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Mixing milk, egg and plant resources to obtain safe and tasty foods with environmental and health benefits

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ARTICLE INFO ABSTRACT Keywords: Background: Since the early 1990s, major health and environmental concerns have developed and driven the Animal/plant mix emergence of diets involving a lower consumption of animal products. However, the transition towards greener Acceptability diets is being hampered by the poor acceptance of vegan foodstuffs among western consumers. Mixed animal/ Nutrition plant alternatives to familiar dairy or egg products offer a new field of innovation. Techno-functional property Scope and approach: This review focuses on innovative mixes of egg or milk with plant ingredients - especially Environment legumes - to develop products in which interactions between animal and plant are not usually expected, such as Risk-benefit ratio dairy or egg gels, emulsions or foams. The opportunities offered by such products in terms of consumer Fermentation acceptance, nutrition, digestibility and techno-functional properties are reviewed and discussed with respect to their risk-benefit ratios. Key findings and conclusions: In many cases, animal/plant mixes offer enhanced protein stability and synergistic interfacial or textural properties that make them a flexible tool for food design. Fermentation offers important prospects for the nutritional and sensorial enhancement of animal/plant mixes, through the multi-criteria application of microbial consortia. Animal/plant mixes enable reduction in animal protein consumption while preserving amino acid and micronutrient intakes and sensory properties. However, their acceptability to consumers and society will also depend on controlled safety, especially regarding allergies or contaminants, on affordability, their degree of novelty or (ultra)processing, their actual environmental footprint and whether they meet consumer expectations for innovative foods in the transition towards greener diets.

1. Introduction: drivers and motives for the emergence of mixed animal/plant foodstuffs

Increasing concerns regarding health and the environment are driving a new phase of food transition in western countries, supported by public policy recommendations to increase the share of plant proteins in order to reduce the consumption of animal resources. In this respect, food policies that recommend more sustainable diets are being effective and alternative diets are emerging that clearly "label" this transition, such as vegetarian or vegan diets and the intermediate flexitarian diets that mainly aim to reduce meat consumption (Aiking & de Boer, 2018; Willett et al., 2019). On the other hand, western societies and consumer habits markedly evolved during the second half of the last century, with considerable reductions in the time and budget spent on home cooking and food. Demand for convenient and affordable foods has been satisfied by intensifying food production and industrializing processing. In western countries, the leading motivations behind food purchasing are price and sensory appeal, followed by health, convenience, taste and familiarity or habits regarding a product (Allès et al., 2017). Demands for "naturalness" and "clean label" foods have emerged more recently but do not yet seem to be affecting the growing share of manufactured foods consumed by households. Therefore, intentions to change and

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Received 10 July 2020; Received in revised form 9 November 2020; Accepted 12 December 2020 Available online 19 December 2020 0924-2244/© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). drivers for the acceptance of a greener diet need to be carefully investigated (Elzerman, Hoek, van Boekel, & Luning, 2011; Niva, Vainio, & Jallinoja, 2017; Vainio, Niva, Jallinoja, & Latvala, 2016). Producing animal protein from eggs or dairy contributes less to water use, land use or greenhouse gas emissions than meat, so they are good candidates to reduce the environmental footprint of food by means of animal/plant mixes. Although egg or dairy products are commonly regarded as sustainable sources of animal protein, there is growing demand for a reduction in animal protein consumption.

In this context, the design of alternative animal/plant mixed foods could open perspectives for innovations to meet this dual demand from society to reduce the environmental footprint of food while offering convenient, healthy and tasty products that offer good value for money (Fig. 1).

Along the same lines as successful flexitarian meat substitutes, animal/plant foodstuffs incorporating dairy or egg ingredients are likely to emerge as a new transitional offer that has yet to find its audience (www. iar-pole.com/evenements/plantbased-animal-hybrid-proteins/). Food companies such as Bongrain, Danone, Fromageries Bel, General Mills, Ingredia, Lactalis, Nestlé or Unilever are already active in using animal/ plant mixes for the manufacture of innovative industrial ingredients or retail beverages, cheese-like food pastes, high-protein foods or frozen desserts. The purpose of this paper is therefore to show how academic research is contributing to the design of safe, healthy and tasty animal/ plant mixed foods by providing knowledge to the public regarding all dimensions of this new and complex field of innovation: molecular interactions (Alves & Tavares, 2019; Schmitt, Silva, Amagliani, Chassenieux, & Nicolai, 2019) and also processing, fermentation, safety, digestibility, nutritional and social aspects. This paper will mainly focus on milk/legume mixes. Research on animal/plant mixes using egg resources is comparatively scarce but will be mentioned throughout. This review of the literature is completed by the position of the INRAE Institut Agro Joint Research Unit, hereinafter referred to as STLO, regarding its opinion on this emerging topic, namely in sections 2, 5 and 6 of the paper.

2. The roots and rise of mixed animal/plant foodstuffs

Traditional mixed animal/plant foodstuffs such as custards, porridges, egg pasta and meatballs have long existed. Some of them are fermented; for example Lebanese kishk, Indian selroti or Turkish tarhana (Tamang, Watanabe, & Holzapfel, 2016). Research activity on mixed animal/plant food products has existed since the 1960s and remained consistent between 1970 and 1990 (Fig. 2), when efforts by countries such as the United States, Canada or India mainly focused on developing low-cost alternatives to dairy products in emerging countries. Mixes of dairy and plant proteins also helped to optimize the body weight gain to cost ratio in ready-to-use foods to combat malnutrition or hunger (Stobaugh, 2018). Another important field of research has been, and remains, that of infant formulas, for which milk proteins are generally blended with vegetable and fish oils to meet nutritional requirements. Finally, research on mixed animal/plant foodstuffs has long existed with respect to specific dietetic products, the design of sports formulas, weight control formulas and medical products for oral/enteral nutrition. In most cases, these products are liquid or ready-to-use powders, pastes or bars, and at least for adults, tend to belong to the category of food substitutes or dietary supplements rather than conventional consumer goods.

A significant increase in the publication of original scientific communications dealing with mixed animal/plant foodstuffs has been clearly noticeable since the 1990s, and even more so since 2010 (Fig. 2). During this period, the new authors mainly came from Europe, Oceania and China. This coincided with the emergence of the important environmental concerns expressed by the first Intergovernmental Panel on Climate Change in 1990. For instance, statements that "animal proteins need to be more widely replaced by plant proteins in food formulation" (Schmitt et al., 2019) or "the partial replacement of animal protein with plant protein in formulated products stands as the beginning for reduced environmental impacts associated with enormous animal protein consumption" (Alves & Tavares, 2019) thus introduced the most recent reviews on mixed animal/plant food design.



Fig. 1. Drivers and constraints supporting the emergence of innovative food products that involve mixed animal and plant resources.



Fig. 2. Research activity on the development of mixed plant/animal foodstuffs worldwide. Left panel: time-scaled increase in the number of Web of Science (WoS) entries and new nations involved per decade. The insert focuses on France (in orange) and the French National Research Institute for Agriculture, Food and the Environment (INRAE, which resulted from the merger of INRA and IRSTEA – in green). Right panel: number of Web of Science (WoS) entries and year of first publication for each nation. The search terms used were (milk OR dairy OR casein* OR whey OR lactoglobulin OR egg) AND (legume* OR pea OR lupin* OR soy* OR chickpea) AND (mix* OR blend*) in TOPIC fields, completed by NOT (graz*) to eliminate most bovine feed studies. The entries were checked individually to eliminate those which did not strictly concern animal/plant foodstuffs, such as animal feeds, analytics or animal *vs* plant comparisons. A total of 406 entries were finally selected. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Among these countries, France started producing significant publications on mixed animal/plant foodstuffs in 2015 with IRD and Montpellier SupAgro, followed shortly by Université du Maine and INRAE (Fig. 2). More publications are anticipated, with a growing number of ongoing projects launched by STLO or elsewhere. For our work, innovative animal/plant foodstuffs are defined as formulated assemblies of milk or egg with plant components, where the animal and plant components, and particularly proteins, interact to constitute new matrices by means of various food processes, including fermentation. Plant components mainly come from legumes, but not exclusively. Because of the expertise at STLO on egg and milk components, this review therefore excludes algae or fungi, as well as meat, insect, fish or other animal resources, as well as vegan products. Distinct from dietary supplements, innovative animal/plant foodstuffs form part of standard vegetarian or flexitarian meals. Our goal is therefore to provide keys to the formulation of safe, innovative mixed animal/plant foodstuffs that present desirable organoleptic qualities such as flavor and texture along with maximized environmental and health benefits.

3. Composition and properties of milk, egg and certain legumes

3.1. Milk

Bovine milk, as a reference, contains 4.5% wt. lactose, 3.9% fat, 3.2% protein and 0.8% ash. The protein consists in 80% wt. casein micelle assemblies constituted of β , α_{s1} , α_{s2} and κ -caseins and 20% whey proteins with a majority of β -lactoglobulin, α -lactalbumin, bovine serum albumin, immunoglobulins and lactoferrin. The casein micelles are stabilized by internal hydrophobic interactions and calcium-phosphate bonds and by external electrostatic and steric repulsion through the carboxyl

moiety of the κ-casein. They are barely heat-sensitive but susceptible to coagulation by rennet or acidification (pI \sim 4.6), based on which cheese and yoghurt making were developed. Conversely, whey proteins are soluble at all pH values but are largely heat-sensitive, undergoing denaturation and forming heat-induced aggregates above 60 °C. During the heat-denaturation process of whey proteins, the free thiol of β-lactoglobulin is extremely reactive in promoting thiol/disulfide exchanges, yielding inter-protein disulfide bonds that strengthen yoghurt gels. Milk proteins can be separated, concentrated and/or isolated to prepare industrial ingredients that make use of their interfacial, thickening or gelation properties in food formulation. Milk proteins are also of excellent nutritional quality, as evidenced by their protein digestibility-corrected amino acid score (PD-CAAS) and digestible indispensable amino acid score (DIAAS) (Fig. 3A; Chalupa-Krebzdak, Long, & Bohrer, 2018). Cysteine and methionine are the less abundant essential amino acids in caseins while valine and histidine are those found in whey proteins (Phillips, 2017).

Essentially embedded in milk fat globules, milk lipids are composed of 98% triacylglycerol and 2% diacylglycerol, free fatty acids, polar lipids and sterols. The fatty acid composition of cow, goat or ewe milks depends on the animal's diet but is notably high in saturated fatty acids within the C4:0-C18:0 range and up to C24:0 in sphingomyelins. Animal milk lipids are generally higher in saturated fatty acids than vegetable oils (Fig. 3B) thus enabling a wide variety of textures in foodstuffs. Milk lipids have valuable nutritional properties in terms of regulating plasma cholesterol or carrying nutrients such as carotenoids or vitamins, etc. Animal *trans* fatty acids nevertheless appear to have detrimental effects on health, with the exception of conjugated linoleic acid which exerts a range of positive biological activities, e.g. in cancer prevention, immunomodulation, and fat or sugar regulation. With respect to sugars, the



(A) essential amino acid composition (% wt of total)

Fig. 3. Composition profiles of some raw animal and legume sources. (A) Truncated Protein digestibility-corrected amino acid score (PD-CAAS), essential amino acid, (B) fatty acid and (C) free sugar compositions of cow, ewe and goat raw milks, raw egg white or yolk, and mature soy, pea, lupin or chickpea whole seeds, in g/100g of each considered fraction. Fig. 3(B) also indicates the saturated fatty acid/unsaturated fatty acid (SFA/UFA) ratio, in g/g. The PD-CAAS of plant proteins concern protein isolates, which include lupin protein isolate that mainly contains globulin-type protein. This leads to a lower PD-CAAS than expected if all lupin proteins had been present. Data were collected from the ANSES CIQUAL and the USDA Food Data Central databases for (A) and (B) and from the academic literature for (C).







great majority in milk is lactose (Fig. 3C), with only traces of other sugars such as oligosaccharides. Milk is also an excellent supply of minerals, and particularly calcium, magnesium and phosphorus.

3.2. Eggs

The edible part of hen eggs is composed of the white (10.5% wt. protein, <1% wt. sugar and small amounts of minerals and vitamins) and the yolk where all egg lipids are concentrated (34.5% wt. fat, 16.1% wt. protein, 0.7-1% wt. sugar and <1% wt. minor compounds). Egg white contains ovomucin fibers which are dispersed in an aqueous solution that mainly contains globular proteins, ovalbumin, ovotransferrin, ovomucoid and lysozyme. Many egg white proteins have vitamin binding and/or bacteriostatic activities that are vital to the developing embryo and can be useful in food formulation. While egg white does not contain lipids, the dry mass of yolk is composed of 68% wt. low-density lipoprotein (LDL), 16% wt. high-density lipoprotein (HDL), 10% wt. livetin as soluble globular proteins, 4% wt. phosvitin and minor proteins. Apoproteins in lipoproteins account for 11–17% wt. or 75-80% wt. of the dry mass of LDL or HDL, respectively. While LDL are dispersible structures. HDL associate with phosvitin through phospho-calcium bridges to form egg yolk granules. Whole egg, egg white and egg yolk all have excellent amino acid profiles (Fig. 3A). The least abundant essential amino acid in hen eggs is histidine (Phillips, 2017). Yolk lipids are composed of 62% wt. triglycerides, 33% wt. polar lipids, <5% wt. cholesterol, <1% wt. free fatty acids and <0.1% wt. carotenoids. The fatty acids in yolk are saturated C16:0-C18:0 (~35% wt. of the total), monounsaturated C18:1 (~45% wt.) and longer chained C18-C22 polyunsaturated fatty acids (~20% wt. - Fig. 3B), including omega-3 fatty acids with bioactivity in brain function and sight. The composition of mono- and polyunsaturated fatty acids is strongly dependent on the hen's diet. Egg yolk is an excellent source of liposoluble vitamins and minerals. On the other hand, iron bioavailability is impaired by its strong binding to phosvitin. The free sugar in egg is essentially glucose (Fig. 3C) but significant amounts of carbohydrates are also found conjugated to proteins, especially ovomucin and ovomucoid.

3.3. Legumes

Legumes are a plant family grown worldwide and include soybean, peas, beans, lentils etc. They are mainly composed of 15-50% dry wt. of storage proteins such as albumins, globulins, glutelins and prolamins. Globulins account for over 50% of the total protein mass and are subdivided into legumin and vicilin/convicilin in pea and chickpea, conglutins in lupin and glycinins/conglycinins in soybean (Bessada, Barreira, & Oliveira, 2019; Hall, Hillen, & Garden Robinson, 2017; Nishinari, Fang, Guo, & Phillips, 2014). Albumins, glutelins and prolamins account for 2-30%, 5-25% and less than 5% of total proteins, respectively. The globulins are barely soluble in water at a neutral pH, unless salts are present. Albumins are soluble in water, but glutelins and prolamins are only soluble in alcohol, acid or alkali solvents. Globulins readily precipitate at pH 4-6 and under processing such as heating, which may decrease the solubility of pea, chickpea or soybean isolates down to as little as 20%. On the other hand, heat aggregation, modulated by salts and/or pH, is desirable to control the gelation properties of legume proteins (Fischer, Cachon, & Cayot, 2020; Nishinari et al., 2014). Glycinin and legumin contain 4-8 cysteine residues involved in disulfide bonds while vicilin and conglycinin lack this feature. The heat aggregation of legume protein tends to be driven by hydrophobic interactions although thiol/disulfide exchanges do occur (Lambrecht, Rombouts, De Ketelaere, & Delcour, 2017). Legume proteins bear a significant surface charge as well as significant hydrophilicity, with soybean and pea isolates being the least and the most hydrophobic, respectively (Fischer et al., 2020; Nishinari et al., 2014). With this, legume proteins have excellent foaming and emulsifying properties that are commonly used in food formulation. Under appropriate processing, legume protein fractions can exhibit elevated PD-CAAS or DIAAS values, but they are not complete essential amino acid sources (Fig. 3A; Chalupa-Krebzdak et al., 2018; Fischer et al., 2020). Cysteine/methionine and tryptophan are the limiting amino acids (Phillips, 2017). Leucine, implicated in muscle synthesis, is not limiting in legume proteins but less abundant than in milk or egg. As storage proteins, legume proteins are highly structured and densely packed, so that proteolysis sites are barely accessible. For these reasons, they are less digestible than animal proteins and require more processing to become bioaccessible. Furthermore, legumes contain anti-nutritional factors such as trypsin inhibitors that can also reduce the protein hydrolysis in the GI tract, tannins, phytic acids or saponins, which reduce their overall quality (Bessada et al., 2019, Fig. 4A). On the other hand, legume proteins also have antioxidant, cellular regulation and bacteriostatic activities, along with the high carotenoid and vitamin content of legumes (Bessada et al., 2019). Lipid contents also vary as a function of species; they are only 2% dry wt. in pea, against 8% in chickpea, 6–20% in lupin and \sim 20% in soybean. They are composed of a majority of phospholipids and triglycerides (20-60% each), free fatty acids and sterols (Hall et al., 2017). Less than 25% of the total fatty acids in legumes are saturated, with a majority of C16:0 then C18:0 (Fig. 3B). Unsaturated fatty acids belong to the C18 family, with a majority of omega-3 C18:2 linolenic acid (Fig. 3B). Carbohydrates are the major components of legumes, accounting for 35-70% of dry mass. Lupin or soybean have the lowest, and chickpea or pea the highest, shares of these components. As well as significant amounts of dietary fiber and starch of nutritional relevance, legumes also contain 3-16% dry wt. of sugars that are important for the digestibility and fermentability of legume sources. Ribose, galactose, glucose and N-acetyl-galactosamine monosaccharides; maltose and saccharose disaccharides as well as galacto-oligosaccharides and a-galactosides in the case of raffinose, stachyose and verbascose (Fig. 3C) are present in soybean, pea, chickpea and lupin. The α -galactosides stachyose, verbascose and raffinose are potentially prebiotics at low doses, but are poorly digestible and can cause digestive discomfort or reduce the digestion of other nutrients or micronutrients. By comparison with milk or even egg ingredients, the composition and properties of legume-based ingredients do not only exhibit considerable diversity but are also markedly affected by the various processes applied. For instance, legume-based flours usually have a more diverse protein composition than legume-based protein isolates (mainly composed of globulins), but anti-nutritional components, and obviously sugar components, are usually more abundant and diverse in the former. This is of primary importance when formulating food products, particularly when fermentation is involved.

4. Complementarity, positive synergies and adverse effects of mixed animal/plant foodstuffs

4.1. Sensory characteristics and preferences

The sensory characteristics of plant products, such as taste, texture, aspect and odor, are important factors when promoting their acceptability among consumers (Niva et al., 2017). Familiarity plays an important role in consumer acceptance. Individual culture and cooking habits are thus the main barriers to increasing the consumption of bean or soybean-based products in western countries. For instance, the appropriateness and acceptability of meat substitutes, yoghurts or gluten-free cookies based on soybean or pea protein were spontaneously rated by consumers in terms of the difference in shape or taste of the substitute when compared to that of the regular product (Elzerman et al., 2011; Jarpa-Parra et al., 2017; Šertović et al., 2019). The partial replacement of animal protein by plant protein in familiar foods, or the development of new mixed animal/plant foods, have been proposed as solutions to encourage the transition towards a plant-protein based diet (Niva et al., 2017). For instance, dairy-flavored tofu may appeal to



Fig. 4. (A) Synergies (left) and adverse effects (right) at play in mixed animal/plant foodstuffs. (B) The powder solubility index, powder dispersibility index and apparent viscosity of the homogenized concentrate of infant formula with 50% wt. replacement of whey protein with pea or faba bean protein were similar to those of the reference dairy infant formula milk (Le Roux, Chacon, et al., 2020). (C) Foaming capacity and foam stability increased in line with the increasing replacement of egg white protein with potato protein, but formed less resistant structures (Lechevalier et al., personal communication). Foaming capacity is expressed in mL foam per 100 g liquid while foaming stability is 100 minus the weight of the liquid draining off 100 g foam over 1 h. (D) At pH 7.5 and ionic strength 10 mM, overall negatively charged milk β-lactoglobulin and positively charged rapeseed napin interacted to form soluble nanoparticles (Ainis et al., 2019).

western consumers. Indeed, the specialized media consider mixed animal/plant foodstuffs, referred to as "hybrid" products, as an interesting niche to promote a flexitarian diet, especially among meat-eaters (Askew, 2020). However, the existence of negative correlations between purchasing motives for health or the environment and motives for tradition (Allès et al., 2017; Pieniak, Verbeke, Vanhonacker, Guerrero, & Hersleth, 2009) calls for a compromise between familiarity and novelty. In other words, consumers who are interested in moving toward healthier and/or sustainable foods may actually look for something completely new rather than a more minor shift from standards.

4.2. Nutrition and digestibility

Animal/plant mixes can both balance the instantly bioavailable essential amino acids (Liu, Klebach, Visser, & Hofman, 2019) and increase targeted protein-driven health effects (Devassy et al., 2017; Wojcik et al., 2016). Similar observations of better-balanced bioavailable fatty acids or sterols have been reported for mixed animal/plant foodstuffs (Hamdan, Sanchez-Siles, Garcia-Llatas, & Lagarda, 2018; Huang, Lee, & Ahn, 2019). Meanwhile, crossed molecular interactions and/or complex co-absorption regulations may lead to positive (but also sometimes negative) side effects that have yet been little investigated (Fig. 4). In a context of acute malnutrition, adding whey or milk protein to plant-based food aid formulas was able to accelerate weight gain and recovery of the gut microbiota (Leng et al., 2019; Stobaugh, 2018). In healthy elderly individuals fed with reconstituted protein meals, their postprandial amino acid plasma profile was best balanced when milk, soybean and pea proteins were ingested together rather than separately (Liu et al., 2019). In particular, leucine and methionine were more available with animal/plant mixes than with plant sources alone, while arginine and glycine were more available with the mixes than with milk proteins alone. Mixing whey, caseins and soybean proteins in a single food has also been recommended for sportsmen and women, as mixing "fast" and "slow" digested proteins with different amounts of leucine is expected to stimulate the synthesis of skeletal muscle protein (Paul, 2009). A mix of soybean, casein and whey proteins was shown to prolong postprandial aminoacidemia and stimulates muscle synthesis in rats, young adult humans or the elderly, more than whey and/or soybean protein alone (Berrazaga, Micard, Gueugneau, & Walrand, 2019). A recent investigation also indicated that infant formulas made with mixes of milk and pea or faba bean proteins were as digestible as the control milk formula and adequately covered requirements for essential amino acids (Le Roux, Chacon, et al., 2020). Mixed animal/plant products were shown to enable a reduction in the intake of animal ingredients without risking deficiencies in unsaturated and particularly omega-3 fatty acids, vitamins such as B12 and D or minerals such as calcium, zinc or iron (Chalupa-Krebzdak et al., 2018). However, the bioaccessibility of these components depends on the structure of the animal/plant protein matrix (Hiolle et al., 2020). Furthermore, the absorption of zinc (but not iron) from rice with a high phytate content increased by 65-78% when women consumed it with milk or yoghurt instead of water (Rosado et al., 2005; Talsma et al., 2017). The hypothesis is that zinc binds preferably to milk proteins rather than to phytate, thus enhancing its bioavailability. The addition of casein

phosphopeptides to phytate-rich soybean-based, oat-based or rice-based infant foods partly counteracted the inhibitory effect of phytate on calcium absorption. However, the effect was less pronounced with whole casein and was not observed with all plant matrices (Hansen, Sandström, & Lönnerdal, 1996). Furthermore, phytate displaces calcium from casein micelles (de Kort, Minor, Snoeren, van Hooijdonk, & van der Linden, 2011) and may decrease the bioavailability of dairy calcium in a mixed dairy/legume food. Phytate or the calcium-phytate complex binds to casein or whey proteins and reduces their digestibility by 4-9% (Carmovale, Lugaro, & Lombardi-Boccia, 1988). However, this drawback can be limited using fermentation (see section 5). Recent evidence has also shown that soaking ground faba beans in Laban fermented milk significantly reduced the occurrence of favism, i.e., acute hemolysis in G6PD-enzyme deficient individuals (Zam & Belal, 2020). In another field, the interaction of saponin with the whey protein α -lactalbumin enhanced its digestibility by trypsin under some conditions (Shimoyamada, Okada, Watanabe, & Yamauchi, 2005), while the binding of tannins was able to inhibit intestinal lactase in young and adult rabbits (Chauhan, Gupta, & Mahmood, 2007). In gluten-free breads, the interaction between egg white protein (used as a gluten replacer) and starch adversely affected the starch digestibility (Sahagún, Benavent-Gil, Rosell, & Gómez, 2020). The specific issue of allergy is treated in section 6.

4.3. Functional properties

The issue of mixing animal and plant proteins to diversify the formation of heat-induced co-aggregates in mixed gels or films or to diversify interfacial properties in foodstuffs has been the focus of various research groups worldwide, such as Corredig et al. at the University of Guelph (Canada), Mession, Saurel et al. at INRAE (France) or Nicolai et al. at the Université du Maine. These studies and others have recently been reviewed in detail (Alves & Tavares, 2019; Schmitt et al., 2019). The present paper focuses on egg/plant and milk/plant mixes and includes other techno-functional properties such as protein solubility, heteroprotein coacervation or protein substitution.

4.3.1. Solubility

The effects of combining denaturing pH cycles, temperature and/or homogenization on mixes of legume and milk proteins have indicated the production of dispersible and/or heat-resistant micro-particles as ingredients with synergistic solubility and stability (Boursier et al., 2014; Kristensen et al., 2020; Zhang et al., 2020). When the animal and plant proteins were oppositely charged, appropriate thermodynamic tuning of the mixing conditions enabled the formation of concentrated nano-droplets of heteroprotein coacervates, using lactoferrin or lysozyme with soybean proteins, or rapeseed napin with β -lactoglobulin or β -casein (Ainis et al., 2019; Schwartz et al., 2015; Zheng, Tang, Ge, Zhao, & Sun, 2020). These coacervates are efficient when developing highly viscous, translucent and highly concentrated protein solutions. Given their characteristics, they have also been considered for the targeted delivery of bioactive compounds.

Furthermore, milk caseins, and particularly β - and α_s -caseins, caseinate or casein micelle assemblies exhibit a chaperone-like property that prevents the chemical or thermal precipitation of globular proteins. This property was effective on hemp proteins (Chuang, Wegrzyn, Anema, & Loveday, 2019) and probably accounted for greater resistance to denaturation and smaller aggregates of pea or soybean globulins when heated in the presence of casein micelles (Alves & Tavares, 2019). Casein also reduced the thiol/disulfide exchanges in gluten (Wouters, Rombouts, Lagrain, & Delcour, 2016). The best thermal stability was reported when replacing 40–60% wt. of the pea or soybean protein with micellar casein (Schmitt et al., 2019). On the other hand, soybean or pea proteins were also found to stabilize micellar casein against heat gelation, possibly as a result of the chelation of ionic calcium by the plant proteins (Schmitt et al., 2019) or by the contaminant plant phytate (de

Kort et al., 2011). Thus, different mixes of micellar caseins and legume proteins all seem to exhibit protective effects against the heat-induced destabilization of others.

4.3.2. Heat-induced gels

When milk is used instead of micellar caseins, whey proteins are also present in the milk protein. In this case, with the heat-induced milk/pea protein isolate or pea protein isolate only, protein gels exhibited similar microstructures and textures (Ben-Harb et al., 2018), because heat-sensitive globular proteins were present in all fractions. At heating, the globular proteins denatured and formed mixed aggregates, depending on the initial protein mix and the respective properties of protein, such as the presence of free sulfhydryl groups and disulfide bonds (Alves & Tavares, 2019; Kato, Watanabe, & Matsuda, 2000). While soybean or pea proteins mostly heat-aggregate through hydrophobic, non-covalent interactions involving few thiol/disulfide exchanges, the presence of whey proteins appeared to drive the heat-induced co-aggregation of all disulfide-containing proteins and yield new aggregate forms (Alves & Tavares, 2019). During this process, the free thiol(s) of β -lactoglobulin, egg ovalbumin or bovine serum albumin seemed to trigger thiol/disulfide exchanges and promote the heat-aggregation of soybean or gluten proteins (Lambrecht et al., 2017; Nozawa, Ito, & Arai, 2016), gel springiness and the water holding capacity of soybean protein isolate/egg white composite gels (Su et al., 2015). Heat-induced gels made of co-aggregated globular proteins exhibited the greatest elasticity, either with an optimum animal/legume protein ratio, or with animal protein alone, while legume proteins alone produced soft and hydrated heat-induced gels (Alves & Tavares, 2019; Ben-Harb et al., 2018).

4.3.3. Acid-induced gels

Acid gels of animal and plant proteins heated together are models for fermented yoghurt-like products. When part of the milk protein was replaced with soybean protein, acid gelation of the co-heated mix started at higher pH values and yielded firmer gels than with milk alone, unless the fraction of soybean protein reached 50% wt. or more. When the milk protein was replaced by pea protein at 50% wt., the final firmness of the mixed gel did not differ significantly from that of pure milk. When the legume protein reached 50% wt. or more, the gels were coarse and soft, thus evidencing the role of the casein micelles in forming the sustaining matrix (Alves & Tavares, 2019; Ben-Harb et al., 2018). Whey proteins (and particularly β -lactoglobulin) are required to mediate the formation of heat-induced co-aggregates of whey and legume proteins, and probably their interaction with the acid casein micelle network (Alves & Tavares, 2019). Preheating the legume or milk protein separately prior to co-acidification appeared to be less favorable to the formation of firm acid gels than heat-induced co-aggregation. Indeed, when preheated milk was partially replaced by preheated pea protein, and then fermented, the firmness of the acid gel was decreased (Yousseef, Lafarge, Valentin, Lubbers, & Husson, 2016). Similarly, no positive interaction was reported when preheated soybean protein was mixed with unheated skim milk (Lin, Hill, & Corredig, 2012). When β-lactoglobulin was absent and the casein micelle content was standardized, a complex picture emerged where the acid gelation behavior of mixed milk/pea protein was markedly dependent on the legume protein and on whether the casein micelles were present or not during preheating (Mession, Roustel, & Saurel, 2017). While legumin, despite its significant cysteine content, was unable to form heat-induced aggregates capable of increasing the firmness of mixed casein/legumin gels, a positive interaction was reported when co-heating vicilin and casein micelles prior to acidification.

4.3.4. Enzymatically-induced gels

Proteolytic enzymes can be used to diversify the texture of mixed animal/plant gels, but this technology has been comparatively little investigated. Rennet gelation has been considered for the desig of animal/plant cheese-like products. Adding rennet at acidification increased the firmness of preheated-soybean acid gels or unheated-milk/ preheated-soybean acid gels (Lin et al., 2012). When milkfat was present, the most appreciated rennet/fermented gels were obtained when homogenization had been conducted in the presence of milk protein rather than soybean protein, and when the casein micelles had been pre-coagulated by adding rennet before the onset of acidification (Gry-gorczyk, Alexander, & Corredig, 2013). Without acidification or preheating, subjecting a mixture of pea protein isolate and milk proteins to a combination of transglutaminase and rennet yielded softer gels than when using each protein type alone (Ben-Harb et al., 2018).

4.3.5. Protein substitution or enrichment

Egg white proteins, whey proteins, milk proteins or collagen are most frequently chosen, together with non-cereal plant proteins, for the design of gluten-free cereal products such as cakes, breads, cookies, pasta or noodles. In general, the incorporation of milk, whey or egg white proteins to replace gluten yielded more cohesive, harder and more brittle cooked products than when legume or potato proteins were used. Meanwhile, plant proteins had a higher water-binding capacity and vielded more viscous batter or dough preparations (Bravo-Núñez, Sahagún, Bravo-Núñez, & Gómez, 2020). In view of the complementary effects of each protein, optimal gluten replacement formulas used binary or ternary mixtures of egg white, whey, soybean and/or pea proteins (Bravo-Núñez et al., 2020). The satisfactory foaming properties of egg white, the elasticity of the heated egg protein network and the hydration properties of pea proteins combined synergistically to make the 50:50% wt. egg white/pea protein mix a good candidate to replace gluten. However, egg white protein adversely affects the sensory properties of bread. A mix of pea and whey protein with only 5% wt. egg white protein sustained optimal formulas (Bravo-Núñez et al., 2020) while mixes of soybean and whey proteins were preferred to make gluten- and egg-free cakes (Julianti, Rusmarilin, Ridwansyah, & Yusraini, 2016). On the other hand, highly texturized protein-rich foodstuffs can be formulated by taking advantage of heat-induced thiol/disulfide, lanthionine and/or lysinoalanine polymerization between gluten and animal proteins. For instance, the principal protein in gluten, gliadin, lacks free thiol groups, and positive co-polymerization can be achieved using glutenin (as a reference) and also with animal proteins containing free thiols, such as egg white protein, defatted egg yolk protein, ovalbumin or bovine serum albumin (Lambrecht et al., 2017). While extensive polymerization is desirable when designing meat analogs, it is detrimental when enriching cereal products with animal proteins, where heat-induced non-covalent hydrophobic interactions are preferred to enable stretchability.

4.3.6. Emulsions

Finally, combining plant and animal proteins with specific interfacial properties offers new perspectives for the diversification of emulsifying or foaming ingredients. Each mix exhibits a specific phase diagram or specific possibilities for chemical interactions such as thiol/disulfide interchanges when concentrated at the interfaces. The replacement of egg yolk by soybean protein decreased the stability of water-in-oil emulsions which nevertheless displayed viscosity similar to that of the control up to 75% wt. replacement (Rahmati, Mazaheri Tehrani, & Daneshvar, 2014). Oil-in-water emulsions made with a 50:50 % wt. mix of sodium caseinate and pea or soybean proteins were optimally stable for up to 6 months and could serve as lipophilic nutrient carriers (Hinderink, Munch, Sagis, Schroen, & Berton-Carabin, 2019; Ji et al., 2015; Li, Feng, Ting, Jiang, & Liu, 2019; Yerramilli, Longmore, & Ghosh, 2017) or in mixed animal/plant ice-cream (Cheng et al., 2016). The oil phase of infant milk formulas was satisfactorily stabilized with a mix of milk and pea or faba bean protein (Le Roux, Mejean, et al., 2020). Mixes of whey/pea proteins presented synergy as emulsifiers (Hinderink et al., 2019) but mixes of whey/flaxseed proteins, or caseinate/saponin, formed unstable emulsions (Kuhn, Drummond e Silva, Netto, & da

Cunha, 2014; Salminen, Bischoff, & Weiss, 2019). Mixes of milk and legume proteins formed antagonistically unstable emulsions when the total protein concentration was too low (Ho, Schroen, San Martin-Gonzalez, & Berton-Carabin, 2018). Emulsions made with micellar casein and pea or soybean proteins were more heat stable than those made with the globular proteins alone, and this stability increased in line with the casein content (Liang, Wong, Pham, & Tan, 2016). When soybean proteins were heat-aggregated prior to emulsification, they formed highly viscous and thereby stable emulsions, properties that were altered if sodium caseinate was present during homogenization (Aoki, Shirase, Kato, & Watanabe, 1984). Alternatively, mixes of whey protein or sodium caseinate with soybean lecithin formed synergistically stable emulsions with cream, soybean oil or thyme oil (Chung et al., 2019; Mantovani, Cavallieri, Netto, & Cunha, 2013; Xue & Zhong, 2014). Mixes of plant saponin and egg lecithin could be used to form oil-in-water emulsions but no synergistic effect was reported (Reichert, Salminen, Bonisch, Schaefer, & Weiss, 2019).

4.3.7. Gelled emulsions

Food products such as voghurt, cheese or processed meat products are gelled emulsions. Homogenizing milk fat or vegetable oil with milk or with a mix of legume and milk proteins resulted in smaller fat droplets and thicker mouthfeel after acid and/or enzymatic gelation compared to homogenization using legume proteins alone (Ben-Harb et al., 2018; Grygorczyk et al., 2013). In all pea, soybean or mixed milk/legume protein formulas, the protein-coated droplets acted as active fillers and reinforced gel firmness, whatever the gelation process (Ben-Harb et al., 2018; Schmitt et al., 2019). Silva's work showed that for thermal emulsion gels, pea or soybean protein could partially or fully replace micellar casein and yield the same final elasticity, although the microstructures varied somewhat (Schmitt et al., 2019). Heating oil-in-water emulsions made with soybean or whey protein in solutions of the counterpart protein showed that β -lactoglobulin propagated thiol/disulfide exchanges with glycinin and reinforced the active-filler property of the emulsion droplets (Manion & Corredig, 2006).

4.3.8. Foams

Whey protein isolate in a 50:50% wt. mix with soybean glycinin displayed a similar foaming capacity to egg albumin but poorer foam stability (Yildirim, Hettiarachchy, & Kalapathy, 1996). The latter could however be improved by reducing the disulfide bonds and cross-linking the two-protein species together using transglutaminase. Films of 50:50% wt. soybean glycinin/β-lactoglobulin or soybean conglycinin/ β -lactoglobulin exhibited higher elasticity than β -lactoglobulin alone, and yielded denser and more stable foams than each protein alone, depending on the pH (Pizones Ruiz-Henestrosa, Martinez, Carrera Sanchez, Rodriguez Patino, & Pilosof, 2014; Yildirim et al., 1996). The addition of modified soybean protein (with pI \sim 10) to egg white resulted in increased foaming capacity and significantly improved stability when compared to adding the same amount of egg white protein (Wang, Troendle, Reitmeier, & Wang, 2012). Hydrolyzed gluten also offers a good replacer for egg white protein. The resulting foams synergistically combined the foaming capacity of gluten hydrolysates and the resistance of egg white to coalescence (Wouters et al., 2018). Meringue, muffins or angel cakes were successfully produced using such modified plant proteins (Wang et al., 2012) or with lentil proteins that partly or wholly replaced milk or egg (Jarpa-Parra et al., 2017).

Whey proteins, caseinate or β -casein in mixtures with saturated or unsaturated monoglycerides or phospholipids usually form phaseseparated films at air-water interfaces (Sánchez, Rodríguez Niño, Caro, & Rodríguez Patino, 2005). Mixes of saturated lipids and globular whey proteins form the most elastic films and the lipids eventually displace the proteins when lateral pressure increases. Soybean phospholipids, and in particular the zwitterionic phosphatidylcholine, bind to sodium caseinate to form new aggregates through electrostatic and hydrophobic interactions. The mixed aggregates exhibited significantly greater foaming stability than caseinate or phospholipids alone (Istarova et al., 2005). At low saponin/lysozyme molar ratios, the two surfactants aggregated and presented synergistic surface activity and greater foaming capacity (Wojciechowski, Piotrowski, Popielarz, & Sosnowski, 2011).

In conclusion, mixes of plant and animal ingredients offer opportunities to formulate foodstuffs containing fewer animal resources while nonetheless meeting the nutritional needs of different consumer populations, from infants to the elderly. Synergies are possible regarding different functional aspects such as digestibility, the stability of globular proteins, and various gelling and surface-active properties. Fig. 4 shows examples of investigations by the STLO on innovative mixes of milk or egg proteins with plant proteins. However, the conditions under which synergies exist are sometimes narrow and often depend on the proteins involved. Systematic investigations are therefore necessary to identify the relevant molecular-mesoscopic-macroscopic scales of the interactions that drive the final functionalities being targeted.

5. Fermentation of mixed animal/plant foodstuffs: a huge opportunity for innovation

Fermentation is a natural process, traditionally used to preserve and transform animal or plant raw materials into edible, safe and tasty foods and to supply various components with high nutritional and health benefits. In addition, it is a natural alternative to supplementation with micronutrients or additives and could help to avoid excessive processing to improve the quality of mixed plant/animal foodstuffs (Tangyu, Muller, Bolten, & Wittmann, 2019, Fig. 5). Different approaches are possible: either the plant and animal fraction(s) are fermented separately and then mixed; or the animal and plant fractions are fermented together, the principal objective being to utilize microbial synergies to promote fermentation of the plant resources by (dairy) microorganisms such as lactic acid bacteria, propionibacteria or yeasts.

5.1. Separate pre-fermentation of the plant fraction

Under the first approach, fermentation is an efficient method to produce vitamins in situ (e.g. belonging to the B family), to reduce the content in anti-nutritional factors, enhance hedonic flavor compounds and/or to reduce the organoleptic defects found in plant-based milk alternatives (Tangyu et al., 2019), prior to mixing with animal ingredients. The lactic acid bacteria or yeasts commonly used as starters in fermented dairy products can be screened and selected on the basis of their enzymatic capabilities, and then used alone or in co-cultures to lower oligosaccharide levels in soybean-based beverages, thus preventing digestive discomfort (Tangyu et al., 2019). However, in this case it is necessary that the milk sugars should not yet be present, as some bacteria would metabolize them for preference. In some other situations, separate fermentation of the animal/plant fractions will also prevent the plant components from being impaired by the activity of microorganisms on the dairy substrate. For example, plant tannins can negatively affect the activity of β-galactosidase, which has been isolated from Kluyveromyces lactis (Kayukawa et al., 2019). Phytate can be converted to free phosphate through fermentation by phytase-positive yeasts or bacteria to recover the bioavailability of calcium and other cations. This effect is enhanced when acidifying bacteria are used; this increases the solubility of phosphate and cations and places the phytases already present in the seed at their optimal pH for activity (Song, El Sheikha, & Hu, 2019). The fermentation of soybean or other plant substrates also reduces the content in trypsin inhibitors (Tangyu et al., 2019) and the fermentation of gluten reduces its allergenicity (El Mecherfi et al., 2019).

5.2. Co-fermentation of animal/plant mixes

The co-fermentation of animal/plant mixes offers new opportunities to drive co-cultures and interactions between microorganisms in order to achieve synergies. In particular, the high carbohydrate content in milk can direct the fermentation of mixed emulsions of skim milk



Fig. 5. (A) Multidimensionality of fermenting mixed animal/plant substrates. Mixing and co-processing animal and plant fractions leads to a diversity of matrices in terms of composition and texture. The fermentation of such substrates adds a new dimension to complexity, involving the respective activities of each strain and their possible interactions. (B) An example of a strategy used to select functionally complementary bacterial strains and assemble them into co-cultures that can provide enhanced organoleptic properties and health benefits (Canon et al. (2020) submitted to Frontiers in Microbiology).

powder/pea protein isolate towards acidification (Ben-Harb et al., 2019). A systematic study showed that yoghurt-type products with satisfactory flavor attributes and limited textural defects such as syneresis could be produced using 30-40% wt. pea protein (as an isolate) mixed with skim milk powder and fermented with Lactobacillus rhamnosus alone or with S. thermophilus in co-culture with L. delbrueckii subsp. bulgaricus, L. acidophilus or L. casei subsp. casei (Yousseef et al., 2016). During the production of Greek voghurt, the replacing a fraction of cow's milk with rice or oat vegetable extract yielded products with good sensory properties; indeed, when oat extract was used, this resulted in enhanced nutritional properties, i.e., reduced lactose, increased fiber and protein (Campos, Garcia, dos, & da Silva, 2018). The supplementation of milk with 2% chickpea flour yielded yoghurt-like products with increased firmness, enhanced bacteria viability over time and satisfactory sensory properties (Chen, Singh, Bhargava, & Ramanathan, 2018). However, the compositional diversity of the plant-based ingredients from extracts to isolates - certainly affected the fermentations. Furthermore, it is not clear whether, or which, components of the legume fraction were metabolized during these studies. No significant proteolysis was reported during fermentation of a mix of 53% wt. faba bean protein isolate and 47% wt. milk protein isolate by S. thermophilus and L. delbrueckii subsp. bulgaricus (Berrazaga, Mession, et al., 2019). However, soybean oligosaccharides and proteins were metabolized during the fermentation of milk/soybean juice mixes with L. acidophilus and yoghurt cultures, and some of the aromas detected during the fermentation of milk/pea emulsion gels by complex consortia of bacteria and yeasts suggested that amino acids were released and metabolized. Green or beany off-flavors due to hexanal or heptanal, for example, were reduced in fermented milk/soybean juice mixes or in skim milk powder/pea protein isolate mixes, and yielded satisfactory products with up to ~50% wt. replacement of the milk fraction (Ben-Harb et al., 2019, 2020; Šertović et al., 2019). Ben-Harb's detailed investigations also indicated that whatever the consortium, some species such as Lactococcus lactis or Geotrichum candidum tended to grow better on mixed rather than on separate milk or pea matrices. Kluyveromyces lactis grew better on the milk/pea mix in a certain consortium, but on milk in another, suggesting that microbial interactions were in play (Ben-Harb et al., 2020). To the authors' knowledge, little information is available on co-fermented egg/plant products. Ovalbumin was not hydrolyzed during the fermentation of wheat flour by S. cerevisiae when making steamed bread (Sang et al., 2018).

To summarize, our ever-increasing knowledge of the metabolic potential of microorganisms, obtained through the use of new generation sequencing, data mining and functional genomics, as well as the smart design of microbial consortia using both *in silico* and *in situ* approaches, offer considerable opportunities for the case-by-case development of fermented animal/plant food products.

Compared to separate intakes, the all-in-one aspect of mixed animal/ plant foods optimizes the supply of essential amino acids (Fig. 6) and increases the share of plant protein in the diet, with less risk of nutritionally or environmentally counter-effective spontaneous substitutions or reductions of animal food (Irz, Jensen, Leroy, Réquillart, & Soler, 2019; Spiteri & Soler, 2018). The acceptability of mixed foodstuffs may be better than that of pure plant alternatives if they succeed in reducing the usual barriers to plant protein consumption in western diets, e.g., unfamiliar taste, faulty texture and poor digestibility.

As an important reason to purchase, food price is key to the protein transition towards a greener diet. Price adversely affects the purchase of healthy products (Allès et al., 2017) especially among consumers who are only starting to change their diet (Vainio et al., 2016). Another aspect of price inequality is that educated people with experience of ethnic foods or chef-led cuisine are more likely to gain sufficient familiarity to increase their own consumption of vegetarian meals (Niva et al., 2017). It is therefore necessary to pay proper attention to propose affordable mixed animal/plant foodstuffs so that they will appeal to a broader population.

Furthermore, mixing animal and plant resources may increase concerns regarding their potentially cumulative adverse effects (Fig. 4A; Fig. 6). In particular, new allergens may be created in co-aggregated or interacting animal/plant proteins, the evaluation of which is not straightforward (Mackie, Dupont, Torcello-Gomez, Jardin, & Deglaire, 2019). On the other hand, co-aggregated plant and animal proteins also create hypo-allergenic structures, as has been reported for co-heated ovomucoid and wheat gluten (Kato et al., 2000) or transglutaminase polymers of soybean, caseinate and whey protein isolates (Li & Damodaran, 2017). The co-processing of animal and plant ingredients may

<u>Strengths</u>	<u>Weaknesses</u>
• Complete range of essential nutrients in one single food	Poorly connected animal and plant food chains
 Balanced input of animal/plant proteins 	Increase in by-products, especially from legumes
 Transitional food towards a more sustainable diet 	 Little knowledge of possible new structures in mixed
• Enhanced acceptability of mixed foodstuffs: better taste	foodstuffs: co-aggregated proteins, co-emulsified
and digestibility and lower risk of neophobia	proteins and lipids, nutrient-matrix interactions, etc
A pathway to enhance solubility of vegetable proteins	 Gap between traditional products and innovative mixed
	foodstuffs
Opportunities	<u>Threats</u>
To reduce the consumption of animal proteins in	Increased exposure to multiple and crossed risks with
western countries	
western countries	animal × plant mixes: allergens, spoilage micro-
 Increasing concern regarding health and nutrition 	animal × plant mixes: allergens, spoilage micro- organisms, anti-nutritional factors
 Increasing concern regarding health and nutrition Increasing concern regarding the environment and 	 animal × plant mixes: allergens, spoilage micro- organisms, anti-nutritional factors Possible cocktail effect of animal × plant contaminants
 Increasing concern regarding health and nutrition Increasing concern regarding the environment and sustainability 	 animal × plant mixes: allergens, spoilage micro- organisms, anti-nutritional factors Possible cocktail effect of animal × plant contaminants Uncertainty regarding classification as "ultra-processed"
 Increasing concern regarding health and nutrition Increasing concern regarding the environment and sustainability Competitive cost of vegetable resources 	 animal × plant mixes: allergens, spoilage micro- organisms, anti-nutritional factors Possible cocktail effect of animal × plant contaminants Uncertainty regarding classification as "ultra-processed" foods
 Increasing concern regarding health and nutrition Increasing concern regarding the environment and sustainability Competitive cost of vegetable resources Introduction of significant biodiversity through the 	 animal × plant mixes: allergens, spoilage micro- organisms, anti-nutritional factors Possible cocktail effect of animal × plant contaminants Uncertainty regarding classification as "ultra-processed" foods Uncertainty regarding classification as "novel" foods
 Increasing concern regarding health and nutrition Increasing concern regarding the environment and sustainability Competitive cost of vegetable resources Introduction of significant biodiversity through the ranges of plant resources and bacterial starters 	 animal × plant mixes: allergens, spoilage micro- organisms, anti-nutritional factors Possible cocktail effect of animal × plant contaminants Uncertainty regarding classification as "ultra-processed" foods Uncertainty regarding classification as "novel" foods Deceptive environmental footprint if animal and/or
 Increasing concern regarding health and nutrition Increasing concern regarding the environment and sustainability Competitive cost of vegetable resources Introduction of significant biodiversity through the ranges of plant resources and bacterial starters available 	 animal × plant mixes: allergens, spoilage micro- organisms, anti-nutritional factors Possible cocktail effect of animal × plant contaminants Uncertainty regarding classification as "ultra-processed" foods Uncertainty regarding classification as "novel" foods Deceptive environmental footprint if animal and/or plant fractions in mixed foodstuffs are produced by

Fig. 6. Proposition of a SWOT analysis of mixed animal/plant foodstuff. The specific issues of mixed animal/plant meat substitutes, e.g. cellular cultures or animal welfare, are not addressed here.

also expose each fraction to chemical contaminants in its counterpart, such as copper, heavy metals, chelating agents, pesticides or antibiotics, a risk that has as yet been little evaluated with respect to cocktail effects on human health or the inhibition of fermentation processes. Spoilage or pathogenic microorganisms such as *Bacillus, Penicillium, Aspergillus, Salmonella* or *Fusarium* and/or their toxins (aflatoxins and ochratoxins) may be supplied to the animal substrate by the plant fraction, depending on purity, quality control and processes such as heat treatment (International Commission on Microbiological Specifications for Foods, 2005). Legume products destined for the manufacture of moist food-stuffs should therefore be closely controlled.

There are also concerns as to how the general public or the authorities might classify innovative mixed animal/plant foodstuffs with respect to "novel food" legislation or the evaluation of "naturalness" or "ultra-processing". According to the European Union, a novel food is defined as a "food that had not been consumed to a significant degree by humans in the EU before 1997" and encompasses "newly developed, innovative food, food produced using new technologies and production processes, as well as food which is or has been traditionally eaten outside of the EU" (ec.europa.eu/food/safety/novel food en). Despite their frequent relevance to standard egg or dairy foods as substitutions, in some cases it may be necessary to apply for authorization to access the EU retail market. Furthermore, some mixed animal/plant products might be perceived as ultra-processed foods under the terminology used by the Food and Agriculture Organization of the United Nations (FAO). For example, this may be the case if thorough fractionation and/or delipidation by organic solvents is involved in the preparation of milk and/or plant ingredients, or if texturing and/or flavoring agents are required to render the mixes more appealing. The same observation could ultimately lead to deceptive life cycle assessments when it comes to evaluating mixed animal/plant foodstuffs in terms of by-product management and energy and water uses, when compared to their purely animal or plant counterparts (Fig. 6). Because motivations to reduce the consumption of animal protein are increasingly linked to environmental concerns, these aspects may become critical for innovators. Previous studies have indicated that purified legume proteins stand the comparison with milk protein concentrates, and can favorably reduce the environmental footprint of meat if 20-40% of it is replaced by soybean protein concentrate (Heusala et al., 2020; Thrane, Paulsen, Orcutt, & Krieger, 2017). However, reaching both nutritional and environmental targets with milk or plant-based alternatives calls for compromises (Grant & Hicks, 2018).

The objective of the STLO is to provide general academic knowledge regarding the native properties of dairy and egg components, their changes during technological processing and their interactions with the environment, including human consumers. Our opinion is that dedicated academic research is necessary to investigate the complexity of animal/plant combinations in terms of opportunities for the design of new safe and tasty foodstuffs with environmental and health benefits. In our view, such associations offer two important pathways for innovation: first, innovative animal/plant foodstuffs will result from controlled, and potentially synergistic, interactions between molecules and molecular structures in the two fractions. To achieve this, details of the impacts of fractionation on the biochemistry and physical chemistry of the systems are required in order to better describe their consequences in terms of sensory and functional properties, as well as benefits and risks. Transformation, process engineering and proper life cycle assessment are other important keys which need to be considered (Hiolle & Lechevalier, personal communication). Second, mixed animal/plant foods offer new options for people who are committed to reducing the proportion of animal products in their diet while meeting their nutritional requirements, encountering familiar sensory properties and/or wish to reduce their environmental footprint. The adaptation of fermentation and other processes in the dairy or egg food chains to these

mixed resources offers attractive perspectives for innovation. The appropriate choice of substrates and microbial consortia will be crucial to the design and control of animal-plant mixtures so that they provide safe and healthy foodstuffs with an extended shelf life, enhanced nutritional value, the desired appearance, texture and flavor, and less intolerance. Finally, it is important to address public expectations and needs so as to ensure that consumer interests are met alongside their acceptability of mixed animal/plant products; more generally, the societal context must be considered throughout future evolutions of our research. In that respect, it is essential that extensive information on the nutritional and environmental impacts of mixed animal/plant foodstuffs should be provided to the general public.

6. Concluding remarks

The ongoing transition towards greener and healthier diets in western countries is paving the way for the development of mixed egg/plant or milk/plant innovative foods to meet the demands of new "flexitarian" consumers and the increasing proportion of vegetarians. Furthermore, mixed animal/plant foods could offer an interesting opportunity for the dairy and egg sectors to deal with and react positively to the decline of animal protein consumption by proposing tasty, nutritious and relatively familiar alternatives to conventional products. Animal and plant combinations thus offer many advantages in terms of their acceptability and nutritional and functional properties, with the existence of possible synergies. Fermentation, which is already largely employed in milk processing, offers huge opportunities for transfer and extension in order to further increase the potentialities of animal/plant combinations by taking advantage of the tremendous microbial biodiversity available. In this context, STLO aims to participate in this emerging field of research thanks to its highly-trained staff, its range of dedicated biochemistry, physical chemistry, microbiology, processing and digestion facilities, the Dairy Platform dedicated to pilot-scale trials of new dairy processes, the Biological Resource Centre on food bacteria (CIRM-BIA) and its history of industrial partnerships. To date, STLO has training and continues to train 10 young scientists who are disseminating their expertise on the formulation of animal/plant foodstuffs and the fermentation of legume substrates using dairy starters, and is committed to considering consumer expectations with respect to animal/plant mixes as a basis for the appropriate orientation of our research.

Declaration of competing interestCOI

Nothing to disclose.

Author statement

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