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Review

Development of "New" Bread and Cheese

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Abstract: Bread and cheese have been a popular combination since early times. Indeed, the history of bread dates back to 8000 BC and that of cheese to 7200 BC. However, new types of breads and cheeses are increasingly popular for several reasons, such as allergies, lifestyles, economy and religion. The major challenge is that food manufacturers are offering new products most of which are not welcomed by consumers. Therefore, recently, researchers have placed importance on their relationships with consumers to boost the success of new products. This short review summarizes the backgrounds of recent trends, processes, and principles to manufacture new bread and cheese products, and discusses future perspectives. The development of additive-free, gluten-free rice bread we have recently done from basic research to commercialization of the products is highly focused in this review. Additionally, ongoing studies on plant-based cheeses are introduced from material selection to suggest future outlooks.

Keywords: gluten-free bread; Pickering foam; soy cheese

1. Introduction

Breadmaking dates back to about 8000 BC [1] or earlier [2]. The first flatbreads were made from the mixture of crushed emmer/einkorn wheat grain and water. The paste was baked on a heated stone into a flat biscuit-like product. The use of yeast to make fermented breads came later and likely resulted from airborne yeast spores contaminating mixtures of flour and water during mixing or while waiting to be baked. The process of cheesemaking dates back 7200 years [3], and was probably discovered accidentally by storing milk in a container made from the stomach of a ruminant, resulting in the peculiar phenomenon in which the milk turned into curd and whey by the residual rennin enzyme in the stomach [4].

Bread and cheese have been a popular combination since early times. The aroma of freshly baked wheat bread sharpens our appetite, meanwhile homemade grilled cheese sandwich pampers our senses. However, some people cannot eat wheat bread because of allergies [5] or celiac disease [6]. Besides, demand for plant-based milk alternatives, such as cheese and yogurt, is growing because consumers want more nutritious and sustainable options [7]. Due to the relatively high cost of animal protein, supplying dietary protein to children is a challenging issue in developing countries. Promoting the utilization of locally available protein-rich crops may reduce the protein-energy malnutrition problem [8]. Plant-based cheese may be a solution.

Thus, the development of gluten-free bread [9,10] and plant-based cheese [11] is in progress in the food processing industry. This short review introduces the backgrounds, new technologies, and future perspectives surrounding such foods.

2. Development of Gluten-Free Bread

2.1. Conventional Wheat Bread

Essentially, conventional wheat bread is made in three steps [12]: mixing, fermentation, and baking. First, wheat flour, water, yeast, salt, and other ingredients such as sugar and oil are kneaded by hand or mechanical mixer until they are distributed evenly. Hydrated wheat flour gradually turns into a viscoelastic dough (Figure 1a), a sign that gluten [13] is being developed. Gluten is made from two major wheat endosperm proteins: gliadin and glutenin. The kneading of wheat dough promotes hydrogen bonding and disulfide crosslinking among these proteins, yielding a highly conformational protein network [14] with excellent gas-holding capacity [15]. By this unique function, gluten has made wheat the world's mainstay crop for breadmaking.

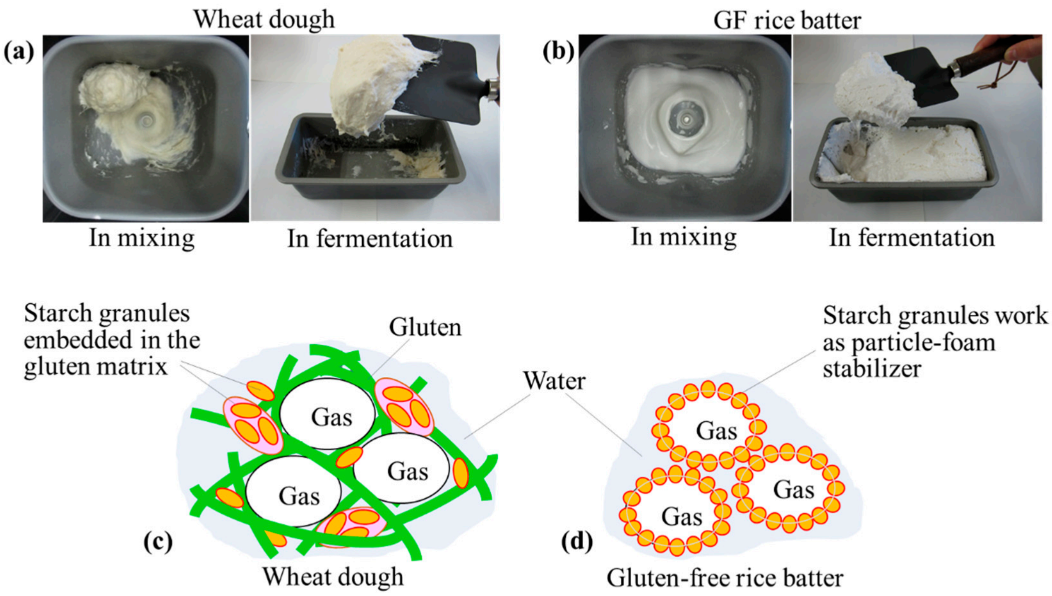

Figure 1. Comparison of wheat dough (**a**) and gluten-free rice batter (**b**) during mixing and fermentation. The drawings show the gas-holding mechanisms of wheat dough (**c**) and gluten-free rice batter (**d**). Adapted from ref. 9 with permission.

The next step is fermentation. In the beginning, yeast multiplies by budding under aerobic conditions. Yeast proliferates rapidly in dough, feeding mostly on supplemented sugar, until the available oxygen in the dough is consumed. Then, its metabolism shifts from aerobic respiration to anaerobic alcoholic fermentation. Yeast produces fermentation gases, mainly carbon dioxide and ethanol. Carbon dioxide inflates small gas cells surrounded by the gluten network. Ethanol and many other volatile compounds such as other alcohols, aldehydes, acids, esters, sulfides, and carbonyl compounds [16] make up the savory aroma of fresh bread.

Here, let us introduce the concept of "damaged starch" [17] of cereal flour. Plant seeds store starch in polyhedral "starch granules", which are major components of the seed endosperm (Figure 2a). Starch is damaged in the process of milling polished grains (Figure 2b), as starch granules are subjected to mechanical as well as heat stresses. In jet milling [18], for example, cereal grains collide with each other in a high-speed jet of compressed air (Figure 2c). While this relatively new type of milling allows the production of fine flour with small particles, it also triggers damage to the starch. The damage includes a changed granular structure, a disrupted crystalline region of starch, and molecular degradation. The extent of damage depends on the milling conditions as well as on the wheat variety (hard vs. soft type) [19]. Damaged wheat flour starch has both positive and negative effects on breadmaking. It helps the intake of water to the starch granules in the mixing and fermentation steps. Local amylases are activated, leading to the production of dextrin and maltose from starch molecules [20]. Consequently, yeast activity increases, as does the final bread volume. Moreover, maltose promotes rich crust browning through the Maillard reaction during baking. However, excessive starch damage results in wet, sticky dough and low-quality bread. Thus, starch damage in wheat flours available on the market is generally controlled to 10–15% [21].

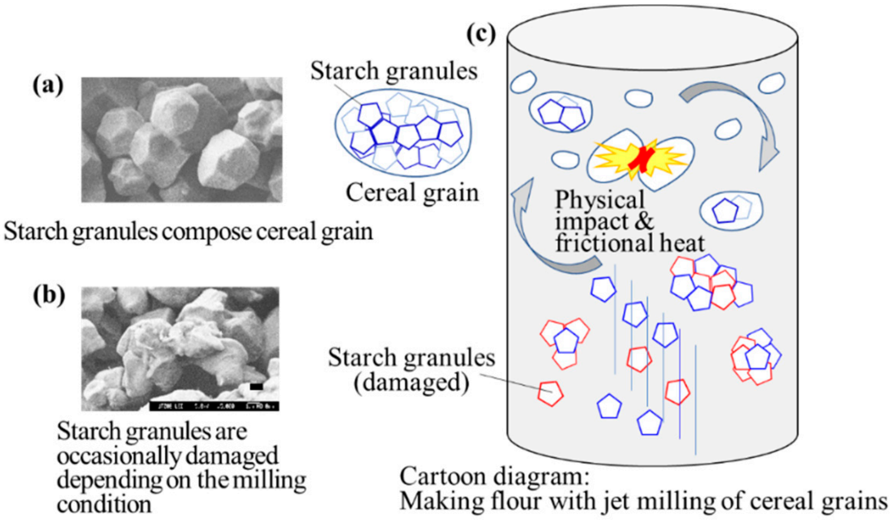

Figure 2. Comparison of starch granules with low (**a**) and high (**b**) starch damage. Drawing of the jet milling process (**c**).

In fermenting wheat dough, gas is confined within the continuous gluten matrix [22], which is composed chiefly of the gluten network, starch granules, and water (Figure 1c). Thus, in the beginning of the fermentation process, many small gas cells appear throughout the dough, like so many tiny balloons (Figure 3a). As the fermentation proceeds, each gas cell grows, collectively making the dough rise. The gas-holding capability of the gluten network is so resilient that bakers sometimes punch the dough to flatten it into a baking pan, but it rises again [23].

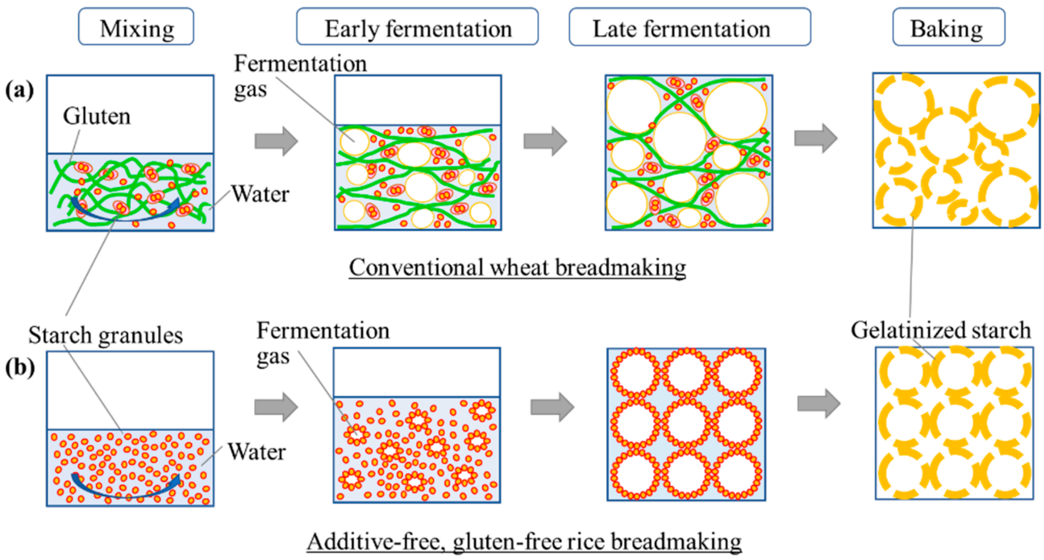

Figure 3. Comparison of breadmaking processes between wheat (**a**) and additive-free, gluten-free rice bread (**b**).

In the following baking process, the gas cells expands further by the intense heat, rapidly increasing the size of the dough in a process called oven spring [24]. The heat gelatinizes the cell membranes, so the gluten matrix forming the envelopes of the "balloons" hardens, thus constructing the stable crumb framework [25]. Increased pressure in the closed gas cells eventually ruptures the cell membranes. The porous structure becomes gradually continuous and open to the outside of the bread, emitting a tantalizing fresh aroma [26]. Concurrently, the crust hardens, creating a sense of crispness when bitten. Meanwhile, the Maillard-type reaction between sugars and amino acids provides browning, creating a dark color and shine on the crust [27].

2.2. Increasing Demand for Gluten-Free Bread

While gluten plays critical roles in wheat breadmaking, some people are allergic to it [5] and others have celiac disease (CD) [6], and thus cannot tolerate wheat. Although the precise prevalence of IgE-mediated food allergy to wheat, such as that confirmed by an oral food challenge, is unknown, it is supposed to be in the range of 0.2% to 1% of the human population [28]. The global prevalence of CD has been estimated to be around 1% and is reported to be increasing. For example, CD among children in Italy has increased significantly over the past 25 years, to more than 1.5% [29]. The demand for gluten-free products is thus expanding, and the global market is projected to reach USD 43.65 billion by 2027, exhibiting a Compound Annual Growth Rate (CAGR) of 9.2% during the forecast period, according to a new report published by Grand View Research [30]. Bakery and confectionary products constitute just over 50% of the market, with cereals and snacks comprising approximately a quarter of the market [31].

2.3. Development of Additive-Free, Gluten-Free Rice Bread

As bread is a representative staple food worldwide, research into the development of gluten-free breads has been intensive [9,32]. The main strategy is to add thickeners such as hydrogels or gums to rice batter as a replacement for gluten. Another approach is to add transglutaminase to promote the protein network among rice proteins. While such additives are effective in helping the rice batter swell, some consumers are concerned by the "mouthfeel" or texture [33] or just the presence of additives [34]. We sought to investigate whether rice bread is producible without using any additives. The ingredients are limited to rice flour, water, yeast, sugar, salt, and oil. Through trial and error rather than strategic approaches, we were finally successful in making such bread [10]. Rice batter was prepared using a bread maker SD-BH105 (Panasonic Corporation, Osaka, Japan). In summary, 160 g of rice flour and 140 g of water were mixed by kneading paddles at 160 rpm for 20 min in a bread bin of the bread maker. Then, 15 g of sugar, 5 g of baker's yeast, 2 g of butter and 1.6 g of salt were added and the batter was mixed again for 20 min. Then, 175 g of the batter was transferred to a square pan case with an 800 mL capacity. Subsequent fermentation (40 °C for about 30 min) and baking (180 °C for 24 min) were done using an EMO-C16C electric oven (Sanyo Electric, Osaka, Japan) by following the supplier's recommendations.

The basic hypothetical mechanism of each step in rice breadmaking is illustrated in Figure 3b for comparison with the steps of wheat breadmaking shown in Figure 3a. Experimental data supporting the hypotheses are provided in our previous papers [9,10]. First, rice flour, water, yeast, sugar, salt, and oil are mixed. Rice flour with less starch damage (<5%) is suitable. The gluten-free rice batter is quite different from the familiar viscoelastic wheat dough. It has the appearance and texture of a slurry with low viscosity (Figure 1b). In fermentation, rice starch granules surround the small gas cells. Each small bubble is a Pickering foam [35], in which starch granules stabilize the boundary between gas and water. It is reminiscent of a soap bubble in which detergent molecules stabilize the boundary between air and water. It has been reported that rice starch granules stabilize the Pickering emulsion by adsorbing onto the interface between oil and water [36]. We reported for the first time that rice starch granules stabilize Pickering foam to realize additive-free, gluten-free rice batter. The fermenting batter is like a meringue (Figure 1b) and is quite different from the classic wheat dough, which is so viscoelastic that its full mass can be lifted with a scoop (Figure 1a). The fermenting batter is so fragile that a small physical shock, such as dropping the container of fermenting batter just a few inches onto a table, can make it sink and never rise again.

The Pickering foam hypothesis explains why rice flour with low starch damage is suitable for this bread. An optimal Pickering agent has an appropriate hydrophobicity/hydrophilicity balance to sit in the oil (or air)/water boundary [37]. If the starch in the granules is highly damaged, the granules in the boundary gradually absorb water, throwing off the balance between hydrophobicity and hydrophilicity, and eventually destabilizing the foam. Generally, starch granules of flour prepared by wet milling has a low damaged starch content [38].

Temperature is another key to successful breadmaking for our bread. As the bubbles are fragile compared to the balloons that form in a gluten network, the environmental temperature should be stable. Fluctuating temperature leads to destabilization of the bubbles, resulting in coalescence between or among them, triggering the breakdown of the swelling batter. The coexistence of large and small bubbles tends to cause their coalescence as well [39]. Thus, air cells in the successful additive-free, gluten-free bread are small and uniformly sized (Figure 4a). In contrast, air cells of wheat bread allow irregular shapes and various sizes [40,41] (Figure 4b).

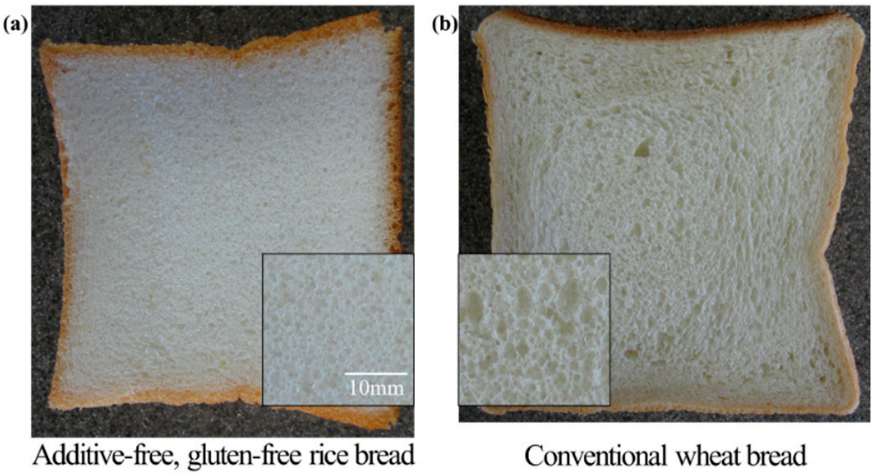

Figure 4. Appearances of additive-free, gluten-free rice bread (**a**) and wheat bread (**b**).

Finally, in the baking process, when the batter is at its most swollen point, it should be baked rapidly. As is well known, the most-inflated soap bubbles are the most fragile. Therefore, starches in the gas cell membrane should be gelatinized by rapid heating before it bursts. In collaboration with the Tiger Corporation and Natural Food, Co. Ltd., we have successfully industrialized our basic research into commercial products: the KBD-X100 home bakery and Wada's Rice Bread (Figure 4a), respectively [42,43].

2.4. Quality Evaluation Studies

The bread made by the KBD-X100 breadmaker was compared with two other commercially available gluten-free breads [44]. Bread A, our bread, was made of rice flour, water, yeast, sugar, salt, and oil. Bread B contained trehalose and polysaccharide in addition to the ingredients in A. Bread C contained egg, milk, and trehalose in addition to A's ingredients. The specific volumes of A, B, and C were 4.0, 2.5, and 2.1 mL/g, respectively. Bread A had small air cells with thin membranes while bread B had larger air cells with thicker membranes (Figure 5a). Bread C had small air cells with thick membranes.

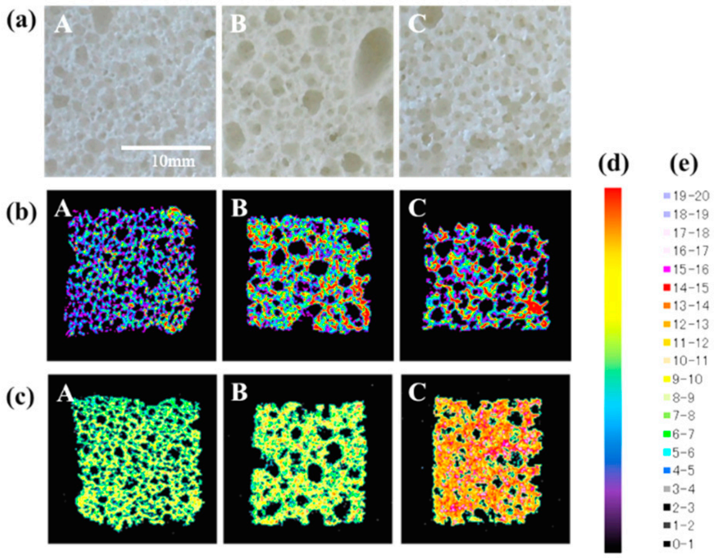

Figure 5. (**a**), Cross sections of breads A, B, and C. Magnetic resonance imaging of proton density weighted images (**b**) as well as T_2 distribution images (**c**) of the three breads. (**d**,**e**), color scale. Adapted from ref. 44 with permission.

The nutrient composition of each bread is shown in Table 1. Bread A contained more water than B and C, while B contained more food fiber (due to the thickener) and lipid than A and C. Bread C contained more protein than A and B due to the addition of egg and milk.

Table 1. Composition analyses of breads.

Items.	Unit	Bread A	Bread B	Bread C
Water	g/100 g	47.6	39.7	40.8
Protein	g/100 g	3.9	3.6	4.5
Lipids	g/100 g	1.5	5.6	4.9
Ash	g/100 g	0.9	1.3	1.0
Carbohydrates	g/100 g	46.1	49.8	48.8
Sugars	g/100 g	45.2	48.0	48.0
Fibers	g/100 g	0.9	1.8	0.8
Energy	kcal/100 g	212	260	256
Na	mg/100 g	338	471	332
Salt	g/100 g	0.859	1.20	0.843

In sensory evaluations, 10 panelists judged A to have the highest “breadness” score, where “breadness” is the essence of the texture of wheat bread (Figure 6a). Moreover, sensory evaluations of the “tactile impression of bread” were conducted with the Check-All-That-Apply (CATA) test [45] using the texture lexicon and the following correspondence analyses. Explained briefly, the CATA method is composed of two steps. First, from the list of easily understood texture terms, each consumer panel selects appropriate terms to describe each bread without any limitation on the number of choices. This test is conducted for all three breads. Subsequent cross tabulation and correspondence analyses portray each bread’s profile according to the impressions of consumers. A comparative profile of the breads is shown on a two-dimensional map, proposing two critical axes (soft/moist vs. hard/dry as well as fine vs. coarse) to evaluate the products (Figure 6b). The 10 panelists evaluated bread A as soft, moist, and fine; bread B as soft, moist, and coarse; and bread C as hard and dry [44]. These sensory impressions should be critical for the evaluation of “breadness” (Figure 6a).

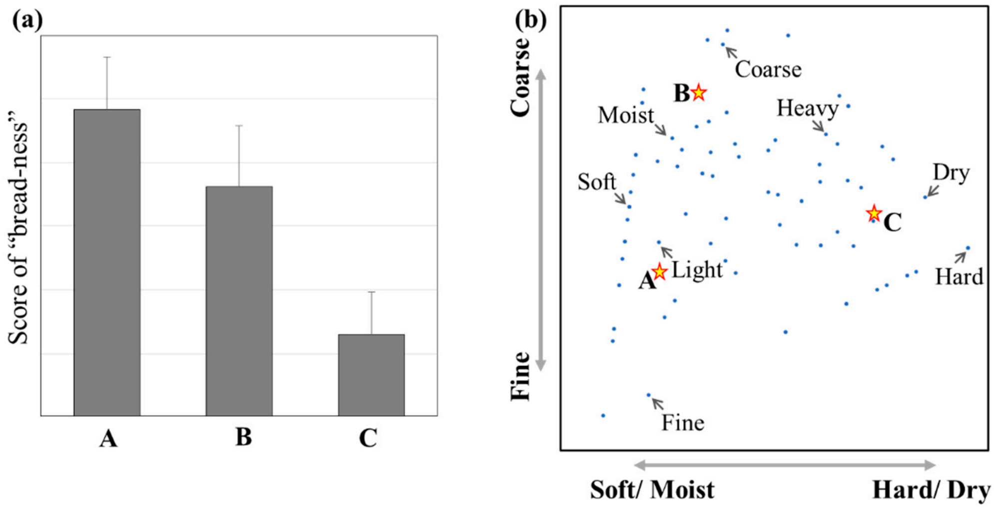

Figure 6. (**a**), Comparative diagram of "breadness" of breads A, B, and C evaluated by panelists. (**b**), Texture map of the three breads. Adapted from ref. 44 with permission.

Magnetic resonance imaging of bread crumbs (Figure 5b,c) suggested that the bubble wall structure and the water distribution therein have important effects on the sensory evaluations. Although water content per weight of bread A is higher than that of B or C (Table 1), the relative water contents per volume of A, B, and C are 1, 1.34, and 1.63, respectively. As water localized in the thicker gas cell membranes in breads B and C, the signal intensity of B or C was considered to be higher than that of A. Additionally, the T_2 relaxation time of A was the shortest. Because A had thinner gas cell membranes and higher specific bread volume, water molecules may have been better distributed, and their mobility may have been lower. In conclusion, comprehensive deliberation of the results obtained by varied analyses was critical to the comparative quality evaluation of the breads.

Recent studies have improved both the quality of additive-free, gluten-free rice bread and researchers' scientific understanding of it. Using 19 rice flour samples with amylose contents ranging from 9.6% to 22.3%, Aoki et al. (2020) [46] found that amylose content was positively correlated with the specific volume of additive-free, gluten-free rice bread. Those authors found that the amylose content of rice flour may affect the hydrophobicity of the starch granules and therefore the stabilization of the Pickering foam in the fermentation process. They also reported that the protein content of the flour did not correlate with the specific volume of the bread.

2.5. Future Perspectives

2.5.1. Better Communication with Consumers

The gap between research and commercial reality in gluten-free breadmaking is well known [47]. Although numerous research studies have worked on improving the manufacture of GFBs, some have adopted approaches far from commercial reality. Indeed, food production companies still face many challenges, one of the most pertinent being the low success rate of new products [48]. As gluten is the key component in conventional breadmaking, the absence of gluten in GFBs looks "fatal". Recent approaches try to overcome this challenge by promoting communication with consumers.

CATA and ODP

Aguiar et al. (2020) [49] compared two rapid techniques, CATA [45] and optimized descriptive profile (ODP) [50], for their ability to describe, discriminate, and identify the drivers underlying a person's enjoyment of sorghum breads. By analyzing the frequencies with which consumers describe a sample by using the sensory terms listed in a questionnaire, CATA provides information about consumers' perceptions of a product, which can be related to the degree to which consumers accept it [49]. However, this method does not allow for quantitative measures, as they are based on frequencies and not ratings [49]. On the other hand, in ODP [50], the evaluation of samples by means of an interval scale allows for the correlation of sensory and instrumental measurements without sacrificing data quality. Rice bread and sorghum bread made with commercially available flour as well as sorghum breads made from six respective flour genotypes were compared. Both methods were successfully applied and provided similar patterns of sample discrimination, whereas the attributes used for sample characterization, as well as those identified as drivers of enjoyment, were generally different. Besides, manufacturers have successfully developed gluten-free bread formulations that consumers have accepted; those breads were prepared with selected sorghum genotypes that are generally better accepted than those prepared with commercial flours. Those authors concluded that this information could prove useful to investigators in the formulation design of gluten-free products for this in-demand market and could guide plant breeders in the development of new genotypes possessing desirable attributes driven by consumers' hedonic responses. Hayward et al. (2020) [51] conducted sensory evaluations to determine the acceptability of, and consumers' sensory perceptions about, GFB made with hemp flour. The first trial instructed participants to assess six fresh bread samples of varying hemp percentages, using a CATA questionnaire [45] and a nine-point hedonic scale. The second one used partially baked bread samples followed by 45 days of frozen storage. Attributes found to drive the liking of bread were smoothness of texture (only for partially baked bread), porosity, moistness, and softness, whereas participants disliked yeastiness and a high density. CATA methods have also been applied effectively to develop other GF foods: protein fortification of Portuguese corn bread (*broa*) [52], traditional confection (*alfajor*) [53], and ladyfinger biscuits [54].

Texture Sensations

In the development of gluten-free products, texture is crucial [55]. Until recently, strategies to modify or improve texture attributes focused on the characteristics of the product itself, such as structural or instrumental texture. However, new food product developers and researchers are becoming interested in oral processing, a recent approach, based on knowledge of product behavior in the mouth, to better understand the mechanisms of texture perception [56,57]. During oral processing, food structures are broken down to form a bolus suitable for swallowing [58]. Fragmentation, agglomeration, hydration, and lubrication take place therein and contribute to the sensory experience in the mouth [59]. It is a complex and dynamic process, like texture perception [60]. Sensations perceived during eating vary continuously because of the breakdown of food structures and the progressive changes in bolus characteristics, releasing stimuli of different sensations [57]. Peutra et al. (2020) [57] studied the food bolus properties of gluten-free breads in relation to the dynamics of sensations perceived during its consumption. They compared bread bolus particle size after three chews, bolus characteristics at the swallowing point, and oral activity among five commercial gluten-free breads and two regular breads. Sensations perceived at the beginning of bread consumption (hard, soft, spongy, dry) were related to the bread's structure and mechanical properties; however, the remaining sensations were explained mostly by oral trajectory features. Crumbly and sandy sensations were related to bread fragmentation in the mouth, the compact sensation to the amount of saliva uptake, the pasty sensation to a cohesive bolus (nonfragmented), and the sticky sensation to a bolus with high dominance of adhesiveness at the end of consumption. The authors remark that, to reformulate or improve gluten-free breads, strategies should take into account the in-mouth behavior of the product, as its breakage pattern in the presence of saliva and oral movements is crucial to modulate texture sensations. Mechanical measurements of oral processing are also in progress. A recently developed "artificial tongue" [61] should work effectively to quantify the complex behavior of food in the mouth.

Discerning Consumer Attitudes

To develop patient-friendly gluten-free products, opinion surveys are essential. However, it is not easy to recruit celiac patients, who represent only 1% of people, to participate in such surveys. Twitter exploration using co-occurrence networks successfully gathered knowledge about what is relevant for gluten-free consumers [62]. When tweeting about gluten-free foods, people mainly talk about five product categories that the lack of gluten crucially affects (bread, cake, cookies, beer, and pizza), share information about how to prepare gluten-free foods (recipes and ingredients) and where to get them (restaurants, supermarkets, food chains), describe the contexts of consumption (social events, places, and occasions), or talk about diet and health aspects of celiac disease. People also use Twitter to share eating or food preparation situations and to exchange tips on obtaining gluten-free products, whether bakery goods, recipes, or ingredients for preparing them. For beer and pizza, recommendations are related to brands, supermarkets, and restaurants.

Understanding the mechanisms underlying consumers' perceptions of gluten-free claims is also relevant. Sielicka-Różyńska et al. (2020) [63] sought to evaluate consumer perceptions of gluten-free claims and of the Crossed Grain symbol on cookie packages. The analysis proved the significant relationship between the number of gluten-free claims and consumers' purchase intention. The respondents paid more attention to verbal gluten-free claims than to nonverbal ones on packaging. In the case of the Crossed Grain symbol, the addition of a verbal statement strengthened the information and decreased respondents' uncertainty level about a given product. Those authors concluded that designing product labels that incorporate the information architecture concerning gluten may help strengthen consumers' attitudes towards gluten-free products and impact their buying behavior.

A comprehensive composition database of cereal-based gluten-free products (Fajardo, 2020) [64] should be helpful. This type of study is a priority, since CD patients include this type of product in their diets, and studies assessing CD patients' diets need to use updated data on GF product composition. Moreover, since nutritional deficiencies have been described for CD patients [65] and it has been shown that GF products have poorer nutritional quality [66], an updated quality assessment of available products is needed in order to further improve GF product development.

2.5.2. Boosting Nutritional Benefits

Although rice-based gluten free breads are generally considered less allergenic, they are often deficient in bioactive compounds. Moreover, whereas rice flour has a higher amino acid score than wheat flour, it also has lower protein content. Numerous studies have reported nutritional deficiencies or imbalances ascribable to a gluten-free diet, especially in children [67]. Therefore, researchers are trying to boost the nutritional value of GFB by adding nutrients such as acerola (antioxidants) [68], acorn (polyphenols) [69], bee pollen (proteins, minerals, polyphenols, carotenoids) [70], chia seed (polyphenols) [71], chlorella (β-carotene, lutein, protein) [72], coffee cascara (dietary fiber) [73], cricket (proteins, antioxidants) [74], curd cheese (minerals, proteins) [75], grasshopper (protein) [76], microalgae (better fatty acid profile, proteins, minerals) [77], unripe banana flour (dietary fiber) [78], and yogurt (proteins and minerals) [79]. More information is available in the recent reviews on the addition of dietary fiber [80], insects [81], and legume flours [82] to boost the nutrition of gluten-free bread.

One interesting trend is that consumers' psychological resistance or fear of insects in food [83] is gradually reducing [84]. This reflects consumers' gradually increasing understanding of the shortage of edible protein available from stock animals because of the growing world population. However, it should also be considered that some people are allergic to insects as food [85].

3. Development of Cheese

3.1. History and Aspects of Conventional Cheese

The earliest cheese was most likely started as an accidental curdling of milk, which was stored and transported in bladders made of ruminants' stomachs, as their inherent supply of rennet would encourage curdling [86]. Over several thousand years, cheesemaking has advanced from an art to wide types and applications. In early times, cheese was made by pressing and salting curdled milk for preservation purposes. When cheesemaking spread in Europe, a great variety of cheeses were invented due to the different climates, which led to the development of aged, ripened, and blue cheeses. Many familiar cheeses (cheddar, gouda, parmesan, camembert, etc.) were first produced in Europe during the Middle Ages [87]. More recently, cheese consumption as an ingredient in foods such as pizza, burgers, and breads has grown rapidly [88]. The liquidity of cheese made foods more enjoyable to eat, as the cheese added stretchability and melting properties in the mouth. Certainly, different processing and ripening times endowed different cheeses with varieties of properties in addition to liquidity, such as firmness and creaminess. In 1916, a method for making processed cheese first emerged in conjunction with an American patent by James L. Kraft [89]. Processed cheese was developed to extend the shelf life of cheese and to find additional avenues to make use of unsold cheese based on emulsification [90]. Recently, processed cheese has become a popular snack food due to its mechanical properties [91–93]. The cheese market was once dominated by varieties of processed cheeses including slices, blocks, and springs, leading customers to pay more attention to the texture than to nutrients.

The mechanism underlying cheese curdling is decided mainly by the hydrophobic bonding of casein micelles (Figure 7). Casein, a major component of cheese, accounts for 80% of a cow's milk protein. It is a family of related phosphoproteins, including α_{s1}-casein, α_{s2}-casein, β-casein, and κ-casein [94]. Casein is relatively hydrophobic and exists as casein micelles in milk due to hydrophobic aggregation. In particular, κ-casein contains the hydrophilic part ofcasein glycopeptide (CGP), which resides at the surface of a casein micelle to keep the micelle stable in the milk. Casein micelles are held together as a colloidal suspension by colloidal calcium phosphate (CCP) bridging. Enzymatic hydrolysis or acidification can coagulate a colloidal suspension of casein micelles to form a continuous and solidified casein network. Conventionally, rennet is widely used to produce cheese by an enzymatic reaction. When rennet is added, CGP residing at the surface of a casein micelle will dissociate due to hydrolysis, whereas casein micelles without CGP will coagulate due to hydrophobic binding to form curd through a nonenzymatic reaction. Generally, cheese will be produced after a final process, including whey separation, pressing, and ripening. In addition, utilization of lactic acid bacteria (LAB) or the lowering of pH can also be used to prepare cheese by building an acidic environment in the milk. The isoelectric point of casein is 4.6, which appears as a negative charge in milk of pH 6.6 [95]. When the charge in milk approaches the isoelectric point of casein, caseins will aggregate and precipitate to form cheese after a process of draining and pressing.

Processed cheese starts as a mixture of several raw, melted cheeses that solidifies by emulsification and cooling [93]. The manufacturing process is regarded as oil-in-water emulsion, with caseins acting as emulsifiers that gel upon cooling [96]. Processed cheese is prepared mainly by smashing raw cheeses; mixing in emulsifying salts, water, and limited amounts of additional ingredients; emulsifying the mixture in a reaction container; filling and packaging; and cooling and storing (Figure 8).

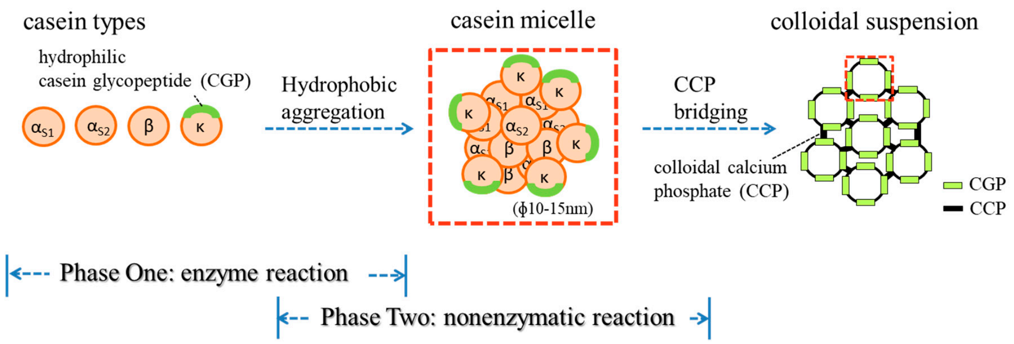

Figure 7. *Cont.*

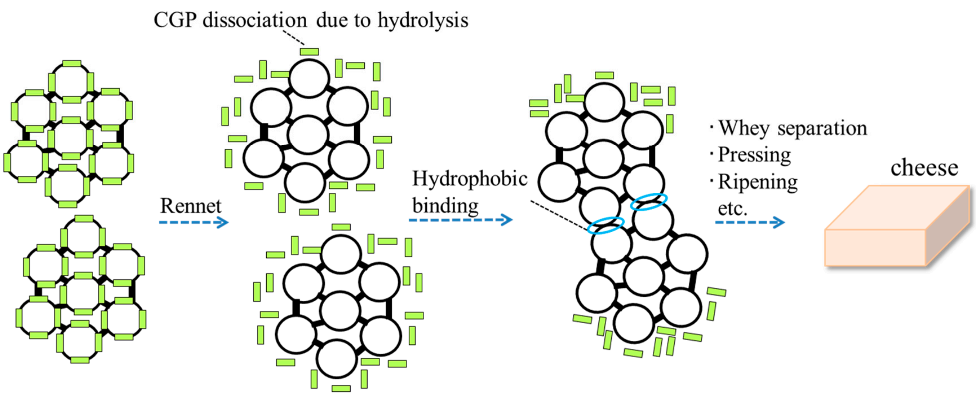

Figure 7. The mechanism of cheese curdling.

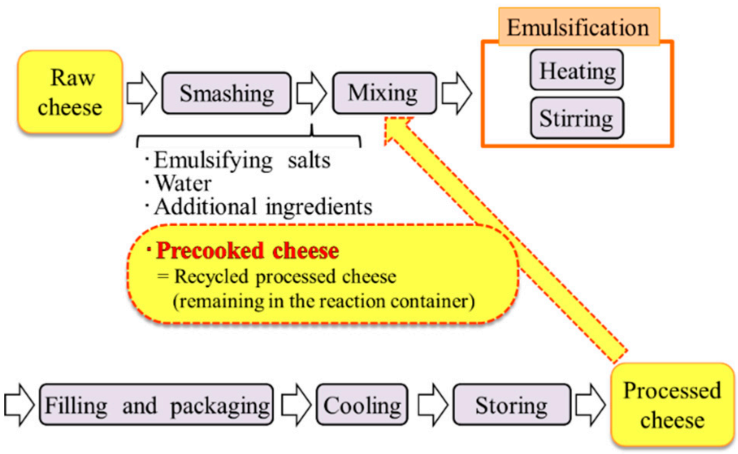

Figure 8. Preparation of processed cheese.

The most important part of the process is heating for an appropriate time with continuous stirring (emulsification) until a homogeneous mass forms. The emulsifying process changes the qualities of processed cheese, especially its viscosity, mechanical properties, and microstructure. The continuous increase in viscosity during emulsification is called the creaming effect [97]. In this process, creaming affects the sizes of milk fat globules and the casein network structure, thereby influencing the viscosity of the melted cheese or the firmness of the final product. Wei et al. demonstrated that increasing the stirring speed reduced the sizes of fat globules and that, after a sufficiently long stirring time, the protein network became fine-stranded, which was thought to be related to the creaming effect, firmness, and appearance of yielding points [90]. Besides, precooked cheese is often used to improve the functional properties of processed cheese during industrial manufacturing. Precooked cheese is essentially recycled processed cheese that remains in the reaction container using in the following production. Precooked cheese can affect the creaming effect and is often used to adjust viscosity or firmness according to purpose. It was suggested that the potency of precooked cheese is influenced mainly by types of emulsifying salts [98]. In general, the potency of precooked cheese will increase when long-chain phosphates or more stirring strength is used [99,100]. The creaming process also depends on the emulsifying salts, which improve the emulsification capacity of caseins [101–105]. During heating, emulsifying salts detach calcium ions from casein micelles and replace them with sodium ions. This ionic exchange converts the insoluble calcium para-caseinate into sodium para-caseinate, which is more soluble and functions as an emulsifier and stabilizer to produce oil-in-water emulsion in the melt (Figure 9). The effects of emulsifying salts (applied singly or in combination) on the production of model processed cheeses are well described in the available literature [106–109].

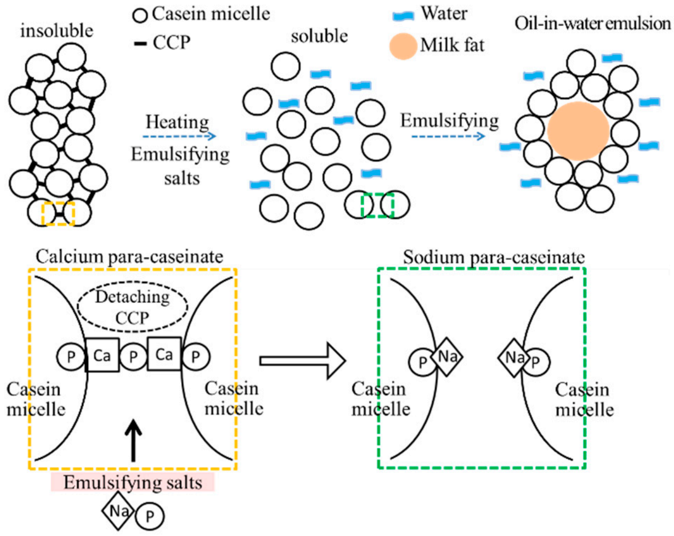

Figure 9. The mechanism by which emulsifying salts function on casein micelles.

3.2. Demand and Trend for Plant-Based Milk Substitutes

Cow milk has been an essential component of human nutrition for thousands of years and is recommended as an important part of the human diet due to its high content of calcium, proteins, and vitamins (A, B_2, and B_{12}) [110,111]. According to the OECD-FAO agricultural outlook 2020–2029, global milk production (81% cow milk, 15% buffalo milk, and 4% for goat, sheep, and camel milk combined) grew by 1.3% in 2019 to about 852 Mt. and is projected to increase to 997 Mt by 2029 (Figure 10). Strong production growth is expected in Africa. However, milk production in the three major dairy exporters (New Zealand, the European Union, and the United States) is increasing slightly and is expected to continue the trend toward slow growth, with predicted rates of 0.36%, 0.35%, and 0.38%, respectively [112].

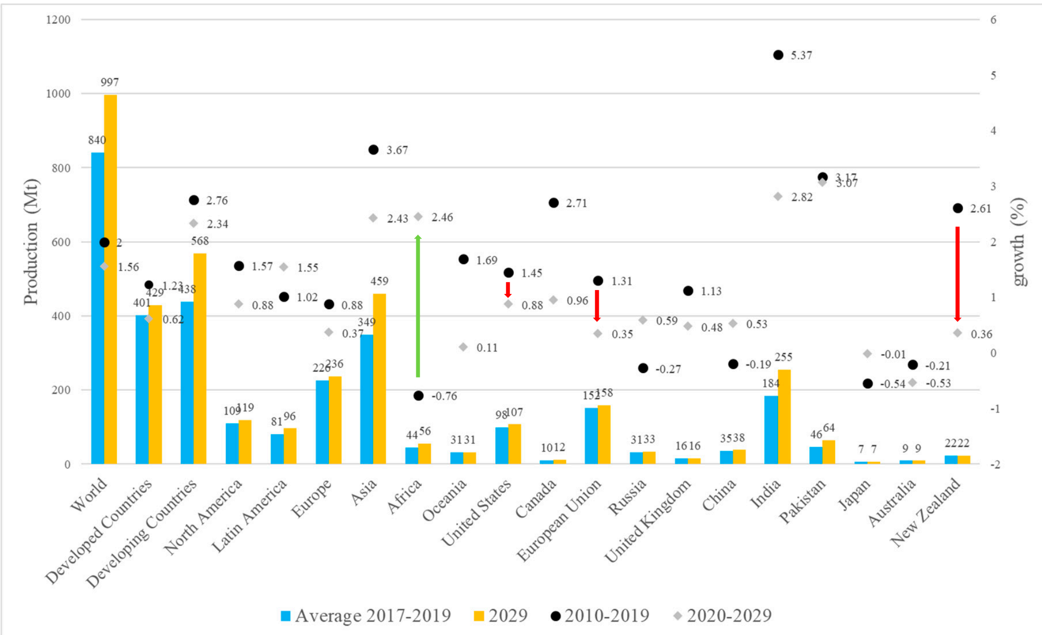

Figure 10. Global milk production and growth in selected regions and countries.

Milk intake has declined sharply in the last decade, particularly in developed countries. Demand for plant-based milk substitutes is induced by ongoing trends ranging from health concerns such as cow milk allergy, lactose intolerance, and cholesterol issues, to animal protection and ecological concerns, as well as lifestyle choices such as vegetarianism and veganism [113–115]. Although awareness varies from country to country, it tends to grow rapidly for people looking for plant-based milk substitutes for a healthy and environmentally friendly lifestyle. According to Plant-Based Foods Association (PBFA) and the Good Food Institute (GFI)' reports [116], plant-based milk substitutes accounted for 40% of the plant-based food market, reaching USD 2 billion with a growth rate of 5% in 2019, which accounted for the largest proportion of plant-based foods in the United States (Figure 11). Besides, correlative products such as plant-based cheese and plant-based yogurt, tended toward rapid growth of 18.3% and 31.3%, respectively. In Europe, plant-based milk substitutes are also growing in status. An estimated 15% of Europeans do not consume dairy products anymore, and the market for plant-based milk substitutes was already growing by 9% in 2015, reaching USD 1.9 billion with 138 different variants [117]. The global plant-based milk market reached an estimated value of USD 8.51 billion in 2016 and is forecasted to see a compound annual growth rate of 12.5%, reaching a market volume of USD 24.6 billion in 2025 [114].

Plant-based milk is a prime ingredient of plant-based cheese. Generally, the materials in plant-based milk substitutes are divided into five categories: cereals (oats, rice, corn, etc.), legumes (soybeans, peas, chickpeas, etc.), nuts (almonds, coconuts, etc.), seeds (sesame, sunflower, etc.) and pseudo-cereals (quinoa, amaranth, etc.) [7,118,119]. Researchers are using these materials to develop products that are as close as possible to cow milk in terms of taste, nutritional value, and appearance. Many plant-based milk products are already on the market, including soy-based, almond-based, coconut-based, and oat-based products [120]. The advantages of the four plant-based milk substitutes are discussed next.

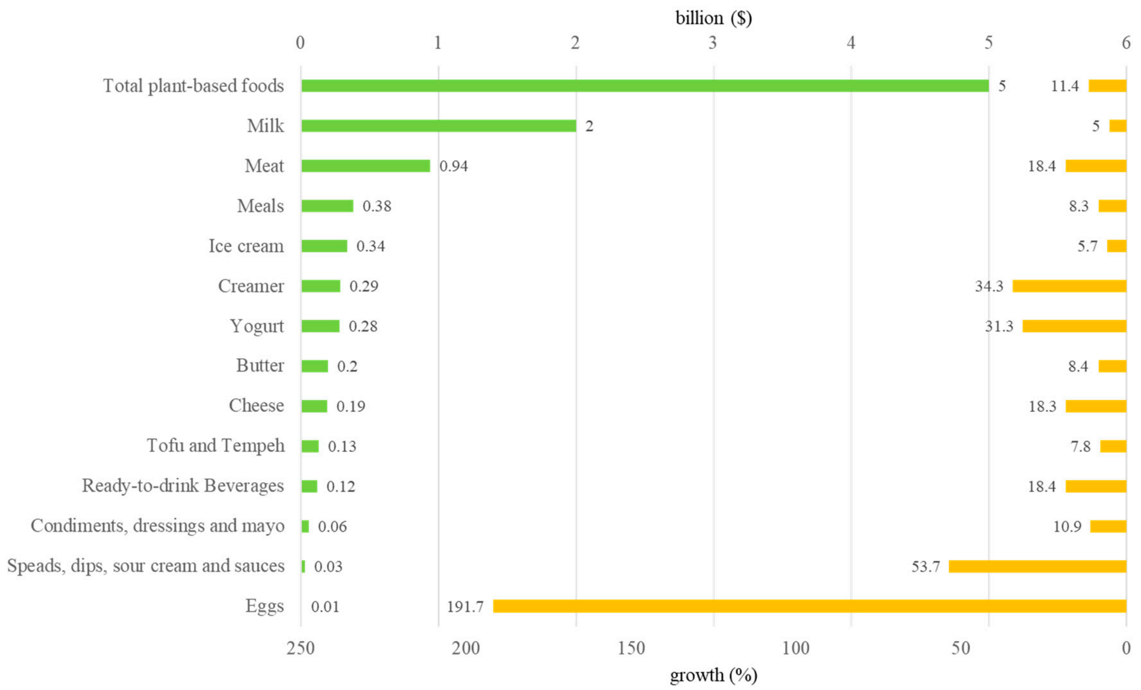

Figure 11. Growth of plant-based food substitutes by category.

3.2.1. Soy-Based Milk

The use of soy milk was first reported about 2000 years ago in China; within the past 100 years, soy milk has appeared on the supermarket shelves of Europe and the United States [115]. Soy milk serves the purpose of providing nutrients to areas of inadequate milk supply and is a milk substitute for people who are allergic to cow milk or are lactose intolerant [118]. Soy milk is the most widely consumed plant-based milk substitute due to soybean's remarkable nutrient profile, and it is a unique plant-based milk substitute that supplies nutritional value closest to that of cow milk. Soybean not only contains high-quality protein comprised of almost all essential amino acids that correspond to those required for humans, and has high digestibility rates of 92–100%, but it contains abundant lipids and carbohydrates [121]. It also contains amounts of polyunsaturated fatty acids, has no cholesterol, and is a rich source of vitamins, isoflavones, and minerals such as calcium, potassium, magnesium, iron, zinc, and copper [113]. Soybean has good therapeutic properties for chronic diseases such as osteoporosis, cancers, and cardiovascular diseases [118]. For this reason, soy milk has been utilized in many products, such as soy cheese, soy yogurt, and tofu, to enhance its consumption [122,123]. However, several negative concerns have limited consumers' acceptance of soy milk, such as its beany flavor due to the action of lipoxygenases on unsaturated fatty acids and the presence of trypsin inhibitors [118]. These factors gave rise to the decline in its consumption. Although soy milk products still dominate the market, the emergence of other plant sources, such as almond, coconut, and oat, have decreased its market share [115].

3.2.2. Almond-Based Milk

Based on its rich nutritional composition, almond milk has become one of the most popular plant-based milk substitutes in North America, Europe, and Australia in recent years, accounting for the largest share of the total nut consumption in those markets [124]. The main components of almond are lipids, proteins, and carbohydrates such as sugars and fibers. The proteins are mostly essential amino acids, and the lipids are mostly unsaturated fatty acids [125,126], of which rich monounsaturated fatty acids are considered helpful in the diet and potentially beneficial to heart health [111,113]. Besides, almond is an excellent source of vitamin E compared to other plant materials. For this reason, almonds are also used as nutrient supplements for vitamin E intake. α-Tocopherol is a functionally active component of Vitamin E and is a powerful antioxidant that plays a pivotal role in fighting free-radical reactions and thus preventing oxidative stress [127]. Additionally, almond milk is suggested to be an effective solution in children who are allergic or otherwise intolerant to cow milk [111].

3.2.3. Coconut-Based Milk

Coconut milk is widely consumed in Southeast Asia and South America. It is not only a beverage but also an ingredient in sweets and recipes [118,128]. Coconut milk has high fat content and is rich in vitamins (C and E) and minerals such as iron, calcium, potassium, magnesium, and zinc. It also contains lauric acid, which is present in breast milk and related to the promotion of brain development [118]. Lauric acid can also contribute to raising the levels of high-density lipoprotein, which helps reduce harmful low-density lipoprotein levels in the blood stream [129]. Coconut milk also promotes digestion and nourishes the skin. These health benefits are associated with the consumption of coconut milk in developed countries such as North America and Europe [130]. However, coconut milk is highly susceptible to chemical deterioration due to the presence of saturated fat. Lipid oxidation and lipolysis can result in off flavoring, which limits its consumption [129].

3.2.4. Oat-Based Milk

Oats are a promising raw material for the preparation of functional plant-based milk owing to the potential therapeutic benefits. Oats are a good source of amino acids, fatty acids, vitamins, minerals, and dietary fibers; in particular, β-glucan in dietary fiber is a functionally active component that benefits the digestive system, fights cancer, and reduces the levels of glucose and low-density lipoprotein cholesterol in the blood [118,131]. In order to diversify oat consumption, attempts have been made to develop oat milk [132]. However, as oat milk is low in calcium, it should be fortified as a milk substitute [118]. Besides, when oat milk is used for food preparation, the lipids can cause various processing problems such as poor flavor and brown color [133]. Another problem for oat milk is the high content of starch. Thermal treatment gives oat milk high viscosity due to the gelatinization of starch, leading to its low acceptability. To prevent gelatinization, the starch can be hydrolyzed [118].

3.3. Technologies in Plant-Based Cheese Making

Plant-based milk substitutes are water extracts of plant materials, which consist of plant proteins homogenized in water together with emulsifiers, stabilizers, flavors, etc., resulting in colloidal suspensions distributed in the range of 5–20 µm, which imitates cow milk in appearance and consistency [7,113]. As described in the section above about conventional cheese, dairy cheese can be achieved by a rennet-induced (via the enzymes from rennet) or acid-induced (acidification) process. When milk clots under normal conditions of pH and protein content, the viscosity does not increase until the enzymatic phase is mostly complete. Furthermore, lowering the pH of milk leads to a reduction in the rennet coagulation time and a faster rate of increase in gel firmness [94]. However, plant-based cheese making follows a proper regime according to the characteristics of plant proteins. Plant proteins have different molecular and functional properties than casein, due to their larger molecular size and complex quaternary structure [134]. Unlike the case with cow milk, rennet does not induce curd formation in soy milk [135]. In the literature, soy milk was coagulated by various proteolytic enzymes, but the product had a weak structure and it was difficult to obtain hard-type cheese [136–138]. Relying on the use of heat and salts (magnesium sulfate, calcium sulfate)/acidifier (glucono delta-lactone) to produce soy gel, the texture of the final product was often unsatisfactory [139]. Therefore, various technologies, such as the application of enzymes, acids, or heat treatments, need to be considered to improve textural acceptability and spreadability to the level of those of dairy cheese.

To attain dairy cheese-like properties, some studies focused on making cheese substitutes with mixtures of cow milk and soy milk, which are called partial dairy cheese analogues. Those studies showed that characteristics of the cheese are reduced significantly and that rennet coagulation time becomes longer as the proportion of soy milk in the blend increases [140,141]. High content of vegetable protein instead of casein was used to prepare a cheese analogue, but the product showed inferior properties such as an adhesive consistency, lower firmness, and off flavoring [142]. Like proteins, fats are also used to determine the properties of plant-based cheese. Fat globules occupy space in the protein matrix and prevent the formation of a dense network that would result in a hard and corky product [134]. To achieve the desired firmness in a cheese matrix, hydrogenated vegetable oils are used. Partial hydrogenation increases the content of saturated fatty acids promoting lipophilic groups of proteins adsorbed on the surfaces of fat globules, and hence improves emulsion and increases matrix density [143]. However, this procedure reduces the nutritional value of plant fats drastically and increases the risk of cardiovascular and coronary heart diseases [144]. Some researchers tried to produce plant-based cheeses with desirable texture and acceptability by simply blending two plant-based ingredients together for nutrient fortification or to incorporate different additives such as pastes, tofu, carrageenan, pectin, etc. [8,145]. However, it is hard to persuade consumers who eschew food additives, and the digestibility of the mixed plant-based ingredients must be ascertained to ensure their contribution to protein intake. The technical breakout for plant-based cheese tends to produce a smooth and uniform texture associated with spreadability, similar to dairy cheese. Using plant-based proteins to match the functionality of casein has proved to be extremely challenging [146]. In general, plant-based meat products contain primarily soy, pea, and wheat proteins for functionalities, but those proteins cannot be used to make meltable plant-based cheese. Exploration of new plant-based protein sources is expected. Mattice and Marangoni evaluated plant-based cheese containing zein to compare its melting and stretching capabilities with those of conventional cheeses [147]. As a hydrophobic prolamin from corn, suitable zein content provided desirable cheese-like functionality relative to conventional cheese products and commercially available plant-based cheeses.

3.4. Fermentation Strategies for Plant-Based Cheese and Outlook

Fermentation is a powerful tool used to improve the sensory properties, nutritional value, and shelf life of plant-based products [115]. The kind and degree of fermentation determine characteristic properties like rheology, texture, flavor, and taste [148]. Sufu, a fermented cheese-like product dating back to ancient China, is produced by coagulating soy milk with coagulate salts like calcium sulfate or calcium chloride, pressing the resulting curd (tofu), and following fungal and/or bacterial fermentation [149]. In contrast to dairy cheese, plant-based cheeses like sufu have attained little scientific interest, but the adaptation of sufu processing technologies, such as the utilization of proper starter culture and the optimization of a fermentation environment, could be an effective method for plant-based cheese production [148]. In order to produce fermented products, the starter cultures should be able to grow and dominate the microflora in the plant-based medium [115]. Lactic acid bacteria (LAB) have been used for cereal fermentation for centuries, and many cereals and pseudo-cereals are known to support their growth [150]. LAB are the key bacteria in the fermentation processes of cheeses, yogurts, and fermented vegetables [151,152]. The roles of LAB in soymilk fermentation are to produce acid and flavor, and to remove any undesirable beany taste [153]. Li et al. (2013) [135] developed an optimal process for making soy cheese by a combination of glucono delta-lactone and LAB fermentation together with enzyme hydrolysis, which resulted in the best spreadability, acceptable sensory properties, and a stable homogeneous structure. Martensson et al. (2000) [154] studied the growth and product characteristics of an oat milk medium fermented with a range of starter cultures. They found that strains of *Leuconostoc mesenteroides*, *Leuc. dextranicum*, *Pediococcus damnosus*, and *Lactobacillus kefiri* produced the highest levels of lactic acid, resulting in a pleasant flavor. In addition, the application of extracellular polysaccharide-producing strains has the potential to improve the firmness and structure of plant-based cheese based on its good expression in dairy cheese [155]. Another important concept is that mixed-culture fermentation can provide synergistic effects to enhance the quality of fermentation in plant materials. In a mixed culture, the two strains stimulate one another's growth, acid production, and compound formation [156]. The combination of amylolytic and probiotic bacterial strains resulted in an increased acidification rate and hence reduced the fermentation time of rice [157].

Increasing awareness of diet's impact on human health has resulted in great demand for plant-based products. Although this trend has lately put a heavy focus on seeking meat analogues, the development of plant-based dairy products, including plant-based cheese, is also greatly desired and is becoming more and more popular all over the world. For this reason, there is still an urgent need to increase the supply of new and sustainable plant-based protein sources as well as that of fortifying nutrients such as the vitamins and the minerals, especially calcium, that are found in cow milk-based products. Fermentation and enzymatic technologies that help to optimize cheesemaking may still be research hotspots for a time. The present authors believe that emulsifying technology and structural engineering have the potential to enable the design and improvement of cheese functionalities to supply varieties of cheese products to meet the strong demand for cheese.

4. Conclusions

Food reflects our society. Until recently, a meat diet has prevailed due to global economic development, but the concurrent rapid population growth has caused a shortage of meat. Additionally, increasing numbers of people are suffering from food allergies [158]. The borderless transfer of people has exposed or diminished food taboos, and as a result, food-related lifestyles are changing. Now, all these issues present a demand for researchers and industry to develop a new type of food. Gluten-free bread and soy cheese are different, but both mirror society.

The marketability of lab-made food samples increases through better communication among consumers, researchers, and industry. Such effort is the most promising way to jump over the "valley of death" in food research.

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