CHAPTER 5

Extrusion Texturized Dairy Proteins: Processing and Application☆

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Contents

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Abstract

The primary proteins in milk, casein and the whey proteins α-lactalbumin and β-lactoglobulin, have a number of health benefits and desirable functional properties. In a twin-screw extruder, mechanical shear forces, heat, and pressure cause considerable changes in the molecular structures of the dairy proteins, a process known as texturization. These changes further impart unique functional properties to dairy proteins, resulting in new protein-based food ingredients. The new functional behavior depends on the extent of texturization and the degree of structural change imparted and is controlled by adjusting parameters such as extrusion temperature and moisture level. Such texturized proteins can be used to produce puffed high-protein snacks. Softer gels and expanded structures can be made using supercritical fluid extrusion and cold extrusion, techniques that avoid elevated temperatures, minimizing possible damage to the nutritive components and functionality of the texturized dairy proteins. The uses of the texturized dairy ingredient in food products with improved functionality and enhanced nutritive profiles are presented.

I. DAIRY PROTEINS

A. Production

Milk from cows contains 3.2% protein, about 80% of which is casein. Casein is isolated by a precipitation process from milk, involving heating, rinsing to remove whey, and drying to a powder. The yield is about 3 kg/100 kg skim milk. Rennet casein is obtained when the casein is precipitated by chymosin enzyme, also known as rennet, and acid casein is produced when precipitation is accomplished by acidification. Acid casein is usually found in the form of sodium caseinate or calcium caseinate, which are water-soluble salts. Caseinates are made by reacting NaOH or CaOH with a slurry of casein curd or powder and then spray drying (Southward, 2010).

Sweet whey is the liquid obtained when milk is coagulated with rennet enzymes, and curds are formed during cheesemaking; making cheese by adding lactic or mineral acid to milk produces acid whey. Over 188×10^6 kg of whey protein concentrate (WPC) and 23.5×10^6 kg whey protein isolate (WPI) were produced in the USA in 2009 (Gould, 2010). Each kilogram of cheese manufactured generates about 9 kg of sweet whey. In the past, much whey was fed to pigs, spread as fertilizer, or simply discarded (Tunick, 2008). Currently, efficient separation technologies create purer forms of whey proteins, making for more effective uses of the lactose and minerals coproducts, while advanced processing techniques such as extrusion texturization are leading the way to greater utilization of the purer forms of whey protein in food products (Morr and Ha, 1993; Onwulata *et al.*, 2010).

The higher protein content whey products are used in many products, and have been mainly promoted for their health benefits. Our contribution is creating extrusion texturized whey products that expands the range of products that can contain whey proteins (Onwulata, 2009; Onwulata *et al.*, 2010).

The range of whey products that are used include, for example, ultrafiltered and dried WPC, which contains between 20% and 89% protein; ion exchange and membrane filtered WPI, which contains at least 90–95% protein (Tunick, 2008); and other whey fraction-enriched products such as β-lactalbumin. These enriched protein whey products can be texturized and used in the manufacture of high-protein content puffed corn products (Onwulata *et al.*, 2010).

B. Health benefits

Dairy proteins are rich in nutrients and occupy a unique place of importance in food and human nutrition because of their wide acceptance in the world. Milk proteins are important in the diet because of the many health benefits associated with their consumption. The proteins have long been recognized as natural sources of health enhancing bioactive peptides because of their structural and physicochemical components as recently reviewed by Livney (2010).

Casein refers to a family of proteins, namely, α_{s1}-, α_{s2}-, β-, and κ-caseins (Table 5.1). Digestion of α- and β-caseins leads to production of peptides that may bind to opioid receptors that exist in the nervous, endocrine, immune, or gastrointestinal system (Kampa *et al.*, 1996; Meisel, 2004). These compounds may modulate absorption processes in the gut and can potentially affect gastrointestinal function through transit

TABLE 5.1 Types of casein (Farrell *et al.*, 2004)

Type	Portion of total caseins (%)	Molecular weight (kDa)	Amino acid residues
α_{s1}	45	23.6	199
α_{s2}	12	25.2	207
β	33	24.0	209
κ	10	19.0	169

time reduction (Meisel and FitzGerald, 2000; Pihlanto and Korhonen, 2003). Peptides derived from α_{s1}-casein have been shown to possess stress-relieving properties (Lefranc, 2001) and have antiproliferative action against T47D human breast cancer cells (Kampa *et al.*, 1996).

Casein-derived phosphorylated peptides are believed to enhance the bioavailability of calcium from milk and dairy products (Pihlanto and Korhonen, 2003), and a phosphopeptide derived from β-casein has been shown to increase iron bioavailability (Bouhallab *et al.*, 2002; Pérès, 1999). Other casein-derived peptides have been found to contain antihypertensive activity in rats (Leclerc *et al.*, 2002; Miguel *et al.*, 2009). A number of casein fragments demonstrate antibacterial activity (Kilara and Panyam, 2003).

The major proteins in whey are α-lactalbumin (α-LA), β-lactoglobulin (β-LG), and bovine serum albumin, as shown in Table 5.2. β-LG, the major whey protein in ruminants, is a good source of the essential amino acid cysteine and has been implicated in hydrophobic ligand transport and uptake, enzyme regulation, and acquisition of passive immunity in infants (Kontopidis *et al.*, 2004). α-LA has branched-chain amino acids used by the muscles for energy and protein synthesis and contains bioactive peptides with antihypertensive, antimicrobial, antioxidative, antitumor, antiulcerative, antiviral, hypocholesterolemic, immune modulating, mineral binding, and opioid activity (Kamau *et al.*, 2010; Morris and FitzGerald, 2008). Bovine serum albumin contributes to osmotic pressure of blood and has a role in transport, distribution, and metabolism of ligands, but its full range of functions has been under investigation for some time.

Whey proteins are known to increase immune response and maintain muscle mass (Phillips *et al.*, 2009). In one instance, when an immunostimulatory vitamin and mineral mixture developed at Tufts University Human Nutrition Research Center on Aging was blended with texturized WPI (TWPI) in an extruded snack bar, immunostimulatory effects were enhanced in young (<5 months) and old (>22 months) mice fed *ad libitum* for 5 weeks. The mineral mixture and TWPI improved T cell proliferation and reduced upregulated production of proinflammatory mediators in

TABLE 5.2 Major proteins in whey (Farrell *et al.*, 2004)

Protein	Portion of total whey proteins (%)	Molecular weight (kDa)	Amino acid residues
α-Lactalbumin	22	14.2	123
β-Lactoglobulin	57	18.3	162
Bovine serum albumin	8	66.4	583

old mice (Wu *et al.*, 2009). The health benefits of whey protein and its appeal to consumers continue to increase, and growing opportunities for its utilization in many foods have been noted (Onwulata, 2010; Smithers *et al.*, 1996). Whey proteins are now more sought after for their many benefits such as ease of digestion and assimilation, in the human body, maintaining muscle mass, and boosting immune functions (Huth *et al.*, 2008; Ward and Bastian, 2007). Recently, researchers have demonstrated that the structures of dairy proteins can be changed to improve their physical and nutritional functionality over the unmodified forms of whey proteins (Hale *et al.*, 2002; Manoi and Rizvi, 2008; Onwulata, 2009; Onwulata *et al.*, 2001a,b).

C. Functional properties

As casein is insoluble in water, it must be converted to caseinate before it can be used in food applications. Caseinates can absorb a great deal of water and are used to modify the texture of dough and baked products and to change the consistency of soup and other solutions. They also form films, which make them ideal for whipped toppings, and are used in foaming and fat/oil emulsification (Southward, 2010).

Whey proteins are relatively more soluble and also impart desirable functional properties to food. Morr and Ha (1993) listed a number of these functional properties along with their chemical and physicochemical interactions such as Maillard and nonenzymatic browning, the result of protein–carbohydrate interactions and ligand binding. Other functional properties of whey proteins such as heat-induced gelation and coagulation result from the extent of denaturation, diffusion, hydration and solvation, molecular unfolding, and protein–protein interactions among the molecules. Emulsification and foaming are results of interfacial activity. Solubility, viscosity, and water binding and retention are other noted functional properties of whey proteins (Morr and Ha, 1993).

Proteins are denatured when the native globular conformations are unfolded or otherwise modified as a result of a physical process such as heating, spray drying, and extrusion, where there is no change in the primary structure and bond cleavages and formation, but functional properties are closely associated with processing. α-LA and β-LG are globular proteins whose structures normally begin to change at 50 and 80 °C, respectively (Farrell *et al.*, 2002). Thermal denaturation of whey proteins in solution occurs in this temperature range over a period of 30 min, with the level of denaturation depending on temperature, time, and pH (Ennis and Mulvihill, 2000). As α-LA and β-LG denature, the proteins unfold and SH groups are exposed and undergo possible interchange (Mousavi *et al.*, 2008; Patel *et al.*, 2006). The heat-induced unfolding behavior of both α-LA and β-LG has been well studied (Farrell *et al.*, 2002;

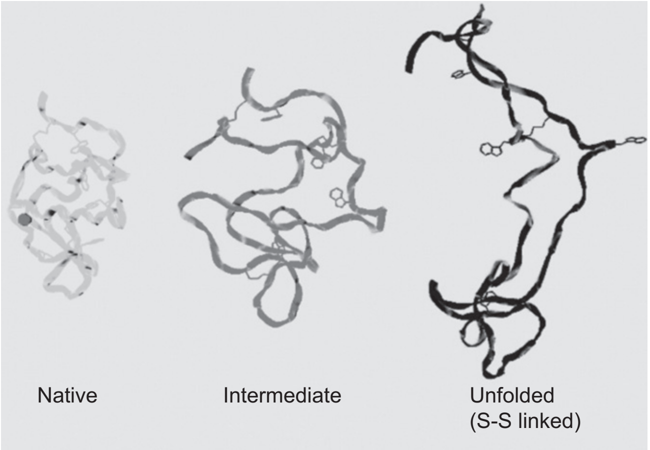

FIGURE 5.1 Rapid molecular simulations of the apoprotein form of α-lactalbumin in vacuo, showing the native holo state and the effect of simulations at 5 and 298 K of the apoform (Farrell *et al.*, 2002).

Kuwata *et al.*, 2001). Figure 5.1 represents one of our own efforts in understanding molecular mechanism of thermal-induced unfolding of α-LA using molecular dynamics simulation techniques. A similar process for β-LG was conducted by Kuwata *et al.* (2001), using hydrogen deuterium (H-D) exchange combined with intrinsic tryptophan fluorescence measurements. Both studies clearly demonstrate that S–S linkage can significantly stabilize the proteins under heating conditions (Fig. 5.2).

Caseins, however, do not display a distinctive conformational transition upon denaturation and texturization; they are considered a family of natively unstructured proteins (Farrell *et al.*, 2006a,b).

There is a continuing interest to improve and extend the functional properties range of dairy proteins to provide both health benefits and their characteristic physical behaviors under different temperature, moisture, and pH conditions so that they may be included in foods that ordinarily do not contain them. One such research area is the extrusion texturization of whey proteins, which have resulted in dairy proteins with new characteristics imparted by a controlled texturization process, depending on the application desired (Hale *et al.*, 2002; Manoi and Rizvi, 2008; Onwulata, 2009; Onwulata *et al.*, 1998). Protein texturization is a two-step process that involves, first, the unfolding of the globular structure (denaturation) and, second, the alignments of the partially unfolded structures in the direction of mass flow in the extruder. The surface characteristics are imparted at the extruder die as the molten mass exits (Onwulata *et al.*, 2003a).

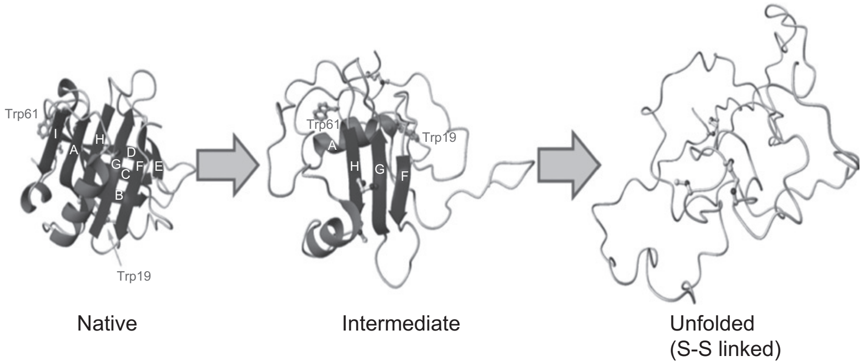

FIGURE 5.2 Schematic representation of the conformational states encountered during folding of β-LG, including the unfolded ensemble, a partially structured intermediate populated on the millisecond time scale and the native state (ribbon diagram based on the X-ray structure 24; PDB code 3BLG). The intermediate contains hydrogen-bonded structure as measured by burst-phase labeling. Side chains are shown for the fluorescence probes, Trp 19 and Trp 61, and five cysteine residues, including two disulfides (Kuwata *et al.*, 2001).

II. PROCESSING

A. Extrusion texturization

Extrusion texturization is a process that uses mechanical shear, heat, and pressure generated in the food extruder to change the structures of food components, including proteins (Harper, 1986). Protein texturization creates filamentous structures, crumbly surfaces, or other physical formations by restructuring or realigning folded or tightly wound globular structures into stretched, layered, or cross-linked mass (Kinsella and Franzen, 1978).

Single-screw extrusion became popular in the food industry starting in 1935, when it was initially used to manufacture pasta products (Kinsella and Franzen, 1978). The first twin-screw extruder was developed in 1869 for sausage manufacture, and the first use of twin-screw extruders for expanded food products began in the 1970s (Hsieh, 1992). Extrusion of caseinate and whey was first reported in the 1980s. In early work, Tossavainen *et al.* (1986) used extrusion of acid casein to produce sodium caseinate without spray drying, and twin-screw extrusion of WPI was investigated as a means of pasteurizing the product (Queguiner *et al.*, 1989).

Twin-screw extruders that contain two internal rotating screws that press material against heated barrel walls and forces the resulting molten mass through a restriction die which aligns the mass in the direction of rotational flow are preferred for protein texturization (Harper, 1986; Kinsella and Franzen, 1978). The process combines transport, mixing, working, and forming in what is basically a low-moisture continuous flow reactor (Camire *et al.*, 1990). Extrusion texturization of proteins is performed with residence times under 2 min (Onwulata *et al.*, 2006). The molten mass in the extruder expands or puffs as it exits the die because of the sudden release of pressure. Heat-sensitive components such as flavors and trace nutrients are therefore added after extrusion (Camire, 1991); else, the vitamins may be destroyed and the mineral may become unavailable if complexed with other materials.

Purely thermal denaturation of proteins requires much longer times: collagen in moist heat below 120 °C needs 30 min to denature (Meyer *et al.*, 2005), wheat glutens must be subjected to 200–215 °C of dry heat for 72 min (Friedman *et al.*, 1987), and as mentioned above, whey proteins require at least 50 °C and 30 min for texturization without the use of extrusion processing.

There are two approaches for extruding whey proteins. One is by direct extrusion. This process involves blending of proteins and cereal carbohydrates in a twin-screw extruder to obtain a final product. Many researchers have investigated the direct extrusion of proteins (Aguilera and Kosikowski, 1976; Harper, 1986; Hale *et al.*, 2002; Holay and Harper, 1982; Kim and Maga, 1987; Matthey and Hanna, 1997; Singh *et al.*, 1991; Smietana *et al.*, 1988). The second approach involves texturizing the protein to produce an ingredient with a desired functionality; the texturized whey protein (TWP) ingredient may then be used subsequently to obtain improved functional properties (Onwulata and Tomasula, 2004; Onwulata, 2009; Onwulata *et al.*, 2010).

Food extrusion generally involves high-temperature (120–170 °C) short-time processing, with most of the energy originating from friction and the heated barrels of the extruder (Harper, 1986). The heat is required to convert water into superheated steam at high pressure, to puff or expand the product. However, whey protein, lipids, and starch can interact when extrusion temperatures increase between 80 and 150 °C, causing the proteins to collapse within the starch or lipid matrix, leading to reduced expansion, and increased hardness of the extrudate (Kim and Maga, 1987; Smietana *et al.*, 1988), and sometimes, unacceptable scores from sensory panels (Onwulata and Heymann, 1994). Texturization reduces the water binding capacity of whey proteins when the extrusion temperature is increased above 60 °C, allowing them to interact better with starch (Onwulata *et al.*, 2001a,b).

The constraint of extruding whey protein above the useful texturization range is keeping the temperature below the point where pyrolysis will occur as evidenced by relatively constant nitrogen content. However, texturized whey products are sometimes extruded at 150 °C to form meat-like stringy structures (Hale *et al.*, 2002; Lin *et al.*, 2000). It is possible then that whey proteins extruded at a temperature higher than 100 °C form very dense fibrous structures. We have seen the evidence of fine structures with transmission electron microscopy (TEM) images at 100 °C in whey isolates. There is further evidence that such texturized whey products could function as food adjuncts. In a consumer taste test, extrusion texturized WPC (80% protein) was shown to be comparable to texturized soy protein (Hale *et al.*, 2002).

We have created structured networks in whey proteins using mild heat and shear, to create reversible TWPs. By understanding on a molecular basis, the effects of shear, ways of creating new functionality can be developed. This will enable development of extrusion parameters that permit controlled denaturation of whey proteins.

Mohammed *et al.* (2000) reported that whey isolates were most denatured by heat among the different proteins they extruded. Soy proteins and gluten are two systems that are generally extruded at high-temperature and low-moisture conditions to form structured products. Their solubility is high, requiring that the hard to break disulfide bonds be dissolved with high-solubility solvents such as β-mercaptoethanol and sodium dodecyl sulfate (SDS). TWPs behaved similar to soy protein and gluten, showing a similar pattern of bonding and cross-linking.

B. Effects on proteins

As mentioned earlier, casein is not denatured with heat. In contrast, whey proteins are modified by chemical reagents, heat, or shear when extruded (Kim and Maga, 1987). Extruded whey proteins are insoluble, resulting in aggregation (Walstra *et al.*, 1999). The reactive groups of the amino acids can be exposed using chemical treatment alone, resulting in changes in the noncovalent forces that influence conformation such as electrostatic interactions, hydrogen bonding, hydrophobic interactions, and van der Waals forces (Kester and Richardson, 1984). Heat and shear alter the conformation of whey proteins through partial denaturation of the protein molecules, exposing groups that are normally concealed in the folded native protein (Kim and Maga, 1987). When heated above 70 °C, cysteine residues undergo thiol–disulfide interchange reactions and thiol oxidation reactions (Gezimati *et al.*, 1997). These reactions, which usually occur within 1 h, lead to cross-linked whey structures resembling gels.

The extrusion process frequently results in realignment of disulfide bonds and breakage of intramolecular bonds. Disulfide bonds stabilize the tertiary structure of protein and may limit protein unfolding during extrusion (Taylor *et al.*, 2006). Flow and melt characteristics were improved when other proteins were extruded with disulfide reducing agents (Areas, 1992), which indicates that disulfide bonds adversely affect the extrusion performance of whey proteins. Intramolecular disulfide bonds also are known to affect the functional properties of whey proteins.

Polyacrylamide gel electrophoresis results suggest that β-LG undergoes a greater conformational loss as a function of extrusion temperature than α-LA, presumably due to intermolecular disulfide bond formation. Atomic force microscopy indicates that texturization results in a loss of secondary structure of around 15%, total loss of globular structure at 78 °C, and conversion to a random coil at 100 °C (Qi and Onwulata, 2011). Moisture has a small effect on whey protein texturization, whereas temperature has the largest effect. Extrusion at or above 75 °C leads to a uniform densely packed polymeric product with no secondary structural elements (mostly α-helix) remaining (Qi and Onwulata, 2011).

Denaturation and aggregation of whey proteins are affected by the pH of extrusion. When extruding WPI, alkaline conditions increase denaturation and solubility, decrease pasting properties, and produce more pronounced microstructural changes (Onwulata *et al.*, 2006). Denaturation in the extruder causes whey proteins to form small primary aggregates that combine to form large clusters. The clusters are then aligned by shear to form fibrous structures.

Texturization is not measured directly but is inferred from the degree of denaturation or decrease of solubility of proteins. The quantities are determined by the difference in rates of moisture uptake between the native protein and the texturized protein (Kilara, 1984), or by a dye-binding assay (Bradford, 1976). Protein denaturation may be measured by determining changes in heat capacity, but it is more practical to measure the amount of insoluble fractions and differences in solubility after physical treatment (Kilara, 1984). The different rates of water absorption are presumed to relate to the degree of texturization as texturized proteins absorb water at different rates. The insolubility test for denaturation is therefore sometimes used as substitute for direct measurement of texturization. Protein solubility is affected by surface hydrophobicity, which is directly related to the extent of protein–protein interactions, an intrinsic property of the denatured state of the proteins (Damodaran, 1989; Vojdani, 1996).

Three different whey protein products extruded at the cook temperature of 75 °C resulted in varying degrees of melt texturization (Table 5.3). Among the whey proteins, WPC (WPC80) was the least texturized. Whey lactalbumin (WLAC) and WPI were both significantly ($p < 0.05$) more texturized, but a wider spread of texturization was observed for WPI, the initial and final values were from 28% to 94.8%, and therefore more emphasis was placed on studying WPI (Onwulata *et al.*, 2006).

Structural changes on the whey proteins from the effect of extrusion cooking were examined by scanning electron microscopy and TEM. Changes in the microstructure of WPI (Fig. 5.3) show the transition from

TABLE 5.3 Extrusion melt temperatures of whey proteins (Onwulata *et al.*, 2003a)

Product	Melt temperature (°C)	Preextrusion (%)	Postextrusion (%)
WPC80	70[b]	40.9[b]	59.9[b]
WLAC	75[a]	68.7[c]	94.4[a]
WPI	74[a]	28.0[a]	94.8[a]

WPC80: whey protein concentrate, 80% protein. WLAC: whey lactalbumin. WPI: whey protein isolate: number reported is mean of three samples. Means with different letters within a column are significantly ($p < 0.05$) different.

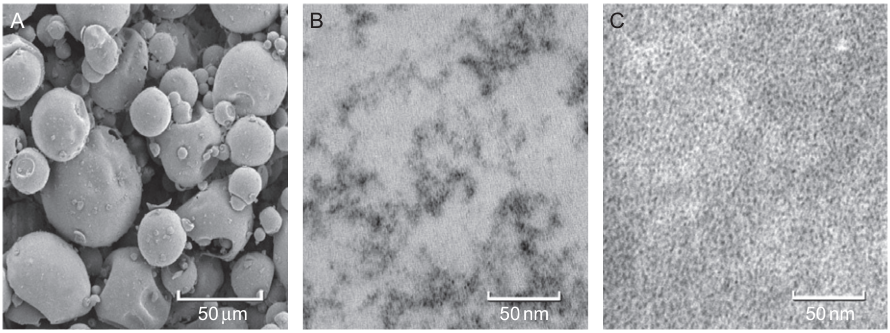

FIGURE 5.3 Electron micrographs of whey protein isolate (WPI). Scanning electron microscopy of dry WPI powder (A). Transmission electron microscopy of WPI stained with uranyl acetate: (B) nonextruded WPI Paste (40% moisture) and (C) extruded texturized WPI (100 °C, 40% moisture) (Onwulata *et al.*, 2003a).

dry powder particles ranging from 10 to 50 µm in diameter (Fig. 5.3A). The structure of a 40% (w/w) nonextruded WPI paste shows the release of protein at the edge of powder particles after brief exposure to water. This state, typical of initial mixing in the extruder (Fig. 5.3B), shows irregular strings and granules corresponding to molecular aggregates ranging from less than 10 nm to over 200 nm. The ultrastructure (Fig. 5.3C) shows a closely packed arrangement of electron dense particles, typical of denatured protein matrix, ranging from approximately 2 to 6 nm in diameter.

Though structural changes may occur in the WPI above 50 °C, the addition of shear in the extruder may cause significant unfolding (denaturation) even below 50 °C. WPI extruded above 50 °C denatured significantly ($p < 0.05$) by an order of magnitude every 25 °C rise in cook temperature degrees (Onwulata *et al.*, 2006). The pH of the suspended protein remained stable as extrusion temperature increased, but measurable nitrogen (protein) increased as shown in Table 5.4. Thermal denaturation and texturization unmask the SH groups (Linden and Lorient, 1999).

TABLE 5.4 Properties of whey protein isolate as function of extrusion temperature (Onwulata *et al.*, 2003a)

Extrusion cook temperature	Melt (°C)*	pH	Protein** (%)	Insoluble (%)	Digestibility (%)
35	39[d]	6.7[b]	90.7	28.4[c]	89.6[a]
50	48[c]	6.8[ab]	90.9	33.3[c]	88.2[ab]
75	66[b]	6.9[a]	91.7	77.7[b]	85.7[bc]
100	92[a]	7.0[a]	91.4	87.2[a]	84.5[c]

WPI, whey protein isolates. Properties of nonextruded WPI: pH 6.8, protein 88.9%, insoluble (denatured) 28.0%, and digestibility 87.7%. Means with different letters within a column are significantly ($p < 0.05$) different.
* Extrusion melt temperature at the die.
** % Protein after drying.

The level of texturization or insolubility at pH 7 depends on the heating temperature, shear conditions, the length of time of protein exposure, and the pH of whey medium (Ennis and Mulvihill, 2000). Though it has been reported that, when heated up to above 80 °C for 30 min, whey proteins in solution lose protein nitrogen, we have observed insignificant changes in protein nitrogen content and digestibility after texturization (Onwulata *et al.*, 2006).

In general, as the amount of protein denatured and texturized increases, with increasing temperature, the overall effect on protein digestibility was minimal (Table 5.4). So, the benefit of whey protein texturization is the interesting result of enhanced functionality without a significant loss of digestibility from extrusion at temperatures below 100 °C. This is not surprising, as extrusion texturization occurs in the short-time order of 45–90 s within the extruder. The short time might explain why extrusion TWPI maintains its digestibility (Onwulata *et al.*, 2006).

In one study, heat-treated WPI evaluated using SDS–PAGE (Fig. 5.4) indicated minimal change in solubility above 75 °C. SDS gels developed without reducing reagent with the protein disulfide bonds intact showed unreduced samples at 35 and 50 °C with somewhat diminished bands for the higher molecular weight whey proteins (Fig. 5.4B). However, at 50 and 75 °C, the samples were of equivalent weight and were fainter than the native whey or whey proteins produced in the lab on the SDS gel (compare lanes 1 and 2 with 6 in Fig. 5.4). In this respect, the SDS gels parallel the solubility data in that increased temperature decreases solubility in SDS alone, confirming sulfhydryl–disulfide cross-linking. When the samples were reduced thoroughly and all disulfide bonds cleaved (Fig. 5.4A), all the extruded whey samples at the different temperatures were similar to each other and to the initial WPI. So conclusively, extruding whey proteins even at the highest temperatures studied did not affect the overall protein ratios. The control nonextruded and extruded whey proteins still have the same amount of the different proteins (Fig. 5.4) and similar total nitrogen values (Table 5.4).

Spatial spectral analyses of TWPI look quite different from the non-texturized proteins at the ultrastructural level (Fig. 5.5A). TWPI extruded at 100 °C had densely packed structures with spacing of 2–6 nm

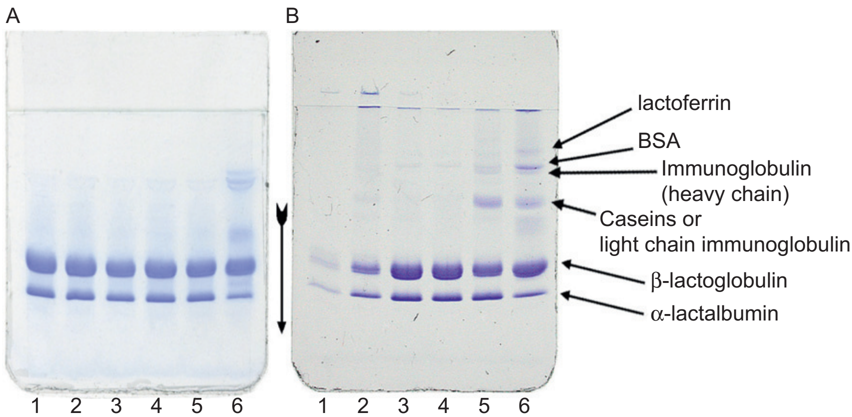

FIGURE 5.4 SDS–PAGE of extruded and nonextruded whey protein isolate. (A) With 2-mercaptoethanol; (B) without 2-mercaptoethanol. The lanes are temperature and product conditions: lane 1, 100 °C; lane 2, 75 °C; lane 3, 50 °C; lane 4, 35 °C; lane 5, native WPI; lane 6, laboratory whey (Onwulata *et al.*, 2003a).

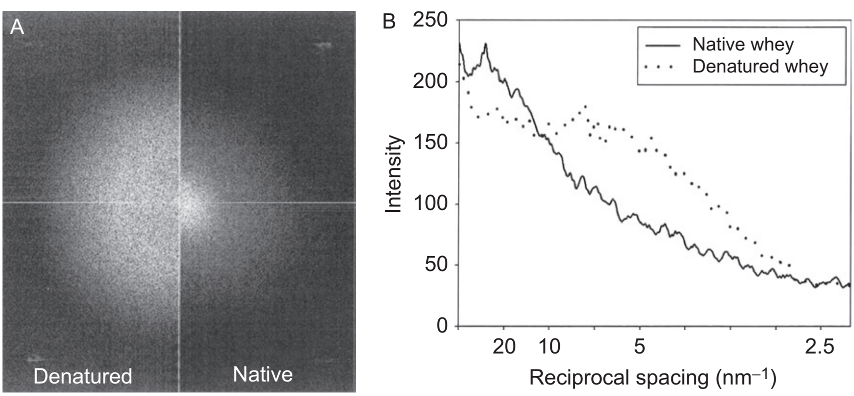

FIGURE 5.5 Electron-density mapping corresponding to the Fourier transforms (A) for denatured (extruded at 100 °C) and native WPI, an (B) inverse reciprocal spacing of electron-density images of native and denatured WPI (Onwulata *et al.*, 2006).

(denatured), while non-TWPI had loosely packed structures with a large spacing of 200–350 nm (native). The differences in fine structure of texturized (denatured) and nontexturized (native) whey protein are illustrated using the distribution of electron density surrounding the hydrating particles in Fig. 5.5. The electron-density profile of native whey proteins shows an open network with clear, electron-lucent spaces ranging from 15 to 40 nm and irregular structures of electron density of similar dimensions. In contrast, texturized (denatured) whey proteins show closely packed fine granules around 3–8 nm in diameter. The corresponding computed Fourier transforms indicate that images of extrudate containing ''native'' whey proteins consist mainly of low spatial frequencies indicating structures with average spacings ranging from 15 to over 40 nm, whereas images of extrudate containing ''denatured'' whey proteins have little intensity at low spatial frequencies, but high intensity corresponding to high spatial frequencies, relating to electron density changes ranging from about 3 nm to less than 10 nm (Fig. 5.5B).

C. Effects on functionality

Varying the extrusion cook temperature and moisture conditions may control the functionality of TWPI. It was found that the degree of denaturation increased from 30% to 60%, 85%, and 95%, respectively, for extruded WPC, WPI, and whey albumin at 35, 50, 75, and 100 °C (Onwulata *et al.*, 2003a). For example, foaming and digestibility were minimally affected by extrusion (Table 5.5). Other physical functional properties of TWPI such as gel strength, foam volume, and stability were significantly affected at 75°C and above. Greater than 30% moisture was needed to extrude the WPIs, but the only significant change in functionality due to moisture content occurred at the extrusion

TABLE 5.5 Physical properties of whey protein isolate as function of extrusion temperature (Onwulata *et al.*, 2003a)

Extrusion cook temperature	Moisture (%)	Gel strength (N)	Foam volume (%)	Foam stability
35	42.5^{a}	114.9^{b}	298.1^{ab}	29.8^{ab}
50	40.9^{b}	145.3^{a}	301.9^{a}	30.2^{a}
75	42.6^{a}	2.8^{c}	173.3^{b}	17.3^{c}
100	38.9^{c}	#	77.1^{c}	7.7^{c}

WPI: Whey protein isolates. Properties of nonextruded WPI: moisture 1.94%, gel strength 52.3 (N), foam volume 288%, and foam stability 28.7%. #: Value not reported. Means with different letters within a column are significantly ($p < 0.05$) different.

temperature of 100 °C (Table 5.5). In particular, whey proteins modified using the extrusion texturization process (TWP) showed the most enhanced benefits. Examples of the benefits are enhanced physical properties, improved digestibility, and protein conversion (Hale *et al.*, 2002; Manoi and Rizvi, 2008; Onwulata, 2009; Onwulata and Tomasula, 2004; Onwulata *et al.*, 2003a,b).

Incomplete texturization or partial denaturation at temperatures below 60 °C significantly increased gel strength, but at 75 °C or above, complete loss of the gelling property resulted. Foam volume remained high up to 50 °C but decreased significantly ($p < 0.05$) above 75 °C. Foam stability followed the same pattern as foam volume, being very stable for an hour below 50 °C. On the contrary, Phillips *et al.* (1990) reported that WPI heated to 80 °C had little effect on foam stability.

D. Effects on flavor and other components

Flavor retention is a concern with extrusion due to thermal degradation in the barrel and volatilization at the die (Riha and Ho, 1996). Moreover, flavor generation from Maillard and other reactions may occur. Maga and Kim (1989) extruded sodium caseinate, WPC, and other proteins with cornstarch and found that low-temperature and high-moisture extrusion resulted in the generation of more flavor compounds than high temperature and high moisture. Flavorings may be added to the material before or after extrusion to enhance desirable flavors and mask unwanted ones generated during extrusion cooking (Maga and Kim, 1989).

Carbohydrates are gelatinized during extrusion, and starch may be degraded to dextrins, which are carbohydrates with lower molecular weight (Bjorck and Asp, 1983). Starch granules gelatinize and melt during extrusion because hydrogen bonding in the polysaccharide chains is disrupted by heat and moisture (Camire *et al.*, 1990). Gelatinization plays an important role in the characteristics of the final product (van de Voort *et al.*, 1984).

Lipids are hydrolyzed by moisture and heat into free fatty acids, though hydrolytic enzymes may be deactivated by extrusion. Also, unsaturated fatty acids may undergo oxidative rancidity (Camire *et al.*, 1990).

Vitamins, microorganisms, and enzymes are susceptible to inactivation or destruction in an extruder. Removal of microorganisms and enzymes is desirable in most cases, but vitamin retention is important for nutritional considerations (Bjorck and Asp, 1983). Survival of vitamins increases if moisture is increased and if temperature, screw speed, and specific energy input decrease (Killeit, 1994). Vitamin loss may be compensated by adding more than the necessary amount of preextrusion or by applying a vitamin coating, filling, or spray postextrusion.

Camire (2002) showed that texturization does not seem to have a great effect on mineral retention and bioavailability. Others have reported increased retention of ascorbic acid in rice- and maize-based snacks (Hazell and Johnson, 1989; Plunkett and Ainsworth, 2007), increased iron diffusibility and absorption of iron-complexed protein (Poltronieri *et al.*, 2000; Watzke, 1998), and no difference in iron and zinc absorption in human subjects fed textured bran-flour (Fairweather-Tait *et al.*, 1989).

Vitamins differ greatly in structure, and degradation of vitamins depends on processing conditions, but minimizing temperature and shear protects most vitamins during processing (Singh *et al.*, 2007). Riaz *et al.* (2009) and Bjorck and Asp (1983) reported losses of vitamins during high-temperature, high-shear extrusion processing at 80–180 °C, but in a more recent review of food extrusion and nutrition, Singh *et al.* (2007) showed that the effect of extrusion on nutritional quality was ambiguous, both beneficial or deleterious depending on processing conditions.

Controlled changes can be induced on proteins by mild heat treatments, pH changes, and shear during food manufacturing to favorably alter them biologically and functionally by modifying specific amino acids (Onwulata *et al.*, 2006). For example, acidic conditions affect glutamine and asparagines, while alkaline conditions affect cysteine, serine, and threonine forming lysinoalanine and D-amino acids. Heating proteins in the presence of reducing sugars results in nonenzymatic browning. Although most thermal denaturation is irreversible, α-LA denaturation is primarily reversible (80–90%) above pH 3.3 depending on the presence of calcium. Below pH 3.3 or in the presence of calcium chelators, its reversibility is reduced (Korhonen *et al.*, 1998).

III. DEVELOPMENT

A. Improving extrudate expansion

To improve the interaction of whey proteins with other food components such as starches, flours, and nondairy proteins, different methods have been explored primarily to increase expansion of the extrudate. For example, extreme extrusion process conditions of high shear and low moisture were used to directly expand high-protein corn meal containing 30 wt.% WPC (Onwulata *et al.*, 2001a,b). In a similar process, an expanded extrudate was made using low temperature (<100 °C) and low shear with supercritical CO_2 extrusion (Rizvi and Mulvaney, 1992). The range of use for unmodified whey proteins in puffed extrudates is extended with difficulty in amounts greater than 10 wt.% (Kim and Maga, 1987; Onwulata *et al.*, 1998). Manufacturing of expanded snacks in large amounts using non-TWPs had been only marginally successful until recently (Singh *et al.*, 1991), but if proteins are texturized prior to adding them to the starch matrix, or if both are texturized together, an improved product with better functionality and preferred texture can be created (Mohammed *et al.*, 2000).

B. Improving functionality

Recent efforts have focused on expanding the functionality of whey protein products for use in other products without using extreme extrusion processing conditions. This is accomplished by a pretexturization step to modify the proteins by chemical, enzymatic, or physical means for enhanced food functionality such as improved solubility. Using primarily physical means, new surface structure effects were created for a range of whey proteins broadening their functionality (Onwulata *et al.*, 2003a). Fibrous structures from extrusion texturization were used as a basis for whey protein-based meat extenders (Hale *et al.*, 2002). Different TWP-based expanded snack products were created by adjusting different extrusion conditions by Onwulata *et al.* (1998, 2001a,b); whey protein cold-setting gels were made by Manoi and Rizvi (2008, 2009). It is known that direct whey texturization intensifies protein–protein networks and improves the matrix network patterns resulting in increased shear modulus (Tunick and Onwulata, 2006). Dairy proteins can be modified structurally at temperatures ranging from 30 to 110 °C, and moisture from 20% to 70% to different degrees of solubility, which is the first step in improving their functionality. Extrusion modifies the structures of the dairy proteins for ease of use in starchy foods to boost nutrient levels. For example, the solubility of WPC with 80% protein (WPC80) or the 95% protein isolate (WPI) can be reduced from 95% to 20% depending on the process conditions selected (Fig. 5.6). The solubility is lost completely at 50 °C for nonfat dried milk (NDM), which has the lowest protein content

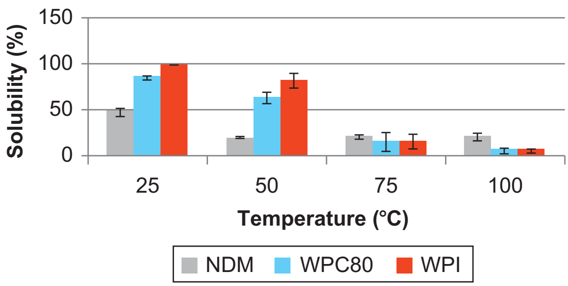

FIGURE 5.6 Solubility of texturized dairy protein products extruded at different temperatures, 25 (control), 50, 75, and 100 °C. Nonfat dried milk (NDM); whey protein concentrate (WPC80), containing 80% protein; and whey protein isolate (WPI), containing 95% protein (Onwulata *et al.*, 2003a).

(<30%). Extrusion texturization minimizes the water binding capacity of dairy protein products, in decreasing order, WPI > WPC > NDM, as temperature increases, making them interact better with starch.

The structures of dairy proteins are easily modified by high temperature, shear, and moisture; in particular, whey proteins can change their globular molecular structures to new unfolded states. The change in protein state is the cause of changes in solubility and the basis for creating new foods. Our studies determined that the extrusion temperature is a more significant change factor than moisture content and that the degree of texturization, or change in protein state, characterized by solubility, affects the viscoelasticity of extruded products.

For example, the consistency of the extruded dairy proteins can range from rigid (2500 N) to soft (3 N). Extruding above 60 °C resulted in significantly increased peak force for WPC (138–2500 N) and minimal increase in peak force for WPI (3–147.1 N). NDM was not fully texturized; the presence of lactose interfered with the texturization. The solubility of WPI products ranged from 72% to 93%.

C. Coextrusion

Coextrusion is the process of extruding two or more materials simultaneously or in tandem. It allows a combination of an ingredient such as wheat flour, which is inexpensive and easily enriched with vitamins and minerals, with dairy protein, which provides functionality and texture. For example, an early coextrusion of wheat flour and rennet casein was performed by van de Voort *et al.* (1984), who obtained products with varying characteristics depending on process parameters.

Coextrusion of whey protein and corn flour was shown to reduce the specific mechanical energy input into the extrusion process and increase the expansion and breaking strength of a protein-enriched extrudate (Onwulata *et al.*, 2001b). Altering the extrusion moisture and shear led to improvement in expansion and breaking strength. When corn, rice, and potato flours were extruded in combination with whey proteins, the whey protein could be substituted for up to 25% of the flour without affecting the quality of the resulting snack product (Onwulata *et al.*, 1998, 2001a,b). Again, the inclusion of whey protein reduced the specific mechanical energy input. Further research using moistures ranging from 20% to 70% by weight and extrusion temperatures of 50, 75, and 100 °C showed that temperature affected the degree of protein texturization (Onwulata *et al.*, 2010). WPC and WPI liquefied when extruded at 50 °C, became soft at 75 °C and high moisture, and were solid at 100 °C. These results reflected the level of denaturation induced by heat and shear (Onwulata, 2009).

Another product, defatted corn germ flour was coextruded at 150 or 170 °C with 5% milk protein to produce a puffed nutrient snack (Peri *et al.*, 1983). The addition of the milk protein at the lower temperature improved the organoleptic characteristics of the extrudates but adversely affected the product expansion and consistency of the samples at the higher temperature.

D. Supercritical fluid extrusion

Low process impact extrusion may be accomplished through the introduction of supercritical CO_2. Supercritical fluid extrusion (SCFX) has been used over a wide range of texturization temperatures for different whey protein fractions with starch. In this process, supercritical CO_2 is injected into dough in the extruder barrel, the temperature and pressure are adjusted to control bubble nucleation, and the degree of cell growth is manipulated by selecting the appropriate die and controlling cooling and drying after extrusion (Rizvi *et al.*, 1995). SCFX mitigates some of the harsh environmental conditions in the extruder, such as destruction of heat- and shear-sensitive compounds. SCFX below 90 °C transforms whey proteins into cold-setting gels (Manoi and Rizvi, 2008, 2009). The process could be used to deposit vitamins directly on a cooked and cooled melt that can be puffed with CO_2 upon exiting the die and then dried to obtain breakfast cereal. The authors suggest that a fast-cooking pasta can be obtained by SCFX, as the product is not precooked in the extruder (Rizvi *et al.*, 1995).

E. Cold extrusion

The elevated cooking temperatures used in normal extrusion lead to discoloration of whey proteins from the Maillard reaction, racemization of protein during cross-linking, destruction of the sulfur-containing amino acids, cysteine and methionine, and other problems (Pordesimo and Onwulata, 2008). As shear without heating has been found to be adequate to induce texturization of particulate whey (Walkenstrom *et al.*, 1998), some investigations have been made in the area of cold or nonthermal extrusion. Cold extrusion is defined as extrusion in which the process temperature is below 50 °C. Molten gel temperatures are not reached in cold extrusion, which produces shear-induced gels similar to cold-set gels (Cho *et al.*, 1997). Cold-denatured proteins are in a state similar to the molten globular state exhibited by heat-denatured proteins (Kunugi and Tanaka, 2002). WPI is denatured in 45–90 s when cold extruded at 50 °C, and the degree of denaturation may be adjusted through manipulation of moisture, shear rate, and temperature. Digestibility, functionality, and protein value are retained when WPI is extruded at $\leq$50 °C (Pordesimo and Onwulata, 2008).

F. Other investigations

Investigating coextrusion of corn meal and WPI, Onwulata *et al.* (2003b) found that the melt temperature of the extrudate was more of an indicator of physical properties than specific mechanical energy. Quality attributes such as breaking strength, color, and expansion index were related to melt temperature measured at the die.

In a different study, it was determined that the concentration range of whey protein required for a fibrous texture suitable for meat extenders. Consumer evaluation showed that 48% whey protein was the optimal level, with no benefits obtained by raising the protein concentration.

The effects of the drying conditions on extruded WPC and WPI were examined by Nalesnik *et al.* (2007), who found no changes in color when extruded material was dried at 40 or 70 °C but did observe differences in force-time curves when performing texture analyses.

IV. APPLICATIONS

A. Puffed snacks

Our group has used twin-screw extrusion to produce many texturized whey-fortified puffed snacks. Whey protein has been blended with barley flour, corn meal, rice flour, and wheat starch prior to extrusion, leading to corn puffs with a protein content of 20% instead of the usual 2% (Onwulata *et al.*, 2001a).

Whey may be substituted for starch by as much as 25% in extruded corn snacks, but the product does not puff as much as corn alone, as the water-holding whey protein does not react with the starch matrix (Onwulata *et al.*, 1998). WPCs or isolates can be added along with starch to create expanded snack foods with boosted nutritional content; however, without texturization, whey proteins in amounts larger than 15% may interfere with expansion, making the products less crunchy. To counter this effect, whey proteins can be texturized with starch to improve their interaction with other food components in a formulation, principally to increase extrudate expansion. In one successful application, between 25% and 35% of the flour was replaced with whey protein (Onwulata *et al.*, 2001a,b).

Texturization enables the creation of more expanded products with boosted protein levels, which are texturally firmer and crispier products, easier to break than the typical cornmeal or cornmeal without TWPI. For example, we developed several directly expanded high-protein corn meal products containing 30 g/100 g WPC (WPC80) and WPI. The prototype products were pretzels, corn chips, and tortilla chips (Onwulata, 2010).

Allen *et al.* (2007) produced puffed snack foods with corn starch and pregelatinized waxy starch, WPC and instantized WPC, and protein concentrations of 16%, 32%, and 40% and showed that the air cell size, extrudate expansion ratio, and water solubility index decreased proportionally as protein and corn starch levels increased. Protein concentration significantly affected total soluble protein, water absorption index, and water-soluble carbohydrate. A covalent complex between amylase and protein formed in the presence of cornstarch, but protein–protein interactions appeared with the presence of low levels of pregelatinized waxy starch.

B. Meat analogs and extenders

Walsh and coworkers at Utah State University have shown that TWP have use as meat analogs and extenders. In one experiment, they texturized WPC by thermoplastic extrusion, rehydrated the fragments, and bound them into patties with wheat gluten, dehydrated egg whites, and xanthan gum (Taylor and Walsh, 2002). They obtained a cohesive patty that withstood baking, freezing, and microwave heating. Sensory analysis revealed that patties containing TWP were as acceptable as commercial soy patties. This group also changed the pH during extrusion and added calcium to the WPC/starch mix before extrusion to obtain extrudates with similar water holding capacity and water soluble protein levels as a mix that was not extruded (Hale *et al.*, 2002). Consumer panels liked beef patties made with $\leq$40% TWP as much as 100% beef patties in flavor, juiciness, tenderness, texture, and overall acceptability. They also found that beef patties formulated with $\leq$40% TWP had higher cook yield and less size reduction than 100% beef patties.

C. Cheese analogs

Calcium caseinate and butter oil have been extruded directly at 50–60% moisture levels to obtain a cheese analog with no surface water or fat (Cheftel *et al.*, 1992). The fat emulsification and melting ability increased with screw speed or barrel temperature. The texture of the extruded analogs was similar to those obtained by batch cooking and was affected by pH (Cheftel *et al.*, 1992) and emulsifying salts (Cavalier-Salou and Cheftel, 1991). The product can be used as adjuncts for hamburger, pizza, and sauces.

D. High-fiber products

Cellulose, oat, and wheat fiber, which are all insoluble, have been incorporated with whey protein into an extruded product (Walsh and Wood, 2010). Increasing the fiber content led to decreases in air cell size, expansion ratio, water-soluble carbohydrate, and water solubility index, and increases in breaking force, extrudate density, moisture content, and water absorption index. Fiber could be added up to a concentration of 18% without seriously affecting the physical and chemical properties of the product as compared with a nonfiber control. High-fiber snack bars containing up to 40% oat bran were produced by extruding with WPC, milk powder, and nonfat dry milk (Onwulata *et al.*, 2000). A nonexpanded flat bread-type snack containing 20% moisture was obtained. Extruding at temperatures up to 140 °C did not affect the texture.

E. Other products

Following research by Tossavainen *et al.* (1986), extrusion is often used to produce insoluble acid casein from skim milk powder and to convert acid casein into sodium caseinate. The procedure is faster than batch mixing (Akdogan, 1999).

Extruded WPI has been used as a fat mimic. The formation of microparticles is required for a creamy sensation in the mouth (Jost, 1993), and this was achieved by extruding at acidic pH (Queguiner *et al.*, 1992).

A cranberry syrup was combined with sucrose, pectin, citric acid, and TWP to obtain a protein-fortified confection (Faryabi *et al.*, 2008).

Extruded whey crisps containing between 30% and 70% protein were developed (Taylor *et al.*, 2005). The whey crisps had a lighter color, lower aroma, and different flavor profile than soy crisps, which allow for easier customization of color and flavor (Taylor *et al.*, 2005).

Nutritional bars containing cold-extruded whey have been developed (Joseph *et al.*, 1995). Extrusion was conducted at 37 °C to produce a low-calorie product with high nutrient value. A weaning food was obtained by extruding WPC, WPI, or α-LA with taro flour, which is derived from a tropical root tuber (Onwulata *et al.*, 2002). The extrudates were pulverized, made into powders, and rehydrated into pastes. WPI coblended extrudates produced the best consistency.

Dairy proteins can be used to boost the protein content of starch-based puffed snacks made from cornmeal; they bind water and form doughy pastes with the starch, but not the non-TWPs. A wide possibility exists for creating new foods with texturized dairy proteins due to the availability of an extensive range of achievable states (Onwulata *et al.*, 2010).

V. CONCLUSIONS

Extrusion is an effective means of denaturing whey proteins to create texturized products. TWP may be used as an ingredient to improve the characteristics of many foods. The production of snack foods with enhanced protein levels is possible by direct extrusion of WPC or WPI. Thermal extrusion at elevated temperatures is usually employed, and coextrusion with flour and other ingredients reduces mechanical energy input. Extrusion using supercritical CO_2 or cold extrusion (at or below 35 °C) is another option. Manipulating the extrusion process may create new food products with enhanced functional properties and nutritional profiles.

Extrusion processing texturizes WPCs, WLAC, and WPI, but the greatest amount of texturing occurred with WPI.

Texturized or denatured WPI retained its native protein value, functionality, and digestibility when extruded below 50 °C; changes in functionality occur at 65 °C and above. Through careful selection of extrusion conditions of temperature and moisture, TWPs with unique functionality can be produced. The degree of texturization increased with increasing temperature, but temperatures higher than 100 °C may be needed to form fibrous structures with WPI.

It is demonstrated here that extrusion is an effective tool for texturing whey proteins to create new functions for dairy proteins and that thermally denatured WPI is a unique ingredient that can be used in large amounts in nontraditional applications for non-TWPI. This review covers the use of extrusion texturized dairy ingredients in foods; however, there are other examples of the successful use of this technique along with the product, TWPI in different types of nonfood applications, such as in biodegradable films, and bioplastics.

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