Compréhension et analyse alimentaire d'un mix fermenté de protéines animales / protéines végétales : influence sur la physico-chimie et l'acceptabilité des produits obtenus

Manhal Yousseef

Université de Bourgogne

AgroSup Dijon

UMR Procédés Alimentaires et Microbiologiques (UMR PAM)
&
Centre des Sciences du Goût et de l'Alimentation (CSGA)

THÈSE
Pour obtenir le grade de
Docteur de l'Université Bourgogne

Discipline : sciences des aliments

Par : **Manhal YOUSSEEF**
Le 09 juin 2017

Compréhension et analyse alimentaire d'un mix fermenté de protéines animales / protéines végétales : Influence sur la physico-chimie et l'acceptabilité des produits obtenus

Jury :

Sylvie MARCHESSEAU	Pr., Univ. Montpellier	Rapporteur
Sylvie CHOLLET	Dr., ISA Lille	Rapporteur
Pascal DEGRAEVE	Pr., Univ. Lyon	Examinateur
Pierre-André MARECHAL	Pr., AgroSup Dijon	Examinateur
Florence HUSSON	Dr., AgroSup Dijon	Directrice de thèse
Dominique VALENTIN	Dr., AgroSup Dijon	Co-directrice de thèse

Remerciements

- Ma thèse a été réalisée au sein de l'Unité Mixte de Recherche en Procédés Alimentaires et Microbiologiques. Je tiens tout d'abord à remercier **Pr. Patrick GERVAIS** de m'avoir accueilli au sein de son équipe de recherche et **Pr. Laurent BENEY** directeur actuel de l'UMR-PAM, pour ses remarques et commentaires très pertinents lors des séminaires de laboratoire.

- Je remercie **Pr. Sylvie MARCHESSEAU** et **Dr. Sylvie CHOLLET**, rapporteurs de cette thèse, ainsi que les autres membres du jury **Pr. Pascal DEGRAEVE** et **Pr. Pierre-André MARECHAL** qui m'ont fait l'honneur de s'intéresser à ce travail et de se réunir pour le juger.

- J'adresse mes remerciements les plus sincères à mes directeurs de thèse :

Dr. Florence HUSSON, Maître de conférences au sein de l'UMR PAM, pour m'avoir confié ce projet passionnant et pour m'avoir fait confiance malgré les connaissances plutôt légères que j'avais au début de cette thèse sur les sciences des aliments. Merci aussi pour sa grande patience. Je dois dire que si j'ai pris autant de plaisir au cours de cette thèse, c'est grâce à tes qualités humaines incomparables.

Dr. Dominique VALENTIN, Maître de conférences au sein de l'UMR CSGA, pour la qualité de son encadrement, ses nombreux conseils précieux, sa continuelle disponibilité, son soutien et ses encouragements qui m'ont permis d'avancer au mieux dans ce travail.

Dr. Samuel LUBBERS, Maître de conférences au sein de l'UMR PAM, pour l'écoute de mes nombreuses questions, les nombreuses discussions que nous avons eues ainsi que sa capacité d'analyse et son enthousiasme m'ont montré que le monde de la recherche pouvait être un univers passionnant.

Je garde d'excellents souvenirs de toutes nos réunions de travail et de tous les instants partagés surtout à Athènes.

- Je voudrais remercier **Dr. Gaëlle ARVISENET**, Maître de conférences au sein de l'UMR CSGA, **Pr. Catherine DACREMONT**, Maître de conférences au sein de l'UMR CSGA et **Céline LAFARGE**, Ingénieur d'études au sein de l'UMR PAM pour ses conseils scientifiques et son aide apportés dans mes travaux de thèse et surtout lors de l'écriture de publications.

- Merci **à Mme Mary Bouley**, enseignante dans le centre des langues à l'Université Bourgogne, pour ces relectures d'anglais des publications dans ce manuscrit.

- Je remercie **Jean-Luc MESSION**, un collègue qui m'a aidé lors que j'ai eu besoin lors de ma thèse.

- Je n'oublie pas de remercier tous les stagiaires avec qui j'ai eu la chance de travailler. L'ambiance de travail a été excellente : **Julien**, **Moulay**, **François**, **Maha**, **Jou-cheng**...

- Merci à tous mes collègues thésard pour les partages de connaissances, d'expériences et des moments sympathiques de la vie de doctorale au laboratoire : **Thu**, **Antonio**, **Sophal**, **Dat**, **Bao**, **Aurore**, **Da** et **Hanh**.

- Mes remerciements aussi s'adressent à tous pour ce qu'ils m'ont aidé pendant mes années de thèse à l'AgroSup Dijon : **Christine**, **Bernadette**, **Claire**, **Alexandra**, **Florence**, **Alexandre**, **Jérôme**, et encore touts les membres de PMB et PCAV.

- Je remercie également tous les amis syriens qui m'ont permis d'oublier momentanément le mal de notre pays dans des soirées, repas, sorties ou autres. Je tiens à remercier tout particulièrement **Bachar** et **Maissaa** qui étaient une vraie famille pour moi surtout durant les derniers mois de cette thèse.

- Mes derniers remerciements iront évidemment à tous ceux qui forment mon "cocon" familial. Je pense tout d'abord à mes parents sans qui l'enfant que j'étais ne serait pas devenu l'homme que je suis. C'est avec émotion qu'à mon tour je leur dévoile le fruit de mes efforts. J'espère être à la hauteur de leur confiance et leur fierté inconditionnelles. Je tiens également à remercier mes beaux-parents pour leur soutien et leur présence sans faille. Après m'avoir offert leur confiance, ils m'ont également réservé une place de choix dans leur cœur. J'ai également une pensée émue pour mon frère, mes sœurs, mes beaux-frères et mes belles-sœurs qui m'ont toujours encouragé.

- Enfin, aucun mot ne peut exprimer ma reconnaissance à mon épouse, **Hiba**, pour sa présence à mes côtés, pour les sacrifices qu'elle a faits et pour son courage de me supporter tous les jours. Je lui remercie à elle ainsi qu'à nos enfants, **Laith** et **Adam**, pour le bonheur qu'ils me procurent au fil des jours. Je vous aime !

Résumé

De nombreux problèmes ont été identifiés lors des tentatives d'incorporation de protéines végétales dans nos aliments. En particulier, les faux goûts, le goût et la texture ont été mis en évidence comme de véritables obstacles à l'acceptabilité des produits végétaux par les consommateurs. Le consommateur lui-même est aussi un déterminant important en ce qui concerne le terme « acceptabilité ». Donc, dans le but de développer un nouveau produit fermenté à base de protéines de pois deux volets ont été étudié : le produit et le consommateur. Afin de comprendre la physico-chimie et l'acceptabilité de produits fermentés à base de protéines de pois, plusieurs facteurs ont été étudiés dans des étapes successives tels que la culture, les allégations positives sur la santé et l'environnement, les cocktails de souches lactiques et les procédés de préparation. Dès les premiers tests sensoriels, il était clair qu'il ne serait pas facile de convaincre les consommateurs de consommer des produits fermentés à base de pois : les consommateurs français n'acceptent pas les produits même à une faible concentration de pois (10 %). Ni la familiarité pour un produit proche de nos produits étudiés, ni l'encouragement des consommateurs à accepter ce type d'aliment en passant des messages positifs sur les protéines végétales n'étaient assez efficaces. Ainsi, une deuxième série d'études a été réalisée afin d'optimiser les propriétés rhéologiques et organoleptiques de ces produits. La meilleure combinaison, 1- cocktail bactérien (*S. thermophilus* + *Lb. bulgaricus*) 2- matière première (globuline de pois isolée dans notre laboratoire) 3- paramètres de préparation (mélange des deux laits avant le traitement thermique à 90 °C) a été sélectionnée afin d'optimiser les produits fermentés en ce qui concerne l'acidité, la fermeté, les profils volatils et peptidiques. Enfin, du point de vue sensoriel, une légère amélioration de l'acceptabilité a été remarquée. 20 % de protéines de pois a donné un produit accepté par la plupart des consommateurs, et 40 % de protéines de pois a été évaluée positivement par certains consommateurs et négativement par d'autres.

Abstract

Many problems have been identified following the attempts to incorporate vegetal proteins in our food. In particular, off-flavor, taste or texture have been highlighted as real barriers to the acceptability of plant products by consumers. The consumer himself is also an important determinant regarding the term "acceptability." So, in order to develop a new fermented product based on pea, two issues were studied: the product and the consumer. To understand the physico-chemical properties and the acceptability of pea protein-based fermented products, several factors have been investigated in successive stages such as culture, positive health and environmental claims, lactic acid bacteria strains and preparation processes. From the first sensory tests, it was clear that it will not be easy to convince consumers to buy the pea-based fermented product: French consumers did not accept products even in a lower concentration of pea (10%). Neither the familiarity to close products nor encouraging consumers to accept this type of food by transmitting positive messages about vegetal protein were efficient enough. Thus, a second series of studies was carried out in order to optimize the rheologic and organoleptic properties of these products. Best combination of 1- starter culture (*S. thermophilus + Lb. bulgaricus*) 2- raw material (pea globulin isolated in our laboratory) 3- preparation parameters (mixing both milk before heat treatment at 90 ° C) were selected to optimize the fermented products in terms of volatile compounds and peptide profiles, acidity and firmness. Finally, from the sensory point of view, a slight improvement in the acceptability was noticed. 20% of pea protein gave a product accepted by most of the consumers, and 40% of pea protein was assessed positively by some consumers and negatively by others.

Valorisation scientifique

Publications

- **Yousseef M.**, Lafarge C., Valentin D., Lubbers S., et Husson F. (2016). Fermentation of cow milk and/or pea milk mixtures by different starter cultures: Physico-chemical and sensorial properties. LWT-Food Science and Technology, 69, 430-437.

- **Yousseef M.,** Arvisenet G., Lin J.C., Dacremont C., Lubbers S., Husson F., et Valentin D. Effect of health and environmental information on the appreciation of fermented pea-cow milk products (Soumise : Food Quality and Preference)

- **Yousseef M.,** Potin F., Valentin D., Husson F., et Lubbers S. Influence of preparation procedures on the quality of yogurt like products from cow milk and pea proteins (à soumettre à “Food Control”)

- **Yousseef M.,** Valentin D., Husson F., et Lubbers S. Plant based yogurts: Physico-chemical, sensory and hedonic characteristics (à soumettre à “Journal of Food Research”)

Communications orales

- **Yousseef M.**, Valentin D., Husson F., et Lubbers S. Évaluation des propriétés physico-chimiques de gels à base de lait de pois fermenté avec différents cocktails bactériens. 22e forum des jeunes chercheurs, Besançon, 16-17 Juin 2016.

- **Yousseef M.**, Lubbers S., Valentin D., et Husson F. From sensory evaluation to food product development: how to fit a new vegetal fermented product to the consumer taste. SPISE, Ho Chi Minh, 29-31 Juillet 2016.

Communications affichées

- **Yousseef M.**, Lubbers S., Valentin D., Lafarge C., et Husson F. Screening of Lactic Acid Bacteria for fermentation of a mix of cow and pea proteins. VITAGORA, Dijon, 3-4 Avril 2014.

- **Yousseef M.**, Lubbers S., Husson F., et Valentin D. Sensory evaluation as a tool in assessing the quality of new fermented products. SPISE, Ho Chi Minh, 25-27 Juillet 2014.

- Lubbers S., **Yousseef M.**, Potin F., Valentin D., et Husson F. Influence of the heat treatment of milk and the fermentation temperature on the physico-chemical properties of dairy gels supplemented with pea proteins (*Pisum sativum*). EFFOST, Athènes, 10-12 Novembre 2015.

- **Yousseef M.**, Valentin D., Husson F., et Lubbers S. Évaluation des propriétés physico-chimiques de gels à base de lait de pois fermenté avec différents cocktails bactériens. 1ères rencontres francophones sur les légumineuses, Dijon, 31 mai-1 Juin 2016.

- **Yousseef M.**, Dobrev I., Lubbers S., Husson F., et Valentin D. Developing sustainable food: The role of consumer liking in optimization of pea yogurt. SPISE, Ho Chi Minh, 29-31 Juillet 2016.

- **Yousseef M.**, Lin JC., Arvisenet G., Lubbers S., Husson F., et Valentin D. Substitution of animal proteins by plant proteins: Effect of health and environmental information on the acceptability of mixed yogurts. EUROSENSE, Dijon, 11-14 Septembre 2016.

Liste des abréviations

β-LG : β-lactoglobuline

GDL : glucono-δ-lactone

LAB : Lactic acid bacteria

Lb. : Lactobacillus

Lc. : Lactococcus

EPS : exopolysaccharides

D° : degré Dornic

ANOVA : analyse de la variance

PCA : analyse en composantes principales

HAC : classification ascendante hiérarchique

MFA : analyse factorielle multiple

SNK & **LSD :** post-hoc tests

V.max : vitesse maximale d'acidification

T.max : le temps auquel la V.max est observée

CPG (GC) : Chromatographie en phase gazeuse

MS : spectromètrie de masse

SPME : Microextraction sur phase solide

Liste des tableaux

Chapitre 1. Revue bibliographique

Chapitre 2. Réactions des consommateurs face au « yaourt » au pois

Proceeding 1: Developing sustainable food: The role of consumer liking in optimization of pea yogurt

Publication 1: Effect of health and environmental information on the appreciation of fermented pea-cow milk products

Chapitre 3. Effet du cocktail bactérien sur les propriétés physico-chimiques et sensorielles

Publication 2: Fermentation of cow milk and/or pea milk mixtures by different starter cultures: physico-chemical and sensorial properties

Chapitre 4. Optimisation et sélection des conditions optimales

Publication 3: Influence of preparation procedures on the quality of yogurt-like products from cow milk and pea proteins

Liste des figures

Chapitre 1. Revue bibliographique

Chapitre 2. Réactions des consommateurs face au « yaourt » au pois

Table des matières

Introduction

Les habitudes de consommation de produits d'origine animale sont bien ancrées pour la population européenne. L'Union européenne est le premier producteur de lait de vache, la France en produisant annuellement environ 23 millions de tonnes. Sa grande consommation provient essentiellement de ses qualités nutritionnelles et sa capacité à fournir des protéines et des minéraux dont le calcium. Au cours des deux dernières décennies, la consommation de lait liquide a diminué d'environ 10 % alors que la consommation des produits fermentés frais a augmenté de plus de 30 % et celle de fromages de 10 %, sur la planète. En Europe, les pays producteurs et consommateurs de fromages (France, Allemagne, Italie) se situent parmi les plus gros consommateurs en équivalent lait. Cependant, des problèmes d'intolérance au lactose et d'allergies aux protéines de lait de vache ainsi que la prise de conscience d'une raréfaction des ressources en protéines animales dans les décennies à venir pourraient devenir des questions majeures. Dans une logique de moindre usage des ressources de protéines animales, il serait intéressant d'allier les bénéfices des protéines laitières à celles des protéines végétales.

Les protéines végétales représentent environ 65 % des apports en protéines pour l'alimentation humaine au niveau mondial. Parmi celles-ci, les plus employées comme substitut des protéines laitières sont les protéines de soja. Elles associent des propriétés technologiques fonctionnelles (fixation de l'eau, liaison, émulsion, texturation, etc.) et nutritionnelles (allègement en matières grasses (et absence de cholestérol), teneur élevée en protéines (40 %) et en lipides (20 %). Cependant, le soja pose le problème de la présence des phytoestrogènes dont les risques pour la santé sont toujours débattus. Il est donc opportun de s'interroger sur la faisabilité de pouvoir trouver de nouvelles sources de protéines végétales.

Dans une étude précédente, une série de yaourts mixtes pour lesquels le lait de vache a été partiellement remplacé par du lait de soja a été fabriquée. Les tests consommateurs menés en France et au Vietnam ont montré que pour les deux pays, le yaourt fabriqué avec une proportion de lait de soja inférieure à 50 % a reçu une note moyenne supérieure à 5 sur une échelle de 1 à 9 (**Tu et al.**, 2012). Cette approche montre qu'il est possible de faire accepter aux consommateurs des produits combinant protéines animales et végétales ; cependant, des efforts doivent être poursuivis afin 1- d'atteindre un niveau d'appréciation équivalent à celui des yaourts traditionnels et 2- d'éviter les inconvénients liés au soja concernant principalement les phytoestrogènes.

Parmi les légumineuses à graines, le pois est une source de protéines qui est particulièrement étudiée depuis une vingtaine d'années (**Owusu-Ansah & McCurdy**, 1991). Cependant son usage en alimentation humaine est encore limité, mise à part la consommation en graines entières. La mise sur le marché d'isolats de protéines de pois (globulines) par plusieurs industriels a permis de dynamiser la R&D des IAA dans la substitution des protéines d'origine animale, les changements de mode d'alimentation du consommateur étant favorables à la mise au point de produit végétarien et flexitarien. Les protéines du pois s'avèrent donc une alternative intéressante au soja mais force est de constater que les extraits de protéines de pois présentent une qualité d'odeur et d'arôme qui est un frein à leur utilisation notamment dans le secteur des produits laitiers.

L'objectif général de cette thèse est d'améliorer les qualités organoleptiques de produits type 'yaourt' fait à partir d'un mix protéine animale/protéine végétale. Une approche pluridisciplinaire combinant fermentation, analyse physico-chimique et compréhension des consommateurs est utilisée. ***L'originalité*** de ce travail repose sur trois choix méthodologiques. 1- notre produit de référence est un yaourt 100 % lait de vache sans matière grasse à 45 g/L de protéines. Tous les gels fermentés seront aussi à 45 g/L de protéines. Nous avons donc opté pour une substitution des protéines de lait de vache par des globulines de pois à des taux variables jusqu'à 100 % de globulines de pois, tous les autres paramètres étant maintenus constants. 2- nous avons adopté une approche macroscopique en travaillant avec des mélanges complexes de protéines et en suivant une démarche proche de celle de l'industrie laitière : caséines et protéines sériques et globulines de pois. En conséquence, la compréhension des phénomènes s'appuiera notamment sur les données de la littérature récemment publiées concernant les agrégats mixtes entre protéines végétales et protéines de lait de vache. 3- Dans l'approche sensorielle, la dimension ''comportement du consommateur'' a été introduite en parallèle de l'analyse sensorielle plus classique.

Chapitre 1 : Revue bibliographique

Dans ce chapitre, les propriétés physico-chimiques des protéines utilisées dans la partie expérimentale de la thèse seront présentées dans une première partie. Dans une deuxième partie, les procédés de fermentation lactique seront présentés. Enfin dans la dernière partie, des éléments sur le comportement des consommateurs appliqués aux produits nouveaux seront abordés.

1.1 Les protéines dans les aliments

Le mot « protéine » est dérivé d'un mot grec signifiant « premier », en raison du rôle fondamental des protéines dans le maintien de la vie (**Morris**, 1992). Les protéines jouent un rôle fondamental dans le maintien de la vie, où elles constituent des composants structuraux et métaboliques majeurs. Pour l'homme, l'apport de protéines par l'alimentation est donc indispensable. La qualité nutritionnelle des protéines alimentaires dépend de plusieurs facteurs notamment de la composition en acides aminés, de la disponibilité en acides aminés essentiels et de la digestibilité de ces protéines (**Li-Chan**, 2004). La composition en acides aminés et la structure de la protéine conditionnent ses propriétés fonctionnelles et la structure de l'aliment qui la contient.

L'Homme ne mange pas des protéines pures mais des aliments complets plus ou moins élaborés. Deux sources de protéines sont disponibles pour l'être humain, les végétaux et les protéines extraites de ces derniers et les produits animaux et les protéines extraites de ceux-ci.

1.1.1 Composition & structure des protéines

La majorité des protéines est constituée de 20 acides L-α-aminés. Chaque acide aminé contient une amine primaire et un acide carboxylique ayant la formule générale : CαH(R)-COOH, ne différant que par R. Chaque protéine se compose d'une chaîne d'acides aminés liés entre eux par des liaisons peptidiques (amide). Lorsqu'elle est synthétisée, une chaîne polypeptidique se replie, et adopte une forme tridimensionnelle spécifique qui est unique à la protéine. Selon **Walsh** (2015) cette conformation est largement stabilisée par des interactions multiples et de faibles énergies. D'autres types d'interactions sont également rapportées dans la littérature. Ainsi, les protéines peuvent être décrites jusqu'à quatre niveaux hiérarchiques de structure (Fig. 1). La structure primaire correspond à la succession linéaire des acides aminés (Fig. 1a), la structure secondaire représente la conformation locale de la chaîne principale d'une protéine (Fig. 1b). La structure tertiaire décrit l'agencement des structures secondaires dans l'espace (Fig. 1c). Enfin, l'association d'au moins deux chaînes polypeptidiques par des liaisons non covalentes produit la structure quaternaire des protéines (Fig. 1d).

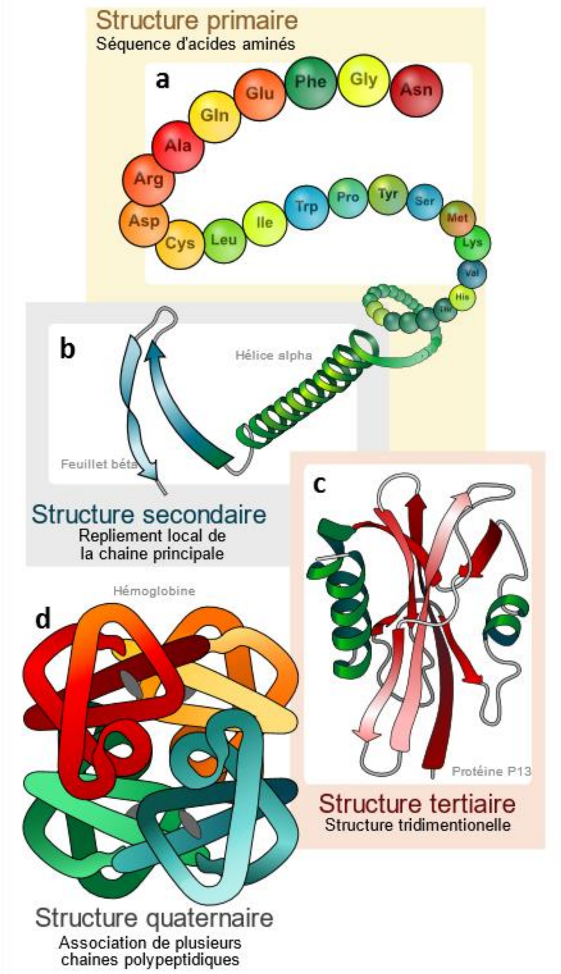


Figure 1. Niveaux de structure des protéines (dehttps://wikipedia.protein_structure_levels)

Cette structuration est commune aux protéines d'origine végétale et d'origine animale. Les différences se trouvent au niveau de la qualité nutritionnelle et au niveau des propriétés fonctionnelles. Cette dernière dimension n'est pas indispensable à la survie de l'homme mais est essentielle pour la formulation des produits alimentaires même peu transformés.

1.1.2 Protéines animales vs protéines végétales

La qualité nutritionnelle des protéines alimentaires est déterminée en grande partie par leur composition en acides aminés essentiels et leur digestibilité. L'avantage des protéines animales réside dans leur richesse en acides aminés essentiels, comme la lysine, la méthionine et le tryptophane (**Swanson**, 1990). Parmi les protéines de référence, on peut citer les protéines de l'œuf de poule ou les protéines sériques du lait de vache. Elles sont considérées comme des protéines idéales sur le plan nutritionnel avec une haute digestibilité proche de 100 % et une composition en acides aminés équilibrée.

Les protéines végétales ne présentent pas de risque sanitaire, sont accessibles en grande quantité, renouvelables, et moins coûteuses que les protéines d'origine animale. Cependant la majorité des protéines végétales peuvent présenter une teneur limitante en certains acides aminés indispensables, la lysine pour les céréales, et les acides aminés soufrés pour les légumineuses. De plus leur digestibilité est généralement moins bonne que celle des protéines animales.

Parmi les protéines dites animales, nous nous focaliserons sur les protéines du lait de vache. Les caséines représentent la protéine animale dominant dans le lait de vache et sont le principal contributeur fonctionnel des ingrédients laitiers qui sont utilisés de façon omniprésente dans l'industrie alimentaire. Les protéines sériques ont aussi d'excellentes propriétés fonctionnelles (**Cayot & Lorient**, 1998).

Certaines protéines végétales ont aussi d'excellentes propriétés fonctionnelles (la solubilité, la rétention de l'eau et des arômes, l'épaississement, l'émulsification, la gélification). Ces dernières ont fait l'objet de nombreuses études notamment comme alternative aux protéines animales, surtout dans le domaine agro-alimentaire. Parmi les protéines de graines de légumineuses, les protéines de soja ont été largement étudiées, ce qui a conduit à l'expansion de leur utilisation dans l'alimentation humaine. La protéine de pois (*Pisum sativum L.*) suscite beaucoup d'intérêt comme alternative au soja en raison de sa faible allergénicité, de sa haute valeur nutritionnelle, de sa disponibilité et de son faible coût (**Shevkani et al.**, 2015; **Stone et al.**, 2015). Cependant, l'utilisation de la protéine de pois comme ingrédient alimentaire est encore limitée du fait d'une qualité d'odeur et d'arôme dominée par des notes verte et haricot sec entre autres (**Murat et al.**, 2013).

1.1.3 Protéines de lait de vache (caséines et protéines sériques) : structure et composition en acides aminés

Le lait contient 32 à 35 g/L de protéines réparties entre 80 % de caséines et 20 % des protéines du lactosérum (**Alais & Linden**, 1997). La fraction protéique majeure du lait est constituée des caséines αs1, αs2, β et κ et est organisée en superstructures sphériques et volumineuses appelées micelles. Leur diamètre moyen est d'environ 180 nm mais peut varier de 30 à 600 nm (**McMahon & Brown**, 1984). La micelle de caséine contient 92 % de protéines et une fraction minérale de 8 %. Les minéraux sont constitués de 90 % de phosphate de calcium et de 10 % d'ions citrate et magnésium (**Horne**, 2009). Le pH isoélectrique de la micelle de caséine est de 4,6 (**Cayot & Lorient**, 1998).

Les protéines du lactosérum se présentent sous forme globulaire et sont solubilisées dans la phase aqueuse du lait. Elles présentent une importante sensibilité thermique. Les protéines majeures du lactosérum tant d'un point de vue quantitatif que technologique sont : la β-lactoglobuline (β-LG) et l'α-lactalbumine.

La β-LG est une protéine globulaire comprenant 162 acides aminés et dont la masse moléculaire avoisine les 18 300 g/mol. En fonction du pH, de la température, de la concentration ou encore de la force ionique du milieu, elle peut exister sous différents états oligomériques allant du dimère au pH naturel du lait à l'octamère. Au voisinage de son point isoélectrique estimé à pH 5,2, elle est très peu soluble dans l'eau (**Pelegrine & Gasparetto**, 2005).

L'α-lactalbumine est une petite protéine globulaire compacte de 123 acides aminés et d'une masse moléculaire de 14 200 g/mol. Cette métalloprotéine présente une structure stabilisée par 4 ponts disulfures et un cation. Son pH isoélectrique est de 4,8 (**Cayot & Lorient**, 1998). Le Tableau 1 présente la composition en acides aminés des protéines de lait (caséines et protéines sériques). Ce tableau montre que l'ensemble des acides aminés essentiels sont présents dans les protéines de lait et en quantité suffisante pour satisfaire les besoins nutritionnels selon les recommandations de la FAO.

Tableau 1. Composition en acides aminés des protéines du lait (en % de total acide aminés) (**Pellegrino et al.**, 2013)

ACIDE AMINE	CASEINES	PROTEINES SERIQUES
ASPARTIC ACID/ASPARAGINE	7.1	10.5
THREONINE	4.9	7.0
SERINE	6.3	4.8
GLUTAMIC ACID/GLUTAMINE	22.4	17.6
PROLINE	11.3	5.9
GLYCINE	2.7	1.8
ALANINE	3.0	4.9
CYSTEINE	0.34	2.3
METHIONINE	2.8	1.7
VALINE	7.2	5.7

ISOLEUCINE	6.1	6.4
LEUCINE	9.2	10.3
TYROSINE	6.3	2.9
PHENYLALANINE	5.0	3.1
TRYPTOPHAN	1.7	2.4
LYSINE	8.2	8.7
HISTIDINE	3.1	1.7
ARGININE	4.1	2.3

La micelle de caséine est un édifice instable en fonction des conditions du milieu. Quand il y a acidification du milieu, la structure de la micelle est progressivement modifiée avec l'abaissement du pH. A partir de pH 5,2 les premiers signes de gélification apparaissent, à pH 5,0 il y a solubilisation totale du calcium et à pH 4,9 la gélification est totale. Le gel ne se forme que si l'acidification est très lente. Le réseau est constitué par des enchainements et des agrégats de caséine et de protéines sériques. La synérèse de ce gel est très faible. Par contre, si l'acidification est brutale, il y a agrégation forte (caillé non homogène) et synérèse importante.

La caséine est aussi sensible à la protéolyse. En industrie laitière, la base de fabrication de caséine dite présure « rennet casein » est opérée par l'action de la chymosine qui hydrolyse la κ-caséine pour produire de la para-κ-caséine et le glycomacropeptide. Suite à cette hydrolyse, une réaction de coagulation qui dépend de la concentration en ions calcium et de la température est engagée. La vitesse d'agrégation est maximale à 40°C. Enfin on constate une séparation entre le caillé et du lactosérum.

Comme toutes les protéines, les caséines et protéines sériques subissent des modifications en fonction de la température. Le fait le plus notable est la possibilité d'interactions entre les deux catégories de protéines : caséines et protéines sériques. Ainsi, au cours d'un chauffage à 90°C, la β-LG vient s'associer avec la kappa caséine des micelles soit par des ponts disulfure si le pH est voisin de pH 6,8 soit par des interactions hydrophobes si le pH est inférieur à pH 6,5 (**Cayot & Lorient**, 1998). Ces interactions inter protéiques contribuent ainsi par la densification du maillage protéique et occasionnent une amélioration de la viscosité et de la fermeté des yaourts (**Beal & Sodini**, 2003).

1.1.4 Globulines de pois : structure et composition en acides aminés

Les graines de pois contiennent généralement 20-25 % de protéines sur une base de poids sec. Ces protéines représentent le deuxième constituant principal de la graine de pois après l'amidon. L'amidon représente environ 50 % de la graine, les fibres 20 % et un taux minoritaire de lipides 2,5 % (**Tzitzikas et al.**, 2006).

Comme toutes les protéines végétales, les protéines de pois peuvent être classées en fonction de leur solubilité. Les albumines sont considérées comme solubles dans l'eau, les globulines sont qualifiées solubles et extractibles en solution saline, les prolamines sont solubles dans des solutions hydroalcooliques et les glutéines partiellement solubles dans les solutions acides et alcalines (**Osborne & Campbell**, 1898). Fig. 2 présent l'ensemble des protéines de pois.

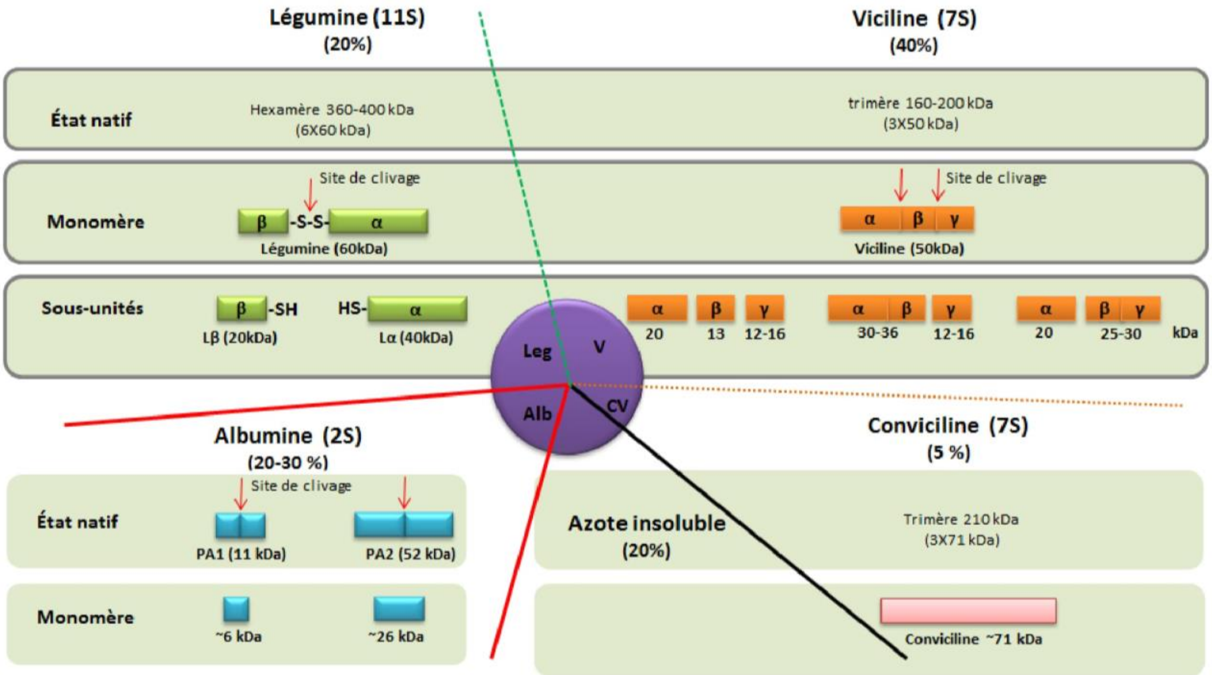


Figure 2. Composition et structure des principales fractions de protéines de pois, et assemblage des sous-unités constitutives des globulines de pois 7S et 11S, et potentiels sites de clivage (**Chihi**, 2016).

Nous avons travaillé dans cette étude avec des extraits industriels de globulines. Les globulines représentent 70 à 80 % de protéines de la graine (**Utsumi**, 1992; **Utsumi et al.**, 1997). Les globulines sont une classe de protéines composée de : viciline 7S et légumine 11S. Les légumines 11S ont des structures quaternaires, hexamères avec des sous unités de masse molaire ≈ 40 000 Da et ≈ 20 000 Da. Les vicilines 7S ont une structure trimérique avec une masse molaire de 175,000-180,000 Da (**Boye et al.**, 2010). Par rapport aux albumines, les globulines de pois contiennent moins d'acides aminés essentiels tryptophane, lysine, thréonine, cystéine et méthionine mais sont riches en arginine, phénylalanine, leucine et isoleucine (**Swanson**, 1990). Le Tableau 2 présente la composition moyenne en acides aminés de pois. On remarque les protéines de pois sont déficitaires en acides aminés soufrés (méthionine et cystéine) selon les recommandations de FAO.

Tableau 2. Composition en acides aminés des protéines de pois (**Boye et al.**, 2010) et globulines de pois (**Jackson et al.**, 1969) (en % de total acide aminés)

ACIDE AMINE	PROTEINE DE POIS	GLOBULINE DE POIS
ASPARTIC ACID/ASPARAGINE	11.16	12.70
THREONINE	4.46	3.23
SERINE	5.71	5.90
GLUTAMIC ACID/GLUTAMINE	18.46	16.50
PROLINE	4.64	--
GLYCINE	4.82	6.20
ALANINE	4.83	5.78
METHIONINE/CYSTEINE	1.60	0.53
VALINE	5.11	4.77
ISOLEUCINE	3.89	4.06
LEUCINE	7.84	7.98
TYROSINE	3.34	2.56
PHENYLALANINE	5.17	4.22
TRYPTOPHAN	0.61	--
LYSINE	6.25	4.40
HISTIDINE	2.33	2.45
ARGININE	7.93	7.50

1.1.5 Caséine vs globuline de pois : propriétés fonctionnelles

Les propriétés fonctionnelles des protéines sont très diverses et peuvent être exploitées dans la formulation des aliments. Elles sont principalement déterminées par la solubilité, la rétention de l'eau, la rétention de l'huile, l'émulsification, le foisonnement, la gélification, l'épaississement et la rétention de composés d'arôme. La composition en acides aminés, la structure, la déformation et l'interaction entre les protéines et les autres composants alimentaires, ainsi que la température et le pH peuvent affecter les propriétés fonctionnelles (**Boye et al.**, 2010). Nous allons passer en revue leurs principales fonctionnalités : la solubilité, la capacité de rétention et la gélification.

1.1.5.1 Solubilité

La solubilité des protéines peut être définie comme l'équilibre entre les interactions protéine-protéine (hydrophobe) et protéine-solvant (hydrophile), exprimées en Protein-Solvent ↔ Protéine-Protéine + Solvant-Solvant. D'autres définitions comprennent le rapport de la protéine présente dans la phase liquide aux protéines présentes dans le surnageant après centrifugation (**Lam et al.**, 2016). Le solvant utilisé dans la plupart des cas est habituellement de l'eau ou un tampon. En plus de facteurs intrinsèques comme la nature et la composition en acides aminés (résidus hydrophiles et hydrophobes), et leur distribution à la surface de la protéine (**Kimura et al.**, 2008), d'autres facteurs influencent la solubilité des protéines dont le pH du solvant, la force ionique, la température et les composants des solvants organiques (**Lam et al.**, 2016) et la méthode d'extraction (**Boye et al.**, 2010). La protéine présente la plus faible solubilité à son pH isoélectrique puisqu'elle porte une charge nette nulle (0), en minimisant les forces de répulsion électrostatiques.

Les caséines ont, en général, un point isoélectrique dans la région de pH 4,6. La modification du pH du lait écrémé à partir de 6,7 (pH naturel) à 4,6 entraine l'agrégation des caséines à une échelle macroscopique (**O'Kennedy**, 2011). Aussi, le point isoélectrique pour la protéine sérique de lait est pI~4.5 à toutes les températures testées (de 40 jusqu'à 60° C) (**Pelegrine & Gasparetto**, 2005).

Le point isoélectrique pour les protéines de légumineuses est généralement compris entre 4 et 6 (**Boye et al.**, 2010)**.** La fraction protéique qui précipite à pI~4,6 est constituée principalement de globulines (**Alamanou & Doxastakis**, 1997), et la valeur de pH pour une solubilité optimale de fractions globulaires de pois est égale à 7,2 (**Chihi**, 2016). Les albumines ont un point isoélectrique plus élevée pI~6,5.

1.1.5.2 Rétention de l'eau et des composés d'arôme

- Rétention de l'eau

La rétention est un terme opérationnel se référant à l'aptitude d'une protéine à maintenir les ingrédients ensemble dans un état solide, semi-solide ou fluide, et dans certains cas, cela implique la prévention ou le retard de la perte de composants volatils, par exemple de l'eau ou des arômes (**Kinsella & Melachouris**, 1976). Cette propriété est importante dans la formulation de nombreux aliments par rapport aux arômes, aux lipides, à l'eau et à d'autres composants. Les valeurs de rétention (soit d'eau ou d'huile) par les isolats protéiques sont influencées par la nature de la protéine et par les conditions de traitement utilisées pour préparer l'isolat.

La β-LG présente des degrés d'hydratation variables, selon la dénaturation, l'agrégation et l'interaction avec d'autres protéines (**Kinsella & Morr**, 1984). Les micelles de caséine sont capables de lier de grandes quantités d'eau (200-400 g/100 g) (**Kneifel & Seiler**, 1993). Lorsque le lait a été chauffé très rapidement à 120 °C en 1 minute, la quantité d'eau retenue par les caséinates est 5 fois supérieure à la quantité retenue par les caséinates témoins non chauffées (**Kneifel & Seiler**, 1993). De même pour les farines de légumineuses, un traitement thermique intense peut augmenter la capacité de rétention de l'eau (**Obatolu et al.**, 2007).

- Rétention des composés d'arôme

Des interactions hydrophobes faibles réversibles et des liaisons covalentes irréversibles peuvent être formées entre des protéines et des composés d'arôme (**Suppavorasatit & Cadwallader**, 2012; **Tromelin et al.**, 2006).

La β-LG de lait est capable de lier des composés d'arôme par des interactions hydrophobes. Généralement, dans la même classe chimique de composés aromatiques, la force de l'interaction augmente avec la longueur de la chaîne (**O'neill & Kinsella**, 1987; **Sostmann & Guichard**, 1998). Plusieurs études ont montré que la β-LG généralement a une capacité de liaison d'arôme (vanilline, benzaldéhyde, d-limonène et 2-nonanone) plus forte que la caséine (**Hansen & Booker**, 1996; **Li et al.**, 2000; **Zhu**, 2003). Dans un système gélifié, des mesures par SPME ont montré une rétention plus élevée des composés d'arôme dans le yaourt avec des teneurs élevées en caséinates (**Saint-Eve et al.**, 2006). Ces auteurs ont suggéré que le réseau plus hétérogène de yaourts enrichis en caséinates ayant de gros pores peut constituer une barrière plus efficace pour le transfert de composés d'arôme que le réseau homogène de yaourts enrichis en protéines de lactosérum (β-LG).

Il existe peu de données sur les interactions protéines de pois et composés d'arôme dans la littérature. Les quelques études réalisées ont mis en évidence la capacité des protéines de pois à piéger des composés d'arôme. Par exemple, **Wang & Arntfield** (2015) ont montré que la rétention d'arômes de fonction aldéhyde (hexanal, heptanal et octanal) est plus élevée avec un isolat de protéine de pois, avec des augmentations entre 14 et 18 % après traitement thermique. Ces observations peuvent être expliquées par la dénaturation thermique qui favorise le déploiement de la protéine révélant ainsi des résidus hydrophobes capables de lier des arômes (**Kühn et al.**, 2006).

1.1.5.3 Gélification

La capacité à former des gels dans des conditions spécifiques est une fonctionnalité importante dans de nombreux aliments. Les gels sont caractérisés par une viscosité, une plasticité et une élasticité relativement élevées (**Kinsella & Melachouris**, 1976). La gélification protéique peut être induite par le pH, les sels, les solvants, les enzymes, le traitement thermique et la pression ou le cisaillement (**Corredig**, 2005). La gélification est un mécanisme en deux étapes impliquant un déploiement initial de la protéine ou une dissociation de la protéine suivie par une agrégation pour former un réseau en gel.

Dans les systèmes que nous avons étudiés, la gélification a été induite par des modifications du pH, avec des traitements thermiques associés.

La dénaturation thermique des protéines est initiée dès 65°C pour les protéines sériques du lait et commence à 80°C (voire 85-90°C) pour les globulines de pois.

Les protéines de pois sont pour la plupart globulaires et tri, hexa, … mériques, il y a donc une phase de dissociation et/ ou de déplissement au cours du chauffage. La présence d'un pourcentage élevé de résidus hydrophobes dans les protéines est généralement favorable à l'obtention d'un coagulum (**Schwenke**, 2001). De plus les conditions de pH durant la phase de dénaturation thermique impactent les propriétés des gels obtenus (**Arntfield et al.**, 1989).

La dénaturation provoquée par la chaleur des protéines sériques est une étape critique dans l'association des protéines sériques avec la caséine. La plupart des chercheurs ont étudié la dénaturation provoquée par la chaleur des protéines sériques à des températures supérieures à 60 °C; cependant, certaines réactions de dénaturation pourraient commencer à des températures aussi basses que 40 °C (**Parris & Baginski**, 1991). Au-dessus de 70 °C, des changements de conformation ont lieu dans les structures secondaires et tertiaires de β-LG, y compris le déploiement et l'exposition de son groupe thiol libre sur le résidu Cys121. L'agrégation de protéines procède par des réactions d'échanges de groupements thiols et de ponts disulfures et des interactions hydrophobes (**Hoffmann & van Mil**, 1997; **Vasbinder & de Kruif**, 2003).

Pour l'interaction entre les globulines de pois et la β-LG, des agrégats mixtes sont formés en faisant intervenir des échanges de liaisons disulfures et /ou des interactions non covalentes (**Chihi**, 2016). A la concentration de 5 mM de NaCl, les liaisons disulfures issues de la β-LG contribuent surtout à la formation d'agrégats mixtes denses (majoritaires) et des agrégats intermédiaires (minoritaires) avec un diamètre réduit par rapport à celui obtenu pour les macro-agrégats de globuline (**Chihi**, 2016). Une acidification lente par la GDL (glucono-δ-lactone) conduit aussi à la formation d'agrégats par réarrangement des oligomères des globulines et de la β-LG via des interactions hydrophobes et des échanges de liaisons covalente (S-/SS) favorisé par la thermodénaturation (**Chihi**, 2016; **Mession et al.**, 2015).

1.2 La fermentation lactique

La fermentation est l'une des méthodes les plus anciennes de conservation et de préparation des aliments. La fermentation apporte aux aliments une variété d'arômes, de goûts, de textures, des attributs sensoriels et des valeurs nutritionnelles et thérapeutiques.

Les bactéries lactiques (LABs) sont traditionnellement utilisées pour obtenir des aliments fermentés à partir de matières premières très différentes comme le lait, les légumes, les céréales et les produits à base de viande (**Gorbach**, 2002). Les LABs sont présentes dans une variété d'aliments et participent au développement de la texture, du goût, de la qualité et de la salubrité de nombreux produits fermentés. Ces micro-organismes sont des bactéries Gram-positives, catalase-négatives, ne sporulent pas, microaérophiles ou anaérobies facultatifs. Ils ont une caractéristique commune qui est la production d'acide lactique à partir du lactose. L'acide généré par la fermentation du lactose donne non seulement un goût acide au produit, mais transforme également le lait liquide au départ ou le mélange laitier en un caillé semi-solide. Dans la matrice de caséine solide, le lactosérum et les autres composants solubles du lait et de la matière grasse du lait sont piégés (**Vedamuthu**, 2006). Les fonctions secondaires de la culture initiale dans les produits laitiers fermentés comprennent la production d'arômes, la production spéciale de texture et l'élaboration de métabolites inhibiteurs divers qui ont des effets de conservation (**Vedamuthu**, 2006). LABs des genres *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* et *Weissella* sont les bactéries les plus importantes dans les fermentations alimentaires. Des auteurs ont criblé des LABs isolées de différentes régions et de différentes plantes et algues au Japon pour leur capacité à fermenter des laits de vache et des laits de soja ; Dix bactéries sur les 138 collectées ont montré des tolérances aux milieux acides et une bonne aptitude de fermentation des laits de vache et de soja. De plus, l'une d'entre elles, *Lc. lactis* Miura-SU2, a permis d'apporter un arôme agréable au lait de soja fermenté. *Lb. plantarum* Tennozu-SUI quant à elle, a permis d'augmenter la capacité antioxydante des laits de soja. Les auteurs ont ainsi pu montrer que certaines souches de LABs étaient capables à la fois d'augmenter la fermeté et d'améliorer la texture du caillé et aussi de permettre d'obtenir des arômes nouveaux et masquant le goût du végétal (**Kuda et al.**, 2016).

1.2.1 L'effet de la fermentation sur la texture des produits

L'effet de la fermentation est très important sur la texture finale du produit. L'exploitation commerciale des exopolysaccharides (EPS) à partir des LABs dans le domaine des produits laitiers fermentés a traditionnellement été destinée à produire des caractéristiques physiques uniques (**Cerning**, 1995), par exemple une viscosité et une rétention d'eau améliorées; Il en résulte que le consommateur perçoit un produit de meilleure qualité en bouche, une texture plus agréable et une persistance plus longue en bouche (**Duboc & Mollet**, 2001; **de Valdez et al.**, 2003). De tels produits sont décrits comme ayant une viscosité plus élevée et un degré de synérèse inférieur (séparation du lactosérum) par rapport aux produits obtenus avec des cultures non productrices d'EPS (**Cerning**, 1990; **Duboc & Mollet**, 2001; **Ruas-Madiedo et al.**, 2002). En outre, la présence d'EPS dans les produits laitiers fermentés conduit souvent à un caractère visqueux du produit (**Cerning**, 1990); c'est pourquoi les cultures bactériennes produisant des EPS sont parfois appelées souches «visqueuses». Cependant, de nombreuses études ont montré que la texture des laits fermentés est loin d'être proportionnelle à la teneur en EPS (**Van Marle & Zoon**, 1995). **Faber et al. (1998)** ont constaté que deux laits écrémés cultivés avec une concentration d'EPS similaire et une structure unitaire répétitive identique de l'EPS différaient en viscosité. La microstructure du gel des produits laitiers fermentés contient des filaments formés par des cultures produisant des EPS qui sont attachées aux cellules microbiennes et aux matrices de protéines (**Hassan et al.**, 2002; **Tamime & Robinson**, 1999). L'effet de la fermentation lactique est bien connu sur les produits laitiers : laits fermentés, yaourts, fromages (**Domínguez-Soberanes et al.**, 2001; **Oliveira et al.**, 2001; **Sodini et al.**, 2004; **Wick et al.**, 2004) ainsi que sur les végétaux : choucroute, olives, carottes…et les laits végétaux : lait de soja, tofu de soja, etc (**Coda et al.**, 2012; **Yang & Li**, 2010). En revanche, les travaux scientifiques ou les applications montrant l'intérêt de substituer les protéines animales par des protéines végétales sont très peu nombreux voire inexistants. Des auteurs ont montrés que des caillés coagulés provenant de lait de vache et contenant des protéines de soja sont plus faibles en fermeté que ceux provenant du lait pur (**Lee & Marshall**, 1979; **Rinaldoni et al.**, 2014). **Ahmed & Hassan** (1995) ont étudié la faisabilité de l'utilisation des protéines d'arachide ou de soja pour la substitution partielle de la caséine dans la fabrication de succédanés de fromages. Les fromages sont devenus plus doux et moins caoutchouteux avec l'ajout croissant de protéines végétales.

1.2.2 L'effet de la fermentation sur la production de composés d'arôme

Les différences observées dans les composés d'arôme du yaourt par rapport à ceux du lait sont produits par le métabolisme des bactéries lactiques. Ces composés aromatiques générés résultent de transformations microbiennes, enzymatiques ou chimiques du lactose, des lipides, de l'acide citrique et des protéines/acides aminés présents dans le lait (**Boelrijk et al.**, 2003; **McGorrin**, 2001).

Le Tableau 3 présente les composés volatils qui ont été identifiés dans un yaourt nature (**Cheng**, 2010). Plus de 90 molécules volatiles différentes ont été identifiées notamment des alcools, des aldéhydes, des cétones, des acides, des esters, des lactones, des composés contenant du soufre, des pyrazines et des dérivés de furane (**Ott et al.**, 1997). Un grand nombre de composés organiques volatils trouvés dans le yaourt ne sont pas produits par les bactéries starters mais proviennent du lait (**Imhof et al.**, 1994; **Tamime & Deeth**, 1980).

Tableau 3. Liste des composés volatils identifiés dans le yogourt nature (**Cheng**, 2010)

Carbonyl compounds	*Alcohols*	*Sulfur compounds*	*Heterocyclic compounds*
Acetaldehyde	Methanol	Dimethyl sulfide	Furan
Acetone	Ethanol	Dimethyl disulfide	Furfural
Propanal	1-Propanol	Dimethyl trisulfide	2-Methylfuran
2-Propanone	2-Propanol	S-methyl thioacetate	2-Pentylfuran
Butanal	1-Butanol	Methional	2-Furanmethanol
2-Butanone	2-Butanol	Tetramethyl thiourea	Pyrazine
Diacetyl	2-Methyl-1-propanol	*Nitrogen compounds*	Methylpyrazine
Acetoin	Cyclobutanol	N,N-dimethylformamide	Pyrrole
Pentanal	1-Pentanol	Lactamide	1-Methylpyrrole
2-Methylbutanal	3-Pentanol	N-ethyl-benzenamine	2-Methyl tetrahydrofuran-3-one
3-Methylbutanal	1-Penten-3-ol	*Hydrocarbons*	2-Methylthiophene
3-Methyl-2-butenal	3-Methyl-2-butenol	Heptane	2-Methyltetrahydrothiophen-3-one
2-Pentanone	3-Methylbutanol	Methylcyclohexane	Benzothiazole
3-Penten-2-one	Pentan-2-one-4-ol	Nonane	Methyl 2-piperidine
2-Hydroxy-3-pentanone	3-Methyl 3-cyclohexenol	Undecane	Furfuralcohol
2,3-Pentanedione	2-Ethyl hexanol	*Aromatic compounds*	1,2-Dihydro-2,2,4-Trimethylquinoline

Hexanal	2-Buthyl octanol	Benzene	2,3-dihidro-1,3,3-trimethyl 1H-Indole
2-Hexanone	Guaiacol	Toluene	***Terpene***
3-Hexanone	***Acids***	Ethylbenzene	L-limonene
Heptanal	Acetic acid	1,3-Dimethylbenzene	***Others–solvent contamination?***
2-Heptanone	Propionic acid	1,4-Dimethylbenzene	Acetonitrile
3-Heptanone	Butyric acid	1,2-Dimethylbenzene	Dichlorometane
Octanal	2-Methylpropanoic acid	Ethenylbenzene	Trichloromethane
3-Octanone	Pentanoic acid	Propylbenzene	
1-Octen-3-one	Isovaleric acid	Trimethylbenzene	
1-Nonen-3-one	Hexanoic acid	1-Methyl ethenylbenzene	
Nonanal	Heptanoic acid	1-Ethyl-4-methylbenzene	
(E)-2-Nonenal	Octanoic acid		
2-Nonanone	Nonanoic acid		
Decanal	Decanoic acid		
Undecanal	Benzoic acid		
2-Undecanone	***Esters***		
2-Dodecanone	Methyl formate		
2-Pentadecanone	Methyl acetate		
γ -Dodecalactone	Ethyl acetate		
δ-Dodecalactone	Butyl acetate		
Benzaldehyde	Diehyl phthalate		
Phenylacetaldehyde			

La composition du lait de vache en glucides comprend du lactose (4,8 % p/v), du citrate (0,18 % p/v) et des traces de glucose, de galactose, etc (**Walstra & Jenness**, 1984). Au cours de la fabrication du lait fermenté et du fromage, LABs transforment le lactose (principalement L) en acide lactique (Fig. 3). L'oxydation du lactate peut également se produire dans le fromage. Au cours de ce processus, le lactate est converti en acétate et en CO_2. L'acétate est présent à des concentrations assez élevées dans le cheddar et est considéré comme contribuant au goût du fromage, bien que des concentrations élevées d'acétate provoquent des faux goûts (**Aston & Dulley**, 1982). *Lc. Lactis biovar diacetylactis* et L*euconostoc* ssp sont capables de métaboliser le citrate dans le fromage cheddar, avec une production de diacétyle et de CO_2. Le diacétyle et l'acétate produits à partir de citrate contribuent au goût des fromages hollandais et du cheddar (**Aston & Dulley**, 1982). Plusieurs espèces de lactobacilles mésophiles et également des leuconostoques métabolisent le citrate avec la production de diacétyle et de formate (**Fryer**, 1970). Les composés d'arôme principaux de yaourt produits à partir du métabolisme du citrate sont l'acétate, le diacétyle (2,3-butanedione), l'acétoïne (3-hydroxy-2-butanone) et le 2,3-butanediol (**Cogan**, 1995) (voir Fig. 3). Le diacétyle est généralement produit en petites quantités, mais l'acétoïne est généralement produite en quantité beaucoup plus élevée (10-50 fois plus élevée que le diacétyle). L'acétate est produit à partir de citrate en concentrations équimolaires (**Singh et al.**, 2007). Les voies générales de dégradation du lactose et du citrate sont présentées dans les Fig. 3 et Fig. 4. Ces deux voies sont représentatives du métabolisme des bactéries lactiques pendant la fermentation lactique et sont impliquées dans la formation des composés d'arôme cités précédemment.

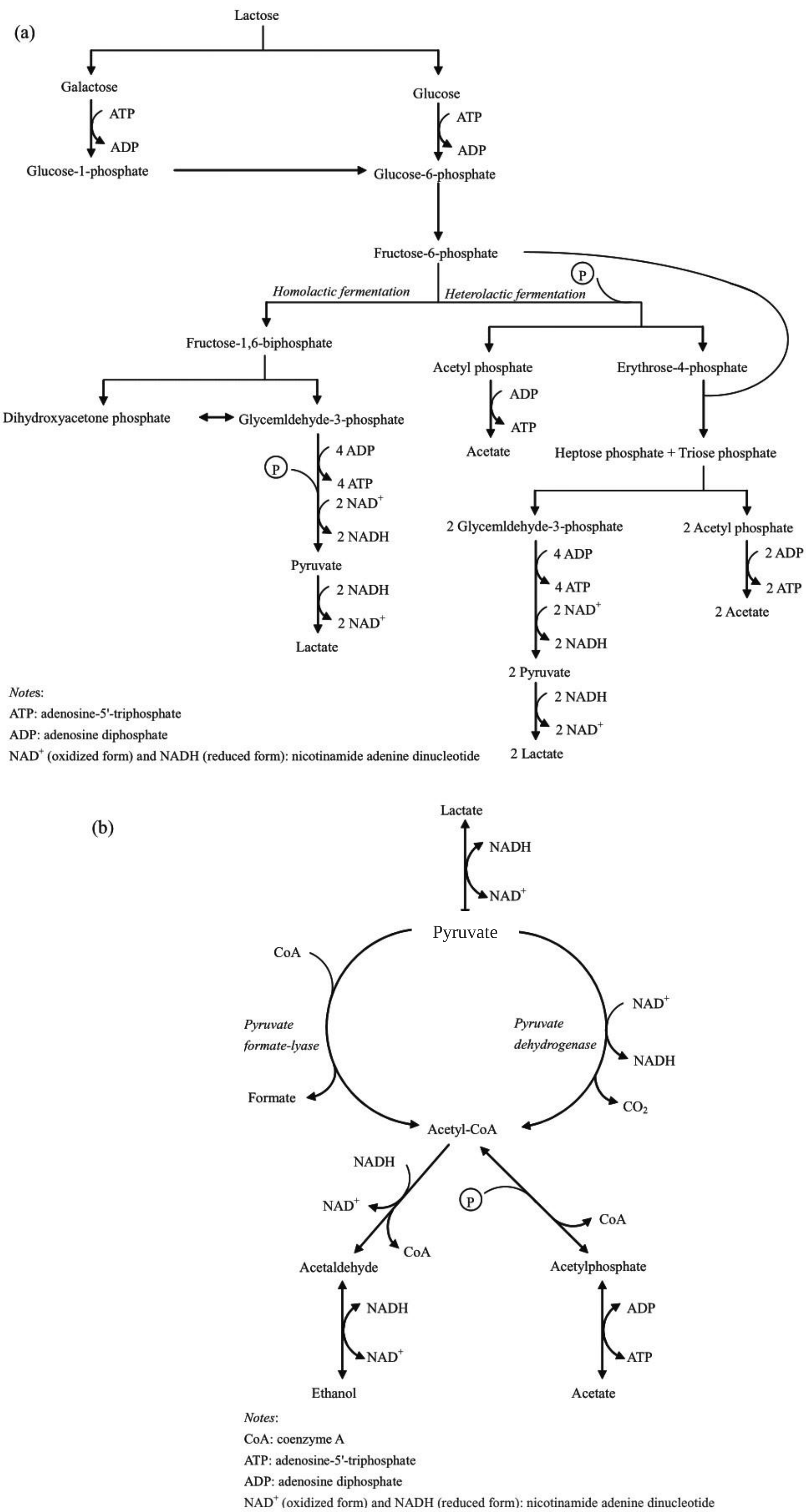


Figure 3. Voie métabolique du lactose (**Cheng**, 2010)

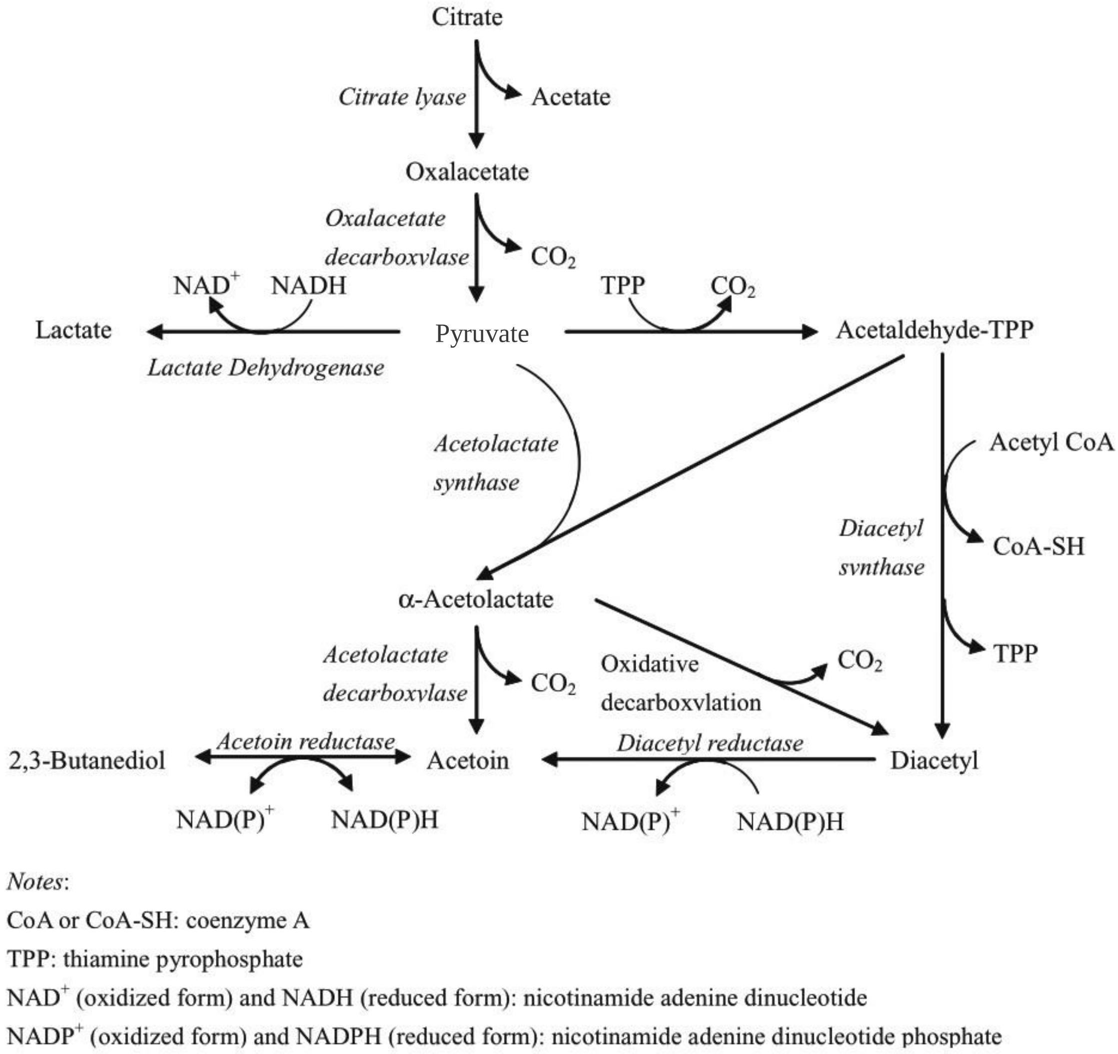


Figure 4. Voie métabolique du citrate (**Cheng**, 2010)

Les « Beany off-flavors » ont empêché les légumineuses dont le pois d'être exploités à leur plein potentiel pour la consommation humaine (**Pattee et al.**, 1983). De nombreuses études ont tenté d'identifier et de réduire les substances volatiles les plus puissantes dans les préparations de soja. L'une des principales molécules est le n-hexanal, une molécule d’arôme dérivée de la dégradation de l’acide linoléique par la lipoxygénase. Pour résoudre le problème, la fermentation avec des souches *Lactobacillus* ou *Streptococci* a été proposée, ou l'utilisation de souches dépourvue de lipoxygénases (**Blagden & Gilliland**, 2005; **Boatright & Lei**, 1999; **Kobayashi et al.**, 1995). **Blagden & Gilliland (2005)** ont testé 2 souches de *Lactobacillus acidophilus* (L1 et C19), 2 de *Lactobacillus casei* (E5 et E10), 3 de *Streptococcus thermophilus (143, OSU-1, and OSU-2) et 1 de Lactobacillus delbrueckii ssp. Lactis* pour fermenter du lait de soja. Les 8 souches ont complètement éliminé l'hexanal dans le lait de soja pendant la fermentation et ont entraîné une réduction significative des concentrations de méthanol. Ainsi, la fermentation lactique a montré son potentiel pour améliorer l'arôme des protéines des isolats de pois en diminuant la teneur en n-hexanal et en réduisant ou en masquant les faux goûts (**Schindler et al.**, 2012).

1.3 Le consommateur

1.3.1 Les modèles du choix des aliments

Le choix des aliments est un phénomène psychologique avec des manifestations physiques et doit être interprété à la lumière d'une théorie du comportement humain telle que la théorie de la construction personnelle (**Gains et al.**, 1988; **Thomson & McEwan**, 1988). Selon cette théorie "people act like scientists in the way they evaluate the world around them: formulating, testing, verifying and updating hypotheses about the world and its relationship to themselves » (**Gains**, 1994, p.52). La gamme de facteurs potentiellement impliqués dans cette construction personnelle du choix des aliments est extrêmement diversifiée et variée. Ces facteurs peuvent être divisés en ceux liés à l'aliment, à la personne qui choisit et au contexte environnemental. Plusieurs auteurs ont essayé de déterminer les relations existant entre les facteurs influençant le choix alimentaire et ont proposé divers modèles (**Gains**, 1994; **Khan & Hackler**, 1981; **Land**, 1983; **Pilgrim**, 1957; **Randall & Sanjur**, 1981; **Shepherd**, 1985). Un des derniers modèles a été proposé par **Gains (1994)**. Selon cet auteur les facteurs liés à l'aliment, au consommateur et au contexte peuvent être divisé en sous facteurs (Fig. 5).

Par exemple, les aliments ont des caractéristiques sensorielles (dépendant du consommateur aussi), des compositions nutritionnelles, des images, des emballages et des coûts, tandis que les consommateurs ont des personnalités, des humeurs, des états physiologiques, des cultures, des habitudes et des souvenirs qui affectent tous leurs réactions envers les différents aliments. Enfin, les aliments ne sont pas consommés dans un vide, mais dans des contextes d'utilisation spécifiques qui affectent largement leurs acceptabilités. Le contexte est le temps, le lieu, les circonstances, le mode de consommation et avec qui et comment les aliments sont consommés. Ainsi, pour comprendre le choix alimentaire, tous ces facteurs doivent être pris en considération.

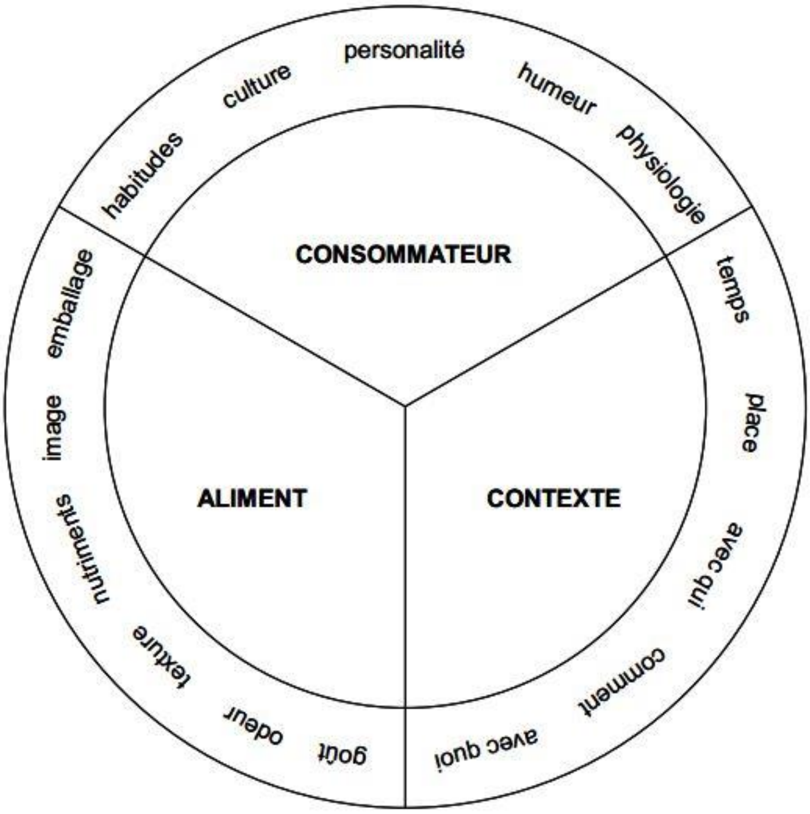


Figure 5. Modèle de Gains (1994)

1.3.2 Mécanismes d'acceptabilité (et de rejet) d'aliments

1.3.2.1 Néophobie

Fallon & Rozin (1983); **Rozin & Fallon** (1980) ont proposé que, chez l'homme, il existe trois dimensions principales sous-jacentes au rejet d'un aliment : (I) l'aversion de ses caractéristiques sensorielles ; (II) le danger, la peur des conséquences post ingestion ; et (III) le dégoût lié à la nature ou à l'origine de l'aliment. Ces dimensions s'appliquent autant aux aliments familiers qu'aux nouveaux aliments. **Pliner et al. (1993)** ont constaté que les participants de leur étude s'attendaient à ce que les nouveaux aliments soient moins agréables que ceux qui sont familiers, et que leurs croyances quant à la palatabilité de ces nouveaux aliments prédisent leur volonté de les goûter. Ces auteurs ont également constaté que les participants ont évalué les nouveaux aliments qui leur étaient présentés au laboratoire comme un peu plus dangereux que leurs équivalents familiers. **Rozin et al (1993)** ont proposé que le dégoût servirait à contrebalancer l'attirance des individus pour les nouveaux aliments et ainsi éviter les risques potentiels liés à ces nouveaux aliments. Ainsi, la néophobie alimentaire (ou réticence à ingérer de nouveaux aliments), caractéristique des animaux omnivores, y compris les humains, serait une force conservatrice et elle permettrait de maintenir le comportement alimentaire de l'organisme « sur une piste sûre » en empêchant ses préférences gustatives de l'éloigner des aliments familiers connus pour être inoffensifs (**Schulze & Watson**, 1995). Selon **Rozin** (1973) l'introduction de nouveaux aliments dans une culture pourrait donc être facilitée en ajoutant un élément familier à ces nouveaux aliments pour diminuer la néophobie qu'ils déclenchent habituellement. Un autre moyen de réduire la néophobie est lié à ce que l'on dénomme l'effet simple exposition.

1.3.2.2 Effet simple exposition

L'essence de la simple exposition est que l'exposition répétée non renforcée à tout stimulus entraîne une augmentation de préférence pour ce stimulus. L'effet d'une simple exposition a été démontrée par **Pliner (1982)** sur des jus de fruits exotique. Plus précisément cet auteur a montré un effet positif du nombre d'expositions sur le choix et l'acceptabilité de jus de fruits non familiers. Ces résultats sont interprétés en termes de « sécurité acquise ». Avec des expositions répétées, les individus apprennent que l'aliment est « sûr » et que son ingestion ne produit pas de conséquences gastro-intestinales négatives. Toutefois cette interprétation de l'effet simple exposition en elle-même ne donne aucune explication sur la nature du changement sous-jacent. Il semblerait que ce changement puisse s'expliquer en termes de familiarité.

1.3.2.3 Familiarité

La familiarité joue un rôle principal dans l'acceptabilité alimentaire. Un manque de familiarité d'un aliment ou de son goût peut affecter négativement l'acceptabilité de cet aliment. Par exemple il a été montré que le soja est généralement rejeté aux Etats unis (**Wansink & Chan**, 2001) ou en France (**Tu et al.**, 2010) car le goût du soja n'est pas familier dans ces pays. De nombreuses études inter culturelles ont révélé l'importance de la familiarité pour façonner la préférence alimentaire. Par exemple, une étude menée en France et au Pakistan a montré que les consommateurs donnent des scores plus élevés à des biscuits provenant de leur pays par rapport aux biscuits provenant de l'autre pays (**Pagès et al.**, 2007).

1.3.3 La consommation de protéine animale et végétale

Les cultures occidentales ont développé ce que **Franklin (1999)** décrit comme un «penchant particulier pour la viande». Ce penchant ne s'appuie pas seulement sur le goût mais aussi sur d'autres facteurs qui ont fait de la viande une part importante des régimes occidentaux. Ces facteurs peuvent être regroupés en trois catégories générales : culturelle, industrielle et gouvernementale (**Fitzgerald**, 2015).

Au niveau culturel, la viande est importante pour deux raisons principales : elle a développé ce que les anthropologues appellent une signification collective (communal significance) représentant le pouvoir, en particulier le pouvoir masculin. Cette signification de la viande peut être retracée dans l'histoire à la pratique de la chasse pour la subsistance (**Fitzgerald**, 2015).

L'influence de l'industrie sur la consommation de la viande est également importante. Depuis plusieurs décennies, les consortiums d'industrie de la viande commercialisent la viande comme une partie essentielle de l'alimentation humaine et favorisent des niveaux de consommation toujours plus élevés. En gardant les coûts de la viande relativement bas, l'industrie est également en mesure d'influencer la consommation (**Azzam**, 1998).

L'industrie influence non seulement les consommateurs de manière directe, mais elle le fait également de manière plus indirecte en faisant pression sur les gouvernements qui à leur tour, influencent la consommation par des politiques spécifiques (**Fitzgerald**, 2015).

A travers les âges, l'alimentation humaine a connu des transitions nutritionnelles. Ces transitions ne sont pas concomitantes à travers le monde et leur durée varie considérablement. En Europe, alors que le changement de transition après la Seconde Guerre mondiale présente une augmentation de la consommation de protéines animales (**Grigg**, 1995), la cinquième transition nutritionnelle a montré un changement vers un régime alimentaire plus sain, contenant plus d'aliments végétaux et moins d'aliments à base d'animaux. Ce changement vers les protéines végétales est une réponse aux problèmes liés à une forte demande pour les protéines d'origine animale. A titre d'illustration, la Fig. 6 montre la projection linéaire des taux d'augmentation de la production de viande et de produits laitiers pour les 40 prochaines années.

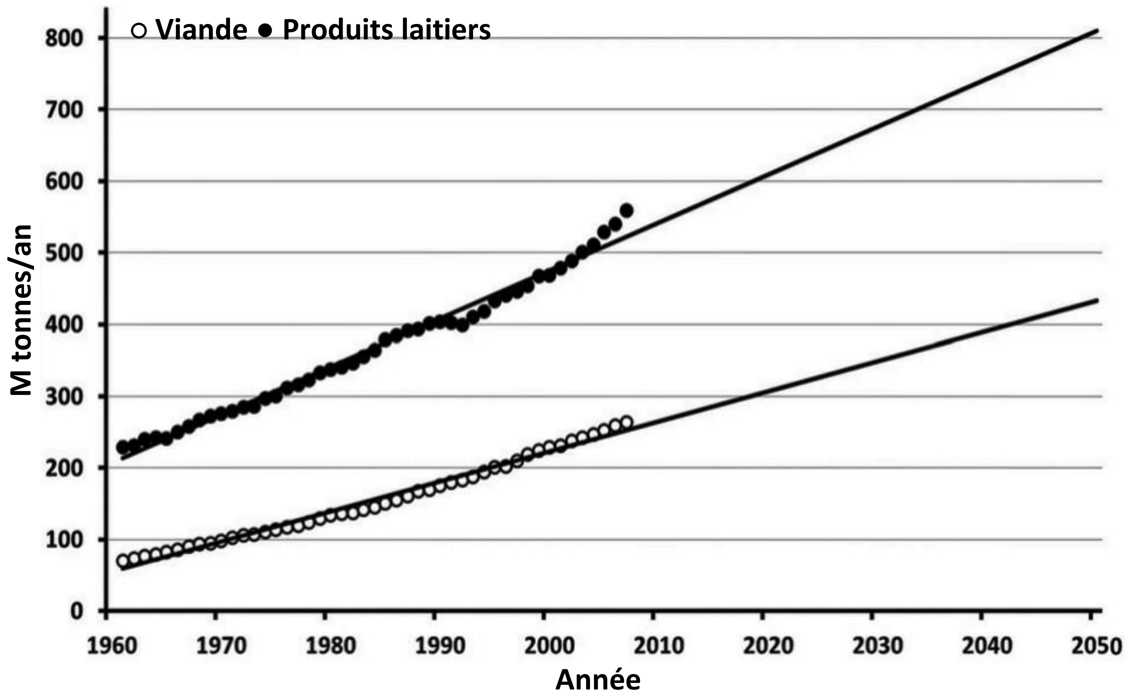


Figure 6. Projection linéaire des taux d'augmentation de la production de viande et de produits laitiers (d'après **Boland et al.**, 2013)

Bien que 1) la conscience des consommateurs européens de l'impact environnemental de la production de viande et 2) leur volonté de modifier leur comportement de consommation de viande (réduction ou substitution) soit étonnamment faible (**Hartmann & Siegrist**, 2017) un changement de comportement alimentaire dans les pays les plus riches est nécessaire pour réduire considérablement les dommages environnementaux causés par les aliments consommés. Cela implique une réduction de la consommation de protéines animales, car elles ont un impact environnemental beaucoup plus important que celui des protéines végétales (**Aiking**, 2011; **Leip et al.**, 2015). Selon **Aiking (2014)**, une réduction de la consommation de viande et de produits laitiers est donc cruciale pour une production alimentaire plus durable. Des substituts de viande constitués entièrement de composants végétaux (par exemple, le tofu) sont disponibles sur le marché. La production de ces substituts est efficace, mais leur goût, leur texture et leur odeur sont différentes de celles de la viande. Par conséquent, la part de marché de ces substituts de viande est limitée aux consommateurs qui sont prêts à consommer des protéines à base de plantes qui sont tout à fait différentes de la viande. Il y a peu d'effort politique pour réduire la consommation de viande et de produits laitiers. Il a été suggéré que la conscience des consommateurs des coûts environnementaux des protéines animales devrait être augmentée, car les consommateurs doivent modifier leur comportement nutritionnel (**Aiking**, 2014). Un changement dans le comportement alimentaire des consommateurs ne sera pas facile, car il est lié aux préférences gustatives, aux normes sociales et aux traditions culinaires (**Sabaté & Soret**, 2014).

1.4 Objectifs et démarche scientifique

L'amélioration des qualités organoleptiques des produits type 'yaourt' peut être envisagée par une meilleure maîtrise de la préparation des laits végétaux et de la fermentation lactique (**Schindler et al.**, 2012). Une compréhension plus fine du rejet ou de l'adhésion vis-à-vis de produits substitués avec des protéines végétales est aussi nécessaire afin d'identifier des leviers pour une plus large introduction de ce type de produit dans notre alimentation.

L'objectif de ce travail est donc

1- de comprendre l'effet de la fermentation des mix de protéines laitières et végétales sur la physico-chimie des produits obtenus : structure, texture, flaveurs.
2- d'analyser le poids respectif des propriétés sensorielles : texture, goût, arôme et des images véhiculées par le produit fini : santé, éco-conception, … sur son acceptation par les consommateurs.

La partie expérimentale de cette thèse se compose de 4 chapitres :

Chapitre 2 : Réactions des consommateurs face au « yaourt » au pois

L'objectif spécifique de ce chapitre est de répondre à la question suivante : est-ce que les gens sont prêts à consommer ce type de produit ?

Le travail a consisté tout d'abord en un test consommateur permettant d'étudier comment le mixte lait vache/pois (0 à 40 % de pois) fermenté pouvait être perçu en France (Europe de l'Ouest) et en Bulgarie (Europe du Sud-Est). Ces deux pays ont la même tradition de consommation de yaourt à base de lait de vache mais diffèrent dans la consommation de produit fermenté végétaux, la consommation de boisson fermentée à base de plantes étant populaire en Bulgarie, mais pas en France. Puis, un test consommateur a été fait avec et sans allégations santé et environnementales afin de savoir si ces allégations permettent d'améliorer l'acceptabilité de ce type de produit.

Chapitre 3 : Effet du cocktail bactérien sur les propriétés physico-chimiques et sensorielles

L'objectif spécifique de ce chapitre est de répondre à la question suivante : est-ce que le cocktail bactérien peut jouer un rôle sur l'acceptabilité des produits ?

Cette partie de l'étude a permis de comprendre l'influence du cocktail des LABs sur les propriétés physico-chimiques et sensorielles des produits. Différents cocktails de souches bactériennes ont été testés vis-à-vis de la cinétique d'acidification. La texture des produits finis et au cours du stockage a également été mesurée. Un profil sensoriel des produits fermentés a été établi pour évaluer les différences perçues entre les souches.

Chapitre 4 : Optimisation et sélection des conditions optimales

L'objectif de ce chapitre est de répondre à la question suivante : est-il possible d'améliorer les qualités organoleptiques de notre produit fermenté concernant la préparation des laits végétaux, les procédures de thermisation et le choix du meilleur cocktail de bactéries lactiques ?

Dans ce chapitre, l'accent a été porté sur les paramètres impactant la qualité physico-chimique des produits fermentés. Différentes modalités ont été testées afin de déterminer les meilleures conditions permettant d'obtenir un produit fermenté partiellement substitué avec des protéines de pois le plus proche en termes d'acidité (pH et degré Dornic), de rhéologie (fermeté, collant), d'arômes et d'amertume d'un yaourt nature témoin (100 % lait de vache).

Chapitre 5 : Validation sensorielle

L'objectif de ce chapitre est de répondre à la question suivante : est-ce que la matière première, le cocktail bactérien et les conditions de préparation sélectionnées conduisent à un produit avec une bonne qualité physico-chimique et accepté par les consommateurs ?

Dans cette partie, les caractéristiques physico-chimiques et sensorielles de produits fermentés réalisés à partir des paramètres optimisés dans le chapitre précédent ont été évalués. Un test d'appréciation a été également réalisé pour valider l'acceptation du produit optimisé.

Chapitre 2 : Réactions des consommateurs face au « yaourt » au pois

2.1 Introduction

Comme l'acceptabilité des consommateurs est le facteur le plus important pour commercialiser de nouveaux aliments, la première étape dans le développement de nouveaux aliments doit être l'étude de l'acceptabilité pour comprendre le besoin d'amélioration. Différents tests consommateurs sont décrits dans ce chapitre. Ces tests visent à évaluer l'effet de différents facteurs incluant l'influence de la culture, de la quantité de sucre et des allégations santé et environnementale sur l'attirance des consommateurs pour consommer des produits fermentés à base de pois (**proceeding 1** et **publication 1**).

2.2 **Proceeding 1**: Developing sustainable food: The role of consumer liking in optimization of pea yogurt

Abstract:

In the development of new food consumers' acceptability is a critical factor. This study aimed at evaluating the possibility to introduce pea proteins as new fermented food. For this purpose, mixtures of cow and pea milks with different ratios of pea (0, 10, 20, 30, 40 and 100%) were fermented with French commercial yogurt-ferment. Consumer acceptability in two European countries: France (Western Europe) and Bulgaria (Southeastern Europe) was investigated. 60 Bulgarian and 70 French panelists were asked to rate: overall acceptability, appearance, odor, taste and texture on a hedonic scale going from 1 (I do not like at all) to 7 (I like very much). The analysis of variance (ANOVA) showed a highly significant effect of products and countries for all acceptability scores. Globally, the Bulgarian consumers gave higher average scores than the French consumers. The mean comparison test showed that the presence of pea protein even in the lowest concentration (10 %) reduced the overall acceptability of yogurts in both countries. These results suggest that replacing animal by vegetal protein will not directly lead to an acceptable product.

Keywords: consumer test, cross-cultural study, vegetal protein, mixed-protein yogurt

2.2.1 Introduction

As a response to the increasing demand for animal protein and the consequences of this issue, it might be interesting to increase the consumption of plant proteins in European diet. To start changing consumer behaviors, the strategy of introducing plant protein as a part of traditional foodstuffs seems promising (**Boland et al.**, 2013). Despite the negative organoleptic properties inherent to plant ingredients, it was shown that consumers can accept dairy-plant products (cow milk and soybean proteins) with a ratio of soybean protein to 50% (**Tu et al.**, 2012). However, soybean might not be the most suitable plant protein source because of its associated allergy and phytoestrogens issues (**Cederroth et al.**, 2010; **Eastham**, 1989). Pea (*Pisum sativum*) may be a better substituent because of its high-quality amino acid profile (**Schneider & Lacampagne**, 2000), its good functional properties (**Bora et al.**, 1994), its lower allergic effect (**Jaffuel et al.**, 2001), and its lower phytoestrogen content (**Mazur**, 1998). Pea protein, like other plant proteins, has some off-flavors (**Murat et al.**, 2012), but sensory characteristics are not the only factor that affects food choice and preference. Non-sensory factors including food familiarity, health claims, price and mood have an influence on food choice (**Prescott et al.**, 2002). **James (2004)** showed that consumers' cultural background could affect these sensory and non-sensory factors.

In last years, many cross-cultural consumer studies have compared variations between cultures in food preference. These studies were usually carried out to assess how product familiarity influence consumers' perception, by comparing the liking of food known and accepted in one culture with another culture which is not familiarized to this food (**Kim et al.**, 2013; **Pagès et al.**, 2007). Because they are omnivore, humans naturally tend to be interested in new food but they are cautious about trying it because of a phenomenon called food neophobia (**Pliner & Hobden**, 1992). Repeated and wide exposure to new food could reduce this phenomenon by increasing consumers' familiarity. One possible way to introduce pea protein to consumers could be dairy products such as yogurt. Yogurt, one of the most popular food for a long time all around the world, is made of cow milk fermented by two specific lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*).

The goal of this study is to know whether it would be possible to introduce pea proteins as fermented new food to European consumers. We investigated how cow-pea fermented milk could be perceived in France (Western Europe) and Bulgaria (Southeastern Europe). While these two countries have a strong tradition of consuming yogurts and have soy yogurt in their markets, the studied product "itself" is not familiar to the French and Bulgarian consumers, however « boza » a cereal-based fermented beverage is popular in Bulgaria. No such plant-based products exist in the French food tradition.

2.2.2 Materials and methods

2.2.2.1 Preparation of samples

Using skim milk powder purchased from Régilait (Saint-Martin-Belle-Roche, France) and pea protein isolate Nutralys® S85F supplied by Roquette (Lestrem, France), cow and pea milks were prepared at the same protein concentration (4.5 g/L). Lactose, calcium and citrate levels were also balanced in the two milks. Before the incubation with starter culture for homemade yogurt production (Streptococcus thermophilus and Lactobacillus bulgaricus) obtained from Alsa (Rueil-Malmaison, France), the cow milk was heated in a water bath at 90 °C for 10 minutes, and the pea milk was autoclaved at 110 °C for 10 minutes. When the milks temperature returned to about 42 °C, the two milks were mixed according to the needed concentrations, inoculated and then incubated at 42 °C for 4-6 hours. Six fermented products with different concentrations of pea protein (0, 10, 20, 30, 40 and 100%) were prepared, stored for 24 hours at 4 °C (Table 1).

Table 1. Gel samples used in the sensory test.

Gel sample (Name)	Milk protein concentration (g/100 g protein total)	Pea protein concentration (g/100 g protein total)
0%	100	0
10%	90	10
20%	80	20
30%	70	30
40%	60	40
100%	0	100

2.2.2.2 Sensory test

- Consumers:

A Bulgarian and a French panel of consumers have evaluated the six fermented products. The Bulgarian panel consisted of 60 students and staff members from the University of Food Technology Plovdiv (73.33% female – 33.33% <30 years and 66.67% >30 years). The French panel consisted of 70 participants recruited during a public scientific event ("Nuit des chercheurs") in the University of Burgundy (64.52% female – 55.88% <30 years and 44.12% >30 years).

- Evaluation procedure:

In both countries, the panel tested the samples in a classroom with enough separation between the tables to avoid any communication between the participants during the test. In France, the test was carried out under red light. For both panels, about 10 g of each sample were served in a white plastic cup that was coded with a random three-digit number. The six samples were presented at the same time. Bottled water was provided to cleanse the palate. Panelists had to rate overall, appearance, odor, taste and texture acceptability on hedonic scales going from 1 (I do not like at all) to 7 (I like very much), with 4 being anchored as neither like or dislike. The questionnaires were written in the native language of each country.

- Statistical analysis:

Three-way ANOVAs were carried out (Dell Statistica version 12) for each acceptability rating with consumer as a random factor, country a fixed between-subject factor and product a fixed within-subject factor. When a significant product effect was observed a student Newman-keuls pairwise comparison test was carried out. For all panelists, a principal component analysis (PCA) on the overall acceptability scores followed by a hierarchical ascending classification (HAC) was performed (SPAD version 8) to identify classes of consumers who were homogeneous in their preference for yogurts. Finally, a multiple factor analysis (MFA) was performed on all the assessed attributes in order to compare the French and Bulgarian panels.

2.2.3 Results

2.2.3.1 Overall acceptability

The ANOVA on the overall acceptability scores from both panels showed a highly significant country effect ($p < 0.0001$) indicating that the two consumer groups evaluated the products differently. On the average, the Bulgarian consumers gave higher scores than the French group as shown Figure 1. This result should, however, be interpreted with caution as it can be due either to a higher preference or to a difference in using the scale as was previously shown in cross-cultural studies (**Prescott et al.**, 2002). No significant product × country interaction effect was observed indicating that the two panels scored the fermented products in the same manner. A highly significant effect of product ($p < 0.0001$) was also observed. The mean comparison test (Table 2) showed that the presence of pea protein even in the lowest concentration (10%) reduced the overall acceptability of yogurts. The overall liking decreases with the increase of pea concentration with a first plateau for 20 and 30% pea protein products and a second one for 40 and 100% pea protein products. We can also note that the average liking score moves from the neutral zone for the 0 and 10% concentration to the negative one for the 20% and above concentration.

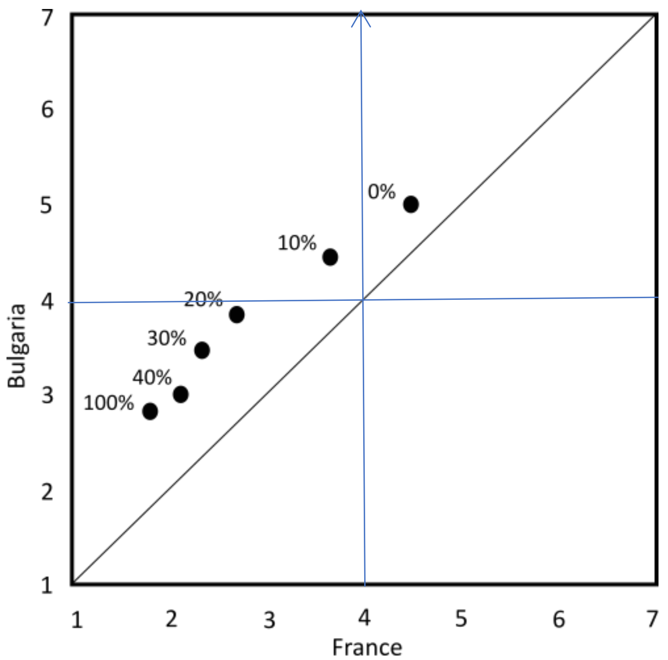


Figure 1. Comparison of means overall acceptability score for all tested products.

Table 2. Mean scores of overall acceptability on a 7-point scale (1: I do not like at all – 7: I like very much) as a function of pea concentration. Means with different superscript letters are significantly different (Newman-Keuls test, $p < 0.05$)

	0%	10%	20%	30%	40%	100%
Overall acceptability	4.76^{a}	4.05^{b}	3.28^{c}	2.94^{c}	2.57^{d}	2.36^{d}

Figure 2 represents the PCA performed on individual overall acceptability scores of all the panelists (French and Bulgarian). The first two PCA dimensions explain 60.52% of the total variance. The first PCA dimension explains 43.96% and is correlated to all products substituted with pea protein (from 10% to 100%) while the second dimension (16.56% of variance) is correlated with the yogurt without pea protein. Most consumers accepted the fermented cow milk but not the yogurt containing pea protein. The third and fourth dimensions (23% of the total variance) explained the difference among the samples depending on pea protein substitution level (data not shown).

The HCA performed on the first four PCA dimensions showed three classes of consumers. Figure 3a shows the distribution of French and Bulgarian consumers in each class and Figure

3b the average liking scores of each class. The first class represents 29.23% of the total number of consumers, 60.53% of consumers in this class are from the Bulgarian panel. The consumers in this class appreciated all the products including the product with 100% pea protein. The second class represents 43.85% of consumers. The proportion of French and Bulgarian panelists in this class are almost equal. The products up to 10% pea protein are in the positive zone and the others in the negative zone. Furthermore, the largest discrepancy between 100% milk yogurt and 100% pea protein occurs in this class. The third class represents 26.92% of all panelists, about 75% of this class are French participants. The consumers in this class scored all the products in the negative zone even the yogurt without pea protein.

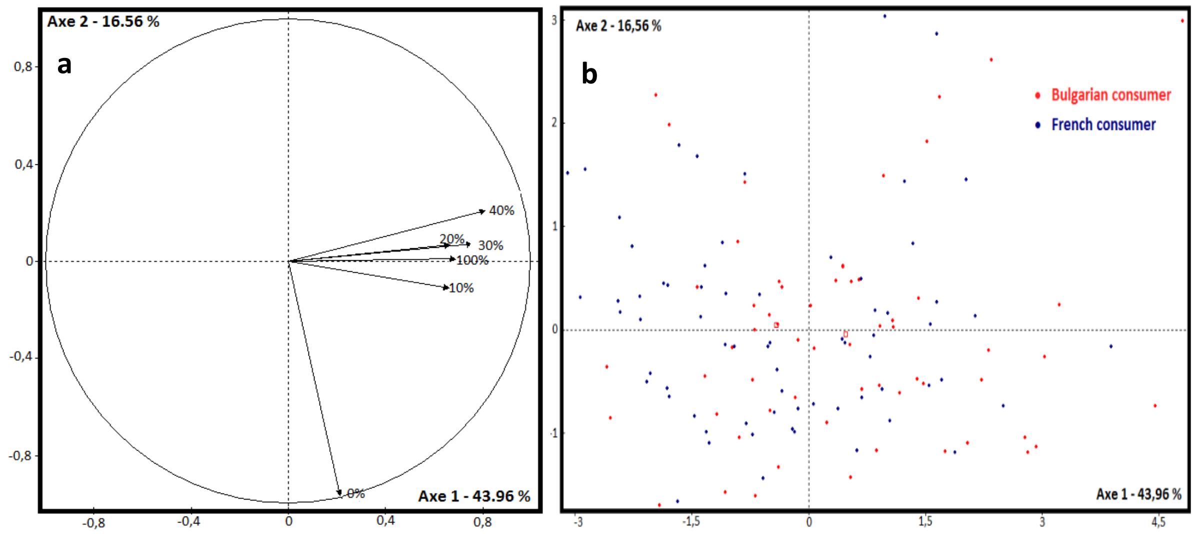


Figure 2. First two dimensions of the principal component analysis (PCA) performed on the overall acceptability. a) correlation circle, b) projections of the panelists. The Bulgarian consumers are represented in red and the French ones in blue.

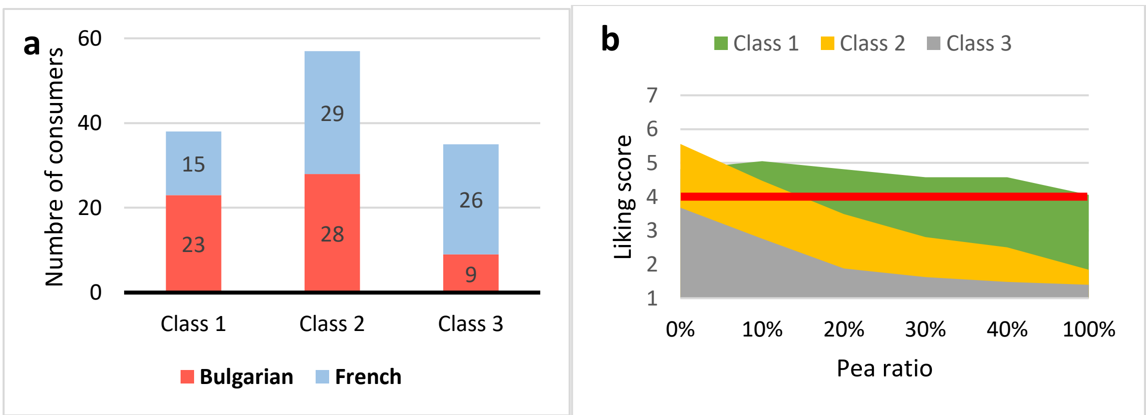


Figure 3. Classes of consumers yielded by the hierarchical cluster analysis performed on the

first fourth dimension of the liking score PCA: a) Distribution of French and Bulgarian consumers in terms of Class b) the three classes of consumers in terms of liking score.

2.2.3.2 Visual appearance, taste, odor and texture

The ANOVA showed the same patterns as for the overall acceptability (Figure 4). Globally, the products with higher pea protein concentration were less appreciated for all the characteristics. Again, for all the attributes, the Bulgarian consumers gave higher scores than the French consumers. Moreover, they generally scored the attributes in the neutral zone for the products that contain till 20% pea protein, unlike French consumers who assessed all the products containing pea protein (even with just 10% pea) in the negative zone.

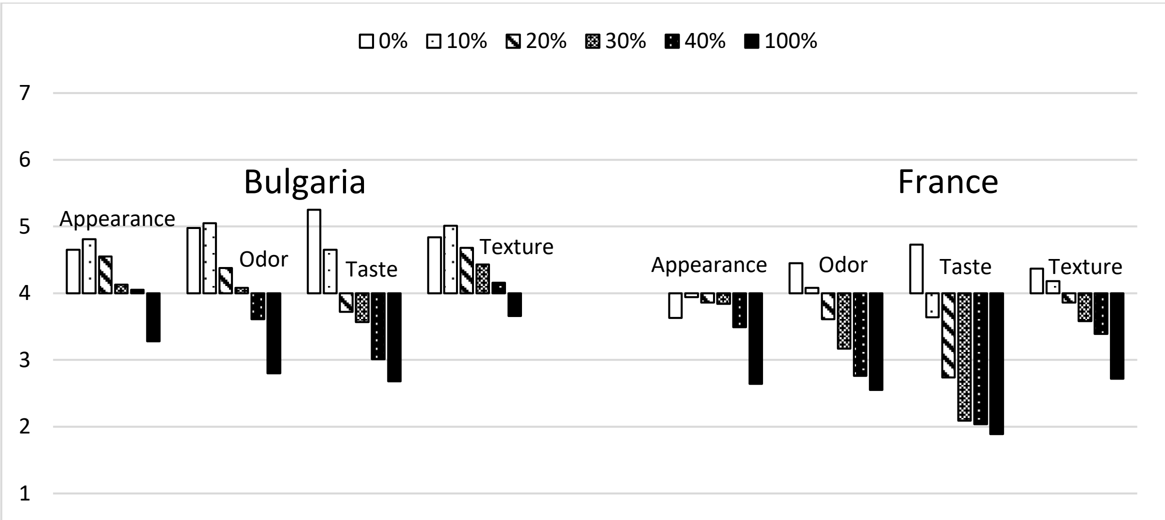


Figure 4. Means of liking scores for visual appearance, taste, odor and texture assessed on hedonic scales ranging from 1 (I do not like at all) to 7 (I like very much), 4 (neither like nor dislike).

The MFA performed on the overall acceptability, appearance, odor, taste and texture scores for the French and Bulgarian panelists is presented Figure 5. The first dimension (89% of variance), is correlated with all scales. As expected, the factors that affected the overall acceptability of products in ascending order are: the taste, the odor, the texture and finally the appearance (Figure 5a). In other words, for both panels, the taste was the most correlated attribute with the overall acceptability and the appearance was the least. The two panels gave the lowest scores to the product with 100% pea protein, possibly because the participants were expecting the fermented milk product to taste like a conventional yogurt (**Tu et al.**, 2010). Although the mean overall acceptability for all the products is highly correlated for the two panels (RV coefficient ≈ 0.98), Figure 5b shows the presence of some differences among the two panels in the assessment of the products from 0% to 40% pea protein. This is due mainly to differences in the assessment of appearance and odor among the French and Bulgarian panels.

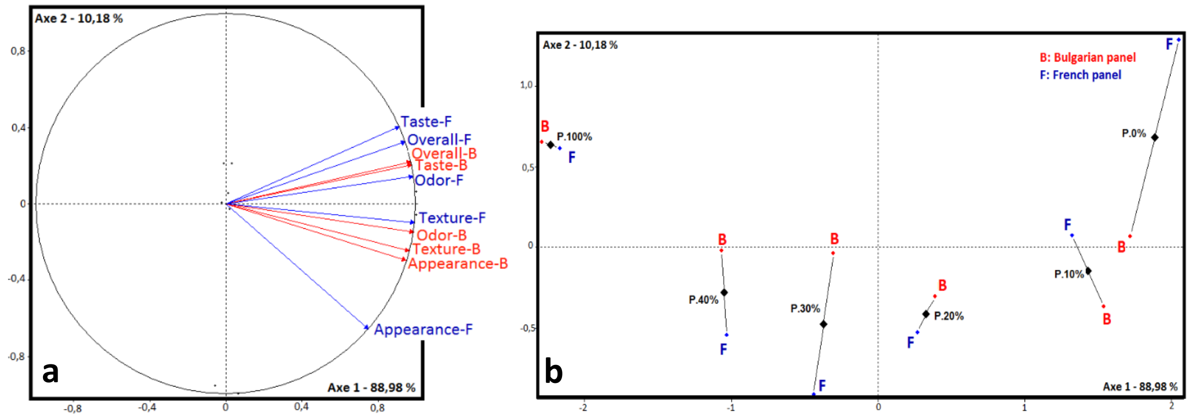


Figure 5. First two dimensions of the multiple factor analysis (MFA) performed on all the assessed attributes: a) correlation circle (the name of the variables is followed by F for the French panel and by B for the Bulgarian panel and b) coordinates of all the products. Lines are drawn between the Bulgarian panel (B) and the French (F) for the corresponding projected products.

2.2.4 Conclusion

As consumers' acceptability is the factor the most important in order to commercialize new food. The first step in developing new food must be the investigation of the acceptability to understand the need of improvements. In this work, the consumers showed clearly that the insertion of plant protein in the European diet need more efforts as the presence of small quantity of pea protein in fermented milk has reduced the acceptability. With just 10% of pea protein, all French consumers and 20% of Bulgarian consumers scored the product in the negative zone. Globally, Bulgarian and French consumers scored the fermented products similarly even though the liking mean scores were higher for Bulgarian. Significant differences between French and Bulgarian were observed for the appearance and odor assessment with French consumers criticizing considerably the appearance and the odor of these products. In the light of these results, more attention must be given not only to the taste but also to the odor and the appearance of these fermented products.

2.3 **Publication 1**: Effect of health and environmental information on the appreciation of fermented pea-cow milk products

Abstract:

The use of plant protein in food products is limited owing to its green/beany off-flavors. The aim of this work was to evaluate whether health and environmental claims improved the appreciation of a fermented pea - cow milk product, formulated with different proportions of pea protein. A pre-test was carried out to assess the impact of health and environmental claims on consumers' willingness to buy yogurts. Health claims - and especially nutritional and cardiovascular risk associated messages - were more persuasive than environmental claims. Two health and two environmental messages were selected from the pre-test to be used in the main study. Four products were prepared by mixing cow and pea milk in two ratios (80:20 and 60:40) and adding sucrose in two proportions (6 and 9.5 g/100 mL). A total of 58 consumers received the four samples, presented with and without the selected messages, and were asked to assess their liking on a 9-point hedonic scale. The results revealed that only samples containing 20% pea milk and 9.5 g/100 mL sucrose were assessed favorably. A significant effect of information was observed only in the health claim condition for the sweetest sample with 20% pea protein. This effect, however, was in the opposite direction than that expected: the health message decreased the liking score, due perhaps to the inconsistency between the message and the high perceived sweetness level.

Keywords: Plant protein. Pea. Fermentation. Health information. Environmental information. Sensory evaluation

2.3.1 Introduction

It is currently acknowledged that a global change towards a diet consisting mainly of plant products would have positive impacts both on the environment and public health (**Esnouf et al.**, 2013). Plant proteins represent approximately 65% of the protein intake for human consumption worldwide (**Young & Pellett**, 1994). In France, this figure is only about 30% (**Halkjær et al.**, 2009), even though nutritionists recommend a contribution of 50% to maintain a balanced diet (PNNS). In January 2017, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) updated its food consumption guidelines for the French population, highlighting the need for a reinforced and regular consumption of pulses and a reduction in animal products, particularly red meat (**ANSES**, 2017). Indeed, plant protein intake has been shown to be a marker of a healthy diet in the French population (**Camilleri et al.**, 2013) and seems to play a role in the prevention of obesity and the reduction in mortality (**Lin et al.**, 2015; **Song et al.**, 2016). Rebalancing diets involves partial substitution of animal protein by plant proteins. This entails not only a reduction in the consumption of meat, but also in the consumption of dairy protein, currently about 23% of the total protein intake in France (**Rousset et al.**, 2003). Animal protein contains all the essential amino acids needed by humans, while plant proteins are generally deficient in one or more essential amino acids. Moreover, animal products are an exclusive source of specific essential micronutrients, such as calcium and heme iron. For these reasons, maintaining a balanced diet while substituting all animal products by plant products implies a true nutritional challenge (**Garnett**, 2013) and is not recommended for the entire population. In order to help people to decrease their consumption of animal protein without completely changing their food practices, it would be advantageous to propose products that combine the benefits of dairy proteins with those of vegetable proteins. Indeed, the main limiting factor in achieving a balance between animal protein and plant protein seems to be the difficulty of changing dietary habits (**Rozin**, 1996).

Eating behavior is influenced by many factors, not only by physiological and nutritional needs. As summarized in Shepherd’s food choice model (**Shepherd & Raats**, 1996), food choice is determined by three classes of factors: foods, individuals and external environment. Various models have tried to integrate these factors. Most of these models are based on socio-psychological research and focus on the relationship between individuals’ beliefs and attitudes and their behavior (**Sobal et al.**, 2006). Beliefs about nutritional benefits or harm in eating specific foods, beliefs about functional and price factors of the food as well as perceived social pressure to eat the food may be more important in determining an individual’s choice than the actual nutritional quality and health consequences. Moreover, beliefs about the food might also modify the perception of the sensory properties of the food. To illustrate this point, **Wansink (2000)** showed that the same nutritional bar was perceived differently according to whether it was labeled with the information “contains 10g of soy protein” or “contains 10g of protein”. Although it did not contain any soy, the bar with a soy labeling was perceived as having a worse taste and texture and a stronger after taste but a higher nutritional value than the bar without soy labeling. Labels can also have a positive value. Descriptive labels such as "tender grilled chicken" compared to "grilled chicken" enhance the perceived quality of the food (**Wansink et al.**, 2005). Prestigious labels can make an ordinary wine (**Brochet & Morrot**, 1999) or champagne (**Lange et al.**, 2002) taste better. Thus, to promote dietary changes it is important not only to focus on the sensory properties of the product itself but also to consider individual consumers and their beliefs and attitudes.

The objective of this work was to evaluate the effect of health and environmental information on the appreciation of new plant-milk fermented products. **Torres-Penaranda et al. (1998)** showed that products derived from plant raw materials are rich in off-flavors (e.g., vegetable taste, chalky) and are characterized by high astringency and bitterness. These off-flavors generally result in a refusal on the part of consumers, even if they are familiar with the class of products or with the ingredients (**Tu et al.**, 2010, 2012). One way to reduce these off-flavors is to use fermentation. A previous study has shown that fermentation considerably improves the aroma profile, resulting in a reduction in or a masking of pea off-flavors (**Schindler et al.**, 2012). The question that remains is would fermentation be enough for the consumer to accept the substitution of milk protein by plant protein in a dairy product? Or would positive information on the benefits of the substitution be necessary for the acceptability? To address these issues, pea protein was selected as a partial substitute for milk protein. Pea (*Pisum sativum*) may be a better substitute than soy, because its high-quality amino acid profile and high biological value (similar to those of soy) (**Schneider & Lacampagne**, 2000) are combined with good functional properties (**Bora et al.**, 1994), lower allergic effect (**Jaffuel et al.**, 2001), and lower phytoestrogen content (**Mazur**, 1998).

Our main hypothesis was that providing positive messages would increase the pea/milk ratio accepted by consumers in yogurts. We were also interested in comparing the persuasive power of health and environmental messages. To test these hypotheses, our approach was threefold: i) we formulated a set of yogurts containing pea milk and performed a sensory test to select four yogurts from this set; ii) we identified health and environmental claims positively perceived by consumers; iii) finally, we tested whether these claims improved the acceptability of yogurts.

2.3.2 Preliminary study 1: Selection of fermented pea-milk samples

2.3.2.1 Objective

The objective was to select four samples that varied in pea protein concentration, texture and sweetness, all of average quality, to be used in the main study.

2.3.2.2 Materials and Methods

- Panel:

Eight panelists (five females and three males, aged from 25 to 50 years old), staff from the AgroSup Institute in Dijon, used to evaluating plant-based fermented products, participated in all sessions.

- Samples:

Cow milk (from skim milk powder purchased from Régilait (Saint-Martin-Belle-Roche, France) and pea milk (from pea protein isolate Nutralys® S85F provided by Roquette (Lestrem, France)) were prepared and fermented with a commercial yogurt starter culture Alsa® (containing lyophilized *Streptococcus thermophilus* and *Lactobacillus bulgaricus*) according to **Yousseef et al.** (2016). The inoculated mixtures were sampled in 1 L glass jars and incubated at 37 °C for 24 hours. The sweetness and texture of the products were modified by adding sucrose (Carrefour, France), Fructooligosaccharide (FOS) Orafti® L85 (Beneo, Germany) and full cream 30% fat (Carrefour, France) after fermentation. The products were stored at 4 °C for one day before the sensory sessions. A set of 25 pea-milk fermented products was obtained using a four-factor (pea, sucrose, FOS and cream) central composite design (Table 1).

Table 1. List of the 25 pea-milk fermented products obtained from a four-factor central composite experimental design

Sample	Pea milk mL/100 mL	Sucrose g/ 100 mL	FOS g/ 100 mL	Cream g/ 100 mL
S.01	0	6	0	0
S.02	0	13	0	0
S.03	40	6	0	0
S.04	40	13	0	0
S.05	0	6	3	0
S.06	0	13	3	0
S.07	40	6	3	0
S.08	40	13	3	0

S.09	0	6	0	2
S.10	0	13	0	2
S.11	40	6	0	2
S.12	40	13	0	2
S.13	0	6	3	2
S.14	0	13	3	2
S.15	40	6	3	2
S.16	40	13	3	2
S.17	20	9,5	1,5	1
S.18	20	6	1,5	1
S.19	20	13	1,5	1
S.20	0	9,5	1,5	1
S.21	40	9,5	1,5	1
S.22	20	9,5	0	1
S.23	20	9,5	3	1
S.24	20	9,5	1,5	0
S.25	20	9,5	1,5	2

❖ Procedure:

The panelists participated in six sensory sessions: one familiarization session and five testing sessions.

- *Familiarization session.* Panelists were asked to taste five yogurts and to write down the negative attributes they found in them. The negative attributes were merged with a set of descriptors obtained from previous studies on the same type of yogurts and the list was reduced to avoid redundancies. The final list included six terms (acidity, sweetness, vegetal taste, astringency, bitterness, presence of particles). Panelists were then familiarized with a four-point quality scale (acceptable, limit acceptable, limit not acceptable and not acceptable). The scale was based on the general quality categories proposed by **King et al. (2002)**.

- *Evaluation sessions.* During the evaluation sessions, panelists were asked to taste from five to seven yogurts and to rate them on the four-point quality scale. Following **King et al. (2002)** procedure If a panelist rated a sample "limit not acceptable" or "not acceptable", the panelist was then asked to rate the intensity of the descriptors that led to this quality score. In each session, yogurts were coded with 3-digit codes and presented in a Williams Latin square arrangement.

❖ Data analysis:

Individual quality scores were coded from 1 (acceptable) to 4 (not acceptable) before being compiled in a products x panelists matrix and submitted to a normalized principal component analysis (PCA). The average intensity of the descriptors previously listed and the experimental factors were added as supplementary variables in the PCA. The coordinates of the products on the first three dimensions (87% of variance) were submitted to a hierarchical cluster analysis (HCA).

2.3.2.3 Results

Figure 1 shows the first two dimensions of the PCA carried out on the individual quality scores for the 25 tested samples. All panelists' quality scores project on the positive side of the first dimension (45% of variance). This dimension can be interpreted as a quality dimension, with products projecting on the positive side being lowest quality and products projecting on the negative side being highest quality. This dimension was positively correlated with the concentration of pea protein. Overall, yogurts with the highest pea concentration were evaluated as having the lowest quality. The second dimension (17% of variance) is correlated with the sucrose concentration and opposed panelists who associated quality with high sucrose concentration to those who associated high quality with low sugar concentration. The HCA yielded five groups of products. Group 1 contains all the samples with the highest concentration of pea protein and no cream, characterized as astringent and considered as being low quality by most panelists. At the opposite end of dimension 1, group 4 contains most of the products without pea and with low or intermediate sucrose concentration. They were considered as higher quality by all panelists. Opposed to these two groups on the second dimension, Group 5 only includes products containing the highest sugar concentration. Two of these products also contain the highest concentration of FOS, which may reinforce their sweet taste. These products were perceived as sweet and with low vegetable flavor by all panelists. A few panelists considered these products as low quality. Group 2 and 3 were located in the center. These products are considered medium quality (neither very high nor very low) and are either products without pea, with high sucrose concentration and no FOS, or products with pea and intermediate sucrose concentration (mostly 9.5%).

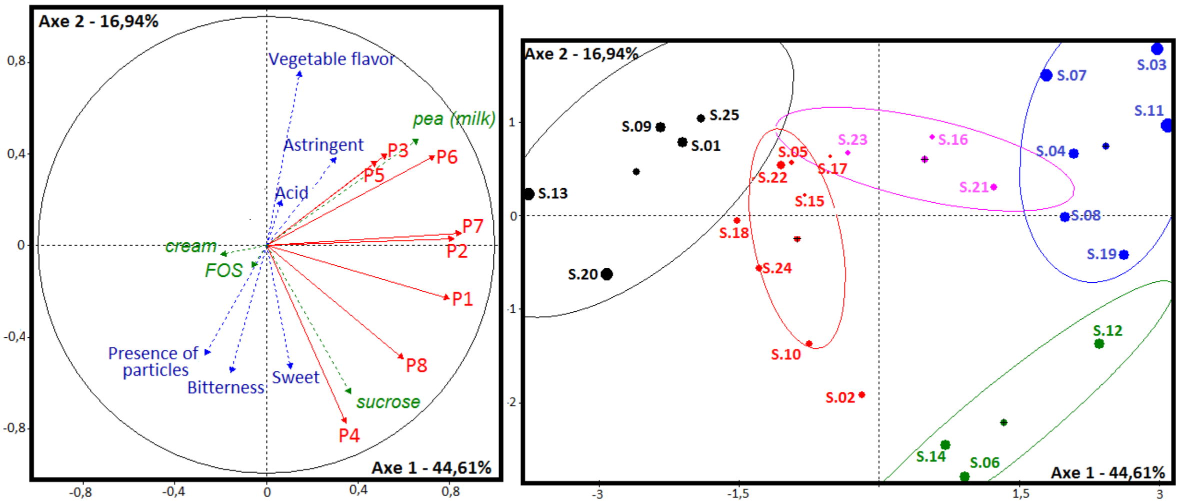


Figure 1. First two dimensions of the principal component analysis performed on the Individual quality scores (1: high quality and 4: low quality). A) Loading plot. Descriptors (in blue), experimental factors (in green) are projected as supplementary variables on the PCA performed on the individual panelists' quality scores (in red)). B) Product map. Colors of the ellipses represent the product groups yielded by the hierarchical cluster analysis

2.3.2.4 Discussion

The pea milk and sucrose content of the yogurts mainly drove our panelists' judgment of quality, and thus related to vegetable flavor. The products considered lowest quality were those containing the highest pea milk proportion, and either the lowest or the highest sucrose quantity. The perceived low quality of high pea-milk products can be understood in the light of previous studies that pointed out the low sensory acceptance of legume based milk alternatives (**Mäkinen et al.**, 2016; **Sethi et al.**, 2016). Sucrose content seems to have modulated the quality judgment of high pea milk products differently according to the individual. For some panelists, high pea concentration products with low sucrose quantity were evaluated as low quality. For others, high pea milk products containing a high quantity of sucrose were considered as low quality. FOS and cream were not in themselves a driver of quality, except for FOS when associated with the higher amount of sucrose. In this case, it tended to decrease the perceived quality, probably by increasing the sweetness of the product, its sweetening power being about 30-50% of that of sucrose (**Protonotariou et al.**, 2013).

These results indicate that the highest concentration of sucrose used in this study was associated with low quality. But when added in intermediate quantity to yogurts containing a high proportion of pea milk, sucrose increased the perceived quality of these products. This observation suggests the occurrence of sensory interactions or masking effects between sweet taste and pea milk flavor. The reasons evoked by panelists to explain their quality judgment of yogurts containing 40% of pea milk tend to confirm this hypothesis. The faults usually attributed to plant milks in the literature, i.e., vegetable flavor, presence of particles, astringency and acidity, are correlated to both the concentration of sucrose and the proportion of pea milk in our study. Products described as having vegetable flavor were those containing a high ratio of pea milk and low sucrose content. Products with particles or described as bitter were not those containing the highest proportion of pea milk.

2.3.2.5 Conclusion

The objective of this preliminary study was to select four samples for the main study. To avoid ceiling or floor effects, we selected these four samples among the products located in the central part of the PCA 2D map, i.e., medium quality samples. Among the selected samples, two consisted of 40% pea protein (S.15 and S.21), and two consisted of 20% pea proteins (S.18 and S.23). In addition, two samples had 6g/100 mL of sucrose (S.15 and S.18), and two had 9g/100 mL of sucrose (S.21 and S.23). Two contained 1.5% FOS (S18 and S21), and two contained 3% FOS (S15 and S23). All contained either 1 or 2% cream.

2.3.3 Preliminary study 2: Determination of the most persuasive messages

2.3.3.1 Objective

The objective of this study was to evaluate the effect of health and environmental claims on consumers' willingness to buy products made with vegetable protein and to select four claims (two health and two environmental claims) with contrasting potential impacts for the main study.

2.3.3.2 Materials and methods

- Panel:

Sixty panelists (56.7% females and 43.3% males; 48.3% were under and 51.7% were over 30 years old) were recruited on the Dijon university campus.

❖ Material:

Thirty-five yogurt labels were designed for the experiment. The labels were identical except for the claim (Figure 2). Among the 35 claims (Table 2), three were related to both health and environment, 16 were related to health and 16 to environment. Health claims were chosen on the basis of the ingredients and nutrients contained in the products, and particularly pea milk. Since the objective was to select claims well accepted by consumers, we chose to test a wide number of claims referring to pea protein properties.

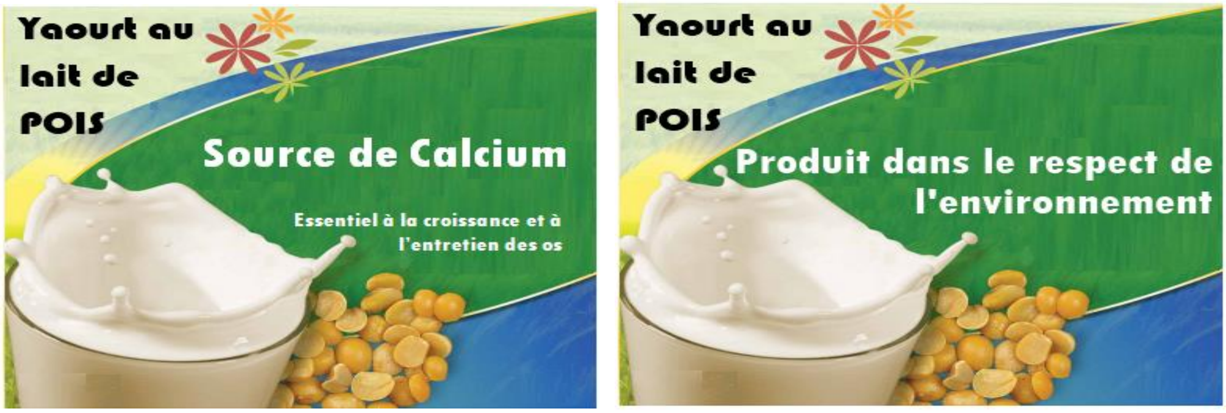


Figure 2. Two examples of labels with 1) environmental claim 2) health claim

Table 2. Claims divided into health, environmental and general information

	General claims
C1	Take care of yourself and preserve the environment
C2	Designed for your health and the environment
C3	Respects your well-being and the environment
	Health claims
C4	Source of Calcium: essential for growth and maintenance of bones
C5	Take your health to heart
C6	Lysine-rich: impact on growth
C7	Pea milk is non-allergenic
C8	Antioxidant: helps to protect cells
C9	Source of vitamins and minerals
C10	Rich in fiber: facilitates intestinal transit
C11	Source of Vitamin B9: Supports the normal functioning of the immune system and reduces tiredness
C12	Antioxidant + Anti-inflammatory + Antibacterial
C13	Decreases risk of cardiovascular disease
C14	Rich in fiber: fiber consumption helps decrease the risk of colorectal cancer
C15	Better control of diabetes
C16	Rich in fibers
C17	Low in sugar, low in calories
C18	Low in cholesterol
C19	Low in saturated fatty acids: a diet high in saturated fat favors weight gain and increases risk of cardiovascular disease

	Environmental claims
C20	This yogurt requires less water for its production
C21	The pea is a natural fertilizer: peas capture the nitrogen from the air and so help to enrich the soil
C22	Peas grown using sustainable agriculture practices: 100% French origin
C23	GMO free
C24	To preserve the planet, let's change our eating habits: eat less animal protein
C25	The pea crop requires little nitrogen fertilizer: reduces the risk of water and air pollution
C26	50% of water pollution is due to intensive farming of animals
C27	More environmentally friendly: reduces CO2 emissions
C28	Contains less animal protein than other yogurts: the production of animal protein is harmful to the environment
C29	Peas grown using sustainable agriculture practices
C30	Environmentally friendly
C31	Pea milk limits the exhaustion of natural resources and the amount of waste
C32	Formulated without animal protein: cattle breeding is responsible for 65% of deforestation
C33	Formulated without animal protein: livestock is responsible for 18% of total greenhouse gas emissions
C34	More respectful of the environment: reduces pollution
C35	Environmentally friendly yogurt: locally produced

- Procedures:

The panelists took part in the experiment individually. They received the 35 labels in a random order and had to sort them into four groups according to their willingness to buy a new fermented plant-dairy product: "vraiment très envie" (*strong desire to buy*), "plutôt envie" (*rather want to buy*), "plutôt pas envie" (*rather not want to buy*) and "pas du tout envie" (*no desire to buy*).

- Data analysis:

Data were analyzed first by counting the number of panelists who sorted each label into each category. Next, an attraction index was calculated for each label by subtracting the number of negative (*rather not want to buy* and *no desire to buy*) from the number of positive assessments (*strong desire to buy* and *rather want to buy*).

2.3.3.3 Results

About 68% of the labels with a health claim were positively evaluated (*strong desire to buy* or *rather want to buy*) versus 46% for the labels with an environmental claim. In agreement with this observation, a Student t-test showed a significant difference (t=4.39, $p < 0.0001$) between the mean attraction indexes obtained for labels with health claims (29.8±12.7) and with environmental claims (7.8±15.5).

Figure 3 shows the average attraction index obtained for the 35 claims. Apart from C6: "*Lysine-rich: impact on growth*" and C7: "*Pea milk is non-allergenic*" health claims were positively evaluated. The most persuasive health claims were C9: "*Source of vitamins and minerals*" and C13: "*Decreases risk of cardiovascular disease*." Among the environmental messages, the most persuasive for the panelists were C23: "*GMO free*" and C30: "*Environmentally friendly*." The least attractive environmental claims were C26: "*50% of water pollution is due to intensive farming of animals*", C32: "*Formulated without animal protein: cattle breeding is responsible for 65% of deforestation*" and C33: "*Formulated without animal protein: livestock is responsible for 18% of total greenhouse gas emissions*." Claims C21: "*The pea is a natural fertilizer: peas capture the nitrogen from the air and so help to enrich the soil*" and C25: "*The pea crop requires little nitrogen fertilizer: reduces the risk of water and air pollution*" were also negatively assessed.

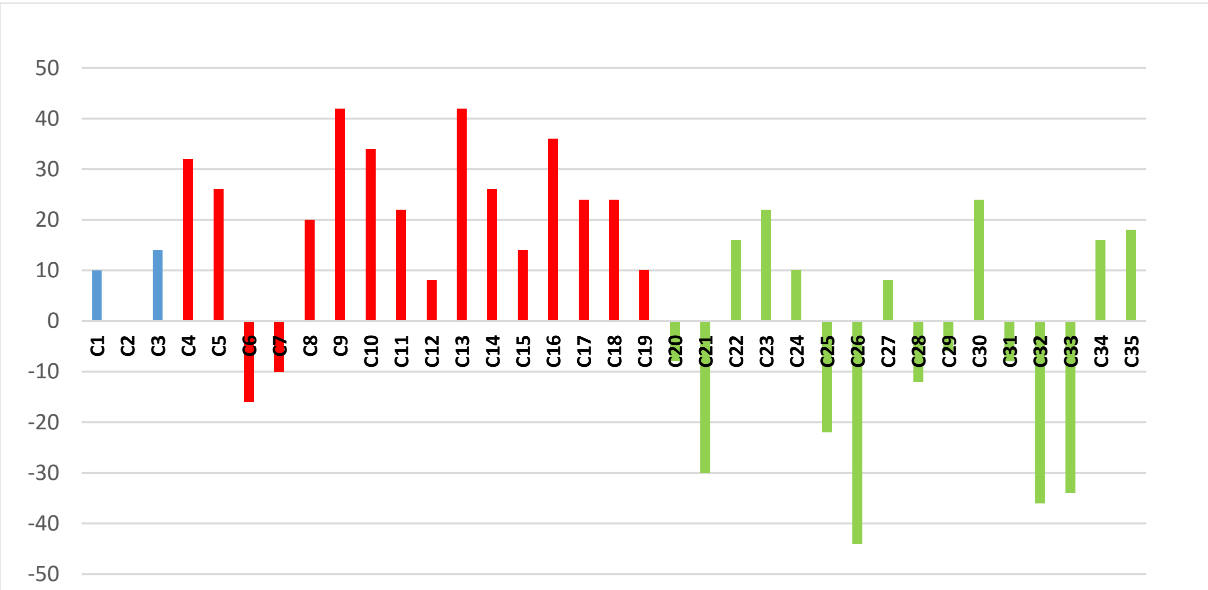


Figure 3. Attraction indexes of the labels: general (blue) health (red) environmental (green)

2.3.3.4 Discussion

Overall, health claims were more positively perceived than environment claims. This tendency has been noted in previous studies. For example, the purchase of organic foods was found to be more strongly related to the perceived benefit for human health than to environmental preservation (**Magnusson et al.**, 2003). These authors stated that egoistic motives are better predictors of the purchase of organic foods than are altruistic motives. The longer use of health claims, compared to environment claims, could also explain consumers' higher concern for such claims. The impact of food on health has long been recognized; this impact was highlighted as far back as Hippocrates ("Let food be medicine"). Health claims have been used for decades, and the development of functional foods in the 1980's increased their number. In comparison, awareness of a relationship between food choice and environmental issues is a relatively recent phenomenon. The link emerged and was studied after the climate change summit of Stockholm in1972 (**Gussow & Clancy**, 1986). In fact, French consumers are not yet very familiar with environment claims as they are not as common as health claims on food labels.

Familiarity may also explain why consumers want to consume products which make claims C9 and C13 ("*Source of vitamins and minerals*" and "*Decreases risk of cardiovascular disease*"), since these two claims are the most widespread in food products in France. Most panelists commented on the fact that they did not understand the word "*Lysine*" which may explain why the attraction index of this claim was the lowest among health claims. In addition to the nature of the information carried by the claim, the architecture of the claim seems to be of major importance in its appropriation by consumers. Indeed, a claim like C13 "*Decreases risk of cardiovascular disease*" was much more appealing to panelists than C19 "*Low in saturated fatty acids: a diet high in saturated fat favors weight gain and increases risk of cardiovascular disease*". The first one is termed a risk reduction claim, while the second is termed a health claim, according to the definitions of Regulation (EC) No 1924/2006. Risk reduction claims were shown to be much more appealing to consumers than health claims (**Dean et al.**, 2012).

Familiarity and level of understanding seem to have been determinant in the attraction indexes of environmental claims as well. It has been previously shown that the quality assessment of products labeled with sustainability claims vary according to the level of consumers' understanding of the claim (**Samant & Seo**, 2016). Thanks to an extremely vague wording, C30 may have been highly accessible to all consumers, even those not well informed about the impact of animal food production on the environment. The environmental claim with the second-best attraction index was C23 ("*GMO free*"). GMOs have been marketed since 1995 in France and their use has generated important controversy in Europe. As a consequence, the "*GMO free*" claim has been used in France for several years (**Saher et al.**, 2006). French consumers are accustomed to reading this claim on the packaging of their everyday products, contrary to other claims tested in this study. Besides not being familiar to consumers, low attraction index claims C26, C32 and C33 refer to livestock and its undesirable effects on water, forests and air. The causal link between pea milk and the preservation of natural resources, water pollution and deforestation may not have seemed obvious for participants, since these arguments are quite new and are not yet used on labels in France. When used, for instance in awareness campaigns about sustainable food choices, these claims mainly refer to the reduction in meat consumption, and not to the reduction in dairy product consumption. The limited appeal of claims related to natural resources and pollution has previously been reported (**Van Loo et al.**, 2014). Moreover, the wording of claims C26, C32 and C33 was negative, highlighting the negative effects of livestock instead of the positive effects of plant based products. This wording may explain the low evaluation of these claims. Finally, in claims C21 "*The pea is a natural fertilizer: peas capture the nitrogen from the air and so help to enrich the soil*" and C25 "*The pea crop requires little nitrogen fertilizer: reduces the risk of water and air pollution*" the word "*fertilizer*" was ambivalent for most of the panelists, which led to the negative assessment of these claims.

2.3.3.5 Conclusion

In light of the above results, four messages were selected for use in the main study: C9: "*Source of vitamins and minerals*", C18: "*Low in cholesterol*", C30: "*Environmentally friendly*", and C35: "*Environmentally friendly yogurt: locally produced".* These messages were selected from the two types of claims (environmental and health) and as having different levels of attraction index.

2.3.4 Main study: Effect of message on the acceptance of plant-based fermented products

2.3.4.1 Objective

The objective of this study was to evaluate whether providing positive messages to consumers is a possible solution to improve the acceptability of plant-protein based fermented products.

2.3.4.2 Materials and Methods

- Panelists:

A total of 58 consumers (25.9% males and 74.1% females; from 18 to 72 years old) participated in the test. All participants consumed yogurt at least once a week.

❖ Samples:

Four samples (S.15, S.18, S.21, and S.23) were selected after the first preliminary study (Table 3) as having an average quality level and variable amounts of pea protein and sucrose. For clarification sake, they were renamed to reflect their content in pea protein and sucrose as follows P40S6, P20S6, P40S9 and P20S9.

Table 3. List of the four products used in the main study

Sample	Code	Pea milk mL/100 mL	Sucrose g/ 100 mL	FOS g/ 100 mL	Cream g/ 100 mL
S.15	P40S6	40	6	3	2
S.18	P20S6	20	6	1,5	1
S.21	P40S9	40	9,5	1,5	1
S.23	P20S9	20	9,5	3	1

❖ Procedure:

The consumer test was conducted over two sessions in individual sensory booths under white light. In the first session, participants assessed their liking for the four products on a hedonic scale ranging from 1 (I do not like the product at all) to 9 (I like the product very much). The samples were served in white plastic cups, coded with random three-digit numbers, without any labels or information. Next, participants completed a questionnaire about their yogurt consumption and purchase habits. In the second session, participants were divided into two groups of 29. Each group assessed two samples carrying environmental messages and two samples carrying health messages, as described in Table 4. The four samples were presented in labeled cups (Figure 4) in a sequential monadic order according to a Williams Latin square design.

Table 4. Selected products with claims presented to each group of participants

	1st group of panelists	2nd group of panelists
Sample	**Message**	**Message**
P40S6 - 40% pea - 6g/100 mL sucrose	Environmentally friendly	Low in cholesterol
P40S9 - 40% pea - 9g/100 mL sucrose	Source of vitamins and minerals	Environmentally friendly yogurt: locally produced
P20S6 - 20% pea - 6g/100 mL sucrose	Environmentally friendly yogurt: locally produced	Source of vitamins and minerals
P20S9 - 20% pea - 9g/100 mL sucrose	Low in cholesterol	Environmentally friendly

Figure 4. Labeled samples

❖ Data analysis:

A repeated measure three-way ANOVA was carried out (Dell Statistica version 12) on the acceptability rating scores with panelists as a random factor, and claim (none vs. health vs. environmental) and product as fixed within-subject factors.

2.3.4.3 Results

The ANOVA showed a significant main effect of product on liking score ($F(3,171) = 33.16$, $p<0.05$). A Student Newman-Keuls test at the 5% significance level (Figure 5) showed that the samples made with 20% pea were preferred to the samples made with 40% pea. More interestingly, the level of sugar had a significant effect in the case of products made with 20% pea protein (P20S9 and P20S6) but not in the case of products made with 40% pea protein. No significant main effect of evaluation condition was found ($F(2,114) = 1.09$, $p<0.3$) but the interaction between product and evaluation condition was significant ($F(6,107) = 2.99$, $p<0.011$). Student Newman-Keuls tests (α=5%) showed that the presence of a health claim decreased the liking score as compared to the blind condition for the sample with 9 g/mL sucrose (P20S9) (Figure 6). Such a decrease was not observed for the sample with 6 g/mL (P20S6), but in the case of this sample, a significant difference was observed between the two claim conditions: liking is significantly higher with a health claim than with an environmental claim.

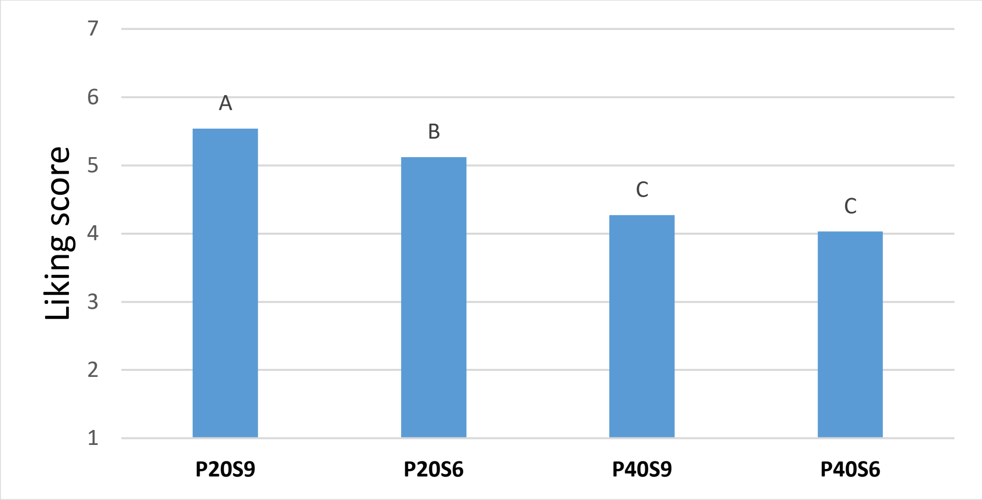


Figure 5. Overall mean liking scores for the four products. Means with different letters are significantly different (Student Newman-Keuls test, $p < 0.05$). 9-point scale (1: I do not like the product at all – 9: I like the product very much)

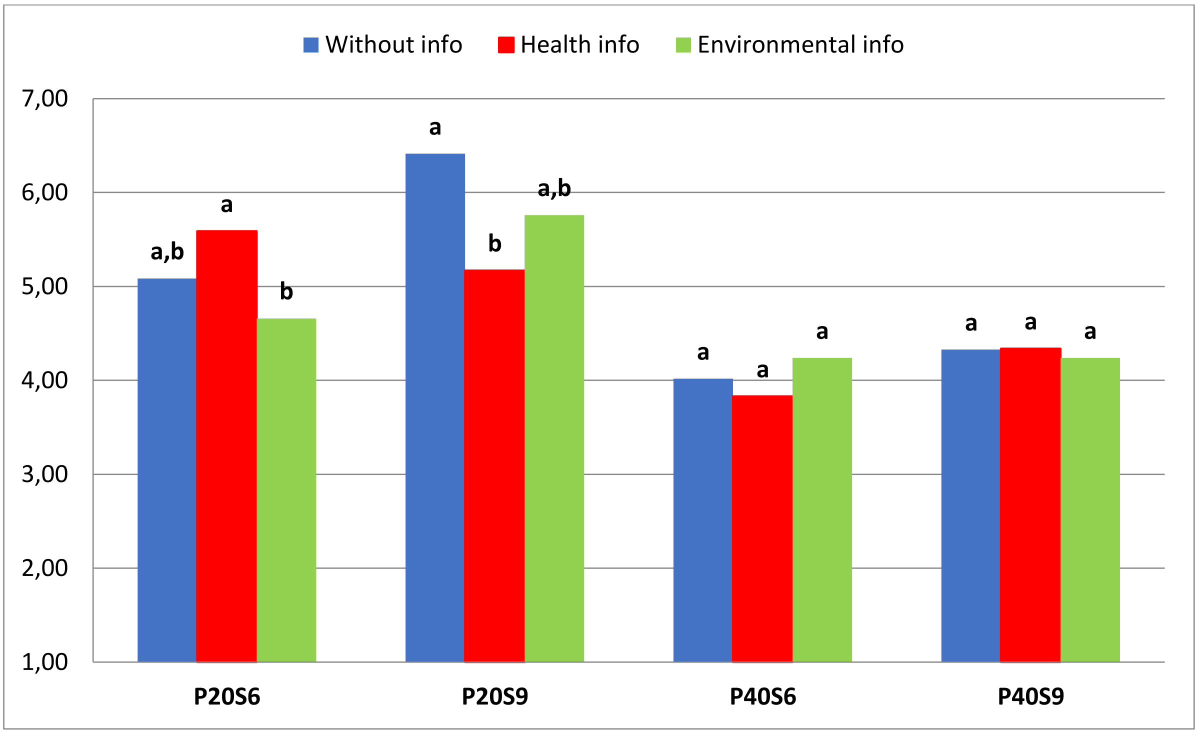


Figure 6. Mean overall acceptability scores on a 7-point scale (1: I do not like the product at all – 7: I like the product very much) for the four tested products, without claims, with health claims and with environmental claims – For each product, means with different superscript letters are significantly different (Student Newman Keuls test, p<0. 05).

2.3.4.4 Discussion

The goal of the present study was to evaluate consumer acceptability of fermented pea-cow milk products made with different pea protein and sucrose concentrations and presented with and without health or environment claims.

Overall, participants did not like products in which 40% cow milk protein was replaced by pea proteins. Adding sugar did not significantly increase liking scores in these cases. A substitution of 20% seemed more acceptable to consumers, especially when 9g of sucrose was added. While the effect of sweetness on yogurt acceptance is well known (**Harper et al.**, 1991; **Johansen et al.**, 2010), it is interesting to note that there are limits to the positive effect of sugar on liking, as sugar had no effect in the cases of the 40% pea protein products.

A significant effect of claim appeared only in the case of 20% substitution. At the level of 40% substitution, the sensory dimension became, in our test, more important than the claim itself, a finding which is in line with the observation that taste has the strongest impact on consumers' food choice and that consumers rarely sacrifice taste for health (**Verbeke**, 2006). Previous work has also reported that claim effects are product dependent. For example, **Kähkönen et al. (1996)** and **Kähkönen et al. (1997)** found a positive effect of information in the cases of low fat ice cream and spread, but not in the case of fat free yogurts. The authors suggested that the absence of effect of a health claim in the case of yogurt was due to the fact that yogurt is already considered as low fat and healthy.

The effect of information may depend on the product category but also on the type of information. Health claims modified consumers' preference but environmental claims did not. Previous work reports that the perceived health benefits of soy foods significantly increase the frequency of their consumption (**Moon et al.**, 2005). The panelists in our study did react to the health claim, but this was dependent on sucrose content. An effect was observed only in the case of higher sugar concentration (9g) and this effect was in the opposite direction than the expected effect: the presence of health claims decreased the liking score of the products. This finding suggests that the expectation created by the claim conflicted with the sensory perception. Two phenomena might be at play here. First, previous studies have shown that positive health information about a product reduces consumer expectations with regards to sensory quality (**Tuorila et al.**, 1994). **Raghunathan et al. (2006)** discuss the unhealthy – tasty intuition: unhealthy food is inherently tastier and inversely, healthy food must be less tasty. Second, sweet foods are not considered to be healthy foods; they are instead consumed for short-term gratification (**Wansink & Chandon**, 2006). In light of these two phenomena, it seems that a conflict occurred, between the expectations derived from the health claims and the actual taste of the sample with 9g of sucrose. Because of this conflict, consumers might have experienced frustration and thus evaluated the product less favorably.

This contrast effect did not occur in the case of environmental claims. Although there has not been much research on the effect of environmental claims on food appreciation, we expected consumers to express the opposite intuition from the one they expressed for healthy foods: good for the planet – good taste, based on the beliefs people have concerning the naturalness of food products. A positive attitude towards naturalness of yogurts was reported by **Hemmerling et al. (2016)** but they found no direct link between this attitude and sensory preferences.

One of the key characteristics of successful claims is perceived immediate personal benefit. Previous work showed for example that environmental awareness encourages consumers to decrease their energy consumption (**Kasulis et al.**, 1981) as consumers perceive an immediate benefit in lowering their consumption. In our study, the link between the taste of pea protein and the benefit for the planet is not directly perceived by the consumers, a fact which may explain the absence of effect of the environment claims. Such claims might have a stronger effect on environmentally aware consumers; the number of participants in our study, however, was not large enough to validate this interpretation.

2.3.4.5 Conclusion

These findings have significant implications for developers of plant protein based products. Products with 20% pea concentration were accepted by our panelists. Is 20% enough? Probably not, but this could be a first step in a progressive replacement in the context of an education program to help consumers become familiarized with the taste of plant protein. In any case, this would require the optimization of certain organoleptic properties. Likewise, the effect of the claim will not be efficient unless: 1) there is improvement in the taste of pea by working at once on the extraction process of plant protein in order to remove off-flavors and on new varieties of peas; 2) there is a match between the taste and the claim; 3) sugar is replaced by green label sweeteners. Indeed, adding sugar is not the proper solution in the current context of the PNNS (Programme National Nutrition Santé) where it is recommended to reduce our salt and sugar consumption. Moreover, consumers are not very sensitive to environmental issues when buying food products and for that reason give little added value to environmental claims. But environmental claims are not all perceived in the same way: some are more accepted than others. The present need is to develop communication strategies to enhance consumers' perception of benefit.

2.4 Conclusion du chapitre 2

Les résultats de ce chapitre ont confirmé que la substitution des protéines animales avec les protéines végétales ne conduira pas directement à un produit accepté. Même avec 10 % de pois, les consommateurs français n'acceptent pas ces produits. De plus, contrairement à l'hypothèse de **Rozin** (1996), la familiarité avec un produit de la même gamme (produit fermenté à base végétale), dans une deuxième culture européenne, la Bulgarie, n'a pas permis d'augmenter l'acceptation d'un produit à base de pois, fermenté avec les ferments classiques du yaourt. L'utilisation d'allégations santé et environnementales n'ont pas davantage permis d'augmenter l'acceptation du produit.

En revanche, la présence de sucre a amélioré l'acceptabilité des produits avec 20 % de protéines de pois, mais cette amélioration reste limitée puisque qu'elle ne s'étend pas aux produits avec 40 % de pois. De plus l'ajout de sucre n'est pas une solution optimale dans une époque où l'on recherche plutôt à diminuer la consommation de cet ingrédient comme en témoigne le slogan « manger mois gras, moins sucré, moins salé ».

Les études consommateurs ayant montré un rejet de la part des consommateurs il est nécessaire d'améliorer la qualité organoleptique de ces produits. Deux tentatives seront faites en vue 1- de diminuer les faux goûts provenant de protéine végétale et 2- d'améliorer la texture. La première consiste à cribler des LABs conférant des caractéristiques spécifiques (texture, arômes) et la seconde à optimiser la fabrication du yaourt à base de pois.

Chapitre 3 : Effet du cocktail bactérien sur les propriétés physico-chimiques et sensorielles

3.1 Introduction

Un bon profil sensoriel est nécessaire pour permettre l'acceptabilité d'un nouvel aliment. Comme mentionné ci-dessus, les souches peuvent affecter les attributs sensoriels du produit fermenté. Certaines souches peuvent acidifier le produit plus ou moins que d'autres. Elles peuvent, donc, produire des produits avec des caractéristiques rhéologiques très différentes. De plus, puisque les souches peuvent agir différemment avec les nutriments disponibles dans le milieu de fermentation (les protéines, les sucres et les faux goûts), les produits fermentés résultants peuvent être plus ou moins proche d'un yaourt traditionnel selon leur capacité de produire les arômes de yaourt et diminuer les faux goûts d'origine végétale. Une étude sur l'effet de dix cocktails bactériens est détaillée dans la **publication 2**.

3.2 **Publication 2**: Fermentation of cow milk and/or pea milk mixtures by different starter cultures: physico-chemical and sensorial properties

Abstract:

Five mixtures of milk and pea protein (0 to 40 g pea protein/100 g total protein) were fermented by ten starter cultures of lactic acid bacteria (LAB)[1] to select the cocktails that can lead to products similar to a conventional yoghurt. Generally speaking, an increase in pea concentration leads to products with higher acidity, higher syneresis and lower firmness. From the sensory perspective, up to 30 g pea protein/100 g total protein, starter cultures show either positive or negative effects. A principal component analysis (PCA)[2] and a hierarchical cluster analysis (HCA)[3] carried out on all variables revealed five groups of products. Two groups of products of 0 g or 10 g pea protein/100 g total protein seemed to be the most similar to conventional dairy products. The third group included products fermented with two starters with negative characteristics such as astringency and bitterness. The last two groups included members made of 10 g, 20 g, 30 g and 40 g pea protein/100 g total protein, among them one group showing a positive sensory profile. From this group, four starter cultures seem promising for the fermentation of milk and pea protein mixtures.

Keywords: Pea protein. Cow milk protein. Lactic acid bacteria. Rheology. Sensory properties

[1] Lactic Acid Bacteria
[2] Principal Component Analysis
[3] Hierarchical Cluster Analysis

3.2.1 Introduction

The use of plant proteins, especially pulse proteins, to extend or substitute for animal-derived products is one way to guarantee food security, sustainable production in environmental safety and health benefits (**Boland et al.**, 2013). Successful uses of plant proteins as food ingredients depend on their functional characteristics and their sensory impacts in the final products. The positive functional properties (gelation, emulsification, foaming …) of this protein source make it a promising food ingredient (**Boye et al.**, 2010; **Ma et al.**, 2011; **Swanson**, 1990). One of the major pulses is pea (*Pisum sativum* L.). Peas are usually consumed as seed, flour, protein isolate (**Aiking et al.**, 2006). Pea protein is easily digestible and has a high-quality amino acid profile as well as high lysine and arginine content (**Schneider & Lacampagne**, 2000), but their use is limited by undesirable sensory characteristics. The main perceived compounds in pea odor profile are described as 'green', 'grassy', 'hay-like', 'pea pod' and are generated by degradation of fatty acids during extraction processes or by microbial activity on seed during storage (**Hansen et al.**, 2000; **Murat et al.**, 2013). Lactic acid fermentation with selected starter cultures could improve the organoleptic characteristics of foodstuff containing pea proteins. The LAB species like *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus, Lactobacillus helveticus…* have shown positive effects on the organoleptic properties of fermented products in terms of flavor and texture (**Marilley et al.**, 2004; **Moslehishad et al.**, 2013; **El Soda et al.**, 1986). Furthermore, exopolysaccharide production has been reported in most of these LAB species, which increase the viscosity and firmness, and improve the texture of yoghurts (**Leroy & De Vuyst**, 2004; **Ruas-Madiedo et al.**, 2002). Mixed cultures have been shown to reduce more lactose in fermented soymilks than a single strain, although sometimes single cultures reduce pH more than mixed cultures (**Wang et al.**, 2002). Acidification by *Streptococcus thermophilus* of soy beverage was faster than that of milk, but the combination of *Streptococcus thermophilus* and different LAB strains did not lead to faster acidification in soy beverage, contrary to what was observed in milk, where symbiosis seems to occur (**Champagne et al.**, 2009). Studies performed on fermented milk supplemented with a small quantity of plant protein (up to 5%) showed positive effects on textural and rheological properties of gels, both with soy protein (**Drake et al.**, 2000) and lentil flour (**Zare et al.**, 2011). On the other hand, negative sensory effects have been noted in products supplemented with plant proteins. The substitution of 50% or more cow milk by soya milk in fermented milk products led to a decrease in liking that might be due, in part, to the fact that consumers were expecting the fermented milk product to taste like a regular yoghurt (**Tu et al.**, 2010). This last observation reveals the difficulty of assessing a new product (mixed soy and milk) when it is similar to a well-identified reference, in this case, conventional cow's milk yoghurt.

The aim of this study is to screen 10 starter cultures of seven LAB species for the fermentation of five ratios of cow protein/pea protein from 0 to 40 g pea protein/ 100 g total protein, in order to determine the combination of starter culture and pea concentration which gives the end product the closest to a traditional yoghurt in terms of physico-chemical and sensory attributes. The pH reduction during fermentation, titratable acidity, syneresis and texture properties of the final product after one, seven and 14 days of storage at 4 °C were measured. Finally, a standard descriptive analysis approach was used to compare the sensory profiles of these products with that of a cow's milk yoghurt fermented with commercial milk ferments.

3.2.2 Materials and methods

3.2.2.1 Ingredients and Cultures

The pea protein isolate Nutralys® S85F was supplied by Roquette (Lestrem, France) and skim milk powder was purchased from Régilait (Saint-Martin-Belle-Roche, France). Lactose monohydrate, sodium citrate and calcium phosphate monobasic were purchased from Sigma (St. Louis, USA). A homemade yoghurt set containing lyophilized *Streptococcus thermophilus* and *Lactobacillus bulgaricus*) were used as a reference (Alsa, Rueil-Malmaison, France). *Streptococcus thermophilus* 102303T, *Lactobacillus delbrueckii* subsp. *bulgaricus* 104365, *Lactobacillus acidophilus* 76.13, *Lactobacillus helveticus* CNRZ 303 and *Lactobacillus casei* subsp. *casei* ATC 334 were obtained from our laboratory collection. *Lactobacillus rhamnosus* CRBIP 24.130 and *Lactobacillus fermentum* CRBIP 24.11 were obtained from the Pasteur Institute laboratories (Paris, France). Each activated culture was inoculated into MRS (Biokar Diagnostics, Beauvais, France), except for *Streptococcus thermophilus* inoculated in M17 and incubated at 37 °C for 12 hours. These cultures were then diluted to 10^7 CFU/mL with 9 g/L sterilized NaCl solution and used as the inoculum of each LAB.

3.2.2.2 Preparation of milks and fermented products

The total amount of proteins was fixed at 4.5 g/100 g of fermented product. Therefore, the concentration in pea proteins were 0, 0.45, 0.9, 1.35 and 1.8 g/100 g of fermented product.

The mixtures of cow and pea milk, prepared as illustrated in Figure 1, were inoculated with different starter cultures (Table 1), sampled in plastic flasks (40 mL in 45 mL flasks) and incubated at 37 °C for 24 hours and stored at 4 °C.

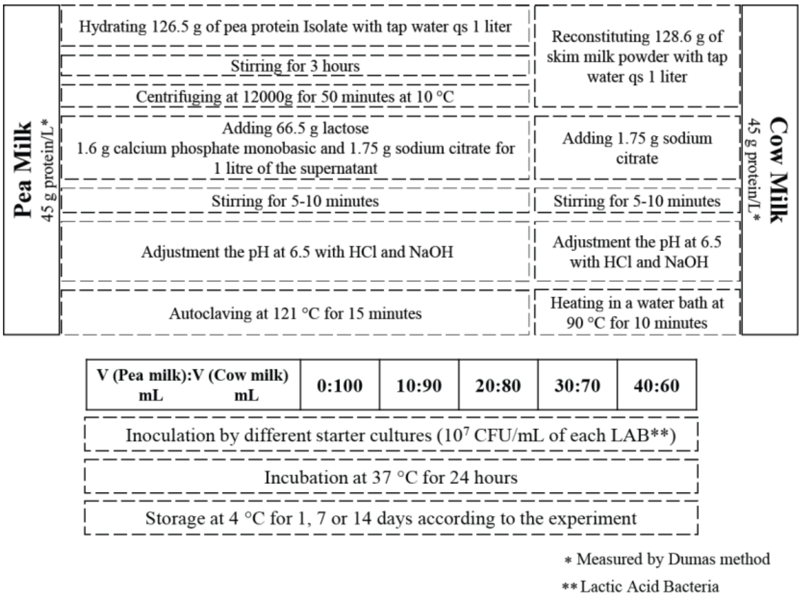


Figure 1. Preparation of pea milk, cow milk and fermented products

Table 1. List of products: Capital letters label the bacteria cocktail used; numbers indicate pea protein concentration (g /100 g total protein)

	Products					**Starter Culture**
A	A00	A10	A20	A30	A40	Alsa (*Streptococcus thermophilus* + *Lactobacillus delbrueckii* subsp. *bulgaricus)*
B	B00	B10	B20	B30	B40	*Streptococcus thermophilus* + *Lactobacillus delbrueckii* subsp. *bulgaricus*
C	C00	C10	C20	C30	C40	*Streptococcus thermophilus* + *Lactobacillus helveticus*
D	D00	D10	D20	D30	D40	*Streptococcus thermophilus* + *Lactobacillus rhamnosus*
E	E00	E10	E20	E30	E40	*Lactobacillus delbrueckii* subsp. *Bulgaricus* + *Lactobacillus helveticus*
F	F00	F10	F20	F30	F40	*Streptococcus thermophilus* + *Lactobacillus acidophilus*
G	G00	G10	G20	G30	G40	*Lactobacillus delbrueckii* subsp. *Bulgaricus* + *Lactobacillus fermentum*
H	H00	H10	H20	H30	H40	*Streptococcus thermophilus* + *Lactobacillus casei* subsp. *casei*
I	I00	I10	I20	I30	I40	*Lactobacillus delbrueckii* subsp. *Bulgaricus* + *Lactobacillus rhamnosus*
J	J00	J10	J20	J30	J40	*Lactobacillus rhamnosus*

3.2.2.3 Product Characterization

- pH and titratable acidity measurements

Changes in pH values during fermentation were determined every two hours at room temperature using a calibrated pH electrode and a pH-meter (Vision 6071, JENCO Electronics LTD, Shanghai, China). Three kinetic parameters were calculated from the curve pH=f(time), using equation 1:

$$V.\max = \left({}^{dpH}/_{dt}\right)$$ Equation 1

where $V.max$[4] is the maximum acidification rate (mUnit pH/min). T.end[5] was the time necessary to reach pH 4.7 (hours); the value pH 4.7 was chosen because it is the pH at which gels begin to form. The value pH.12h[6] was the pH obtained after 12 hours of fermentation (Unit pH); 12 hours was selected because there were significant differences in pH values among the different mixtures (cow milk and pea milk) in most of the studied cultures. Titratable acidity was determined according to the Dornic degree (D°) method (**Robinson & Wilbey**, 1998). Three replicate experiments were carried out for each measurement.

- Syneresis measurement

The degree of whey separation was determined according to a siphon method (**Amatayakul et al.**, 2006). Syneresis after one, seven and 14 days of storage at 4 °C was measured in triplicate and expressed as a percentage of total weight of the product.

- Rheological analysis

Rheological characteristics were evaluated by Texture Analyzer TA.HD *plus* (Stable Micro Systems, Godalming, England) with Exponent software version 6. A single penetration test was performed using a cylindrical probe of 10 mm diameter, a 5Kg-load cell with a return distance of 85 mm, a test speed of 1.00 mm/sec and 9 mm as the distance of penetration. From the curve, four values were collected representing “Firmness”: maximum force value, “Consistency”: positive area under the curve, “Index of Viscosity/Consistency”: minimum force value and “Cohesiveness”: negative area under the curve. This test was performed in triplicate after one, seven and 14 days of storage at 4 °C.

- Sensory evaluation

Ten training sessions were conducted with 12 panelists selected for their ability to detect tastes and odors in pea "yogurt" as well as their verbal fluency. Two sessions were carried out to generate a preliminary list of attributes based on five samples selected from our products. This list was then reduced to obtain a final list based on the ISO 11035:1994 norm (**ISO**, 1994). The next four sessions were dedicated to training. Panelists agreed upon

[4] Maximum acidification rate
[5] The time necessary to reach pH 4.7
[6] The pH after 12 hours of fermentation

definitions, references and procedures for each attribute and were trained to rank different water and yoghurt solutions containing substances that conferred the required attributes. In the last three sessions, ten new products were presented in duplicate to determine whether the panel was homogeneous, discriminating and repeatable. Panelists had to rate the intensity of all attributes for each product on a structured interval scale ranging from 1 (low) to 10 (high). After training, 10 trained panelists (two participants abandoned the panel due to availability issues) evaluated the 50 products in duplicate.

- Statistical Analysis

In order to estimate the significance of differences between means, factorial ANOVAs[7] were conducted, followed by a LSD[8] test at the 5% level with Statistica version 10 (StatSoft, Tulsa, USA). Additionally, a PCA and a HCA were conducted with SPAD version 8.0 (Coheris, Suresnes, France).

3.2.3 Results and discussion

3.2.3.1 Fermentative behavior

Determining the acidifying capacity of starter culture is the classical way to estimate its activity. In this study, three kinetic parameters were considered: V.max, T.end and pH.12h. The results concerning the effects of pea concentration and starter culture on the kinetic parameters and Dornic degree are summarized in Table 2.

The maximum acidification rate ranged from 1.58 to 15.33 mUnit pH/min depending on pea ratio and bacteria cocktail. A minimum of 6.55 hours was needed to obtain pH 4.7, and this was not obtained in six samples, even after 24 hours of fermentation. In this case, when fermentation is slow or the time to reach the desired pH is over 24 hours, it could be considered problematic for industrial applications as contamination may occur. According to **Beal et al. (1989)**, a medium rate of acidification is preferable because it results in non-excessive acid production, a more homogeneous structure of the coagulum and a greater viscosity of the final product.

[7] Analysis Of Variance
[8] Least Significant Difference

Table 2. Effect of different starter cultures and pea concentrations on the kinetic parameters of acidification (V.max, T.end, pH.12h) and Dornic degree of final products. V.max was the maximum acidification rate (mUnit pH/min), T.end was the time required to reach pH=4.7 (hours) and pH.12h was the pH obtained after 12 hours of fermentation.

The sample	V.max mUnit pH/min	T.end hour	pH.12h	Dornic degree	The sample	V.max mUnit pH/min	T.end hour	pH.12h	Dornic degree	The sample	V.max mUnit pH/min	T.end hour	pH.12h	Dornic degree	The sample	V.max mUnit pH/min	T.end hour	pH.12h	Dornic degree	The sample	V.max mUnit pH/min	T.end hour	pH.12h	Dornic degree
A00	7,5±0,48	7,1±0,06	4,4±0,02	93,4±2,52	C00	3,1±0,39	*	5,7±0,03	140,8±3,47	E00	3,7±0,08	15,2±0,03	4,8±0,03	107,5±4,20	G00	6,3±0,10	8,7±0,06	3,9±0,03	89,3±3,96	I00	1,6±0,06	*	5,8±0,03	100,1±4,94
A10	7,6±0,40	7,0±0,06	4,3±0,01	93,8±3,27	C10	2,3±0,15	20,4±0,04	5,6±0,01	141,1±4,08	E10	4,0±0,17	12,3±0,09	4,7±0,03	133,4±2,76	G10	5,8±0,14	8,8±0,10	4,1±0,02	106,2±2,01	I10	2,3±0,08	*	5,5±0,04	118,1±6,91
A20	7,8±0,61	6,6±0,04	4,1±0,01	102,1±4,01	C20	7,6±0,43	10,8±0,06	4,1±0,01	147,6±2,23	E20	6,9±0,18	6,6±0,06	4,2±0,03	171,4±3,09	G20	6,4±0,04	8,6±0,07	3,9±0,01	113,8±3,47	I20	3,2±0,13	*	5,2±0,04	124,6±4,48
A30	7,1±0,34	8,0±0,07	4,4±0,02	95,2±1,80	C30	6,7±0,19	11,4±0,04	4,4±0,02	156,8±4,19	E30	4,9±0,15	12,5±0,06	4,7±0,01	162,2±3,46	G30	6,2±0,12	8,5±0,04	3,8±0,02	114,1±4,30	I30	3,0±0,11	22,6±0,08	5,2±0,06	127,6±4,48
A40	7,5±0,31	9,0±0,09	4,4±0,01	101,7±3,48	C40	8,9±0,27	11,3±0,04	4,3±0,01	146,5±3,76	E40	5,8±0,21	8,2±0,04	4,3±0,02	159,5±5,53	G40	6,0±0,16	8,7±0,05	3,9±0,03	110,7±4,39	I40	3,9±0,10	22,7±0,09	5,0±0,04	128,9±6,17
B00	4,7±0,43	16,5±0,05	5,0±0,01	90,7±1,88	D00	15,3±0,25	11,2±0,03	3,9±0,02	81,5±1,82	F00	2,7±0,11	*	5,6±0,01	68,6±2,19	H00	2,8±0,12	17,7±0,06	5,1±0,04	91,3±2,88	J00	3,8±0,05	22,7±0,09	5,6±0,01	91,4±3,81
B10	5,6±0,32	11,1±0,07	4,4±0,02	97,2±3,20	D10	15,0±0,24	11,0±0,07	3,8±0,01	94,3±2,76	F10	2,7±0,16	*	5,4±0,02	62,5±3,51	H10	3,3±0,13	14,5±0,06	4,9±0,03	92,3±3,47	J10	4,7±0,05	21,3±0,04	5,4±0,03	98,3±4,78
B20	8,5±0,23	10,4±0,06	3,9±0,01	105,3±3,16	D20	10,7±0,21	10,5±0,05	3,7±0,02	96,5±3,63	F20	4,0±0,15	21,2±0,07	5,2±0,03	69,3±2,62	H20	2,8±0,05	16,5±0,05	5,0±0,05	95,6±2,87	J20	4,3±0,08	18,1±0,12	5,4±0,02	107,8±4,41
B30	7,3±0,34	10,7±0,07	4,1±0,01	100,6±4,55	D30	10,2±0,21	10,4±0,07	3,7±0,02	98,3±2,46	F30	5,2±0,11	19,7±0,07	5,1±0,01	74,9±5,73	H30	3,3±0,11	12,7±0,05	4,8±0,03	101,2±2,88	J30	4,8±0,05	17,4±0,05	5,1±0,05	113,0±4,41
B40	6,4±0,29	10,2±0,04	4,0±0,02	106,0±1,17	D40	8,7±0,23	10,2±0,08	3,7±0,02	94,9±4,98	F40	6,2±0,14	19,7±0,09	4,9±0,02	69,8±2,65	H40	4,0±0,10	12,4±0,06	4,7±0,03	101,7±3,35	J40	4,6±0,07	15,3±0,09	5,0±0,03	106,7±3,42

* Indicates samples in which pH 4.7 was not achieved even after 24 hours

- Means followed by the confidence intervals α=0.05

ANOVAs showed that pea concentration, starter culture and the interaction between the two factors have significant effects on the three parameters of kinetics and the Dornic degree (P< 0.05). Globally, an increase in pea milk ratio caused a decrease in the time required to obtain a gel and an increase in the products' acidity. However, the effect of pea ratio was not linear for all bacteria cocktails. The LSD test showed that the most acidifying group includes two starter cultures. Starter cultures C and E exhibit the highest Dornic degrees: these are the two starters containing *"Lactobacillus helveticus*" that is known to have the most efficient proteolytic system among the LAB species (**Griffiths & Tellez**, 2013). It is also one of the most used LAB species for lactic acid production because it is acidotolerant with optimum survival pH 5.5, and is relatively insensitive to product inhibition by lactic acid (**Roy et al.**, 1987). On the other extreme, starter culture F (*Streptococcus thermophilus* + *Lactobacillus acidophilus*) produced the lowest acidity.

3.2.3.2 Syneresis

Syneresis is considered as a defect that may be reduced by increasing the level of milk solids, especially protein **(Tamime & Deeth, 1980)** or by using starter cultures producing exopolysaccharides that help decrease the level of liquid separation (**Hassan et al.**, 1996). The amounts of liquid separation resulting from the different mixtures and starter cultures after one, seven and 14 days of storage at 4 °C are presented in Figure 2. Syneresis values ranged from 0.22 to 4.65% after 1-day storage, from 0.32 to 7.82% after 7-day storage and from 0.83 to 8.5% after 14-day storage. Factorial ANOVA showed that pea concentration, starter culture and the interaction between the two factors had significant effects on syneresis (P< 0.05). Additionally, the increase in pea protein seemed to favor syneresis in most samples. For the highest pea milk ratio, only two starters (A and E) gave gels with syneresis levels of less than 5% after 14-day storage. No correlation was found between the parameters of acidification and syneresis. Thus, fast acidification did not produce a gel characterized by more syneresis.

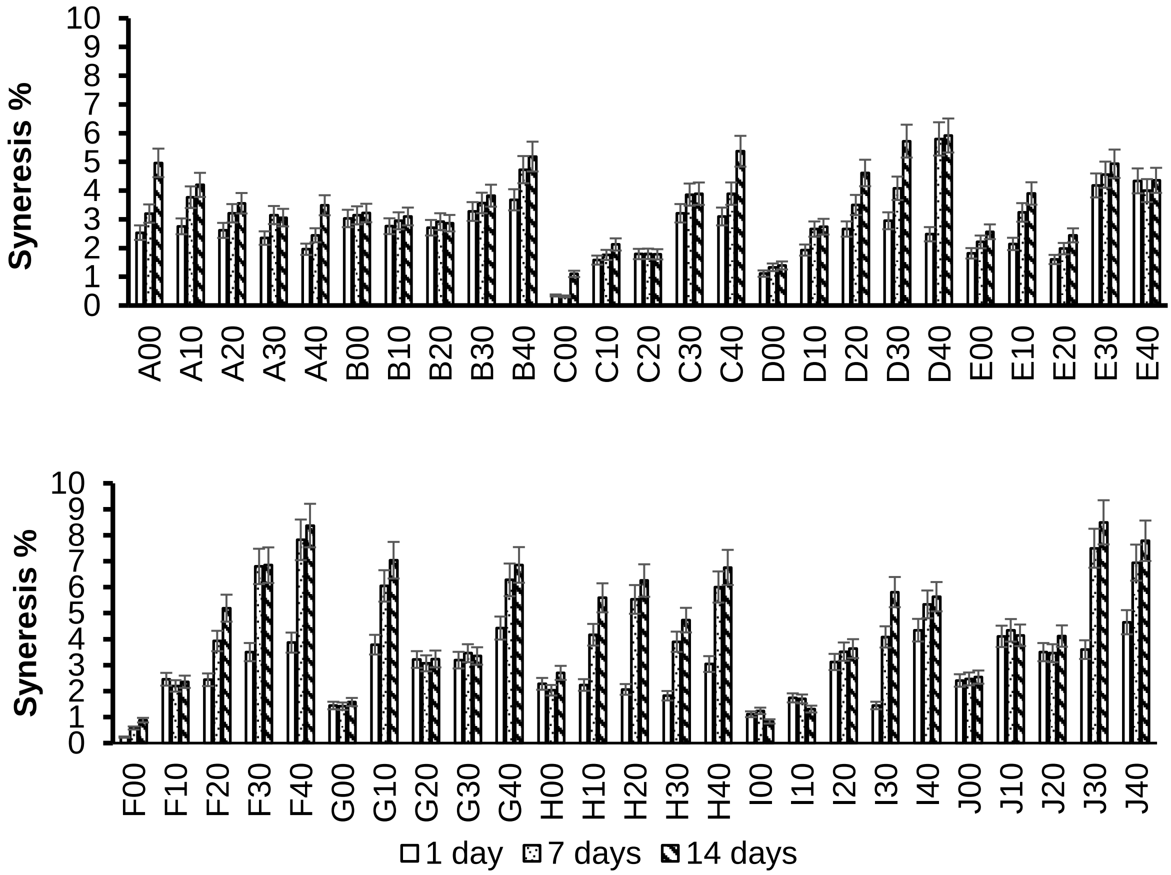


Figure 2. Syneresis of 50 samples stored at 4 °C for 1, 7 or 14 days. Capital letters refer to the starter culture and numbers to the pea protein concentration (g/100 g total protein)

Although the phenomena occurring during syneresis are not fully understood, it is agreed that increased syneresis with storage time is usually associated with major casein network rearrangements (**Van Vliet et al.**, 1997), that promote whey expulsion. In our study, the same phenomena may be at work with the possible protein network composed of pea and cow milk proteins.

3.2.3.3 Rheological properties

The products were submitted to a penetration test after three different storage times at 4 °C. As expected, firmness was correlated with consistency (R^2 = 0.935) and anti-correlated with the viscosity/consistency index (R^2 = 0.794). Factorial ANOVA showed that the firmness of products after 14 days of storage was significantly higher than that after one and seven days of storage ($P < 0.05$). Additionally, ANOVA showed that starter cultures, pea concentration and the interaction between them had significant effects on rheological parameters ($P < 0.05$). For purposes of clarity, only results concerning firmness after 14 days of storage at 4 °C are presented in Figure 3. For all starter cultures, the presence of pea milk induced a decrease in firmness. The highest decrease in firmness was observed with the H starter culture in yogurts without pea milk and in those with 40 g pea protein/100 g total protein at 14 days of storage.

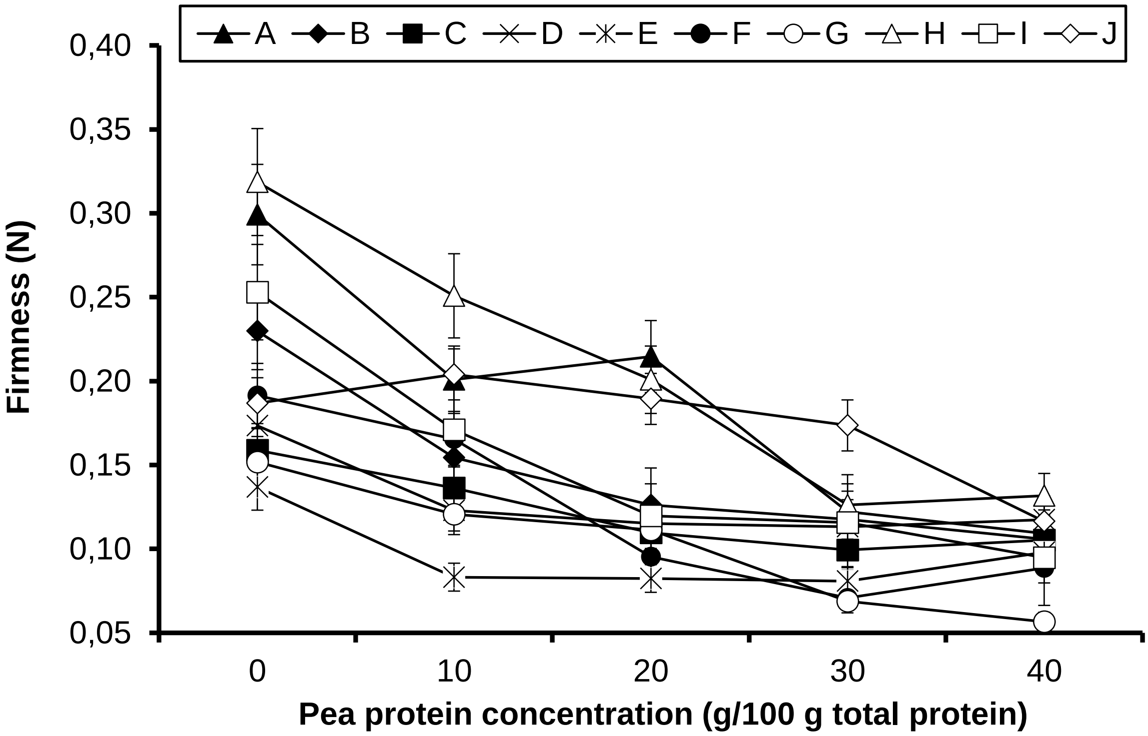


Figure 3. The firmness of products after 14 days of storage at 4 °C, according to the starter culture and the pea protein concentration (g/ 100 g total protein)

Figure 4 shows the values of product firmness in the two extreme conditions: 0 and 40 g pea protein/100 g total protein, 1 and 14 days of storage according to starter culture. In cow milk, product firmness increased with storage time. The gain in firmness during storage from 1 to 14 days was important in starter cultures H, I, J, A and B. In the presence of 40 g pea protein/100 g total protein, product firmness was dramatically reduced. However, the gain in firmness during storage still occurred.

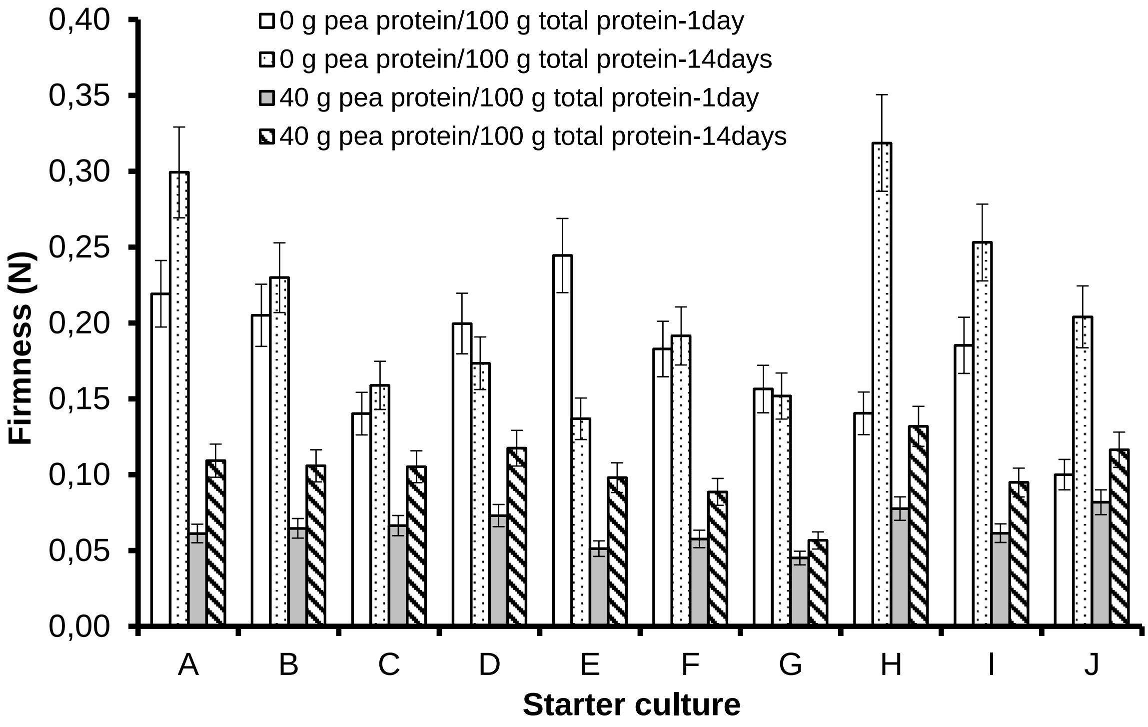


Figure 4. Values for the firmness of products made with 0 and 40 g pea protein/100 g total protein at 1 and 14 days of storage at 4 °C

This increase in firmness can be explained by the post acidification residual activity of lactic bacteria in the products. The lactic acid production and the ability of strains to produce exopolysaccharides leads to the stabilization of casein micelles and a reinforcement of the strength of the protein network (**Guzel-Seydim et al.**, 2005; **Saint-Eve et al.**, 2008). The presence of pea protein did not favor the firmness of the products. Pea proteins seemed to hinder the implementation of the casein network, given the assumption that the milk protein network (casein and whey) may be formed first. Thus, the coagulum of pea protein may settle into the free spaces in the casein network and weaken it. Indeed, the possible molecular interactions between pea globulins and casein are not clearly established in the literature. Especially in dairy products, the structure of the gel composed of plant proteins, casein and whey protein is not fully understood. Certain authors have reported that the firmness or hardness of different fermented products increased with weak supplements of plant ingredients: up to 3% lentil flour in yoghurt (**Zare et al.**, 2011), up to 8% sesame protein isolate in soft cheese (**Lu et al.**, 2010), up to 3% soy protein in rennet curd formation “Mozzarella” (**Hsieh et al.**, 1993). On the contrary, greater pulse supplementation leads to a more fragile coagulum and so to a lesser degree of firmness or “hardness”: 20% soy protein in rennet curd formation (**Lee & Marshall**, 1979), 12% sesame protein isolate in soft cheese (**Lu et al.**, 2010). The last authors reported that sesame proteins in low concentration aggregated as clusters of small poly-particles covering the surface of cheese fractures, but when sesame concentration increased, the sesame protein aggregated as a different protein phase in the casein gel matrix.

3.2.3.4 Sensory profile

The sensory profiles of the 49 formulated products were compared with the profile of cow milk fermented with a commercial lactic bacteria starter (Alsa). The lexicon included three odor (vinegar, earth, vegetal), three aroma (smoked, dairy, pea), three taste (in mouth: bitter, acid, and sweet), and two mouthfeel (creamy, astringency) attributes. Vinegar, earth, vegetal, astringency, bitter, acid, smoked and pea were regarded as negative descriptors for the overall smell and taste in yoghurt, whereas creamy, sweet and dairy were regarded as positive descriptors. A three-way repeated ANOVA measurement with panelists as a random factor and pea concentrations and starters as fixed factors showed a significant effect of bacteria starters for all descriptors except smoked (Table 3). Globally, bacteria starters A, G, D and J tended to have higher intensities for positive descriptors such as creamy, dairy and sweet, and lower intensities for negative descriptors such as vegetal, earth and vinegar. On the other hand, bacteria starters E, I and C were associated with higher intensities for negative descriptors such as acid and astringent but rather low intensities for the negative descriptors pea and earth. Bacteria starters such as A, D and J, which are associated with the highest intensities in negative descriptors such as pea or vegetal, also have high positive effects, leading to descriptors such as creamy. A significant effect of pea concentration was also found for seven descriptors (vinegar, earth, creamy, acid, smoked, dairy and pea). Among these descriptors, as expected, the intensity of the negative descriptors pea, earth, vinegar, and smoked increased with pea concentration, whereas the intensity of the positive descriptors creamy and dairy decreased with pea concentration. In addition to these main effects, significant interactions between starter culture and pea concentration were found for all descriptors except dairy and vegetal. An effect of pea concentration was observed for five negative descriptors (earth, smoked, pea, acid and fluid) as well as for one positive descriptor (creamy) for most starter cultures. As for the other descriptors (vinegar, bitter, sweet and astringent), we observed a pea concentration effect in only a small number of starter cultures.

Table 3. The effect of starter culture, pea concentration and the interaction between starter culture and pea concentration on sensory profile by using three-way ANOVA

	Starter culture		**Pea concentration**		**Starter & pea interaction**	
	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
Odor **vinegar**	5.54	<.0001	2.92	0.0343	1.73	0.0076
Odor **earth**	2.57	0.0121	8.66	<.0001	1.73	0.0074
Odor **vegetable**	5.32	<.0001	2.41	0.0675	1.42	0.0615
texture **creamy**	7.96	<.0001	10.12	<.0001	5.49	<.0001
sensation **astringent**	4.73	<.0001	1.05	0.3950	1.50	0.0387
Taste **sweet**	7.48	<.0001	1.16	0.3443	2.77	<.0001
Taste **bitter**	3.21	0.0023	2.66	0.0485	1.50	0.0386
Taste **acid**	29.14	<.0001	4.42	0.0052	4.39	<.0001
Aroma **smoked**	1.20	0.3041	24.31	<.0001	1.56	0.0249
Aroma **dairy**	2.88	0.0055	17.28	<.0001	1.17	0.2377
Aroma **pea**	6.03	<.0001	36.17	<.0001	1.68	0.0113

3.2.3.5 PCA and HCA for all measurements

Figure 5 a, b, c and d represent the first three PCA dimensions (70.27 % of total variance). The first dimension (37.48 % of variance) opposes the negative aroma attributes pea, earth and smoked as well as the negative texture attributes fluid, viscosity and cohesiveness to the positive aroma attribute dairy and positive texture properties firmness and consistency. It represents a gradient in pea concentration ranging from 0 g to 40 g pea protein/100 g total protein. Negative attributes are mostly associated with the highest pea concentration products and positive attributes to the products without pea protein. The second dimension (18.99 % of variance) opposes the sweetness attribute to the negative attributes astringent, acid and bitter. It opposes starter cultures A and D yielding positive attributes to starter cultures E yielding negative attributes, independently of pea concentration. The third dimension (13.80 % of variance) opposes the kinetic parameters of acidification: V.max, T.end and pH.12. It opposes starter cultures A and D yielding good acidification profiles to starter cultures F and G yielding negative profiles, independently on pea concentration.

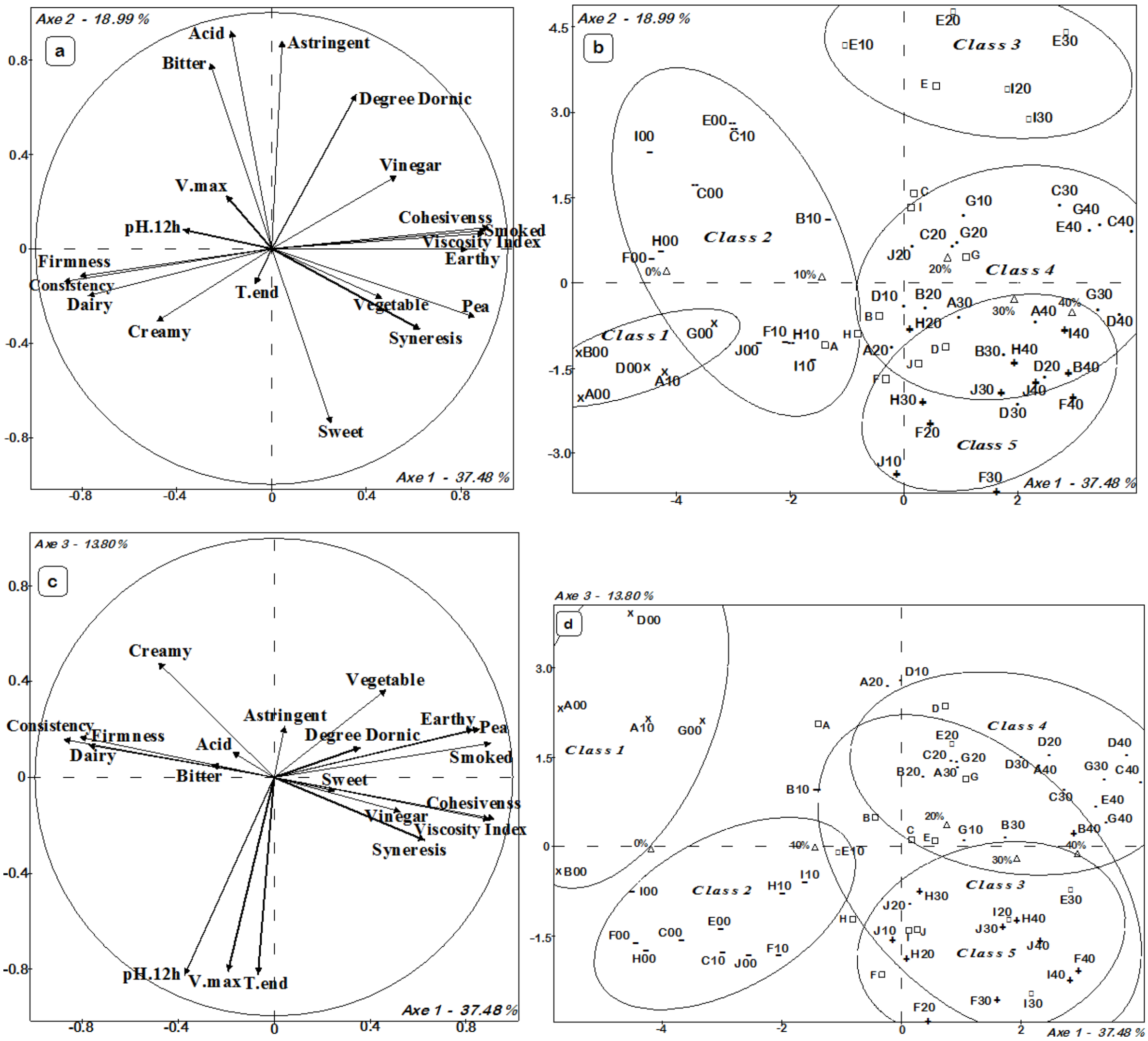


Figure 5. First three dimensions of the principal component analysis performed on the attributes by product matrix a) correlation circle for axes 1 and 2, b) projections of the products, c) correlation circle for axes1 and 3, d) projections of the products. The two variables are pea concentration (Δ) and starter culture (□), where the members of the first group are represented by (×), the second by (–), the third by (⊠), the fourth by (•) and the fifth by (+)

The HCA performed on the first three PCA dimensions showed that the 50 products (49 + control) could be divided into five classes (Table 4 & Figure 5). The first class includes five products, four of them without pea protein. These products are characterized by good texture and were described with positive attributes similar to the attributes of traditional yoghurts usually consumed by the panel (**Tu et al.**, 2010). Moreover, the fifth product in this group, fermented by traditional bacteria starter A, has the same texture as traditional yoghurt and is neither bitter nor astringent although it is composed of 10 g pea protein/100 g total protein. The second class includes 11 products, six of them without pea protein and five with only 10 g pea protein/100 g total protein. This group was described as having dairy and creamy characteristics as well as good firmness. Also, the negative properties of this group, such as long fermentation time and bitter taste, could be related to starter culture. The third group is composed of five products associated with the negative characteristics vinegar, astringent, acid and bitter and with the absence of dairy taste. This third group includes products fermented with just two starter cultures, E (*Lactobacillus delbrueckii* subsp. *Bulgaricus* + *Lactobacillus helveticus*) and I (*Lactobacillus delbrueckii* subsp. *Bulgaricus* + *Lactobacillus rhamnosus*) with the only *Bacillus* without *Coccus.* The fourth class includes 18 products with 10, 20, 30 or 40 g pea protein/100 g total protein, with only negative sensory characteristics such as pea, earthy and smoked taste and negative rheological characteristics such as low values of firmness and consistency. The promising fifth class includes products without negative sensory characteristics. Although this class contains 8 from 11 products with 30% or 40% pea and without products 0% pea protein, the average notes (10-point scale from low to high) of acid, astringent and bitter attributes in this class were 2.8, 3.3 and 2.4 instead of 4, 4 and 3 in overall average.

Table 4. The characteristics of the five classes of hierarchical cluster analysis performed on the first three dimensions of the principal component analysis

Class 1		**Class 2**		**Class 3**		**Class 4**		**Class 5**	
A00- A10- B00- D00- G00		B10- C00- C10- E00- F00- F10- H00- H10- I00- I10- J00		E10- E20- E30- I20- I30		A20- A30- A40- B20- B30- C20- C30- C40- D10- D20- D30- D40- E40- G10- G20- G30- G40- J20		B40- F20- F30 –F40- H20- H30- H40- I40- J10- J30- J40	
Over-represented variable	Under-represented variable	Over-represented variable	Under-represented variable	Over-represented variable	Under-represented variable	Over-represented variable	Under-represented variable	Over-represented Variable	Under-represented variable
Dairy	Earthy	pH.12h	Vegetable	Acid	Creamy	Pea	Consistency	Syneresis	Consistency
Firmness	V.max	V.max	Viscosity In.	Astringent	Dairy	Smoked	Firmness	T.end	Acid
Consistency	Vinegar	Consistency	Cohesiveness	Dornic	Sweet	Earthy	T.end	Sweet	Astringent
Creamy	Pea	Firmness	Syneresis	Bitter		Vegetable	V.max	Viscosity In.	Bitter
	Smoked	Bitter	Earthy	Vinegar		Viscosity In.	pH.12h	Cohesiveness	
	Cohesiveness	dairy	Smoked			Cohesiveness			
	Viscosity In.	T.end	Pea			Vinegar			

3.2.4 Conclusion

The partial substitution of milk protein with pea protein did not enhance the physico-chemical characteristics of the dairy gels studied. In order to improve the firmness of these fermented products, a study on the procedure of preparation that may favor the interactions between pea and milk proteins must be performed. Moreover, future investigations are needed to understand the architecture of the pea/milk protein network. From the sensory point of view, the substitution of animal protein with pea protein dramatically reduced the quality of products regarding their texture and flavor profiles. Whatever the strain, the intensity of five negative descriptors increased with the pea protein ratio. In these conditions, the products composed of more than 20 g pea protein/100 g total protein were far removed from the sensory profile of conventional yoghurts. Considering both the sensory characteristics and fermentation parameters, four starter cultures (A, B, F and H) seem promising for the production of yoghurts with pea proteins. It should be noted that these four starters all contain *Streptococcus thermophilus*.

3.3 Conclusion du chapitre 3

Quel que soit le cocktail bactérien, l'intensité des attributs sensoriels négatifs augmente et la fermeté des produits diminue avec l'augmentation de la concentration en protéine de pois. Dans ces conditions de préparation, les produits contenant plus de 20 % de pois, ont un profil sensoriel éloigné de celui du yaourt classique.

Afin d'améliorer la texture de ces produits fermentés, une étude sur les procédés de préparation pouvant favoriser les interactions entre les protéines de lait et les globulines de pois est nécessaire. La matière première utilisée comme source de ces globulines doit également être prise en considération.

Chapitre 4 : Optimisation et sélection des conditions optimales

4.1 Introduction

Une stratégie de plan d'expérience a été mise en place afin de sélectionner les conditions de mise en œuvre les plus favorables ; les mesures effectuées dans cette partie sont des mesures physico-chimiques (pH, Dornic et texture). Lors de notre travail, il a été constaté que la thermisation conjointe des deux laits (vache et pois) a un impact sur la texture du produit fermenté. La possibilité d'interactions entre protéines de lait et globulines de pois est donc envisagée à ce stade. De même, les températures de fermentations induisent la production de composés d'arôme et de petits peptides. Nous avons fait l'hypothèse qu'elles pourraient contribuer soit positivement soit négativement à la qualité organoleptique des produits finaux. Ce travail a mis en évidence l'importance de la qualité de la protéine végétale pour la rhéologie des gels. Or nous avons travaillé jusqu'à cette étape avec un isolat industriel de globulines qui sont partiellement dénaturées par le processus d'extraction. De plus, nous savons que les interactions protéines-protéines sont dépendantes d'une dénaturation préalable de ces dernières. Afin de maitriser cette étape première de dénaturation (lors de la thermisation des laits), nous avons décidé pour la suite de notre travail d'utiliser un isolat de globulines obtenu au laboratoire dans des conditions non dénaturantes. Dans cette **publication 3**, nous avons étudié l'effet de quatre paramètres de préparation sur les produits à base de pois fermentés avec les ferments classiques du yaourt.

4.2 **Publication 3**: Influence of preparation procedures on the quality of yogurt-like products from cow milk and pea proteins

Abstract

To increase the consumption of plant proteins in Europe, the strategy of supplementing or substituting traditional foodstuff with plant protein seems promising. However, the manufacturing processes must be adapted to guarantee a quality level close to that of traditional products. On the basis of a dairy gel with 45 g/L protein amount, four factors were studied using two levels experimental design. Dairy-plant gels were made by changing two different levels of pea protein substitution ratio in cow milk (20% and 40%), milk heat treatment (90 °C and 110 °C), blend of pea proteins in milk (before or after heat treatment) and incubation temperature (37 °C or 42 °C). The values of pH, titratable acidity and gel firmness were used as responses to a complete three replicates experimental design. The heat treatment at 90 °C significantly favored the firmness of yogurt whatever the pea protein substitution. Also, the simultaneous heat treatment of pea proteins with milk proteins had a positive impact on the firmness of gels, which suggested possible interactions between caseins and pea proteins (globulins). Under the optimal conditions to assure maximum gel firmness, and an acceptable acidity, i.e. mixing cow and pea milks before the heating at 90 °C, the tyrosine index and some volatile molecules were measured as indicators for bitterness, lactic fermentation and pea aroma. Finally, the sample exhibiting properties the closest to those of cow milk yogurt was obtained with a substitution of 20% of pea protein in cow milk, simultaneous heat treatment at 90°C and 42 °C fermentation temperature.

Keywords: yogurt quality; pea protein; fermentation; rheology; heat treatment; aroma

4.2.1 Introduction

Taking into consideration the increase in demand for animal protein and its consequences regarding food security, environment and economy, there is a demand for an increased in plant proteins consumption. One promising strategy to run down animal products towards more sustainable foods is the supplementation or substitution protein in traditional foodstuff with plant protein. Furthermore, using plant protein, especially pulse protein, has also some health benefits (**Bazzano**, 2008; **McCrory et al.**, 2010; **Rizkalla et al.**, 2002). Pea (*Pisum sativum*), one of the most important pulses, has a high-quality amino acid profile with a high content of lysine and arginine (**Schneider & Lacampagne**, 2000), and some appreciated functional properties such as texture, viscosity, gelation, emulsification, water and oil absorption capacities and foaming (**Bora et al.**, 1994; **Dagorni-Scaviner et al.**, 1987). To obtain acceptable food products, these positive nutritional and functional properties must overcome pea protein limiting characteristics such as the "green, beany" related with some off-flavor/ volatile molecules (**Klein & Raidl**, 1986; **Murat et al.**, 2012), "bitter" from some peptides (**Lemieux & Simard**, 1992), and fragile coagulum caused by the interaction between plant protein and casein (**Lee & Marshall**, 1979). Therefore, the successful use of plant protein as food ingredients depends on the sensory and rheological characteristics of the final product that could be affected by the manufacturing processes. Thus, the factory processes must be adapted to guarantee a quality level close to that of traditional products. Several authors have tested different parameters in yogurt manufacturing processes such as milk base, inoculation conditions, heat treatment and storage time, and their effects on the rheological and sensory properties of fermented cow milk (**Sodini et al.**, 2004). Moreover, studies were performed on fermented milk supplemented with a plant protein either with soy protein (**Drake et al.**, 2000) or lentil flour (**Zare et al.**, 2011). Although many studies have focused on the effects of different processing parameters on the quality of fermented milk, no study focused on the quality of products in the cases of substituted milk fermentation, despite there is a need for improvements in this type of products. The objective of this research was to determine the parameters that will give a dairy product close to a traditional yogurt. So, an investigation was undertaken to compare and understand the effects of heat treatment (90 °C and 110 °C), mixing of cow milk and pea milk before or after heat treatment, and incubating the starters at two different temperatures (37 °C and 42 °C) for two concentrations of pea protein (20% and 40%). Then, the products under the most favorable conditions for firmness and Dornic degree were studied to estimate the bitterness and green taste by determination the tyrosine index and the quantification of some important molecules either in plant ingredient or in dairy products.

4.2.2 Materials and methods

4.2.2.1 Ingredients and starters

Skim milk powder was purchased from Regilait (Saint-Martin-Belle-Roche, France) and pea protein isolate Nutralys® S85F was supplied by Roquette (Lestrem, France). Lactose monohydrate, sodium citrate and calcium phosphate monobasic purchased from Sigma (St. Louis, USA) for preparing milks. Lactic acid bacteria for homemade yogurt production (containing lyophilized *Streptococcus thermophilus* and *Lactobacillus bulgaricus*) were obtained from Alsa (Rueil-Malmaison, France).

4.2.2.2 Preparation of cow milk and pea milk

From skim milk powder and pea protein isolate, two different milks were prepared (cow milk and pea milk) with the same concentrations of protein, lactose, calcium and citrate. 128.6 g of skim milk powder was reconstituted with tap water to obtain one liter of cow milk (45 g/L protein concentration). Finally, 1.75 g of sodium citrate was added. Pea milk was obtained by reconstituting 126.5 g of pea isolate with tap water to obtain one liter. The suspension was gently stirred for 3 hours to obtain complete rehydration of the powder. The pea milk was then centrifuged at 12000g for 50 min at 10 °C to release the insoluble particles. The protein concentration of the supernatant was 45 g/L (as measured by Dumas method). Finally, 1.6 g of calcium phosphate monobasic, 1.75 g sodium citrate and 66.5 g of lactose were added to one liter of supernatant. The cow milk and pea milk were stirred for 10 minutes, and the pH of each preparation was adjusted at 6.5 using HCl or NaOH.

4.2.2.3 Design of experiments (DOE) approach and prepared samples

A 4 factors X 2 DOE including : *"pea amount"* percentage of substitution with pea protein in cow milk (20%, 40%), *"heat temp"* heat treatment temperature of milks (90 °C, 110 °C for 10 min), *"mix"* blend of pea proteins in cow milk before or after heat treatment and *"incubation temp"* lactic acid fermentation temperature (37 °C, 42 °C) was used.

Sixteen samples (1-16) were generated once within the DOE (Table 1). In addition, the central point condition was prepared (30% pea protein, heat treatment 100 °C, half quantity mixed before heating and the rest after heating, incubation at 39.5 °C). For further comparisons, four other samples were prepared: 100% cow milk heated at 90°C, incubated at 37 °C or 42 °C and 100% pea milk heated at 90 °C, incubated at 37 °C or 42 °C.

Table 1. The sixteen tested samples and the control sample with the different parameters of preparation.

Sample name	**Pea amount (%)**	**Heat temp (°)**	**Mix**	**Incubation temp (°)**
0 (center point)	30	100	½ before + ½ after	39.5
1	20	90	After	37
2	40	90	After	37
3	20	110	After	37
4	40	110	After	37
5	20	90	Before	37
6	40	90	Before	37
7	20	110	Before	37
8	40	110	Before	37
9	20	90	After	42
10	40	90	After	42
11	20	110	After	42
12	40	110	After	42
13	20	90	Before	42
14	40	90	Before	42
15	20	110	Before	42
16	40	110	Before	42

4.2.2.4 Fermentation of two milk mixtures

Two grams of lyophilized powder of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were added directly to one liter of milk (two milk mixture in our case) according to product instructions. Different inoculated mixtures were sampled in plastic flasks (40 mL in 45 mL flasks) and incubated for 24 h, either at 37 °C or 42 °C. The samples were stored for 24 h before analysis.

4.2.2.5 Characterization of DOE samples

- pH and titratable acidity measurements

pH of gel samples was determined in triplicate using a calibrated pH electrode and a pH-vision 6071 microcomputer (JENCO Electronics LTD, Shanghai, China). Lactic acid quantity or titratable acidity was determined according to the Dornic degree (D°) method (**Robinson & Wilbey**, 1998).

- Rheological analysis

Firmness and consistency were evaluated by Texture Analyzer TA.XT2*i* (Stable Micro Systems, Godalming, Surrey, England). These rheological measurements were performed using a single compression test, using a 5Kg-load cell with a cylindrical probe of 10 mm diameter. The test speed was 1.00 mm/sec, the trigger force was 0.010 N, the return distance was 85 mm, and the distance of penetration was 9 mm. The analyzer was linked to a computer that recorded the data via the Exponent software version 6,1,7,0. From recorded curves, two values were collected representing “Firmness”: maximum force value, “Consistency”: positive area under the curve. This test was performed in triplicate after one-day storage at 4 °C.

4.2.2.6 Characterization of samples with the most favorable conditions

- Tyrosine index

The Tyrosine index has been used for bitterness evaluation as described by (**Singh & Ranganathan**, 1978).

- Volatile compounds

Solid phase microextraction (SPME) was carried out according to (**Murat et al.**, 2012) with some modifications. Five grams of fermented product placed in a vial with a total volume of 20 ml. The vial was placed in an oven at 60 °C for 5 min. An SPME fiber divinylbenzene (DVB), carboxen (CAR), polymethylsiloxane (PDMS) (2 cm–50/30 µm, Supelco, USA) was exposed to the headspace, for 60 min under the same conditions. Preliminary experiments were done to optimize time and temperature parameter. The SPME extracts were injected into a splitless injector and analyzed using a gas chromatography (GC) model 7890A (Hewlett–Packard, Palo Alto, CA, USA) equipped with a DB-WAX column (30 m, 320 µm, 0.5 µm, JW Scientific Agilent). The fiber was injected at 250 °C in the splitless mode. Helium was used as the carrier gas in the constant flow mode with a linear velocity of 42 $cm.s^{-1}$. The chromatograph temperature was programmed from 38 °C, with an isotherm of 1 min, to 240 °C at a rate of 4 °C min^{-1} with a final isotherm of 10 min. Mass spectrometry was performed on a mass selective detector model 5975C (Agilent Technologies, Palo Alto, CA) operated in the electron impact mode at 70 eV. The mass spectrometer scanned masses from m/z 29 to 350. The ionization source was set at 230 °C and the transfer line at 240 °C. The data are presented as a mass spectrum. Volatiles were identified by comparison of the mass spectra with the spectra database (NIST databases). Butane-2,3-dione (diacetyl), 3-hydroxybutan-2-one (acetoin) and benzaldehyde were followed as indicators for yogurt aroma and lactic fermentation (**Imhof et al.**, 1995; **Widyastuti & Febrisiantosa**, 2014). Furthermore, hexanal and hexan-1-ol (1-Hexanol) were considered as markers of pea aroma resulting from lipid oxidation (**Schindler et al.**, 2012). Although acetaldehyde is an essential marker of aromatic quality of yogurt, this compound has been difficult to quantify by SPME. Indeed, the chromatogram was always very 'noisy' in the identification zone. The data could not be used.

4.2.2.7 Data analysis

Experimental design analyzed by DOE++ version 10.0.4 (ReliaSoft, USA). The same software was used to do a simple optimization to determine the most favorable conditions for firmness and Dornic degree of the gels. Finally, to estimate the significance of differences between means, factorial ANOVAs were conducted, followed by a Newman-Keuls test at the 0.05% level with Statistica version 12 (StatSoft, Tulsa, USA).

4.2.3 Results and discussion

4.2.3.1 pH and Dornic degree

As expected, the Dornic degree and the pH of fermented gels were correlated (R^2=0.82): the higher the pH, the higher Dornic degrees. The four studied factors have a significant effect on pH and titratable acidity ($P<0.05$). Regarding Dornic degree (Fig. 1), the greatest changes were observed when the fermentation temperature changed from 37 °C to 42 °C. The fermentation at 42 °C has significantly increased the amount of the produced lactic acid, possibly due to growth, survival and activity optimal for the used starter culture at 42 °C. The remaining factors affected the values of Dornic degree according to their importance as follows: the pea concentration in milk, the heat treatment conditions and finally the moment of mixing the two milks (Figure 1). As the lactic acid bacteria require nitrogen source for their growth (**Zourari et al.**, 1992), the changes in amino acid levels may explain the effect of the factors "pea amount" and "heat temp" on the Dornic degree. An optimal growth of LAB depends on sufficient levels of free amino acids and oligopeptides. Thus, to reach an optimal concentration, proteolysis is needed to release additional amino acids and small peptides from proteins. This phenomenon is well known in cow milk (**Neviani et al.**, 1995). However, the globular structure of pea proteins and their amino acid content are quite different in comparison with caseins or whey proteins which are usually used in yogurt. Thus, some of the amino acids, primarily required by *Streptococcus thermophilus* as methionine, cysteine and tryptophan (**Zourari et al.**, 1992), exist in low levels in pea protein. Furthermore, the increase in heat treatment temperature from 90 °C to 110 °C produced a lightweight brownish color caused by Maillard reaction. More Maillard reaction occurred leading to more amino acids participation in this process (**Shimamura & Ukeda**, 2012), so leaving less amino acids available for lactic acid fermentation and thus less lactic acid formation. All that may explain why the higher the pea protein concentration was, the lower the Dornic degree.

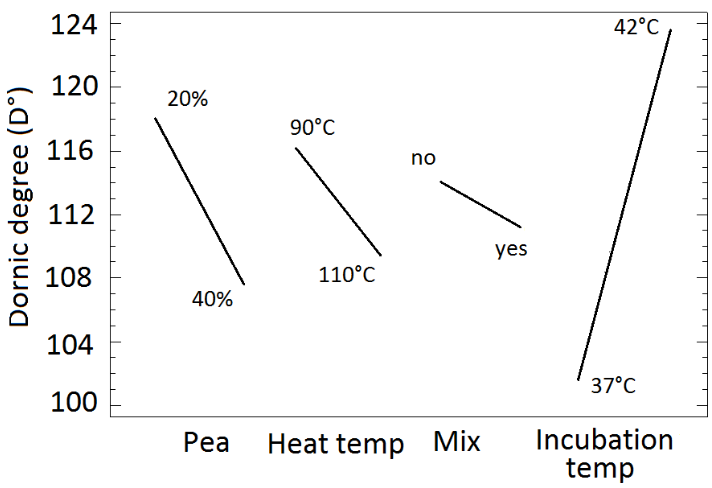


Figure 1. The main effect for Dornic degree obtained from the experimental design.

4.2.3.2 Rheological measurements

Four values were extracted from the compression curve. The measures of firmness and consistency were highly correlated (R^2=0.99), thus only firmness measures were discussed here. The four technological factors: pea amount, heat temp, mixing, incubation temp, induced a significant effect on the firmness of the gels ($P<0.05$). Mixing the two milks before heat treatment increased the firmness of gels to the highest level. This is possibly due to the formation of a primary interaction between the vegetable and animal proteins before the fermentation process took place. The next three factors affecting the firmness in order of importance were: the pea amount, the heating temperature and the incubation temperature (Figure 2). For the pea amount, substituting 40% of milk protein with pea protein generated a significant decrease in the firmness of gels in comparison to gels with 20% of pea protein. This is in accordance with our previous results indicating that the firmness of gels decreases with the increase of milk protein substituted by pea protein, even with different starters (**Yousseef et al.**, 2016).

For the two milks heated at 90°C and fermented at 42°C (the most favourable conditions), average firmness decreased from 100% cow's milk: 0.345 +/- 0.017 N to (i) 20 % pea milk: 0.264 +/- 0.013 N, (ii) 40% pea milk: 0.178 +/- 0.008 N, and (iii) 100% pea milk: 0.116 +/- 0.008 N. No statistical difference was observed in gel firmness between the cow milk sample and the 20% pea milk sample. The gelation of conventional yoghurt results from interactions between milk proteins: caseins and whey proteins (β-lactoglobulin, α-lactalbumin). It is well known that high heat treatment of milk, which causes significant denaturation of whey proteins, significantly increased the firmness and viscosity of resulting gel compared with gel made from unheated milk (**Donato et al.**, 2007; **Lucey & Singh**, 1997). Pea proteins, at a low level, in cow milk seemed to favor, or not disturb, the formation of this three-dimensional network. The formation of disulfide bonds and noncovalent interactions between β-lactoglobulin and pea globulin subunits after a thermal treatment was described by **Chihi et al.** (2016). The heat-treated initial mixtures of the two proteins provide more elastic acid gels with a more regular and less porous fibrillary structure. (**Lu et al.**, 2010) also showed that sesame proteins increased the hardness in soft cheese but when sesame concentration increased, the sesame protein aggregated as a different protein phase in the casein gel matrix leading to a more fragile coagulum. Heating at the highest temperature (110 °C) seems to denature caseins and whey proteins completely without helping to make stronger bonds between milk proteins and pea proteins for the structure of gels. The incubation at 42 °C instead of 37 °C allowed the formation of firmer gels. A temperature of 42 °C was favorable to a rapid acidification of medium by lactic acid production, which contributes to a higher gelation of cow milk and pea proteins and an enhanced firmness. The enhanced rheological properties of yogurt formed at high temperature (40-45°C) are attributed to a progressive increase in the extent and strength of hydrophobic association between proteins (**Haque et al.**, 2001; **Lee & Lucey**, 2004).

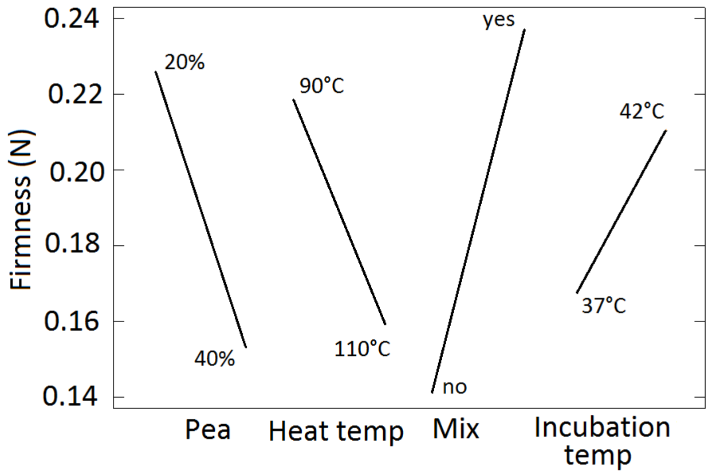


Figure 2. The main effect on firmness obtained from the experimental design.

4.2.3.3 Tyrosine index

Under the most favorable conditions for firmness and Dornic degree of the gels (mixing cow and pea milks before the heating at 90 °C), the tyrosine index was studied to understand the effect of pea amount and incubation temperature on the changes in proteolysis intensity. Indeed, an excessive proteolysis in dairy gels results in more bitter taste (**Chandan**, 2015). The results were compared with values obtained from 100% cow milk. ANOVAs showed that the pea amount, the incubation temperature and the interaction between these two factors have a significant effect on the tyrosine index value ($P<0.05$). Whatever the type of ''milk'': 100% cow milk, 20% pea milk and 40% pea milk, the tyrosine index was significantly lower with incubation at 42 °C than at 37 °C (Figure 3). On the other hand, a 40:60 pea and cow milk ratio gave the highest index of tyrosine, regardless of the incubation temperature. Also, pea globulin appeared to be more easily degraded by proteolysis than casein and whey proteins. However, more investigations are needed to verify this hypothesis. Thus, gels rich in pea protein would be perceived as bitterer than those from cow milk. And so, according to this index, the gel with 20% pea protein fermented at 42 °C was the closest product to the traditional yogurt.

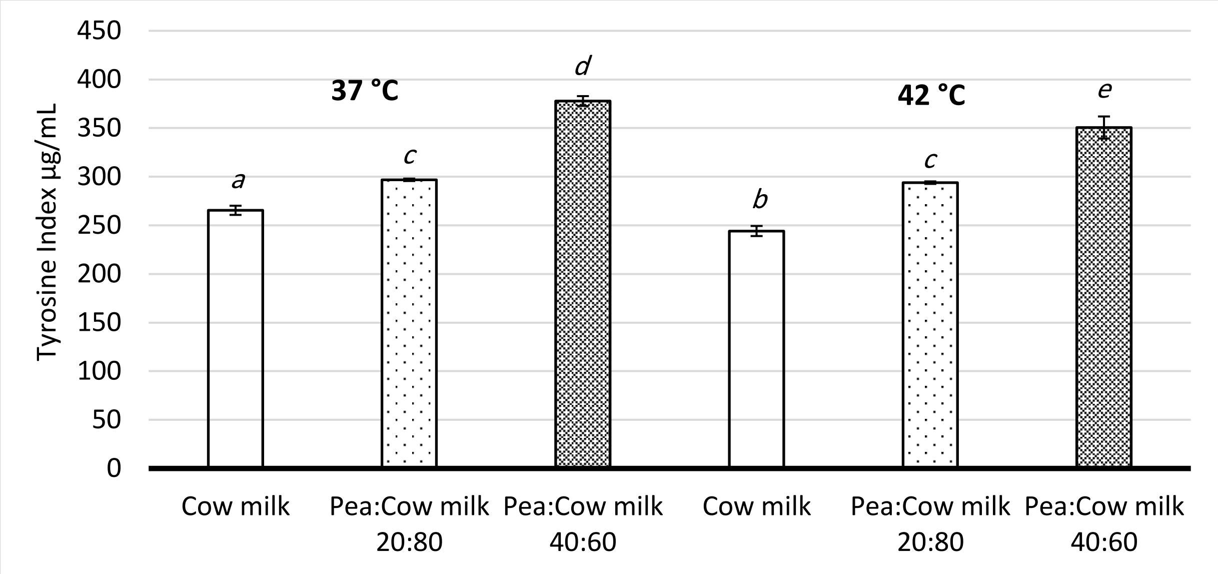


Figure 3. Tyrosine index in gels as function of pea protein amount and lactic fermentation temperature - Two products with different letters are significantly different.

4.2.3.4 Volatile compounds

Fifty-two molecules were detected in the fermented products. The molecules detected using SPME-GC-MS are listed Table 2. Although acetaldehyde was detected, the results cannot be exploited due to the poor quality of the baseline in the detection zone of this compound. The amounts of five key aroma compounds, markers of lactic fermentation and pea flavor were followed. The results were compared with values obtained from 100% cow milk.

Table 2 List of the volatile compounds identified in the fermented products by GC-MS from SPME extracts (using data in NIST WebBook).

Acids	**Carbonyl compounds**
Acetic acid	Acetaldehyde
Butanoic acid	Acetone
Isovaleric acid	2,3-Butanedione (diacetyl)
Butanoic acid, 3-methyl-	2,3-Pentanedione
Hexanoic acid	3-Hexanone
Heptanoic acid	hexanal
Octanoic Acid	Dodecane
Nonanoic acid	2-Heptanone
n-Decanoic acid	2-Butenal, 3-methyl-
Benzoic acid	2-Heptanone, 4-methyl-
Alcohols	2-Heptanone, 4,6-dimethyl-
3-Methylbut-2-en-1-ol	2-Octanone
1-Pentanol	Octanal
2-Butanol	2-Butanone, 3-hydroxy- (acetoin)
2,5-Hexanediol, 2,5-dimethyl-	2-Buten-1-ol, 2-methyl-
1-Hexanol	2-Nonanone

1-Heptanol	Nonanal
2-Ethylhexanol	3-Octen-2-one
1-Octanol	1-Hexanol, 2-ethyl-
1-Nonanol	Decanal
4-Methylbenzyl alcohol	Benzaldehyde
Aromatic compounds	3,5-Octadien-2-one
Benzene, 1,3-bis(1,1-dimethylethyl)-	2-Undecanone
Toluene	Benzaldehyde, 4-methyl-
Heterocyclic compounds	Benzaldehyde, 2-methyl-
2-Pentylfuran	Oxime-, methoxy-phenyl-_
Furfural	2-Tridecanone
Sulfur compounds	
Dimethyl trisulfide	

ANOVAs showed that the factors pea amount, incubation temperature and the interaction between these two factors had significant effects on the "quantity" (express in total ion current, arbitrary unit) of the five keys molecules ($P<0.05$). An increase in pea milk ratio caused a decrease in the amount of diacetyl and acetoin and an increase in the quantity of benzaldehyde, hexanal and 1-hexanol (Figure 4).

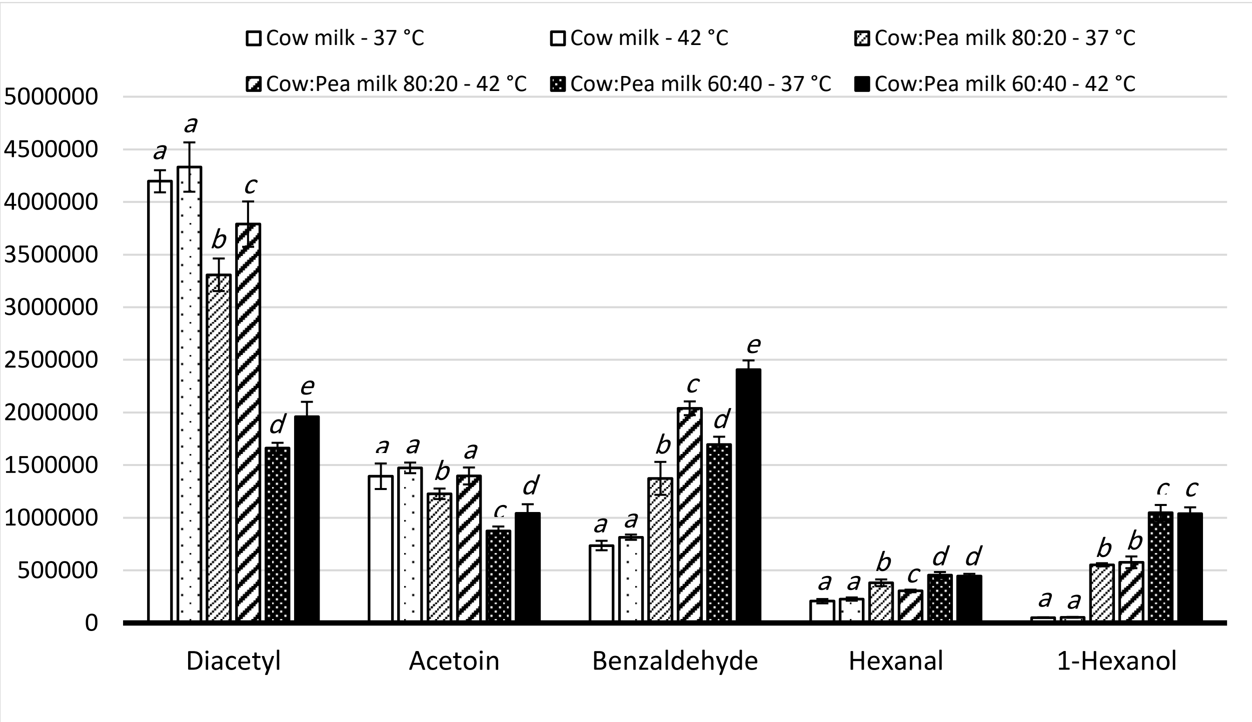


Figure 4. The amount of five aroma compounds (TIC, arbitrary unit) at one day of storage (4°C) in term of pea protein concentration and incubation temperature. For each molecule, two products with different letters are significantly different.

The contents of lactic fermentation flavor compounds (diacetyl and acetoin) in gels with 20% peas were quite similar to those measured in the cow milk gels (Fig. 3). In contrast, a content of 40% pea in milk gave gels with lower levels of diacetyl and acetoin than those obtained in cow milk; these aroma compounds are favorable for the aromatic note "dairy". For benzaldehyde, the amount was higher in gels with pea proteins in comparison with 100% cow milk. This compound has an olfactory note "bitter almond" (**Burdock**, 2009), and its presence is not conducive to "dairy" notes even though it was detected as one of yogurt aroma (**Cheng**, 2010; **Routray & Mishra**, 2011). Finally, the incubation temperature slightly affected the amount of flavoring compounds bound to the metabolism of the fermentation. At 42 ° C, the production of diacetyl, acetoin and benzaldehyde was the highest higher.

In contrast, the amount of aromatic compounds (hexanal, 1-hexanol) derived from the pea material was not affected. It should be noted that hexanol and 1-hexanal are also produced by LAB during fermentation. Neither the doubling of the pea protein amount nor the incubation temperature affects the amounts of these two compounds.

Diacetyl combined with acetoin imparts a mild, pleasant, buttery taste, and they are critical to the rich perception of yogurt. Diacetyl gives the buttery flavor and it may improve yogurt flavor quality. Diacetyl is a diketone, derived by the fermentation of citrate present in milk and dairy mixes (**Cheng**, 2010). Acetoin has a mild creamy, butter-like flavor that is similar to that of diacetyl. Acetoin is readily converted from diacetyl by the enzyme diacetyl reductase (**Cheng**, 2010). The production of these two aromatic compounds depends on the metabolism of citrate and therefore on the LAB fermentative behavior in cow milk or in mix of cow and pea milks. The incubation temperature affects the intensity of fermentation activity (more or less important production of lactic acid) which necessarily impacts the production of secondary metabolites such as aroma compounds. The availability of nitrogen precursors as a function of the nature of proteins (pea or cow) is also to be taken into account. A more detailed study of the relationship between protein type (pea or cow) and aroma compound production during lactic fermentation should be carried out.

4.2.4 Conclusion

Insertion of plant protein in European food diet, via traditional foodstuff as yogurt, may need some modifications in the manufacturing processes to guarantee a quality level close to that of traditional products. This study showed that the partial substitution of proteins in milk by pea globulins led to major changes in the physico-chemical characteristics of obtained gels.

Moreover, the behavior of lactic acid bacteria was affected by the presence of pea proteins during the fermentation process. From a technological point of view, a heat treatment of milks at 90°C significantly favored the firmness of yogurt whatever the pea protein substitution. Likewise, the combined heat treatment of pea proteins with cow milk had a positive impact on the firmness of gels, which suggested possible interactions between caseins, whey proteins and pea protein (globulins). As expected, an increase in fermentation temperature from 37 °C to 42 °C resulted in an increase of the final Dornic degree, and a decrease in the tyrosine index. Finally, the sample exhibiting properties the closest to those of cow milk yogurt was obtained with a substitution of 20% of pea protein in milk, a heat treatment at 90 °C and an incubation temperature at 42 °C. These results suggest a promising use of pea proteins as a protein substitute in dairy gel process with the need for more work on used raw materials and the effect of storage on these products.

4.3 Conclusion du chapitre 4

Les paramètres testés dans cette étape dite d'optimisation, nous permettent de conclure que 1-Mélanger les deux types de lait (pois et vache), avant traitement thermique (90°C), permet d'obtenir des gels significativement plus fermes qu'avec un traitement thermique des deux laits séparés. Nous supposons d'après la littérature que des interactions entre les protéines de lait de vache te les globulines de pois sont à l'origine de ce réseau plus dense.

2-De même une fermentation plus rapide à 42°C est plus favorable à la qualité du produit avec une production plus importante d'arômes type yaourt et une réduction des faux goûts de pois.

Ce travail a mis en évidence l'importance de la qualité de la protéine végétale pour la rhéologie des gels. Or nous avons travaillé jusqu'à cette étape avec un isolat industriel de globulines qui sont partiellement dénaturées par le processus d'extraction. Or nous savons que les interactions protéines-protéines sont dépendantes d'une dénaturation préalable de ces dernières.

Afin de maitriser cette étape première de dénaturation (lors de la thermisation des laits), nous avons décidé pour la suite de notre travail d'utiliser un isolat de globulines obtenu au laboratoire dans des conditions non dénaturantes. De même nous avons montré que les profils d'arômes, des peptides et de l'Indice de Tyrosine, pour le cocktail F « *Streptococcus thermophilus* + *Lactobacillus acidophilus* » donne le meilleur produit. Nous l'avons donc retenu pour la suite de notre étude (**Annexe 1**).

Chapitre 5 : Validation sensorielle

5.1 Introduction

Après avoir testé différentes solutions (de l'étude de 10 cocktails bactériens à l'optimisation de la matière première et jusqu'aux procédures de fermentation) afin de résoudre les problèmes de texture et de faux goûts liées à la substitution des protéines de lait par les globulines de pois, ce dernier chapitre s'est intéressé à valider sensoriellement grâce à un test consommateur et un test CATA une gamme de produits obtenus avec le cocktail F sélectionné précédemment. Six produits avec différentes concentrations de pois ont été étudiés dans la **publication 4.** Nous avons évalué en parallèle les propriétés physico-chimiques et sensorielles de ces produits optimisés pour définir la concentration la plus élevée de protéine de pois pouvant être acceptée.

5.2 **Publication 4**: Plant based yogurts: Physico-chemical, sensory and hedonic characteristics

Abstract

Off-flavors are commonly described in plant-based yogurts. The reduction of these defects depends on finding the most appropriate lactic acid bacteria strain. A starter composed *Streptococcus thermophilus* and *Lactobacillus acidophilus* have shown their capacity to reduce the bitterness and the off-flavors of fermented pea proteins. To confirm the quality of this strain with a broad range of concentrations of plant proteins, six different ratios of pea protein in cow milk (20 to 100%) were fermented and characterized from a physical point of view. A consumer test (check-all-that-apply test) was then used to evaluate the acceptability and sensory profile of the samples. From a physical point of view, all samples showed characteristics close to those of a conventional yogurt (pH, Dornic degree, syneresis, firmness). From a sensory point of view, a ratio of 20% of pea protein in cow milk gave a product accepted by most of consumers. The 40% ratio discriminates between two consumer groups, with half of the participants accepting the sample and the other half rejecting it. For higher pea protein ratios (80-100%), products were negatively assessed by most participants.

Keywords: Pea protein, Cow milk protein, Lactic acid bacteria, Physical properties, Sensorial properties

5.2.1 Introduction

One of the main problems that hinder the incorporation of vegetal proteins in processed food is that vegetal raw materials provide off-flavors (e.g., vegetal taste, chalky) and are characterized by a high astringency and bitterness (**Murat et al.**, 2013; **Schindler et al.**, 2012; **Torres-Penaranda et al.**, 1998). Fermentation might be seen as a way to reduce this problem. Lactic fermentation, for example, plays a major role in the texture and the organoleptic characteristics of fermented products. As a consequence researchers focus their efforts to find potential starter cultures to improve the texture and flavor quality of fermented product (**Widyastuti & Febrisiantosa**, 2014). Some authors tried to introduce little quantities of plant ingredients in traditional fermented foods: soy protein (**Drake et al.**, 2000), lentil flour (**Zare et al.**, 2011), sesame protein (**Lu et al.**, 2010). In these studies, the usual starter cultures were used while other authors seek to improve the quality of fermented products by using different starter cultures (**Yousseef et al.**, 2016; **Zare et al.**, 2013). **Yousseef et al.** (2016) tested ten lactic acid cultures to ferment different mixtures of cow and pea milk. This screening test showed the effect of starter cultures on the organoleptic quality of fermented products: Some starters yielded products with more positive properties than the other ones. Another preliminary study conducted in our laboratory showed that a LAB cocktail (called "F") including *Streptococcus thermophilus* & *Lactobacillus acidophilus* reduced the bitterness and the off-flavors in the fermented products.

The present study was a follow up of this previous work our objective was to validate the ability of LAB cocktail "F" to mask the bitterness and off flavor brought by pea proteins. Six yogurts with different ratios of pea protein in cow milk (0%, 20%, 40%, 60%, 80% and 100%) were made with the selected starter culture and characterized using both a physico-chemical and a sensory approach.

Conventionally in sensory evaluation, Quantitative Descriptive Analysis (QDA) is used to obtain product sensory profiles. However, although this method is very efficient it has the drawbacks of not reflecting consumers' perception and of being time and money consuming. Recently new methods that take into account consumers, are more rapid and less expensive has been developed to obtain product description (**Ares & Varela**, 2014; **Valentin et al.**, 2012). Among these methods, check-all-that-apply (CATA) has become very popular as it is well suited to work with consumers (**Ares, Barreiro, et al.**, 2010; **Dooley et al.**, 2010; **Jaeger et al.**, 2013; **Lado et al.**, 2010). We combined this approach with a consumer test to evaluate the efficiency of the LAB cocktail "F", and to determine the optimum ratio of cow and pea milk that could be used to develop plant based yogurts.

5.2.2 Materials and methods

5.2.2.1 Production of fermented products

Cow milk from skim milk powder Régilait® and pea milk from laboratory pea globulin isolate were prepared at 45 g/L of protein amount (**Yousseef et al.**, 2016). The pea globulin isolate preparation was adapted from (**Chihi et al.**, 2016); the defatted step was only done with ethanol. Six samples of fermented cow and/or pea milk were prepared. Cow milk and pea milk were mixed to obtain six concentrations (v:v) of pea milk in cow milk (0%, 20%, 40%, 60%, 80% and 100%). These mixtures were heated at 90 °C for 10 mins, then inoculated with the selected starter F (*S. thermophilus + Lb. acidophilus*) and incubated at 37 °C for 10 h to obtain products with pH= 4.6±0.1. The products were stored at 4 °C for one day before performing the CATA and liking tests.

5.2.2.2 Instrumental characterization

Changes in pH values during fermentation were determined in triplicate with an automated lactic starter monitoring and characterization with the CINAC-System (Ysebaert, Frepillon, France). pH electrodes were calibrated with two standard buffers at pH 7 and pH 4 before each experiment. From the pH curve over time (pH = f (time)), two kinetic parameters were determined: *V*max (the maximum acidification rate (×10^{-3} Unit pH/min)), and *T*max was the time (hours) where the acidification achieve its maximum rate. The titratable acidity was determined according to the Dornic degree (D°) method which quantifies the lactic acid present in dairy products (**Robinson & Wilbey**, 1998). Ten mL of the product with two drops of phenolphthalein solution (2% in ethanol 96%) was titrated with standardized NaOH (0.111 mol/L) until the pink color change lasted 30 seconds. This measurement was conducted on all the samples after 1, 14 and 28 day of storage at 4°C. Three replicate experiments were carried out for each measurement. The measurement of the degree of whey separation as well as syneresis and firmness of the fermented product were determined after 1, 14 and 28 days of storage at 4°C in cold chamber was measured. All analyses were carried out in triplicate (**Yousseef et al.**, 2016).

5.2.2.3 Sensory characterization: CATA and liking test

- Panelists

Sixty consumers (32 men and 28 women) aged from 18 to 59 years participated in the sensory test. They were recruited from the student and the staff of the University of Burgundy, Dijon, France.

❖ Procedure

Ten grams of each sample were served to the panelists in closed plastic cups labeled with three-digit random numbers. Samples were presented monadically following a Williams Latin square. Mineral water was used for rinsing between samples. Participants were asked to test each sample and to score their overall liking using a 9-point scale going from (1) "dislike very much" to (9) "like very much." After rating their overall liking, participants completed a CATA question with 21 terms related to sensory characteristics of yogurts and vegetal protein (Table 1). The attributes were selected based on previous studies (**Ares and Jaeger**, 2014; **Youssef et al.**, 2016). After completing the CATA question, consumers were asked to check all the terms they considered appropriate to describe their ideal vegetal yogurt. The order of terms was randomized between participants but the same order was kept for each panelist.

Table 1. List of attributes used in CATA

Astringent	Chalk	Aftertaste	Thick
Vinegar	Bitter	Liquid	Heterogeneous
Sour	Sweet	Smooth	Homogeneous
Earth	Creamy	Viscous	
Vegetal	Taste of cream	Firm	
Smoked	Taste of yogurt	Consistency	

5.2.2.4 Data analysis

❖ Physico-chemical properties

All results were analyzed using one-way ANOVA with pea protein ratio as a fixed factor. A Student Newman-Keuls (SNK) test (α = 0.05) was used to compare the samples when there was a significant effect of pea protein ratio.

❖ Sensory properties

Liking scores were analyzed using two-way repeated measures analysis of variance (ANOVA) with consumer as random and sample as fixed factors. A Student Newman-Keuls test (α = 0.05) was used to compare the samples. *CATA data* were analyzed by counting the number of consumers that used each word to describe each fermented product. A correspondence analysis (CA) was performed on the frequency counts. Consumer overall liking scores and ideal product evaluation were projected as supplementary data (**Ares, Deliza, et al.**, 2010).

5.2.3 Results and discussions

5.2.3.1 Acidification trend during the fermentation and pH evolution during storage

The effects of milk protein substitution with pea protein on acidifying kinetics are summarized in Table 2. The ANOVA showed a main effect of pea protein ratio on these two values (*V*max & *T*max) ($P<0.05$). The *V*max varied from 7.1×10^{-3} pH unit.min^{-1} for 100% pea milk to 11.6×10^{-3} pH unit.min^{-1} for cow milk. *T*max ranged from 2.1 h for cow milk to 3.4 h for pea milk.

Table 2. Effects of cow milk substitution on acidification characteristics of cow milk substituted with 0%, 20%, 40%, 60%, 80% and 100% pea protein. Means in the same column with different letters are significantly different (SNK test, $P<0.05$)

Pea protein ratio	*V*max ($\times10^{-3}$ pH unit min^{-1})	*T*max (h)
0%	9,98^{c}	2,09^{a}
20%	9,97^{c}	2,63^{e}
40%	11,37^{d}	2,48^{d}
60%	11,58^{e}	2,24^{b}
80%	9,56^{b}	3,36^{f}
100%	7,07^{a}	2,42^{c}

Several authors studied the effect of different factors on acidification kinetics, such as incubation temperature, heat treatment, milk source, milk supplementation and culture composition (**Beal et al.**, 1999; **Bezerra et al.**, 2012; **Medeiros et al.**, 2015; **Oliveira et al.**, 2001). All these factors have generally an effect on acidification kinetics. For example, **Oliveira et al.** (2001) showed that culture composition has an effect on *V*max, and the value of *V*max is higher with mixed cultures than with pure ones. Values of *V*max and *T*max in our study (11.9 $\times10^{-3}$ pH unit min^{-1}, 2.8 h) are close to those reported for cow milk supplemented with milk protein and fermented by *S. thermophilus + Lb. acidophilus* (**Oliveira et al.**, 2001). Contrary to what we expected the SNK test did not show a linear relationship between the pea protein ratio and the acidification kinetics neither for *V*max nor for *T*max. Vmax increased from 20% to 60% and then decreased. Tmax increased from 0% to 20% then decreased up to 60% and increased again from 60 to 80% and then decreased. This pattern of results could be explained by the differences in the availability of some constituents (especially organic acids, salts and proteins), mainly after heat treatment of the two-milk mixtures. This variation in components leads to difference in buffering capacity which is essential for acidification and its kinetics (**Bezerra et al.**, 2012).

Most authors stop the fermentation when pH=4.5 or 4.6 (**Bezerra et al.**, 2012; **Božanić et al.**, 2011; **Chumchuere & Robinson**, 1999; **Sodini et al.**, 2005; **Zare et al.**, 2013). Previous study in our laboratory showed that the gelation of pea and cow milk mixtures occurred when pH=4.7 (**Yousseef et al.**, 2016), which is proximate to the isoelectric point of pea globulins (**Mession et al.**, 2012). So, as the pH of all samples was in the 4.5 to 4.7 pH range after 10 hours this time was selected as the optimum time of incubation. The pH varied weakly in a small range of 0.2 pH unit from samples made with 0% to samples made with 100% pea protein. The fermentative behavior has been followed by the measurement of lactic acid production. Titratable acidity for all the samples, after one and 28 days of storage at 4°C, are illustrated in Figure 1. The ANOVA showed also that pea protein concentration has a significant effect on the Dornic degree of products ($F(5,12) = 1426.3$, $p<0.05$). The Dornic degree decreases from samples made with 0% (cow milk) to samples made with 100% pea protein. In this study, increasing pea protein ratio up to 100% has a weak effect on pH but induce a decrease from 100° (cow milk) to 55° (pea milk) on Dornic degree. It seems that the presence of high concentration of pea protein hinder the formation of lactic acid leading to low values of Dornic degree. As expected, after 28 days of storage the samples tended to be more acid than after 1 day of storage (Figure 1). The differences between pH and Dornic variations could be explained by a high buffer capacity of pea proteins.

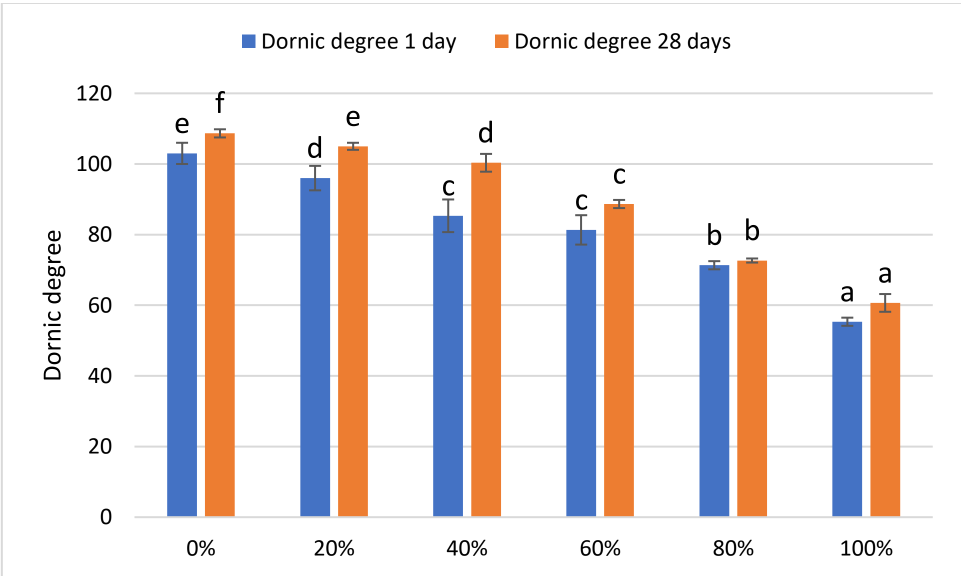


Figure 1. Effects of cow milk substitution and storage for 28 days on titratable acidity of cow milk substituted with 0%, 20%, 40%, 60%, 80% and 100% pea protein and fermented with *S. thermophilus* and *Lb. acidophilus*. Means for same storage time with different letters are significantly different (SNK, P<0.05)

5.2.3.2 Syneresis during storage

Syneresis is the spontaneous liquid separation on the surface of fermented milk. It is regarded as a defect. After one day of storage, the highest syneresis was observed for the samples made with 100% cow milk, followed by the sample substituted with the lowest quantity of pea protein (20%) (Figure 2). Thus, the ratio of substitution in samples significantly affected the syneresis (F (5,12) = 2733.2, *p<0.05*). After 28 days of storage, the samples made with 100% pea protein were subject to the greater change in syneresis and become the product with the highest syneresis value (Figure 2). Water retention capacity of protein gel is dependent on the nature and stability of protein-protein and protein-water interactions (**Campbell et al.**, 2009). It has been shown that 100% globulin gels have a more porous structure resulting in a lower water retention capacity (**Chihi**, 2016). In the case of cow and pea protein mixtures, the presence of disulfide bonds (**Chihi et al.**, 2016) between β-lactoglobulin and pea globulin subunits could explain a lower syneresis by a greater water retention capacity. Indeed, the establishment of covalent bonds in the stabilization of the protein gel structure, which leads to a more rigid structure of the system, correlates with a greater water retention capacity (**Alting et al.**, 2000; **Campbell et al.**, 2009).

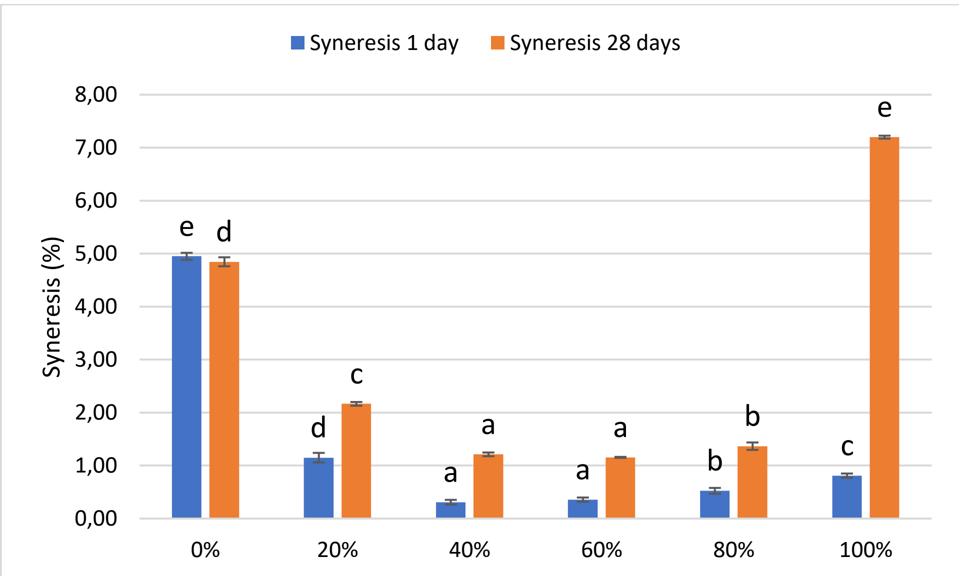


Figure 2. Effects of cow milk substitution and storage for 28 days at 4°C on syneresis of cow milk substituted with 0%, 20%, 40%, 60%, 80% and 100% pea protein and fermented with *S. thermophilus* and *Lb. acidophilus*. Means for same storage time with different letters are significantly different (P<0.05)

5.2.3.3 Rheological properties during storage

After 1 and 28 days of storage at 4°C, the ratio of substitution has a significant effect on the firmness of the samples (Figure 3). After one day of storage, firmness increased from 0.25 N for 0% to 0.57 N for 80% of pea protein, and decreased to 0.53 N for 100%. The firmness of yogurt is usually highly related to total solids and protein content, and to the type of protein (**Oliveira et al.**, 2001). As in this study, protein content and total solids are constant (protein content = 45 g/L and dry matter ≈ 11.4% for all fermented samples) the quality of the texture of the samples made with pea protein (from 20% to 100%) can be attributed to the effect of starter culture on the protein mixtures. The molecular interactions between casein and pea globulin are not well known, especially in dairy products. Recently, the formation of disulfide bonds and noncovalent interactions between β-lactoglobulin and pea globulin subunits after a thermal treatment was described by **Chihi et al.** (2016). So pea protein, at a specific level, in cow milk seemed to favor the formation of this three-dimensional network. Others studies showed that the firmness or hardness of the fermented samples increased with supplements of plant ingredients up to 3% lentil flour in yogurt (**Hsieh et al.**, 1993; **Zare et al.**, 2011), while other supplementations yielded a fragile coagulum (**Lee & Marshall**, 1979; **Lu et al.**, 2010). After 28 days of storage, the firmness significantly increased for all samples (Fig.3). This gain in firmness, although low, occurred in a similar ratio for all samples (R^2=098). This behavior is explained by a phenomenon of the gel structure reinforcement (**Oliveira et al.**, 2001).

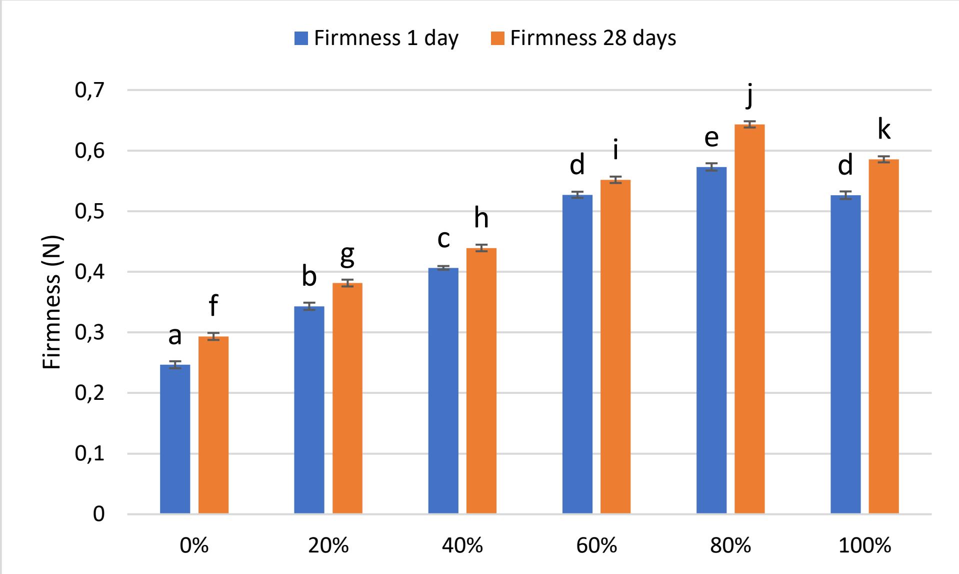


Figure 3. Effects of cow milk substitution and storage for 28 days at 4°C on firmness of cow milk substituted with 0%, 20%, 40%, 60%, 80% and 100% pea protein and fermented with *S. thermophilus* and *Lb. acidophilus*. Means with different letters are significantly different (P<0.05)

5.2.3.4 Sensory properties (CATA and liking scores)

❖ Liking scores

The ANOVA showed that as expected pea concentration has a significant effect on the acceptability of the products ($P< 0.05$). Figure 4 shows the average liking scores as a function of pea protein concentration. The newman-Keuls test indicates that the sample without pea is significantly preferred. The liking score of the remaining samples decreases as the amount of pea increases, but in all cases, correspond to a negative evaluation (liking score < 5).

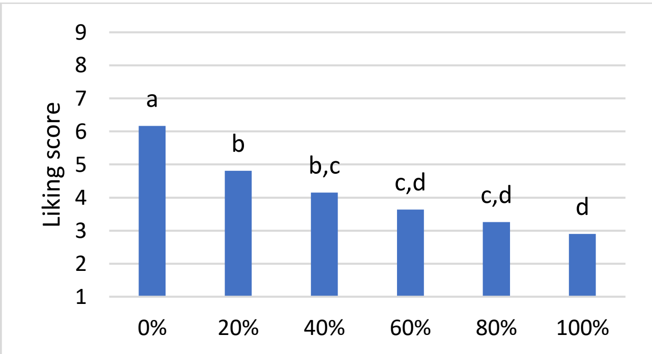


Figure 4. Mean scores of liking as a function of pea concentration. Two products with different letters are significantly different (Student Newman-Keuls test α=0.05).

To analyze consumer individual differences, we plotted the liking score frequency histogram for each sample (Figure 5). Most consumers scored the samples made with 0 and 20 % pea protein in the positive zone of the scale, and the samples made with 60% to 100% pea protein in the negative one. In contrast, a disagreement is observed for the samples made with 40% pea protein. Some consumers assessed it positively, and others assessed it negatively. Based on this result, we divided the panelists into two groups: G+ with consumers who positively assessed the samples made with 40% pea protein (liking score above or equal to 5), and G- with consumers who gave a liking score below 5. We analyzed the CATA results separately for the two groups.

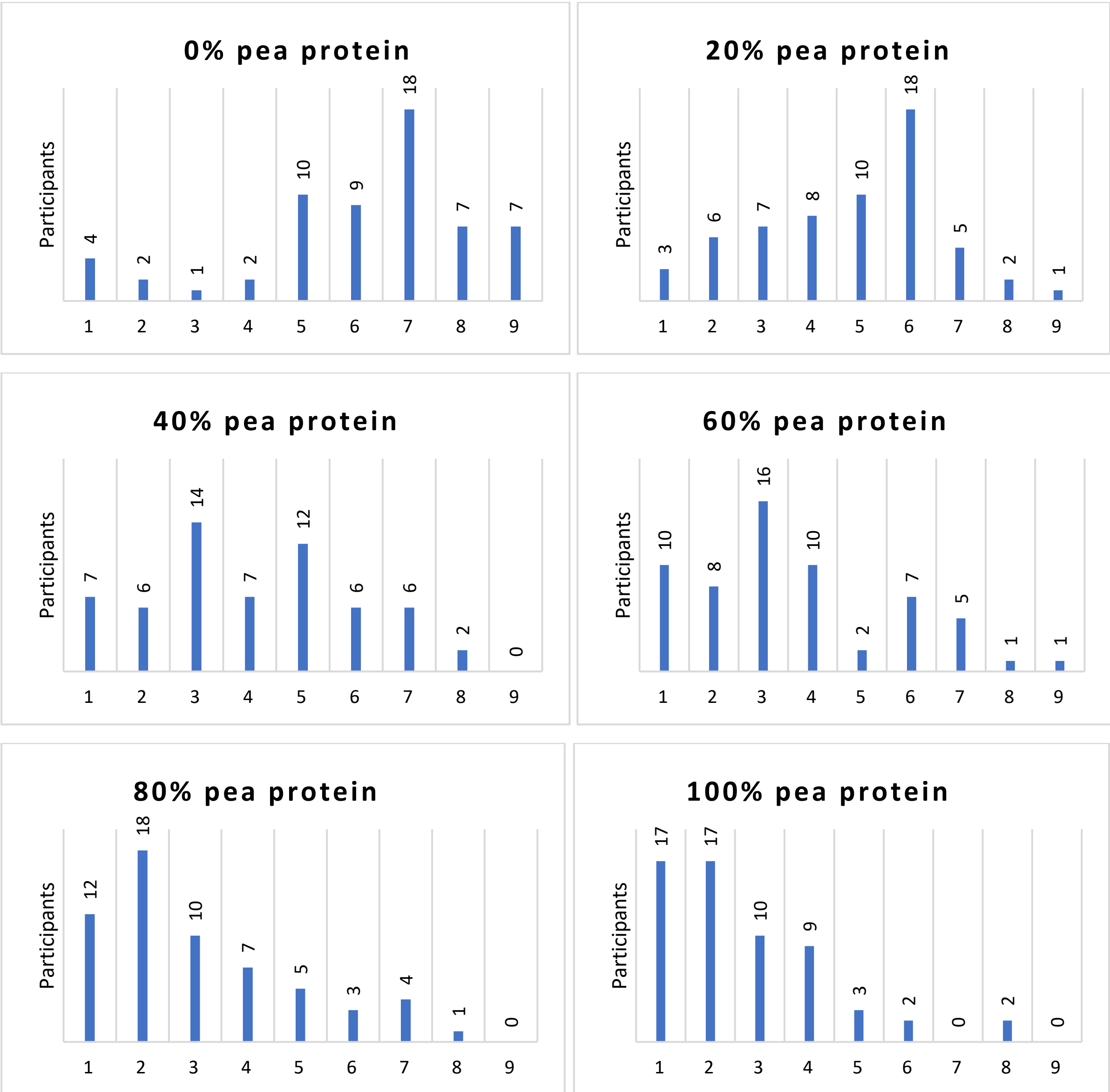
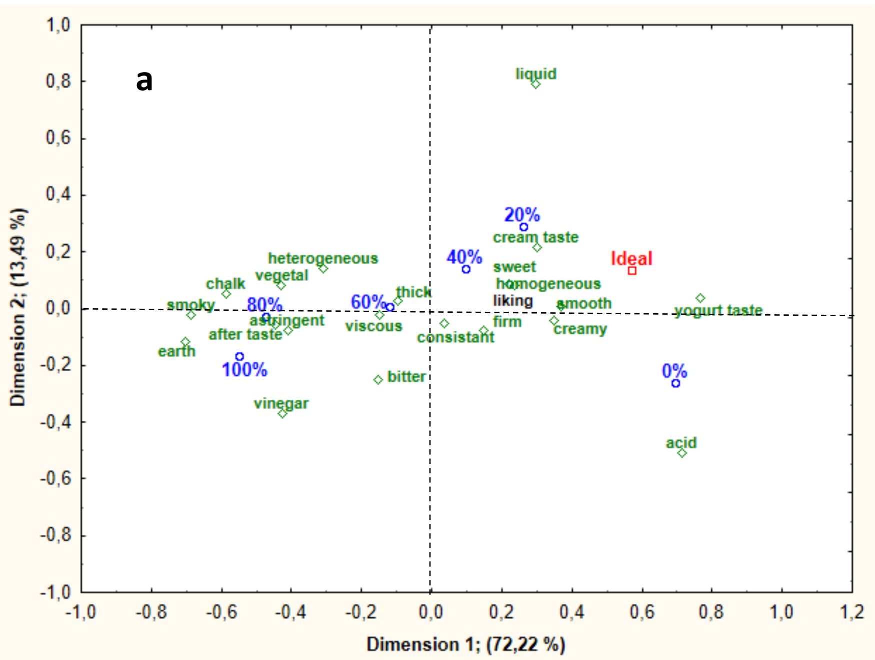


Figure 5. Frequency of consumers liking scores (from (1) “dislike very much” to (9) “like very much”) for each product

❖ CATA

(Figure 6A) shows the CA first two dimensions (85.7% of variance) for the group G+. The first dimension is correlated with liking. It opposed the terms: smoked, astringency, aftertaste, thick, viscous, vegetal and chalk, to the terms: smooth, creamy and taste of yogurt. The samples are well spread out along the first dimension with the sample made with 0% pea being acid and yogurt taste, the samples made of 20 and 40% creamy taste, homogeneous, sweet and more liquid that the samples made with 0% pea and the other samples made with pea protein. The samples made with more than 40% pea protein are associated with negative descriptors. The ideal product project in between the samples made with 0%, 20 and 40% pea protein. The second dimension is negatively correlated to firm and positively to liquid. Liking scores are positively correlated to the term taste of yogurt and delicious, and negatively correlated to the terms: smoked, earth, vinegar.

For the group G- (Figure 6B), the first two dimensions of the CA represent 86.8% of the variance of the experimental data. The distribution of descriptors for this group is similar to that of the CA carried out for G+. However, the distribution of samples is different among the two groups. The sample made without pea protein (0%), describe as yogurt taste, creamy and acid, is the closest to the ideal product for the consumers in this group. This product is very different from all other products which are associated with negative terms. As for G+ the 20% pea product is described as more liquid, cream taste and somewhat sweet. However, it is quite far from the ideal product.



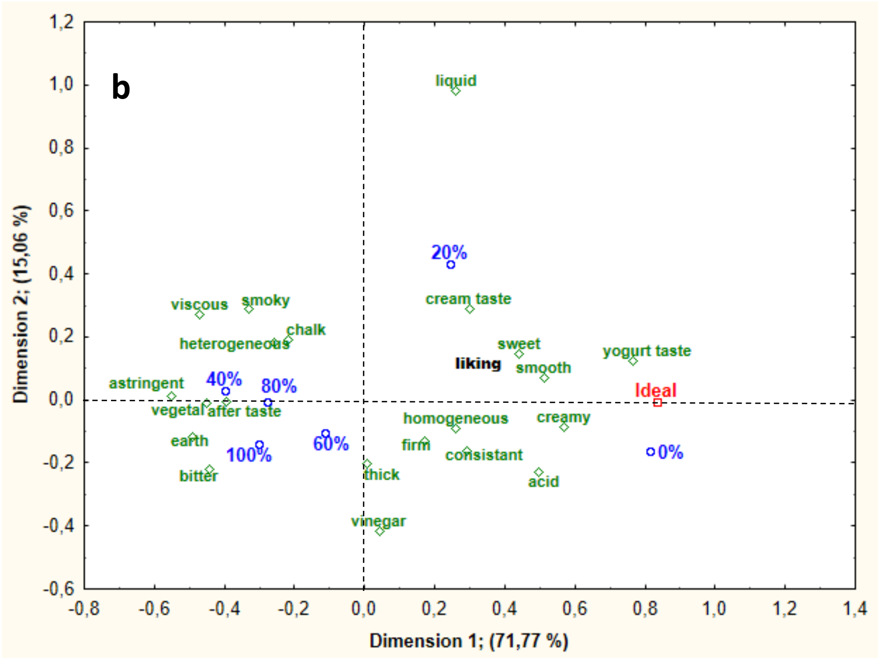


Figure 6. First two dimensions of the Correspondence Analysis carried out on the CATA frequency Table analysis for. (A) group G+ and (B) group G-. Liking and ideal product were projected as supplementary data. The size of the dots representing the six fermented products is proportional to their average liking scores.

5.2.4 Conclusion

Some changes in people eating habits are required to face the increasing animal protein consumption and to avoid the associated environmental problems. Fermentation could be a possible solution to facilitate the introduction of vegetal protein in food as starter cultures might have a beneficial effect on the physical, textural and sensory properties of fermented products. In this study, based on some preliminary results, a combination of *S. thermophilus* & *Lb. acidophilus* was used to ferment six mixtures of cow milk and pea milk (from 0% to 100% pea milk, with variation level 20%). Sensory and physical properties were investigated along with consumers' acceptability. From a physical point of view, all the products showed characteristics close to conventional yogurt. From sensorial point of view, 20% of pea protein gave a product accepted by most of consumers. On the other hand, for 40% there was a bimodal distribution of responses as this product assessed positively by some participants and negatively by others. Furthermore, products with 60%, 80% and 100% negatively assessed by most of participants. On the basis of our work, we could consider the production of yogurt with a reduced animal protein content of 20%, which could help to rebalance the ratio animal/ plant in our protein consumption.

5.3 Conclusion du chapitre 5

Malgré toutes les étapes d'optimisation présentées dans les chapitres précédents, l'analyse sensorielle n'a montré qu'une faible augmentation de l'acceptation de notre produit par les consommateurs. La plupart des consommateurs ont accepté le produit obtenu avec 20 % de protéines de pois, et certains participants ont accepté celui contenant 40 % de protéines de pois. Ces résultats ont donc permis de montrer qu'un yaourt mixte contenant jusqu'à 40 % de protéines de pois pouvait être apprécié par une partie des consommateurs mais la majorité des consommateurs n'est pas prête à consommer ce type de produit.

Discussion générale

L'idée de faire un yaourt de lait de soja a initialement été conçue en 1910. Beaucoup d'études et d'efforts ont été faits pour développer ce type de produit. Le premier yaourt au soja a été vendu en 1977 sous différentes flaveurs : nature, framboise, fraise et pêche.

Lors des dernières années, l'idée de supplémenter des yaourts à base de protéines animales par des protéines végétales a émergé. Certains auteurs ont testé des supplémentations ou des substitutions de protéine animale avec des protéines de légumineuses dans les produits fermentés (**Drake et al.**, 2000; **Zare et al.**, 2012, 2013). La plupart de ces études ont montré les effets négatifs de l'utilisation de protéines végétales même avec une supplémentation de 1 à 5 %. Beaucoup d'efforts sont donc encore nécessaires pour améliorer ce type de produits.

Lors de ce travail, une série d'études a été effectuée afin d'optimiser un nouveau produit fermenté à base de pois et pour comprendre les facteurs qui influencent l'acceptabilité de ce produit par les consommateurs. Nous nous sommes intéressé à la fois à des facteurs liés aux produits et des facteurs liés aux consommateurs.

Dans une première étude (**Proceeding 1**) nous avons cherché à évaluer l'impact des habitudes de consommation de produits fermentés à base de protéines végétales sur l'acceptation de notre produit. Conformément aux études précédentes, des consommateurs français, n'ayant pas l'habitude de consommer ce type de produits n'ont pas accepté les produits avec le plus faible taux de substitution testé (10 %). De façon plus surprenante, des consommateurs bulgares davantage familiers avec des produits fermentés à base végétale, n'ont pas davantage accepté les produits même avec le plus faible taux de substitution testé.

De nombreuses études ont montré que les allégations santé peuvent augmenter l'intention d'achat des consommateurs sur des produits à base de soja (**Moon et al.**, 2011). Dans une seconde étude (**Publication 1**), nous avons montré que si des allégations positives sur la santé attirent davantage les consommateurs que des allégations positives environnementales, les deux types d'informations n'ont pas permis d'améliorer l'acceptabilité des produits fermentés à base de pois. Cette absence d'effet des allégations est causée principalement par la présence de défauts liés à la protéine végétale utilisée, sa qualité et son interaction avec les protéines de lait. Dans cette publication, nous avons vu que l'utilisation de sucre a augmenté partiellement l'acceptabilité des produits fermentés à base de pois, mais cela ne peut pas être considéré comme une solution viable pour améliorer la qualité organoleptique à une époque où l'on cherche à diminuer les quantités de sucre ingérée par les consommateurs.

Ensuite, puisqu'il est connu que différentes souches lactiques peuvent conférer des caractéristiques spécifiques aux produits fermentés nous avons cherché à optimiser la qualité de notre produit en sélectionnant de nouvelles souches. **Schindler et al.** (2012) ont montré que différentes souches peuvent réduire la quantité d'hexanal et d'hexanol de la protéine de pois. Dans une troisième étude (**Publication 2**), dix cocktails des LABs ont été testés pour comparer les profils sensoriels et les propriétés physico-chimiques des produits fermentés afin de sélectionner les cocktails qui pourraient donner des produits acceptés. Bien que les cocktails (*Streptococcus thermophilus* + *Lactobacillus bulgaricus*) (*Streptococcus thermophilus* + *Lactobacillus acidophilus*) et (*Streptococcus thermophilus* + *Lactobacillus casei*) aient conduit à des produits fermentés « avec 30 et 40 % de pois » sans les attributs négatifs : l'amertume et l'astringence, cette publication a révélé la nécessité d'améliorer la texture de ces produits fermentés. En effet, la présence de protéines de pois a réduit de façon importante la fermeté des produits fermentés. Cette diminution de fermeté a soulevé la question de la qualité des globulines de pois utilisé dans cette étude et des paramètres de préparation des produits fermentés.

En ce qui concerne la qualité des globulines de pois, une comparaison entre le produit à base d'isolat industriel de pois (Nutralys) et celui à base de globulines de pois isolé au laboratoire a montré que ce dernier possède une structure plus ferme et plus stable (cohésion importante). Cela est probablement dû à la préservation de la fonctionnalité des protéines pendant l'extraction. Le produit fermenté à base d'isolat commercial est également apparu comme étant plus riche en molécules volatiles, avec une quantité plus élevée d'hexanal, que celui à base de globulines de pois isolé au laboratoire. Cette différence est peut-être due au procédé d'extraction protéique utilisé ainsi qu'aux conditions de stockage.

Pour tester cette hypothèse dans une quatrième étude (**Publication 3**) une série d'optimisation de production de produits fermentés ont été faites pour sélectionner les meilleures conditions. Mélanger les deux laits (pois et vache), avant le traitement thermique, semble permettre d'obtenir un réseau de gel plus ferme qu'un traitement séparé des deux laits. Si l'on chauffe les deux laits à température plus élevée, les protéines subissent une dénaturation plus importante et les agrégats deviennent plus fragiles. D'autres facteurs pourraient également affecter les produits fermentés comme la température d'incubation l'effet de ces facteurs dépendrait de plus du cocktail bactérien utilisé lors de la fermentation.

Du point de vue physico-chimique, le cocktail *Streptococcus thermophilus* + *Lactobacillus acidophilus* a réussi à donner un produit fermenté à base de pois proche du yaourt traditionnel avec une bonne fermeté, des niveaux faibles d'hexanal et d'hexanol, et une teneur élevée en composants typiques de l'arôme de yaourt. Une cinquième étude a été mise en place (**Publication 4**) pour obtenir une validation sensorielle des résultats précédents. Les résultats de cette étude ont montré que le cocktail *Streptococcus thermophilus* + *Lactobacillus acidophilus* avec 20 % de protéines de pois a donné lieu à un produit accepté par la plupart des consommateurs. Le produit avec 40 % de protéines de pois a été évalué positivement par certains panélistes et négativement par d'autres.

Conclusion et Perspectives

Ces travaux de recherche ont donné quelques résultats importants, et ont aussi permis d'identifier certaines pistes qui pourraient être intéressantes pour le développement de futurs produits :

- ❖ Dans cette étude, une comparaison entre un isolat industriel de globuline de pois et des globulines extraite d'une farine de pois a été effectuée. La claire différence observée entre ces deux sources de globulines de pois souligne l'importance de la source des protéines végétales et suggèrent quelques pistes de réflexion pour la suite de ces travaux. Avant de lancer de nouveaux travaux il serait important entre autres de :
    1. Faire le choix entre différentes variétés de pois. Quelques variétés peuvent révéler des profils d'arômes plus intéressants que d'autres, avec moins de faux goûts d'origine végétale.
    2. Optimiser une méthode d'extraction afin d'obtenir les globulines les plus fonctionnelles, et les moins riches en faux goûts.
    3. Réduire au maximum l'effet négatif du stockage en appliquant des conditions permettant de minimaliser l'oxydation qui conduit à la production de faux goûts.

- ❖ Dans cette étude, l'effet de la culture et de la familiarité d'une boisson proche de ce produit fermenté n'ont pas été mis en évidence. Cela peut être dû au fait que les produits végétaux fermentés en Bulgarie sont des céréales pas du pois et que ces deux types de produits végétaux n'ont pas les mêmes caractéristiques organoleptiques. Une étude avec une autre légumineuse comme le pois chiche, très consommé dans les pays du Moyen-Orient, pourrait davantage mettre en évidence l'effet positif de la familiarité sur l'acceptabilité et ainsi conduire à la proposition d'un nouveau produit végétal fermenté plus facilement acceptable.

- ❖ Une dernière piste de réflexion pourrait concerner l'ajout d'arômes naturels pour rendre le produit plus palatable tout en préservant la dimension développement durable.

Références

Ahmed, N.S. & Hassan, F.A.M. (1995), "Utilization of plant proteins in manufacture of cheese analogs", *Egyptian Journal of Food Science (Egypt)*.

Aiking, H. (2011), "Future protein supply", *Trends in Food Science & Technology*, Elsevier, Vol. 22 No. 2, pp. 112–120.

Aiking, H. (2014), "Protein production: planet, profit, plus people?", *The American Journal of Clinical Nutrition*, Am Soc Nutrition, Vol. 100 No. Supplement 1, p. 483S–489S.

Aiking, H., de Boer, J. & Vereijken, J. (2006), *Sustainable Protein Production and Consumption: Pigs or Peas?: Pigs Or Peas?*, Vol. 45, Springer Science & Business Media.

Alais, C. & Linden, G. (1997), "Abrégé de biochimie alimentaire. 4éme Edition Masson", *Paris,(119-123)*.

Alamanou, S. & Doxastakis, G. (1997), "Effect of wet extraction methods on the emulsifying and foaming properties of lupin seed protein isolates (Lupinus albus ssp. Graecus)", *Food Hydrocolloids*, Elsevier, Vol. 11 No. 4, pp. 409–413.

Alting, A.C., Hamer, R.J., de Kruif, C.G. & Visschers, R.W. (2000), "Formation of Disulfide Bonds in Acid-Induced Gels of Preheated Whey Protein Isolate", *Journal of Agricultural and Food Chemistry*, Vol. 48 No. 10, pp. 5001–5007.

Amatayakul, T., Sherkat, F. & Shah, N.P. (2006), "Syneresis in set yogurt as affected by EPS starter cultures and levels of solids", *International Journal of Dairy Technology*, Vol. 59 No. 3, pp. 216–221.

Ares, G., Barreiro, C., Deliza, R., Giménez, A.N.A. & Gambaro, A. (2010), "Application of a check-all-that-apply question to the development of chocolate milk desserts", *Journal of Sensory Studies*, Wiley Online Library, Vol. 25 No. s1, pp. 67–86.

Ares, G., Deliza, R., Barreiro, C., Giménez, A. & Gámbaro, A. (2010), "Comparison of two sensory profiling techniques based on consumer perception", *Food Quality and Preference*, Vol. 21 No. 4, pp. 417–426.

Ares, G. & Jaeger, S. (2014), "Check-all-that-apply (CATA) questions with consumers in practice. Experimental considerations and impact on outcome", *Rapid Sensory Profiling Techniques: Applications in New Product Development and Consumer Research*, Elsevier, pp. 227–245.

Ares, G. & Varela, P. (2014), "Comparison of novel methodologies for sensory characterization", *Novel Techniques in Sensory Characterization and Consumer Profiling*, CRC Press, pp. 365–390.

Arntfield, S.D., Murray, E.D., Ismond, M.A.H. & Bernatsky, A.M. (1989), "Role of the Thermal Denaturation-Aggregation Relationship in Determining the Rheological Properties of Heat Induced Networks for Ovalbumin and Vicilin", *Journal of Food Science*, Wiley Online Library, Vol. 54 No. 6, pp. 1624–1631.

Aston, J.W. & Dulley, J.R. (1982), "Cheddar cheese flavour", *Australian Journal of Dairy Technology*, Dairy Industry Association of Australia, Vol. 37 No. 2, p. 59.

Azzam, A.M. (1998), “Competition in the US meatpacking industry: is it history?”, *Agricultural Economics*, Elsevier, Vol. 18 No. 2, pp. 107–126.

Bazzano, L.A. (2008), “Effects of soluble dietary fiber on low-density lipoprotein cholesterol and coronary heart disease risk”, *Current Atherosclerosis Reports*, Springer, Vol. 10 No. 6, pp. 473–477.

Beal, C., Louvet, P. & Corrieu, G. (1989), “Influence of controlled pH and temperature on the growth and acidification of pure cultures of Streptococcus thermophilus 404 and Lactobacillus bulgaricus 398”, *Applied Microbiology and Biotechnology*, Springer-Verlag, Vol. 32 No. 2, pp. 148–154.

Beal, C., Skokanova, J., Latrille, E., Martin, N. & Corrieu, G. (1999), “Combined effects of culture conditions and storage time on acidification and viscosity of stirred yogurt”, *Journal of Dairy Science*, Elsevier, Vol. 82 No. 4, pp. 673–681.

Beal, C. & Sodini, I. (2003), “Fabrication des yaourts et des laits fermentés”, *Techniques de L’ingénieur. Bioprocédés*, Techniques de l’ingénieur, No. F6315.

Bezerra, M.F., Souza, D.F.S. & CORREIA, P. (2012), “Acidification kinetics, physicochemical properties and sensory attributes of yoghurts prepared from mixtures of goat and buffalo milks”, *International Journal of Dairy Technology*, Wiley Online Library, Vol. 65 No. 3, pp. 437–443.

Blagden, T.D. & Gilliland, S.E. (2005), “Reduction of levels of volatile components associated with the ‘beany’ flavor in soymilk by lactobacilli and streptococci”, *Journal of Food Science*, Wiley Online Library, Vol. 70 No. 3, pp. M186–M189.

Boatright, W.L. & Lei, Q. (1999), “Compounds contributing to the ‘beany’ odor of aqueous solutions of soy protein isolates”, *Journal of Food Science*, Wiley Online Library, Vol. 64 No. 4, pp. 667–670.

Boelrijk, A.E.M., de Jong, C. & Smit, G. (2003), “Flavour generation in dairy products”, *Dairy Processing: Improving Quality. Part 1: Dairy Product Safety and Quality*, Woodhead Publishing Ltd.

Boland, M.J., Rae, A.N., Vereijken, J.M., Meuwissen, M.P.M., Fischer, A.R.H., van Boekel, M.A.J.S., Rutherfurd, S.M., et al. (2013), “The future supply of animal-derived protein for human consumption”, *Trends in Food Science and Technology*.

Bora, P.S., Brekke, C.J. & Powers, J.R. (1994), “Heat Induced Gelation of Pea (Pisum sativum) Mixed Globulins, Vicilin and Legumin”, *Journal of Food Science*, Blackwell Publishing Ltd, Vol. 59 No. 3, pp. 594–596.

Boye, J., Zare, F. & Pletch, A. (2010), “Pulse proteins: Processing, characterization, functional properties and applications in food and feed”, *Food Research International*, Vol. 43 No. 2, pp. 414–431.

Božanić, R., Lovković, S. & Jeličić, I. (2011), “Optimising fermentation of soymilk with probiotic bacteria”, *Czech Journal of Food Sciences*, Vol. 29 No. 1, pp. 51–56.

Brochet, F. & Morrot, G. (1999), “Influence of the context on the perception of wine cognitive and methological implications”, *Journal International Des Sciences de La*

Vigne et Du Vin, VIGNE ET VIN PUBLICATIONS INTERNATIONALES, Vol. 33 No. 4, pp. 187–194.

Burdock, G.A. (2009), *Fenaroli's Handbook of Flavor Ingredients*, CRC press.

Camilleri, G.M., Verger, E.O., Huneau, J.-F., Carpentier, F., Dubuisson, C. & Mariotti, F. (2013), "Plant and animal protein intakes are differently associated with nutrient adequacy of the diet of French adults", *The Journal of Nutrition*, Am Soc Nutrition, Vol. 143 No. 9, pp. 1466–1473.

Campbell, L.J., Gu, X., Dewar, S.J. & Euston, S.R. (2009), "Effects of heat treatment and glucono-δ-lactone-induced acidification on characteristics of soy protein isolate", *Food Hydrocolloids*, Elsevier, Vol. 23 No. 2, pp. 344–351.

Cayot, P.H. & Lorient, D. (1998), *Structures et Technofonctions Des Protéines Du Lait*, Arilait Recherches.

Cederroth, C.R., Auger, J., Zimmermann, C., Eustache, F. & Nef, S. (2010), "Soy, phyto-oestrogens and male reproductive function: a review", *International Journal of Andrology*, Wiley Online Library, Vol. 33 No. 2, pp. 304–316.

Cerning, J. (1990), "Exocellular polysaccharides produced by lactic acid bacteria", *FEMS Microbiology Reviews*, The Oxford University Press, Vol. 7 No. 1–2, pp. 113–130.

Cerning, J. (1995), "Production of exopolysaccharides by lactic acid bacteria and dairy propionibacteria", *Le Lait*, EDP Sciences, Vol. 75 No. 4–5, pp. 463–472.

Champagne, C.P., Green-Johnson, J., Raymond, Y., Barrette, J. & Buckley, N. (2009), "Selection of probiotic bacteria for the fermentation of a soy beverage in combination with Streptococcus thermophilus", *Food Research International*, Vol. 42 No. 5, pp. 612–621.

Chandan, R.C. (2015), "Dairy Processing and Quality Assurance: An Overview", *Dairy Processing and Quality Assurance*, John Wiley & Sons, Ltd, pp. 1–40.

Cheng, H. (2010), "Volatile flavor compounds in yogurt: a review", *Critical Reviews in Food Science and Nutrition*, Taylor & Francis, Vol. 50 No. 10, pp. 938–950.

Chihi, M.-L., Mession, J., Sok, N. & Saurel, R. (2016), "Heat-induced soluble protein aggregates from mixed pea globulins and β-lactoglobulin", *Journal of Agricultural and Food Chemistry*, ACS Publications, Vol. 64 No. 13, pp. 2780–2791.

Chihi, M.L. (2016), "Etude des interactions dans la formation d'agrégats thermiques mixtes entre globulines de pois et béta-lactoglobuline: application à l'élaboration de gels acides", Université de Bourgogne.

Chumchuere, S. & Robinson, R.K. (1999), "Selection of starter cultures forthe fermentation of soya milk", *Food Microbiology*, Elsevier, Vol. 16 No. 2, pp. 129–137.

Coda, R., Lanera, A., Trani, A., Gobbetti, M. & Di Cagno, R. (2012), "Yogurt-like beverages made of a mixture of cereals, soy and grape must: microbiology, texture, nutritional and sensory properties", *International Journal of Food Microbiology*, Elsevier, Vol. 155 No. 3, pp. 120–127.

Cogan, T.M. (1995), “Flavour production by dairy starter cultures”, *Symposium Series-Society for Applied Bacteriology (United Kingdom)*.

Corredig, M. (2005), “Protein-Protein Interactions in Food”, *Ingredient Interactions*, CRC Press, pp. 283–308.

Dagorni-Scaviner, C., Gueguen, J. & Lefebvre, J. (1987), “Emulsifying properties of pea globulins as related to their adsorption behaviors”, *Journal of Food Science*, Vol. 52 No. 2, pp. 335–341.

Dean, M., Lampila, P., Shepherd, R., Arvola, A., Saba, A., Vassallo, M., Claupein, E., et al. (2012), “Perceived relevance and foods with health-related claims”, *Food Quality and Preference*, Elsevier, Vol. 24 No. 1, pp. 129–135.

Domínguez-Soberanes, J., García-Garibay, M., Casas-Alencáster, N.B. & Martínez-Padilla, L.P. (2001), “Instrumental texture of set and stirred fermented milk. Effect of a ropy strain of Lactobacillus delbrueckii subsp. bulgaricus and an enriched substrate”, *Journal of Texture Studies*, Wiley Online Library, Vol. 32 No. 3, pp. 205–217.

Donato, L., Alexander, M. & Dalgleish, D.G. (2007), “Acid gelation in heated and unheated milks: interactions between serum protein complexes and the surfaces of casein micelles”, *Journal of Agricultural and Food Chemistry*, ACS Publications, Vol. 55 No. 10, pp. 4160–4168.

Dooley, L., Lee, Y. & Meullenet, J.-F. (2010), “The application of check-all-that-apply (CATA) consumer profiling to preference mapping of vanilla ice cream and its comparison to classical external preference mapping”, *Food Quality and Preference*, Elsevier, Vol. 21 No. 4, pp. 394–401.

Drake, M.A., Chen, X.Q., Tamarapu, S. & Leenanon, B. (2000), “Soy protein fortification affects sensory, chemical, and microbiological properties of dairy yogurts”, *Journal of Food Science*, Vol. 65 No. 7, pp. 1244–1247.

Duboc, P. & Mollet, B. (2001), “Applications of exopolysaccharides in the dairy industry”, *International Dairy Journal*, Elsevier, Vol. 11 No. 9, pp. 759–768.

Eastham, E.J. (1989), “Soy protein allergy”, *Carnation Nutrition Education Series (USA)*.

Esnouf, C., Russel, M. & Bricas, N. (2013), *Food System Sustainability: Insights from duALIne*, Cambridge University Press.

Faber, E.J., Zoon, P., Kamerling, J.P. & Vliegenthart, J.F.G. (1998), “The exopolysaccharides produced by Streptococcus thermophilus Rs and Sts have the same repeating unit but differ in viscosity of their milk cultures”, *Carbohydrate Research*, Elsevier, Vol. 310 No. 4, pp. 269–276.

Fallon, A.E. & Rozin, P. (1983), “The psychological bases of food rejections by humans”, *Ecology of Food and Nutrition*, Taylor & Francis, Vol. 13 No. 1, pp. 15–26.

Fitzgerald, A.J. (2015), *Animals as Food:(re) Connecting Production, Processing, Consumption, and Impacts*, MSU Press.

Franklin, A. (1999), *Animals and Modern Cultures: A Sociology of Human-Animal Relations* *in Modernity*, Sage.

Fryer, T.F. (1970), “Utilization of citrate by lactobacilli isolated from dairy products”, *Journal of Dairy Research*, Cambridge Univ Press, Vol. 37 No. 1, pp. 9–15.

Gains, N. (1994), “The repertory grid approach”, *Measurement of Food Preferences*, Springer, pp. 51–76.

Gains, N., Krzanowski, W.J. & Thomson, D.M.H. (1988), “A comparison of variable reduction techniques in an attitudinal investigation of meat products”, *Journal of Sensory Studies*, Wiley Online Library, Vol. 3 No. 1, pp. 37–48.

Garnett, T. (2013), “Food sustainability: problems, perspectives and solutions”, *Proceedings of the Nutrition Society*, Cambridge Journals Online, Vol. 72 No. 1, pp. 29–39.

Gorbach, S.L. (2002), “Probiotics in the third millennium”, *Digestive and Liver Disease*, Elsevier, Vol. 34, pp. S2–S7.

Griffiths, M.W. & Tellez, A.M. (2013), “Lactobacillus helveticus: the proteolytic system”, *Frontiers in Microbiology*, Vol. 4:30, pp. 1–9.

Grigg, D. (1995), “The nutritional transition in Western Europe”, *Journal of Historical Geography*, Academic Press., Vol. 21 No. 3, p. 247.

Gussow, J.D. & Clancy, K.L. (1986), “Dietary guidelines for sustainability”, *Journal of Nutrition Education*, Vol. 18 No. 1, pp. 1–5.

Guzel-Seydim, Z.B., Sezgin, E. & Seydim, A.C. (2005), “Influences of exopolysaccharide producing cultures on the quality of plain set type yogurt”, *Food Control*, Vol. 16 No. 3, pp. 205–209.

Halkjær, J., Olsen, A., Bjerregaard, L.J., Deharveng, G., Tjønneland, A., Welch, A.A., Crowe, F.L., et al. (2009), “Intake of total, animal and plant proteins, and their food sources in 10 countries in the European Prospective Investigation into Cancer and Nutrition”, *European Journal of Clinical Nutrition*, Nature Publishing Group, Vol. 63, pp. S16–S36.

Hansen, A.P. & Booker, D.C. (1996), “Flavor interaction with casein and whey protein”, ACS Publications.

Hansen, M., Jakobsen, H.B. & Christensen, L.P. (2000), “The aroma profile of frozen green peas used for cold or warm consumption”, in Schieberle, P. and Engel, K.H. (Eds.), *The 9th Weurman Flavour Symposium Proceedings: Frontiers of Flavour Science*, Deutsche Forschungsanstalt für Lebensmittelchemie, Garching, pp. 69–73.

Haque, A., Richardson, R.K. & Morris, E.R. (2001), “Effect of fermentation temperature on the rheology of set and stirred yogurt”, *Food Hydrocolloids*, Elsevier, Vol. 15 No. 4, pp. 593–602.

Harper, S.J., Barnes, D.L., Bodyfelt, F.W. & McDaniel, M.R. (1991), “Sensory Ratings of Commercial Plain Yogurts by Consumer and Descriptive Panels”, *Journal of Dairy Science*, Elsevier, Vol. 74 No. 9, pp. 2927–2935.

Hartmann, C. & Siegrist, M. (2017), “Consumer perception and behaviour regarding sustainable protein consumption: A systematic review”, *Trends in Food Science &*

Technology, Elsevier.

Hassan, A.N., Frank, J.F. & Qvist, K.B. (2002), "Direct observation of bacterial exopolysaccharides in dairy products using confocal scanning laser microscopy", *Journal of Dairy Science*, Elsevier, Vol. 85 No. 7, pp. 1705–1708.

Hassan, A.N., Frank, J.F., Schmidt, K.A. & Shalabi, S.I. (1996), "Textural properties of yogurt made with encapsulated nonropy lactic cultures", *Journal of Dairy Science*, Vol. 79 No. 12, pp. 2098–2103.

Hemmerling, S., Canavari, M. & Spiller, A. (2016), "Preference for naturalness of European organic consumers", *British Food Journal*, Emerald, Vol. 118 No. 9, pp. 2287–2307.

Hoffmann, M.A.M. & van Mil, P.J.J.M. (1997), "Heat-induced aggregation of β-lactoglobulin: role of the free thiol group and disulfide bonds", *Journal of Agricultural and Food Chemistry*, ACS Publications, Vol. 45 No. 8, pp. 2942–2948.

Horne, D.S. (2009), "Casein micelle structure and stability", *Milk Proteins: From Expression to Food*, Academic Press/Elsevier Amsterdam, pp. 133–162.

Hsieh, Y.I.N.L., Yun, J.J. & Rao, M.A. (1993), "Rheological properties of Mozzarella cheese filled with dairy, egg, soy proteins, and gelatin", *Journal of Food Science*, Vol. 58 No. 5, pp. 1001–1004.

Imhof, R., Glättli, H. & Bosset, J.O. (1994), "Volatile organic aroma compounds produced by thermophilic and mesophilic mixed strain dairy starter cultures", *LWT-Food Science and Technology*, Elsevier, Vol. 27 No. 5, pp. 442–449.

Imhof, R., Glättli, H. & Bosset, J.O. (1995), "Volatile organic compounds produced by thermophilic and mesophilic single strain dairy starter cultures", *LWT - Food Science and Technology*, Vol. 28 No. 1, pp. 78–86.

ISO. (1994), *Sensory Analysis: Identification and Selection of Descriptors for Establishing a Sensory Profile by a Multidimensional Approach*, International Organization for Standardization.

Jackson, P., Boulter, D. & Thurman, D.A. (1969), "A comparison of some properties of vicilin and legumin isolated from seeds of Pisum sativum, Vicia faba and Cicer arietinum", *New Phytologist*, Wiley Online Library, Vol. 68 No. 1, pp. 25–33.

Jaeger, S.R., Chheang, S.L., Yin, J., Bava, C.M., Gimenez, A., Vidal, L. & Ares, G. (2013), "Check-all-that-apply (CATA) responses elicited by consumers: Within-assessor reproducibility and stability of sensory product characterizations", *Food Quality and Preference*, Elsevier, Vol. 30 No. 1, pp. 56–67.

Jaffuel, D., Demoly, P. & Bousquet, J. (2001), "Les allergies alimentaires", *Revue Française D'allergologie et D'immunologie Clinique*, Elsevier, Vol. 41 No. 2, pp. 169–186.

James, D. (2004), "Factors influencing food choices, dietary intake, and nutrition-related attitudes among African Americans: Application of a culturally sensitive model", *Ethnicity & Health*, Vol. 9 No. 4, pp. 349–367.

Johansen, S.B., Næs, T., Øyaas, J. & Hersleth, M. (2010), "Acceptance of calorie-reduced yoghurt: Effects of sensory characteristics and product information", *Food Quality and Preference*, Vol. 21 No. 1, pp. 13–21.

Kähkönen, P., Tuorila, H. & Lawless, H. (1997), "Lack of effect of taste and nutrition claims on sensory and hedonic responses to a fat-free yogurt", *Food Quality and Preference*, Vol. 8 No. 2, pp. 125–130.

Kähkönen, P., Tuorila, H. & Rita, H. (1996), "How information enhances acceptability of a low-fat spread", *Food Quality and Preference*, Vol. 7 No. 2, pp. 87–94.

Kasulis, J.J., Huettner, D.A. & Dikeman, N.J. (1981), "The feasibility of changing electricity consumption patterns", *Journal of Consumer Research*, The Oxford University Press, Vol. 8 No. 3, pp. 279–290.

Khan, M.A. & Hackler, L.R. (1981), "Evaluation of food selection patterns and preferences", *Critical Reviews In Food Science & Nutrition*, Taylor & Francis, Vol. 15 No. 2, pp. 129–153.

Kim, Y.-K., Jombart, L., Valentin, D. & Kim, K.-O. (2013), "A cross-cultural study using Napping®: Do Korean and French consumers perceive various green tea products differently?", *Food Research International*, Vol. 53 No. 1, pp. 534–542.

Kimura, A., Fukuda, T., Zhang, M., Motoyama, S., Maruyama, N. & Utsumi, S. (2008), "Comparison of Physicochemical Properties of 7S and 11S Globulins from Pea, Fava Bean, Cowpea, and French Bean with Those of Soybean French Bean 7S Globulin Exhibits Excellent Properties", *Journal of Agricultural and Food Chemistry*, ACS Publications, Vol. 56 No. 21, pp. 10273–10279.

King, S., Gillette, M., Titman, D., Adams, J. & Ridgely, M. (2002), "The Sensory Quality System: a global quality control solution", *Food Quality and Preference*, Elsevier, Vol. 13 No. 6, pp. 385–395.

Kinsella, J.E. & Melachouris, N. (1976), "Functional properties of proteins in foods: A survey", *C R C Critical Reviews in Food Science and Nutrition*, Taylor & Francis, Vol. 7 No. 3, pp. 219–280.

Kinsella, J.E. & Morr, C. V. (1984), "Milk proteins: physicochemical and functional properties", *Critical Reviews in Food Science & Nutrition*, Taylor & Francis, Vol. 21 No. 3, pp. 197–262.

Klein, B.P. & Raidl, M.A. (1986), "Use of Field-Pea Flours as Protein Supplements in Foods", in Ory, R.L. (Ed.), *Plant Proteins: Applications, Biological Effects, and Chemistry*, Vol. 312, American Chemical Society, Chicago, pp. 19–31.

Kneifel, W. & Seiler, A. (1993), "Water-holding properties of milk protein products-A review", *Food Structure*, Vol. 12 No. 3, p. 3.

Kobayashi, A., Tsuda, Y., Hirata, N., Kubota, K. & Kitamura, K. (1995), "Aroma constituents of soybean [Glycine max (L.) Merril] milk lacking lipoxygenase isozymes", *Journal of Agricultural and Food Chemistry*, American Chemical Society, Vol. 43 No. 9, pp. 2449–2452.

Kuda, T., Kataoka, M., Nemoto, M., Kawahara, M., Takahashi, H. & Kimura, B. (2016), "Isolation of lactic acid bacteria from plants of the coastal Satoumi regions for use as starter cultures in fermented milk and soymilk production", *LWT-Food Science and Technology*, Elsevier, Vol. 68, pp. 202–207.

Kühn, J., Considine, T. & Singh, H. (2006), "Interactions of Milk Proteins and Volatile Flavor Compounds: Implications in the Development of Protein Foods", *Journal of Food Science*, Blackwell Publishing Inc, Vol. 71 No. 5, pp. R72–R82.

Lado, J., Vicente, E., Manzzioni, A. & Ares, G. (2010), "Application of a check-all-that-apply question for the evaluation of strawberry cultivars from a breeding program", *Journal of the Science of Food and Agriculture*, Wiley Online Library, Vol. 90 No. 13, pp. 2268–2275.

Lam, A.C.Y., Can Karaca, A., Tyler, R.T. & Nickerson, M.T. (2016), "Pea protein isolates: Structure, extraction, and functionality", *Food Reviews International*, Taylor & Francis, pp. 1–22.

Land, D.G. (1983), "What is sensory quality", *Sensory Quality in Foods and Beverages. Chichester: Ellis Horwood*.

Lange, C., Martin, C., Chabanet, C., Combris, P. & Issanchou, S. (2002), "Impact of the information provided to consumers on their willingness to pay for Champagne: comparison with hedonic scores", *Food Quality and Preference*, Elsevier, Vol. 13 No. 7, pp. 597–608.

Lee, W.J. & Lucey, J.A. (2004), "Structure and physical properties of yogurt gels: Effect of inoculation rate and incubation temperature", *Journal of Dairy Science*, Elsevier, Vol. 87 No. 10, pp. 3153–3164.

Lee, Y.H. & Marshall, R.T. (1979), "Rennet curd from milk plus soy protein mixtures", *Journal of Dairy Science*, Vol. 62 No. 7, pp. 1051–1057.

Leip, A., Billen, G., Garnier, J., Grizzetti, B., Lassaletta, L., Reis, S., Simpson, D., et al. (2015), "Impacts of European livestock production: nitrogen, sulphur, phosphorus and greenhouse gas emissions, land-use, water eutrophication and biodiversity", *Environmental Research Letters*, IOP Publishing, Vol. 10 No. 11, p. 115004.

Lemieux, L. & Simard, R.E. (1992), "Bitter flavour in dairy products. II. A review of bitter peptides from caseins: their formation, isolation and identification, structure masking and inhibition", *Le Lait*, EDP Sciences, Vol. 72 No. 4, pp. 335–385.

Leroy, F. & De Vuyst, L. (2004), "Lactic acid bacteria as functional starter cultures for the food fermentation industry", *Trends in Food Science & Technology*, Vol. 15 No. 2, pp. 67–78.

Li-Chan, E.C.Y. (2004), "2 - Properties of proteins in food systems: an introduction A2 - Yada, R.Y. BT - Proteins in Food Processing", *Woodhead Publishing Series in Food Science, Technology and Nutrition*, Woodhead Publishing, pp. 2–26.

Li, Z., Grün, I.U. & Fernando, L.N. (2000), "Interaction of vanillin with soy and dairy proteins in aqueous model systems: a thermodynamic study", *Journal of Food Science*, Wiley Online Library, Vol. 65 No. 6, pp. 997–1001.

Lin, Y., Mouratidou, T., Vereecken, C., Kersting, M., Bolca, S., De Moraes, A.C.F., Cuenca-García, M., et al. (2015), “Dietary animal and plant protein intakes and their associations with obesity and cardio-metabolic indicators in European adolescents: the HELENA cross-sectional study”, *Nutrition Journal*, BioMed Central, Vol. 14 No. 1, p. 1.

Van Loo, E.J., Caputo, V., Nayga, R.M. & Verbeke, W. (2014), “Consumers’ valuation of sustainability labels on meat”, *Food Policy*, Elsevier, Vol. 49, pp. 137–150.

Lu, X., Schmitt, D. & Chen, S. (2010), “Effect of sesame protein isolate in partial replacement of milk protein on the rheological, textural and microstructural characteristics of fresh cheese”, *International Journal of Food Science & Technology*, Vol. 45 No. 7, pp. 1368–1377.

Lucey, J.A. & Singh, H. (1997), “Formation and physical properties of acid milk gels: a review”, *Food Research International*, Elsevier, Vol. 30 No. 7, pp. 529–542.

Ma, Z., Boye, J.I., Simpson, B.K., Prasher, S.O., Monpetit, D. & Malcolmson, L. (2011), “Thermal processing effects on the functional properties and microstructure of lentil, chickpea, and pea flours”, *Food Research International*, Vol. 44 No. 8, pp. 2534–2544.

Magnusson, M.K., Arvola, A., Hursti, U.-K.K., Åberg, L. & Sjödén, P.-O. (2003), “Choice of organic foods is related to perceived consequences for human health and to environmentally friendly behaviour”, *Appetite*, Elsevier, Vol. 40 No. 2, pp. 109–117.

Mäkinen, O.E., Wanhalinna, V., Zannini, E. & Arendt, E.K. (2016), “Foods for special dietary needs: Non-dairy plant-based milk substitutes and fermented dairy-type products”, *Critical Reviews in Food Science and Nutrition*, Taylor & Francis, Vol. 56 No. 3, pp. 339–349.

Marilley, L., Ampuero, S., Zesiger, T. & Casey, M.G. (2004), “Screening of aroma-producing lactic acid bacteria with an electronic nose”, *International Dairy Journal*, Vol. 14 No. 10, pp. 849–856.

Van Marle, M.E. & Zoon, P. (1995), “Permeability and rheological properties of microbially and chemically acidified skim-milk gels”, *Nederlands Melk En Zuiveltijdschrift*, Centen, Vol. 49 No. 1, pp. 47–65.

Mazur, W. (1998), “11 Phytoestrogen content in foods”, *Baillière’s Clinical Endocrinology and Metabolism*, Vol. 12 No. 4, pp. 729–742.

McCrory, M.A., Hamaker, B.R., Lovejoy, J.C. & Eichelsdoerfer, P.E. (2010), “Pulse consumption, satiety, and weight management.”, *Advances in Nutrition (Bethesda, Md.)*, Vol. 1 No. 1, pp. 17–30.

McGorrin, R.J. (2001), “Advances in dairy flavor chemistry”, *SPECIAL PUBLICATION-ROYAL SOCIETY OF CHEMISTRY*, Royal Society of Chemistry; 1994, Vol. 274, pp. 67–84.

McMahon, D.J. & Brown, R.J. (1984), “Composition, Structure, and Integrity of Casein Micelles: A Review1”, *Journal of Dairy Science*, Vol. 67 No. 3, pp. 499–512.

Medeiros, A.C., Souza, D.F. & Correia, R.T.P. (2015), “Effect of incubation temperature, heat treatment and milk source on the yoghurt kinetic acidification.”, *International Food Research Journal*, Faculty of Food Science and Technology, Vol. 22 No. 3, pp. 1030–

1036.

Mession, J.-L., Assifaoui, A., Cayot, P. & Saurel, R. (2012), "Effect of pea proteins extraction and vicilin/legumin fractionation on the phase behavior in admixture with alginate", *Food Hydrocolloids*, Elsevier, Vol. 29 No. 2, pp. 335–346.

Mession, J.-L., Chihi, M.L., Sok, N. & Saurel, R. (2015), "Effect of globular pea proteins fractionation on their heat-induced aggregation and acid cold-set gelation", *Food Hydrocolloids*, Vol. 46, pp. 233–243.

Moon, W., Balasubramanian, S.K. & Rimal, A. (2005), "Perceived Health Benefits and Soy Consumption Behavior: Two-Stage Decision Model Approach", *Journal of Agricultural and Resource Economics*, Western Agricultural Economics Association, Vol. 30 No. 2, pp. 315–332.

Moon, W., Balasubramanian, S.K. & Rimal, A. (2011), "Health claims and consumers' behavioral intentions: The case of soy-based food", *Food Policy*, Vol. 36 No. 4, pp. 480–489.

Morris, C.G. (1992), *Academic Press Dictionary of Science and Technology*, Gulf Professional Publishing.

Moslehishad, M., Mirdamadi, S., Ehsani, M.R., Ezzatpanah, H. & Moosavi-Movahedi, A.A. (2013), "The proteolytic activity of selected lactic acid bacteria in fermenting cow's and camel's milk and the resultant sensory characteristics of the products", *International Journal of Dairy Technology*, Vol. 66 No. 2, pp. 279–285.

Murat, C., Bard, M.-H., Dhalleine, C. & Cayot, N. (2013), "Characterisation of odour active compounds along extraction process from pea flour to pea protein extract", *Food Research International*, Vol. 53 No. 1, pp. 31–41.

Murat, C., Gourrat, K., Jerosch, H. & Cayot, N. (2012), "Analytical comparison and sensory representativity of SAFE, SPME, and Purge and Trap extracts of volatile compounds from pea flour", *Food Chemistry*, Elsevier, Vol. 135 No. 3, pp. 913–920.

Neviani, E., Giraffa, G., Brizzi, A. & Carminati, D. (1995), "Amino acid requirements and peptidase activities of Streptococcus salivarius subsp. thermophilus", *Journal of Applied Bacteriology*, Wiley Online Library, Vol. 79 No. 3, pp. 302–307.

O'Kennedy, B.T. (2011), "2 - Caseins BT - Handbook of Food Proteins", *Woodhead Publishing Series in Food Science, Technology and Nutrition*, Woodhead Publishing, pp. 13–29.

O'neill, T.E. & Kinsella, J.E. (1987), "Flavor Protein Interactions: Characteristics of 2-Nonanone Binding to Isolated Soy Protein Fractions", *Journal of Food Science*, Wiley Online Library, Vol. 52 No. 1, pp. 98–101.

Obatolu, V.A., Fasoyiro, S.B. & Ogunsunmi, L. (2007), "Processing and functional properties of yam beans (Sphenostylis stenocarpa)", *Journal of Food Processing and Preservation*, Wiley Online Library, Vol. 31 No. 2, pp. 240–249.

Oliveira, M.N., Sodini, I., Remeuf, F. & Corrieu, G. (2001), "Effect of milk supplementation and culture composition on acidification, textural properties and microbiological stability of fermented milks containing probiotic bacteria", *International Dairy Journal*, Elsevier, Vol. 11 No. 11, pp. 935–942.

Osborne, T.B. & Campbell, G.F. (1898), "PROTEIDS OF THE PEA.", *Journal of the American Chemical Society*, American Chemical Society, Vol. 20 No. 5, pp. 348–362.

Ott, A., Fay, L.B. & Chaintreau, A. (1997), "Determination and origin of the aroma impact compounds of yogurt flavor", *Journal of Agricultural and Food Chemistry*, ACS Publications, Vol. 45 No. 3, pp. 850–858.

Owusu-Ansah, Y.J. & McCurdy, S.M. (1991), "Pea proteins: A review of chemistry, technology of production, and utilization", *Food Reviews International*, Taylor & Francis, Vol. 7 No. 1, pp. 103–134.

Pagès, J., Bertrand, C., Ali, R., Husson, F. & Lê, S. (2007), "Sensory analysis comparison of eight biscuits by French and Pakistani panels", *Journal of Sensory Studies*, Wiley Online Library, Vol. 22 No. 6, pp. 665–686.

Parris, N. & Baginski, M.A. (1991), "A rapid method for the determination of whey protein denaturation", *Journal of Dairy Science*, Elsevier, Vol. 74 No. 1, pp. 58–64.

Pattee, H.E., Salunkhe, D.K., Sathe, S.K., Reddy, N.R. & Ory, R.L. (1983), "Legume lipids", *Critical Reviews in Food Science & Nutrition*, Taylor & Francis, Vol. 17 No. 2, pp. 97–139.

Pelegrine, D.H.G. & Gasparetto, C.A. (2005), "Whey proteins solubility as function of temperature and pH", *LWT-Food Science and Technology*, Elsevier, Vol. 38 No. 1, pp. 77–80.

Pellegrino, L., Masotti, F., Cattaneo, S., Hogenboom, J.A. & de Noni, I. (2013), "Nutritional Quality of Milk Proteins", in McSweeney, P.L.H. and Fox, P.F. (Eds.), *Advanced Dairy Chemistry: Volume 1A: Proteins: Basic Aspects, 4th Edition*, Springer US, Boston, MA, pp. 515–538.

Pilgrim, F.J. (1957), "The components of food acceptance and their measurement", *The American Journal of Clinical Nutrition*, Am Soc Nutrition, Vol. 5 No. 2, pp. 171–175.

Pliner, P. (1982), "The effects of mere exposure on liking for edible substances", *Appetite*, Elsevier, Vol. 3 No. 3, pp. 283–290.

Pliner, P. & Hobden, K. (1992), "Development of a scale to measure the trait of food neophobia in humans", *Appetite*, Vol. 19 No. 2, pp. 105–120.

Pliner, P., Pelchat, M. & Grabski, M. (1993), "Reduction of neophobia in humans by exposure to novel foods", *Appetite*, Elsevier, Vol. 20 No. 2, pp. 111–123.

Prescott, J., Young, O., O'Neill, L., Yau, N.J.. & Stevens, R. (2002), "Motives for food choice: a comparison of consumers from Japan, Taiwan, Malaysia and New Zealand", *Food Quality and Preference*, Vol. 13 No. 7–8, pp. 489–495.

Protonotariou, S. V, Karali, E., Evageliou, V., Yanniotis, S. & Mandala, I. (2013), "Rheological and sensory attributes of cream caramel desserts containing fructooligosaccharides as substitute sweeteners", *International Journal of Food Science & Technology*, Wiley

Online Library, Vol. 48 No. 3, pp. 663–669.

Raghunathan, R., Naylor, R.W. & Hoyer, W.D. (2006), “The Unhealthy = Tasty Intuition and Its Effects on Taste Inferences, Enjoyment, and Choice of Food Products”, *Journal of Marketing*, Vol. 70 No. 4, pp. 170–184.

Randall, E. & Sanjur, D. (1981), “Food preferences—their conceptualization and relationship to consumption”, *Ecology of Food and Nutrition*, Taylor & Francis, Vol. 11 No. 3, pp. 151–161.

Rinaldoni, A.N., Palatnik, D.R., Zaritzky, N. & Campderrós, M.E. (2014), “Soft cheese-like product development enriched with soy protein concentrates”, *LWT - Food Science and Technology*, Vol. 55 No. 1, pp. 139–147.

Rizkalla, S.W., Bellisle, F. & Slama, G. (2002), “Health benefits of low glycaemic index foods, such as pulses, in diabetic patients and healthy individuals”, *British Journal of Nutrition*, Cambridge Univ Press, Vol. 88 No. S3, pp. 255–262.

Robinson, R.K. & Wilbey, R.A. (1998), “Tests for acidity and chemical analysis in process control”, *Cheesemaking Practice*, Springer, pp. 81–97.

Rousset, S., Patureau Mirand, P., Brandolini, M., Martin, J.-F. & Boirie, Y. (2003), “Daily protein intakes and eating patterns in young and elderly French”, *British Journal of Nutrition*, Cambridge University Press, Vol. 90 No. 6, p. 1107.

Routray, W. & Mishra, H.N. (2011), “Scientific and technical aspects of yogurt aroma and taste: a review”, *Comprehensive Reviews in Food Science and Food Safety*, Wiley Online Library, Vol. 10 No. 4, pp. 208–220.

Roy, D., Leduy, A. & Goulet, J. (1987), “Kinetics of growth and lactic acid production from whey permeate by Lactobacillus helveticus”, *The Canadian Journal of Chemical Engineering*, Vol. 65 No. 4, pp. 597–603.

Rozin, E. (1973), *The Flavor-Principle Cookbook*, Hawthorn Books, available at: https://books.google.fr/books?id=DrxHGQAACAAJ.

Rozin, P. (1996), “The socio-cultural context of eating and food choice”, *Food Choice, Acceptance and Consumption*, Springer US, Boston, MA, pp. 83–104.

Rozin, P. & Fallon, A. (1980), “The psychological categorization of foods and non-foods: A preliminary taxonomy of food rejections”, *Appetite*, Elsevier, Vol. 1 No. 3, pp. 193–201.

Rozin, P.H., Haidt, J. & McCauley, C. (1993), “Disgust”, *Handbook of Emotions New York: Guilford*, pp. 575–594.

Ruas-Madiedo, P., Hugenholtz, J. & Zoon, P. (2002), “An overview of the functionality of exopolysaccharides produced by lactic acid bacteria”, *International Dairy Journal*, Vol. 12 No. 2, pp. 163–171.

Sabaté, J. & Soret, S. (2014), “Sustainability of plant-based diets: back to the future”, *The American Journal of Clinical Nutrition*, Am Soc Nutrition, Vol. 100 No. Supplement 1, p. 476S–482S.

Saher, M., Lindeman, M. & Hursti, U.-K.K. (2006), “Attitudes towards genetically modified and organic foods", *Appetite*, Elsevier, Vol. 46 No. 3, pp. 324–331.

Saint-Eve, A., Juteau, A., Atlan, S., Martin, N. & Souchon, I. (2006), "Complex viscosity induced by protein composition variation influences the aroma release of flavored stirred yogurt", *Journal of Agricultural and Food Chemistry*, ACS Publications, Vol. 54 No. 11, pp. 3997–4004.

Saint-Eve, A., Levy, C., Le Moigne, M., Ducruet, V. & Souchon, I. (2008), "Quality changes in yogurt during storage in different packaging materials", *Food Chemistry*, Vol. 110 No. 2, pp. 285–293.

Samant, S.S. & Seo, H.-S. (2016), "Quality perception and acceptability of chicken breast meat labeled with sustainability claims vary as a function of consumers' label-understanding level", *Food Quality and Preference*, Elsevier, Vol. 49, pp. 151–160.

Schindler, S., Zelena, K., Krings, U., Bez, J., Eisner, P. & Berger, R.G. (2012), "Improvement of the aroma of Pea (Pisum sativum) protein extracts by lactic acid fermentation", *Food Biotechnology*, Taylor & Francis, Vol. 26 No. 1, pp. 58–74.

Schneider, A. & Lacampagne, J.P. (2000), "Peas: a European production of protein-rich materials for feed and food", *Industrial Proteins*, Vol. 8 No. 1, pp. 3–6.

Schulze, G. & Watson, N. V. (1995), "Comments on 'Flavor neophobia in selected rodent species'", *Biological Perspectives on Motivated Activities. Ablex Publishing Corporation, Norwood, New Jersey*, pp. 229–230.

Schwenke, K.D. (2001), "Reflections about the functional potential of legume proteins A Review", *Molecular Nutrition & Food Research*, Wiley Online Library, Vol. 45 No. 6, pp. 377–381.

Sethi, S., Tyagi, S.K. & Anurag, R.K. (2016), "Plant-based milk alternatives an emerging segment of functional beverages: a review", *Journal of Food Science and Technology*, Springer, Vol. 53 No. 9, pp. 3408–3423.

Shepherd, D. (1985), "Dietary salt intake", *Nutrition & Food Science*, MCB UP Ltd, Vol. 85 No. 5, pp. 10–11.

Shepherd, R. & Raats, M.M. (1996), "Attitudes and beliefs in food habits", *Food Choice, Acceptance and Consumption*, Springer, pp. 346–364.

Shevkani, K., Singh, N., Kaur, A. & Rana, J.C. (2015), "Structural and functional characterization of kidney bean and field pea protein isolates: A comparative study", *Food Hydrocolloids*, Elsevier, Vol. 43, pp. 679–689.

Shimamura, T. & Ukeda, H. (2012), "Maillard reaction in milk–effect of heat treatment", *Milk Protein, InTech, Rijeka, Croatia*, pp. 147–158.

Singh, J. & Ranganathan, B. (1978), "A comparison of the activity of Lactobacillus bulgaricus and one of its mutants in different types of milk", *Journal of Dairy Research*, Cambridge Univ Press, Vol. 45 No. 1, pp. 123–125.

Singh, T.K., Cadwallader, K.R. & Drake, M. (2007), "Biochemical processes in the production of flavor in milk and milk products", *Handbook of Food Products Manufacturing*, A

John Wiley&Sons Inc, New Jersey, Vol. 2, pp. 715–748.

Sobal, J., Bisogni, C.A., Devine, C.M. & Jastran, M. (2006), “A conceptual model of the food choice process over the life course”, *Frontiers in Nutritional Science*, CABI PUBLISHING, Vol. 3, p. 1.

El Soda, M., Abd El Wahab, H., Ezzat, N., Desmazeaud, M.J. & Ismail, A. (1986), “The esterolytic and lipolytic activities of the Lactobacilli. II. Detection of the esterase system of Lactobacillus helveticus, Lactobacillus bulgaricus. Lactobacillus lactis and Lactobacillus acidophilus”, *Le Lait*, Vol. 66 No. 4, pp. 431–443.

Sodini, I., Montella, J. & Tong, P.S. (2005), “Physical properties of yogurt fortified with various commercial whey protein concentrates”, *Journal of the Science of Food and Agriculture*, Wiley Online Library, Vol. 85 No. 5, pp. 853–859.

Sodini, I., Remeuf, F., Haddad, S. & Corrieu, G. (2004), “The relative effect of milk base, starter, and process on yogurt texture: a review”, *Critical Reviews in Food Science and Nutrition*, Taylor & Francis, Vol. 44 No. 2, pp. 113–137.

Song, M., Fung, T.T., Hu, F.B., Willett, W.C., Longo, V.D., Chan, A.T. & Giovannucci, E.L. (2016), “Association of animal and plant protein intake with all-cause and cause-specific mortality”, *JAMA Internal Medicine*, American Medical Association, Vol. 176 No. 10, pp. 1453–1463.

Sostmann, K. & Guichard, E. (1998), “Immobilized β-lactoglobulin on a HPLC-column: a rapid way to determine protein—flavour interactions”, *Food Chemistry*, Elsevier, Vol. 62 No. 4, pp. 509–513.

Stone, A.K., Karalash, A., Tyler, R.T., Warkentin, T.D. & Nickerson, M.T. (2015), “Functional attributes of pea protein isolates prepared using different extraction methods and cultivars”, *Food Research International*, Elsevier, Vol. 76, pp. 31–38.

Suppavorasatit, I. & Cadwallader, K.R. (2012), “Effect of Enzymatic Deamidation of Soy Protein by Protein–Glutaminase on the Flavor-Binding Properties of the Protein under Aqueous Conditions”, *Journal of Agricultural and Food Chemistry*, American Chemical Society, Vol. 60 No. 32, pp. 7817–7823.

Swanson, B. (1990), “Pea and lentil protein extraction and functionality”, *Journal of the American Oil Chemists’ Society*, Springer-Verlag, Vol. 67 No. 5, pp. 276–280.

Tamime, A.Y. & Deeth, H.C. (1980), “Yogurt technology and biochemistry”, *Journal of Food Protection*, Vol. 43 No. 12, pp. 939–977.

Tamime, A.Y. & Robinson, R.K. (1999), *Yoghurt: Science and Technology*, Woodhead Publishing.

Thomson, D.M.H. & McEwan, J.A. (1988), “An application of the repertory grid method to investigate consumer perceptions of foods”, *Appetite*, Elsevier, Vol. 10 No. 3, pp. 181–193.

Torres-Penaranda, A. V, Reitmeier, C.A., Wilson, L.A., Fehr, W.R. & Narvel, J.M. (1998), “Sensory Characteristics of Soymilk and Tofu Made from Lipoxygenase-Free and Normal Soybeans”, *Journal of Food Science*, Wiley Online Library, Vol. 63 No. 6, pp.

1084–1087.

Tromelin, A., Andriot, I. & Guichard, E. (2006), “9 - Protein-flavour interactions BT - Flavour in Food”, *Woodhead Publishing Series in Food Science, Technology and Nutrition*, Woodhead Publishing, pp. 172–207.

Tu, V.P., Husson, F., Sutan, A., Ha, D.T. & Valentin, D. (2012), “For me the taste of soy is not a barrier to its consumption. And how about you?”, *Appetite*, Vol. 58 No. 3, pp. 914–21.

Tu, V.P., Valentin, D., Husson, F. & Dacremont, C. (2010), “Cultural differences in food description and preference: Contrasting Vietnamese and French panellists on soy yogurts”, *Food Quality and Preference*, Vol. 21 No. 6, pp. 602–610.

Tuorila, H., Cardello, A. V & Lesher, L.L. (1994), “Antecedents and Consequences of Expectations Related to Fat-free and Regular-fat Foods”, *Appetite*, Vol. 23 No. 3, pp. 247–263.

Tzitzikas, E.N., Vincken, J.-P., de Groot, J., Gruppen, H. & Visser, R.G.F. (2006), “Genetic variation in pea seed globulin composition”, *Journal of Agricultural and Food Chemistry*, ACS Publications, Vol. 54 No. 2, pp. 425–433.

Utsumi, S. (1992), “Plant food protein engineering”, *Advances in Food and Nutrition Research*, Elsevier, Vol. 36, pp. 89–208.

Utsumi, S., Matsumura, Y. & Mori, T. (1997), “Structure-function relationships of soy proteins”, *FOOD SCIENCE AND TECHNOLOGY-NEW YORK-MARCEL DEKKER-*, MARCEL DEKKER AG, pp. 257–292.

de Valdez, G.F., Torino, M.I., De Vuyst, L. & Mozzi, F. (2003), “Food-grade heteropolysaccharides: ongoing research and future trends of biopolymers from lactic acid bacteria”, *APPLIED BIOTECHNOLOGY FOOD SCIENCE AND POLICY*, TIM HILL, Vol. 1, pp. 223–234.

Valentin, D., Chollet, S., Lelièvre, M. & Abdi, H. (2012), “Quick and dirty but still pretty good: a review of new descriptive methods in food science”, *International Journal of Food Science & Technology*, Blackwell Publishing Ltd, Vol. 47 No. 8, pp. 1563–1578.

Vasbinder, A.J. & de Kruif, C.G. (2003), “Casein–whey protein interactions in heated milk: the influence of pH”, *International Dairy Journal*, Elsevier, Vol. 13 No. 8, pp. 669–677.

Vedamuthu, E.R. (2006), “Starter cultures for yogurt and fermented milks”, *Manufacturing Yogurt and Fermented Milks*, Blackwell Publishing Professional Ames, IA, p. 89.

Verbeke, W. (2006), “Functional foods: Consumer willingness to compromise on taste for health?”, *Food Quality and Preference*, Vol. 17 No. 1–2, pp. 126–131.

Van Vliet, T., Lucey, J.A., Grolle, K. & Walstra, P. (1997), “Rearrangements in acid-induced casein gels during and after gel formation”, *SPECIAL PUBLICATION-ROYAL SOCIETY OF CHEMISTRY*, Vol. 192, pp. 335–345.

Walsh, G. (2015), “Proteins and Proteomics”, *Proteins*, John Wiley & Sons, Inc., pp. 1–23.

Walstra, P. & Jenness, R. (1984), *Dairy Chemistry & Physics.*, John Wiley & Sons.

Wang, K. & Arntfield, S.D. (2015), "Binding of selected volatile flavour mixture to salt-extracted canola and pea proteins and effect of heat treatment on flavour binding", *Food Hydrocolloids*, Elsevier, Vol. 43, pp. 410–417.

Wang, Y.-C., Yu, R.-C. & Chou, C.-C. (2002), "Growth and survival of bifidobacteria and lactic acid bacteria during the fermentation and storage of cultured soymilk drinks", *Food Microbiology*, Vol. 19 No. 5, pp. 501–508.

Wansink, B. & Chan, N. (2001), "Relation of soy consumption to nutritional knowledge", *Journal of Medicinal Food*, Mary Ann Liebert, Inc., Vol. 4 No. 3, pp. 145–150.

Wansink, B. & Chandon, P. (2006), "Can 'Low-Fat' Nutrition Labels Lead to Obesity?", *Journal of Marketing Research*, Vol. 43 No. 4, pp. 605–617.

Wansink, B. et al. (2000), "How soy labeling influences preference and taste", *The International Food and Agribusiness Management Review*, Vol. 3 No. 1, pp. 85–94.

Wansink, B., Van Ittersum, K. & Painter, J.E. (2005), "How descriptive food names bias sensory perceptions in restaurants", *Food Quality and Preference*, Vol. 16 No. 5, pp. 393–400.

Wick, C., Nienaber, U., Anggraeni, O., Shellhammer, T.H. & Courtney, P.D. (2004), "Texture, proteolysis and viable lactic acid bacteria in commercial Cheddar cheeses treated with high pressure", *Journal of Dairy Research*, Cambridge Univ Press, Vol. 71 No. 1, pp. 107–115.

Widyastuti, Y. & Febrisiantosa, A. (2014), "The Role of Lactic Acid Bacteria in Milk Fermentation", *Food and Nutrition Sciences*, Scientific Research Publishing, Vol. 5 No. 4, p. 435.

Yang, M. & Li, L. (2010), "Physicochemical, textural and sensory characteristics of probiotic soy yogurt prepared from germinated soybean", *Food Technology and Biotechnology*, Prehrambeno-biotehnološki fakultet, Sveučilište u Zagrebu, Vol. 48 No. 4, pp. 490–496.

Young, V.R. & Pellett, P.L. (1994), "Plant proteins in relation to human protein and amino acid nutrition.", *The American Journal of Clinical Nutrition*, Am Soc Nutrition, Vol. 59 No. 5, p. 1203S–1212S.

Youssef, M., Lafarge, C., Valentin, D., Lubbers, S. & Husson, F. (2016), "Fermentation of cow milk and/or pea milk mixtures by different starter cultures: Physico-chemical and sensorial properties", *LWT - Food Science and Technology*, Vol. 69, pp. 430–437.

Zare, F., Boye, J.I., Champagne, C.P., Orsat, V. & Simpson, B.K. (2013), "Probiotic Milk Supplementation with Pea Flour: Microbial and Physical Properties", *Food and Bioprocess Technology*, Springer-Verlag, Vol. 6 No. 5, pp. 1321–1331.

Zare, F., Boye, J.I., Orsat, V., Champagne, C. & Simpson, B.K. (2011), "Microbial, physical and sensory properties of yogurt supplemented with lentil flour", *Food Research International*, Elsevier, Vol. 44 No. 8, pp. 2482–2488.

Zare, F., Champagne, C.P., Simpson, B.K., Orsat, V. & Boye, J.I. (2012), "Effect of the addition of pulse ingredients to milk on acid production by probiotic and yoghurt starter cultures", *LWT - Food Science and Technology*, Vol. 45 No. 2, pp. 155–160.

Zhu, X.Q. (2003), “Interactions between flavor compounds and milk proteins”, MSc Thesis. Massey University, Palmerston North, New Zealand.

Zourari, A., Accolas, J.P. & Desmazeaud, M.J. (1992), “Review article Metabolism and biochemical characteristics of yogurt bacteria . A review”, pp. 1–34.

Annexe 1

ÉVALUATION DES PROPRIÉTÉS PHYSICO-CHIMIQUES DE GELS À BASE DE LAIT DE POIS FERMENTÉ AVEC DIFFÉRENTS COCKTAILS BACTÉRIENS

MANHAL YOUSSEEF [1], DOMINIQUE VALENTIN [2], FLORENCE HUSSON [1], SAMUEL LUBBERS [1]

1 UMR Procédés Alimentaires et Microbiologiques, AgroSup Dijon, Univ. Bourgogne Franche-Comté, 21000 Dijon, France

2 Centre des Sciences du Goût et de l'Alimentation, CNRS, INRA, Univ. Bourgogne Franche-Comté, 21000 Dijon, France

INTRODUCTION

L'utilisation de protéines de légumineuses dans la formulation d'aliments est limitée en raison de certains problèmes liés à l'odeur (notes verte, terreuse…) et au goût (astringence, amertume). La fermentation lactique pourrait aider à résoudre ces problèmes (1), mais pour cela il est nécessaire de sélectionner les ferments lactiques les mieux adaptés à ces nouvelles protéines. Au cours de la production du coagulum par les bactéries lactiques, des composés d'arôme ainsi que des peptides sont formés résultant de l'hydrolyse de protéines de légumineuses.

OBJECTIF

L'objectif de cette étude est de comparer la capacité de 4 cocktails de bactéries lactiques à réduire les faux-goûts et les peptides amers et à augmenter les arômes « type yaourt » et la fermeté du gel formé.

MATÉRIELS ET METHODES

Farine de pois → Délipidation → Extraction de globuline (2) → Suspension de « 45 g globuline/L » → Ajout de Lactose, Calcium et citrate → Traitement thermique 90°C – 10 min → "Lait" de pois

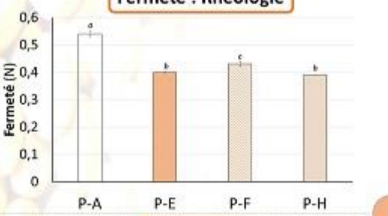
4 cocktails bactériens : A(*S. thermophilus + Lb. bulgaricus*) - E(*Lb. bulgaricus + Lb. helveticus*) - F(*S. thermophilus + Lb. acidophilus*) - H(*S. thermophilus + Lb. casei*)

Acidité (pH & degré Dornic) | **Fermeté** : pénétromètre | **Profils peptidiques :** HPLC et indice de tyrosine | **Composés d'arôme** (faux-goûts: hexanal et 1-hexanol / arômes yaourt : 3-hydroxy-2-butanone et 2,3-butandione) : SPME GC-MS

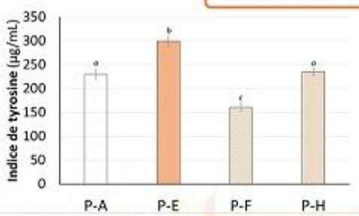
RÉSULTATS

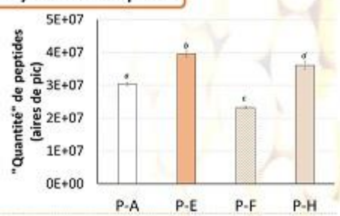
P-A : Lait de pois fermenté avec le cocktail A - **P-E** : Lait de pois fermenté avec le cocktail E - **P-F** : Lait de pois fermenté avec le cocktail F - **P-H** : Lait de pois fermenté avec le cocktail H - **P-non fermenté** : Lait de pois non fermenté - **V-A** : Lait de vache fermenté avec le cocktail A « culture pour le yaourt traditionnel »

Fermeté : Rhéologie

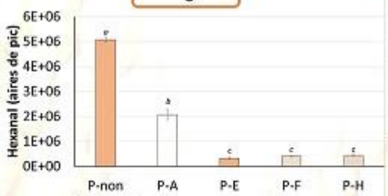


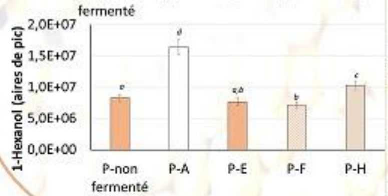
Potentiel d'amertume : Tyrosine et Peptides



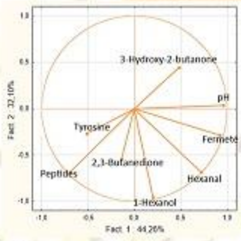


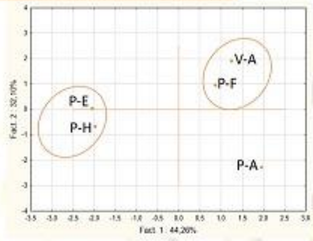
Faux-goûts





Analyse en composantes principales (ACP)





L'ACP a montré que:

- Les cocktails « E » et « H » ont donné les produits avec les valeurs les plus élevées de l'indice de tyrosine et de la quantité des peptides, ce qui suggère des produits potentiellement les plus amers.
- Le cocktail « A » a donné le produit le plus ferme, mais sa capacité à réduire la quantité d'hexanal (off-flaveur) est la plus faible entre les quatre cocktails étudiés.
- **Le cocktail « F »** a donné le produit le plus proche du yaourt traditionnel « lait de vache fermenté », avec la plus forte teneur en 3-hydroxy-2-butanone (arôme yaourt).

CONCLUSION ET PERSPECTIVE

Pour minimiser la quantité de faux-goûts et des petits peptides amers et maximiser les arômes « type yaourt » et la fermeté du gel formé, le cocktail bactérien F (*S. thermophilus + Lb. acidophilus*) a donné les meilleurs résultats. Le gel de lait de pois fermenté formé avec ce cocktail se rapproche le plus des caractéristiques du yaourt traditionnel au lait de vache. Pour valider ces résultats, un test sensoriel devra être effectué.

Références :

(1) Yousseef M, Lafarge C, Valentin D, et al. (2016) Fermentation of cow milk and/or pea milk mixtures by different starter cultures: physico-chemical and sensorial properties. LWT - Food Science and Technology 69:430–437.

(2) Nguyen, T. D., Lafarge, C., Murat, C., Mession, J.-L., Cayot, N., & Saurel, R. (2014). Partition of volatile compounds in pea globulin–maltodextrin aqueous two-phase system. Food Chemistry, 164, 406–412.