Protein Functionality Modification by Extrusion Cooking[1]

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Cooking extruders process high-protein materials into palatable foods. New applications have been developed for protein extrusion during the past decade. Improvements in functional characteristics of proteins may be achieved through modification of temperature, screw speed, moisture content, and other extrusion parameters. Flavors and odors may be removed during expansion at the extruder die. Extrusion can improve the digestibility of proteins, while reducing gossypol, proteinase inhibitors, allergens, aflatoxins, and other undesirable compounds. In the future the use of extruders as chemical reactors for both animal and plant proteins will provide new food ingredients as well as novel, nutritious foods.

KEY WORDS: **Extrusion, nutritional quality, protein functionality, protein modification.**

Extrusion-cooking has been used to process foods in the United States for over fifty years (1), yet many new applications have been found only in the past decade. In this review the effects of extrusion on proteins and protein foods will be discussed in addition to possible new directions for protein extrusion based upon advances in other areas.

Extrusion has become the major processing method for textured vegetable protein (TVP), ready-to-eat breakfast cereals, animal feeds, and snack foods (2). During the past decade extruders have been used to commercially produce other foods, including various dairy and meat products. These applications are summarized in Table 1. The advantages of extruders as bioreactors for starches and grains have been demonstrated (3–5), but the application of this technology to proteins has not yet been proven. Although extruders have long been used to manufacture films from plastics, this technology has not been exploited for the production of extruded edible films from protein-rich materials.

EXTRUSION PROCESS

Today, extruders can be found in a variety of sizes and types, but in general they consist of a fixed metal barrel through which material is transported. The barrel contains one or two screws which convey the food material from the feed end of the barrel to the die, which determines final product shape. Heat is usually applied to the barrel, but heat due to friction may be sufficient to cook the material. The temperature is usually increased along the barrel from the feed to the die end. The dimensions and geometry of the barrel, the screw compression ratio and position of elements on modular screws are additional variables which affect shear and pressure within the extruder. The speed of screw rotation also affects the degree of shear developed and the length of residence time within the extruder.

Several characteristics of the raw material influence the final product. These have been summarized by Phillips (6) and include chemical composition, prior thermal history, particle size, and moisture level. The feed moisture content may be adjusted prior to the process or water may be metered into the extruder at the feed port. Thermolabile compounds, such as flavors, may be injected into the barrel near the die end to minimize exposure to heat. Typically, flavors and other heat-sensitive materials are added post-extrusion because volatile compounds are steam-stripped as the material leaves the extruder. Some volatiles may become bound to protein or starch, and thus are not sensed. Pressure builds within the extruder barrel as the plasticized material progresses towards the die. As the food exits the die, steam is flashed due to the pressure differential. The steam acts as a leavening agent, stretching the still-plastic material. Expansion occurs both longitudinally and radially, and hardening may occur within seconds.

TABLE 1

Extruded Protein Applications

Traditional	Recent	Future
TVP®[a]	Caseinate	Hydrolysates
RTE[b]	Processed cheese	Soluble collagen
Animal feed	Restructured meat	Chemical reactors
Snacks	Infant foods	Edible films

[a]Texturized vegetable protein.
[b]Ready-to-eat breakfast cereals.

PROTEIN DENATURATION

Native proteins are denatured during extrusion. The forces which stabilize the tertiary and quaternary structures of the proteins are weakened by a combination of increased temperature and shear within the extruder. Individual protein molecules unfold and align themselves with the flow of material towards the die (7). These changes are presented schematically in Figure 1. The

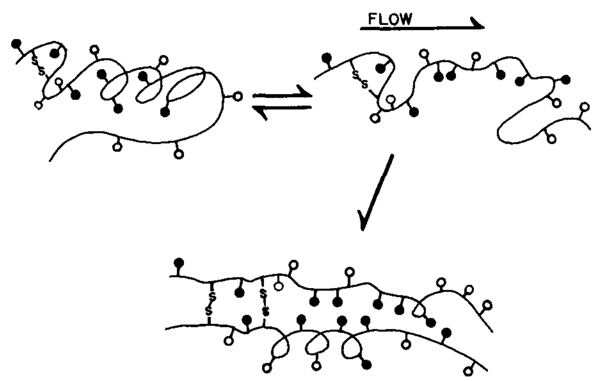

FIG. 1. Schematic diagram of a protein molecule unfolding, aligning with the flow in the extruder barrel, and forming new bonds with another molecule. Open circles represent hydrophilic amino acid residues; closed circles, hydrophobic residues; s-s, disulfide bridges.

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situation is further complicated by the presence of many different types of proteins of varying molecular weights and amino acid compositions within a food material.

Previously hidden amino acid residues become exposed and are free to react with reducing sugars and other food components. The exposure of hydrophobic residues, such as phenylalanine and tyrosine, reduce the solubility of extruded protein in aqueous systems. Intermolecular bonds may form between protein molecules prior to leaving the die. Intermolecular disulfide and other hydrophobic bonds appear to be the major type of bond that occurs, but peptide bond formation during extrusion may also influence extrudate characteristics (8). However, many protein-rich materials have already undergone some denaturation prior to extrusion. Soy meal is generally heated to evaporate residual hexane used in oil extraction, therefore full-fat soy flour will have a different nitrogen solubility from defatted soy flour.

Other food components also influence denaturation. Cell wall components, high fat and/or moisture levels may protect cellular proteins from thermal excesses by preventing the desired mass temperature levels from being achieved. Cheftel (9) has suggested that minimal protein deterioration occurs during high-moisture (>25% weight basis) extrusion for this reason.

The extent of denaturation is most often estimated by measuring the change in the nitrogen solubility of the protein after extrusion. Protein in cornmeal snacks had low solubility in pH 6.8 phosphate buffer, but considerably more protein was solubilized by a 1% sodium dodecyl sulfate (SDS) solution (10). Additional protein was extracted with a mixture of 1% SDS and 1% 2-mercaptoethanol. The mercaptoethanol disrupted disulfide bonds, thereby increasing solubility in the aqueous system. Extrusion conditions of low barrel temperature, low screw speed, and low moisture increased denaturation as measured by protein solubility, as compared with high temperature, screw speed and moisture. Slower screw speed may have resulted in a longer residence time and, in combination with the low water content of the feed material, may have increased thermal damage to the protein.

FUNCTIONAL CHARACTERISTICS

Solubility. The solubilities of a protein in water or buffer solutions of neutral pH depends not only on the extent of denaturation (reversible or not), but also on the isoelectric point of the protein, which is the pH at which the net global charge on the protein is zero. An imbalance of charges is necessary to keep the protein suspended in water or dilute salt solutions. Thus, the protein precipitates at the isoelectric point.

The final product use of a protein must be considered when evaluating the effect of extrusion on nitrogen solubility in water or buffer solutions of neutral pH. Acid casein is a by-product of cheese manufacture that is neutralized with alkali for use in coffee whiteners and other liquid products. Tossavainen and co-workers (11) compared casein neutralized by a traditional batch process with extruded casein. Sodium bicarbonate was added at the extruder at levels of 2.3%, 4%, and 6% (weight basis). The effect of increased sodium bicarbonate on pH and solubility of the caseinate is shown in Figure 2. Complete solubility was achieved with the use of 4% sodium bicarbonate.

Millauer, Wiedmann, and Strobel (12) neutralized acid casein with sodium hydroxide and different feed moisture levels. A post-extrusion drying step was necessary for the viscous caseinate extruded at 50% moisture with 25% NaOH. However, at 6–20% moisture and 20–40% NaOH, stable, low-moisture strands were produced directly from the extruder. Potassium and sodium casein salts produced in this manner were water-soluble with high water absorption capacities at low temperatures. Calcium caseinates were colloidal and suitable for use in baked goods.

Emulsification. Proteins have varying abilities to form emulsions with lipids. The distribution of lipid droplets within a protein matrix depends upon both the amount of oil present and the prior thermal history of the protein. Defatted soy flour with up to 15% oil added could be texturized successfully, while full fat soy flour containing 20% oil could not (13). Lipids are less readily extracted after extrusion due to binding with proteins (14) and carbohydrates (15).

Zuber *et al.* (16) evaluated fat emulsification in extruded processed cheese. Increased screw speed and the use of a high-shear screw profile produced finer emulsions. The use of polyphosphates in cheeses produced by batch or extrusion cooking was essential to emulsification. The average size of fat droplets in extruded cheese decreased with increasing fat content. These findings have implica-

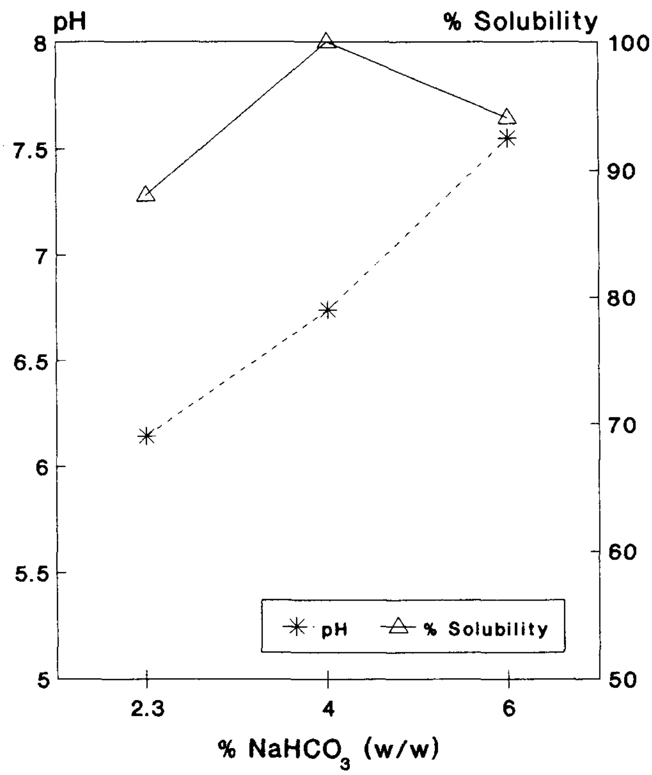

FIG. 2. pH and percent solubility of acid casein extruded with increasing amounts of sodium bicarbonate. Extrusion conditions were: Creusot-Loire BC45 twin screw extruder (Clextral, Inc., Tampa, FL), 600 mm long screws, 150 rpm screw speed, moisture content of 24%, mass temperature 80-84°C. Adapted from Tossavainen *et al.* (11).

tions for other emulsified products such as extruded sausages and feeds.

Gelation. The retention of gel-forming capabilities is important for many extruded protein products, particularly high-moisture materials processed with twin-screw extruders. The gel rigidity, elasticity, and water retention of extruded whey protein isolate were not impaired at temperatures below 133°C in a 1000 mm-long Clextral BC-45 extruder (Clextral, Inc.) or below 145°C in a 500-mm barrel (17).

Gel properties are improved by the addition of carbohydrates and other compounds. Firm gels were produced from soy protein isolates to which starches or gums had been added prior to extrusion (18). Mechanically deboned chicken meat could not be extruded as a gel without the use of binding or gelling agents (19). Flying fish meat with added salt was texturized into a kamaboko-like sol by cooling the extruder barrel with ice or tap water (20). The extruded fish was heated in water after extrusion to induce gelation, but no information was available on protein changes.

Texture. Extrusion has been used as an alternative to alkaline dope spinning for the production of fibers from soy that resemble meat (7,21). This texturized soy material is later hydrated to improve palatability. Smith (22) observed that reduced pH resulted in a chewier soy product with reduced water absorption capacity and smaller cell size. A less chewy product with increased water absorption could be produced by adjustment to a pH slightly more alkaline than normal.

Fiber formation is inhibited by soy carbohydrates (23) present in soy flour and concentrate. Soy protein isolate also increases expansion when extruded (10,24,25). This characteristic can be exploited when an expanded product is desired from a base material that expands poorly.

Loss of volatiles. Expanded products are often blander in both flavor and odor than the original feed material. This phenomenon may be advantageous for some products, but in other foods additional flavoring will be required. Prior to extrusion volatile compounds may be bound to proteins or polysaccharides. During extrusion these ligands are loosened or broken. Some volatiles are lost with water during the expansion process (26). Extruded products containing soy or other oilseeds may develop beany or grassy odors and flavors during storage because additional bonds between volatiles and macromolecules are weakened.

In addition to the losses that occur as a result of expansion, several other factors may contribute to increased blandness in extrudates. Volatiles may react with macromolecules and become less easily perceived. Palkert and Fagerson (27) studied the retention of added volatile compounds to soy and concluded that thermal degradation, oxidation, and polymerization of these chemicals may also occur at higher extruder temperatures. There may also be losses due to evaporation during post-extrusion drying operations.

The type of protein used also affects the loss of volatile materials. Maga and Kim (28) examined the effects of extrusion parameters on volatiles and sensory evaluation of extruded cornstarch-protein blends. Higher levels of protein (5%, 15%, 30%, 50% w/w) resulted in stronger flavor scores by the sensory panel. Higher moisture and lower dough temperature were associated with the production of blander samples. Figure 3 shows the differences between protein sources at both temperature levels and low moisture. The differences between defatted soy flour (DSF) and soy protein concentrate and between whey protein concentrate (WPC) and sodium caseinate may be due to the presence of carbohydrates in the DSF and WPC. A greater number and concentration of volatiles were detected by the gas chromatograph for the samples which received bland scores. This aspect, as well as the use of extruders as reactors for flavor production deserve further attention.

NUTRITIONAL CHANGES

The losses in protein and micronutrient quality due to extrusion have been a major concern, and several reviews on the subject have been published (2,15,29–33). In general, extrusion causes no more damage to protein quality than do other methods of thermal processing.

Digestibility. The unfolding of protein molecules (Fig. 1) during denaturation renders them more susceptible to digestion by proteolytic enzymes. Thus, extrusion can enhance the digestibility of proteins through denaturation and by inactivation of protease inhibitors (32), but these improvements are generally less than 5%. The true digestibility (TD) by rats of four plant protein blends was increased significantly by extrusion (34). The TD of a fifth blend of sesame, soybean and peanut meals was slightly decreased compared with the raw material.

The *in vitro* digestibility of extruded corn gluten meal (CGM) and CGM-whey blends was higher when extruded at pH 7.00 than at pH 3.85, and also at higher screw speeds (35). Higher barrel temperature and larger ratios of fish:wheat contributed to small increases (<6%) in the *in vitro* digestibility of the extruded blends over the nonextruded minced fish and wheat flour (36). The higher temperature was assumed to increase denaturation, while the improved protein quality of the fish, as compared with the wheat, was responsible for better digestibility. Increased temperature and pH also improved the *in vitro* digestibility of extruded sorghum (37).

Available lysine. Since lysine is the limiting amino acid in cereals and some oilseeds, the effect of extrusion on this nutrient has been studied extensively. During heating of a food, reducing sugars or other carbonyl compounds may react with the ε-amino group of lysine, thereby reducing its biological availability. The loss of available lysine can be minimized by removing reducing sugars such as glucose and lactose from the formulation, but starch and sucrose may be hydrolyzed into reducing sugars by the shear during extrusion (38,39).

Higher temperatures during extrusion may increase the rate of Maillard reactions, but this effect may be offset by increased moisture content (Fig. 4) (40). Noguchi and co-workers (38) suggested that a mass action effect may occur at higher moisture contents. The effect of increasing screw speed is unclear since two opposing effects take place simultaneously: increased shear may favor the reaction, while reduced residence time may limit the exposure of the food to adverse conditions (36).

This situation is further complicated by a lack of agreement between *in vitro* and *in vivo* methods of assessment (40). The Hunter L values for wheat-based breakfast cereals produced by extrusion and other methods was

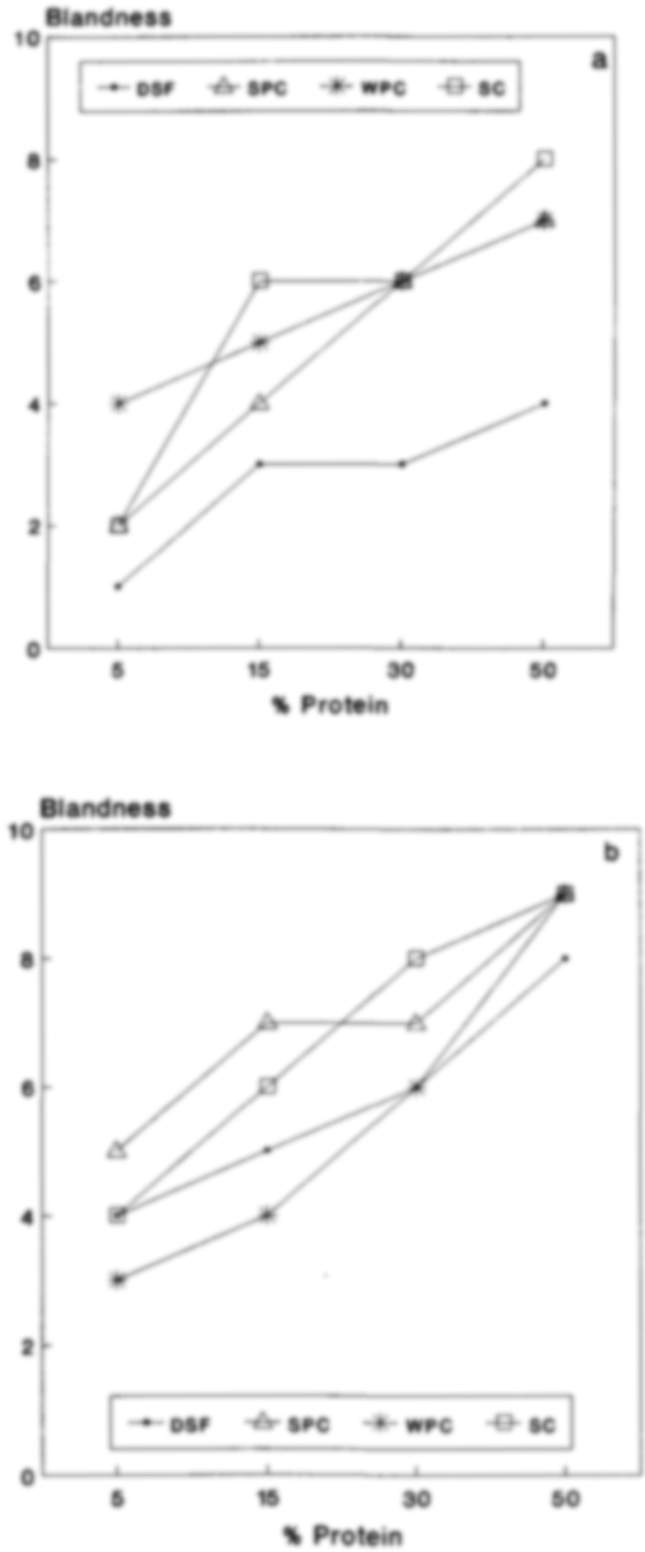

FIG. 3. Sensory blandness scores of various protein materials blended with corn starch at levels of 5–50% (dry weight) using a Brabender Plasticorder Model PL-V500 (C. W. Brabender Instruments, S. Hackensack, NJ) with screw speed of 120 rpm and a 3:1 screw compression ratio. Scale: 1=bland, 10=strong. a. Samples extruded at 120°C and 15% moisture; DSF, defatted soy flour; SPC, soy protein concentrate; WPC, whey protein concentrate; SC, sodium caseinate. b. Samples extruded at 150°C and 15% moisture. Adapted from Maga and Kim (28).

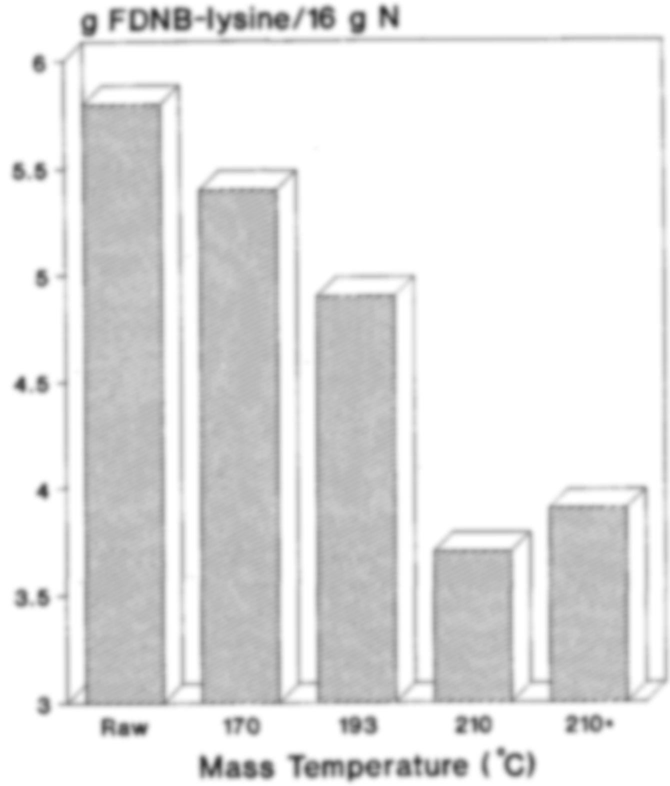

FIG. 4. Effects of extrusion temperature and moisture content on available lysine retention in wheat flour. Raw=unextruded wheat flour; 210* had a moisture content of 18%; all other extruded samples had 13% moisture. A Creusot-Loire BC-45 extruder was used. Adapted from Bjorck *et al.* (40).

highly correlated with available lysine and *in vitro* digestibility (41). Color measurement may offer a solution for the determination of lysine loss in quality assurance programs.

Lysinoalanine. Amino acids may crosslink during food processing, particularly under alkaline conditions. Lysinoalanine (LAL) is the most common of these compounds, which interfere with growth in rats. There is less LAL formed in extruded foods as compared to similar products prepared by traditional methods (21). The LAL content of caseinate extruded with 2.3% sodium bicarbonate was zero; less than 95 μg per gram were recovered from caseinate extruded with 6% sodium bicarbonate (11). This level was well below the maximum found in commercial caseinates at that time. Extrusion did not significantly increase the content of LAL or lanthionine in field bean and soy bean concentrates (42).

Antinutritional factors. Many seeds which have high protein contents also contain compounds that interfere with protein digestion or otherwise impair health. Extrusion, like other thermal processes, may reduce the amount or the activity of such compounds (Table 2). Cheftel (9) has summarized much of the research conducted in this area. Trypsin inhibitors and other relatively large proteinaceous toxic compounds can be denatured during extrusion at moisture contents of approximately 20% by weight.

TABLE 2

Toxic Factors Reduced by Extrusion

Toxic material	Foodstuff
Protease inhibitors	Legumes
Gossypol	Cottonseed
Glycoalkaloids	Potato
Ricin	Castor bean
Glucosinolates	Rapeseed
Aflatoxins	Peanuts

The other compounds listed in Table 2 are smaller and less thermolabile (9). Less of these materials have been recovered after extrusion, but for some compounds there is some doubt whether they are destroyed or merely bound to other molecules, and thus less easily extracted. For example, extrusion reduces the level of free gossypol in cottonseed flour or meal because the gossypol reacts with lysine. The bound gossypol may not cause adverse effects in itself, but the PER of extruded blends containing cottonseed was reduced due to the loss of available lysine via gossypol binding (43). Higher retention of available lysine and reduced free gossypol was achieved with increased water and additional passes through the extruder (44). These studies were conducted with low-cost single screw machines, and improved quality may be possible using twin screw extruders if capital investment in equipment is not a limiting factor.

Some substances are inactivated during extrusion by increased exposure to neutralizing agents. The extrusion of castor bean meal with lime reduced the levels of ricin and CB-1A allergen compared to dry extrusion alone (45). The total glucosinolate content of rapeseed-soybean mixtures was reduced by 80% by extrusion at 150°C with the addition of 5% alkali and 1% ferrous sulfate, but toxic levels of nitriles developed as a result of glucosinolate hydrolysis (46). Extrusion alone decreased total glucosinolates by about 20–35% (46,47).

The presence of aflatoxins in peanuts, corn, cottonseed, and other seeds poses a serious health threat, especially in tropical areas. Ammoniation of feed is permitted to detoxify aflatoxins, and peanut meal with 0–2.5% ammonium hydroxide extruded at 175°C or 185°C had residual aflatoxin contents that were 20% or less of the initial levels (48). Aflatoxin destruction was slightly greater at the higher temperature.

FUTURE DEVELOPMENTS

Many new uses for twin screw extruders are possible. Improved hydrolysis procedures for starch have been demonstrated (5), and this type of process may be applicable to the production of protein hydrolysates for flavorings or for special diets. Thermostable enzymes could be mixed with protein in the barrel, then incubated with additional enzymes post-extrusion to produce hydrolysates. A combination of low barrel temperature to facilitate enzymatic digestion and minimize protein damage, low pH to increase hydrolysis, and high shear could sufficiently break down vegetable proteins and animal by-products into value-added hydrolysates.

The production of protein films could be improved by the use of extruders, but apparently this concept has not yet been attempted. Plastic films are extruded, and the take-away equipment could be modified to process films from gelatin, soy, or other proteins. Expansion would be discouraged to prevent bubbles and other defects. A slit die would develop the necessary geometry, and rollers could adjust the tension of the still-pliable film. These and other projects remain to challenge the protein chemists and engineers in this dynamic area of research.

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