Properties of legumes, egg or milk products and new opportunities for mixes

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Bibliographic synthesis in biology and biotechnology

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« Le tuteur chercheur a pour rôle de conseiller l'étudiant, l'orienter dans ses recherches bibliographiques, l'aider à comprendre les articles, en faire une synthèse de manière logique et rigoureuse. Il ne peut vérifier toutes les citations et interprétations de l'étudiant. Il ne peut donc s'engager vis à vis d'éventuelles erreurs ».

Abstract

In order to respond to the global food security challenge that is associated with an increasing world population, many ways to design new variety of foods are currently being studied by the scientific community. While animal products like meat, fish, milk or egg are the main source of protein intake, several other ingredients could be used in a partial or total substitution in order to reduce the environmental footprint and advantageously increase the nutritional balance of the western diet. Over the vegetal reign, legumes such as soy, pea, chickpea and lupine are currently subject of a growing interest. With high protein and fiber contents, they have nutritional benefits that make them good substitute candidates to animal protein. Furthermore, they have technological properties comparable to milk or eggs to make foams, emulsions or gels. This synthesis provides information about the existing ways to design new functional food systems using both animal and legume protein by exploiting their nutritional and technological properties.

Summary

Abbreviation list

B-Lg: Beta lactoglobulin

GDL: glucono-δ-lactone

LGC: least gelling capacity

G’: storage modulus

G’’: shear modulus

WHC: water holding capacity

LDL: low density lipoprotein

HDL: high density lipoprotein

FS: foam stability

FE: foam expansion

FC: foam capacity

EW: egg white

EY: egg yolk

FFA: free fatty acids

SFA: saturated fatty acids

USFA: unsaturated fatty acids

MUSFA: monounsaturated fatty acids

PUSFA: polyunsaturated fatty acids

FAA: free amino acids

TGase: transglutaminase

WPI: Whey protein isolates

SEM: scanning electron microscope

MC: micellar casein

SP: soy protein

PP: pea protein

GP: globular protein

Summary of tables and figures

I. Introduction

Since the advent of the consumerism in the last century, most of the western countries have achieved to create an efficient agriculture system in order to feed their ever-growing population. However, several regions of the world didn’t reach this state, especially in the lower-middle income countries in Asia, Africa or South America. In an ever-growing human population, food security is a challenge that these countries need to address. In addition, it is well known that mass production of animal protein in western countries does have an impact on the environment. While trying to reduce their environmental footprint, the world richest countries also began to search for more eco-friendly foods.

Mixed foods are one of the multiple solutions that could respond to the requirement of reducing animal farming for environmental and health issues, with little impacts on western food habits. Furthermore, it can increase access to nutritionally balanced food in emerging countries. However, mixed foods are sparingly used to date. In France, the National Research Institute of Agronomy (INRA), works on government directives who calls in a new way to sustain the increase in the world population and the food demand. Among others, the egg and milk science and technology lab (STLO), Rennes, France is currently trying to design mixed foods that can combine both animal and vegetable products, in order to keep the benefits of an animal nutrient income while lowering the environmental, health and production costs by using nutritive and affordable plants. In that way, legumes like soy, pea, chickpea or lupine are subject to a growing interest. Thanks to their high protein content, evidence is growing that combining animal and plants products makes it possible to create a nutrient rich and affordable food with reduced environmental and health drawbacks.

Therefore, many researches have thought deemed necessary to understand the molecular interactions between the animal and vegetable systems. It is the first step that will allow recreating these complex molecular interactions. Gelation, for instance, implicates the formation of networks between reactive proteins to turn a solution into a solid, involving fermentation or not. By modulating the pH of the medium, it is actually possible to obtain different kind of gels (Grygorczyk, Alexander et Corredig 2013). It also depends on the ratios between animal and plants products (Park et al. 2005), which have an impact on the rheological behavior of the mixes and on their sensory profile.

This review presents a current state-of-knowledge on the researches made on food based on mixes of animal (egg or milk only) and plant (legumes only). First, we will establish a fine composition of all individual ingredients that are possibly used to make animal-plant based food, their physical and nutritional behavior. Then, several animal-plant mixes made under different operational conditions will be reviewed, in regards of the properties previously described, including the maximum possible combinations of ingredients. Since proteins are mainly involved in those physical behaviors, this review will focus mainly on these components, although other biomolecules like fats also have an impact on physical interactions. Some information about nutritional properties of the components will also be shown, in order to highlight the potential benefits of the mixes.

II. Nutritional composition and properties, and related rheological behavior of each ingredient of interest

A. Composition and nutritional assets of animal and vegetal products selected

The ingredients targeted for the elaboration of the different mixes come from two different origins: plants and animals. We selected for this review two animal products: cow's milk and hen eggs. Both are widely part of the nowadays western human diet. The global production of cow's milk is about 827 million tons in 2018 (FAO 2018) while the global production of eggs approaches 80 million tons. We chose to select legumes as plant resources for this study. Legumes are plants from the family Fabaceae (Turland et al. 2018). They include peas, soy, beans, lentils, lupine and other plants that constitute high source of vegetal proteins and are low in fat. In addition, they represent a source of other components, like complex carbohydrates (including fibres), phytosterols and natural antioxidants (Messina 1999). However, they also contain anti-nutritional factors such as phytic acid, trypsin inhibitors and phenolic compounds that can lower the protein digestibility and the bioavailability of minerals (Kalogeropoulos et al. 2010). Legumes represents in 2017, a production above 80 million tons worldwide (http://www.lafranceagricole.fr/actualites/cultures/monde-la-production-de-legumineuses-en-hausse-1,2,160468745.html). They are commonly farmed in middle- and low-income region of the world, for their sustainability and high nutritional profile. The legumes that we will investigate for this review are lupines, peas, chickpeas and soy.

In order to better understand the nutritional values of the ingredients selected, an average composition table will be showed for each. All values come from the CIQUAL tables established by the French national food security agency (ANSES) (« Ciqual Table de composition nutritionnelle des aliments », https://ciqual.anses.fr/).

Table 1 : Overall nutritional composition of cow milk, egg and several legumes.

Ingredient	**Composition (g/100g)**					
	Energy (kcal/100g)	**Water**	**Proteins**	**Carbohydrates**	**Fats**	**Fibres**
Cow milk (semi-skimmed)	45.8	89.7	3.28	4.8	1.53	0
Egg white (raw)	48.1	87.6	10.8	0.85	0.19	0
Egg yolk (raw)	307	55	15.5	1.09	26.7	0
Pea, P*isum sativum* (split pea, dry)	344	8.33	22.8	52	1.44	15.58
Chickpea, C*icer arietum* (raw seed, dry)	351	8.99	20.5	47.5	5.85	13.3
Soy, G*lycine max* (raw seed)	419	7.77	34.5	20.8	19.2	13
Lupine, *Lupinus albus* (raw seed)	356	10.4	36.2	21.5	9.74	18.9

Reference : (« Ciqual Table de composition nutritionnelle des aliments » s. d.)

A. Overview of the main components

Each ingredient selected for this review has either nutritional or rheological assets or both depending on some key components. Previous data have identified some components responsible for those assets at a molecular level and had linked them to one or the other properties. The main ones will be first showed in table 2.

Table 2 : Overview of the key components and their nutritional or rheological properties.

Ingredient	**Component Name**	**Functional properties**	**Nutritional properties**	**References**
Proteins				
SOY	Glycinin (11S)	Aggregating Gelling Emulsifying	N.A	(A. Grygorczyk et Corredig 2013; Roesch et al. 2004; Malaki Nik et al. 2009; Lin, Hill, et Corredig 2012) (Benjamin et al. 2014)
	β-Conglycinin (7S)	Aggregating Gelling Emulsifying	N.A	(Roesch et al. 2004; Malaki Nik et al. 2009; Benjamin et al. 2014; Lin, Hill, et Corredig 2012)
PEA	Legumin (11S)	Emulsifying Aggregating Gelling Salt soluble	Hypoallergenic	(Benjamin et al. 2014; Boye, Zare, et Pletch 2010; Amagliani et Schmitt 2017; Ben-Harb et al. 2018)
	Vicilin (7S)	Emulsifying Aggregating Gelling Salt soluble	Hypoallergenic	(Benjamin et al. 2014; Boye, Zare, et Pletch 2010; Amagliani et Schmitt 2017; Ben-Harb et al. 2018)
	Convicilin (7S)	Gelling	N.A	(Ben-Harb et al. 2018)
LUPINE	α-Conglutin (11S)	Emulsifying	N.A	(Kohajdová, Karovičová, et Schmidt 2011)
	β-Conglutin (7S)	Emulsifying	N.A	(Kohajdová, Karovičová, et Schmidt 2011)
MILK	Casein and casein micelles	Aggregating Gelling	High digestibility	(Roesch et al. 2004; Alexandra Grygorczyk, Alexander, et

MILK		Foaming Emulsifying		Corredig 2013; Boye, Zare, et Pletch 2010)
	Albumin	High Solubility in water Foaming	Low digestibility	(Boye, Zare, et Pletch 2010; Ghumman, Kaur, et Singh 2016)
	β-Lactoglobulin	Emulsifying Gelling Foaming	Antihypertensive, antioxidant, antimicrobial, rich in essential amino-acids	(Benjamin et al. 2014; Amagliani et Schmitt 2017; Sharma 2019; Destribats et al. 2014)
EGG	Ovalbumin	Gelling, Aggregating Foaming	N.A	(Mine 1995)
	Ovotransferrin	Gelling, Aggregating Foaming	Iron transport	(Mine 1995)
	Ovomucoid	Gelling, Aggregating Foaming	N.A	(Mine 1995)
	Ovomucin	Gelling, Aggregating Foaming	N.A	(Mine 1995)
	Lysozyme	Gelling, Aggregating Foaming	antimicrobial	(Mine 1995)
	Globulin (white egg)	Gelling Aggregating Foaming	N.A	(Mine 1995)
Fats				
LEGUMES	Phytosterols	N.A	Protection against low density lipoprotein oxidation, anti-oxidant, anti-inflammatory	(Kalogeropoulos et al. 2010)

	Squalene	Emulsifying	Anti-oxidant	(Kalogeropoulos et al. 2010; Kim et al. 2017)
Carbohydrates				
LEGUMES	Raffinose	N.A	Gases, growth-promoting effect on bifidobacteria	(A. Grygorczyk et Corredig 2013; Olson et al. 1981; Mitsuoka 1982)
	Stachyose	N.A	Gases, growth-promoting effect on bifidobacteria	(A. Grygorczyk et Corredig 2013; Olson et al. 1981; Mitsuoka 1982)
	Lactose	N.A	Intolerance, low-absorption, lower the risk of diabetes	(Ismail 2016; Favier et Dorsainvil 1985)
Others				
SOY	Isoflavones	N.A	Lower the hormonal disease risk	(Jiang, Cai, et Xu 2013)
ALL	Vitamin A	N.A	Essential for life	(Favier et Dorsainvil 1985)
	Vitamin B2	N.A	Essential for life	(Favier et Dorsainvil 1985)
	Vitamin B12	N.A	Essential for life	(Favier et Dorsainvil 1985)
LEGUMES	Phytic acid	N.A	Reduction of mineral bioavailability, inhibitor of trypsin and pepsin, *in vitro* decrease in protein digestibility (IVPD) Antioxidant	(Deshpande 2002; Lajolo et al. 2004; Messina 1999; Graf 1990; Kohajdová, Karovičová, et Schmidt 2011)
	Oxalic acid	N.A	Inhibitor of calcium absorption	(Weaver et Plawecki 1994)

	Phenolic compounds	N.A	Antioxidant	(Ismail et al. 2017; Kohajdová, Karovičová, et Schmidt 2011)
	Alkaloid quinolizidine group	Water-soluble	Anti-nutritional	(Kohajdová, Karovičová, et Schmidt 2011; El-Adawy et al. 2001)

N.A : not analyzed

B. Rheological properties

The “functional” properties of an ingredient are defined as properties that affect the behavior of their components during chemical or physical processes (Boye, Zare, et Pletch 2010). Beneath those properties, the rheological ones specifically affect the structure, texture, or the shape of the ingredients under various physicochemical conditions of temperature and pH (Ghumman, Kaur, et Singh 2016). In the scope of this review, four functional properties are mainly concerned, given the fact that they have direct application to food industry nowadays: emulsification, foaming, aggregation and gelation. Those functional properties are affected mainly by proteins, because they can interact to form elaborated structures like gels, but also by non-protein molecules such as salt (NaCl) and other minerals, fats, carbohydrates and phenolic compounds. Factors such as the amino-acid composition, size, structure and conformation of the proteins have an influence on the functional properties. Physicochemical conditions like temperature, pH and ionic strength are environmental factors that modify the protein conformation and their stability (Boye, Zare, et Pletch 2010). Taken together, those factors drive the proteins conformation and interactions with other proteins or other molecules.

1. Emulsification

When two immiscible liquid phases are present, like water and oil, emulsification is a process that will eventually disperse one of the phases into another, forming droplets of one liquid suspended into the other, known as an emulsion. Most often, oil droplets are suspended in water or other aqueous medium (Varjani et Upasani 2017). Components which facilitate that process have the name of emulsifiers. They act by placing themselves at the interface between the two phases, thereby relieving tension forces. Many compounds are tensioactive. Phospholipids for example are present in large amounts in both plant and animal products and are constituent of cells membranes. The polarity of those component makes them very good emulsifiers, because they can protect well the fat droplets, forming a barrier to prevent coalescence. Thanks to that, they’re commonly used in the food or pharmaceutical industry (Kim et al. 2017). Proteins are another main nutrient responsible for the emulsification behavior. Due to their amphiphilic profile, they form a protective layer around the fat droplets and prevent coalescence (Boye, Zare, et Pletch 2010; Karaca, Low, et Nickerson 2011). The emulsification characteristic of the proteins depends on their structure, size, solubility, conformational flexibility and are directly affected by the hydrophobicity/hydrophilicity ratios of their amino-acid constitution (Boye, Zare, et Pletch 2010; Benjamin et al. 2014).

Among the ingredients selected in table 2, globulins from legumes and from whey has been described to have high emulsifying activity (Benjamin et al. 2014; Amagliani et Schmitt 2017; Destribats et al. 2014). Furthermore, they easily form a protective layer around the droplets, preventing coalescence (Ghumman, Kaur, et Singh 2016; Amagliani et Schmitt 2017).

In fact, the soluble globular proteins such as legume and whey globulins partly unfold at the interface between droplets and aqueous medium in order to expose their interior hydrophobic amino-acids to the oil phase. Thus, they become amphiphilic and improve their interactions with other nearby proteins at the interface (Benjamin et al. 2014). In general, 7S globulins are better emulsifiers than 11S globulins, because of their smaller size and the presence of glycosylated groups that enhance their flexibility (Benjamin et al. 2014). β-lactoglobulin (β-Lg) and lupine globulins have both an emulsifying power, but a higher concentration of lupine globulin is needed to obtain the same droplets size as with β-Lg (Benjamin et al. 2014). Nevertheless, it shows that legume proteins especially lupine ones (α or β-conglutins) could partially or totally replace animal protein for stabilizing low-fat emulsions (Benjamin et al. 2014; Kohajdová, Karovičová, et Schmidt 2011). Here is an example of the pea (*Pisum sativum*) protein emulsification process which is representative of the other legume proteins listed above.

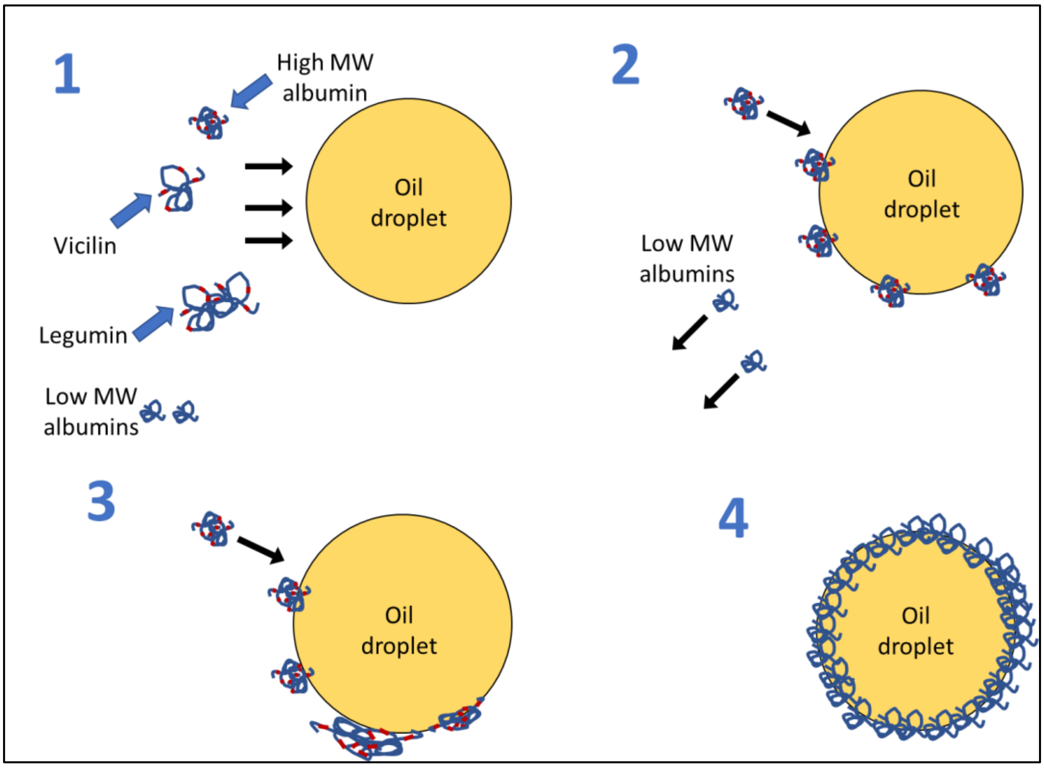

Figure 1: Behavior of the pea proteins at the oil-water interface in a sequence of (1) migration, (2) adhesion, (3) partial denaturation and reorientation, and (4) formation of a viscoelastic film at the interface (Burger et Zhang 2019). In red there are hydrophobic patches on the protein surface that promote the adsorption.

2. Aggregation

The aggregation phenomenon is commonly obtained by heating globular proteins above their denaturation temperature. Thus, those proteins eventually create variable sized particles. This applies to a variety of globular proteins, either coming from animal or plants sources. Different parameters could modulate the size and the structure of the aggregates, such as protein concentration, heating protocol, pH, nature and concentration of salts (Amagliani et Schmitt 2017). Typically, aggregation is promoted when protein concentration, salt concentration and/or heat are increased. Divalent ions, like calcium, accelerate aggregation more than monovalent sodium. Aggregation is generally faster at the vicinity of the isoelectric pH of proteins. When those soluble proteins are heated, they unfold, expose their hydrophobic regions and irreversibly aggregate by forming hydrophobic interactions, hydrogen bond between acidic and basic polypeptides and thiol/disulfide exchanges (Malaki et al. 2009; Benjamin et al. 2014). Various kinds of structures can be obtained by a heating treatment: spherical particles, flexible strands and semi-flexible fibrils. These constitute the first step to create a gelation process that will be explain later. That is important to say that the formation of aggregates depends on the level of electrostatic repulsions (Amagliani et Schmitt 2017) and on the amino-acid composition of the globular proteins, especially the presence of cysteins with free thiol groups (Hoffmann et al. 1997).

According to literature, this behavior applies to soy globular proteins which represent 90% of the total protein content of soy. Soy globular proteins are constituted of β-conglycinin and glycinin which are 7S and 11S proteins respectively. When these globulins are heated, soluble aggregates begin to form thanks to comparable hydrophobic interactions observed for the whey proteins (Amagliani et Schmitt 2017).

Pea proteins contains 55-65% of globulin consisting of 7S vicilin and convicilin and 11S legumin (Amagliani et Schmitt 2017). As expected, those protein also create aggregates upon heat treatment, by forming hydrophobic interactions and disulfide bonds (Chihi, Sok, et Saurel 2018; Ben-Harb et al. 2018). Interestingly, the presence of sulfhydryl amino acid has an impact on the size of the particles, given that they are crucial to create disulfide interactions. In that way, authors assessed that polypeptides who lacks cysteine residues in their structure couldn’t show as good aggregating properties as the others.

Globulins are also present in egg white and have the same behavior as globulins found in other sources. They are known as lysozyme but during the thermal treatment process, not only globulins react to create aggregates. Egg white proteins like ovotransferrin and ovalbumin also react to this treatment by aggregating themselves into β-sheets structures and/or by undergoing thiol/disulfide exchanges. This particular conformation exposes hydrophobic residues that allows stacking and interaction between the β-sheets (Mine 1995).

In milk, caseins that are not globular proteins, are not affected by thermal treatment while the globular whey proteins are (Ben-Harb et al. 2018). Another way to obtain aggregation is to use acidification agents such as the glucono-delta-lactone (GDL) or citric acid, or to use sugar fermentation by lactic acid bacteria to form lactic acid, to reach the isoelectric pH of unfolded acidic proteins, such as the caseins or the denatured globular proteins such as whey proteins. GDL hydrolyses into gluconic acid, releasing protons in the process (Ben-Harb et al. 2018). Several studies have been made with caseins from milk (Roesch et al. 2004) and demonstrated that casein aggregates begin to form at pH 5.3. Using rennet is another way to form aggregates commonly used in the cheese industry. This protease hydrolyses the Phe_{105}-Met_{106} bond of the κ-casein, one of the four caseins that is mainly present at the surface of the casein micelles, cleaving off a casein fragment responsible for electrostatic repulsion and creating an hydrophobic zone that allows aggregation (Ben-Harb et al. 2018; Grygorczyk et Corredig 2013).

Although most of the aggregation studies have been made on heat-denatured whey proteins for their high solubility and aggregating power, fewer literature exists on the acid-induced aggregation of denatured legume proteins. However, it has been shown that during acidification, the concentration of protons increases, and near pH 5.8 (Malaki et al. 2009), gradually neutralize surface charges of the soy glycinin and β-conglycinin. Then, the basic subunit of glycinin folds into a particle, while the acidic sub-unit and the α and α' subunits of β-conglycinin comes over it (Grygorczyk et Corredig 2013) To sum up, the globulins which eventually destabilize, and begin to form hydrophobic interactions (Malaki Nik et al. 2009) by unfolding themselves and finally aggregate into particles (Grygorczyk et Corredig 2013).

3. Gelation

Gelation is an important reaction widely used in food industry and could be defined by a reaction leading to an infinite aggregation stabilized by several kind of interactions including electrostatic and hydrophobic interactions, hydrogen and/or disulfide bonds (Ma et al. 2011). An indicator known as the least gelling concentration (LGC) is commonly used to define the lowest concentration of gelling agent required to form a stable gel (Boye, Zare, et Pletch 2010). The gelation point is also known to be related with two indicators: the storage modulus that represents the capability of a matter to be more solid-like and noted G', and the shear storage that represents its capability to be more liquid-like, noted G''. Usually the gelation point is defined as the moment when G''>G' (Malaki et al. 2009). The water holding capacity (WHC) represents the capability of a food to trap water and is often used as an indicator of gel quality. Protein gels work the same way and consist in a formation of a three dimensional network that traps large amount of water with a small amount of proteins (Mine 1995). Gelation is affected by the same factors as aggregation: pH, temperature, and ionic strength (Zhang, Jiang, et Wang 2007; Grygorczyk et Corredig 2013) and also depend on the protein concentration, type of proteins but also the presence of non-protein components like carbohydrates or lipids (Kaur et Singh 2007; Ma et al. 2011; Ben-Harb et al. 2018). In pea gels, the addition of vegetal fats like rapeseed oil increases G'. When gels incorporate emulsion droplets, the presence of emulsifying proteins at the droplets' interface helps connecting them as active fillers inside the gel's structure (Ben-Harb et al. 2018).

The structure of the final gel depends on the structure and size of the aggregates that built it, which themselves depend on physicochemical conditions like pH and salt concentration. At least , three types of aggregates are formed by heat-induced aggregation : spherical particles, flexible strands and semi-flexible fibrils (Amagliani et Schmitt 2017), leading to different gelation behaviors and different gel structures. In fact, a second aggregation could occur between those three types of aggregates. Spherical particles and flexible strands associates to create larger aggregates and eventually form a turbid or a translucent gel, respectively (Amagliani et Schmitt 2017; de la Fuente et al 2002; Foegeding et al 1998; Renkema et al 2000). A microgel is a particle, composed of a stable network consisting in cross-linked molecules maintained by covalent bond(s) and intermolecular interactions (Amagliani et Schmitt 2017). In soy "milk", heat-induced aggregation may lead to this particular structure as reported by Chen et al (2014). Its structure is composed by a hydrophobic core containing β subunits and some 11S basic polypeptides enveloped/surrounded by α and α' subunits of 7S and acid polypeptides of 11S. The core is stabilized by strong disulfide bonds and hydrophobic interactions.

Using GDL with addition of calcium sulfate is a common way to make soy gels resulting in tofu (Malaki et al. 2009). By using GDL to acidify the media, a gel may result as an aggregation of the previously heat-denatured soymilk proteins. But contrary to the heat-induced method, gels made by acidification are more stabilized by non-covalent interactions like salt-bonding, hydrogen bonding and Van der Walls forces, rather than by thiol/disulfide bonds (Grygorczyk et Corredig 2013). Environmental pH plays a major role into gel formation and allow setting the proteins at their neutral charges. Once the pH of the media approaches the isoelectric point of the proteins, β subunits of β-conglycinin are the first to destabilize followed by α and α’ subunits of β-conglycinin who participate to form a stable gel network. Neutralizing the repulsive charges allows the proteins to come in close contact. The formation of hydrogen bonds at this moment will determine the gel stiffness (Malaki et al. 2009). Malaki et al (2009) reported that modifying the GDL concentration does not affect the gelation point; however, it does greatly impact G’ by affecting the time needed to the proteins to arrange together to form a gel. Finally, higher ratios of globular protein compositions between glycinin (11S)/β-conglycinin (7S) may increase the storage modulus G’ of the gels formed, where G’ represent the stiffness of the gel (Yagasaki, Kousaka, et Kitamura 2000). In fact, gels formed around pH 5,6-5,8 with higher proportion of glycinin were firmer than gels made of pure β-conglycinin, because glycinin formed larger aggregates (Malaki et al. 2009). However, the isoelectric point of β-conglycinin (4.5-5.0) is lower than that of glycinin (6.3-7.0), due to more negatively charged subunits. Therefore, the media need more H^+ protons to aggregate β-conglycinin and the gelation point at acidic pH depends mainly on the proportion of that protein. β-Conglycinin and glycinin are the main globular proteins implicated in the formation of soy gels, but it is actually glycinin that affects the firmness of the gels. Under these conditions, it shows that soy cultivars with little β-conglycinin amount both improve the firmness of gels and require less acidifier (Kohyama et Nishinari 1993).

It is also possible to acidify soy “milk” with lactic acid bacteria. Proceeding this way, it has been shown that the pH measured at the gelation point is higher than that using the GDL way (Grygorczyk et Corredig 2013). This could be explained by the slower fermentation time of soy “milk” by bacteria that allows more protein rearrangement during acidification. However, it does not affect the final gel structure.

During acidification of milk proteins, neutralization of the casein micelles (pHi = 4.6) leads to the formation of a gel network. When unheated milk is used, gelation occurs at pH 5. That is important to note that undenatured whey proteins, mainly constituted of β-lactoglobulin do not impact the gelation process (Roesch et al. 2004). In contrast, in heated milk, the whey proteins are denatured and form aggregates that increase the gelation point to a higher pH. Thus, it has been shown that whey proteins play an important role in gelation mechanisms of heated milk by reinforcing the gel network (Roesch et al. 2004).

In egg white, gelation properties are also due to globular proteins and obey the same rules as previously described for the other globular proteins (Mine 1995) with respect to pH, ionic strength, protein concentration and/or heating method. The aggregation process can produce the same types of aggregates than those reported by Amagliani and Schmitt (2017) : spherical particles, flexible strands and semi-flexible fibrils. Several methods have been reported to be effective for aggregation and gelation. Heat treatment leads to the denaturation and aggregation of ovotransferrin, lysozyme and ovalbumin (Mine 1995). The addition of salts like iron or aluminum increases the denaturation temperature of the egg white, maybe due to the ovotransferrin capabilities to increase its stability by binding metallic ions (Mine 1995). The gelation process for egg white is defined by the three steps described below. First, the heat treatment induces the partial unfolding of egg white proteins and thus, the formation of spherical aggregates via hydrophobic interactions. Second, the already formed aggregates reinforced themselves through disulfide bonds that even though not necessary for gelation can increase the stability of the structure. Finally, upon cooling, a large formation of hydrogen bonds leads to an increase of the structure elasticity. It has been also reported that ovalbumin gels, formed by a thermal treatment are the result of cross-linked β-sheet structures, stabilized by hydrophobic interactions (Mine 1995).

Since alkali treatment is a relatively old conservation technique for eggs, it is also useful to study aggregation and gelation behavior of egg white at high pH values. As observed at other pH values, heat causes a denaturation of the egg white proteins followed by an unfolding and an exposition of their hydrophobic structure. Thus, inter-proteins interactions could be made as well as disulfide bonds and eventually lead to gel formation. Under these conditions, no hydrogen bonds can however be created (Gharbi et Labbafi 2018).

The yolk is constituted by non-soluble protein aggregates known as granules, hosted in the plasma which is the yellow fluid. Thus, granules could easily be separated from plasma by centrifugation. Those granules count for approximatively 50% of yolk proteins, the other 50% being contained in the plasma (Valverde et al. 2016). Plasma is rich in low density lipo-proteins (LDLs) and livetins, while granules are rich in high density lipoproteins (HDLs) and phosvitin. Upon heating, egg yolk proteins denature the same way as the egg white proteins, but form a much harder and dense gel, due to its higher solid content. Thus, the egg yolk protein gels are commonly accepted to be among the hardest ones (Zhang, Jiang, et Wang 2007).

4. Foaming

Foams are defined by air bubbles suspended in a continuous phase that is usually an aqueous medium (Amagliani et Schmitt 2017; X. Li et al. 2019). Making foams is usually achieved by mechanical whipping at high speed (Amagliani et Schmitt 2017). Quality of foams can be characterized by three indicators, the foam stability (FS), the foam expansion (FE) and the foam capacity (FC). FC and FE are expressed as the volume (%) of foam produced with a volume of initial liquid (FC) and the volume increase depending on the foaming protocol (FE). Meanwhile, FS is measured as the increase of the liquid phase in time units due to drainage, and is an indicator of the ability of the foaming agent to form a protective layer to retain water and prevent foam coalescence (Boye, Zare, et Pletch 2010; Ma et al. 2011). Foaming properties of a mixture are affected by aeration conditions, temperature, pH and ionic strength and are also dependent on the protein content as well as other components like carbohydrates that may modify the viscosity of the aqueous phase. As emulsions, foams are thermodynamically unstable systems that could be stabilized by the amphiphilic properties of the proteins. By unfolding themselves, they rapidly adsorb at the air-water interface and rearrange it in order to create a protective film that relieves interfacial tension (Boye, Zare, et Pletch 2010; Mine 1995). Likely, they act as a protective layer around the air bubbles forming a widely range of interactions such as hydrogen bonds, electrostatic and hydrophobic interactions (Ma et al. 2011). Size, solubility, and ratios of protein isolates affect the foaming properties of a mixture as well. Several studies report the foaming abilities of unfolded whey protein aggregates. In fact, heat induced (85°C-15min) whey proteins aggregates at a pH of 5.9 could act as foams stabilizers by a similar mechanism than emulsions (Amagliani et Schmitt 2017). β-Lactoglobulin (β-lg) is by far the globular protein from whey the most capable to make foams. Thermal aggregates of β-lg were found to be effective to create foams, as they provide a cohesive interfacial film between air and the aqueous medium. It has also been reported that FS is function of the aggregate size.

Egg white contains proteins that show high foaming abilities. This ingredient is widely used for a long time in cooking or in the food industry to make cakes, cookies, mousses and other air suspensions (Li et al. 2019). Foaming properties of egg white have been shown to be correlated to globulins as well as ovalbumin (Mine 1995). Like other protein sources, electrostatic and hydrophobic interactions are essential for FC of egg white proteins. In addition, the ability of the egg white proteins to create disulfide bonds can impact the foaming properties since those bonds makes a less flexible structure (Mine 1995). That is important to note that only the egg white contains foaming agents. Even in small amount, egg yolk contamination can dramatically reduce the foaming properties of egg white. This could partially be explained by the high amount of fat in egg yolk, who act as a defoaming agent and greatly impact FC and FE.

Lupine protein possesses foaming capabilities as reviewed by Kohajdová, Karovičová, and Schmidt (2011). In this paper, authors assessed that lupine flour has comparable foaming capabilities to the egg albumin located in egg white. Prior to heat-treatment by boiling, these flours form a consistent foam which is like uncooked egg white, in terms of texture and microstructure. Nevertheless, fats in lupine could be a problem for FC and FE. In fact, polar lipid such as glycolipid and phospholipids absorb at the air-liquid interface and consequently are foam stabilizers. But non-polar lipids like triglycerides often create weaker layers at the interface and can be desorbed more easily (Kohajdová, Karovičová, et Schmidt 2011). Other studies have reported that fats in general are considered as a defoaming agent (Li et al. 2019). Fortunately, the defatting processes does not affect the lupine protein and consequently increases the FC.

C. Nutritional properties implicated and health attributes

The selected ingredients have specific nutritional properties, depending on their animal *vs* vegetal origin.

Human organism needs essential amino-acids for a good functioning. According to the OMS, these needs are shown in table 3 and are compared with the contribution of the different ingredients selected for this review. Thus, it will serve as a reference to evaluate the nutritional quality of protein profiles of these ingredients.

Table 3: Comparison of essential AA amounts in animal and vegetal products with basic human needs

	Amount of essential AA (in % of a 70kg adult need)									
	Phe	**Leu**	**Met**	**Lys**	**ILe**	**Val**	**Thr**	**Try**	**His**	**Ref.**
Cow milk (semi-skimmed)	10%	13%	8%	12%	15%	12%	14%	16%	13%	1
Egg white (raw)	7%	37%	38%	38%	47%	41%	43%	45%	41%	2
Egg yolk (raw)	39%	51%	36%	58%	62%	48%	65%	63%	59%	3
Pea, *Pisum sativum* (split pea, raw)	66%	62%	19%	84%	70%	53%	77%	57%	84%	4
Chickpea, *Cicer arietum* (raw seed, dry)	63%	54%	26%	66%	63%	44%	73%	71%	81%	5

Soy, *Glycine max* (raw seed)	121%	121%	52%	129%	236%	104%	168%	211%	157%	6
Lupine, *Lupinus albus* (raw seed)	82%	100%	24%	92%	115%	77%	127%	103%	147%	7
Needs for a 70kg adult (in mg/day)	**1750**	**2730**	**1050**	**2100**	**1400**	**1960**	**1050**	**280**	**700**	10

Phe: phenylalanine ; Leu : Leucine ; Met : Methionine ; Lys : Lysine ; Ile : Isoleucine ; Val : Valine ; Thr : Threonine ; Try : tryptophan ; His : Histidine. (1) (Favier et Dorsainvil 1985) ; (2-9) (« USDA » s. d.) ; (10) (Joint Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition et al. 2007). Three color gradient scale: 0% = red ; 50% = yellow ; 100% = green

Milk is known to be a source of balanced amino acids essential to newborns, but actually poorer than other animal products like egg white and yolk that is usually taken as a reference. Milk also contains ~4% fats where saturated fatty acids are much more important than unsaturated ones. Thanks to food engineering, it is possible to modulate fat content by creaming processes. Milk fat is also a good source of cholesterol as well as vitamins. Lactose is subject to discussions, since it is the major carbohydrate present in milk and has been recognized to be less digestible by the human adult organism. This intolerance to lactose can be reduced and even removed by consuming yogurt, in which the bacterial β-galactosidase is able to replace the human deficient enzyme during digestion. Nevertheless, the galactose released during the lactose hydrolysis has health benefits, since it is involved in the synthesis of cerebrosides, an important lipid for the nerve tissue constitution. Although milk is a poor source of oligo-elements, the calcium and phosphorus are important source to the bone growth and development. Caseins from milk have been shown to be highly digestible (98.3% w/w), and often serve as a reference to measure this parameter in other food systems. Protein digestibility of legumes selected are the follow : Soy bean : 71.8% ; Pea ; 60.4-66.5% ; Chickpea : 89% (Boye, Zare, et Pletch 2010) ; Lupine : 90.8% (Regina Pereira Monteiro et al. 2014). Besides those nutritional properties, milk proteins, and especially whey proteins contain biologically encrypted sequence of bioactive peptides that are released during food processing by endogenous milk enzymes or bacterial proteolytic enzymes, and/or during digestion. The α-lactalbumin is a rich source of tryptophan that is the precursor of the serotonin in brain content (Sharma 2019). Whey proteins also have the ability to regulate the food intake, by regulating the satiety signal (Sharma 2019). Mohanty et al. (2016) reported that casein and β-lg proteins also contain bioactive peptides that have health benefits such as radical scavenging activity and inhibition of lipid peroxidation.

In general legume proteins show an interesting nutritional profile with high amount of essential amino acids such as lysine, leucine as well as aspartic acid glutamic acid and arginine. However, low amounts of methionine, cysteine and tryptophan have been detected, since these are contained in the albumin fraction which is minor regarding to the overall globulin content (Boye, Zare, et Pletch 2010). In particular, soy proteins have been shown to have high digestibility (71,8%) and a well-balanced amino acid profile (Boye, Zare, et Pletch 2010; Amagliani et Schmitt 2017). Pea proteins contains more essential amino than animal products except for methionine and tryptophan, which are much more present in eggs (see table 3). In addition, some soy and lupine proteins have been reported to be potential allergens. Studies have reported that children who are subject to peanut allergy are also allergic to lupine and soy, since the three are belonging to the legume family. However, other legumes like pea and chickpea could represent an alternative for those allergic people up to now (Boye, Zare, et Pletch 2010), since the protein they contain (Legumin and Vicilin) are hypoallergenic and have a high nutritional value (Amagliani et Schmitt 2017). Nevertheless, no one can exclude that a higher contact of such legume proteins to a larger number of people will also provoke allergy in the future. It is also important to note that legumes are non-gluten plants which constitute a good consumption alternative to cereals products, in addition of their other nutritional properties (Kohajdová, Karovičová, et Schmidt 2011). Legumes and cereals can be used in combination to fulfil a complete essential amino-acid profile.

Legumes often contains good amounts of antioxidant molecules such as tocopherols which prevent lipid peroxidation. In that way, tocopherols are particularly high in chickpeas (Kalogeropoulos et al. 2010). Soy is also a good source of phenolic compounds which possesses strong antioxidant properties (Ismail et al. 2017). In addition, soybean also contains large amount of isoflavones, a molecule from the phytoestrogen family, that has been shown to reduce the risk of hormonal and age related diseases (Jiang, Cai, et Xu 2013; Ismail et al. 2017). Lupine has quite an interesting antioxidant activity, because of the presence of phenolic compounds which are mainly tannins and flavonoids. As reported by Kohajdová, Karovičová, et Schmidt (2011) lupine has slighter less anti-nutritional compounds like trypsin inhibitors, phytic acid and complex carbohydrates, lectins and saponins than other legumes. Also, phytosterol content of the legumes is quite interesting since they are present in relatively large amount in those plants, especially in chickpeas and lupines. They are known to compete with the dietary cholesterol thus limiting absorption by the intestine. Thus, they could offer a protective effect against LDL oxidation and preventing cardiac diseases. They also possesses anticancer, anti-inflammatory and antioxidant activities (Kalogeropoulos et al. 2010). Squalene is an intermediate in the biosynthesis of sterols. It has been reported that this component contributes to the inhibition of several forms of cancers such as lung, colon and skin cancer. Although squalene exists in very low amount in legumes, lupine is a relatively good source of it (Kalogeropoulos et al. 2010).

However, legume seeds may contain some anti-nutritional factors such as enzyme inhibitors like trypsin, chymotrypsin inhibitors, phytic and oxalic acid, complex carbohydrates and phenolic compounds. In fact, these components may cause a diminution of protein digestibility and mineral bioavailability. Complex carbohydrates contained in legumes, such as stachyose and raffinose, have a particularly low digestibility and are often the cause of intestinal uncomfort, while they are also bifidogenic factor implied in the microbiote homeostasis. If not previously treated by heating, dehulling or soaking, such anti-nutritional compounds can have an impact on the overall digestibility of legumes proteins and their mineral bioavailability (Boye, Zare, et Pletch 2010; Kalogeropoulos et al. 2010; Messina 1999; Weaver et Plawecki 1994). Furthermore, phytic acid is known to lower the calcium bioavailability. On the other hand, heating dramatically reduces the rates of trypsin inhibitors, lectins, and undigestible oligosaccharides, and improves the overall legume proteins digestibility except for phytic acid, which is not particularly affected by this treatment (Jiang, Cai, et Xu 2013). It has also been reported that phytic acid contributes to reducing the cancer risk, thanks to its antioxidant properties (Messina 1999). In lupine, the major anti-nutritional components are alkaloids from the quinolizidine group such as lupinine, lupanine, sparteine and others (Kohajdová, Karovičová, et Schmidt 2011) that are partially or even completely removed through selection of “sweet” lupin varieties (*Lupinus albus* and *Lupinus angustofolius*).

III. Properties of mixes between animal and vegetal products

In the context of a need for a more balanced animal to vegetal protein ratio in human diet, mixes between milk or egg products with legumes are an emerging field of research in food industry and food design. Most of the articles are actually very recently published in this area. The main interest is relied on technological properties of proteins and namely of legume proteins. Actually, rheological properties of certain legumes look like those commonly exploited in dairy products. In addition, the nutritional properties of legumes could provide some added values to the products made this way, with respect to their also known anti-nutritional properties. Several data have shown that is it possible to combine those products to make a stable mixture and exploit their rheological assets to create a wide range of applications. In the part of this review, we expose some mixes that have already been proposed, their benefits and limits regarding to the method employed and their potential application for food industry.

Ismail et al (2017) have attempted to produce mixes between 25% soy “milk” with 75% buffalo or cow milk in order to create a yoghurt-like product. Then, the mix was inoculated with a commonly-used yoghurt starter containing both *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Several parameters were measured such as the chemical composition (Total solids, fats, total nitrogen and ash content), the free fatty acid content (FFA), the saturated (SFA) and unsaturated (USFA) fatty acids, the monounsaturated (MUSFA) and polyunsaturated fatty acids (PUSFA) content, the short chain fatty acids (C_8-C_{12}), the medium chain fatty acids (C_{14}-C_{16}), the long chain fatty acid ($<C_{16}$), and the free amino acids content (FAA). A microbial analysis and a sensory evaluation of yoghurt were also performed. Results showed that the incorporation of soy “milk” can produce a highly nutritional yoghurt, with high amount of USFA and essential amino acids, without reaching the contents of pure milk samples yet. In yoghurt-like product containing 0.5, 10 or 15% of soy “milk”, (Altahir et al., 2014) showed that the proteins and fat content were significantly lower in the 15% soy/milk yoghurt, probably because of the lower protein and fat content contained in soy, and legumes in general. They also showed that depending on the bacteria strain used for fermentation, different acidification rates can be obtained. Thus *L. bulgaricus* that was used in this study has a low ability to ferment the complex soy carbohydrates commonly founded in legumes. However, the sensory evaluation showed that the fermentation contributed to eliminate the “beany” flavor of soy and thus, the organoleptic properties of the final product were quite acceptable.

Lin, Hill, and Corredig (2012) investigated the formation of a mixed gel containing soy “milk” and reconstituted bovine skim milk with a final protein concentration of 3,4% and a ratio of 60:40 soymilk proteins to milk proteins. All gelation processes were conducted at 30°C, without previous thermal treatment. The gelation method was induced by GDL acidification and/or rennet addition. Results showed that it was possible to create gels with this kind of mixture. Particle size distribution of the mixtures was comparable to those with soy “milk” alone. Gels formed with GDL alone or in combination with rennet were comparable in terms of structure with those formed by soy “milk” alone. However, no gelation process was observed when only rennet was used. This highlights the importance of the acidification process and confirms that rennet, that reacts on very specific amino acid sites and is known to destabilize casein micelles, has no effect on soy proteins, regarding gelation. However, rennet was able to react on caseins in soy-casein mixes and aggregates were formed trapping soy and milk proteins together, forming unique microstructures. The gelation point for the mix occurred at pH 6.1, instead of 5.0 for milk. It was concluded that the gelation process was driven by the soy proteins, because the gelation occurs although the pH was insufficiently low to cause aggregation of milk proteins. More specifically, the behavior of pre-heated (90°C) soy "milk"-whey protein isolates (WPI) aggregates has been investigated during acidification by GDL (Roesch et al. 2004). Different ratios were tested from 100:0 (w/w) WPI/soy proteins to 0:100 (w/w) with 70/30, 50/50, 10/90 and 30/70 intermediates, all with a 6% final protein content, in presence of 0,1M of NaCl. The gels formed were analyzed both by dynamic oscillatory measurement and confocal microscopy. Results showed that all mixes formed gels upon heating. Moreover, WPI seems to play a major role in the network formation. No gelation was observed with pure soy proteins or at high soy protein content (90/10). The confocal microscopy also showed that heat treatment (90°C) increases the size of aggregates particles and proteins clusters before acidification. G' was the highest for gel formation with a milk/soy protein ratio 70/30 and does not increase with further addition of soy proteins anymore.

Ben-Harb et al (2018) compared the gelation process suspension of pea proteins, milk proteins or a ratio of 1:1 pea/milk proteins, with a constant protein concentration of 14.8%. More precisely, three gelation techniques were used for gelation: a GDL treatment without heating, an enzymatic treatment with chymosin or transglutaminase (TGase) without heating and a thermal gelation (80°C – 1h and cooling to 20°C). The rheological properties were followed by a rheometer and the microstructure by confocal laser microscopy in order to analyze the gelation process and the gel's microstructure. Results showed that thermally induced pure pea gels and mixed gels had comparable rheological and microstructural properties. It has also been shown that addition of fats reinforces the mechanical properties of the pea/milk gels. In the thermally induced gelation, the presence of pea protein led to much more stiffer gels, perhaps because of increased protein crosslinking with covalent bonds. Like soy, pea proteins drove the gelation process, creating bonds with milk proteins to form a three-dimensional network. In contrary to milk/soy gel, the pea/milk gel have a much greater elasticity as well as the pure pea and milk gels alone. Also, the study reported that higher concentration of pea proteins yielded to a much stiffer gel after the thermal treatment. In pea/milk gels, those findings mean that the stiffness depends on the whey protein concentration. Nevertheless, mixed gels were firmer than the pure milk gels and equal to pure pea gels. In the enzymatically-induced gelation, the G' of the mixed gels was lower than the pure milk or pea gels, probably because the TGase is more susceptible to form bonds between similar proteins than different proteins. In the acid-induced gelation, the pure pea and mixed suspensions gelled quicker than the pure milk suspension, due to a higher gelation point of the pea proteins. All these findings show that the heat-induced gelation works well for mixed suspensions, as G' was the greatest among all gels. That could be explained by a co-gelation phenomenon between pea and milk proteins, creating covalent bonds in the process. However, the nature of those bonds has not been explained. Also, the enzymatic treatment produces more elastic and strain resistant gels than the other gelation techniques. The addition of fat into the suspensions has also been studied, as it conducts to an increase of G' for all gels, probably because the droplets act as fillers in the network.

Another study investigated more specifically the acid gelation of mixed thermal aggregates of pea globulins and β-Lg (Chihi, Sok, et Saurel 2018). The acid gelation was conducted by GDL and the authors measured the rheological properties, the microstructure and the WHC of the formed gels. Results showed that the gels formed by thermally-induced aggregates of pea globulins and β-Lg have better elasticity properties and WHC than these containing pure non-thermal induced aggregates of pea globulin and β-Lg. More fibrillate structures were found in those gels, conducting to a much more porous and irregular structure with reduced elasticity and WHC. Those finding show that is better to heat both types of proteins prior to an acid induced gelation process. In addition, it has been reported that an increase in β-Lg amount leads to enhanced gelling properties.

Zhao et al (2018), investigate the properties of recombined soymilk and bovine milk gels by acidification of the media with different levels of GDL (0,1% ; 0,15% ;0,20%, 0,25% ; 0,30% ; 0,30%). Gels contained 20% bovine milk and 80% soymilk and were heated to 100°C for 10 min before addition of GDL. The gel formation was conducted at 75°C. Measurements were made on protein composition, WHC, textural properties, SEM (scanning electron microscope) analysis, particle size test, sensory evaluation and attributes analysis. Results showed that hydrophobic interactions and disulfide bonds were the main driving forces to the gelation process in mixtures with bovine milk and soymilk. After 2h of cooling, textural analysis was made. Texture properties were driven by GDL, creating protons H^+ that cause a pH decrease in the media. Results suggested that addition of GDL greatly affects the structure of the gels made. Springiness, which is the ease of the gel to be broken into smaller pieces was at maximum for a value of 0.25% GDL. Hardness was a dependent of the concentration of GDL, with much harder gel as the concentration of GDL increases. WHC does not shown significant differences regarding the GDL concentration. However, it is to note that the formation of a network improves the WHC of the gels. Regarding the microstructure, the minimum concentration of GDL to efficiently form a gel with protein aggregation was 0.15%. Adding more GDL did not result in a more uniform and homogenous structures, as well as the particle size distribution, where the aggregates ranged from 10.3 to 14.3 µm. The sensory evaluation of gels formed depends significantly of the amount of GDL added. Gels with 0.25% GDL tends to have the highest scores at color and flavor. Finally, the attributes analysis revealed that mixed gels showed a protein value (3.7%) between pure bovine milk gel (3%) and pure soy gel (3,. %), as well as a crude fat content (1.8%), located between pure bovine milk gel (3.3%) and pure soy gel (1.6%).

Silva et al (2018), studied the gelation behavior of aqueous micellar casein (MC), supplemented with some soy (SP) and pea (PP) globular protein (GP), in comparison with a standard : addition of whey protein (WP). Gels contained 6% w/w of MC supplemented with the different globular protein content ranged between 0-6% w/w. pH of the suspension was 5.6-6. Heat induced gelation ramped with 5°C/min from 20°C to 90°C and was held at 90°C for 1h and then cooled as the same rate. Different parameters were measured: protein composition by SDS-PAGE, and rheological properties such as stiffness. Results suggested that the different suspension gelation point temperature was dependent on the quantity of GP added but also vary between GP sources. The gelation critical temperature was higher in the order of SP>PP>WP. This could be explained by the fact that GP have properties to bind the calcium present, reducing by the way the availability of it for the casein to make bridges. The stiffness of the gels were significantly stronger at 90°C for MC-WP gels only. With supplementation with SP, the gel stiffness increased not significantly at pH 5.6 and 5.8 and decreased at pH6.

Li and Damodaran (2017) investigated the *in vitro* digestibility and IgE reactivity of enzymatically cross-linked heterologous protein polymers. The suspensions tested were as follows: WPI, soy protein isolate (SPI) and casein (CN). Each suspension was a 2% of SPI, WPI or CN. For the mixes: WPI-SPI, WPI-CN and SPI-CN, 2% of each protein sources were mixed together at 1:1 (w/w) ratio to give a solution with 1% of each. Gelation process used TGase without pre-heating treatment. *In vitro* digestion, protein composition, and immunoassay analysis were conducted. Results explained that the TGase catalyzes the formation of isopeptide bonds between primary amines groups and carboxamide group of glutamine residues, resulting in a formation of a crosslinked network. The network is composed by branched-brain strains that were unique depending the different sources of protein used. The gelation process partially covers IgE binding sites which lower the potential allergenicity of the proteins. Since the network formation mainly depends on the glutamine to lysine ratio and the distribution of these residues in the protein's structure, some proteins like β-Lg who has been reported to have a low ratio Glu-Lys could presents large gaps in the resulting network formation, allowing IgE to bind. Thus, authors noted a decrease in IgE reactivity in SPI-WPI and SPI-CN gels to 10% of their original reactivity. This was not the case in WPI-CN gels. This ability must relate to the high glutamine content of SPI which leads in an effective high polymerization with high branched chains who can disrupt epitopes of WPI and CN. The *in vitro* digestion shows that peptides released form SPI and CN in all polymer products did not affect the IgE reactivity, which dropped to 0 after the gastric phase digestion. Counter to that, peptides released from WPI (single or mixed) showed a residual 5-20% IgE reactivity during the entire digestion. Taken together, those results suggest that GDL gelation led to a better disruption of IgE epitopes in gels with CN, SPI, and SPI-CN than in WPI systems.

Silva et al. (2019) investigate the heat-induced gelation of mixtures of micellar caseins and plant proteins in an aqueous solution. Gelation process were made using different mixtures with MC and plant protein (SP, PP) ranged from 100/0 to 0/100 MC/SP, PP for a total protein content of 4%, 6% and 8% at pH 5,8 and 6. The gelation was made by heating treatment starting at 20°C with a 5°C/min rate to 90°C. Then the mixtures were held at 90°C for 1h and then cooled from 90°C to 20°C at a rate of 5°C/min. Rheological properties of mixes and structural analysis by confocal microscopy were made to qualify the resulting gels. The solubility each kind of proteins was also calculated in aqueous solution with 2% protein content. Results shown that in water, plant proteins have a lower solubility than MC and as a function of the pH. In fact, solubility of SP and PP decreased below pH 6 and 6.5 because it approaches the isotonic point of protein which are 4.4 and 4.5 for soy protein isolate (SPI) and pea protein isolate (PPI) respectively. At pH> 6.5 SPI showed a better solubility than PPI. Authors explain that these results limit the use of plant proteins in food systems but only when the proteins were not thermally treated. Regarding the rheological properties in MC suspensions, G' increased quickly around 50-60°C for a concentration of 4% (w/w). Then, the critical gelation temperature decreased when the MC concentration increased (up to 40°C at 8% w/w) and at lower pH values. When MC was replaced by SP and PP the critical temperature of gelation increased at the same pH values independently of the final protein content. This effect could be explained by the fact that plant proteins better chelate calcium than MC, bridging peptides more effectively. The effect was stronger for SP compared to PP. Confocal microscopy revealed that MC and plant protein do not aggregates one another/each other, forming independent networks. Thus, stiffness of the mixed gels at a given total protein content was lower when MC or plant proteins were replaced by their respective counterparts (MC, PP or SP). Gel stiffness was minimal when mixture contained 40% SP or 70% PP of the total protein at pH 5.8. However the formation of interpenetrated networks from MC and plant proteins should increase the WHC (not measured in this study), as the authors report an inhibition of the syneresis in the mixed gels, which not the case with the individual protein gels.

IV. Conclusion and perspectives

Preparing mixtures with both animal and legumes component is achievable and could have a great impact on the development of food industry and a great interest in a near future. Legumes are sustainable and affordable resources, their proteins are also valuable and show an interesting nutritional profile. Rheological properties of legumes and milk are compatible and make interesting synergies, mainly in terms of aggregation and gelation protocols.

Pea/milk mixes and soy/milk mixes are currently the most represented mixes in this literature review, and no data was found about designing mixes with egg as the animal product. Making gels which involve soy and milk proteins is a good start and could be achieved by several techniques, including those already used in the dairy industry. In those mixtures the gelation process is mainly driven by soy proteins. Acid-induced gels show the best results in terms of stability and firmness and the gels made this way often have a strong storage modulus, near the pure milk gels which makes it more solid-like. According to the literature varying the concentration of both soy and milk proteins may also lead to the creation of a wide range of gels and unique microstructural profiles.

Making gels with pea proteins and milk proteins (caseins and whey proteins) are also possible, even if it does not lead to the creation of a product on the market now. It could however conduct to several food application in terms of texturation. For now, it is possible to assess that mixed pea/milk gels have comparable rheological and microstructural properties. Fat can play a role on the gelation process as they increase the stiffness of the products. Like the soy proteins, pea protein leads to the gelation process of mixes. Pea proteins also show a greater elasticity which could conduct to food applications different than that showed with the soy/milk mixes.

Although legumes show good rheological properties, they are also known for their nutritional properties and health benefits and it is important to remember it when designing new food applications. Because of their relatively good amount of antioxidant molecules and phytosterol they could contribute to reduce the risk of developing several diseases. However, legumes also contain some anti-nutritional factors that can lower the digestibility and are often cause of intestinal discomfort. Fortunately, the heating processes and fermentation often achieved to reduce the amount of the anti-nutritional components in the final product. Apart from sensory evaluation and crude composition analysis, studies reviewed on mixes do not report health benefits for now, as they are mainly focused on more technological aspects.

Despite of the rheological properties, fermentation by lactic bacteria leads on more concrete products. Soy/milk mixes yoghurt-like product has already been made under laboratory conditions, using common fermentation techniques and the same bacterial species that are already used in the dairy industry that is often taken as the reference. Although it was not possible to obtain a nutritional profile superior or identical to pure milk yoghurts, products made this way show however an interesting nutritional profile (ref ?). This may depend on the species and even the strain used for the fermentation which also could affect in turn the sensory profile of the product, removing the undesirable “beany” flavor to a much creamy sensation. However, little is known about fermentation of mixes between animal and vegetal products yet, (Ben Harb et al 2019).

Therefore, there is room for investigation and this review gives clues about legumes and their properties and how it is possible to use them in order to design novel food. In that way both affordable and sustainable, mixes could offer an interesting response to the current demand of proteins, in order to nourish an always growing population.

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