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Hybrid Yogurts: Exploring the Functional, Sensory and Nutritional Potential of Dairy-Plant Protein Combinations

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ABSTRACT
As global demand for sustainable food options grows, hybrid products combining animal- and plant-based proteins, emerge as an innovative solution to balance environmental concerns and consumer preferences. Within the wide range of hybrid food options, yogurts have been featured for their worldwide popularity. Moreover, the health and sensory benefits associated with the fermentation process could help overcome some of the potential drawbacks of plant proteins. This review explores the advances in the development of hybrid yogurts and their physical, sensory, and nutritional properties. The effects of the inclusion of plant-based ingredients on the fermentation process, as well as the synergistic effects of plant and dairy proteins on the texture, flavor and nutritional value of the yogurts are considered. This work aims to provide valuable insights into the development of hybrid yogurts, offering a deep understanding of how combining plant-based and dairy proteins can enhance product quality, meet consumer demands, and contribute to more sustainable food systems.

KEYWORDS
Acceptability; animal/vegetal mix; fermentation; sustainability

Introduction

In 2024, the world population reached 8.2 billion people and is expected to continue growing to a peak of around 10.3 billion people in the mid-2080s.[1] Global population growth is increasing the overall protein demand. While animal sources remain the main protein source, environmental sustainability concerns are driving a shift towards more plant-based diets.

Developing plant-based food products is not without challenges. From a nutritional perspective, animal-based proteins such as dairy, meat, and eggs are complete protein sources. They contain all nine essential amino acids in adequate proportions and have good digestibility, as measured by the Digestible Indispensable Amino Acid Score (DIAAS) and Protein Digestibility-Corrected Amino Acid Score (PDCAAS). In contrast, most plant proteins lack sufficient lysine and threonine and exhibit lower digestibility.[2,3] In addition, as food ingredients, proteins play not only a nutritional role but also a techno-functional one. The structural versatility and amphiphilic nature of proteins allow the interaction between proteins and other compounds in food products, and thus the stabilization of colloidal food systems.[4,5] Animal-based proteins, and in particular milk proteins, exhibit excellent functionality, including emulsifying, foaming and gelling properties, which are widely exploited in the food industry.[6,7] On the other hand, the low solubility of most plant-based proteins constitutes a main barrier to replacing dairy ingredients by plant-based proteins in food products.[5] Furthermore, sensory attributes play a key role in consumer acceptability. In that sense, the development of plant-based foods faces additional challenges, including lack of familiarity and presence of off-flavors and off-odors, together with sociocultural factors and rooted cooking habits.[8]

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Although the food industry has incorporated plant-based proteins into animal-based products for decades, primarily to reduce costs, the concept of hybrid foods has recently evolved. The term hybrid foods refers to products strategically formulated to promote the use of plant proteins, while addressing their possible nutritional, sensory and techno-functional limitations.[8] The concept of hybrid foods opens a new range of innovation, as these products could potentially be shaped into any category of product (beverages, emulsions, gels, meat analogues, etc.) and blends of animal protein sources (meat, egg, dairy) and plant protein sources (legumes, cereal, oilseeds), and could be particularly appealing to consumers following a flexitarian diet. Recent developments in hybrid foods formulated with both dairy and plant ingredients (dairy-like hybrids) have reached the market. Examples include a powdered infant formula blending milk and soy proteins launched by Danone, a milk and soy protein instant powder mix from Nestlé, and a range of products blending milk and oats (butter, cheese and milk) from Kerry Group's brand Smug.[9–11]

Within the dairy-like hybrids, the category of fermented products has been identified as a promising alternative, as the fermentation process has the potential to increase digestibility, reduce the content of anti-nutritional factors, decrease off-flavors and enhance hedonic flavor compounds.[8] Fermented dairy products include cheeses and fermented milks. Yogurt is the most popular fermented milk worldwide, due to its convenience and recognized health benefits. Hence, the present review focuses on the advances made in hybrid yogurts, providing an overview of the types and levels of plant proteins incorporated into dairy yogurt matrixes and their implications on sustainability, the fermentation process, and the physical, sensory and nutritional properties of the product.

Raw materials in hybrid systems

A new challenge for food formulation research stems from the ongoing so-called protein transition, which aims at reducing the consumption of traditional animal-based proteins, such as meat or dairy, while promoting the use of alternative food proteins, mainly plant-based proteins.[5,12] Formulating new functional foods requires a full understanding of the structural features of the individual proteins and of the mechanisms behind how animal- and plant-based proteins can interact.

Proteins from milk sources

Among the potential animal-based proteins that can be combined with the plant ones, milk proteins stand out due to their high production, easy isolation and purification by membrane filtration systems, stability in the dry form, techno-functionality in dairy and non-dairy products and good consumer acceptability. Milk proteins are one of the most studied systems over the years, caseins being the major group (80%), followed by whey proteins. The ability to manipulate and transform these proteins is crucial to generate a variety of food products, such as yogurt, cheese, or ice cream.[8,13]

Caseins form a supramolecular structure, known as casein micelle, of approximately 200 nm of hydrodynamic diameter, 105 kDa and isoelectric point of 4.6 in natural conditions. They are bound together by hydrophobic and electrostatic interactions and especially by calcium phosphate nanoclusters. These proteins are widely used in the food industry to improve viscosity, stabilize emulsions and foams and enhance the nutritional properties of different products. Caseins are good emulsifiers due to their flexibility, lack of tertiary structure, and ability to quickly unfold and then reorient at the interface.[4,13,14] On the other hand, whey proteins are mainly composed of β-lactoglobulin (60% w/w) and α-lactalbumin (20% w/w). Unlike caseins, these globular proteins have well-defined structures that are influenced by various environmental factors, such as pH, ionic strength, and temperature, as well as treatments like pressure, ultrasound, and pulsed electric fields. In the food industry, the main products obtained from whey processing are whey protein concentrates (WPCs) and whey protein isolates (WPIs). Those products can be used as ingredients in food formulations due to their ability to strengthen food gels and/or stabilize emulsions and foams.[13,15] In general, milk proteins have excellent nutritional quality, evidenced by their protein digestibility-corrected amino acid score (PD-CAAS) and digestible indispensable amino acid score (DIAAS).[8]

Proteins from plant sources

Plant proteins can biosynthesize many proteins, which can be classified into "metabolic" and "storage" proteins. While the first are responsible for plant development, the second are reservoirs of vital amino acids to sustain plant life and are an important source of nutrients. Plant proteins are characterized by a different structure and morphology than animal proteins, which highly influences their functionality.[16,17] More than 30 plant protein sources are currently used in food formulations, and overall, they can be organized into three general groups: legumes, cereals, and oilseeds.[13] Among the legumes, soybean and green peas are the most employed, but also proteins from other beans such as lupin and chickpea are used in the food industry.[18–20] Regarding the cereals group, the main sources of proteins are provided by wheat, oat and rice, while proteins from oilseeds are separated from the oil, starch, and fibers of products such as canola and hemp.[21–24]

Legume proteins can be classified according to their solubility (Osborne fractionation), in globulins, albumins, prolamins and glutelins. Salt-soluble globulins and water-soluble albumins constitute the predominant groups, ranging from 38–75% to 8–31% of total proteins, respectively. According to their sedimentation coefficient, two main globulin protein fractions can be distinguished: legumin-(11S protein type) and vicilin-like (7S protein type) globulins. Legumins have hexameric quaternary structures with a basic-acid subunit (α- and β)- linked by a disulfide bond, with a total molecular weight between 350 and 400 kDa. Legumins are not usually glycosylated, apart from lupin, which contains covalently linked carbohydrates.[25,26] On the other hand, vicilins are oligomers with a molecular weight between 150 and 190 kDa, lacking the sulfur-containing amino acids which prevent the formation of disulfide bonds, so their association – dissociation balance is closely dependent on pH and ionic strength. Vicilin is a more flexible protein than legumin, thus having better interfacial activity. Proteins from different pulses generally show higher solubility at alkaline ($pH > 5.0$) and acidic pH values ($pH < 4.0$) and are much less soluble at pH around their isoelectric point. The structure and solubility of the proteins may be affected by thermal processing, pH variation, ionic strength or the presence of salts.[4,15,25,27] Legume proteins have excellent foaming and emulsifying properties that are used in food formulations. They can exhibit elevated PD-CAAS or DIAAS values, but they are not a complete essential amino acid source, being cysteine, methionine and tryptophan the limiting amino acids.[8]

Cereals already constitute one of the most important protein sources in human diets. The protein content of cereal grains varies between 7 and 18% of dry matter, depending on the species and variety. Similar to the legumes, cereal proteins are classified based on their solubility in water (albumins), saline (globulins), aqueous alcohol (prolamins), or acid/base solutions (glutelins).[28] While legumes contain predominantly albumins and globulins, cereal protein is dominated by poorly soluble prolamins and glutelins, which explains why non-dairy milk substitutes made from cereals are very low in protein.[29] Among cereals, oat, maize and rice proteins are usually used for functional food formulations.[28,30] Most cereal proteins are typically deficient in lysine whereas pulse proteins are deficient in methionine, cystine, and tryptophan. In addition, an important feature of some cereals is the high content of arabinoxylans and β-glucans, known as prebiotic fiber.[31,32]

Oil processing by-products rich in oilseed proteins of excellent nutritional and bioactive properties, are interesting raw materials that offer possibilities to develop healthy products or new functional foods. Hemp cake or meal, a by-product obtained after oil extraction from hemp seeds, is abundant in high-quality storage proteins. Hemp seed protein is well known for its excellent digestibility and desirable essential amino acid composition. In fact, the amino acid profile of hemp seed has a nutritional quality similar to soy with good digestibility but with lower lysine content.[2] Hemp seed protein consists mainly of globulin (edestin) and albumin. Edestin represents 60–80% of the total protein content, with a molecular weight of approximately 300 kDa, and consists of six identical subunits. Each subunit contains an acidic and a basic component linked by a disulfide bond. The albumin fraction constitutes about 25% of hemp seed storage protein and contains fewer disulfide-bonded proteins and a less compact structure with greater flexibility than the globulin fraction.[33–35] Canola meal, a by-product of canola oil extraction, is a highly rich raw material with about 35–40% protein. The two major protein fractions in canola are cruciferin and napin. Cruciferin is a 12S hexameric globulin with a molecular weight of 300–360 kDa, linked by disulfide bonds. Meanwhile, napin is a 2S albumin of around 17 kDa. Napin has about 45% of its hydrophobic amino acids mainly located in one distinct domain whereas the hydrophobic amino acids are widely distributed across the protein surface in cruciferin. Canola protein is recognized for its balanced amino acid profile, providing all essential amino acids.[17,36]

Beyond high protein content and quality, the plant matrices have antinutritional factors like saponins, phytates and tannins that influence the protein digestibility and organoleptic properties, as well as the bioavailability of other nutrients such as minerals.[8,15,30]

Combining dairy and plant proteins for the development of hybrid yogurt-like products

The development of hybrid yogurts, combining dairy- and plant-based proteins, represents an innovative approach to meeting consumer demand for nutritious, sustainable, and sensory appealing foods (Fig. 1). The successful formulation of these products requires an in-depth understanding of key factors, including fermentation dynamics, structural and physical properties, and the interactions between dairy and plant proteins. Additionally, consumer perception and sensory acceptance play a critical role in market success.

Fermentation processes in the production of hybrid yogurts

Yogurt is the most popular fermented milk product and can be typically classified into set and stirred or drinking type.[37] The essential microflora of yogurt consists of the thermophilic lactic acid bacteria

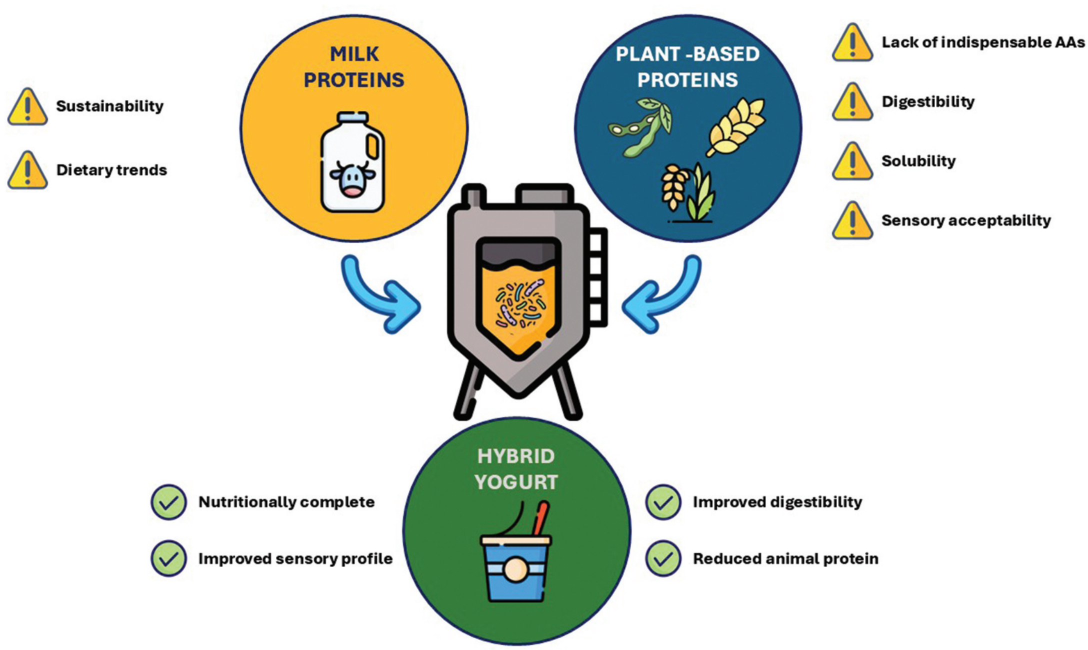

Figure 1. Drivers for the development of innovative hybrid yogurts.

Streptococcus thermophilus and *Lactobacillus delbrueckii* subsp. *bulgaricus*. The production of yogurt consists mainly of: (1) standardization of the milk or preparation of the mix, (2) homogenization, (3) heat treatment, (4) cooling, (5) inoculation of the starter cultures, (6) incubation in bulk for stirred-type yogurt or in packages for set-type yogurt, and (7) cooling.[38]

In the case of hybrid yogurts, as complex systems, the first step of mix preparation must ensure a stable protein dispersion. Key factors such as protein solubility, particle size, and viscosity should be carefully considered as they affect the final product.[13] Different studies dealing with mixes of milk and plant proteins have shown that physical treatments such as heat, homogenization or high pressure could be applied to improve the solubility of the proteins.[14,39,40] In traditional dairy yogurt production, heat treatment is applied for microbiological reasons but also to denature whey proteins and promote whey protein-casein aggregations, which is believed to increase the viscosity and enhance the water-binding capacity of the coagulum.[37] Furthermore, in the case of hybrid yogurts the heat treatment step can also promote the denaturation of the plant-based proteins, enhance their solubility and promote interactions with the milk proteins. Protein denaturation can lead to aggregation or crosslinking by exposing hidden hydrophobic residues. Denatured forms of albumins and globulins (for example, pea albumins and β-lactoglobulin found in milk) exhibit enhanced ability to adhere to the interfaces when compared to their native counterparts. They can also form gels through interactions involving disulfide bonds and hydrophobic forces. Processes conducted at moderate temperatures and pH, such as fermentation, show considerable promising results in enhancing protein functionality without compromising nutritional value.[2]

Fermentation is a traditional biotechnological process that has been used to improve nutritional, safety and sensory properties of foods. The lactic acid bacteria (LAB) fermentation process involves acidification, protein hydrolysis, flavor formation and production of metabolites, which are beneficial for food processing. Moreover, LAB are generally regarded as safe (GRAS) food-grade microorganisms.[41] LAB are usually used in the fermentation of different mixed protein products. This heterogenous group of Gram-positive bacteria ferment carbohydrates into lactic acid (homofermentation); or into lactic acid, ethanol and CO_2 (heterofermentation).[42] *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* are the most commonly used strains in the fermentation of hybrid yogurts.[43–51] These vastly studied strains have a synergic relationship: *S. thermophilus* provides peptides for *L. bulgaricus* growth by using free amino acids in the mixture. As the pH decreases, *L. bulgaricus* grows faster and produces a higher amount of lactic acid leading to a significant reduction in pH.[47] During this acidification, the electrostatic and steric repulsion between proteins decreases (near the isoelectric point), which causes approximation between them, formation of new interactions, aggregation, and ultimately formation of a continuous three-dimensional network.[13] The fermentation behavior in hybrid milk-plant matrixes depends on the proteins involved in the process. In general, the replacement of milk proteins by plant-based proteins increases the acidity and reduces the fermentation time. Martinussi et al.[43] reported that the replacement of milk proteins by plant-based proteins (almond and pumpkin seed) favored lactic acid bacteria metabolism, suggesting that plant-based proteins are more susceptible to the hydrolysis from *L. bulgaricus* proteolytic system, facilitating amino acid release. The authors discussed that the increase in LAB count in the new protein matrix enhanced the conversion of lactose into lactic acid, leading to increased acidity. Yousseef et al.[52] evaluated 10 starter cultures of seven LAB species in yogurts with five pea/milk protein ratios. In those systems, altering the pea/milk protein ratio from 0:100 to 40:60 at 4.5% total protein led to faster gel formation and increased acidity due to the reduction in the casein content, which has buffer capacity. Šertović et al.[46] studied the effect of cow milk and soy beverage combination fermented with probiotic bacteria (*L. acidophilus* La5) and yogurt starters. They reported that as the soy beverage concentration increased, the pH decreased and the acidity increased, reducing the fermentation time. The probiotic bacteria could grow well in all samples, regardless of the type of milk or their ratio. Soy beverages have oligosaccharides that act as prebiotics contributing to the growth of *Lactobacillus*. The addition of probiotic bacteria in hybrid milk-plant matrix is an added value in the functional product. "Symbiotic" products, which combine prebiotics and probiotics, are of interest in functional food product development as it is expected that this association would enhance the health benefits.[53] Plant-based ingredients in hybrid yogurt formulations are rich in proteins that influence flavor precursor formation and key physicochemical properties, including water-holding capacity and gelation. Additionally, they often contain starch and non-digestible carbohydrates, such as galacto-oligosaccharides and β-glucans, which contribute not only to stimulate the probiotic growth but also to promote their viability over time.[15,18,45,46]

LAB activity extends beyond just acidification; it also facilitates efficient proteolysis, boosts various bioactive compounds, reduces anti-nutritional factors (ANFs), and enhances sensory quality.[30] Some LAB are capable of producing exopolysaccharides during fermentation. Exopolysaccharides' synthesis is a strain-dependent metabolic characteristic, affected by the composition of the matrix and fermentation settings, that has been identified as crucial for obtaining optimal texture.[54] Indeed, the presence of exopolysaccharides in hybrid yogurts improves not only the rheological properties but also the sensory and mouth-feel characteristics, water-holding capacity and stability of the product.[55] These are all relevant properties and will be discussed in the following section. Furthermore, by selecting the adequate LAB strains, not only texture, but also the flavor and aroma profile of the yogurts could be tuned to increase acceptability.[56]

Structural and physical properties of hybrid yogurts

During yogurt production, the heat treatment of milk at high temperature induces the denaturation of whey proteins and their interaction with the κ-casein located on the surface of the casein micelles. Then, during fermentation, as pH decreases, the casein-casein attraction increases, leading to gelation as caseins reach their isoelectric point (pH = 4.6).[57] Each yogurt type, namely set, stirred or drinking yogurt, exhibit distinctive physical characteristics.[37] Set-type yogurts are fermented inside the packages without further stirring and develop a continuous gel texture that is expected to be firm and spoonable.[58,59] Stirred yogurts are fermented in tanks under continuous mild stirring, developing a viscous creamy smooth texture, while drinking yogurts are stirred yogurts with low viscosity.[37,58] In all types of yogurts, water holding capacity is a desirable property of the gel, as opposite to syneresis, which results from the shrinkage of the protein gel network, expelling the liquid phase.[49] Syneresis is affected by dry matter content, cooling temperature, shaking and agitation.[13,48]

Physical properties of yogurts, including texture, rheological properties and syneresis, i.e. serum separation, play an important role in quality and consumer acceptability, and are strongly influenced by the gel structure. Gelling properties are related to protein features such as molecular weight, isoelectric point, denaturation temperature, as well as to extrinsic factors, such as pH, temperature and ionic strength. Therefore, understanding the gelation process during fermentation and the effects of added plant proteins is essential for controlling the physical properties of hybrid yogurts.

Table 1 summarizes the results of previous studies investigating the physical properties of yogurts produced from mixes of milk and different plant protein sources, such as lupin, chickpea, pea, soy or rice.

Vieira et al.[50] showed that a lupin-enriched yogurt (90% semi-skimmed milk, 6% skimmed milk powder, 4% lupin flour) had a higher total solids content, which enhanced the formation of cross-linking bridges between milk casein and other added proteins. This resulted in a firmer yogurt with higher viscosity than the control yogurt (94% semi-skimmed milk, 6% skimmed milk powder). Similarly, Raza et al.[60] reported that the firmness of yogurt increased with the level of addition of chickpea flour.

Other studies have reported that the addition of plant proteins led to a decrease in the gel strength. Youssef et al.[52] evaluated the effect of the pea/milk protein ratio in yogurts with a fixed total protein content of 4.5 g/100 g and found that the firmness decreased with the pea protein concentration. The authors hypothesized that the pea protein settles into the free spaces of the preformed casein network, weakening the coagulum. Cow milk yogurts were reported to have higher hardness and water-holding capacity than yogurts made from soy milk or blends of cow and soy milk.[49] Also, Šertović et al.[46]

Table 1. Physical and sensory properties of hybrid yogurts.

Milk protein source	Plant protein source	Total protein content (%, w/w)	Milk-to-plant protein ratio	Total solids (%, w/w)	Physical properties	Sensory attributes	Reference
SMP	SPI, PPI, RP, WG	3.10–6.80	0.5% plant protein added	6.52–7.06	All plant proteins increased syneresis and all plant proteins, except WG reduced viscosity.	Yogurts with 0.5% plant proteins (except for RP) showed equal or better sensory attributes than control (100% dairy).	48
SMP + WPI	LPI	6.6	50:50 and 67:33 (milk:lupin protein ratio)	NR	Increasing lupin protein proportion (50:50) reduced apparent viscosity, firmness and syneresis.	Higher lupin protein proportion yogurts (67:33) were less acceptable than 50:50 ones.	56
UHT milk	UHT soy milk	NR	UHT milk:UHT soy milk ratios = 100:0, 67:33, 50:50, 33:67, 0;100	NR	Adding soy reduced hardness and increased syneresis of yogurts.	NE	49
UHT milk	Soy beverage	3.00–3.43	UHT milk:soy beverage ratios = 100:0, 75:25, 50:50, 25:75, 0:100	8.20–12.23	Adding soy milk reduced viscosity.	Acceptability decreased with soy beverage level inclusion.	46
UHT milk	Roasted chickpea flour	NR	1–5 g/100 g of flour added	NR	Firmness increased and syneresis decreased with chickpea flour concentration.	5% chickpea flour addition presented the highest score in texture while 2% chickpea flour addition had the highest overall acceptability.	60
Organic semi skimmed milk + SMP	De-hulled organic lupin flour	7	4% lupin flour added	16.8–21.7	Yogurt enriched with lupin flour was the stiffest and had the highest viscosity.	Addition of 4% lupin flour was undetected by triangular test.	50

(*Continued*)

Table 1. (Continued).

Milk protein source	Plant protein source	Total protein content (%, w/w)	Milk-to-plant protein ratio	Total solids (%, w/w)	Physical properties	Sensory attributes	Reference
SMP	PPI	4.5	100/0; 90/10; 80/20; 70/30; 60/40	NR	Increase in PPI decreased firmness and favored syneresis.	Increase in PPI increased negative attributes: vinegar and earth odors, and pea and smoked aromas; and decreased positive attributes: dairy aroma and creamy mouthfeel.	5
Whole milk + WPI	SPI	NR	1, 3, 5% fortification with WPI or SPI.	11.99–17.98	Higher hardness, gumminess and chewiness in yogurts fortified with WPI than with SPI.	NE	61
WMP	Almond protein concentratePumpkin seed protein concentrate	NR	26–74% of WMP replaced by a mixture of 25:75, 33:67, 50:50, 67:33 or 75:25 almond: pumpkin seed protein concentrates	13	Higher WMP replacement by plant proteins resulted in lower viscosity, firmness and consistency decreased and higher syneresis increased.	26% of WMP replacement by 25:75 almond-to-pumpkin seed protein concentrates mixture resulted in 30% purchase intention. Appearance and flavor acceptability as well as purchase intention improved by green apple flavor addition.	43

LPI: lupin protein isolate; NE: not evaluated; NR: not reported; PPI: pea protein isolate; RP: rice protein; SMP: skim milk powder; SPI: soy protein isolate; UHT: ultra high temperature; WG: wheat gluten; WMP: whole milk powder, WPI: whey protein isolate.

prepared probiotic drinks from cow milk, soy milk or blends of both types of milks and found that the inclusion of soy milk decreased the apparent viscosity of the beverages. Canon et al.[56] reported that set-type yogurts produced with 67:33 milk-to-lupin protein ratio presented higher firmness, viscosity and water-holding capacity than 50:50 milk-to-lupin protein ratio yogurts. Mitra et al.[61] found that milk yogurts fortified with whey proteins were harder than yogurts fortified with the same level of soy proteins, probably due to whey protein-casein aggregates formed through disulfide bonds. Akin and Ozkan[48] reported that fermented milks (drinking yogurts) elaborated by mixing milk with soy, pea or rice proteins showed lower apparent viscosities than the control (100% dairy), and the yogurt containing rice proteins had the lowest viscosity. On the other hand, the hybrid yogurt produced with wheat gluten showed the highest apparent viscosity. The authors attributed the different viscoelastic properties to the differences in the density of the protein matrixes inside the macro-gel structure. Rice proteins formed a less viscous gel with lower water-absorbing capacity and higher syneresis, while wheat gluten produced a highly viscoelastic gel with greater water-holding capacity.

It should be noted that in studies taking a plant protein supplementation approach rather than substituting milk proteins with plant proteins, not only the protein composition but higher dry matter contents could be responsible for increased firmness and viscosities of the yogurts. Moreover, the purification level of the added proteins should be considered when analyzing the effects of plant protein ingredients on the syneresis or water-holding capacity of yogurts, since the presence of other components such as starch or fibers exerts a significant effect. Indeed, a positive impact on yogurt syneresis (reduction) was observed in the yogurts fortified with roasted chickpea powder, which could be enhanced by the presence of dietary fiber absorbing whey.[60]. On the other hand, different results were found when the hybrid yogurts were formulated considering a replacement approach via fixing the total solids (TS) content. This was the case in the work conducted by Martinussi et al.,[43] who used a rotational central composite experimental design to investigate the effects of the percentage of replacement of whole milk powder (WMP) by a mixture of almond and pumpkin seed protein concentrates and the ratio of plant proteins concentrations on the physical properties of hybrid yogurts. The authors found that the level of replacement (reducing WMP concentration) decreased the viscosity, firmness and consistency of the yogurts while increasing the syneresis. The TS of the yogurts was fixed at 13% and no improvement was observed in the physical properties, despite the plant proteins used in the formulations had 13–16% (w/w) of fiber. This result highlights the importance of the milk protein network on the texture and mouthfeel of the products. The protein profile of the fermented milk plays a crucial role in their physical properties. Plant proteins have different properties depending on their intrinsic parameters such as the presence of specific protein fractions, their isoelectric point, denaturation temperature, surface hydrophobicity, and the presence of thiol groups. The individual gelling properties of the proteins, as well as the interactions between the different protein fractions affect the microstructure of the gel in terms of compactness, regular/irregular distribution of the protein network, or gel strength. Therefore, important features of hybrid yogurt-like products such as texture, rheological behavior and syneresis, will be extremely dependent not only on the type of protein and presence of other components but also on the production process, with heat treatment, and acidification and proteolysis during fermentation playing an important role.[49]

The development of hybrid yogurts presents a significant challenge due to the intricate balance required between maintaining desirable textural properties and integrating plant proteins, which often alter the gelation process fundamental to yogurt's quality. This challenge comes from the diverse physical and chemical properties of plant proteins such as differences in isoelectric points and denaturation temperatures, which can significantly affect yogurt's viscosity, firmness, and syneresis. Addressing these challenges demands a deep understanding of protein interactions within the yogurt matrix which necessitates advanced formulation techniques to optimize both sensory and structural qualities. To successfully innovate in this space, manufacturers must employ targeted research to tailor protein blends that harmonize the functional attributes of both dairy and plant sources ensuring product acceptance.

Nutritional quality of hybrid yogurts

Protein-rich foods vary in their nutritional quality, i.e., their ability to meet human requirements for amino acids. Factors influencing nutritional quality include protein content, proportions of different amino acids, and digestibility. Among the 20 amino acids crucial for human metabolism, 9 are classified as essential as they cannot be produced by the body: leucine, isoleucine, valine, lysine, threonine, tryptophan, methionine, phenylalanine, and histidine. The essential amino acids must be consumed as part of the diet, and the quantity of digestible indispensable amino acids therefore limits the nutritional value of food proteins.[2,8,62]

Animal-based protein sources, such as milk, whey, casein and eggs, are generally considered complete protein sources for supporting indispensable amino acid requirements for human growth and development. Although soy and quinoa are also complete proteins, they are usually more difficult to digest when compared to animal proteins.[2,62,63] Combining two or more plant proteins can improve amino acid balance, but the challenge of low digestibility remains, which can have a significant impact on human health, especially among infants and young children. An effective strategy to address these limitations is blending plant-based proteins with animal-based proteins, which has shown immediate potential to enhance both amino acid balance and digestibility.[2,64] In this context, mixing milk with plant proteins helps to balance the diet, optimize dietary structure, and improve nutritional status. van Dam et al.[65] found that a 60:40 casein/pea protein blend presented higher plasma amino acid availability than pea protein. The digestibility of plant and milk protein blends has also been assessed. Mixes of soy protein isolate (SPI), rice protein isolate (RPI) or pea protein isolate (PPI) with milk protein concentrate (MPC) showed higher *in vitro* digestibility than the individual plant proteins.[23] Wang et al.[66] compared the dynamic *in vitro* digestion behavior of oat milk and an oat milk-bovine skim milk blend. They found that the presence of bovine milk affected the gastric emptying rate and the delivery of macro and micronutrients. Moreover, not only the source of plant-based ingredients but also the ingredient type plays a role in the digestibility of the product. Plant protein ingredients such as concentrates and isolates undergo processing that eliminates or reduces antinutrients. As a result, their digestibility is typically much higher than the digestibility of the native protein embedded in the whole food matrix. For example, the protein digestibility of soy protein isolate is 96% or higher while the protein digestibility of soy flour is only 84%.[62]

Shifting the focus to fermentation, it is essential to examine how this process further enhances the nutritional quality of protein blends. Fermentation plays a critical role in enhancing both the digestibility and bioavailability of nutrients by breaking down complex molecules. The improved protein digestibility in yogurt compared to raw milk is well known. Yogurts are less immunoreactive than milk and have bioactivity and properties that induce immune or anti-inflammatory processes.[67] Some studies have investigated the nutritional quality of fermented hybrid products. Akin and Ozcan[48] compared the amino acid profile of non-fat fermented milks produced from cow milk or mixes of milk with SPI, PPI, rice protein (RP) and wheat gluten and found that fermented milks containing SPI had the highest essential amino acid content with lysine, leucine, isoleucine, methionine and threonine being the most prevalent amino acids. Berrazaga et al.[68] studied the gelation process of faba bean and caseins produced either by chemical acidification (glucono-δ-lactone) or by lactic acid fermentation and its repercussions on *in vivo* nutritional parameters in young rats. Compared to their acidified counterparts, the fermented gels had lower carbohydrate content. Lactic acid bacteria metabolism led to a decrease of ~ 28% of the initial reducing sugar content (lactose).

During microbial fermentation, microorganisms produce enzymes, such as esterases, amylases, lipases, oligopeptidases, pectinases, and peptide hydrolases. These enzymes break down the complex protein crosslinks, altering the structure and nutrient composition by releasing free phenolic compounds, carbohydrates, proteins and amino acids. Fermentation enhances the nutritional value and protein digestibility. It also promotes an increase in the concentration of organic acid and short-chain fatty acids, enhancing, in turn, the absorption and solubility of minerals (calcium, iron, and zinc) and vitamins.[69] Fermentation has many health advantages: it is a source of beneficial lactic acid bacteria, enhances digestion, increases nutrient availability, potentially boosts mood and behavior, and may contribute to heart health. Additionally, fermented milks offer health-promoting effects such as antihypertensive and antioxidant activities, relief from lactose intolerance, improved protein digestibility, and enhanced vitamin content and probiotic activity.[70]

The presence of antinutritional factors may limit the use of plant proteins in food systems as they can impair the sensory properties, protein digestibility, and bioavailability of other nutrients.[35] Some of the important adverse impacts of antinutrients may include leaky gut and autoimmune effects (e.g.

lectins and some saponins), protein maldigestion (trypsin and protease inhibitors), carbohydrate maldigestion (α-amylase inhibitors), and mineral malabsorption (phytates, tannins, and oxalates). Fermentation promotes nutritional properties not only by improving digestibility but also by degrading the antinutritional components.[62,63] El-Menawy et al.[71] studied the fermentation of quinoa and cow milk. They found an improvement in the nutritional profile due to quinoa milk enhanced the protein content and increased the proportion of essential amino acids like lysine and leucine. Furthermore, the antioxidant capacity increased by adding quinoa to cow milk. Fermentations with commercially available probiotic culture containing *Bifidobacterium* sp., *Lactobacillus acidophilu*s, and *Streptococcus thermophilus* significantly increased the total phenolic content and antioxidant activity in the final products.[71,72] As mentioned above, the microorganisms (starters and probiotics) produce proteolytic enzymes that hydrolyze proteins in free amino acids and simple peptides enhancing their digestion and absorption, which exhibit health benefits, such as antioxidant or antihypertensive capacity.[73] More studies *in vitro* and *in vivo* are needed to evaluate the nutritional value of fermented milk products as a hybrid of plant and animal protein sources.

The combination of plant- and animal-based proteins in the development of hybrid yogurts improves their nutritional value by optimizing amino acid profiles and improving protein digestibility. This combination preserves essential amino acids like lysine and leucine which are crucial for human growth. In addition, when the blends undergo fermentation, the nutritional benefits are further enhanced by breaking down complex proteins and antinutritional components, thus increasing nutrient availability for better absorption. Fermented hybrid products represent an advantageous choice for conscious consumers as they not only contribute to meeting the nutritional/health demands but also support sustainability by reducing dependence on animal-based ingredients.

Sensory properties and consumer perception

Studies have shown that plant-based products often exhibit sensory characteristics that differ significantly from their animal-based counterparts, generally showing less attractive sensory properties. These sensory properties can result in lower consumer acceptance, posing a potential barrier to the broad adoption of plant-based alternatives.[74,75] In the same way, substituting cow milk may cause challenges due to the generally favorable consumer perception as a good source of protein and calcium, convenient to use, and highly palatable.[48] As a result, replacing dairy products may require effort, even among consumers who view plant-based alternatives as more sustainable. In this sense, hybrid products can be a good alternative since they mitigate the impact of vegetable proteins on sensory characteristics. As shown in Table 1, quality information on the sensory attributes of hybrid yogurts is scarce. Fermented foods formulated with up to 10% vegetable flour had no impact on the sensory properties compared to those made with only milk protein.[50,60] However, in formulations containing higher levels of plant protein ingredients, negative sensory attributes, such as off-odors and off-flavors or loss of creaminess, start to emerge.[52,56] Even so, not all vegetable proteins show the same sensory impact. Šertović et al.[46] found that in fermented milks with up to 25:75 soy beverage-to-cow milk ratio the sensory acceptability was not impaired compared to pure cow milk although samples with 50% or more soy beverage were not accepted by consumers. Greis et al.[76] reported that a blend of 75% dairy and 25% plant-based yogurt had no significant differences in liking compared to 100% dairy yogurt. They found that hybrid yogurts had more “dairy-like” sensory characteristics and perform better than existing plant-based products. Yogurts with a higher proportion of dairy were preferred over the samples with a lower proportion despite a segment of young consumers who accept plant-based yogurts that are different from typical dairy yogurts. Their findings show the importance of texture and mouthfeel in consumer acceptance, suggesting that achieving a dairy-like texture in plant-based yogurts can significantly enhance their acceptability. The most relevant sensory properties identified in this study were creaminess, thickness, and the absence of undesirable traits like sliminess and watery texture. Creaminess was found to be the primary driver of textural liking for plant-based yogurts, significantly influencing consumer preference. Furthermore, maintaining an optimal thickness while minimizing negative attributes such as sliminess enhances the overall sensory appeal. Studies examining the sensory properties of hybrid food products suggest improved acceptance compared to the plant-based versions, and better results in formulations with up to 25% of plant-based protein substitution.

When it comes to the consumer perception, there are not many published studies on hybrid products. Marlapati et al.[77] evaluated consumer preferences on hybrid yogurt products prepared with dairy and plant proteins. The research identified key factors influencing consumer choices, and some of them were protein source and content, flavor, price, and packaging claims. The findings revealed that consumers generally prefer hybrid and dairy yogurts over fully plant-based options, mainly due to their superior taste and texture. This underscores the potential of hybrid products to balance sustainability concerns with consumer acceptance. Curutchet et al.[78] investigated Uruguayan consumers' perceptions of hybrid yogurts and beverages made from a mix of milk and plant-based proteins such as canola, hemp, and soy. The study evaluated purchase intention, environmental friendliness, and other product attributes. The findings revealed that consumers showed a clear preference for yogurt over beverages, with canola being the most favored plant-based protein and hemp the least favored one. While sensory appeal and health attributes like protein content positively influenced purchase intention, environmental friendliness did not significantly affect consumer choices, particularly among young people. Both studies' results suggest opportunities for hybrid products in niche markets although traditional dairy remains preferred by most consumers.

The attitudes and beliefs of French consumers toward hybrid yogurts were investigated by Drigon et al.[79] The authors found that consumers with self-centered motivations or driven by altruistic food choice criteria (environmental sustainability, animal welfare) were less likely to purchase hybrid yogurts than omnivorous consumers, for whom the mixed products could act as transitional products toward plant-based diets. Furthermore, the study revealed that the beliefs and representations of the consumers depended on the nature of the plant-based ingredient, and unfamiliarity was not the main barrier to the adoption of these products. Östlund et al.[80] examined factors influencing consumer willingness to buy plant-based yogurt analogues. They found animal welfare was a key motivator for vegans whereas curiosity about new foods drove omnivores. All consumer groups agreed that these products were environmentally beneficial. However, the primary barriers were the unpleasant taste and a lack of reason to switch from traditional dairy yogurt. Respondents consuming more animal-based foods had slightly higher demands on product sensory characteristics than respondents who consume more plant-based foods.

Plant-based products often have less appealing sensory properties than animal-based ones, affecting consumer acceptance and adoption. However, hybrid products combining dairy and plant-based proteins have shown desirable characteristics like creaminess and thickness. The effectiveness of these hybrid products in maintaining desirable sensory properties significantly depends on the type and level of plant-based proteins used, influencing overall consumer acceptance and product success. These hybrids potentially bridge the gap between traditional dairy and plant-based alternatives, and they are appealing to consumers seeking sustainable options without compromising on taste or texture. The review also shows a preference for hybrid over purely plant-based products, and highlights the ability of these hybrid products to meet consumer expectations and support sustainability goals.

Sustainability of hybrid yogurts

There is an increasing emphasis on understanding consumer motivations in food choices, particularly as sustainability and environmental impact become a key concern for many. Customizing products to align with consumers' specific needs and values is essential.

Carlsson et al.[81] analyzed the data from 21 peer-reviewed articles about the differences in emissions and resources used between various plant-based alternatives to dairy and dairy products. The comparison shows that the plant-based dairy alternatives have lower or much lower impacts in almost all cases with two exceptions: water use for almond drinks (several studies) and emissions of ozone-depleting substances for margarine (one study). This analysis shows the potential of hybrid products to blend the benefits of plant-based sustainability with dairy attributes offering a viable solution for reducing environmental footprints in the dairy industry.

Environmental impact is commonly evaluated using Life Cycle Assessment (LCA), which measures the use of resources and emissions throughout the entire production process, from extraction to disposal.[82] The LCA processes have been standardized by ISO 14,040:2006[83] and ISO 14,044:2006,[84] which are useful tools for evaluating the environmental impact of foods. Vieira et al.[50] demonstrated that incorporating lupin flour into dairy products significantly enhanced their nutritional profile while reducing their environmental impact. Sustainability was assessed using the LCA approach by quantifying the resources used and environmental emissions associated with the production of the ingredients and the final product. The assessment allowed the comparison of the environmental impact of lupin-enriched products versus conventional yogurts.

de Jong et al.[85] proposed a new approach to compare the sustainability of food categories based on their CO_2 emissions in relation to the nutrients' density and considering their availability and uptake in the consumer's body, and environmental costs. The case study compared semi-skimmed milk (SSM) with several plant-based beverages (PBBs). The results showed that the PBBs had lower carbon footprint per unit of mass than SSM. However, when the carbon footprint was calculated in relation to nutrient content, all PBBs, with the exception of soy-based beverage, had a higher footprint than SSM. The authors highlighted the general need of PBBs fortification (except for soy) in order to attain reasonable sustainability footprints.

Information on the carbon footprint and other sustainability indicators of hybrid yogurts and hybrid products in general is almost zero, and therefore studies along these lines are required. In their review, Guyomarc'h et al.[8] highlighted the poor connection between animal- and plant-based food chains as a possible weakness of hybrid products. Therefore, if steps are taken to minimize these weaknesses, the reduced carbon footprint of plant-based ingredients together with the high nutrient density of milk products, suggest that their combination in hybrid products could be a promising strategy for the development of sustainable foods.

Concluding remarks and future perspectives

Fermentation of blends of milk and plant to obtain yogurt-like hybrid products is emerging as a promising opportunity to develop appealing sustainable foods with adequate nutritional quality that can be embraced by consumers. However, progress in this area is still in its early stages. To ensure successful developments, further research is needed to gain a deeper understanding of the interactions between the different proteins during the fermentation process, and their potential synergistic effects and implications at physical, nutritional and sensory levels.

The current review on hybrid yogurts shows considerable variability in terms of product type, protein sources, strains used for fermentation, and the concentration of both total and plant-based proteins. The available literature on hybrid yogurts mainly focuses on the inclusion of soy proteins, and to a lesser extent pea and lupin proteins, while other sources of plant proteins are less explored. In addition, even in studies focusing on the same source of plant proteins, different types of ingredients (protein concentrates, isolates or flours) and concentrations (0.4–5%) are used. The type of protein ingredient can affect not only the protein content but also the levels of other nutrients such as fibers, fats, and carbohydrates. These inconsistencies make it difficult to draw universal conclusions about the effects of hybrid formulations on fermentation parameters and consumer acceptability. Further work is required on treatments that enhance the techno-functional properties of plant-based proteins and on exploring new protein sources to optimize these hybrid formulations. Additionally, more research is needed to evaluate the long-term environmental benefits of hybrid products, particularly through comprehensive life cycle assessments.

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