des muscles de l'œsophage.

Au travers de ces phases, le goût chez l'être humain est une combinaison complexe de sensations (Fig.2), ressenties aux différents stades de la déglutition. Les sensations sont :

- l'impact initial : l'aspect visuel et l'odeur,
- la perception pendant la mastication : la texture, la température et les goûts primaires (sapidité : salé, sucré, amer, acide...), et une partie des arômes libérés qui transitent vers le nez dans le cas de l'ouverture du voile du palais (Buettner et al., 2001), captés par les capteurs olfactifs,

- la perception après la déglutition, produite par les différents composés aromatiques libérés des résidus déposés sur les parois du pharynx, qui transitent vers les capteurs olfactifs lors de l'expiration post-déglutition, dite « swallow-breath » (Buettner et al., 2001). ». Ce flux

d'air (5 à 15 ml) d'environ 3 secondes pour un débit de 1 à 2 l/min venant des poumons remonte vers le nez. Les arômes issus de la couche déposée sur les parois sont libérés et transportés vers le nez, où un pic de sensation est alors perçu. Les sensations peuvent perdurer au fil des expirations, tout en perdant en intensité dans le temps.

Dans le cas de l'ingestion d'aliments « fluides », la libération aromatique se concentre sur la partie rétronasale. En effet, une barrière physiologique formée par la base de la langue et le velum (palais souple) empêche ces derniers de passer (Buettner et al., 2001). Dans le cas des aliments semi-solides ou solides, l'ouverture du velum dépend de la consistance de l'aliment et des individus, car il a pour fonction première de protéger le pharynx et les fonctions respiratoires au cours de la mastication. Alors que l'individu respire en mâchant, une partie des aliments pourrait venir se loger dans le larynx, ou la trachée ou même les poumons, trouble appelé dysphagie, au passage une cause de mortalité majeure notamment chez les personnes âgées. En cas d'ouverture, une partie des arômes vient à passer dans la cavité nasale, entraînant une perception aromatique au cours de la mastication. Ainsi les arômes, dans le cas des aliments semi-solides ou solides, peuvent être détectés lors de la mastication, et après la déglutition. La libération aromatique doit donc être étudiée au cours de ces deux instants.

Une meilleure compréhension de la perception des arômes doit prendre en compte la dynamique de libération des saveurs (Piggott, 2000). Malgré les nouveaux développements pour mesurer *in vivo* la libération de molécules au cours de la mastication des aliments en utilisant l'API-MS (Taylor et al., 2000), les causes des différences observées dans la perception sensorielle entre matrices avec différentes structures ne sont pas bien définies, ce qui suggère un effet combiné des essais physico-chimiques, mécanistiques et cognitifs. La libération des arômes est un processus séquentiel complexe impliquant le type de denrée alimentaire, le transfert des matières volatiles à la phase gazeuse, le transfert des arômes aux voies aériennes supérieures et la persistance de la saveur (Linthorpe and Taylor, 2006). La libération des arômes dans la cavité nasale atteint sa concentration maximale au cours de la déglutition, en raison de paramètres physiologiques (Buettner et al., 2001; Saint-Eve et al., 2006). Les différences observées dans les modèles physico-chimiques de libération peuvent s'expliquer en partie par des modes de consommation particuliers (Mestres et al., 2006). Il a été aussi montré que des produits de duretés différentes induisent des comportements

masticatoires conduisant à des différents modes de libération *in vivo* (Gierczynski et al., 2007).

Certaines études ont été menées pour corrélérer les données provenant de la perception sensorielle, des paramètres physiologiques et de la libération des arômes *in vivo*, obtenues sur le même sujet. Mais, les relations entre la physiologie de la mastication, les flux salivaires, la libération des arômes et des goûts primaires et leur perception ne sont pas bien établies (Pionnier et al., 2004). Ces résultats soulignent la nécessité de mieux comprendre les mécanismes de libération de l'arôme au travers d'une caractérisation rhéologique.

Nous cherchons au travers du présent article à identifier quels paramètres gouvernent la quantité de bol déposée dans le cas d'aliment semi-solide. Cet article met en lien les propriétés rhéologiques des bols alimentaires avec leur structure. À partir des résultats précédents, l'influence de la phase suspendante au sein des bols alimentaires sur la libération sera analysée. De plus, des données de libération aromatiques sont mesurées au travers d'une méthode développée par l'équipe Flavi du CSGA. Les données rhéologiques et sensorielles proviennent d'un panel de personnes sélectionnées pour l'étude. Enfin, les liens entre la rhéologie des bols alimentaires et la libération des arômes sont analysés et discutés dans l'article.

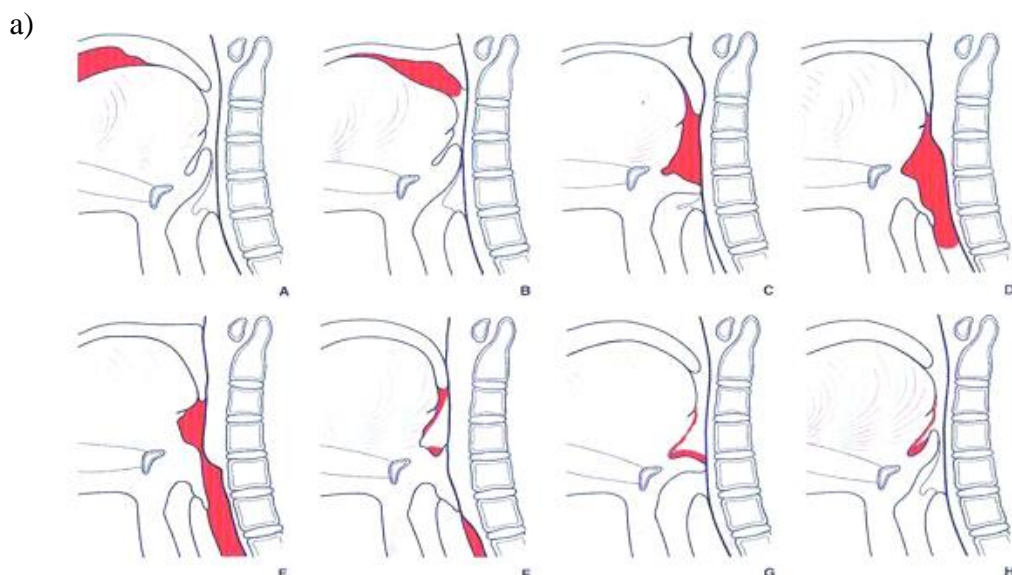




Fig.1 : (a) Séquence du transport du bol alimentaire au travers des phases schématisées de la déglutition où une partie du bol (en rouge) se dépose sur les muqueuses pharyngées. (b) La vidéofluoroscopie permet de visionner la séquence par l'ingestion d'une solution de baryte en noir. (c) Le dépôt des résidus à l'origine de la libération des arômes est simulé avec un bol coloré sur une muqueuse porcine.

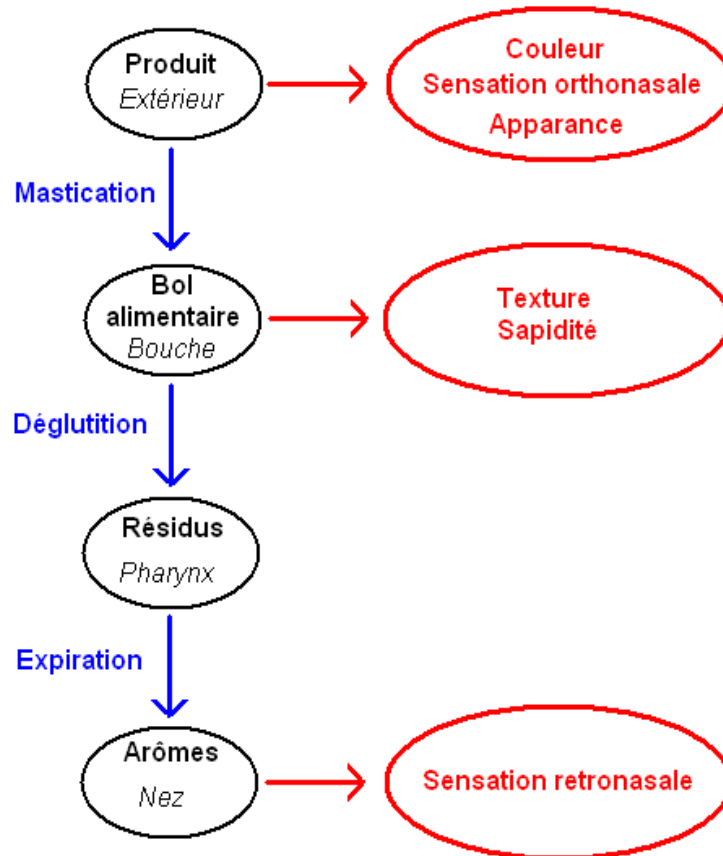


Fig.2 : Génération du goût. En noir les états du produit, en bleu les procédés qu'il subit, en rouge les sensations captées.

Rheology of heterogeneous boluses from cheese: effects on *in vivo* aroma release in human

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Abstract:

The aim of this work was to clarify the influence of the bolus rheological properties from a solid processed model cheese on *in vivo* aroma release while considering the hydrophobicity of aroma compounds, on a large number of well characterized subjects. *In vivo* aroma release was studied on 28 subjects who freely consumed four processed model cheeses flavoured with the same concentration of nonan-2-one and ethyl propanoate. Cheeses had two levels of firmness and two levels of fat content, making four cheeses. Globally, an increase in yield stress of the bolus induced an increase in total amount of aroma released. The release clearly differed between the two aroma compounds. Ethyl propanoate presented a higher release for low-fat cheese and was more released during the mastication step, whereas nonan-2-one was more released in case of high-fat cheeses, due to its higher hydrophobicity. Aroma release is related to the breakdown behaviour of the original cheese: firm cheeses tend to create heterogeneous suspension and soft cheeses create continuous pasty bolus. The yield stress of the bolus suspending matrix was found related to the intensity of aroma release, notably for firm cheeses. The results could be helpful to better understand the relative influence of the parameters related to products and subjects in order to reformulate foods with good sensory acceptability.

1. Introduction

Many studies in recent years have dealt with texture and aroma release from semi-fluid food bolus (Buettner et al., 2001; de Wijk et al., 2003a, 2003b; Feron et al., 2014; Lucas et al., 2002; Repoux et al., 2012a; Yven et al., 2012). Solid foods, like cheese meat or bread, tend to generate heterogeneous bolus with millimetric-size particles (Chen, 2009; Peyron et al., 2004; Prinz and Lucas, 1997, Patatin et al., 2014b, 2014c). In consequence, relevant rheometry on semi-fluid bolus with particles must be carried out with specific test conditions (Patarin et al., 2014a, 2014c); and results are dependent of on panelists physical characteristics and masticatory behaviour (Buettner et al., 2001; Gierczynski et al., 2008; Mishellany-Dutour et al., 2012).

Concerning aroma release, reliable data on perceived olfactory sensations are also extremely difficult to obtain during ingestion of food (Gierczynski et al., 2011). As most aroma compounds are more soluble in fat than in water, an increase in fat content decreases the amount of aroma compounds in the vapour phase (Guichard, 2002) and also the amount of aroma released during the in-mouth process. This can be explained by a modification of the partitioning of aroma compounds between the different phases present in the mouth, depending on their physico-chemical characteristics (Brauss et al., 1999; Miettinen et al., 2004; Tarrega et al., 2008). Nevertheless, the literature has produced heterogeneous or contrasting results regarding the effects of food matrix texture on aroma release (Gierczynski et al., 2008), showing for example a higher effect for hard products such as cheeses than for soft products such as yogurts (Mei and Reineccius, 2007). Besides the diversity of the physical structure of matrices (i.e. liquid, semi-solid or solid) and aroma compounds, different consumption protocols (Aprea et al., 2006; Lethuaut et al., 2004) were used and different parameters extracted from the release curves (Boland et al., 2006; Repoux et al., 2012a; Weel et al., 2002). Moreover different between-subject aroma release patterns have often been observed (Gierczynski et al., 2008; Mestres et al., 2006) which could be related to differences in velum opening (Buettner et al., 2001; Repoux et al., 2012c). However other in-mouth processing parameters, such as the chewing characteristics, quantity and composition of saliva and the rheological properties of the bolus, are involved in this dynamic phenomenon (Salles et al., 2010) and could also explain differences in aroma release.

In the case of cheeses, previous studies have demonstrated that the rheology of bolus can be correlated with the masticatory behaviour of panelist (Yven et al., 2012), in-mouth

residues (Repoux et al., 2012b) and aroma released from the matrix (Feron et al., 2014). A mechanical approach has been describe by Doyennette et al. (2014) and Trelea et al. (2008) to explain the aroma release process from bolus. The bolus may spread around the mouth and create exchange areas with the in-mouth air during a certain residence time. The initial composition of the cheeses, i.e. the fat content and water content, may impact the aroma release (Guichard, 2002).

The present study aims to evaluate the rheological properties of different cheeses, and to evaluate their effects on aroma release during the mastication and swallowing steps over a panel of 28 persons. We also aim to propose an explanation to the different correlations, considering the suspension nature of the bolus: food particles immersed in a non-Newtonian fluid matrix. The results could be helpful to better understand the relative influence of the parameters related to products and subjects in order to reformulate foods with good sensory acceptability.

2. Materials

2.1. Cheese base

Four cheese bases were provided by two industrial companies, Fromagerie Bel (Paris, France) and Soredab (La Boissière-Ecole, France). Table 1 lists their names and their attributes. They are melt cheeses and do not contain any texturizing agent. Two broad categories of cheese were used, featuring two different fat contents adjusted by added butter. The two categories will be referred to subsequently as "low-fat cheeses" (l) and "high-fat cheeses" (h) respectively. Within each category, there are two levels of hardness: soft and hard. The firmness was adjusted with the water content. The pH ranged from 5.27 to 5.55. The rheological characterization was carried following the vane test methodology proposed by Patarin et al. (2014b). Results are also display in Table 1.

Cheeses were flavored as described in Repoux et al. (2012c). Two aroma compounds were used with different hydrophobicity ($\log P$) and vapour pressure P_v : nonan-2-one ($\log P=2.9$; $P_v=83.5$ Pa at 25°C), labeled NO; and ethy propanoate ($\log P=1.4$; $P_v=4790$ Pa at 25°C), labeled EP.

2.2. Panel

Twenty-eight panelists (males and females from 20 to 60 years old) were recruited following a medical questionnaire and bucco-dental examination from a group of 174 volunteers. They were selected on their good dental status and on physiological parameters (salivary flow, respiratory flux, masticatory behaviour, saliva composition, oral volume...) for their normality and repeatability. All subjects gave informed consent. The protocol of the study was submitted to the local ethical committee and approved on 17th April 2008 by the Comité de Protection des Personnes Est-1 (N°2008/15) and on 8th August 2008 by the Direction Générale de la Santé - France (N° DGS2008-0196).

3. Methods

3.1. Bolus rheology

Compression rheometry was used to characterise the rheological properties of the semi-fluid bolus, following the methods described in Patarin et al. (2014c). The panelists were given 3 g of cheese to chew and spat out the bolus freely when they felt ready to swallow. The bolus samples were created with the truncated syringe into which the bolus had previously been spat out. A volume of $\Lambda=3\text{mL}$ of bolus was then tested to extract the rheological properties, while the bolus flowed between the two plates of the compression device. The temperature of the samples was measured systematically before compression. Less than 30 seconds elapsed between the bolus being spat out and the start of the test.

The boluses are assumed to be non-thixotropic yield-stress fluids. They will be considered to be homogeneous, modeled by a Herschel-Bulkley fluid with a constitutive law given by the expression:

$$\tau = s + K\dot{\gamma}^n \quad (1)$$

where n is the power-law index, K the consistency, s the yield stress, $\dot{\gamma}$ the shear rate and τ the shear stress.

Analytical solutions have been proposed for the change in compression force. For Herschel-Bulkley fluids, at constant volume and with wall adherence conditions, Covey and Stanmore (1981) proposed a model where the compression force is related with the height between the plates, to evaluate the rheological properties. Details are given in Patarin and al. 2014c.

Examination of the compression curves reveals three phases (Fig.1). For each phase, the model of Covey and Stanmore is fitted on experimental data, to extract the parameters of Herschel-Bulkley model. The first phase, called “loading”, corresponds to the phase where the compression flow is established at a height called h_{flow} . Boluses of the same volume exhibit indeed different heights before compression (Fig.2), and those were correlated with the yield stress of the cheese using the consistometry technique (Patarin et al. 2014c).

The second phase is the “flow phase”, the bolus flows in bulk and the suspended particles are reorganized upon a squeeze flow. The phase is representative of the flow that occurs in mouth. The parameters determined by fitting Covey and Stanmore model are named n_{flow} , s_{flow} and K_{flow} . Its yield stress is naturally below than the yield stress of the associated cheese (s_{cheese}). The dimensionless yield stress $[s_{\text{flow}}] = s_{\text{flow}} / s_{\text{cheese}}$ is defined, to create a parameter independent of cheese.

In the last phase, called “particles phase”, particles govern the mechanical behaviour, and the viscous component becomes negligible in comparison to the yield stress component s_{part} . The phase begin at a height called h_{part} , which has been previously positively correlated to the maximum diameter of the particles inside the bolus (Patarin et al. 2014c). In the case of cheese, the particles have been softened during the mastication process with the action of saliva. The yield stress s_{part} is therefore lower than s_{cheese} , and the dimensionless yield stress $[s_{\text{part}}] = s_{\text{part}} / s_{\text{cheese}}$ is defined. The phase end at a heigh called h_{end} , while the bolus is compressed at a force of 50N.

The percentage of water content was determined using an infrared dryer and a balance. The volume fraction of the boluses Φ_v was therefore calculated by measuring the dry matter of the bolus DM_{bolus} and knowing the dry matter of the cheese DM_{cheese} . The calculation has been carried out with the assumption that the volume fraction Φ_v is nearly equal to mass fraction, based on equivalent density between the water and cheese. The volume fraction Φ_{bolus} is estimated by the equation:

$$\Phi_v \approx \Phi_{\text{mass}} = \frac{DM_{\text{bolus}}}{DM_{\text{cheese}}} \quad (2)$$

the rheological properties of the matrix can be determined applying a suspension model.

As proposed in Patarin et al. (2014c), a model from Chateau et al. (2008) is applied on suspensions of soft particle suspended in a yield stress matrix. The model offers relationships

between the Herschel-Bulkley parameters of the suspension (s ; K ; n), and parameters of the suspending matrix (s_0 ; K_0 ; n_0), with Eq.3 or Eq.4:

$$s = s_0 \sqrt{(1 - \phi) \left(1 - \frac{\phi}{\phi_m}\right)^{-[\eta]\phi_m}} \quad (3)$$

$$K = K_0 \sqrt{(1 - \phi)^{1-n} \left(1 - \frac{\phi}{\phi_m}\right)^{-[\eta](n+1)\phi_m}} \quad (4)$$

Φ_m is representative of the ability of the suspension to create a compact structure, and $[\eta]$ is representative of particle shape. Based on this model, n was assumed independent of the volume fraction, i.e. $n=n_0$.

3.2. Flavour release

In vivo aroma release was assessed using Atmospheric Pressure Chemical Ionization–mass spectrometry (APCI-MS), with the same procedure and device used by Feron et al. (2014), Giercynski et al; (2008), Repoux et al. (2012a) and Doyonnette et al. (2014). Both compounds were monitored according to their protonated molecular ion (MH⁺): EP ($m/z = 103$) and NO ($m/z = 143$).

Each subject was asked to position a plastic tube in one nostril and to breathe normally. After an initial swallow, the subject was instructed to intake the piece of cheese (6 g), and to consume it freely while keeping the lips closed. The times of swallowing were recorded using a sensor placed on the subject's throat. Bread, apple and water were used as mouth cleansers between two tests.

After smoothing the curves to eliminate signal fluctuations due to the subject's breathing pattern, two release phases were identified (Fig.3). The mastication step extended from placing the cheese in the mouth to the first swallowing, and the post-swallowing step extended from the first swallowing to the time at which the signal returned to its baseline level. For both release phases and for each aroma compound, three main parameters were extracted from each individual release curve: the area under the curve A (a.u. : arbitrary unit) representing the quantity of aroma released and the maximum intensity I_{max} (a.u.), for both release phases and for both aroma.

3.3. Statistical analyses

ANOVA tests with Prism (GraphPad Software, USA) were performed on rheological parameters for each panelist. For correlation analyses, Pearson tests were performed on a different set of parameters, including the uncertainties and variations due to repetitions.

4. Results

4.1 Inter individual analysis

Huge inter-individual differences are found regarding the volume fraction, the rheological properties and the aroma release, whatever the cheeses. The individual behaviors are preponderant face to cheese nature, and the ANOVA analysis show $p > 1$. As an illustration, data presented in Table 2 are the means and standard deviations of parameters over the 28 panelists. However intra-individual variability was correct, and mean values were then used to compare of one parameter to another. This type of analysis gives significant results which are exhibited in the next sections. No significant differences were found regarding the results of men and women in terms of bolus rheology and aroma release.

4.2. Rheological behaviour

The boluses behave as a concentrated suspension of cheese particle into a yield stress matrix. The rheological properties s_{flow} and K_{flow} depend therefore upon the volume fraction Φ_v (Fig.4). This relation is related with the cheese nature, and the influence of Φ_v is more important on s_{flow} , with a variation over 3 decades, than on K_{flow} which values varies on 1 to 2 decades. This fact can explain why more correlations were found with s_{flow} in the rest of the analysis, as its variations are greater than the inter-individual variability. On Fig.4 n_{flow} is also shown as a function of Φ_v .

The rheological test can also provide the properties of the particles, extracted from the “particle phase”: yield stress s_{part} and granulometry, represented by h_{part} . A linear correlation between $[s_{\text{flow}}]$ and $[s_{\text{part}}]$ is found (Fig.5) with different slopes according to the cheese nature. Firm cheeses present higher slopes than soft cheeses, as the rheological properties of bolus from firm cheeses may be more dependent from the rheology of its suspended particles. Beyond the relation between the particles rheological properties and the bolus rheological properties, the consistency of the soft particles do not entirely govern the rheology of the suspension. The bulk rheology of the bolus is highly related to the rheological properties of

the suspending matrix. Knowing those properties may be useful to fully understand the aroma release process. However its properties cannot be directly obtained through the measurement method, because the fluid cannot be easily separated from the bolus without modifying its rheological properties. As proposed in Patarin et al. (2014c), the rheological properties of the matrix can be estimated by applying a suspension model, like the Chateau model. Those calculations give an evaluation of the matrix yield stress s_0 and consistency K_0 , with the assumption that the flow index $n_0=n_{flow}$. Φ_m is representative of the ability of the suspension to create a compact structure, and $[\eta]$ is representative of particle shape. The choice of both parameters for model fits may be questionable. They may depend on the cheese and the individual behaviour, on which we have a lack of information. In first approximation, we choose $[\eta] = 2.5$ as this factor is related to the shape of particles, with are considered as spherical, and a Chateau model is fitted for each cheese (Fig.4). Different Φ_m were obtained, which correspond to a maximum packing fraction of particle. Φ_m are higher for high fat cheese, as the particle may be more deformable and then may create denser pack. Firm cheeses present suspending matrix with higher yield stress s_0 . The consistency K_0 varies on a smaller interval in the same way. To better fit the and to take into account the individual variability, we choose to apply the Chateau model on each individual, to generate $(s_0 ; K_0)$ based on related $(n_{flow} ; s_{flow} ; K_{flow})$, $[\eta]=2.5$ and particular Φ_m of the cheese. The fluid matrix may be a great source of aroma, notably once the bolus has been swallowed, as it may stick to the mucosa. Thus, its rheological properties may be related to the aroma release. Those correlations will be discussed in the proper section.

The rheological properties of high-fat cheeses have higher $[s_{part}]$ than the low-fat cheeses, whereas their $[s_{flow}]$ ratios are lower, as shown on Table 2. However, the particle size distribution represented by h_{part} appears to be heavily dependent on the hardness of the initial cheese. This provides information on the two types of bolus structure, illustrated by Fig.6. Low-fat cheeses consist of an assembly of relatively unaffected particles suspended in a distinct fluid phase, which ensures the cohesion of the system (Fig.6 a & c). High-fat cheeses consist of particles that are much more broken down by saliva, with a hardly observable fluid phase owing to the fact that the saliva is well mixed with the dissolved cheese. The bolus thus appears visually to be much more homogeneous, as the particles, which are indeed present, are more blended into the bulk (Fig.6 b & d).

4.3. Aroma Release

All rheological parameters are tested with aroma release parameters, and few correlations were found. Among these, s_{flow} can be correlated with aroma compound I_{max1}, depending on their hydrophobicity (Fig.7): I_{max1} of Ethyl-Propanoate (EP), the aroma with the lowest hydrophobicity, is correlated low-fat cheese Fl and Sl; I_{max1} of nonan-2-one (NO), the aroma with the highest hydrophobicity, is correlated high-fat cheese Fh and Sh. The slopes of the relations tend to decrease with a decreasing firmness of the cheese, which means firmness may increase the aroma release. This observation was previously made by Repoux et al. (2014c). In contrast, no clear linear or other kind of relation ($R^2 < 0.3$) was made with I_{max2}, the post-swallowing aroma release parameter, as well as A1 and A2. The relation between the cheese fat content and the I_{max} of each aroma was previously noted (Repoux et al., 2014c), but those new results defend the idea that the yield stress of the bolus may influence quantitatively the aroma release, depending on the aroma hydrophobicity and cheese fat-content. No linear relations ($R^2 < 0.2$) were found between the aroma release parameter and K_{flow} , which stands for the viscosity and the dynamic behaviour of the bolus, as s_{flow} stands for yield stress and the pseudo-static behaviour. The lack of correlations is related either to the individual variability, or the fact that K_{flow} varies on a too small interval in the case of cheese boluses. Indeed, K_{flow} varies over one decade on Fig.4, whereas s_{flow} varies over 3 decades.

As seen in the previous section, the bolus yield stress depends upon the volume fraction, the particle shape, size and yield stress. To clarify the relation, s_0 was plotted with the different I_{max1} (Fig.8). The yield stress of the suspending matrix from low fat cheeses (Fl and Sl) appear to be well correlated with the EP release during the mastication process, while no linear correlation ($R^2 < 0.20$) is obtained for high fat cheeses (Fh and Sh). The origin of this trend will be debated in the next section. Like K_{flow} , small linear correlation ($R^2 < 0.30$) was found with K_0 and the aroma release, keeping in view the same explanation.

5. Discussion

During eating, foods are submitted to two main oral processes: chewing, including biting and crushing with teeth, and progressive impregnation by saliva resulting in the formation of a cohesive bolus to be swallowed (de Wijk et al., 2011). During the mastication step, an increase in firmness of the food products induces an increase in chewing duration and quantity

of saliva incorporated into the bolus, and the relation moisture content (Repoux et al., 2012b; Tarrega et al., 2011). An increase in the firmness of the cheese was related to an increase in the rate of release (Repoux et al., 2012b), whereas the opposite was observed on gelatin and pectin gels, which do not need any great chewing activity (Boland et al., 2006). This effect can be explained by the mode of breakdown of the cheeses rather than by the chewing activity. Using a model mouth, van Ruth and Buhr (2004) showed no effect of the rate of mastication on the rate of release and only an effect on the maximum intensity. In the present study, we observed that firm cheeses are rapidly broken into particles (Fig.6a-c), and the suspending matrix create a greater exchange area, which favor aroma release into the gas phase ; whereas the soft cheese create more continuous bolus with less suspending matrix (Fig.6b-d). The suspending matrix may coat the mucosa more easily, as it may be trap between the roughness and then be a source of aroma release. To illustrate the coating process, a colored bolus was coat on a pharyngeal mucosa from a pig (Fig.9). We use the pigs as a living model, because this part of the throat is difficult to access in case of humans, and the pig is a good physiological model for humans. Moreover, the roughness shape is equivalent in term of length in pigs and humans. Once the mucosa is coated, the yield stress of the suspending matrix σ_0 may counter the natural clearance, that is why the positive correlation was found between this rheological property and the aroma release in the results section (Fig.8). In contrast, no correlation is found between the viscosity parameter K_{flow} and K_0 , because the variation of K_{flow} or K_0 may not be significant enough in comparison with individual variability. Those observations tends to demonstrate that yield stress of the matrix may increase the aroma release after a coating process, as it was previously show with mechanistic models (de Loubens et al., 2011, 2010).

The effect of the yield stress on the total amount of aroma released highly depends on the aroma compound, maybe because of the fat content of the cheese (Table 2). Ethyl propanoate is more volatile and less hydrophobic than nonan-2-one. The more the chewing duration, the more the release during the mastication step of ethyl propanoate, as a result of either the increase in the exchange surface during the in-mouth transformation of the product (Gierczynski et al., 2007) or to the duration of the mastication leading to an increase of aroma transfer between the oral cavity and the nose cavity. In particular, jaw and teeth movements during mastication induce velopharynx opening and air flow between the pharynx and the mouth (Buettner et al., 2001; Matsuo et al., 2005). A previous study conducted on the same

cheese and the same panel showed that hard and low-fat cheeses required more muscle work and longer chewing time to be eaten than soft and high-fat cheeses (Yven et al., 2012). Results of bolus rheological properties tended to show that hard and low-fat cheeses, which required a high total muscle work to be eaten, gave harder boluses or boluses with a lower level of breakdown than soft and high-fat cheeses. In parallel, lower volume fractions, related to moisture content, imply smaller amounts of ethyl propanoate released, probably due to the dilution of this more hydrophilic compound.

Swallowing induces the opening of the velum (Buettner et al., 2008) and thus the transfer of aroma compounds from the oral cavity to the pharynx. It is thus at the time of this post-swallowing step that the major part of the aroma is released (Buettner et al., 2008; Repoux et al., 2012d). The higher yield stress of the suspending matrix, the higher the quantity of aroma released at the swallowing step, whatever the fat content. The effect of matrix yield stress s_0 on the quantity released after swallowing is more important for ethyl propanoate than for nonan-2-one. For ethyl propanoate, this increasing of release with yield stress is directly related to both the amount of saliva incorporated into the bolus and the persistence in the mouth. Similar results were found for ethyl butyrate by increasing gel firmness (Boland et al., 2006), which was attributed to a higher coating of hard gels on the back of the tongue, leading to more persistence of aroma. The cheeses varied both in firmness and fat content and only fat content significantly modified mouth coating (Repoux et al., 2012b). The more hydrophobic and less volatile compound, nonan-2-one persists longer in the breath than ethyl propanoate. This could be explained first by its lower vapour pressure, as observed by Buffo et al. (2005) for different aroma compounds in human breath after consuming an aqueous mixture. However, the dynamic of aroma release is also influenced by matrix properties. In the case of nonan-2-one, yield stress increases the quantity released (Fig.7) related to the fat content. As fat content increases, more product remains in the mouth and this higher mouth coating will induce a higher persistence of the more hydrophobic compound. It was thus demonstrated that fat has not only an effect on bolus properties but also on the persistence of hydrophobic aroma in the breath.

6. Conclusion

This study was performed using a free protocol of solid cheese consumption by 28 subjects. It enabled us to draw some general observations regarding aroma release in function of

rheological properties. Correlations indicated that the more volatile and less hydrophobic aroma compound, ethyl propanoate, was more influenced by yield stress of the bolus and the less volatile and more hydrophobic aroma compound, nonan-2-one, was more influenced by the fat content. Globally, variations in yield stress affected the kinetics of aroma release less than variations in fat content. An increase in yield stress increased the total amount of aroma released mainly during the mastication step, through chewing duration and amount of saliva incorporated into the food bolus. Variations of consistency are too small in the case of studied bolus to reveal any significant correlations: the consistency varies over one decade, whereas the yield stress varies over 3 decades. The bolus yield stress may be the most significant parameter to follow, which can be also estimated under conditions using the consistometry technique (Patarin et al., 2014c).

These results showed that the influence of food matrix properties on aroma release is linked to the physicochemical properties of the aroma compound considered. Therefore, in order to gain a clearer understanding of *in vivo* aroma release, our work emphasized the importance of considering not only the texture and composition of initial food, but also food oral processing events, and the amount of product remaining in the mouth after swallowing. The effects of cheese firmness and fat content in processed model cheeses are significant whatever the large inter-individual variations observed between our 28 subjects. This means that the obtained results could help cheese manufacturers to predict aroma release in function of cheese formulation. Conclusions will be helpful to reformulate products with the aim, for example, to reduce fat content or modify the texture while keeping the same amount of aroma release.

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7. Figures

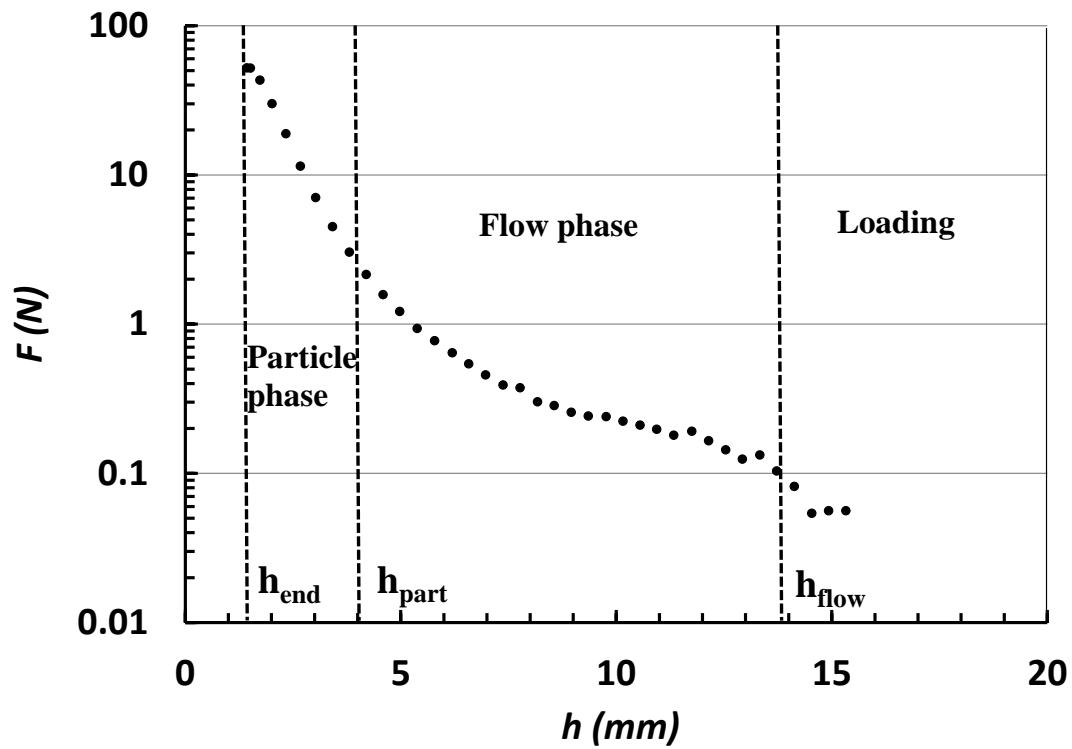


Fig.1: Description of the succession of phases through a compression test, delimited by dotted line, for a human bolus from cheese. The compression curve (\bullet) is from a panelist after eating a cheese sample. The loading area corresponds to the beginning of the squeeze, where the top surface of the sample is not yet fully in contact with upper plate. (from Patarin *et al.* 2014)

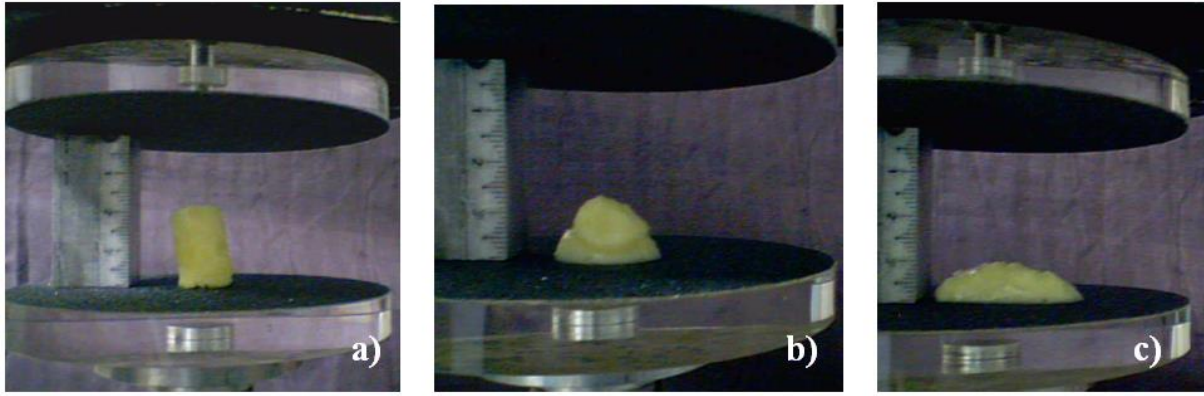


Fig.2: Photographs of samples before the start of the compression test. Three different samples configurations may be distinguished: (a) The sample has not collapsed, gravity is not sufficient to cause the bolus to flow; (b) and (c) The sample has collapsed to different degrees under its own weight.

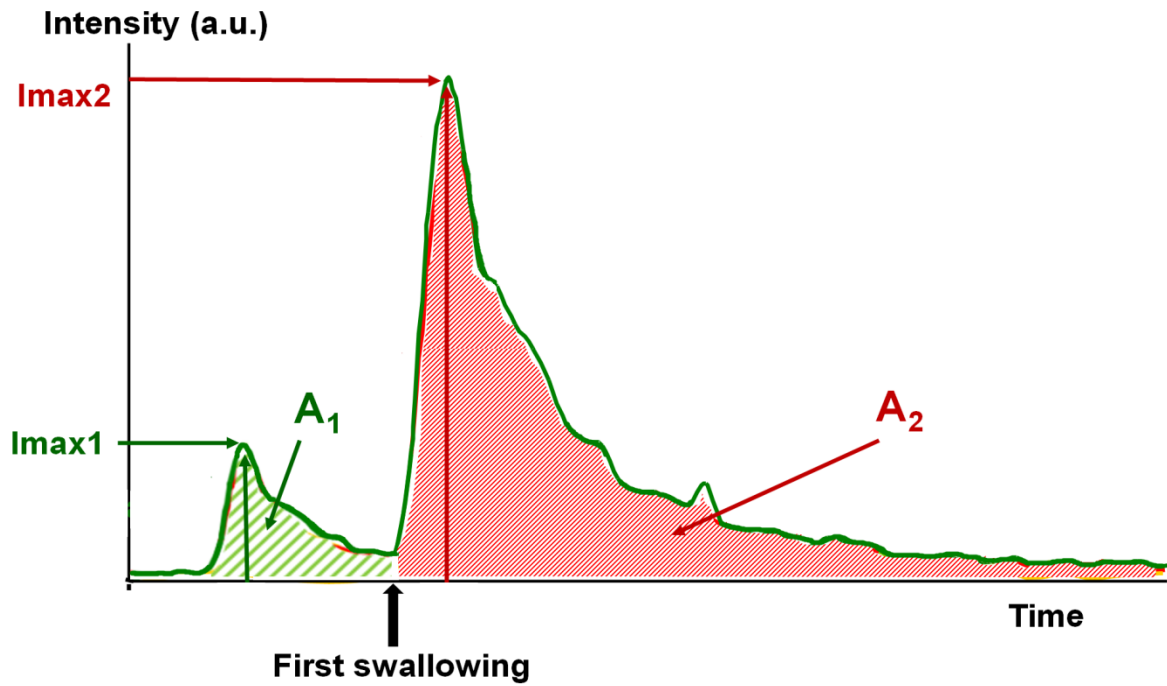
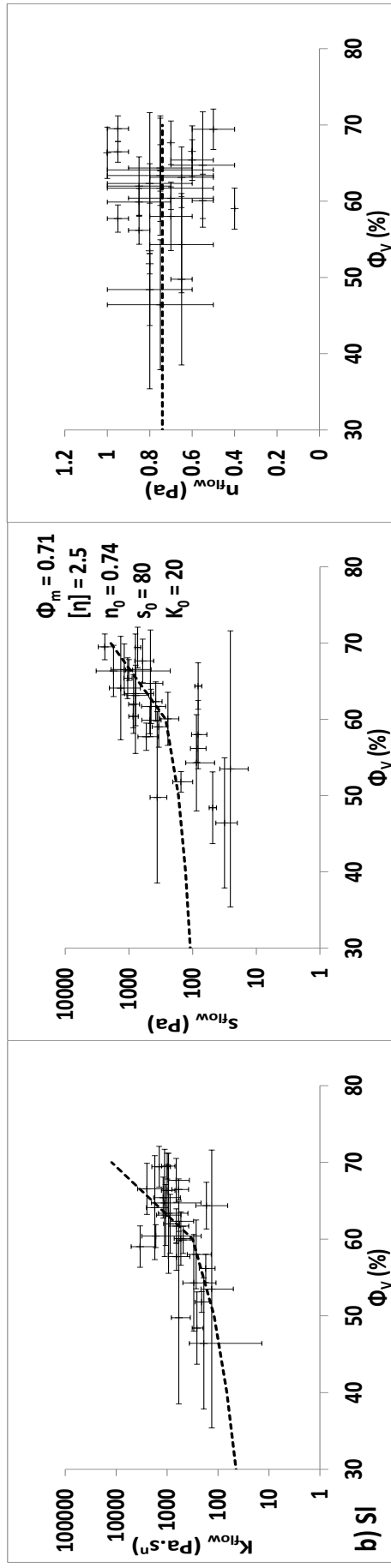
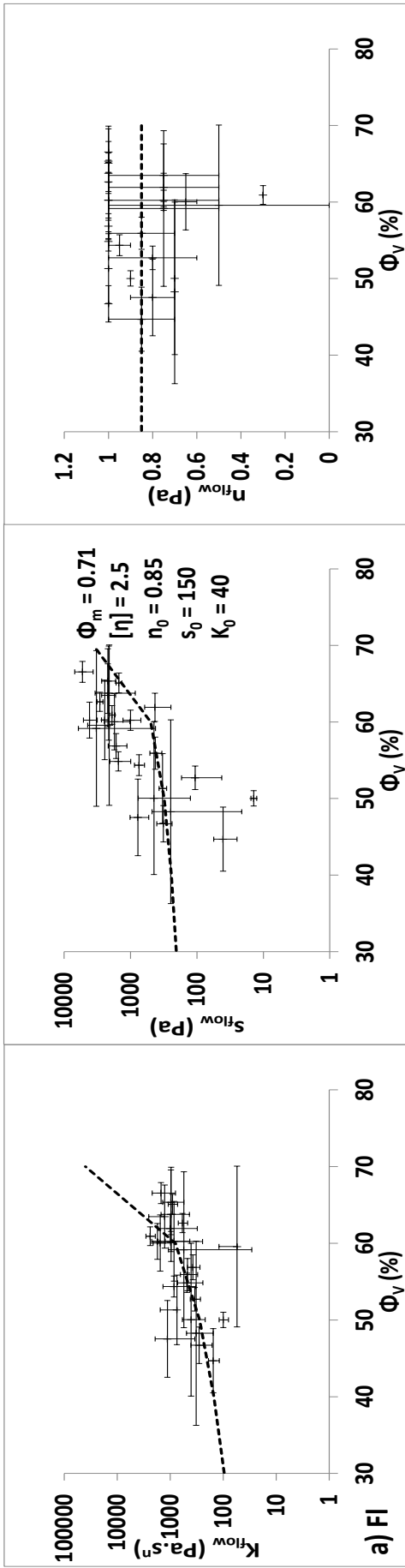


Fig.3: Typical aroma release curve profile. The release profile was separated in two release phases: before (green) and after (red) first swallowing. The quantity of aroma released (A_1 & A_2) and the maximum intensity ($Imax1$ & $Imax2$) were extracted from the curve for each release phase. (adapted from Feron *et al.* 2014)



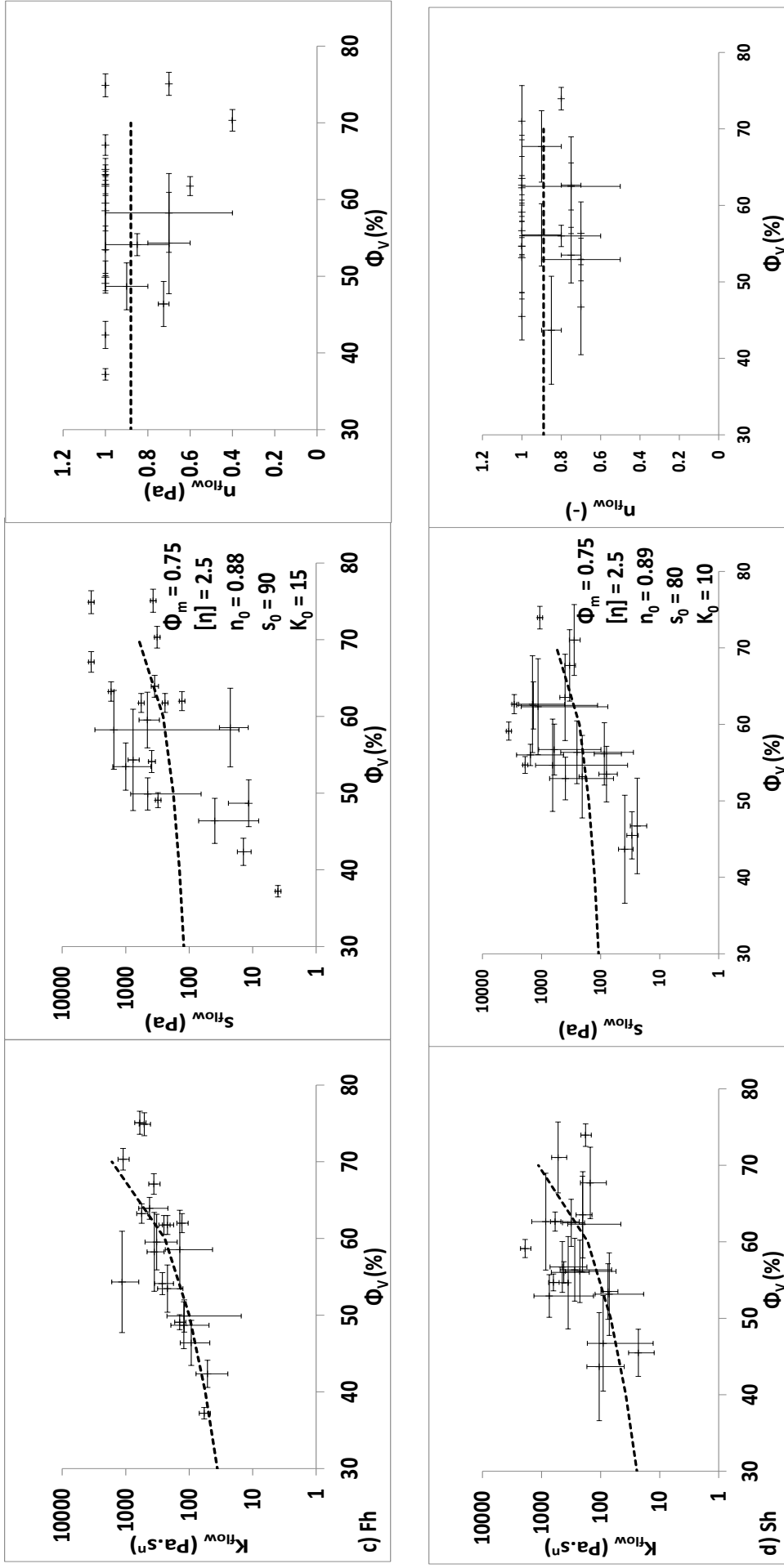
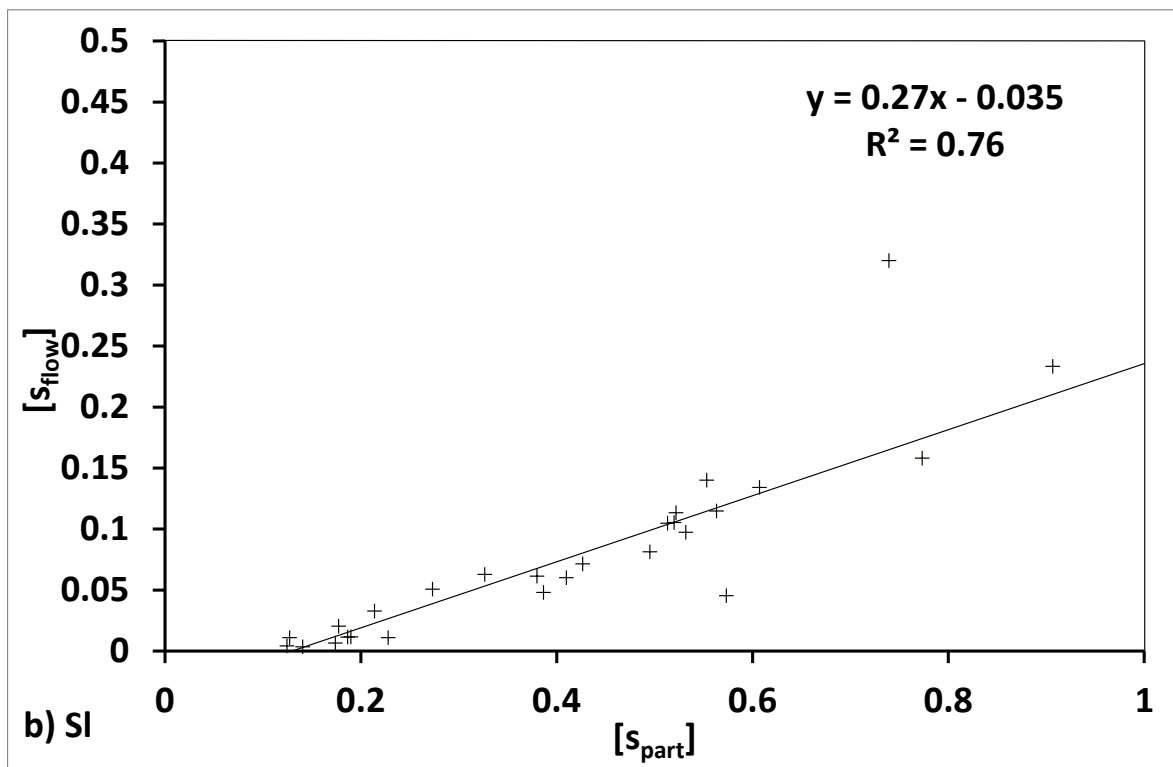
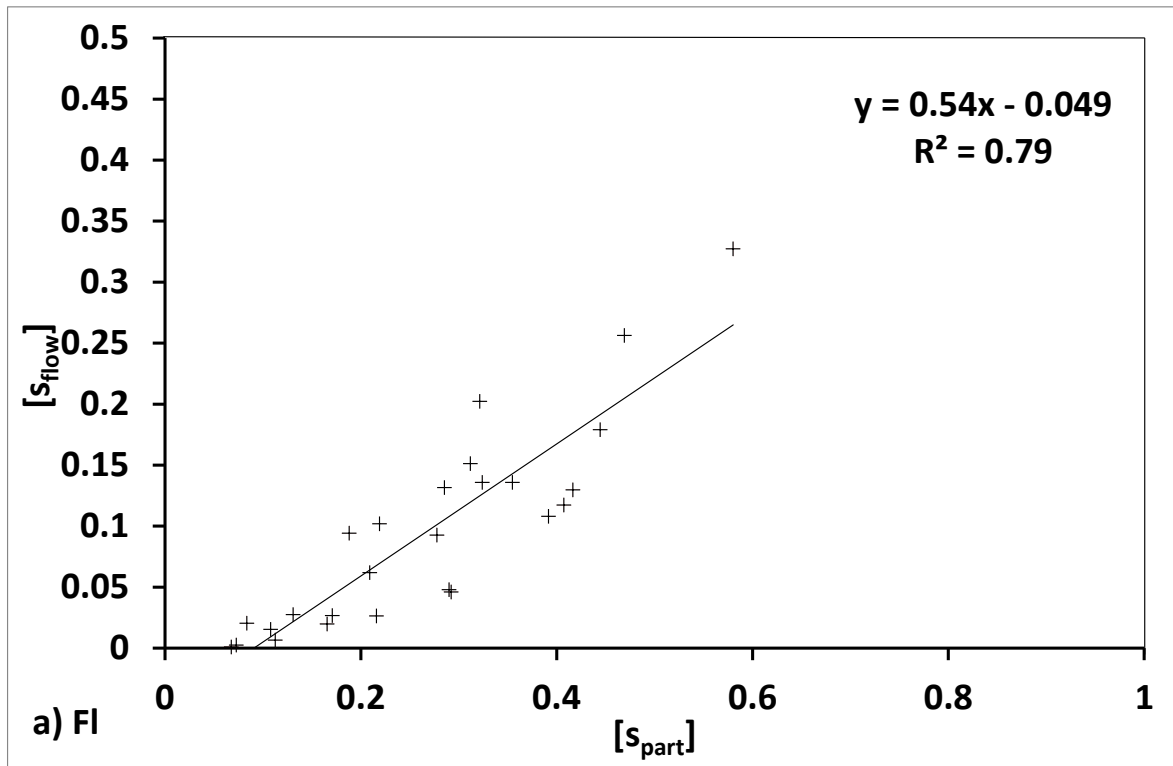


Fig.4: Consistency Kflow and yield stress sflow and Flow index nflow are displayed according to Φ_V from human bolus, for all cheeses (a,b, c & d). Values are the mean of 2 repetitions over 28 panelists. Dotted lines are a fit of Chateau model for each cheese over all panelists, using the parameters displayed on the central graph.



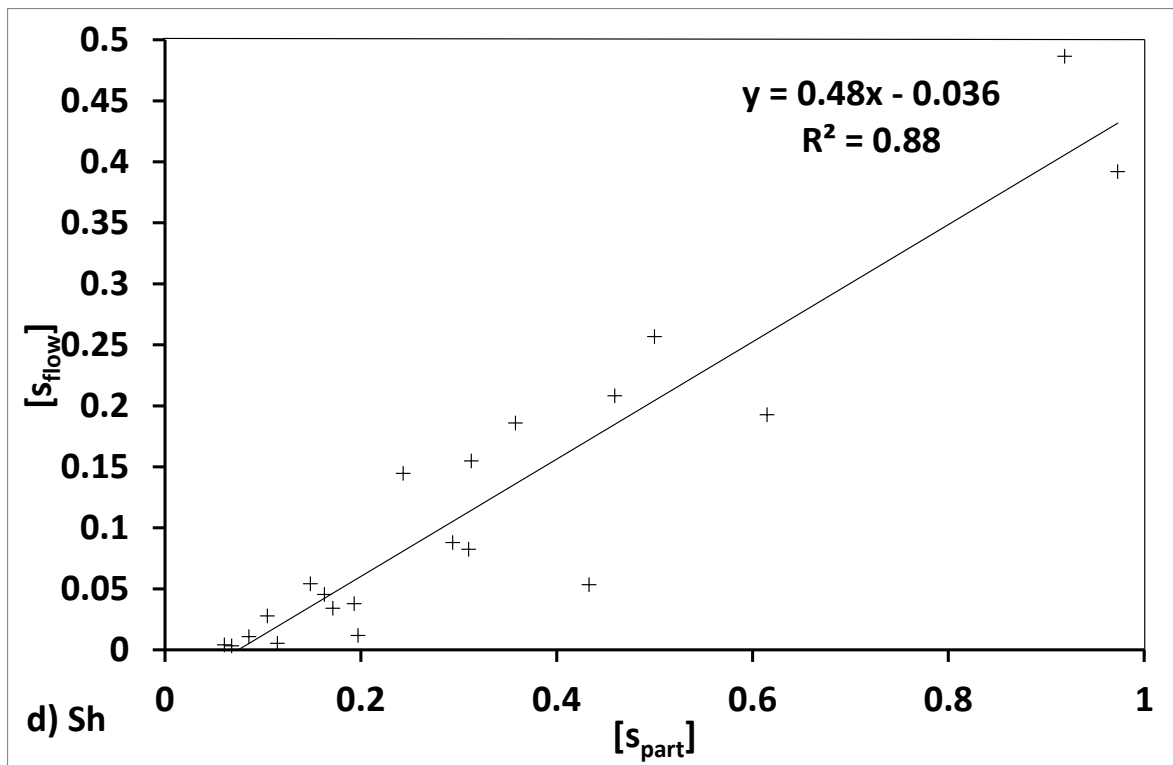
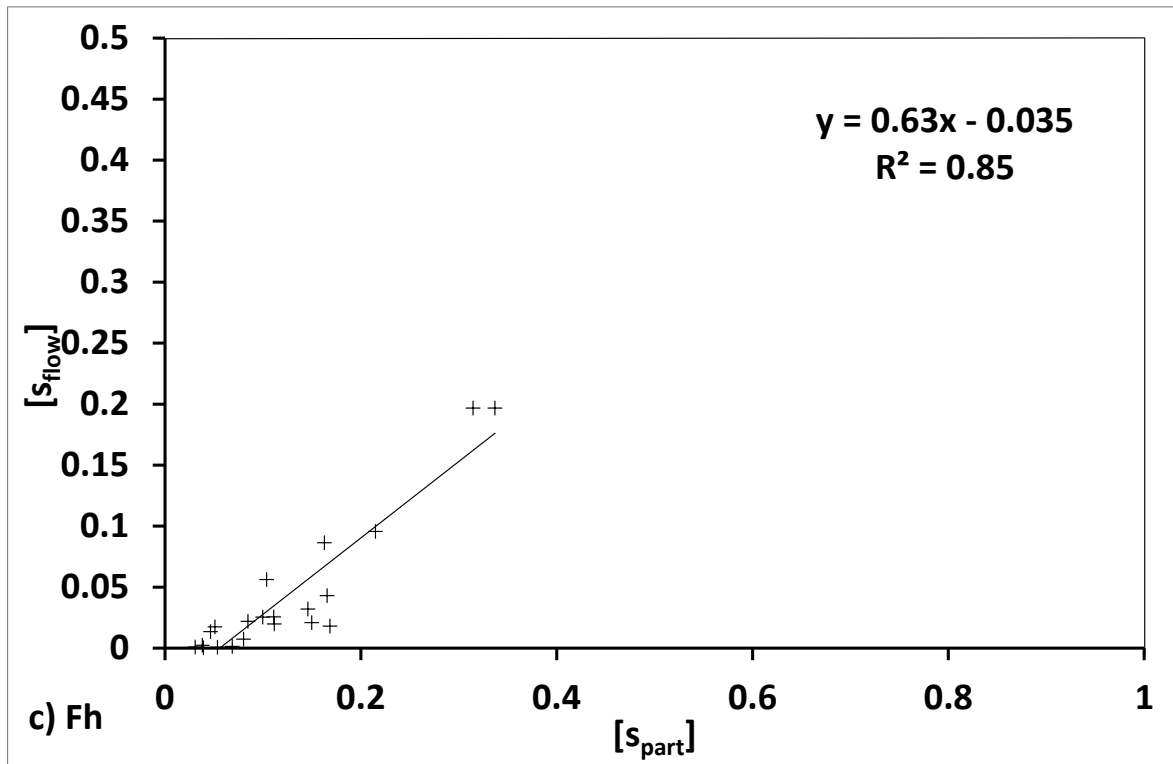


Fig.5: Relation between [s_{flow}] and [s_{part}] for the four cheeses. All panelists are display. Solid lines are the results of a linear regression.

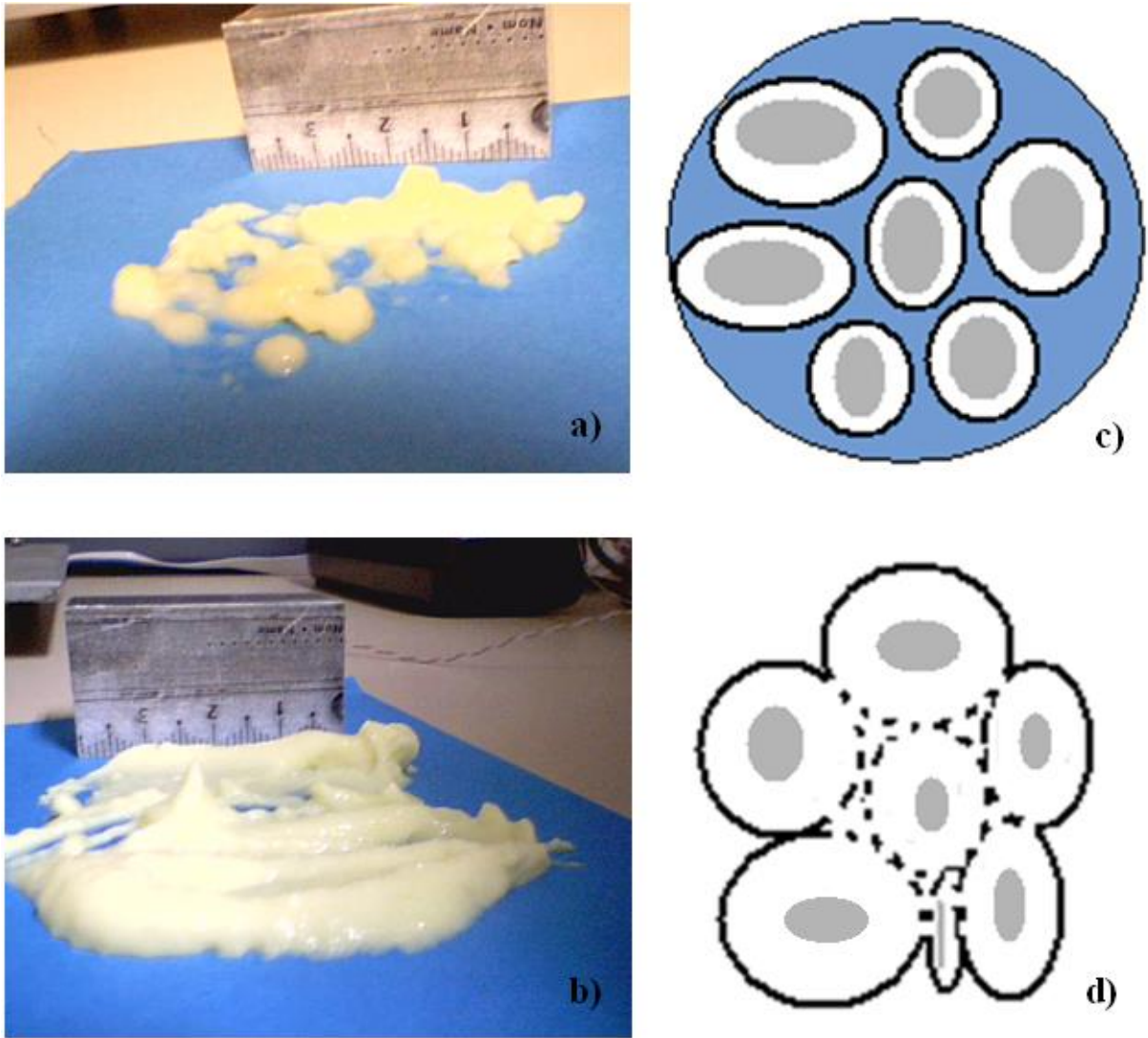
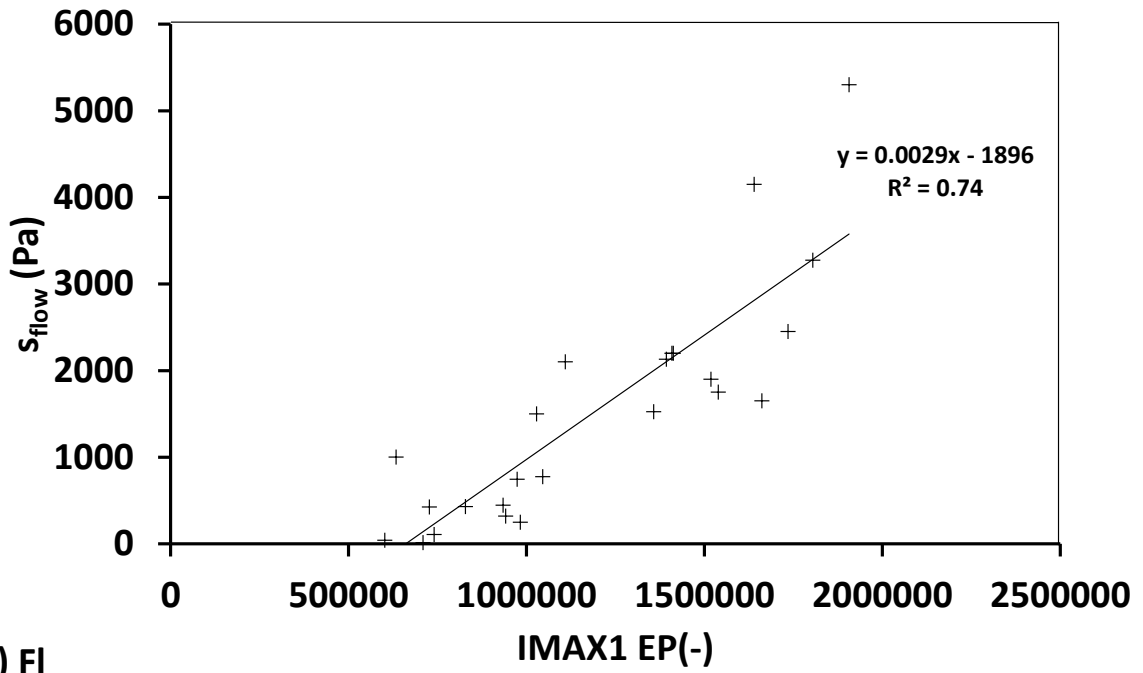
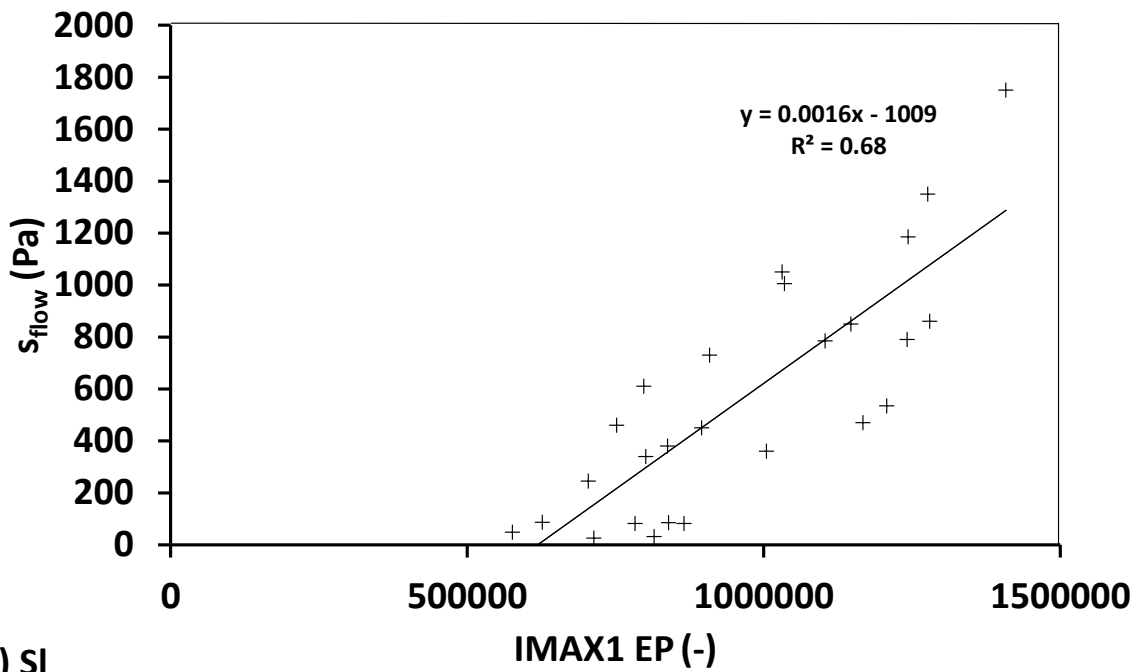


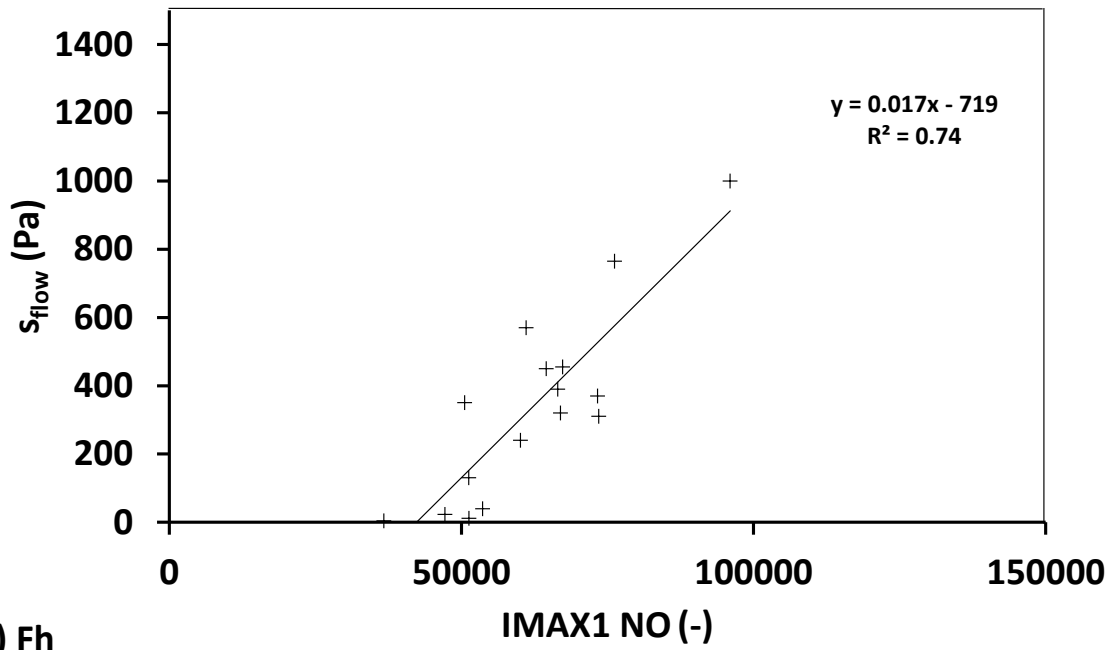
Fig.6: Photographs of a bolus derived from a low-fat cheese (a) and high-fat cheese (b). Diagram (c) represents the structure of a "low-fat" bolus with distinct and hardly broken down particles. Diagram (d) represents the structure of a "high-fat" bolus with mixed and well broken-down particles.



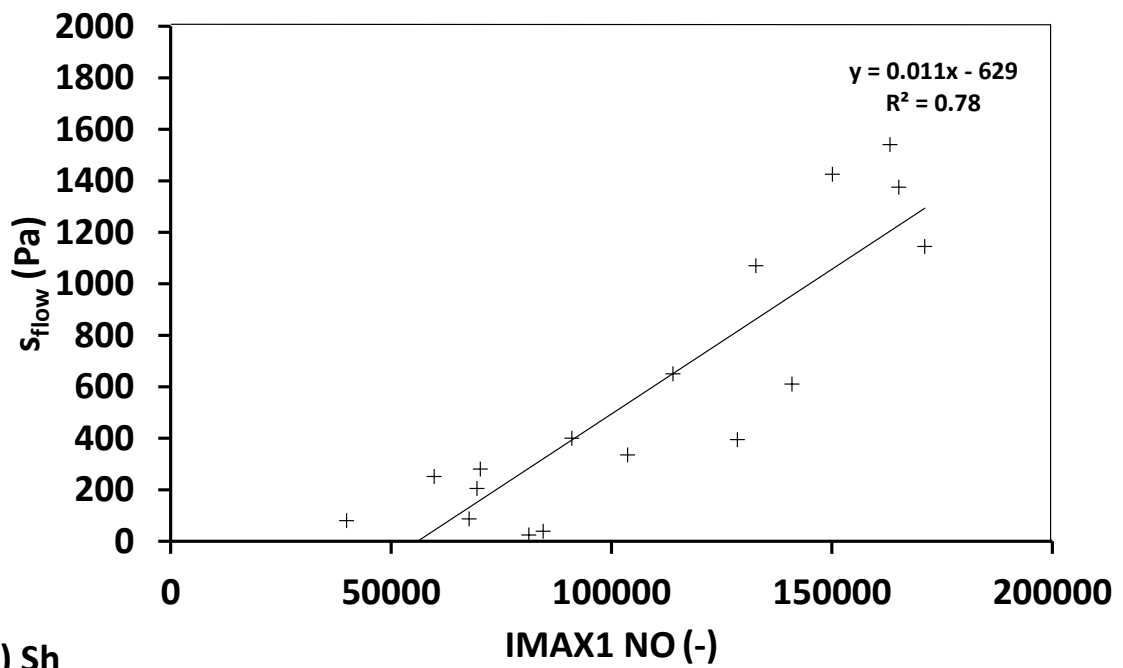
a) FI



b) SI

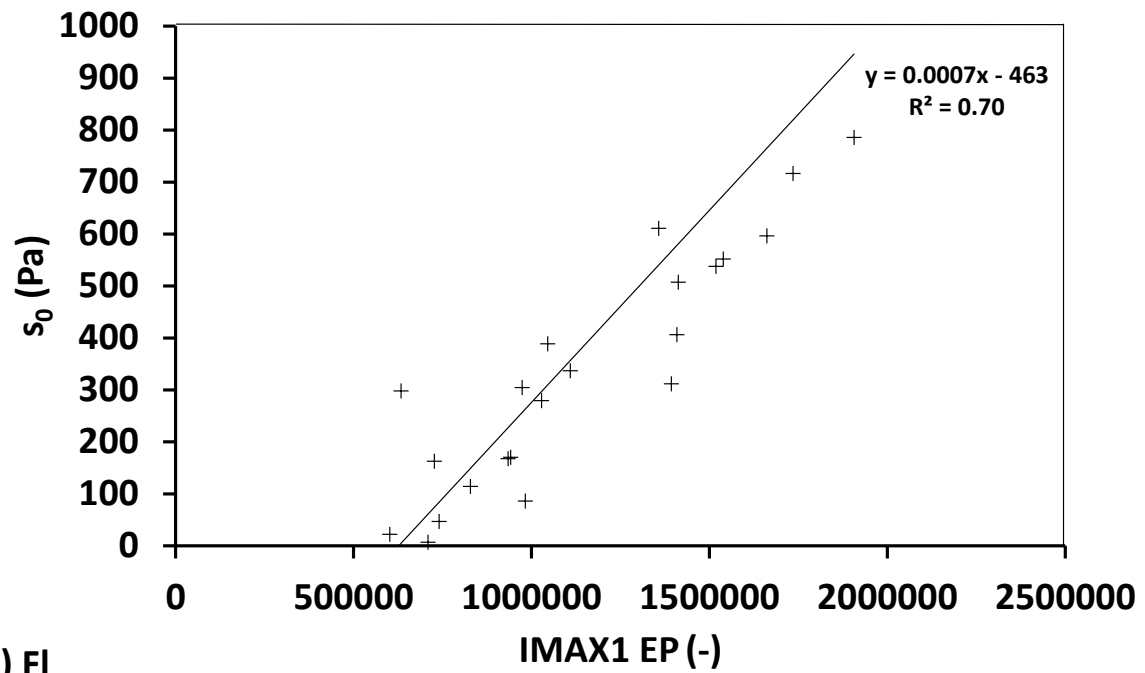


c) Fh

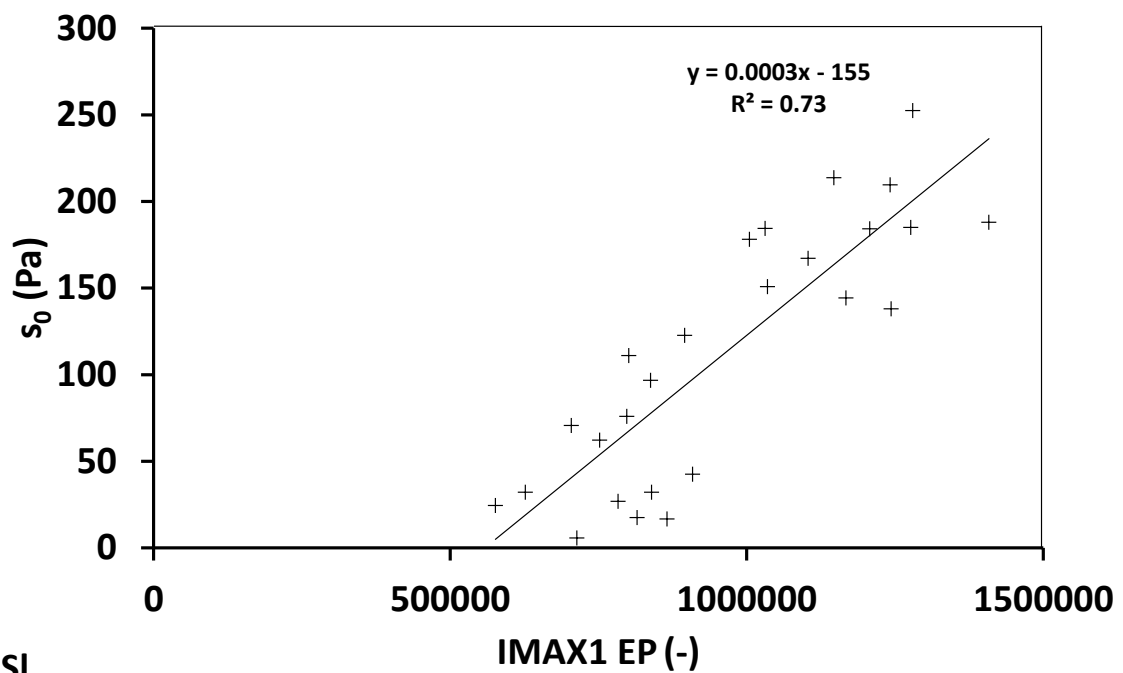


d) Sh

Fig.7: Yield stress s_{flow} is displayed according to aroma release from human bolus, for all cheeses. Solid lines are the results of a linear regression.



a) FI



b) SI

Fig.8: Yield stress s_0 of the suspending matrix is displayed according to aroma release from human bolus, for low-fat cheeses. Solid lines are the results of a linear regression.

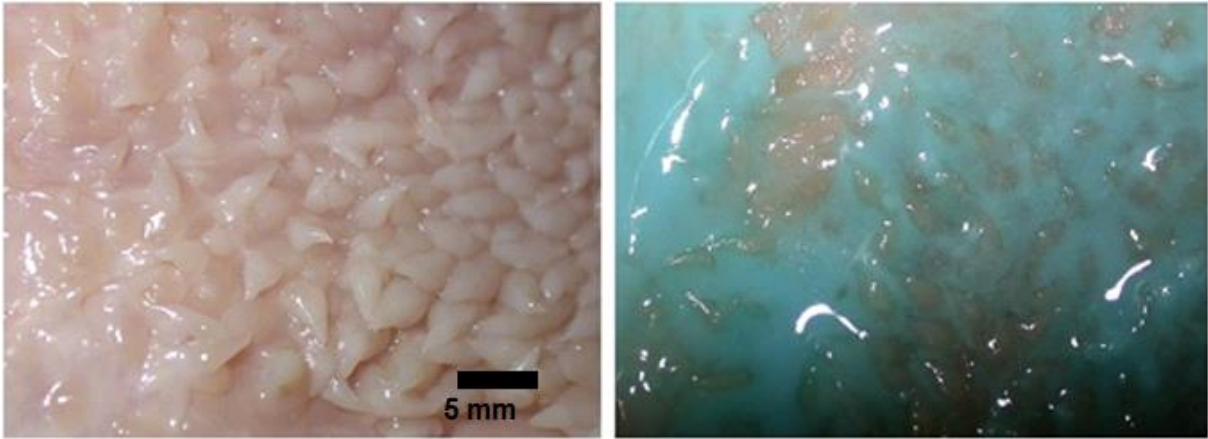


Fig.9: Coating simulation of a porcine mucosa with a colored bolus.

Table 1:

Characteristics of cheese bases

Code	Description	Dry extract (%)	Full-fat/Dry (%)	Failure threshold (Pa)	Critical Strain (Rad)
Fl	Hard, Low-fat	49.4	25	16200±1200	0.84±0.05
Fh	Hard, High-fat	51.5	48.3	17800±2300	0.35±0.06
Sl	Soft, Low-fat	45.6	25	7500±400	0.80±0.06
Sh	Soft, High-fat	45.5	48.4	7400±300	0.27±0.02

Table 2:

Herschel-Bulkley parameters of human boluses and related data, extracted from compression curves, as well as Herschel-Bulkley parameters of the suspending matrix calculated with the suspension model proposed. Aroma release values are also displayed. Standard deviations are calculated over a panel of 28 individuals.

Parameters	η_{flow}	S_{flow}	K_{flow}	S_{part}	h_{part}	S_{end}	ϕ_v	S_0	K_0	EP Imax 1	EP Imax 2	NO Imax 1	NO Imax 2
Unit	(-)	(Pa)	(Pa.s ^{n_{flow}})	(Pa)	(mm)	(mm ²)	(%)	(Pa)	(Pa.s ^{n_{flow}})	(-)	(-)	(-)	(-)
Fl	0.85	1535	809	4308	3.2	1742	57.0	420	59	1.1E+06	1.4E+06	1.1E+05	1.9E+05
SD (%)	0.18	1339	559	2193	0.7	496	6.4	350	78	1.5E+05	2.5E+05	2.5E+04	3.6E+04
Fh	0.88	747	342	2188	2.8	2243	57.7	137	21	9.5E+05	1.2E+06	9.8E+04	1.8E+05
SD (%)	0.18	1026	305	1509	0.7	724	10.0	156	33	1.7E+05	3.0E+05	2.8E+04	2.7E+04
Sl	0.74	759	873	3243	2.9	2004	60.4	137	57	9.4E+05	1.3E+06	5.7E+04	1.3E+05
SD (%)	0.14	940	749	1813	0.6	629	6.3	132	96	9.0E+04	1.9E+05	6.1E+03	1.3E+04
Sh	0.89	873	399	2370	2.5	2436	57.9	302	30	9.5E+05	1.2E+06	9.5E+04	1.7E+05
SD (%)	0.12	978	416	1905	0.3	777	7.8	357	30	7.0E+04	2.8E+05	3.0E+04	1.1E+04

PARTIE II :

Rhéologie des jus gastriques issus de pains et rôle de la rhéologie dans la vidange gastrique

L'obésité est un problème de santé publique dans les pays occidentaux et des politiques publiques, basées sur la nutrition, sont mises en place. Le PNNS (Plan National Nutrition Santé) recommande la consommation de produits céréaliers avec un faible index glycémique. L'objectif premier du projet ANR NOMAC (Nouvelles ressources pour maîtriser le devenir digestif des nutriments des produits céréaliers) était de cribler la variabilité naturelle du blé pour identifier des variétés à forte teneur en amylose, et d'étudier les effets quantitatifs et qualitatifs de l'amylose sur les aspects cinétiques de la digestion, dont la viscosité du jus gastrique, à travers un modèle porcin *in vivo*. Les retombées socio-économiques sont attendues dans la sélection de variétés naturelles panifiables, ainsi que dans l'amélioration de la santé et du bien-être des consommateurs.

Dans la littérature, une importance particulière est accordée à l'étape de la digestion au niveau de l'estomac. Les études passées sont fondées sur l'hypothèse que la phase gastrique est sans incidence sur le taux de digestibilité de l'amidon et sa cinétique. Avec une seule protéase, la pepsine, jouant un rôle au niveau de l'estomac, c'est bien l'absence d'amylase qui valide cette hypothèse. L'incidence de la cinétique de la vidange gastrique est alors souvent négligée alors qu'elle influe directement sur la digestion. Après avoir consommé un repas solide typique, il existe un temps de latence de 20 à 30 min où la vidange gastrique est minime. Elle est suivie par une phase dans laquelle la vitesse de vidange est à peu près linéaire, à la différence d'une vidange à vitesse exponentielle pour les liquides (Achour et al., 2001). La teneur en lipides du bol gastrique est le facteur le plus connu qui affecte la vidange gastrique. Dans le cas des études suivantes, ce facteur est à proscrire vis-à-vis de l'objectif. En effet, la consommation d'un produit de céréales avec une désintégration lente a été retenue dans ce projet de recherche, afin de ralentir la vitesse de la vidange gastrique en modifiant les taux d'hydrocarbonate. Ce choix est basé sur les résultats de la littérature dans lesquels on se propose de modifier les cinétiques de la vidange gastrique en modifiant la viscosité de repas, afin de ralentir la digestion et l'absorption des nutriments, dans le but d'améliorer la gestion de l'intolérance au glucose et l'obésité (Marciani et al., 2000).

Il a été montré que les fibres alimentaires (DF) solubles retardent l'absorption du glucose, ce qui entraîne des réponses glycémiques et insuliniques modifiées suite à un repas de glucides, à la fois chez l'homme et chez les animaux (Ebihara et al., 1981; Jenkins et al., 1978). Cet effet est censé être gouverné par une vitesse réduite de la vidange gastrique et/ou par un effet direct sur la diffusion et l'absorption des nutriments dans l'intestin grêle (Ebihara et al., 1981;

Jenkins et al., 1987). A cet égard, la capacité de DF solubles pour augmenter la viscosité dans l'intestin est jugée incontournable (Blackburn et al., 1984; Jenkins et al., 1978). Cependant, les effets de DF solubles sur la vidange gastrique sont ambigus, mais bien établis dans le cas de l'ajout de DF solubles à des repas liquides, basé sur des modèles glucose (Ebihara et al., 1981; Holt et al., 1979; Torsdottir et al., 1991). La forme sous laquelle les DF ont été introduites dans les repas et leur niveau d'hydratation peut expliquer en partie les différences dans les résultats obtenus (Low, 1990). En outre, les résultats publiés se rapportent plus à la viscosité de la farine et rarement à la viscosité de la matrice à l'endroit où la fonction physiologique est censée avoir lieu (Cherbut et al., 1990). Cela peut fausser les conclusions sur le rôle physiologique de viscosité de manière assez significative pour certains types de DF solubles (Edwards et al., 1987). La structure et la taille des particules sont aussi deux paramètres importants influençant la vidange gastrique (Johansen et al., 1996).

Les rares études de suivi de la vidange gastrique sont classiquement réalisées par marquage de la nourriture avec le technétium (Chao and Vandenplas, 2007), méthode compliquée et coûteuse. Ces travaux *in vivo* dans des locaux spécifiques nécessitent une structure d'accueil de taille considérable, comprenant les soins apportés aux animaux. Le contrôle de la vidange gastrique par échantillonnage dans l'estomac est délicat, car le cisaillement au travers de la canule gastrique peut endommager la structure du jus, notamment les particules (Knudsen et al., 2006; Rudi et al., 2006).

Ainsi, le passage des produits à base de céréales dans l'estomac est un phénomène peu connu et les travaux publiés dans ce domaine sont réalisés en règle générale *in vitro*, avec des dispositifs qui simulent le fonctionnement de l'estomac, mais sans prendre en compte les "contractions" gastriques (Bednar et al., 2001; Hoebler et al., 1999). Cependant, certaines ont été menées *in vivo* sur des chevaux (Julliand et al., 2006), des ruminants (Hogan and Flinn, 1999), des rats (Holm et al., 1988) et des porcs (Knudsen et al., 2006; Weber and Ehrlein, 1998). Il est connu que la digestion de l'amidon se poursuit ensuite dans l'intestin par l'action des amylases sécrétées, et enfin par l'absorption des micro-nutriments dans l'intestin grêle. L'impact de la teneur en amylose sur la fonction gastrique est évalué sur deux groupes de porcs. Cet animal a été choisi comme un modèle humain en raison de ses similitudes dans l'anatomie et la fonction du segment gastrique (Malbert and Horowitz, 1997).

Dans un premier article, la méthodologie de rhéométrie de jus gastriques de porcs issus d'un pain sera présentée. La rhéométrie a été réalisée sur un instrument dédié aux mesures sur les

jus gastriques (Fig.1). Il est composé d'un viscomètre VT550 (Haake, Allemagne), d'une caméra (Logitech, USA) pour le suivi des champs de déformation, d'un capteur ultrason (PEPPERL & FUCHS, Allemagne) pour identifier automatiquement la profondeur d'enfoncement de l'outil de rhéométrie, et d'un logiciel développé à façon sur Labiew (NI, USA) afin de rendre la procédure de mesure la plus ergonomique possible. Des outils de trois diamètres différents (35, 17.25, 13.125 mm) ont été réalisés pour éventuellement s'adapter aux variations de consistance. Au final, l'outil de 35mm de diamètre a été utilisé systématiquement. Seule la mie des pains tests (Fig.2) a été donnée aux porcs (Fig.3), préparés et soignés par l'équipe du Senah. En effet, ces derniers ont subi une intervention chirurgicale afin de leur implanter une canule gastrique, au travers de laquelle sont extraits les jus gastriques (Fig.4). L'enjeu de la rhéométrie était de réaliser l'essai immédiatement après le prélèvement, au sein d'un réservoir en plexiglas qui limite le refroidissement de l'échantillon. Les courbes d'écoulement ont été construites à l'aide d'une suite d'échelons croissants de vitesses permanentes (2 vitesses par décade, de 0.01 à 30 rad/s). Les propriétés rhéologiques ont été enrichies des données sur la teneur en eau et sur la structure macroscopique à l'aide de prises de vue. Nous avons pu ainsi statuer sur le comportement rhéologique des suspensions de bols alimentaires en sortie d'estomac, décrit dans le chapitre 2.1 de cette partie, au travers d'un article consacré à la méthodologie.

Dans le chapitre 2.2, les facteurs de coordination de la vidange gastrique, et donc l'alimentation des nutriments dans le duodénum et le jéjunum, sont étudiés chez le porc en utilisant l'imagerie par scintigraphie gamma de l'estomac, par l'équipe du Senah. La vidange gastrique est évaluée après l'ingestion de pain radio-marqué. L'enjeu de l'étude est d'évaluer si l'adjonction d'amylose à la farine servant à la panification est, à elle seule, suffisante pour modifier les caractéristiques rhéologiques dans l'estomac et donc la vitesse d'évacuation du repas par l'estomac. Pour cela, trois pains, dont les concentrations en amylose et amylopectine varient, ont été fabriqués. D'un point de vue physiologique, l'objectif final est, au travers de cette modification des cinétiques de vidange de l'estomac, de réduire l'excursion glycémique et donc l'index glycémique du pain.



Fig.1 : Dispositif de rhéométrie construit.



Fig.2 : Coupe d'un pain après panification



Fig.3 : Vue du porc dans sa cage métabolique



Fig.4 : Photographie d'un jus gastrique après prélèvement

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Chapitre 2.1

Rhéométrie des jus gastriques issus de pains

Rheological characterization of gastric juices from bread with different Amylose/Amylopectin ratios

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Abstract:

The purpose of this paper is to present a methodology for characterizing the rheological properties of heterogeneous gastric contents, for the first time, to our knowledge. Pigs were used as a model because their gastric system is similar to that of humans. Bread was chosen as a high-calorific, solid test meal. The bread was made with flours offering a wide range of amylose/amylopectin ratios. A specific method of extraction and measurement was developed. For the study, the vane test technique was chosen in order to take into account the heterogeneity and short lifetime of the boluses. The variation in viscosity values for each gastric system was compared with their moisture contents, different residence times in the stomach and various amylose/amylopectin ratios. The viscosities decrease significantly as the residence time increases. Furthermore, we suggest that the amylose/amylopectin ratio (Am/Ap) determines the moisture content, which then provides the viscosity level, since the gastric contents behave as a concentrated suspension. Pigs add variability to the results because the structure of the bolus depends on mastication.

1. Introduction

The rheological properties of gastric contents, such as viscosity, play an important role in gastric emptying, the kinetics of starch digestion and glycemic response (Björck et al., 1994; Edelbroek et al., 1993; Horowitz et al., 1993; Jebb, 2007). Gastric juices containing bread tend to create a semi-fluid medium with large swollen lumps. It is therefore quite a challenge to monitor the relevant rheological properties during digestion and take into account the real in vivo conditions (Johansen et al., 1996; Kong and Singh, 2008; Lindberg et al., 2003). The main objective of the present study is to overcome these difficulties. Most published results relate to the viscosity of the meal and rarely to the viscosity of the in vivo gastric juices, where the physiological function is supposed to take place (Cherbut et al., 1990). Because human gastric contents cannot easily be extracted, pigs are used here, as their gastric system is similar to that of humans. Breads were chosen as the original meal because the modification of their carbohydrate content provides a wide spectrum of bolus consistency. A dedicated device had to be designed to take into account the multiple characteristics of the gastric juices, such as large particles, temperature and short lifetime of the sample, owing to the rapid changes in physical and chemical properties of the bolus.

The measurements aim for evaluating the impact of carbohydrate on the rheological properties of the gastric contents, by using different carbohydrate compositions within the bread. The influence of fibers, such as carbohydrates, has been noted in several studies (Deshpande and Cheryan, 1984; Brown et al., 1988; Potkins et al., 1991; Guerin et al., 2001). In some papers, authors proposed to increase the bolus viscosity with addition of different fibers, to reduce the glycemic response and to increase satiety (Granfeldt et al., 1994; Åkerberg et al., 1998; Marciani et al., 2000). In such context, carbohydrates, especially amylose and amylopectin, have been used to modify the rheological properties of in-vitro

solutions (Morita et al., 2002; Dikeman et al., 2006; Van Hung et al., 2006; Sandhu and Singh, 2007; Juhász and Salgó, 2008; Xie et al., 2009; Sulaiman and Dolan, 2013). In this study, we want to evaluate the impact of the amylose/amylopectin ratio, denoted by Am/Ap, on the rheological properties of the in vivo gastric juices during digestion. This investigation is carried out by monitoring two major parameters, namely the residence time and the moisture content.

The objective of our work is therefore to present the sampling and measurement methodology that concern extraction, sample loading, measurements and calculations. As soon as the rheological behavior of the gastric juices is described, the data are then correlated with the moisture content, the gastric residence time and Am/Ap. Finally, we propose to investigate the role of Am/Ap at different stages of digestion, and to analyze the variability induced by pig mastication behavior.

2. Material and Methods

2.1. Material

2.1.1 Animals

In the present study, eight animals (large white female pigs, 35 ± 3 kg) were considered. A large gastric cannula (ID 1.2 cm) was surgically inserted into the proximal stomach to obtain access to the gastric contents. The large diameter was chosen so that large particles in the gastric juice could be sampled without any possible skimming of the gastric content. This protocol has been approved by the French veterinary services (authorization numbers A35-622 and 01894).

The pigs were pre-anaesthetized with ketamine (5 mg kg^{-1} , i.m.). A surgical level of anesthesia was obtained with halothane (3–5%, v/v) delivered by a mechanical ventilator (SAL 900, Siemens, Saint-Denis, France). The oxygen fraction (F_{iO_2}) and tidal volume were adjusted such that the spO_2 , measured by pulse oximetry (Ohmeda, Limonest, France), and the $spCO_2$, measured by an IR capnometer (Amstrong, Coleraine, N. Ireland), were found to be larger than 98% and lower than 5%, respectively. A venous cannula was inserted into the

marginal vein of the ear to infuse Ringer-lactate (500 mL h⁻¹) during surgery to prevent dehydration. A T-shaped silicon cannula (1 cm ID) was introduced in the fundus, 15 cm distal from the lower esophageal sphincter and exteriorized in the left flank.

2.1.2. Breads

Bread samples, referred as Bread 1, Bread 2 and Bread 3, were made with three different flours distinguished by their Am/Ap. The characteristics of the breads and flours are shown in Table 1. They consist of a mix of flours based on standard T55 flour (Girardeau, France), HI-maize 260 amylose powder (National Starch and Chemical Company, Bridgewater, USA) and Alice amylose-free waxy flour (Limagrain, Saint-Beauzire, France).

The breads were obtained by using a Moulinex bread machine (SEB, Ecully, France) incorporating a 2h30' cooking program. When starting the process, the following ingredients were inserted into the manufacturing vessel at the beginning of the program: 216ml of water, 5g of NaCl, 360g of flour and 4.7g of yeast. Each test session was performed with breads made 12 hours before the experiment.

2.2. Methods

2.2.1. Bread characterization

Once cooked, the bread loaves were practically of same size, although the texture of each bread was visually different (Fig.1). To appreciate the different textures, we chose to measure the ratio of trapped air and the average size of air bubbles in the crumbs by the means of tomographic analysis.

In practice, each loaf of bread was placed in the tunnel of a medical X Computerized Axial Tomography Scan (Hi-Speed NTxi, GE Médical System, Meylan, France). A set of 3-mm-thick transversal slices was obtained at a voltage of 120 kV with a X tube current of 40 mA. The CAT was calibrated before each imaging session with air and water density. An assessment of the spatial resolution was also performed by using a suitable phantom surrounded by water.

Each axial section was transferred to the Image J software for quantitative image analysis. A binary threshold, based on the Hounsfield scale, was chosen and the air value was set to -1000. The mask thus created was then analyzed. The results are compiled in Table 1.

Bread 3 was found to be significantly more compact than the two other samples, with smaller air inclusions and a reduced proportion of trapped air.

2.2.2. Rheometry

From their visual appearance, the gastric contents are suspensions of soft particles of varying numbers and sizes (Fig.2). Particles of bread are inflated with a pool of water, saliva and gastric secretions. Numerous constraints have been required for rheological characterization of the gastric juices. Depending on their residence time, gastric juices may be described as ranging from a highly concentrated non-Newtonian suspension to a Newtonian fluid containing a reduced number of particles, leading to explore a broad range of viscosities for fluids containing soft and deformable particles. The maximum particle size was found to be approximately 2 mm, evaluated from images of the samples, as shown in Fig.2 for Bread 2. To satisfy conditions of a continuous medium, we adopted a large gap in our rheometry experiments. Furthermore, owing to the rapid physical, chemical and thermal changes taking place in the bolus, a fast and simple method was carried out. Additionally, the volume of the sample may vary with residence time. These constraints finally led us to adopt the vane test technique for the experiments, along with suitable measurement and analysis protocols.

An experimental device has been set up for gastric juice rheology, using a Couette cell combined with a VT-550 viscometer (Thermo, Karlsruhe, Germany), consisting of a fixed cup filled with the sample and a vane half-inserted in the sample. The depth of insertion of the vane depended on the available volume of gastric juice extracted from the pigs (between 100 and 300ml). To impose an adhesion at the wall, the inner surface of the cup was roughened to produce a grid with a 5 mm mesh size. The gap between the vane and the cup was 12.5 mm, which is more than 6 times longer than the maximum diameter of particles. Such procedures were essential to satisfy the continuity assumption of the medium during the test, requiring the use of a large gap geometry for the rheology experiments and involving corrections and specific calculations.

The shear rate $\dot{\gamma}$ was calculated by means of Eq.1 (Macosko, 1994), where θ is the rotational velocity, R_v the radius of the vane and R_c the radius of the cup.

$$\dot{\gamma} = 2\theta / (1 - (R_v/R_c)^2) \quad (1)$$

Owing to the pasty nature of the fluids, characterized by a yield stress, the entire gap may be not sheared, especially at low velocities. Accordingly, the value of the radius of the true sheared zone, R_s , was used instead of the cup radius. R_s was extracted from the videos. Thus, Eq.1 becomes Eq.2, as follows.

$$\dot{\gamma} = 2\theta / (1 - (R_v/R_s)^2) \quad (2)$$

The shear stress was calculated with the hypothesis under the assumption of no secondary flow between the blades. The calculations related to (Eq.3)

$$\tau = M / (\pi D^3/2 \cdot (H/D+1/6)) \quad (3)$$

took into account the fact that the vane was not fully inserted into the fluids (Yoshimura et al., 1987). The apparent viscosity η was therefore calculated with Eq.4.

$$\eta = \tau / \dot{\gamma} \quad (4)$$

2.3. Protocols

2.3.1 Meal intake

The animals were allowed one week to recover from surgery before starting experiments. During the second week, the animals were presented with the bread test meal to accustom them to it. The animals were housed in modified metabolism crates to restrict their mobility. The trials were carried out after a fasting of 12 hours. Immediately after the test meal and during the experimental session, the animals were not allowed to drink water.

The bread was cut into cubes of approximately 2cm³. To obtain extractable gastric juices through the gastric cannula, the test meal consisted of 500 kcal cubes of bread (171g for Bread 1, 182g for Bread2, and 177g for Bread 3) mixed with 150ml of water. The quantity of bread was adapted to obtain isocaloric meals. The animals ate the meals in less than 5 minutes.

Three samplings of the gastric contents were carried out at 30, 60, and 120 minutes after starting the test meal. To limit any alteration in viscosity due to experimental constraints, each sampling operation was performed once during an experimental session, i.e., there was no reintroduction of gastric chyme after the full sampling of all the gastric contents. After

opening the cannula, the gastric contents were completely poured into a container by gravitational flow. The maximum available quantity of juices was then isolated and immediately transferred into the measuring cell.

2.3.2 Test sequence

The temperature of the samples was measured systematically before and after the rheological tests. Less than 2 minutes elapsed between the extraction of the gastric juice from the pig and the start of the rheological test. The cup was maintained at 39°C before filling. The thick plastic wall of the cup prevented major heat loss during the test. The average heat loss measured was 2.2°C, with a standard deviation of 0.9°C.

Several rotational velocity steps were applied to the sample, from 0.01 to 30 rad/s. For each velocity, the test was stopped after reaching permanent flow in order to limit the total strain applied to the sample. Flow curve acquisition lasted less than 3 minutes to minimize changes in physical, chemical and thermal characteristics of the gastric juice. Flow at the free surface was recorded simultaneously.

During the rheological test, a video of the free surface was taken at each step. A tracer powder was dropped on the free surface before beginning the test. The flow at the free surface was considered to be representative of the bulk flow. For each test, the strain fields were extracted from the videos. At low shear rates, typically under 0.5 s^{-1} , the strain imposed by the vane could not propagate from the vane to the cup wall. In the unsheared area, the stress level is below the yield stress. The true shear rate was therefore corrected using a new gap, in which the fluid was sheared from the edges of the vane to the unsheared area. The radius of the virtual wall (defined by R_s in Eq.2) was monitored by videos, which also determine the fluid velocity at the wall, to check the adherence conditions.

After performing the rheological tests, the weight of the gastric juices are measured and desiccated. The moisture content was then determined using the ratio of the dry weight to the initial weight.

3. Results

3.1. Influence of residence time on rheological properties and moisture content

For Bread 1, Fig.3a shows the changes in viscosity according to shear rate, for 3 residence times in the stomach, Fig.3b gives the change in the corresponding shear stress. The flow curves demonstrate the pasty behavior of the gastric contents for short residence times (30 and 60 minutes). In fact, the shear stress for the lowest the shear rate tends to a plateau value. This stress value is representative of the yield stress of the gastric contents. When the shear rate increases, the viscosity decreases non-linearly. In this state, the gastric contents are therefore shear-thinning yield stress fluids. When the residence time increases (120 minutes), the level of shear stress decreases significantly, when compared to those corresponding to 30 and 60 minutes. The shear stresses then have a linear correlation with the shear rate and the viscosity is nearly constant. The gastric contents are no longer pasty but exhibit a Newtonian behavior. The viscosity level is still at least 100 times the viscosity of water (>100 mPa.s). These viscosity data have been measured previously using gastric contents from liquid meals (Guerin et al., 2001). Moreover they cannot be explained simply by the presence of saliva, the viscosity of which is at most 10 times the viscosity of water (Schipper et al., 2007). Carbohydrates may completely cover the non-Newtonian behavior of saliva and other gastric proteins.

For a short residence time of 30 min, large particles of bread created by chewing are observed (Fig.2a). The gastric juice is a concentrated suspension of deformable lumps swollen with water. Contacts between particles are lubricated to varying degrees depending on the available water quantity. The yield stress may be due to particles jamming together while they are still rigid. When increasing the residence time up to 60 min, a certain amount of liquid is added from the pig's stomach, which dilutes the gastric juice (Fig.2b). The lumps also dissolve in the liquid medium, and the particles tend to be softer. In addition, the amylase breaks starch down into sugars and thereby reduces the molecular weight. Those three mechanisms contribute to liquefy the suspension and reduce viscosities, until it practically reaches a liquid state at 120min, with few immersed particles (Fig.2c).

For further analysis, we chose a viscosity at a low shear rate, i.e. 0.03 s^{-1} , because flow through the duodenum is quite low and the order of magnitude of the velocity is small. The

low viscosities of Newtonian juices at 0.03 s^{-1} result in low torques, which cannot be measured by our device, on account of the transducer sensitivity. Therefore, as Newtonian fluids exhibit a constant viscosity regardless of shear rate, the viscosity was extrapolated from data obtained at higher shear rates.

Fig.4 shows the change in viscosity (Fig. 4a) and in moisture content (Fig. 4b) according to residence time for the three breads. Moisture content increases with residence time. At 30 min, the moisture content and viscosity are constant regardless of the breads. The moisture is directly related to the water content of the original meal before ingestion, leading us to decide that the pigs should not have access to drinking water during the experiments. After ingestion, the pigs add a certain amount of liquid themselves depending on digestive activity. The moisture content therefore tends to increase at 60 min, and the viscosity decreases as a consequence. The trend is influenced by the different gastric secretions and the breads, owing to the differences in Am/AP. This increase in moisture content and decrease in viscosity have been noticed previously (Johansen et al., 1996). Finally, a viscosity reduction of the order of 2-3 decades is recorded after a residence time of 120 min, with an increase in moisture content.

It should be pointed out that the overall change in viscosity versus the residence time clearly depends on the individual pig. This can be explained by the differences in oral and gastric secretions, and by those concerning chewing behavior. The drop in viscosity confirms the mechanism of dilution, lump dissolution and amylase action on starch.

3.2. Relation between moisture content and rheological properties

The rheological properties are very sensitive to the moisture content of the gastric juice, as shown in Fig.5. The decrease in rheological properties with increasing moisture content confirms the fact that the gastric juices are suspensions. Indeed, the viscosity is related to the moisture content according to an exponential law, fitted over the entire digestion period. Such correlation highlights the assumption that the rheological behavior of gastric juices with particles is rather governed by the particle concentration, and therefore by their dissolution and dilution. The rheological behaviour of the gastric juices may be also considered as depending on the shape and size of the particles, but our rheological approach based on bulk measurement cannot discriminate this effect.

3.3. Influence of Am/Ap on rheological properties and moisture content

The Am/Ap is the main chemical parameter that changes with the composition of the bread. As shown in Fig.4a, the viscosities at 30 min and 60 min are not significantly different with any of the breads. For those residence times, the rheology of the gastric juices may be governed more by the mechanical structure, i.e. the macroscopic particles immersed in a yielded suspension (Fig 2a &b), than by the chemical properties of the carbohydrates. On the other hand, a significant difference in viscosity can be noted at 120 min, especially in the case of Bread 1. Therefore, we provide in Fig. 6a the viscosities versus the Am/Ap ratio. The increase of viscosity with Am/Ap is quite significant at 60 min, but more significant at 120 min, as the particles have been largely dissolved (Fig.2c) and the gastric juice is no longer a yield stress fluid (pasty). In parallel, a significant drop in moisture content as a function of Am/Ap can be observed only in the case of a long residence time, 120 min (Fig.6b). Thus, the two breads with higher amylose content appear to maintain a higher viscosity at 120 min as they absorb less moisture. The origin of the trend will be discussed in the next section of the present article.

4. Discussion

During the digestion process, gastric juices behave as a concentrated suspension, as visually illustrated by Fig.2 and by rheological data of Fig.5, that depicts the exponential relation between viscosity and moisture content. This relation is not significantly affected for breads containing various Am/Ap. During the process, the suspensions switch from a yield stress behavior of jammed particles to a less concentrated suspension, with smaller and softer particles. Previous results (Fig.6) show that the Am/Ap does not significantly modify the moisture content and the rheology at the beginning step of digestion. After a certain residence time, the suspensions became “soft” enough to perceive the effect of Am/Ap on their rheological properties (Section 3).

In this section, we wish to propose an explanation for the difference in viscosities between the breads observed after 120 min. This requires an accurate analysis of the continuous process from ingestion to digestion from mechanical and chemical points of view, in light of the rheological data.

Initially, the breads have an alveolar sponge-like structure, depending on the flour, as shown in Fig.1. The breads have various crumb densities due to the Am/Ap ratio, and therefore different porous media (Table 1). The bread cubes are swollen with water before ingestion but the porous medium is not saturated. During mastication, the cubes are reduced to pieces of different sizes. Once ingested, the gastric juices are a structure of large swollen lumps of bread lubricated with water (Fig.2a). The lumps are progressively dissolved by the action of enzymes and water through the porous medium, which differs from bread to bread. The measurements carried out at 30 min show that the moisture content and viscosity are similar regardless of the Am/Ap (Fig.4a - 4b), which proves that the porous media induced by Am/Ap does not affect the way the lumps are dissolved. As observed in the previous section, the rheology of the gastric juices is determined more by the lumps jamming up to 30 min.

As dissolution takes place in the stomach, a slight rise in the moisture content is observed at 60 min in the case of the bread with the lowest Am/Ap (Fig4.b). Several studies have suggested that once solubilized, amylopectin retains a larger quantity of water (Tester and Morrison, 1990; Eastwood and Morris, 1992; Morita et al., 2002; Van Hung et al., 2006; Singh et al., 2010). But the difference in moisture content is not significant at 60 min, maybe because the suspension is not sufficiently degraded to solubilize a large amount of amylopectin. Indeed the juices have nearly the same appearance at 30 min (Fig.2a) and 60 min (Fig.2b). The slight decrease in viscosity observed at 60 min in the case of the bread with the highest Am/Ap (Fig4a and Fig 6a) may be correlated with the rise in moisture content, not with the Am/Ap itself from a chemical point of view.

After 120 min, the lumps have practically disappeared (Fig.2c). Measurements show that the moisture content is much higher in the case of high Am/Ap bread, but the corresponding low viscosity cannot be explained only by the moisture content, because the gastric juice is visibly no longer a concentrated suspension (Fig.2c). The amylopectin is more rapidly degraded by the amylase action, and no longer contributes to the consistency of the juice. In contrast, the amylose is more resistant to degradations in stomach, and rather degraded in the intestines (French, 1984; Black, 2001; Lindberg et al., 2003; Kim et al., 2005). The amylose therefore helps to increase the viscosity of the liquid phase with a long residence time.

The pig's chewing behavior and gastric secretion affects the results, as shown in Fig.4 and Fig.6. Those may change the particle size distribution and moisture content of the concentrated suspension. Their influence is stronger in the case of a short residence time, where the particles determine the behavior of the suspension. In the case of a long residence time, lump dissolution reduces the effect of the initial chewing, but the effect of secretion is still apparent, leading to variable moisture contents and viscosities. However, some important observations can be made at 120 min. The initial meal could be homogenized by mechanical mashing. This would reduce the influence of the individual pigs in the case of short residence times, but the experiment would be far from representing real bread consumption by humans.

5. Conclusions

The main objective of this study was to present a methodology for rheological characterizing gastric contents from a solid meal. This characterization was carried out by a vane test associated with the proper experimental conditions and a suitable analysis. Despite the constraints linked to the suspensions studied and the strong influence of the pigs' behavior, the results revealed that the rheology of gastric contents is dictated by their suspension nature up to a residence time of 60 min. The consistency of the concentrated lump suspension produced by particle jamming almost surpasses any effects caused by the different A_m/A_p ratios. The viscosity decreases with residence time in the stomach, which is correlated with an increase in the moisture content. We suggest that the A_m/A_p only influences the moisture content and viscosity in the case of a long residence time, after 120 min. The gastric contents are no longer concentrated lump suspensions, since the lumps of bread are digested through amylase action. The viscosity is then dictated by the molecular weight of the amylopectin and amylose in solution. As the amylopectin is rapidly degraded, the amylose contributes more to the viscosity because it is less susceptible to amylase action and therefore maintains a higher molecular weight and higher viscosity.

Rheology may be a relevant method for understanding the changes in gastric juices during residence in the stomach, and the influence of amylose and amylopectin on the process. It should be noted that the variability in viscosity between the different animals suggests that the way the meal is broken down by chewing has an influence on the rheological behavior of the gastric juice, especially after short digestion periods.

Acknowledgments

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6. Figures



Fig.1 CAT scan of the three breads. The size of the air inclusion is visibly different, mostly in the case of bread 3.

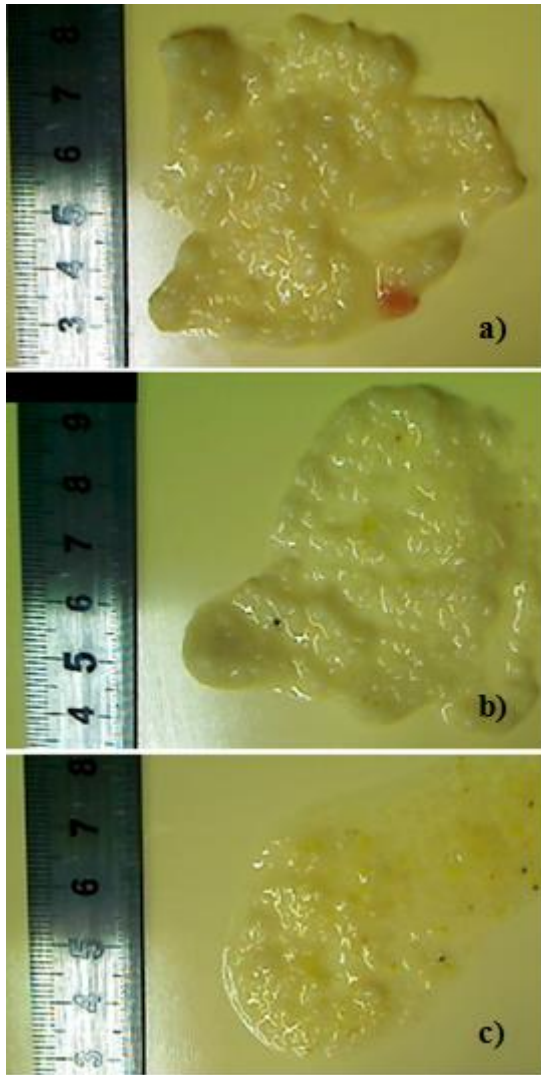


Fig.2 Picture of gastric content for each residence time: 30 min (a), 60 min (b) and 120 min (c).

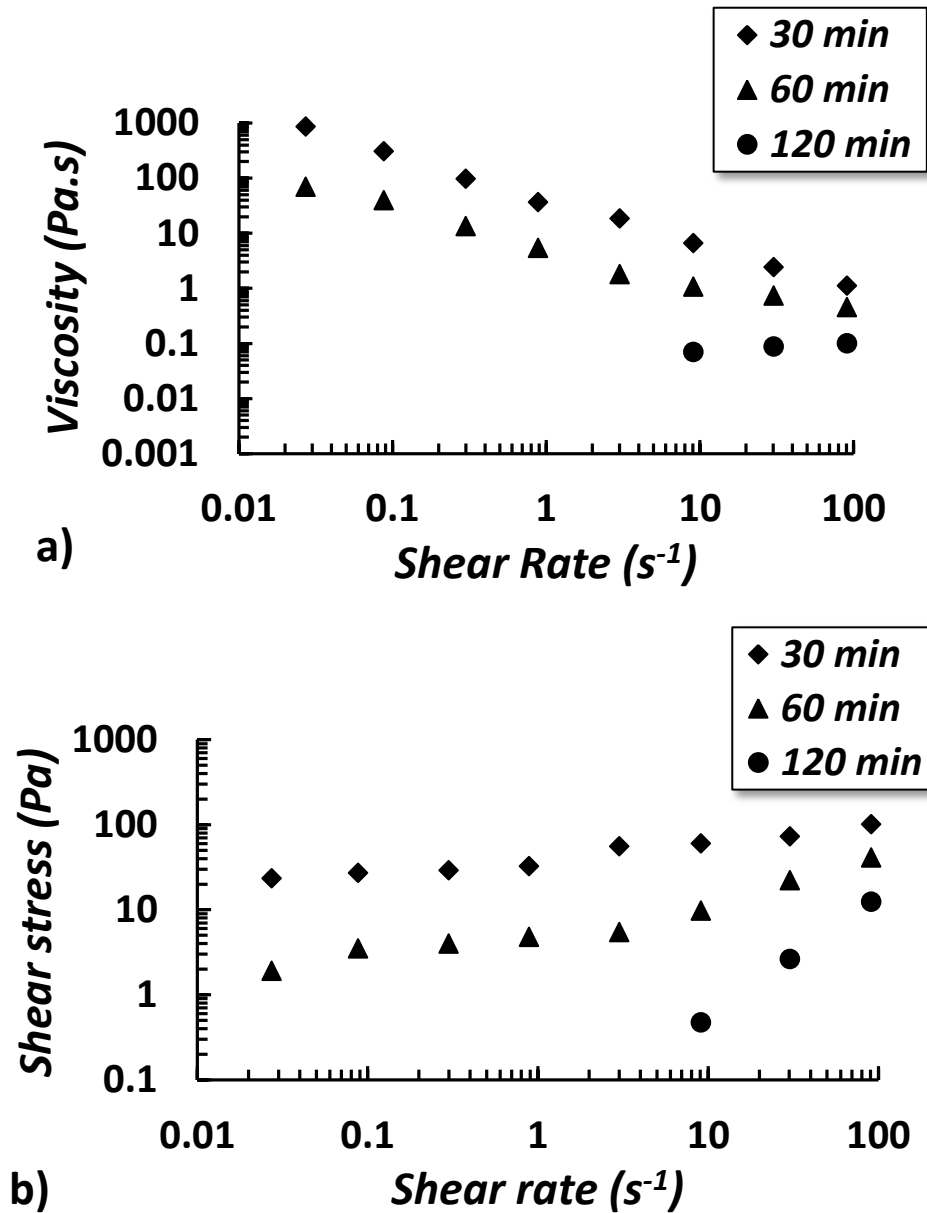


Fig.3 Flow curves of gastric juices extracted after different residence times: 30, 60 and 120 minutes. Gastric juices of Bread 1 are extracted from a single pig. Graphs show the change in shear stress (a) and viscosity (b) according to shear rate.

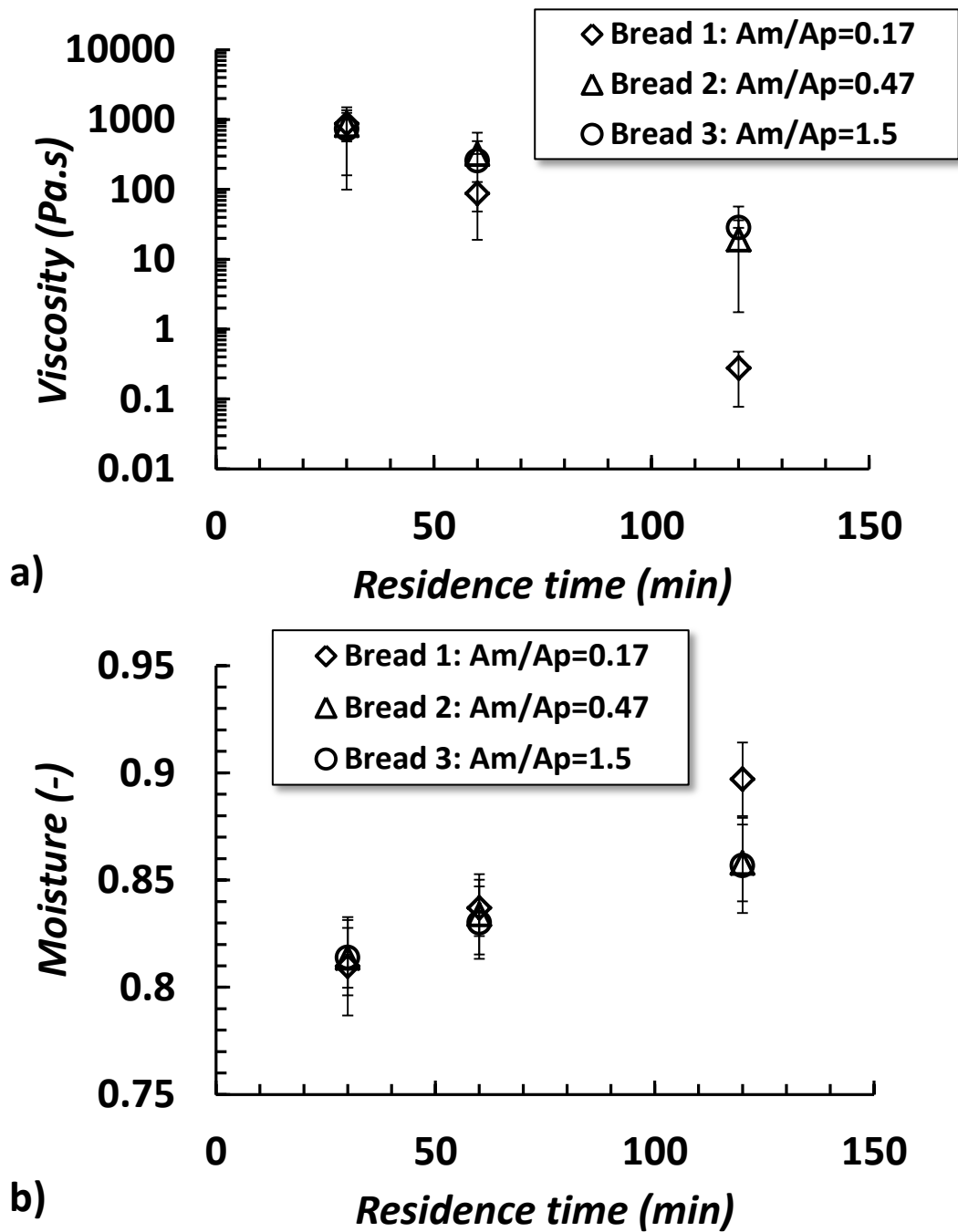


Fig.4 Change in viscosity at low shear rate (a) and moisture content (b) according to residence time for all pigs (n=8). Each type of bread is presented.

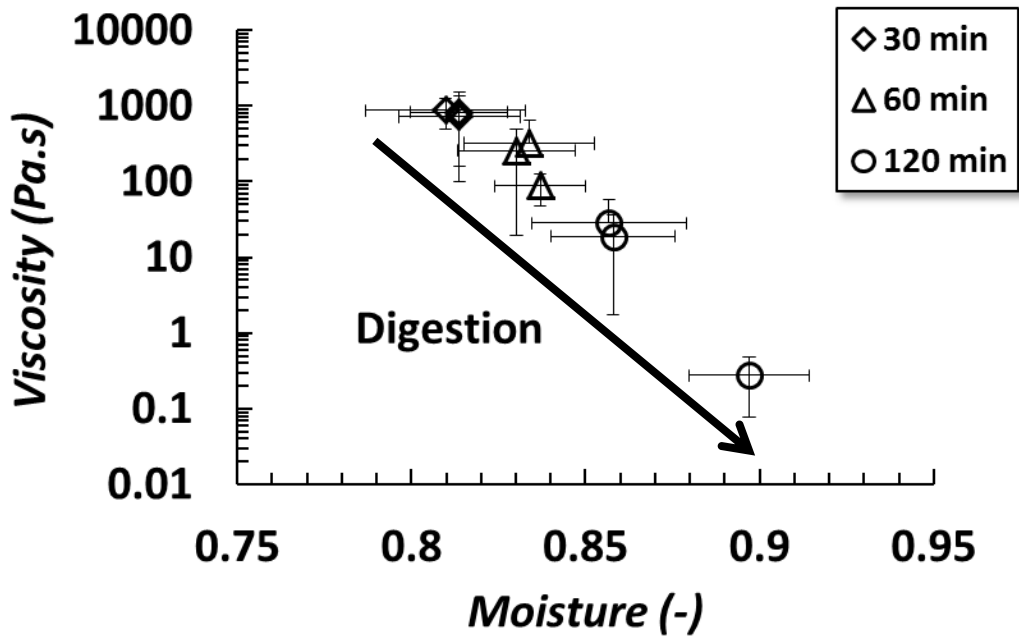


Fig.5 Change in viscosity at low shear rate according to moisture content for all pigs (n=8). Residence times have been provided on the graph to show the effect of the digestion process on the properties of the suspension.

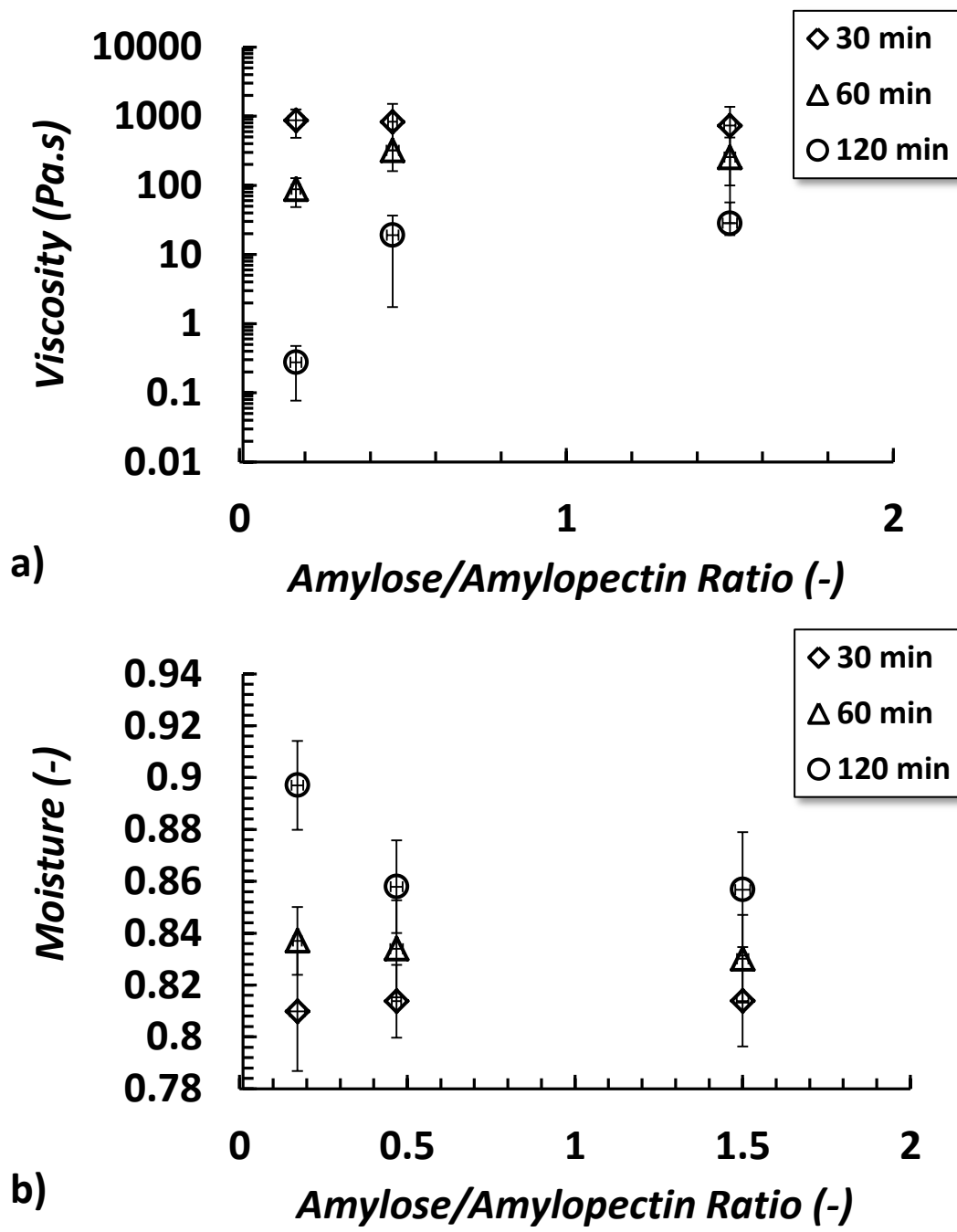


Fig.6. Change in viscosity at low shear rate and moisture content according to Am/AP for all pigs (n=8). Each residence time is presented.

Table 1:

Bread codification, formulation and characteristics. Data of air distribution through the breads

Bread reference	Flours mix	Amylose (g/100 g)	Amylopectin (g/100 g)	Energy (kcal)	Intake mass (g)	Amylose/Amylopectin ratio (-)	Average size of inclusions in the crumb (mm ³)	Trapped air ratio (%)
Bread 1	50% Wheat flour + 50% Waxy floor	10±0.1	58±0.1	500±3	162±1	0.17±0.1	1.9±0.1	14±1
Bread 2	100% Wheat flour	22±0.1	47±0.1	500±3	124±1	0.47±0.1	1.4±0.1	12.5±1
Bread 3	80% Wheat flour + 20% Amylose	27±0.1	18±0.1	500±3	215±1	1.5±0.1	0.7±0.1	5±1

are given as the mean of the acquisitions of 10 slices.

Chapitre 2.2

Rôle de la rhéologie des jus gastriques sur la vidange gastrique

Gastric kinetic of emptying following a meal of bread and the relationship with the rheological properties of gastric contents and the fiber content

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1. Introduction

To slow down the glycaemia and insulinemic kinetics, various technological solutions exist: use of partially crushed grains (Fardet et al., 2006; Juntunen et al., 2002), where the intact vegetable walls decrease the accessibility of the substrates to the degradation enzymes, the addition of exogenous fibres (whole-wheat bread, bran bread, whole and complete flours) (Jenkins et al., 1986; Leinonen et al., 1999) or the use of less refined flours (T 70, 75, 80, remilling). These products look like those marketed in Northern Europe for their sensory characteristics. Whereas the influence of the technological processes (Calderón-Domínguez et al., 2005; Katina et al., 2006; Primo-Martín et al., 2007; Shittu et al., 2007) and the aspects of the composition were shown (Batifoulier et al., 2006; Hartmann et al., 2005), the studies on genetic diversity exist but are insufficient. In these works, a major place is given to the stage of ileal digestion; they are founded on the assumption that the gastric stage has no effect on the rate of digestibility of the starch and its kinetics. The incidence of the gastric kinetic of emptying is then often neglected whereas it directly influences the digestion (Gray, 2005). After consuming a typical solid meal, there is a lag time of 20 to 30 min in which there is minimal gastric emptying. This is followed by a phase in which the rate of emptying is roughly linear, in contrast of the exponential and quick rate for liquids (Achour et al., 2001). Moreover, the rheological properties of gastric contents during digestion may have an important role in gastric emptying, which can impact the kinetic aspects and the glycemic response (Björck et al., 1994; Edelbroek et al., 1993; Horowitz et al., 1993; Jebb, 2007). Thus, the first aim of this work is to monitor the effect of gastric contents rheology on gastric kinetic of emptying in the case of solid food such as breads.

The lipids content of the gastric bolus is the most known factor that affects gastric emptying. Of course, this factor is to be proscribed in the aim in view. On the other hand the consumption of a cereal product with slow disintegration is one of the assumptions chosen in this study, to increase viscosity and then maybe to decrease the speed of gastric emptying and rates of intraluminal carbohydrate hydrolysis. This choice is based on results in the literature in which it is proposed that slowing gastric emptying by means of increasing meal viscosity might slow digestion and absorption of nutrients, thus improving the management of glucose intolerance and obesity (Marciani et al., 2000). Indeed it was shown that soluble dietary fibre delay absorption of glucose, resulting in attenuated post-prandial peripheral glucose and

insulin responses to carbohydrate meals in both man and animals (Ebihara et al., 1981; Jenkins et al., 1978; Nunes and Malmjöf, 1992). This effect is thought to be mediated by a reduced rate of gastric emptying and / or a direct effect on the diffusion and absorption of nutrients in the small intestine (Ebihara et al., 1981; Jenkins et al., 1987). In this respect, the ability of soluble dietary fibre to increase gut viscosity is generally considered to play a major role (Blackburn et al., 1984; Jenkins et al., 1978). However, reports on the effect of soluble dietary fibre on gastric emptying are ambiguous and well established in case of the addition of soluble dietary fibre to liquid glucose test meals (Ebihara et al., 1981; Holt et al., 1979; Torsdottir et al., 1991). The form in which the dietary fibers (DF) has been induced in the meals and their level of hydration may partly explain differences in the results obtained (Low, 1990). Furthermore, most published results relate to the viscosity of the meal and rarely to the viscosity of the matrix at the site where its physiological function is supposed to take place (Cherbut et al., 1990); this can bias conclusions about the physiological role of viscosity quite significantly for some types of soluble dietary fibre (Edwards et al., 1987). Structure and particles size are two important parameters in relation to gastric-emptying rates (Johansen et al., 1996). Thus the second aim of this study is to assess the effect of fibers content in a cereal based product, bread, on gastric contents rheology and on their kinetics through the duodenum.

To achieve these two objectives, the methodology we adopted is as follows. The tests campaign will be carried out on two groups of pigs with three types of bread. This animal was selected as a human model because of its similarities in anatomy and function of the gastric segment (Malbert and Horowitz, 1997). The gastric emptying through the duodenum and the jejunum will be studied in pigs by using gamma scintigraphic imaging of the stomach. Gastric emptying and motility (propagated gastric contractions) will be evaluated after spontaneous ingestion of radio-labelled bread supplied in an isocaloric manner so to cancel a possible effect of different caloric contents between breads. The rheological properties of gastric contents will be performed following the methodology described in Patarin et al. (2014).

2. Materials and Methods

2.1. Materials

2.1.1. Breads

Breads were made from three different flours distinguished by their amylose/amylopectin ratios (Am/Ap). The flour characteristics are shown in table 1. They are mix of standard flour T55 (Girardeau, France), amylose powder l'HI-maize 260 (National Starch and Chemical Company, Bridgewater, USA) and amylose-free waxy flour Alice (Limagrain, Saint-Beauzire, France).

Aiming to evaluate stomach emptying with gamma scintigraphy, a strategy bread making was developed. A short life time radio isotope was mixed into the bread paste thanks to the water with 70 MBq of colloidal ^{99m}Tc . Breads were then made using a Moulinex bread machine (SEB, Ecully, France) with a 2h30 cooking program. Breads were made of 216 ml of water, 5 g of NaCl, 360 g of flour and 4.7 g of yeast. Each test session was performed with breads made 12 hours before the experiment, and breads had at least 10 MBq (^{99m}Tc has a half-life of 6h).

2.1.2 Animals

The impact of the amylose contents on gastric function will be evaluated in three groups of pigs (female pigs, large white, 35 ± 3 kg). Animals from the first group (Group 1, n=8) were trained for one week in a Pavlov stand to ingest the bread meal they would eat during the test campaign. This adaptation period was followed of a recovering period of two weeks, aiming to suppress any residual effect of the ingested meal. These animals were used the measure the gastric emptying kinetics.

Animals from the second group (Group 2, n=8) endured a surgical intervention to locate a cannula in the proximal stomach, and were allowed a period of recovering (Patarin et al., 2014). These animals were used for the rheological tests on their gastric juices. Gastric juices were gathered through the cannula.

2.2. Methods

2.2.1. Bread intake

The gastric emptying studies were carried out using each animal as his own control. The trials were performed after 12 hours fasting. For groups 1 and 2, animals received sequential portion for a random bread crumb cut in 2 cm³ cubes, to provide 500kcal portion (171 g for bread 1, 182 g for bread 2, and 177 g for bread 3) mixed with 150 ml of water. The animals ate the meals in less than 5 minutes. Immediately after the meal and during a period of 120 minutes, the animals do not have access to drinking water.

For group 3, animals received portion for a random bread crumb cut in 2 cm³ cubes, to provide 50g equivalent glucose portion (74.4 g for bread 1, 73.9 g for bread 2, and 80.8 g for bread 3). The animals ate the meals in less than 5 minutes. Immediately after the meal and during a period of 120 minutes, the animals do not have access to drinking water.

2.2.2. Image acquisition and processing

For animals of the first group, the meals were radiolabeled with 99mTc colloid. The measurements are performed on scan for 120 minutes following the waning of gastric emptying.

Quality control, acquisition and signal processing provided by the gamma camera comply with the standards set by The Nuclear Medicine Society (General Imaging and Specific recommendation for gastric emptying and motility). In practice, the images of the lateral projection of the stomach were acquired through a high-resolution low-energy collimator. Data from a matrix of 64x64 pixels are stored as images representing 120 seconds of acquisition on a dedicated computer (Apex, Elscint, Haifa, Israël). These images were subsequently transferred to a treatment console using a program coded specifically for gastric emptying measurements (LabView-IMAQ, National Instruments, Austin, USA). Movements of the animal during the measurement are compensated with image alignment function. The program performed also the compensation due to radioactive decay and ultimately adjusted the amount of radioactivity present in the stomach region of interest, according to an exponential power law. Gastric volume ratio were calculated for a residence time, dividing the actual gastric volume ratio by the initial gastric volume at $t = 0$ min.

2.2.3. Gastric juices rheometry

The samplings of the gastric contents and the rheological measurements have been performed following the methodology previously developed by Patarin et al (2014) on the group 2. Gastric juices from bread tend to create semi-fluid suspensions with large swollen lumps. Viscosities of the juices are measured with controlled environmental conditions (temperature, time, strain, ...) and as a function of the residence time (30, 60, and 120 minutes) and the Am/Ap.

2.2.4. Statistical analysis

Graph Pad Software (Prism, USA) was used for statistical analyses (mean and standard deviations) and ANOVA test. For analyzing correlations, Pearson tests were performed on different set of parameters, including the uncertainties and variations due to repetitions.

3. Results

3.1 Gastric contents rheology

Pigs have a causative effect on the rheological properties of the juice in the early digestion time (30 minutes). Chewing has a strong influence on the structure of juice. The structure and thus the rheological behavior are ruled by jammed bread lumps, swollen with water. The size depends on the masticatory activity of each pig. The effects of Am/Ap on viscosity and moisture are embedded in Fig.1a. At this stage, the juices have a pasty aspect, behave as a suspension and exhibit a yield stress behaviour.

After 60 minutes, the viscosities of the juices fall down. The decrease is slightly stronger for low Am/Ap, maybe due to higher moisture content (Fig.1b). Indeed high amylopectin concentration in solution is known to increase the moisture in the stomach (Tester and Morrison, 1990; Eastwood and Morris, 1992; Morita et al., 2002; Van Hung et al., 2006; Singh et al., 2010).

After 120 minutes, the viscosities of all juices decrease at least one decade, compared to those at 60 minutes. The viscosity of low Am/Ap solution is one decade lower the viscosities of other juices (Fig.1c). The cause may be the increase of moisture for higher concentration of amylopectine, or the lower concentration of amylose which is more slowly digested, and may then participate in the increase of viscosity for the other juices.

3.2. Gastric emptying kinetics

Fig.2 shows typical emptying curves of one pig for the 3 breads. In this example the shape of the curves are quite similar; that exhibit an exponential decay. This shape is related to solid meals, whereas liquid meals exhibit a linear shape.

To get a comparison with viscosities values at different residence time, gastric volume ratio were plotted according to A_m/A_p for residence times of 30 min (Fig.3a), 60 min (Fig.3b) and 120min (Fig.3c), and every pigs. No significant dependency of the gastric volume ratio with fibers content can be shown at 30 and 60 min. As the A_m/A_p has also no clear effect on viscosity. We are not able to conclude in those cases : the consistency of the gastric juices, governed by jammed bread lumps, tends to level off fibers effects on rheology.

At 120 minutes, although a dependency between viscosity and fibers content was shown but no consequence on gastric volume can be brought out from Fig.3c. If the gastric emptying was a pumping process, viscosity should modify the flow rate and the gastric volume. Thus the flow rate seems to be regulated. The parameter which may regulate the flow rate is the energy content. The meals ingested by the pigs were set to be isocaloric. We can make the hypothesis that the energy density of the meal may regulate the flow rate even if the viscosity of the gastric content from a solid meal is different.

4. Conclusions

Rheometry results show differences in terms of viscosities between gastric juice from breads with different fibers contents for long residence time, despite the initial effects of chewing process. According to the kinetics of gastric emptying, gastric content volumes at different times of residence time exhibit on average no significant variations with varying fiber contents. It should be noted that meals in the case of viscosity tests were mix of water and bread, while no water was added to meals for gastric emptying monitoring. In spite of this difference, those experiments suggest that different rheological properties do not involve fundamentally different kinetics of emptying in this case the solid meal, notably for long residence time. The regulation of gastric emptying may be then operated exclusively by the energy charge in the gastric juice. Other measurements have to be performed to confirm this hypothesis, keeping the same protocol meal intake for viscosity tests and gastric emptying monitoring.

Acknowledgements

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5. Figures

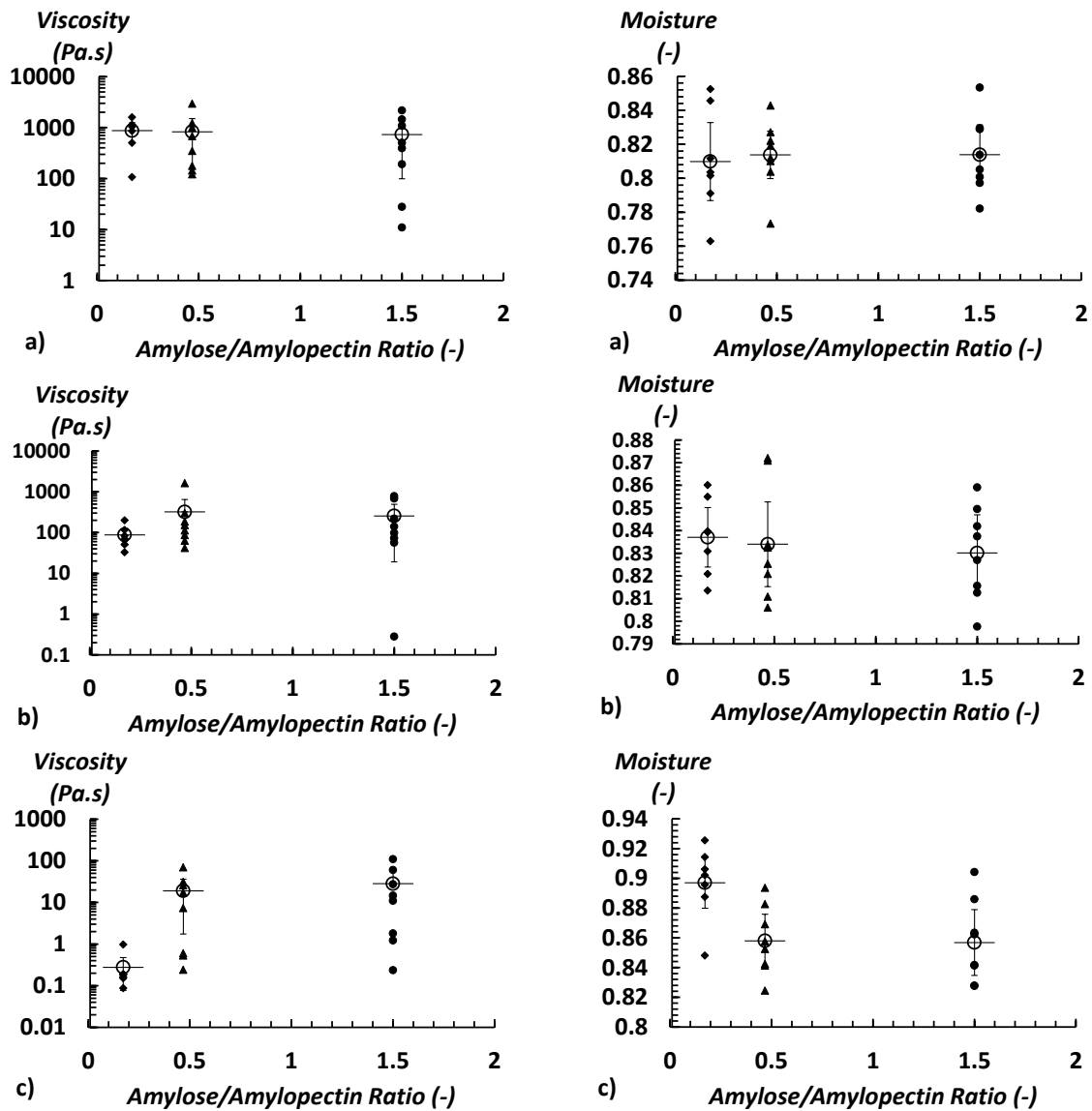


Fig.1 Change in the viscosity at a low shear rate and moisture content according to the Am/Ap for 8 pigs from group 1. Each residence time is presented, respectively 30 min (a), 60 min (b), 120 min (c).

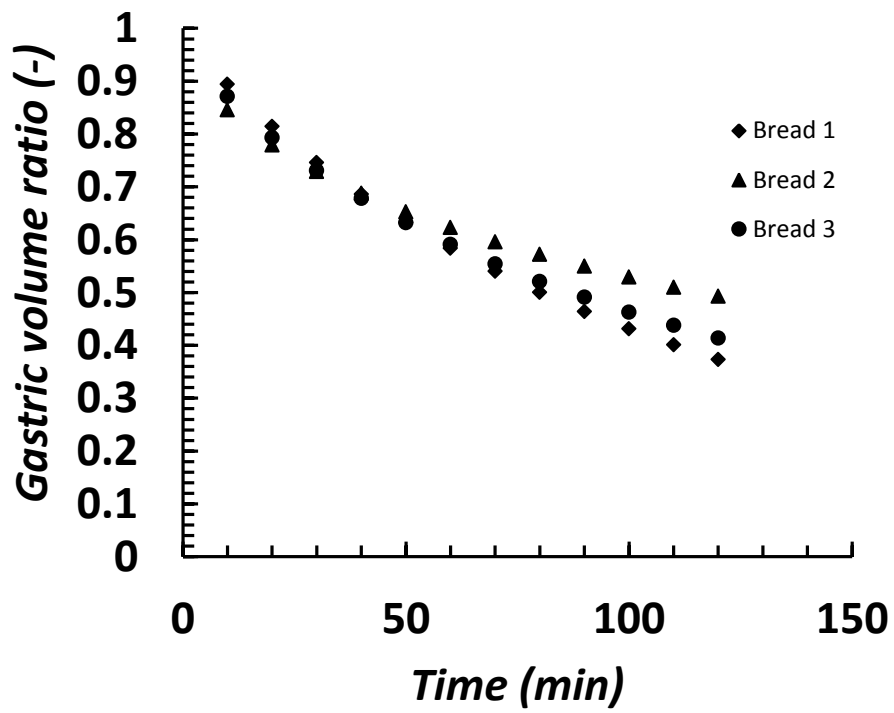


Fig.2 Evolution of gastric volume ratio with time for the three breads for one pig.

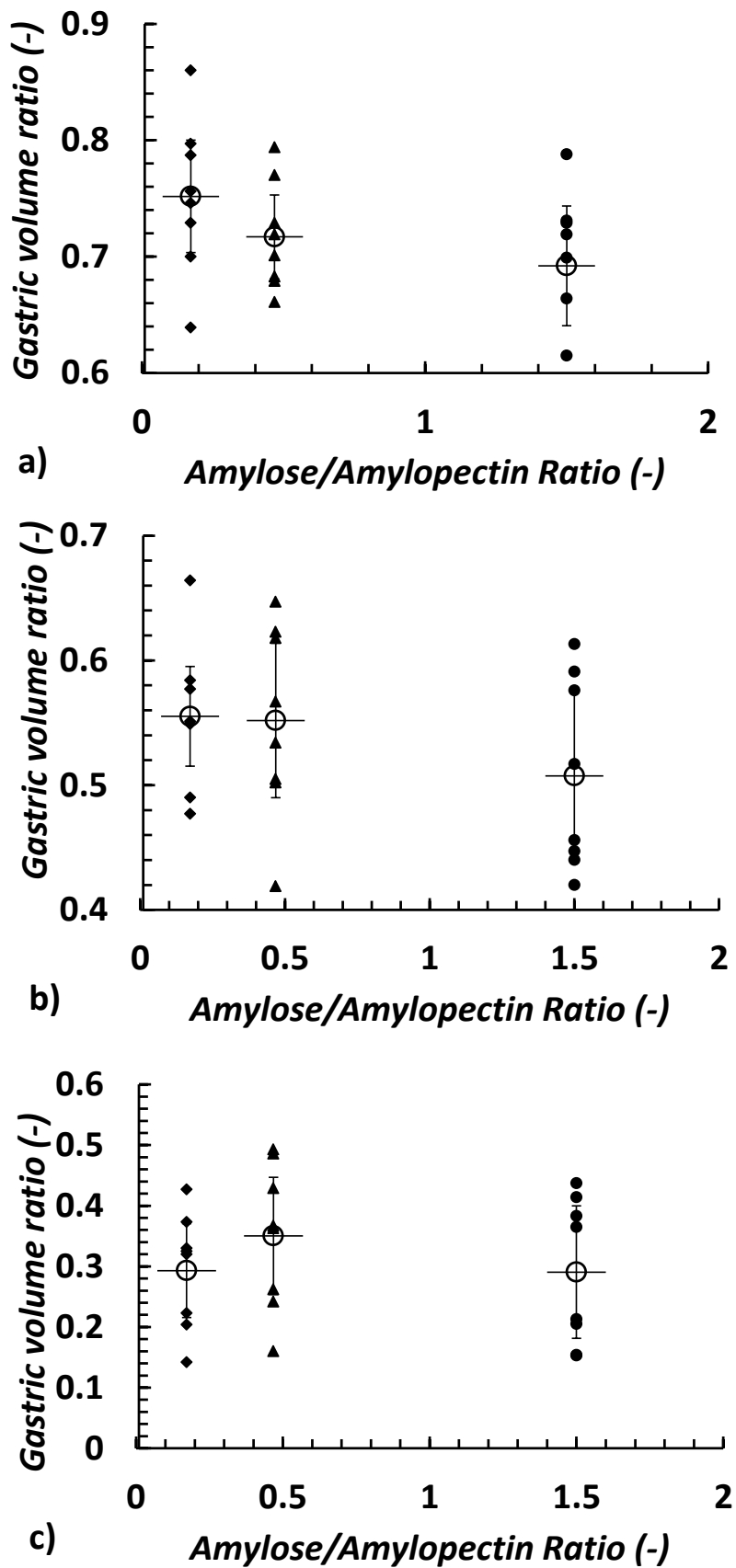


Fig.3 Change in the gastric volume ratio according to the Am/Ap for 8 pigs from group 1.

Each residence time is presented, respectively 30 min (a), 60 min (b), 120 min (c).

Table 1:

Bread reference	Flours mix	Amylose (g/100 g)	Amylopectin (g/100 g)	Energy (kcal)	Intake mass (g)	Amylose/ Amylopectin ratio (-)	Average size of inclusions in the crumb (mm ³)	Trapped air ratio (%)
	50% Wheat flour							
Bread 1	+ 50% Waxy floor	10±0.1	58±0.1	500±3	171±1	0.17±0.1	1.9±0.1	14±1
Bread 2	100% Wheat flour	22±0.1	47±0.1	500±3	182±1	0.47±0.1	1.4±0.1	12.5±1
Bread 3	80% Wheat flour + 20% Amylose	27±0.1	18±0.1	500±3	177±1	1.5±0.1	0.7±0.1	5±1

Bread codification, formulation and characteristics. Data of air distribution through the breads are given as the mean of the acquisitions of 10 slices.

Conclusion générale et perspectives

Ce manuscrit présente le résultat de travaux menés par le Laboratoire Rhéologie et Procédés dans le cadre de deux projets (Sensimouth et Nomac) financés par l'Agence National de la Recherche sous la direction scientifique d'Albert Magnin.

Deux types de suspensions concentrées transitant dans le système digestif ont été étudiés : les bols alimentaires issus de la consommation de fromages et des jus gastriques issus de la consommation de pain.

A l'origine, les produits de base sont ingérés. Leurs caractéristiques rhéologiques et structurelles sont fondamentales pour appréhender le comportement rhéologique des bols alimentaires et des jus gastriques. Bien qu'elle soit a priori plus aisée, car elle concerne des milieux continus, cette caractérisation nécessite la mise en place de protocoles particuliers afin de mener une rhéométrie pertinente. Les résultats du premier chapitre de la partie I mettent en valeur l'importance de contrôler l'adhérence du produit à l'interface avec l'outil de mesure, et de garantir un effort normal minimal sur l'échantillon. Ces précautions permettent d'accéder à des propriétés rhéologiques en cisaillement reproductibles, sûres, et affranchies de tout artéfact de mesure.

Dans un second temps, la rhéométrie des aliments transformés en bouche demande un compromis entre la mécanique des milieux continus, et le fait qu'on veuille tester une suspension de morceaux millimétriques hétérogènes. D'autres contraintes viennent s'ajouter : des faibles volumes (~10 ml), des temps de vie *ex vivo* courts (~1min) et une température de 37°C. Ces contraintes ont abouti à la création de deux méthodologies de mesure distinctes, pour les bols alimentaires issus de fromages (chapitre 2 de la partie I) et pour des jus gastriques issus de pains (chapitre 1 de la partie II). Notons que dans chaque situation, nous avons dû créer une instrumentation de « terrain » originale pour aller au plus près de la génération des échantillons : les panelistes en condition clinique pour les bols alimentaires et les porcs dotés de canule gastrique pour les jus gastriques. Une méthode de compression a été tout d'abord créée et validée sur des suspensions modèles. La scissométrie a été utilisée pour la caractérisation des jus gastriques, technique au préalable déjà éprouvée dans la littérature pour caractériser des suspensions concentrées. Dans les deux cas, l'enjeu était d'extraire des données physiques relatives aux propriétés de volume, qui peuvent être utilisées dans des modèles physiques et physiologiques.

Dans le cas des bols alimentaires issus de fromage, l'aliment est transformé en bouche sous l'effet de la mastication. Lors de cette étape, de nombreux stimuli sont libérés dans la sphère orosensorielle. La libération de ces stimuli génère une dynamique de perception sensorielle, responsable pour une grande part de la qualité organoleptique du produit. La méthode d'analyse utilisée a validé le comportement de suspension des bols et a permis d'extraire des paramètres issus d'un modèle d'Herschel-Bulkley. Un modèle de Château a été alors utilisé pour extraire une évaluation des paramètres rhéologiques de la phase suspendante, particulièrement performant pour les fromages fermes et maigres créant des particules discrètes dans une phase liquide de consistance plus faible. Dans le cas des fromages mous et gras, les différences de rhéologie entre la phase suspendante et les morceaux deviennent si ténues que les limites de cette approche sont atteintes. Au travers du chapitre 3 de la partie I, les paramètres rhéologiques extraits ont été en partie corrélés à certaines données issues de la libération aromatique. L'influence du comportement masticatoire des individus est importante et seule une analyse au cas par cas a permis de montrer l'influence du seuil de contrainte à l'écoulement du bol alimentaire sur le pic de libération aromatique, notamment la contrainte seuil de la phase suspendante dans le cas de fromage maigre. Cette observation valide l'importance du seuil de contrainte à l'écoulement dans les procédés d'enduction des muqueuses buccales et pharyngiennes au cours des phases de mastication et de déglutition, vis-à-vis de la libération aromatique. De plus, de la synthèse bibliographique, nous avons pu identifier que cette interaction influençait plus particulièrement la génération de surface de contact entre le produit et la salive lors de la mastication, et l'enduction des muqueuses du pharynx.

Dans le cas des jus gastriques issus de pains, et contrairement aux bols alimentaires issus de fromages chez l'humain, le volume d'échantillon issu de l'estomac des porcs est suffisamment conséquent pour utiliser la scissométrie comme méthode de mesure pour caractériser ces suspensions. Cependant, le développement d'un instrument spécifique a encore été nécessaire pour travailler sur un échantillon de faible durée de vie, en température, tout en contrôlant les champs de déformation. Les méthodes de mesure ont permis d'identifier les propriétés rhéologiques des jus gastriques et leurs évolutions en fonction d'autres paramètres. Quelle que soit la composition du pain, la viscosité et les seuils de contrainte à l'écoulement diminuent avec l'augmentation du temps de résidence dans l'estomac, à cause de l'attaque enzymatique graduelle et la dissolution des fibres dans la matrice suspendante,

suivie grâce à une mesure du taux d'hydratation. Les jus gastriques passent alors d'un comportement viscoplastique à un comportement newtonien. Ces variations temporelles de viscosité se sont montrées très significatives face aux variations dues aux formulations des pains, notamment à 30 et 60 minutes de digestion. Ainsi l'effet du rapport d'amylose/amylopectine ne se fait sentir qu'à partir de 120 minutes, où l'encombrement dû aux particules de pain gonflées est moindre. La viscosité est alors plus forte pour les jus contenant plus d'amylose, car la dégradation de ce glucide n'est opérée qu'à partir de l'intestin grêle. Les résultats issus de la rhéométrie ont été ensuite comparés aux résultats issus de l'analyse des cinétiques de vidanges gastriques. Alors que l'influence de la composition en fibre est notable et significative à 120 minutes sur la viscosité des jus gastriques, cette composition n'a que peu d'effet sur les cinétiques de vidanges. Sachant que les repas donnés aux porcs étaient isocaloriques pour tous les pains, ces observations supportent la théorie que le débit en sortie d'estomac serait régulé par la charge calorique du repas, et non pas par sa viscosité. Cela suppose l'existence d'un système de régulation basé sur la « mesure » de la charge énergétique du bol, induisant une rétroaction sur le débit au travers du jéjunum.

D'un point de vue général, les campagnes de caractérisations rhéologiques sur des fluides biologiques issus d'humains ou d'animaux imposent forcément de trouver un compromis entre la précision de la mesure liée à l'application de la théorie des milieux continus, et les contraintes dues à la structure, à la température, et aux faibles durées de vie des dits fluides. Par ailleurs, l'influence du panel est notable, particulièrement en raison du comportement masticatoire variable des individus, mais aussi des animaux. Aux vues de ces variabilités, il a été pour nous plus que nécessaire de travailler individu par individu pour prendre en compte leur spécificité. Dans le contexte de l'agroalimentaire et des essais, les protocoles libres peuvent injecter une complexité supplémentaire dans l'étude de systèmes déjà complexes, et pourtant encore éloignés de la réalité : qui mange du fromage ou du pain sans accompagnement ? De plus, la notion de « libre » est sociologiquement peu précise. Un individu auquel on laissera le loisir de mâcher librement, pourra dans la situation de l'expérience, mâcher lentement et longtemps par désir de bien faire, ou bien rapidement par volonté d'expédier l'essai en laboratoire. Dans un contexte réel, un même consommateur n'aura de toute manière pas le même comportement masticatoire lorsqu'il consommera un sandwich entre deux rendez-vous que lorsqu'il dégustera une assiette de fromage un

dimanche midi ensoleillé. Ainsi plutôt qu'adapter un produit à une classe d'individu donné (âge, sexe, situation, localisation,...), il serait peut-être judicieux de l'adapter au contexte de sa consommation (fast-food, slow-food, en pleine santé, malade,...).

En guise de perspectives, des études sur de nombreux types d'aliments semi-fluides réels peuvent faire l'objet de mesure de rhéologie avec une visée gustative ou nutritive. La rhéologie gouverne en partie les surfaces d'échanges de sapides et d'arômes à l'origine des sensations gustatives. Une modélisation est envisageable aux démarches de caractérisations développées et aux résultats obtenus. Le contrôle du comportement masticatoire au cours des essais pourrait permettre de mieux comprendre l'effet de ce comportement et de pouvoir adapter la formulation des aliments en fonction.

Dans un contexte médico-social, les troubles de la déglutition et la sécheresse des muqueuses peuvent avoir un impact sur la santé des personnes. Pour des personnes saines, les muqueuses du pharynx sont toujours lubrifiées par la salive et le régime d'enduction est probablement toujours dans un régime où l'influence de la viscosité est faible. Des modèles pourraient aider à formuler des produits pour des personnes présentant des pathologies telles que des cancers de la gorge, des syndromes de sécheresses des muqueuses, des BPCO et de la dysphagie.

Une démarche transdisciplinaire pourrait alors être mise en place, faisant appel à la rhéologie, à la modélisation mathématique, à l'agronomie, à la sociologie et à la médecine. Un tel projet pourrait s'inscrire dans le défi sociétal #7 « sécurité alimentaire » du programme européen Horizon 2020, comme le SFS-17-2014 à titre d'exemple, et y trouver un financement adéquat et d'envergure.

Résumé / Abstract

L'enjeu des travaux contenus dans cette thèse est la caractérisation rhéologique de suspensions concentrées d'objets viscoplastiques suspendus dans une matrice viscoplastique. Trois contraintes physiques se présentent : l'hétérogénéité des objets, la taille des objets au regard de la taille du système étudié, et la proximité entre la consistance de la phase suspendante et celle des objets. Dans notre contexte alimentaire, il faut ajouter des contraintes d'ordre biologique : les faibles durées de vie des produits, les prélèvements *in vivo* et la conservation des conditions de températures et d'humidité. L'ensemble de ces contraintes aboutit à une *rhéométrie spécifique*, aux frontières de la mécanique des milieux continus, avec pour objectif de mesurer des propriétés rhéologiques pertinentes. Pour cela, deux instrumentations originales ont été construites pour effectuer la caractérisation rhéologique le plus vite possible et au plus près de la génération des échantillons.

Appliquée aux bols alimentaires issus de fromage, leur rhéologie vient gouverner les écoulements en bouche et induire la création de surface d'échange en bouche, lors de la mastication. Au travers de ces interfaces, les arômes et les sapides sont libérés et transportés vers les récepteurs sensoriels. Les résultats montrent le lien entre le seuil de contrainte à l'écoulement du bol et la libération d'arômes de différentes hydrophobicités, fonction de la fermeté et du taux de matières grasses du fromage. Plus particulièrement, la phase suspendante fluide, semble jouer un rôle majeur dans la création et la persistance des surfaces d'échanges.

Appliquée aux jus gastriques issus de pain, l'enjeu est de savoir si des modifications des contenus en glucides pouvaient impacter la rhéologie des jus gastriques, modifier les cinétiques de vidange en sortie d'estomac, et diminuer l'excursion glycémique. Les résultats montrent l'effet d'un ajout d'amylose sur l'augmentation de la viscosité aux temps de digestion longs, lorsque la rhéologie n'est plus gouvernée par l'encombrement des particules de pain gonflées d'eau. Cependant, l'amylose ne semble pas avoir d'impact sur les cinétiques de vidange, ce qui vient conforter que le débit en sortie d'estomac est régulé par la charge énergétique contenue dans le repas.

The challenge of this work is to carry out the rheological characterization of concentrated suspensions of viscoplastic objects suspended in a viscoelastic matrix. Three structural constraints are faced: the heterogeneity of the particles, the size of particle in relation to the size of the whole system studied, and the proximity between the consistency of the suspending phase and that the consistency of the particles. In the food context, some biological constraints are added: small lifetimes of bolus, *in vivo* sampling and test conditions of temperature and humidity. All these constraints lead to a compromise in rheometry, at the edge of continuum mechanics, aiming to measure useful rheological properties. To carry out the rheological characterizations quickly and efficiently, two original devices has been designed trough this work.

Applied to food bolus from cheese, rheology comes to govern flows in the mouth and induce the creation of exchange area in the mouth, during the chewing process. Through these interfaces, the savors and flavors are released and transported to the sensory receptors. The results show the relationship between the yield stress of the bolus and flavors release of different hydrophobic aromas, depending on the firmness and fat content of the cheeses. Specifically, fluid suspending matrix appears to play a major role in the creation and persistence of exchange area.

Applied to gastric juice from bread, the issue is whether changes in carbohydrate content could impact the rheology of gastric juice, modify the kinetics of stomach flow rate, and decrease the glycemic excursion. The results show the effect of amylose addition on increasing the viscosity for long digestion time, when the rheology is no longer governed by the jamming effect of the particles made of water-swollen bread. However, amylose does not seem to have any impact on the kinetics of emptying, which reinforces that the stomach flow rate is regulated by the caloric charge contained in the meal.