Alternative Ways to Processing Whey

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Introduction

Yesteryears have witnessed significant potential of science in resurrecting whey. Not too long back, whey; the greenish colored liquid discharge from manufacturing of various mainstream dairy products; was a potential threat to the ecology and problem to the industrial dispense because being high on biological oxygen demand. But with science and technology intervening, the bane turned itself into boon with technofunctional and biofunctional abilities. Given the persistent increase in demand of the products; i.e. cheese in prime; resulting in rising voluminous generation of whey, more growth in utilization of it is not only expected but inevitable too. Whey processing and application today are yielding a wealth of quality products that are increasingly seen as ingredients in formulations that have recognized positive health benefits (Onwulata. 2008). Due to the presence of scarce and indispensable amino acids (lysine, tryptophane, methionine, threonine and cystein), whey staffs itself as an extremely important by-product of dairy produces (Kondratovych *et al*., 2013). Qualitative composition of amino acids present in whey have conferred various health benefits and are pouring in reports and studies in scientific community. Innovative processing techniques are ousting older methods, yielding products with better applicability. Bypassing of cheese-making step is possible for producing separate protein fractions from skim milk by use of correct pore sizes in microfilters.

High Hydrostatic Pressure Processing

According to Pascal's law, pressure acts instantly, isostatically and homogenously, independent to the size and shape of the material. High pressure processing of milk, on microflora, appeared in literature in 19th century (Hite, 1899). Thereafter, the medieval years did not witness much of high pressure processing of milk. After 1903, the reports of high pressure processing started appearing at frequent intervals and it was only in the latter half of 20th century when studies on this field started becoming frequent. The main reason of the long intermission in the field of high pressure investigation was the lack of appropriate equipment (Huszar, 2008). High-pressure processing has been shown to have marginal influence on the nutritional characteristics of milk, hydrolysis, or stability of vitamins. Several publications support β-Lg being more sensitive to pressure over α-La. Forfeiture in solubility at pH 4.6 explains denaturation of whey proteins. With HP method α-La was denatured at pressures higher than 400 MPa, and β-Lg at pressures higher than 100 MPa. Given that there are four intra-molecular disulphide bonds, the superior barostability of α-La is explained over β-Lg tat has only two. Also, β-Lg was found to denature more by HP than α-La in milk, but less so in whey. However, removal of colloidal calcium phosphate from milk negatively impacted HP-induced denaturation of α-La and β-Lg. Huppertz *et al.*, (2005) explained denaturation of β-Lg and α-La at pressures greater than 100 MPa, with increase in their association within the serum phase milk fat globule membrane. Reversible effects have been seen on β-Lg for pressures up to 300 MPa and non-occurence of Maillard browning for pressures up to 600 MPa. Digestibility remains unaffected due to denaturation of proteins resulting from HP processing (Messens *et al.*, 2003). Though, at higher pressures (400-800 MPa), occurrence of relatively little further denaturation has been given by Scollard (2000), the extent of HP-induced denaturation of α-La and β-Lg has been shown to increase with increase in holding time, temperature, and pH of milk by several researchers (Huszar, 2008). Unfolding of β-Lg due to HP processing exposes its free sulphydril group. Felipe *et al.*, (1997) suggested formation of small aggregates of denatured β-Lg during HP treatment of milk whereas workers like Needs *et al.*, (2000) and Scollard *et al.*, (2000) suggested interaction with casein micelles. Adding to the suggestion by Felipe *et al.*, (1997), Dumay *et al.*, (1994) and Van Camp *et al.*, (1997) suggested partial reversibility of aggregated β-Lg, on subsequent storage. In HP treating of whole milk may result in association of some α-La and β-Lg with the milk fat globule membrane (Ye *et al.*, 2004). Discussed next is the mechanism for high pressure induced denaturation of α-La and β-Lg in milk as well as in whey as given by Huppertz, 2006 and produced by (Huszar, 2008): β-Lg unfolds under high pressure, which results in the exposure of the free sulphydryl group in β-Lg. This free sulphydryl-group can interact with other milk proteins (κ-casein, α-La or β-Lg, and perhaps αs_2-

casein), through sulphydryl-disulphide interchange reactions. On release of pressure, unfolded α-La and β-Lg molecules, that have not interacted with another protein, may refold to a state closely related to that of native form of these proteins. The close structural similarity of monomeric untreated, and HHP treated β-Lg indicates that the sulphydryl-disulphide interchange reactions occur *during* HHP treatment, since the free sulphydryl-group of β-Lg is not available for interaction after high pressure treatment. Different pressure stability of isoforms A and B of β-Lg were suggested by Botelho *et al.*, (2000) with B form being more sensitive to pressure than A. The possible explanation was rested on the existence of of a core cavity in β-Lg B.

Ultrasound Processing

As given by Mason *et al.*, (2005), the major mechanical effects of ultrasound are provided when the power is sufficiently high to cause cavitation. Similar to any sound wave, ultrasound too propagates via a compression and rarefaction wave series induced in the molecules of the passage medium. When the power is sufficiently high, the attractive forces of the molecules of the liquid are exceeded by rarefaction cycle, thus forming cavitation bubbles. These bubbles continue to grow and this phenomenon; known as rectified diffusion, is explained by entering of small amounts of gas or vapor from the medium in the bubble during its expansion phase and incomplete expulsion during compression. The bubbles that are distributed throughout the liquid grow over the period of a few cycles to an equilibrium size for the particular frequency applied. If the bubbles were only subject to that particular frequency they would remain as oscillating bubbles, however, the acoustic field that influences an individual bubble among the many thousands generated in a cavitating fluid is not uniform. Each bubble will slightly affect the localized field experienced by neighbouring bubbles. Under such circumstances the irregular field will cause the cavitation bubble to become unstable and collapse. It is this collapse that generates the energy for chemical and mechanical effects. For example, in aqueous systems at an ultrasonic frequency of 20 kHz, each cavitation bubble collapse acts as a localized 'hotspot' generating temperatures of about 4000 K and pressures in excess of 1000 atmospheres. This bubble collapse, distributed through the medium, has a variety of effects within the system depending upon the type of material involved. Sonication, has been applied to milk products for varying applications. Ultrasound was used to break bacterial "clump" which was masking total bacterial count in milk. Huhtanen, (1966) suggested sonication at elevated temperatures improved the desired isolation of bacteria in raw milk. The treatment of milk with low frequency sonication increased the total bacterial counts, but the heat produced by ultrasonic treatment did not account entirely for its effect. In presence of heat, the synergistic effect of processing on protein increases the denaturation of whey proteins α-La and β-Lg; but caseins remain unaffected as the highest temperatures reported are below 76ºC. Electrical and sonic forces have been shown to modify the filtration performance of membrane filtration of whey. Reduction in membrane fouling and thus enhancement in flux, have been demonstrated by Muralidhara *et al.*, (1986), Tarleton *et al.*, (1992) and Tarleton (1988) by both electric and ultrasonic fields. They observed a synergistic effect when both the fields were applied simultaneously. Effective use of stand-alone ultrasound has been demonstrated by various other researchers to enhance the permeate flux (Kobayashi *et al.*, 2003; Lamminen *et al.*, 2004). Muthukumaran *et al.*, (2005a) showed restoration of initial permeate fluxes; during membrane processing; with the assistance of ultrasound. They also concluded that ultrasound does not damage the membrane surface or increase the pore size of the membranes (Lamminen *et al.*, 2004). Their yet another work revealed the effectiveness of ultrasonic enhancement by use of spacers irrespective of flow current concluding the possibility of doubling of permeate flux with the combined effect of spacers and ultrasound. Increase in the acoustic streaming and mechanical vibration, were utilized by them for resting the main mechanisms involved in flux enhancement. However, the influence of acoustic cavitation cannot be completely excluded. The ultrasonic irradiation acts to reduce the resistance of both the initial protein deposit and the growing cake, reducing the compressibility of these deposits. The mass transfer coefficient within the concentration polarization layer also increases. The low power levels for sonication also imply that damage to the membrane surface itself can be minimized and indeed. (Kondratovych *et al.*, 2013) showed whey ultrasonic treatment as an effective method of product disinfection, allowing the doubling of its storage time. They described the kinetics of microorganism destruction in whey is by first order kinetic equation and concluded that ultrasound treatment of whey destructs the polymers, increases the number of amino and carboxyl groups due to the protein hydrolysis and does not causes evident oxidation of organic compounds. In their work, the polypeptides molecular mass decreased from 17.10^3 to 5.10^3 g/mol under ultrasound treatment.

Membrane Processing

The membrane technology is a novel, non-thermal, environmental friendly technology with a minimum adverse effect on product. The "membrane filtration" is a separation process where specific semi-permeable membrane filters are used to concentrate or fractionate a liquid (Winston and Sirkar, 1992) by selective permeation of some compounds through membrane and retaining the others. The liquid that is able to pass the membrane is known as "permeates" and the retained liquid is known as"retentate" or "concentrate". The hydrostatic pressure gradients or the trans-membrane pressure across the membrane and concentration gradient of the liquids determine the efficacy of membrane. Occasionally, membrane efficacy is also affected by electric potential (Winston and Sirkar, 1992). Widely used membrane separation technologies are micro-filtration, ultra-filtration (Balannec *et al.*, 2005), nano-filtration (Vourch et al., 2005) and reverse osmosis (Balannec *et al.*, 2005; Vourch *et al.*, 2005). MF membranes are generally used to separate fine particles of 0.1 to 10.0µm size whereas, in UF membranes with 1 to 100 nm pore size, proteins and other macromolecules are retained and (Van and Zydney, 2007) water and low molecular weight solutes pass through the membrane. The molecular cut-off of UF is 10,000 MW and operating at 40 psig, and at 50 to 60°C with polysulfone membranes. Nano-filtration has a membrane of slightly smaller pore size than UF. It retains the divalent ions. Reverse osmosis membrane pore size allows only small amounts of very low molecular weight solutes to pass through the membranes and is used mainly for concentration. Diafiltration (DF) is another specialized type of ultrafiltration process in which deionized water is added in the retentate continuously to reduce the concentration of lactose and mineral in retentate with an increase in the concentration of retained components. Membrane filtration has provided a wide scope for whey processing such as concentration, fractionation, purification etc.

Modification of Whey Proteins for Health Benefits

Past two decades have witnessed steady growth in the studies related to the physiological benefits from specifically defragmented amino acid sequences, better known as Biologically Active Peptides. The term Peptidome have often been associated with such kind of studies. These sequences or peptides remain encrypted within the parent protein chain and are released by processes as enzymatic/ acid/ alkali hydrolysis or microbial action. The released peptides have been confirmed to confer various health benefits as being hypotensive, immunomodulating, antioxidative, anticarcinogenic, mineral binding, etc. *In vitro*, *in vivo* and human trials as well have been conducted with positive results. Biologically active fractions of whey; β-Lg, α-La, lactoferrins, glycomacropeptides, and immunoglobulins; function as antioxidants, antihypertensive, antitumor, hypolidemic, antiviral, antibacterial, and chelating agents. β-Lg carries small hydrophobic molecules and retinoic acid modulating lymphatic response (Marshall, 2004). Depression in body fat accumulation, acceleration of loss in weight and fat and satiation enhancement has been shown by whey proteins. Calcium mediates the mechanism of accomplishment of adiposity function by whey protein. (Ha and Zemel, 2003). Korhonen, (2002) reported success in deriving anti-hypertensive peptides from whey protein hydrosylates. It is thought also that the iron binding capacity of lactoferrins reduces oxidative damage caused by unbound iron in tissues (Marshall, 2004). Apart biologically active peptides, distinctive constituents of whey proteins such as Ig, Lf, GMP & LP improve shield against cellular oxidative stresses thereby benefiting cardiovascular system. Lactoferrins sequester iron, interact with microbial cell wall components, and cellular receptors through its highly positively charged N-terminus (Nuijens *et al.*, 2005). Modulation in immune functions is brought about by immunoglobulins, and they act as antibacterial agents. Inhibition of bacterial growth by catalyzing thiocynates and other halides and by depletion of hydrogen peroxides is carried out by lactoperoxidases. GMP and bovine serum albumin (BSA) are available amino acids (Ha and Zemel, 2003).

Future Possibilities

Whey proteins and components of whey along with modified forms foster useful nutritional and other supplements with health maintenance and healing being more pressing. Contemplation foreruns that advanced processes for purifying and modifying whey products upon development can potentially increase the numerals of products that can be made. They can smoothly fit into new products such as beverages, confectionery items (e.g., candies), convenience foods, desserts, baked goods, sauces, infant food and formulae, geriatric foods, animal feeds, and as drug constituents, and plastics.

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Consumers' Acceptance For Buffalo Milk

The Indian population has a great liking for buffalo milk, which forms a thick cream layer (malai). This layer thickens further after boiling and storage. The high viscosity of buffalo milk exerts an additive influence on the consumer's preference. It is known to impart a distinct whitening effect to tea and coffee because of higher quantity of whey proteins and casein. Boiling of buffalo milk causes the release of high amounts of sulphydryl compounds, which contribute to nutty, cooked flavour leading to its high acceptance as a drink. Full cream buffalo milk is sold at premium price because of its flavour and its ability to produce good quality products (Rajorhia, 2000).