Chapter

Stability Aspects of Non-Dairy Milk Alternatives

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Abstract

In recent years, plant-based milk products, commonly called as non-dairy milk alternatives have gained high popularity due to concerns associated with bovine milk like lactose intolerance, allergies, hypercholesterolemia, and pesticide and antibiotic residues. Important strategies for manufacture of non-dairy milk alternatives involve disintegration of plant materials in aqueous medium; its homogenization and addition of some additives to attain a consistency and appearance similar to that of bovine milk. Different range of ingredients are added to non-dairy milk alternatives such as oils, emulsifiers, thickeners, antioxidants, minerals etc. The main problem associated with non-dairy milk alternatives is generally linked with its stability. Stability is a crucial factor that governs the sensory properties and overall acceptance of non-dairy milk alternatives. Differences in processing parameters and molecular interaction mechanisms affect the stability of emulsions as well as the stability of non-dairy milk manufactured thereof. Various treatments like thermal treatment, non-thermal processing (ultra high pressure homogenization, pulsed electric field, ultrasonication), addition of emulsifiers are effective in achieving the stability of non-dairy milks. The present chapter aims to summarize the various factors contributing to the physical stability of non-dairy milk alternatives like appearance, consistency, emulsion stability, and the approaches required to maintain it.

Keywords: non-dairy milk alternatives, emulsifiers, thickeners, ultra high pressure homogenization, stability

1. Introduction

Food has served multitude of functions for humans since ages, such as satiating hunger, quenching the palate with different savory food products, promoting well-being and socializing on one side of the equation, and providing the basis of energy production for regulating physiological needs, acting as a source of health promoting bioactive components, and antioxidants, on other. Among the foods, animal based products like bovine milk and beef are by far the most commonly consumed ones in the world. Apart from reasons of health and wellbeing, consumers nowadays are interested in reducing their intake of animal products because of moral and environmental reasons. Different issues underlying the negative attitude towards the manufacture of animal based products include environmental pressures from the vast amounts of agricultural produce and water essential for feeding animals, habitat loss deforestation, animal exploitation, species extinction, and pollution in production and transportation of the food until it reaches the consumer.

Since in recent years the animal based diet is being negatively associated with the individual's health and the environment, people have started looking for other food options [1]. Consequently, the plant based diet has become a favorite among people because of its potential to promote health, to improve food security, and to decrease pollution, land use, and water use [2].

Because of the increase in the global urban population, and with the consumers having more purchasing power and health awareness nowadays, the demand for healthier, tastier, and newer food products has risen tremendously. Furthermore, research for various innovative and novel food product developments in the last decade has been focused on meeting the emerging needs and adapting to existing market demands by providing newer food choices and alternatives. Therefore, the plant based diets like non-dairy milk alternatives, in particular, seem to have experienced a surge in the market. Besides, there is increasing negative perception related to the consumption of bovine milk among consumers as it has been linked adversely to many diseases such as bovine milk allergy, lactose intolerance, anemia, and coronary heart diseases for the past many years [3–5] and also due to issues that have raised concern in recent years, like the presence of toxic chemicals, antibiotics, contaminants, and greenhouse gas emissions. Nondairy milk alternatives possess health beneficial components, including antioxidant, antimicrobial, dietary fibers, unsaturated fatty acids; and hence, are desirable among consumers [6–8]. Nonetheless, the market for non-dairy milk alternatives is still emerging and currently, the range of products available in the market include hazelnut, peanut, sesame, soy, almond, oat, rice, hemp, and walnut milk; issues regarding the stability and nutritional value is still a concern among consumers. For successful commercialization of non-dairy milk alternatives, processors are often interested in the technological interventions and ingredients that can help maintain the physical stability of the final product. Physical stability refers to the maintenance of inherent attributes of suspension in relation to its viscosity, appearance, consistency, color, and resistance to destabilization mechanisms like sedimentation, phase separation, flocculation, creaming, etc. The general manufacturing process involves soaking the raw material (nut, legume, cereals, pseudocereal) in water, disintegrating moist material, separating oil bodies, adding different additives, heating for killing the harmful microorganisms, homogenization, and aseptic packaging [9]. Technological interventions are required to manufacture milk substitutes equivalent to bovine milk in their appearance, flavor, stability, and nutritional components. Most of these milks are unstable during manufacturing and storage; they tend to undergo phase separation and spoilage on long term storage. For these reasons, various methods have been employed to achieve stability in these non-dairy milks, for instance, by incorporation of different types of additives, such as gums, thickeners, emulsifiers, and by application of new technologies, like ultra-high-pressure homogenization, ultrasound, and pulsed electric fields. Therefore, while formulating non-dairy milk alternatives, it is necessary to endeavor towards utilizing the beneficial properties of plant materials and employing appropriate technologies for manufacturing non-dairy milks such that they are stable, display functional characteristics and sensory attributes similar to those of bovine milk. The aim of this chapter is to discuss the processing steps, mechanisms underlying the physical instability and to explore possible solutions with regard to use of different additives and advanced technological interventions in manufacture of non-dairy milks.

2. Need for stability of non-dairy milk alternatives

Bovine milk is nature's most complete food [10] with different components present in heterogeneous mixture like carbohydrates, whey proteins and minerals in solution; fat globules in emulsion while casein micelles and some minerals are distributed in colloidal phase, giving the bovine milk its typical composition and structure [11]. Being a rich source of nutrients, bovine milk is a perishable food item and is often subjected to heat treatments, like pasteurization to extend shelf life for a week on refrigeration; UHT for shelf life extension to several months at ambient temperature [12]. In general, different processing operations, like heat treatment and homogenization are greatly influenced by the structural design of bovine milk components conferring it suitability for use in different food systems [13]. However, in case of the plant-based milk alternatives, sales trend suggest that the customers are hesitant to buy them because they display undesirable behavior when served hot or on blending with the hot drinks.

Therefore, the beneficial qualities of the bovine milk must be closely reproduced by plant based milks, if they are to be perceived better than or equal to them. During the formulation of plant-based milk substitutes, it is essential to adopt effective technologies and suitable ingredients to achieve the stability to overcome the problems of unacceptable flavor and phase separation on storage, commonly associated with the beverages. Different novel technologies that have been applied for achieving stabilization involve reduction in particle size, decrease in viscosity, and decrease in microbial count [14–19].

It has been demonstrated that size of dispersed phase particles in plant-based milks is one of the important factors governing their stability [15, 20, 21]. Plant-based milks are colloidal dispersions consisting of wide range of components such as fat globules, ground raw material, proteins and carbohydrates etc. They often contribute to unstable product on storage as they tend to exhibit phenomena like creaming, sedimentation and phase separation. Besides, non-dairy milks are often associated with sandy, gritty or chalky mouthfeel and tend to develop off flavors during storage [22, 23]. Also, during formulation of non-dairy milk substitutes, bovine milk fat globule is an ideal candidate that needs to be simulated due to its significant contribution to the creaminess, texture and flavor of dairy products. To develop non-dairy milk alternatives, fat phase is incorporated either through addition of oil bodies [24] or fabrication of fat globules from plant sources [25].

It is essential to take different aspects in account, such as kind of raw material, shelf stability, processing operations and various electrostatic interactions underlying phase destabilization (creaming, flocculation, sedimentation, coalescence) while manufacturing non-dairy milks. With regard to stability of non-dairy milk alternatives, one fundamental attribute that is relevant to most of the products is their colloidal nature since other features like composition, and structure often vary markedly among different brands. Therefore, different characteristics that need to be monitored accurately in non-dairy milks, include properties of colloidal particles such as their size, charge density, surface charge, and surface properties, the nature of the continuous aqueous phase (the pH, ionic concentration, components, density, and viscosity), and the extent of exposure to external environment during its shelf life (storage temperature and time). Plant based milks not only undergo objectionable changes in physicochemical properties but also show signs of microbial spoilage on long term storage. Some of the necessary ingredients, processing techniques, and phenomena governing the physical stability of plant-based milks during manufacture and storage have been discussed below.

3. Processing steps for manufacturing non-dairy milk alternatives

During the manufacture of non-dairy milk alternatives, they are often subjected to various preprocessing treatments like dehulling, soaking, sprouting, blanching etc., to assist in subsequent processing. In general, the processing of milk from plants involves two main methods, namely, wet and dry. Otherwise, product is formulated by reconstitution using protein isolates or concentrates, water and other ingredients like oils, sugars, salts and stabilizers [26]. In the wet process, plant based raw material is soaked and ground with the water into a slurry, while in the dry method, the plant based material is ground into flour and then extracted with water. Such material is then subjected to filtration to remove insoluble or coarse particles to obtain aqueous phase. Afterwards, the processing steps followed include the addition of ingredients like oil, sugar, salts, colors, flavors, and stabilizers; homogenization and thermal processing treatments to yield non-dairy milk alternatives with desirable attributes.

3.1 Preliminary processing treatments

3.1.1 Dehulling

Dehulling operation involves the mechanical breaking of thick and hard seed coats of plant based raw materials before soaking to facilitate hydration. The strength of binding of the hull to endosperm governs the time required for dehulling procedure. Since the hull has a hydrophobic nature due to its association with hemicellulose and pentosans, it tends to lower down the hydration capacity of plant material. The polysaccharides present in the hull often lead to off-flavor, and foaming during processing, hence, their removal improves the processing operation and organoleptic properties of product. Also, microorganisms and enzymic activity associated with hulls reduces on dehulling. The traditional method includes initially exposing the raw material to the sun for drying and then dehulling with mortar and pestle. Alternatively, they are dehulled using the mills, and may also be milled using splitting machine, which employs both splitting and dehulling simultaneously. The milk prepared from dehulled raw material allows for production of a shelf stable and appealing final product.

3.1.2 Roasting

Roasting is a thermal process encompassing dehydration of raw material [27] for its improved flavor, aroma, and milling properties. Decrease in protein, starch content and improved extraction yield of roasted pulses and grains have been reported by many authors [28, 29]. Studies have shown that roasting leads to improved protein digestibility, and reduction in antinutritional compounds found in raw pulses and nuts. The decrease in protein content has been ascribed to the partial loss of amino acids, as well as of some nitrogenous compounds, and the reduction in starch to the solubilization of starch during the thermal process. Also, roasting has been shown to increase the water absorption capacity and water absorption index. An increase in WAC and WAI is related to the denaturation of proteins and starch gelatinization, which contribute to enhanced water imbibition [30]. Therefore, the flours with higher WAC are likely to result in the more viscous non-dairy alternative compared to untreated ones. Thermal processing during roasting results in partial disruption of the raw material [31, 32], thereby facilitating efficient particle size reduction required for stable suspension of non-dairy milk alternatives. For manufacturing milk from nuts and seeds, which contain high levels of unsaturated fatty acids, roasting should be carried out in controlled conditions of time and temperature to improve their nutritional properties and for prevention of off flavor development due to oxidation of unsaturated fatty acids. Inactivation of lipoxygenase during the process improves the flavor of non-dairy alternatives like soy milk, peanut milk, melon milk, sesame milk [33–37]. Roasted plant material becomes drier and brittle, and the non-dairy alternatives obtained from them are likely less-creamy [36]. In the study for manufacture of sesame milk, it was studied that the roasting process decreased acidity, total solids content and improved sensory profile by decreasing bitterness and a chalky taste associated with the milk [37]. The product obtained upon roasting has improved nutritional and sensory properties.

3.1.3 Sprouting

Sprouting refers to the soaking of seeds in water for specified time (1–14 hours) depending on the kind (variety, size, shape) of food grains in order to hydrate them for breaking their dormancy. The soaked grains are subsequently drained and rinsed at regular intervals to enable sprouting. Sprouting results in the initiation of series of metabolic changes in seeds (legumes, cereals, nuts & oilseeds) that improves the nutritional quality by inactivating the anti-nutritional factors such as trypsin inhibitor and phytic acid [38]. The improvement in nutritional value occurs due to enhanced activities of hydrolytic enzymes, which cause the conversion of stored chemical compounds, such as protein, starch and lipids into simple compounds; thereby, increasing the levels of total proteins, fat, certain essential amino acids, total sugars, B-group vitamins and decreasing the levels of starch. Therefore, the sprouting of raw material assists in the development of non-dairy milk alternatives, which are generally prepared using the heat treatment to decrease anti nutrient factors. Because sprouting is a natural biochemical process involving enzymatic activity, the treatment yields the improved quality of final product in terms of the nutrient and sensory value. Such a treatment decreases the intensity of heat treatment required for the manufacture of the product. Sprouting ensues improved protein solubility and reduced fat content for raw materials, which decreases the viscosity of non-dairy milk alternatives [38, 39]. Also, improvement in sensory properties takes place due to absence of beany flavor.

3.1.4 Blanching

Blanching with hot water is employed to inactivate enzymes like lipoxygenase and trypsin inhibitors for improvement of the flavor and nutritional value of the non-dairy milk alternatives [40]. Such a treatment has been reported to be effective in diminishing the beany, grassy, bitter, and rancid flavor; it also prevents suspension instability and chalkiness in non-dairy milks prepared from peanuts, soybean, almonds etc. [41–45]. Blanching with hot water (85–100 °C for 2–5 min) is commonly used for skin removal of raw materials and overcoming off flavors in non-dairy milk alternatives. Like roasting, blanching inactivates enzymes, reduces possible microbial contamination, and aids in deskinning in processing by wet or dry methods [46]. Pressure blanching (at 121 °C, 15 psi for 3 min) has been found to be effective for developing peanut milk with desirable sensory and physicochemical properties [47]. The treatment yields the milk with pleasing sensory attributes because blanching treatment for suitable time decreases the total solids and nutty flavor associated with peanut milk. The treated milk has improved consistency as well as decreased soaking time.

3.2 Wet processing

3.2.1 Soaking

The process for manufacturing of non-dairy milk alternatives involves the soaking of raw material in the proper volume of water contained in large stainless steel containers. Soaking is done to hydrate the raw material (cereals, legumes, nuts, or seeds) for grinding and further processing. Time required for soaking depends on the nature of raw material and temperature of the soaking water. At an ambient temperature, soaking requires longer time, and souring may take place due to bacterial growth, whereas if the temperature is raised up to 50–80 °C, soaking time is decreased, and hydration is accelerated. It has been demonstrated that during the soaking of lentils at different temperatures (20, 50, and 80 °C), rate of hydration at 50 and 80 °C was four to six fold higher than at 20 °C [48]. Softening due to soaking at higher temperatures could be related to the heat-induced modification in biomolecules, including starch, pectin, and protein, and the moisture for making the biomolecules susceptible to the changes. Different processes for preparation of non-dairy milks like peanut, soy, almond milk include soaking the raw material for 12 to 18 h before grinding it either in the mixer grinder or in colloidal mill [45]. Soaking facilitates the inactivation of enzyme inhibitors, improves digestibility and bioavailability of nutrients [49]. In case of pulses and grains, soaking step reduces the polyphenols and eliminates the alkaloids (e.g., in lupin) present in some of them; decreases the cooking time; improves the protein bioavailability and assists in peeling or dehulling [50, 51]. Soaking in acidic or basic solution is done to facilitate peeling of walnuts, almonds, tiger nuts, Brazilian nuts etc. Studies have shown that basic solution (1–2% NaOH) is suitable for peeling of walnuts and Brazil nuts [52–53] while citric acid is effective for peeling tiger nuts [54].

3.2.2 Wet milling or grinding

The procedure involves the grinding or milling of the plant material with the use of water for the split opening of the exterior hull. Wet grinding consists of grinding of fresh raw materials with the water to result in a suspension. The wet grinding method tends to produce finer particle size of the ground material [55] that results in more stability of non-dairy milk alternatives, and therefore, is more commonly used for their manufacture. In general, a colloid mill is used for reducing the particle size of raw material in suspension. Initially, the coarse grinding of raw material is done, which is followed by fine grinding. During the wet milling with the colloid mill, the rotor generates a substantial amount of stress by the rotation of the rotary stirrer, which can effectively accomplish the creation of submicron particles. In addition to disintegration, the colloid milling performs broad spectrum of functions like mixing, blending, and homogenizing effects [56]. In the manufacturing of the non-dairy milk alternative, this technique is mostly used for homogenization and emulsification [57]. The optimization of colloidal milling process parameters improves the physical stability of non-dairy milk alternatives by efficiently reducing the size of dispersed particles [58]. Different studies have shown that the amount of water added for wet milling depends on the kind of raw material, for instance, almond milk (1:9; almond& water), Sesame (1:5; sesame & water), Peanut (1:9; Peanut: water), soybean (1:5; soy: water) [15, 36, 37, 59]. Wet milling contributes the formulation of stable product where different factors like rotor speed, temperature, ratio of raw material and water can be fine-tuned to achieve any kind of non-dairy milk alternative.

3.3 Dry processing

The dry process comprises drying the raw materials and milling them into flours. For improving the efficiency of dry grinding, the raw material should be dried to minimum water content. The flour may be subsequently treated to yield different fractions: the protein, the starch and fiber. The protein concentrate or isolate, afterwards, is often utilized in formulation of non-dairy beverage. Therefore, dry processing mostly leads to development of product with higher protein contents.

3.3.1 Dry milling or grinding

The dry milling is mostly employed to reduce the particle size of the dried raw materials into their respective powder forms. The ground material is then mixed with water to form paste. However, during the manufacturing of the non-dairy alternative from pastes, solids tend to settle out down in the container, thereby resulting in the incomplete transfer of the content to the homogenizer and its wastage as well. For ensuring the efficient fuctioning of the dry grinding process, the important factors to be considered are the particle stiffness and feed size. Although the dry grinding decreases water wastage and energy consumption, yields a product with higher quality of protein, carbohydrates, fat, and minerals, it is less popular due to handling problems, like dust and wastage of raw material.

3.4 Extraction

For manufacturing the non-dairy milk alternative, the raw material, once it has been subjected to preliminary processing treatments, is extracted with water. The extraction efficiency can be improved by variation of pH or enzymatic treatment.

3.4.1 Extraction by variation of pH

The pH during extraction dictates the efficiency of protein extraction and stability of emulsion in non-dairy milk. Globulins comprise a major fraction of plant proteins, while albumins represent a minor fraction [60–62]. The pI for globulins is near pH 4.5, whereas the pI of albumins is around pH 6. The pI for different plant proteins lies between these values. Different studies have also demonstrated that plant proteins like pea, lentil, chickpea, soy etc. have a low net charge around pH 5 [63]. The plant proteins are mostly stable to pH changes at all pH values except at pH 5, which is around the pI where the droplets carry no charge and tend to display phase destabilization phenomena like aggregation and flocculation. During extraction, proteins should a have a high net charge at pH values well above or below their isoelectric point, which solubilizes them to increase the yield. Extraction in alkaline pH exhibits improved protein extraction yield, which may then be followed by neutralization step. For achieving the higher yield of the process, the efficiency of this step may be improved by alkalization of the medium using bicarbonate or NaOH.

3.4.2 Extraction using enzymatic treatment

Enzymatic treatment for hydrolysis of proteins and polysaccharides is mostly employed to improve the extraction yields. Disruption of plant cell wall components like cellulose, hemicelluloses, and pectin is facilitated by enzymes to improve the yield. The efficiency of protein and oil extraction is closely related with cell wall disruption of plant based material [64]. Studies have shown that cell wall degrading enzymes with pectinolytic activity like polygalacturonase, pectate lyaese, or pectin methyl esterase enhance the extractability of protein, fat, and antioxidant activity [65–67]. Also, upon application of cell wall degrading enzymes (cellulase, hemicellulase, pectinase) after homogenization step helps reduce the particle size, thereby facilitating suspension stability [68]. Because of the reduced particle size of suspended material, the enzyme treated non-dairy milks exhibit improved physical stability and flavor. Rosenthal et al. [68] reported that enzymatic treatment (1.2% of Celluclast) decreased the tendency of soymilk to undergo sedimentation on storage and improved sensorial attributes in terms of improved viscosity and lack of chalkiness. Proteolytic enzymes tend to improve the extraction yield and suspension stability [69]. A high solubility is required for the proteins because it governs their functional properties, for instance, emulsification, which subsequently affects the colloidal stability of the emulsion. The extraction of protein also increases due to improved solubility of proteins. Other enzymatic treatments involving the use of carbohydrate degrading enzymes like amyloglucosidase, amylases etc. have been demonstrated to improve the carbohydrate recovery and stability of non-dairy milk alternatives. Depending upon the plant based material containing appreciable amount of starch, for instance, in case of cereals & pseudocereals, liquefaction with α-and β-amylases is done for starch hydrolysis [70–72]. Upon heating, starch gelatinizes to set as thick gel during heating, and hence enzymatic treatment is required to maintain the non-dairy milk in the liquid state. The liquefaction treatment increases the yield due to hydrolysis of starch into maltodextrin, thereby improving the viscosity for the non-dairy milk alternative. Since it facilitates the filtration, the enzyme treatment is often employed during or before filtration; however, it might also be used after filtration, subject to the conditions. Studies have shown that starch liquefaction using amylases generally improves the viscosity and overall acceptability in non-dairy milk alternatives like oat milk, quinoa milk, rice milk, [72–74].

3.5 Filtration

Following the extraction step, removal of okara (the water-insoluble portion) from the slurry is done to obtain aqueous portion for manufacturing non-dairy milk alternative. The separation step is achieved by employing either batch process using filter cloth or continuous process like centrifugation [75, 76]. In general, two stage centrifugation is carried out to improve the efficiency of separation. In two stage clarification, separation of okara is carried out in first stage while fine particles are separated in second stage. Efficient filtration enables the retention of fine particles in the aqueous phase which assists in achieving the suspension stability. Different studies have shown that filtration treatment through a decanter or continuous filtration system (20–80 μm) during the manufacturing process of non-dairy milks improves the physical stability of milk because of removal of suspended particles [68, 77]. These days membrane separation is becoming popular as it allows for efficient separation of aqueous portion from okara. In case of manufacturing milk from fat rich raw material, the surplus fat is separated using a separator as is done in dairy processing with cream separator.

3.6 Addition of ingredients

Once aqueous phase or base material is obtained upon extraction and filtration, other ingredients are blended in the aqueous phase in optimum levels for successful manufacturing of non-dairy milk alternatives. These ingredients include fat, vitamins, sugar, flavorings, salt, oils and stabilizers etc. Since physical poses a challenge for the successful development of any non-dairy milk alternative, different range of additives (emulsifiers and stabilizers) have been explored for their use in the milks. Various emulsifying agents such as alginates, gelatin, xanthan gum, gum Arabic, locust bean gum, and gellan gum in a range of 0.5 to 1% by weight demonstrate improved emulsion stability. The destabilization due to settling of solid particles in the emulsion may be overcome by addition of alkalizing agents, such as disodium phosphate or sodium bicarbonate. Maghsoudlou et al. [16] achieved stability of almond milk by using lecithin, modified starch and agar at 0.09%, 1.31% and 0.15% levels respectively. Nor (2012) suggested that addition of lecithin (0.03% w/w) at the time of the milling during manufacture of almond milk was beneficial for its stability. Hinds et al. [78] reported good results with the use of 0.02–0.04% carrageenan as stabilizer in peanut milk. Bernat et al. [20] established that addition of 0.05 g/100 mL xanthan gum before the heat processing was suitable for developing hazelnut milk substitute as it causes thickening of the hazelnut milk substitute and enhances the colloidal stability of the final product. Processing operations should be performed carefully, since non-dairy milk alternatives are fortified with minerals and vitamins which may compromise the stability of emulsion. This is because vitamins are known to exhibit instability in relation to environmental conditions like high temperature, light and exposure to oxygen. In addition, mineral fortification might result in destabilization of emulsion; therefore, their fortification is accompanied with the addition of chelators like citric and EDTA. Based on the dispersibility and solubility of mineral sources, the salts that are commonly used for the mineral fortification include ferric gluconate, ferric ammonium citrate and ferric pyrophosphate as iron sources and calcium citrate, tricalcium phosphate and calcium carbonate as calcium sources [79, 80].

3.7 Homogenization

Homogenization is employed for size reduction of the dispersed phase components in the range of 0.5–30 μm by application of shear forces. The particles of the dispersed phase like protein, starch, fiber, and other cellular materials tend to sediment at the bottom when allowed to stand for some time; however, with the contribution of size reduction due to homogenization and addition of emulsifying agents or hydrocolloids, the stabilization of suspension is achieved during manufacturing of non-dairy milk alternatives. For carrying out homogenization, a pressure range of 20–60 MPa has been employed to improve the suspension stability during manufacture of non-dairy milk alternatives like rice, hemp, coconut milk [81–83]. The process assists in subdivision of fat globules to prevent phase separation and facilitates development of creamier and homogenized product.

3.8 Heat treatment

High temperature treatments like pasteurization, sterilization or UHT are employed to increase the shelf life of non-dairy milks by destruction of microorganisms. Several studies have reported application of sterilization treatments at 121 °C for 15–30 min in various non- dairy milks like almond, soy and peanut milks [20, 81, 84, 85]. Also, UHT treatment in range of 134–140 °C for 2 to 20 seconds has been applied in different non-dairy milks like peanut, coconut and almond milk [69, 86]. However, high temperature treatments have been reported to destabilize non-dairy milk alternative by resulting in coagulation of proteins. This is because proteins at high temperatures unfold to expose nonpolar amino acid residues, which participate in protein–protein interactions and consequently, exhibit aggregation, sedimentation, or gelling phenomena. Homogenization treatment after heat processing improves suspension stability by disruption of aggregates and reduction of particle size distribution [87]. The gelling and thickening of non-dairy milks due to presence of starch is addressed by enzymatic treatment for hydrolyzing the carbohydrate. Apart from enhancing physical stability, these heat treatments cause simultaneous destruction of pathogenic microbes in plant based milk alternatives resulting in increased storage stability of these beverages. Maria *et al.* [88] reported that the pasteurization treatment improved the quality characteristics of almond milk. Likewise, Khodke *et al*. [89] evaluated the effect of sterilization on shelf life of soymilk. It was observed that sterilized samples were acceptable up to 90 days at ambient storage while at refrigerated storage, the shelf life of milk samples increased up to 170 days. In another study, the effect of ultra-high temperature treatment on quality attributes of soymilk was investigated. It was concluded that single step UHT process (143 °C/60 s) can result into a commercially sterile soymilk with thiamin retention up to 93%, reduced trypsin inhibitor activity and improved acceptable sensory properties [90].

3.9 Aseptic packaging

Aseptic packaging of non-dairy milk alternative into sterile packaging material is done to increase the shelf life of the product.

4. Influence of ingredients on the stability of non-dairy milk alternatives

4.1 Important ingredients

4.1.1 Fat phase

In formulation of nondairy milks, fats are standardized in products either as oil bodies obtained from plants or are fabricated synthetically through homogenization. Oil bodies consist of a fatty acid core made up of triacylglycerol and a surrounding monolayer of phospholipids and unique proteins (oleosins), thus which imparts a structure composition similar to that of milk fat globule [91]. Extraction of oil bodies from plant seeds is generally achieved by employing physical processes, like soaking and crushing to enable their separation from adjacent tissues [9]. Oleosins play important role in stabilization of oil bodies by preventing their coalescence [92], preventing their hydrolysis by phospholipases [20] and by balancing of PUFA to MUFA ratio [93]. Even though plant based milks are similar to bovine milk, they may exhibit a distinct flavor, perceptible as nutty or beany, and may not be as desirable compared to flavor of milk [24] which is mild and unique owing to its typical aroma and taste profile [94].

Owing to differences between the dispersed and continuous phases in colloidal dispersions, there is a net movement of particles between two phases under the influence of gravitational force; creaming occurs if density of particles in dispersed phase is lower compared to dispersion medium whereas sedimentation is evident, if the case is otherwise. Both these phenomena tend to destabilize a colloidal dispersion. With respect to non-dairy milks, oil bodies tend to exhibit upward movement, while raw material fractions and being heavier, tend to settle down at bottom resulting in sedimentation, and is usually overcome by homogenization. Besides, simulated fat may also be stabilized by use of emulsifiers and homogenization, thereby imparting to non-dairy milk alternatives the characteristics similar to those of bovine milk in terms of consistency, appearance, flavor, and mouthfeel [95]. Fat phase in plant based milks is derived from different oil sources like coconut, palm, sesame, flaxseed, sunflower, olive, and soybean which contribute to different attributes like solid fat index, melting/crystallization pattern, viscosity, sensory and physicochemical properties. These features have important implications on processing of non-dairy milk alternatives such as presence of molten state of fat prior to homogenization and subsequently size of oil droplets created. However, presence of unsaturated fatty acids in lipid phase of these milks renders them more prone to lipid oxidation and rancidity. In case the ratio of unsaturated to saturated fatty acids is high, it contributes positively to human health. Numerous studies over the years have associated the consumption of plant-based oils with beneficial health effects, such as anticarcinogenic, anti-inflammatory, anti-dyslipidemia, antioxidant and in particular, improved cardiac health status has been attributed to intake of unsaturated fatty acids [96, 97].

Because of the density difference that exists between the dispersed phase and continuous phase, gravitational separation is a phenomenon commonly observed in non-dairy milk alternatives. In order to overcome phase separation, the density difference may be diminished either by incorporating in the milk alternatives the fat with the higher solid fat index or by adding some weighting agents, surfactants, and biopolymers that can hold onto the oil bodies by completely surrounding them. Creaming is controlled either by formation of tenacious films by proteins on oil droplets or by increase of viscosity of the medium, for instance by addition of thickening agents like hydrocolloids and polysaccharides to the dispersion medium. It is because when there is incomplete coverage of the oil body, partial coalescence may take place, and aggregation occurs in the fat bodies in such cases. In general, the difference between the density of the aqueous and fat phase may be adjusted by the use of weighting agent like brominated vegetable oil. However, brominated oil is not commonly used in food emulsions since it has been shown to negatively affect the fat metabolism in rats [98]. Addition of brominated vegetable oil to regular vegetable oil at 25 wt% level diminishes the density difference between oil phase and aqueous phase [99]. Therefore, in order to achieve stability in milk alternatives, it is essential that lipid bodies may be designed either using fats with proper solid proportion to increase the density of dispersed phase or using suitable biopolymers for ensuring efficient coverage.

Flocculation is a phenomenon that involves the weak association of oil droplets due to net attractive forces resulting in formation of flocks. The characteristics of the flocks vary with the extent of the net force of attraction between the droplets and the oil volume fraction. In the cases when the net attractive forces are not strong, weak flocculation occurs, while large aggregates formation takes place due to strong attractive forces in the non-dairy milk alternatives. Flocculation of oil droplets leading to instability of the milk substitutes is governed by non-covalent interactions which may be either attractive (van der Waals forces) or repulsive (electrostatic forces and steric forces) and can be manipulated by using appropriate surfactant or biopolymer. The additive should present the properties capable of generating stronger repulsive forces compared to attractive forces to overcome aggregation. Surfactants, cationic or anionic in nature, upon formation of films, generate electrostatic forces, which stabilize the oil droplets against aggregation due to net repulsive forces. However, proteins are quite effective in stabilization against aggregation owing to strong steric repulsive forces associated with them.

Adsorption of fat droplets by proteins, causes overlap of the outer portion, which entails an osmotic pressure gradient; thereby, generating the repulsive forces which prevent droplet aggregation. This leads to decrease in entropy and overall stabilization of non-dairy milk.

4.1.2 Emulsion stabilizer

Since non-dairy milk alternatives are typically oil in water emulsions present in complex multi-component systems entailing fats, proteins and polysaccharides, additives, water, sugars, flavors, other small molecular-weight compounds, and are inherently unstable exhibiting phenomena like aggregation, creaming, coalescence, sedimentation. Therefore, it is essential to select relevant emulsion stabilizer (surfactants, emulsifiers and hydrocolloids) for improvising the stability of milk substitutes.

4.1.3 Different types of emulsifiers

Emulsifiers are usually surface active molecules that act by adsorbing to the surfaces of the droplets of dispersed phase by creating a protective coating around them to prevent their aggregation. They may be categorized in different forms like, low molecular weight compounds: synthetic (monoglycerides, polyglycerol esters) or natural (phospholipids) and high molecular weight biopolymers (proteins and polysaccharides) [100–102]. As to the stability of emulsions imparted by emulsifiers, it is mainly related to formation of viscoelastic films around dispersed droplets. Several studies have suggested that the main cause of stabilization of emulsions is related to the capacity of emulsifiers to efficiently adsorb on dispersed droplets, size of the droplets, concentration of emulsifier, and generation of repulsive forces as well as considerable reduction of surface tension [95, 103, 104].

4.1.4 Low molecular weight surfactants

Food industry has always shown interest in use of suitable emulsifiers in different formulations as various features of food are influenced like stability, mouthfeel, color, flavor, appearance, texture and shelf life of food. Low molecular weight surfactants (phospholipids, monoacylglycerol) are more efficient than proteins in reducing the interfacial tension between two phases of an emulsion because of their property of quick diffusion and adsorption to interface [105–114]. Proteins on account of being bulky are slow to diffuse to interface and hence, exhibit lower surface activity [105]. This might be attributed to the complex structure of a protein consisting of both hydrophobic and hydrophilic groups present variably throughout its primary structure, and as separate patches in tertiary structures with no clearly defined head and tail region, which, are essentially distinct in case of small surfactants. Moreover, due to the absence of conformational constraints for rearrangement at the interface, low molecular weight surfactants, at sufficiently high concentrations, are more successful than proteins prevent adsorption to oil droplets. In the case of emulsions, when the protein to surfactant ratio is low, protein displacement into the continuous phase takes place due to the surfactant molecule, based on the orogenic mechanism [102]. The mechanism suggests that the protein molecules are unable to pack completely, and adsorb homogeneously on the interface because of steric hindrance, thereby creating a void space. The void spaces are primarily occupied by the surfactant domains, which enlarge gradually creating pressures, that compress the nearby protein film, and finally resulting in its desorption in the continuous phase [113].

4.1.5 Proteins

Proteins adsorb to oil droplets by undergoing partial denaturation to position themselves such that buried hydrophobic residues are exposed to the oil phase while hydrophilic residues align towards the aqueous phase [13]. On diffusing to the interface, proteins form tenacious viscoelastic films which are not apparent with the surfactants. The films are able to withstand mechanical stress and impart electrostatic as well as steric stabilization corresponding to type of protein and solvent conditions [115]. In these emulsions, stability may also be attributed to presence of "loops and trains" in protein chain conformation [116–118].

Among the natural class of emulsifiers, proteins represent very interesting emulsifiers due to their film forming ability and amphipathic nature [119]. Generally, animal proteins have been popular in food industry due to their excellent emulsifying abilities. These include bovine milk and egg proteins such as casein, whey protein isolate, bovine serum albumin, ovalbumin, ovotransferrin [120–128]. However, during recent years, plant proteins have experienced increasing popularity among manufacturers because of their association with several beneficial properties such as stability, sustainability non-allergenicity, non-toxicity, low-cost, biodegradability, functional properties, and consumer acceptance due to the clean label status ascribed to them [129, 130]. Different plant proteins that have gained acceptance as emulsifier in various emulsion based food systems include soy proteins, chickpea, lentil, cowpea, pea proteins wheat gluten, rice glutelin and flaxseed protein [131–134]. In order to stabilize emulsions successfully, it is necessary that emulsifiers should not only prevent droplet aggregation but also be stable to exterior stresses like temperature, pH, salt concentrations, sugars, etc. Biopolymers such as proteins and polysaccharides vary in stability with respect to external conditions. Plant proteins (pea, legume, faba bean) lack stability at pH close to their pI, high temperature and high salt concentrations [135], whereas polysaccharides exhibit stability under similar conditions [136, 137].

Plant proteins are generally globular, like soy, pea, chickpea and cereal protein which undergo entropy changes on adsorption at interface through structural rearrangement in secondary and tertiary changes [138]. Likewise, in bovine milk, the whey proteins are globular in nature. β-lactoglobulin usually has much the unordered structure and α-lactalbumin helical structure. In contrast, the complex globular proteins from plant sources have ordered structure. For instance, the legume proteins such as glycinin and legumin have well-ordered and greatly conserved structure due to their rigid quaternary conformation. The quaternary structure undergoes conformational deformation at tertiary and secondary configuration on getting adsorbed at the interface. Proteins that have inherently disordered structures show better surface activity compared with ordered proteins. Unstructured proteins like casein, which have open random coil structure, exhibits conformational rearrangement as an emulsifier causing fast changes compared to globular proteins. Studies have shown that the competitive adsorption of proteins takes place at the oil–water interface in non-dairy milks, and among the mixture of proteins, some proteins adsorb more effectively compared to others based on their structure and the partitioning of hydrophobic and hydrophilic residues. Moreover, as plant proteins are globular, the exposed hydrophobic groups tend to adsorb to nonpolar groups of oil droplets in non-dairy alternatives, ensuing strong and long-range hydrophobic attractive forces, which overcome the repulsive forces, so that the net effect is particle aggregation. Therefore, the important aspect for control, in the viewpoint of the manufacturers to ensure the stability, is hydrophobicity, which is the inherent characteristic of the globular proteins, and besides, it becomes more pronounced due to thermal or surface denaturation. In order to prevent hydrophobic flocculation, it is necessary to select suitable proteins, which are less hydrophobic, and to avoid the processing procedures that encourage protein denaturation.

Therefore, to achieve stability in plant based milks, certain protein modification strategies may be applied. As discussed above, globular proteins are susceptible to denaturation, their surface activity and solubility may be altered during processing of non-dairy alternatives [139]. Physical, chemical and enzymatic modifications can be used to enhance the functional properties of proteins. In physical modification, proteins are subjected to controlled heating and shear conditions that lead to unfolding or partial denaturation of these macromolecules [140, 141]. Chemical modification involves acylation, sulfitolysis, phosphorylation and alkylation, which alters the secondary, tertiary and quaternary structure of proteins alongwith their hydrophilicity-hydrophobicity balance [142–144]. Enzymatic modification is an effective approach to enhance the functionality of proteins by means of hydrolysis and polymerization reactions catalyzed by proteases (pepsin, chymotrypsin & trypsin) and transglutaminases. The controlled hydrolysis generates smaller oil droplets than intact proteins and also increases the emulsifying activity index [145, 146].

4.1.6 Hydrocolloids

For achieving stability in the non-dairy milk alternatives, the addition of hydrocolloids like guar gum, locust bean gum, Gum Arabic, carrageenan, xanthan gum, and so on, is often carried out to prevent creaming and phase separation [147–149]. The charge on polysaccharides impacts their ability to inhibit the aggregation of oil bodies or fat droplets as well as of proteins by the formation of a protective coating around them. For instance, carrageenan, an anionic hydrocolloid, adsorbs to cationic regions on surfaces of aggregating proteins and hence, prevents aggregation near their isoelectric point by creating strong electrostatic or steric repulsive forces [150]. However, studies suggest that while hydrocolloids are capable of promoting stability at high concentrations, they tend to create instability in emulsions at low concentrations. Different mechanisms have been hypothesized to elucidate this phenomenon. When two droplets covered with a surfactant are in close vicinity, a link between the droplets develops which creates a connection between droplets [151, 152]. Development of numerous contacts of this type tend to encourage flocculation and increase the creaming rate. This is generally identified as "bridging flocculation," and it is more common when the hydrocolloid is a weak emulsifier [153]. Therefore, the success of emulsion stabilization depends on the choice of proper biopolymers that lack the attraction to the dispersed phase droplets.

Other mechanism, proposed as "depletion flocculation," was initially suggested by Asakura and Oosawa [154, 155] and was supported by many scientists later on [156–158]. According to mechanism, upon addition of any nonadsorbing hydrocolloid to a reasonably concentrated emulsion, elimination of the hydrocolloid might occur in the area between droplets, because of its hydrodynamic size, and thereby leads to development of local osmotic pressure gradient. The osmotic force results in the aggregation of oil droplets. The extent of the attractive force is related to the molecular weight and conformation of the hydrocolloid and varies proportionally with the concentration of the nonadsorbing hydrocolloid. Such kind of instability may be prevented by mixing the polysaccharide in less quantity so that aggregation does not occur.

4.2 Advanced processing techniques in relation to stability of non-dairy milk alternatives

4.2.1 Ultra high pressure homogenization (UHPH)

Ultra high pressure homogenization is an emerging technology which can be utilized to enhance the stability of plant based milk alternatives by reducing the colloidal particles. UHPH produces more uniform sized particles and improves the physicochemical characteristics of food products without affecting their nutritional properties [159]. Apart from reducing the particle size, this technique can also be applied to improve the shelf life of plant based milk alternatives by means of simultaneous destruction of microorganism [160]. UHPH involves the use of high pressure in the range of 200–600 MPa and temperatures between 30 and 85 °C [161]. The use of UHPH also displays an important role in reduction of allergenic character of plant-based milk alternatives. Briviba *et al.* [21] investigated the effect of UHPH treatment (350 MPa at 80 °C) on physicochemical properties of almond milk. No significant reduction in vitamin B_1 and B_2 contents was reported while the mean particle size increased threefold. A reduction (99.8%) in almond protein antigens response was also observed. The effect of UHPH treatment (300 MPa at 80 °C) and UHT treatment (142 °C, 6 s) on microbiological, physical and sensorial properties of soymilk was evaluated [162]. The study showed a reduction in hydroperoxide index and microbial growth throughout the storage period for both treatments. Slight differences in sensory characteristics were observed between treatments; however, panelists did not consider these differences to compare treatments.

4.2.2 Pulsed electric field (PFF)

Pulsed electric field is another promising technology that involves the use of short electricity pulses to inactivate microorganisms in food products while causing minimal changes in color, flavor, taste and nutritional components [163]. In this technology, food is placed between two electrodes and electric fields (5–50 KV/Cm) are generated with the help of short high voltage pulses (microseconds) between the electrodes. The voltage range can be used for development of non-dairy milk alternatives according to the requirements of size reduction. The experiment carried out by Xiang [164] investigated the effect of pulsed electric field treatments with different electric field intensities and number of pulses on structural modification and rheological properties of soymilk. Pulse electric field treatments at electric field intensities (18, 20 and 22 kV/cm) and number of pulses (25, 59, 75 and 100) increased the apparent viscosity of soymilk (6.62 to 7.46) as compared to control (not treated). The changes were attributed to the PEF induced coagulation of the soy protein and reduction in size of fat globules and their distribution in soy milk. Similarly, Cortes *et al*. [165] explored the impact of treatment time (100–475 μs) and electric field intensity (20–35 kV/cm) on the quality attributes of horchata (a Spanish vegetable beverage) during 5 days at refrigerated storage (2–4 °C). The study revealed that PEF treatment significantly decreased the peroxidase activity and a negative correlation was found among peroxidase activity and pH. The increase in pH was proportional to increasing treatment time in the same electric field intensity.

4.2.3 Ultrasound processing

Ultrasound processing is an effective non-thermal technology applied for processing and preservation of foods. Ultrasound processing is based on the phenomenon of acoustic cavitation i.e. rapid expansion and contraction of bubbles of gas/vapors. This generates intense local heating and high pressures that causes disintegration of microbial cells and reduces the size of colloidal particles as well. In the study conducted by Iswarin and Permadi [166], the effect of ultrasound on droplet diameter of coconut milk was evaluated. The beverage was subjected to different combinations of power levels (2.5 to 7.0 W) and exposure times (5 to 25 minutes) and a reduction in particle size of coconut-based milk was observed as the US power and time increased. Similarly, Maghsoudlou et al. [19] studied the effect of ultrasonication treatment on physical stability of almond milk when applied at a power level of 300 W for the time periods of 0, 2.5 and 5 min. It was revealed that exposure time for 5 minutes was sufficient to manufacture a desirable product. The study demonstrated a decrease in sedimentation tendency of milk as well as decreased viscosity of almond milk. The improved stability has been attributed to cavitation induced fragmentation of colloidal polysaccharide molecules into smaller size particles. Size reduction of plant cellular material keeps them in suspension and hence, aids in improved stability.

5. Future prospect and conclusion

Being a fast-growing segment of food market, the plant-based milk substitutes need to be extensively explored by using advanced processing and innovative technologies to produce a nutritionally complete beverage with high overall acceptability. Plant-based milk substitutes lack cholesterol, milk allergens, lactose, antibiotics, and saturated fatty acids that make them convenient to be considered nutritious, economical, health promoting, palatable dairy-free beverage. To meet consumer's needs, it is essential to produce high quality beverages having good physical stability and desirable sensory attributes. Addition of stabilizers and processing are crucial steps in determining the stability and shelf life of plant-based milk alternatives. Manufacturers and consumers are more interested in clean label options for use as additives. Since synthetic stabilizers are generally added for improving the stability of milk substitutes, the natural substitutes could present a plausible solution to consumers. Some advanced food processing techniques including ultra-high pressure homogenization, pulsed electric field processing, ultrasound processing and high pressure processing can be employed to overcome instability factors responsible for limiting success of these beverages. Progressive efforts are required for improving product quality through research and development activities.

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