Chapter

Technological and Biological Properties of Buttermilk: A Minireview

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Abstract

Buttermilk is a by-product obtained from the batting insertion in the process of obtaining cream and butter and it is constituted by fat globules which are surrounded by milk fat globule membranes (MFGMs). During the stirring process, the membrane is ruptured and the various components present therein are released. Because it has a high nutritional content and low cost, buttermilk has drawn attention in the prospect of new forms of application. In addition, its disposal is expensive and not biologically viable. The objective of this work is to present a compilation of the technological and biological activities of buttermilk. Among the technological properties, it is worth mentioning its application as in the production of functional foods, a conduit for the incorporation of probiotics, inhibition of bacterial adherence on industrial surfaces, as well as the encapsulation of easily degraded activities and fermentative processes. Among the biological properties, its antioxidant, hypocholesterolemic, antimicrobial, and anticancer activities stand out. In conclusion, the reuse of buttermilk is economically and sustainably viable and encourages increasing research related to its use.

Keywords: buttermilk, technological properties, biological properties, butter, milk fat globule membranes

1. Introduction

From the batting inserted in the manufacturing process of the cream of fresh milk and butter, it is possible to obtain a by-product derived from the aqueous phase, called buttermilk [1, 2]. Buttermilk is made up of fat globules which are surrounded by membranes called milk fat globule membranes (MFGMs). Such membranes avoid its coalescence and its enzymatic degradation [1, 3]. During agitation, the fat globules are disrupted releasing various components dispersed into the aqueous medium such as polar lipids and fragments of MFGMs, which are possessing high functional potential due to their nutritional and technological characteristics [1, 2]. Exact quantity of production of buttermilk is not assessed; however, the quantity of production of buttermilk can be predicted on the basis of production of butter. Approximately 6.5–7.0% of total milk produced worldwide is used for the preparation of butter that yields high bulks of buttermilk as a by-product (around 3.2 million tons/annum) [4].

MFGM is rich in polar and neutral lipids, phospho- and sphingolipids, as well as mono-, di-, and triglycerides, as well as cholesterol [1]. Due to their technological and biological properties, the search has been increased by methods that concentrate and isolate MFGM [1, 3].

The use of buttermilk is justified due to its high nutritional value and low cost of obtaining it [5]. Its disposal in wastewater is uneconomical because it results in high BOD value (biochemical oxygen demand) [6]. Moreover, the lack of opportunities by not using this by-product in other industrial segments forces the dairy producers to continually invest in new technology for an environmentally correct disposal for this waste such as bioremediation [7].

On this front, the dairy industry is increasingly interested in using its by-products in new applications. The application of buttermilk is facilitated by the application of techniques such as pasteurization, concentration, and spray drying. Considering these aspects, the objective of the work was to gather studies that present the technological and biological properties of buttermilk.

2. Extraction, concentration, and analysis of phospholipids from the MFGM of buttermilk

Dairy glycerophospholipids, phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol are the phospholipids commonly found in buttermilk. Of the class of sphingolipids, the main ones, found in buttermilk, are sphingomyelin, glucosylceramide, and lactosylceramide. The most commonly used phospholipid concentration processes are microfiltration (MF) and ultrafiltration (UF) [8]. The importance of extracting and concentrating these phospholipids is summarized by their bioactivity and their ability to act as emulsifiers.

MF has some limitations due to the size of casein fragments and MFGM phospholipids, which are very similar. Regarding the optimization of UF, studies performed pretreatments for the elimination of casein, predicting UF, precipitating it with acid, as well as the addition of agents that dissociate casein micelles such as citrate [8].

Isolation of MFGM has been hampered by the interaction of its components with other proteins during processing. The formation of the aggregates begins by ionic attraction between positively charged amino acid residues of the protein and the polar grouping of the lipids [9]. Researchers found that spraying, aiming at the drying of buttermilk, may induce the formation of MFGM protein and phospholipid complexes, making extraction of phospholipids difficult. It was further verified that in pasteurized buttermilk, there was an almost three times increase in the amount of MFGM-bound proteins compared to raw buttermilk. In addition, there was a high incorporation of β-lactoglobulin (β-LG) in MFGM isolated after heat treatment, and β-LG constitutes the major MFGM protein separated from pasteurized cream [10].

According to previous studies, the whey proteins of cheese may undergo thermal aggregation in the presence of concentrated buttermilk. In conclusion, its study showed that the phospholipids of the buttermilk membrane contributed to the formation of heat-induced aggregates with whey proteins [9]. Other analyzes have reported that most mechanisms of protein aggregation depend on nucleation and, as a consequence, the onset is done through the formation of an aggregation nucleus. These protein aggregates can be used as fat mimics in low-fat cheese or as yoghurt texture modifiers [11].

A study developed a gradient solvent system for high-performance liquid chromatography (HPLC) coupled to the ELSD detector (evaporative detector with light scattering) to separate the lipids present in buttermilk. The ELSD detector is sensitive only to the mass of the vaporized analyte, not being limited by solvent flow or ambient temperature, thus allowing better analysis time and adequate level of sensitivity. Among the standards used for analysis in HPLC were cholesterol, monostearin, diolein, phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, sphingomyelin, and other neutral lipids. In this study, separation with high reproducibility and accuracy of all classes of lipids present in buttermilk powder, including phospholipids, was observed, with no previous fractionation stage. The total concentration of polar lipids found in buttermilk was about 30%, the predominant phospholipid being phosphatidylcholine, followed by phosphatidine ethanolamine and phosphatidylserine, and with a lower concentration of sphingomyelin [12].

3. Technological properties of buttermilk

3.1 Biofilm formation

The incorporation, using different proportions of corn starch and buttermilk to obtain biopolymers, as well as the influence of temperature on the polymeric structure, is reported in the literature [13]. The study was justified by the application of the biopolymer in sustainable packaging, replacing polymers derived from petroleum, and reusing the by-product of the manufacture of butter. To obtain the biofilm, the author incorporated the phospholipids present in the milk derivative into polytetrafluoroethylene. The plates, containing the polymers, were dried during the minimum period of 24 hours in two different temperatures at 2% relative humidity. The thickness of the biofilms was monitored, which remained as a surface density of 55 g/m^2. The samples remained in desiccators under temperature control. The results indicated that in the polymers in which the buttermilk was incorporated, there was initially a separation of the phases, resulting from a certain incompatibility between buttermilk and starch, which significantly affected the modulus elasticity and tensile strength. The heating of the films, added with buttermilk, promoted a positive impact in relation to the resistance, which is justified by the gel formation that, during drying of the film, reduces the critical concentration for its formation. The permeability to water was not altered by the addition of up to 50% of buttermilk. Under heating, there was a decrease in the mass transport property due to the greater adhesion of the components of the polymer mesh. Finally, the rheological study classified the polymer as Newtonian, in addition to having antioxidant properties when subjected to heat treatment by the release of active peptides, and without antimicrobial activity under *Listeria innocua* [13]. In general, buttermilk, rich in proteins, has good film-forming ability by having plasticizers such as lactose.

3.2 Beverage production

A study investigated the use of buttermilk from fresh buffalo milk for the production of carbonated beverages flavored with fresh mango, orange, and pineapple fruit, varying the fruit juice concentration between 18, 20, 22, and 24% v/v of sugar between 8, 10, 12, and 14% m/v. The buttermilk used was 0.8% acidity, being first prefiltered to remove casein clots, and then filtered in millipore systems to obtain ultrafiltered buttermilk. Fresh fruit juices were also filtered to remove possible fibers. After the filtration processes, the buttermilk acquired low viscosity, since a large fraction of the proteins and lipids were removed from it. Among the analyzed flavors, the drink with 24% v/v of fruit juice flavored pineapple and 12% of sugar was the most accepted during sensory analysis, possibly due to the smaller amount of total solids in relation to the others. However, the higher the total solids content, the lower the solubilization of CO_2, which made the carbonation process difficult. The use of the fruit juice in the formulation helped to mask the astringent, sweet, and/or sour taste of buttermilk color, taste, aroma, and palate, as well as the overall appearance and acceptability of the product. The beverage produced had a higher concentration of proteins, vitamins, and minerals than market samples, as well as better physicochemical properties, and therefore had a better nutritional quality than the other samples analyzed [6].

3.3 Buttermilk as an additive in the composition of culture media for fermentative processes

Acidic bacteria (AB) are widely used in the production of fermented products. The use of buttermilk as a growth medium for probiotic lactobacilli has been reported. Probiotic foods are those that have probiotic bacteria in their formulation which, when given in sufficient quantities, promote health benefits for the user [5, 14]. The benefits are related to both the direct effect of probiotics and active metabolites produced during the fermentation process. These products should have between 106 and 107 CFU/mL of probiotics, being *Lactobacillus* and *Bifidobacterium* the most commonly used probiotics [14].

Although buttermilk presents a carbon source, there is a lack of nitrogen, which is necessary for the growth of AB. In this regard, an investigation used yeast extract as a source of nitrogen and obtained a good performance of the three strains tested in a medium containing buttermilk and yeast extract [15]. Another study, with a small concentration of yeast extract (0.3%), also perceived a good performance in the growth of *Lactobacillus* in buttermilk [16].

3.4 Application of buttermilk in the treatment of industrial surfaces

The procedures for controlling bacterial adhesion on industrial surfaces are of extreme importance, since a short time of contact with the surface is enough for the bacteria to begin biofilm formation. In this sense, buttermilk may aid in the inhibition of the formation of bacterial biofilms on industrial surfaces due to the high concentration of MFGM, which possesses polar lipids, which in turn affect the adhesion of bacteria on the industrial surface, preventing the formation of bacterial biofilms [17]. Studies indicated that buttermilk inhibited the adherence of microorganisms—*Lactococcus lactis, Leuconostoc cremoris*, *and Lactobacillus casei*—on stainless steel surfaces for 720 minutes, while other products, such as skim milk, were able to reduce bacterial adherence for about 30 minutes of exposure, which is considered a short time for the function [17].

3.5 Buttermilk encapsulation properties

A research examined the property of encapsulation of buttermilk in order to entrap omega-3 and thus be able to stabilize O/W emulsions. Omega-3 is a fatty acid with high demand due to its functional properties. A problem in their manufacture is their sensitivity to oxidation that hinders the delivery process to their place of absorption. Therefore, one of the strategies used to maintain its stability is encapsulation, causing its components to be confined within a matrix or within a small capsule, keeping them isolated from the external environment until, through an external stimulus, being released. In addition, buttermilk has emulsifying properties that aid in encapsulation. This emulsifying property is due to the presence of, for example, phospholipids and proteins, and other components such as liposomes that can be obtained from MFGM from buttermilk [18].

Buttermilk is still a good carrier of curcuminoid substances due to the presence of proteins and lipids in its composition, including phospholipids from MFGM. Curcuminoids are polyphenols from turmeric (*Curcuma longa*), which contain about 70–80% curcumin, 15–25% desmethoxycurcumin, and 3–10% bisdemetoxyc-curcumine, with anti-inflammatory, antioxidant, anti-HIV and as they are protectors against Alzheimer's disease, cystic fibrosis, and colon cancer [19]. A difficulty found in its clinical use is its low bioavailability, since it is poorly absorbed by the intestine because it has low aqueous solubility and low stability in pH near the neutral, present in the intestine, causing its hydrolysis to occur in smaller compounds [19, 20]. Curcumin has the ability to interact with MFGM lipids and proteins [19]. Some authors studied the influence of the use of buttermilk as a carrier for the curcuminoids. During storage of the curcuminoid with buttermilk and with buffer solution only, there was less degradation of the curcuminoids that were stored with buttermilk [19, 20]. This can be explained by the interaction of the same with the hydrophobic region of buttermilk that prevents its hydrolysis in aqueous environment. The encapsulation of actives has emerged as suitable vehicle for overcoming pharmacokinetic limitations associated with conventional drug formulations. Oftentimes, these features include incorporation of active targeting moieties for enhanced uptake in specific cells or constituent components for stimulus-responsive release (e.g., pH-sensitive, thermosensitive and ultrasound). Considering the contents discussed in topic (Buttermilk encapsulation properties).

Like curcuminoids, transresveratrol is a bioactive compound found mainly in strawberry, red grape, and wine, but it has limitations on its clinical application due to its low solubility and stability in aqueous environment. Among its benefits, antioxidant, cardioprotective, anticancer, and anti-inflammatory activities are mentioned. The solubility of resveratrol in aqueous phase is 13.6 μg/g in phosphate buffer (pH 7.4), considered low, and a higher solubility in oils, 179.8 ng/g. Therefore, the association of transresveratrol with lipids, such as those of MFGM, as well as proteins present in buttermilk, helps in increasing their bioavailability [21].

4. Biological properties

Among the biological properties found in buttermilk, some are as follows:

4.1 Antioxidant activity

Peptides released during the enzymatic or thermal hydrolysis of buttermilk proteins have known antioxidant properties. An investigation studied samples of biofilms, with antioxidant potential, produced from buttermilk. The results were expressed as trolox equivalent antioxidant capacity (TEAC), that is, the concentration of buttermilk in g/L that produces the same inhibition of a Trolox solution (analogous solution of vitamin E) at 1 mmol/L. When analyzing the antioxidant character of the biofilms, it was observed that the effect was only noticed in the samples that went through heating, which was justified by the release of the peptides during the heat treatment of the biofilms [13].

4.2 Hypocholesterolemic activity

The pathophysiology of coronary diseases is well established in the literature and is related, among other factors, to high cholesterol and low-density lipoprotein (LDL).

Considering the importance of the control of serum levels for greater clinical benefit, different studies have been looking for nonpharmacological measures in patients of low risk, among them the consumption of buttermilk [22, 23]. The hypocholesterolemic action of buttermilk was studied using different methodologies that demonstrated a reduction in total cholesterol and other variables with consumption of the dairy derivative in the short term [22–24]. The effect is explained by phospholipids present in buttermilk that may be responsible for the hypocholesterolemic effect. The effect occurs through an intervention in the pathway of cholesterol synthesis, as well as in the intestinal absorption of cholesterol, lowering their blood levels. A study conducted a double-blind, randomized, placebo-controlled study. Volunteers between 18 and 65 years and body mass index (BMI) of 35 kg/m^2 were submitted to two consecutive treatments of 4 weeks each, in random order. Participants should maintain their diet, medication, weight, alcohol consumption, and smoking habits normally, with the exception of the days before blood sampling for the exams. Vitamins and food supplementation products were banned from interfering with the research. The formulations tested and ingested by participants contained 22.5 g buttermilk or placebo, which should be mixed with 250 mL water and sucralose in a shaker provided to the participants. The placebo contained varied dairy ingredients; however, such ingredients do not have in their MFGM composition present in the milk fluid. The usual food intake was evaluated through a specific questionnaire for dairy foods in three moments: at the beginning, after the first treatment, and after the second treatment. After the blood draw procedure volunteers, tests of total cholesterol, triglycerides and serum concentrations of LDL and HDL, C-reactive protein, latosterol, β-sitosterol, campesterol and plasma levels of PCSK9, also called convertase 1, an enzyme that interacts directly with LDL receptors, decreasing its metabolism [25]. In conclusion, consumption of short-term buttermilk significantly reduced total serum cholesterol and triglyceride concentrations.

A manuscript evaluated 100 participants aged from 18 to 65 years regarding the lipid profile of individuals who ingested buttermilk. The study was randomized and placebo-controlled for a period of 12 weeks. The buttermilk drink was traditionally prepared with increments such as vanilla sugar in order to increase the acceptability of the dairy product. Participants were instructed not to change their usual diet, level of physical activity, and alcohol use. Total cholesterol, HDL, LDL, triglycerides, liver, and kidney function parameters, as well as cholesterol precursors, were quantified using HPLC. The author also noted a reduction in cholesterol and LDL levels [23].

4.3 Antimicrobial action

According to the World Health Organization (WHO), 30% of the population of industrialized countries in 2007 suffered from foodborne intestinal infections transmitted by food, creating, consequently, a public health problem. Due to the increased resistance of pathogenic organisms to antibiotics, new methods for gastrointestinal prevention and control are under investigation [26]. An investigation conducted an *in vivo* mouse study of the anti-infective effect of MFGM on *Salmonella enteritidis* and *Listeria monocytogenes,* a Gram-negative and a Gram-positive pathogen, respectively. The study demonstrated that ingestion of MFGM-rich buttermilk powder increased resistance to *L. monocytogenes* compared to skim milk with low amount of MFGM. This increase of resistance can be explained by the products of the phosphoglyceride and sphingolipid digestion, which showed to have antimicrobial activity *in vitro*. Another likely mechanism for resistance to *L. monocytogenes* colonization is that MFGM proteins promote inhibition of pathogen adhesion in the intestinal mucosa [26].

4.4 Anticancer action

Some authors studied the antiproliferative effect of several isolated fractions of buttermilk obtained from food and nonfood solvents on some types of human cancer cells. Fractions, rich in phospho- and sphingolipids, have strong antiproliferative action on colon and ovary cancer cells. These observations allow to hypothesize the use of these phospho- and sphingolipids as functional food additives [27].

5. Conclusion

A wide variety of applicability for buttermilk can be found in literature. Once obtained during the production process of the butter, it can be reused in different ways with technological or biological approaches. Its technological properties focus on the food and pharmaceutical industry as production of functional foods, a vehicle for the incorporation of probiotics, inhibition of bacteria adherence on industrial surfaces, as well as the encapsulation of easily degraded activities and fermentation processes. The biological properties focus on antioxidant, hypocholesterolemic, antimicrobial, and anticancer action. Thus, the wide variety of research presented, in different areas, stimulates new research related to this by-product.

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