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RESTRUCTURING OF MECHANICALLY DEBONED CHICKEN USING

TWIN-SCREW EXTRUSION

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RESTRUCTURING OF MECHANICALLY DEBONED CHICKEN USING TWIN-SCREW EXTRUSION

by

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ABSTRACT

RESTRUCTURING OF MECHANICALLY DEBONED CHICKEN USING TWIN-SCREW EXTRUSION

By

Valente Alvarez-Barrera

A twin-screw cooking extruder was used to restructure mechanically deboned chicken (MDC) in combination with three non-meat binders, corn starch (CS), soy protein isolate (SPI) and wheat gluten (WG) at concentrations of 10-30%. Extrudates were evaluated by apparent tensile strength (ATS), Warner Bratzler shear stress (WBSS), proximate composition and reheat yield. SPI and WG were less effective than starch for increasing the ATS and WBSS of extruded MDC. The effect of die temperatures between 71-115°C on chemical and textural properties of extrudates containing 10 and 15% starch were investigated. The ATS and WBSS increased as a function of temperature up to 104°C. Fat content and lipid oxidation decreased as extrusion temperatures increased from 71 to 104 °C.

Proximate composition, textural properties and microstructural changes of mechanically deboned chicken/corn starch extrudates were evaluated as a function of die and barrel temperature in a twin-screw extruder. Moisture and protein content did not change significantly, but fat content decreased as die temperature increased. When die temperature increased from 71°C to 104°C, apparent stress at

failure of extrudates containing 10% corn starch increased by 12 kPa while those containing 15% corn starch increased by 44 kPa. Textural changes in extrudates were correlated to changes in the protein matrix, fat globules and starch granules observed by scanning electron microscopy.

Protein extractability in 0.9% NaCl and starch gelatinization of mechanically deboned chicken:15% corn starch extrudates were determined as a function of position and temperature in the barrel. Protein extractability decreased and starch gelatinization increased as product passed through the barrel. Minimum extractability occured in section 5 at 104°C and maximum starch gelatinization occurred in section 3 at 112 °C. The number of protein bands decreased from 15 in the first barrel section at 48 °C to 5 in the last barrel section at 104 °C. The relative protein molecular weights were in the range of 13000-201000.

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I. INTRODUCTION

Introduction to Research

Mechanically deboned poultry meat (MDPM) has been used in the United States since about 1965. The rapid development of its use has been stimulated by low cost and nutritional advantages (Field, 1988). Restructuring into value-added products is of considerable interest to the poultry meat industry as a means of upgrading the value of MDPM.

The use of MDPM has been limited by the final characteristics of the product after deboning. Mechanically deboning disrupts muscle fibers and alters the protein and lipid composition of meat, which may result in flavor instability, protein denaturation, lipid oxidation and formation of undesirable characteristics such as paste-like and grainy texture (Johnson et al., 1974; Dawson and Gartner, 1983). The use of mechanically deboned chicken (MDC) may cause problems such as poor water holding capacity, poor binding between meat pieces and a mealy, coarse texture in finished processed products (Lampila et al., 1985).

Mechanically deboned chicken is used in a variety of further processed products. The usage level in poultry products has been dependent on technological capabilities and its use has reached 100% in products like bologna and frankfurters. The United States Department of Agriculture permits the use of up to 15% poultry meat in comminuted red meat products (Froning et al., 1971; Field, 1988). Products in which MDC has been used with success include frankfurters, bologna, salami, turkey rolls, ground raw patties, cutlets, diced turkey, dry soups and casserole dishes (Froning, 1981).

Studies on extrusion and texturization of MDPM have reported acceptable products using single screw extruders or by addition of non-meat proteins, although the need for improvement in the binding of meat pieces in restructured MDC was indicated (Lampila et al., 1985). In the last decade, twin screw cooking extruders (TSCE) have received significant attention in the food industry. Twin-screw extrusion technology has the advantages of multiple controls and ability to accomplish a variety of food processing operations (Morgan, 1988). Twin-screw cooking extrusion technology has been used extensively to prepare meat analogs from defatted vegetable proteins in the United States and to texturize cereals and to produce bread analogs in Europe (Gwiazda et al., 1987).

The use of TSCE was suggested to improve the texture of mechanically deboned chicken MDC (Lingle, 1987), however, few studies have been reported on the twin-screw extrusion of MDPM or red meat derivatives. Further research on restructuring of MDPM was suggested due to limitations in the extruder system utilized (Megard et al., 1985). The extrusion of pure meat was difficult due to feed and instability problems (van Zuilichem and Jager, 1990). Feed problems were related to the high water content in the meat. Addition of pigskin collagen or corn starch produced stable extrusion process at 40% (w/w) moisture content. Upon achieving stable extrusion process the formation of a structure consisting of meat particles enveloped in a starch matrix was suggested. The objectives of this research were:

Study 1. To assess the effect of twin-screw cooking extruder process conditions and non-meat binders on restructured MDC product quality; specifically texture, reheat yield and lipid oxidation.

Study 2. To investigate changes in texture and microstructure of restructured MDC/corn starch as a function of extruder barrel and die temperature.

Study 3. To determine changes in extractability of 0.9% NaCl soluble proteins and starch gelatinization during extrusion of MDC/CS mixtures as a function of barrel position and temperature at constant extrusion conditions

Dissertation Organization

This dissertation contains five chapters. The first chapter is a common review of the literature for the entire dissertation. The second chapter is a detailed materials and methods section in which every method used in the research is completely described along with the study in which it is used.

Chapters three through five are the three studies which are the dissertation research. Each study was organized using the format of the Journal of Food Science and contains the following sections: Introduction, Literature Review, Materials and Methods, Results and Discussion. Chapters six and seven are the overall conclusions of the research and recommendations for future research, respectively. The final chapter contains the references for the entire dissertation.

II. LITERATURE REVIEW

MECHANICALLY DEBONED CHICKEN MEAT

Characteristics

Different terms have been used to describe the product resulting from the mechanical separation of meat from bones. The history and the regulations have been reviewed by the USDA (1987). This type of meat product has been referred to as mechanically deboned meat (MDM), tissue from ground bone (TFGB) and mechanically processed (species) product. The USDA calls the meat products obtained from chicken and turkey, mechanically deboned poultry (MDP). Mechanically separated meat (MSM) is the term that was adopted at the 10th session of the Codex Committee on Processed Meat and Poultry Products in Copenhagen in 1978 and by the USDA in 1982. European countries use the name of mechanically recovered meat (MRM) for red meat and poultry (Field, 1988).

Production of Mechanically Deboned Poultry Meat

Mechanical deboning has become a widely accepted and economical means of removing meat from poultry carcasses (Lampila *et al.*, 1985). Mechanically deboned poultry meat (MDPM) has been used in the United States since about 1965.

The use of MDPM in further processed products reached 1.8 x 10^8 kg in 1984 (Schuler, 1985). Restructuring of mechanically deboned chicken in new forms to produce value-added products is of considerable interest to the poultry meat industry as a means of upgrading the value, which is currently sold for about 46-53 cents/kg (Urner Barry's, 1989).

MDPM is used generally in products such as dry soup mixes, boned turkey rolls, casserole dishes, bologna, frankfurters and other types of sausages (Froning et al., 1971). One problem involving the use of MDC in processed products is protein binding between pieces of meat and development of acceptable texture (Lampila *et al.,* 1985). The mechanism of binding is very complex. Binding includes both the cohesive force exerted between the binding matrix and the meat pieces and the strength of the binding matrix itself. The main factors that determine the efficacy of binding are protein extraction, mechanical treatment, presence and concentration of added salts, pH and the temperature of heating (Trout and Schmidt, 1984). Myofibrillar proteins are the most important muscle proteins for achieving binding between pieces of meat (Bouton et al., 1981).

Process of Mechanical Deboning

Different deboners have been developed for separation of poultry meat. Mechanical deboners can be classified in two groups. One forces the meat from the outside to the inside of a perforated drum, leaving the bone residue on the outside of the drum. The other group separates the meat from the bone by forcing the meat from the inside to the outside of a perforated cylinder, leaving the bone residue on the inside (Froning, 1976 and 1981). In both cases, the principle of the process of mechanical deboning involves grinding meat and bone together through a 1.3 to 3.0 cm plate. To remove the meat, the ground material is forced through a fine screen or slotted surface of a mechanical deboner. Bone particles are separated and become a part of the waste residue (Froning, 1981).

Composition and Structural Characteristics

The variability in composition of different anatomical parts of the animal causes the composition of MDM to vary considerably. Posati (1979) found that broiler or fryer carcasses contain 15% fat on a raw basis, whereas stewing chicken carcasses average 20% fat. The average fat content in MDPM is about 18.5% (Field, 1988).

The amount of myofibrillar protein was lower in mechanically deboned turkey (MDT) than hand-boned meat products. MDT showed higher water-holding-capacity but lower emulsifying capacity than hand-boned products (McMahon and Dawson, 1976). MDPM has a protein quality comparable to hand deboned meat. The amino acid composition and 2.5 Protein Efficiency Ratio (PER) value of MDPM are similar to hand deboned meat from the same sources (MacNeil et al., 1978; Babji et al., 1980). The PER value was comparable to the 2.9 of the casein control observed by Crawford et al. (1972).

Using different pressures (40-150 lb/in^2) to mechanically debone whole roaster breast, significant differences in chemical composition, lipid oxidation and fatty acid content were observed (Barbut et al., 1989). Mechanically deboning alters the lipid and protein composition of the resultant meat paste (Gruden et al., 1972; Janky and Froning, 1975). MDPM contained lower protein and higher fat, than hand deboned meat of the same source (Froning, 1970; Froning and Johnson, 1973). The variability in fat content and chemical composition has been attributed to factors such as age of the bird, bone-to-meat ratio, cutting methods, deboner settings, skin content and protein denaturation (Froning, 1976).

Initial studies reported no differences fatty acid content of MDPM as compared to hand deboned meat. Moerck and Ball (1973) found that bone marrow lipid is high in polyunsaturated fatty acids which have a lower melting point than lipids from hand-boned meat. They also found that chicken bone marrow fatty acid composition is similar to that of hand deboned products. Substitution of MDPM for hand boned products did not influence the fatty acid content of the product (Murphy et al., 1979). Froning (1981) reported that high amount of lipids and heme components, which are released from the bone marrow during deboning, produce differences in the chemical composition of MDPM and hand deboned meat.

The amount of bone in MDPM is limited to 1% (USDA, 1969). Studies showed that bone content in MDPM was not a health problem and bone particles were substantially smaller than those from hand-deboned sources (Grunden and MacNeil, 1973; Froning, 1979; Murphy et al., 1979). Overall bone content of commercial MDPM varies widely depending on the processing methods and raw materials used (Patashnik et al., 1974; Field, 1988).

Stability and Microbiology

Mechanical deboning disrupts the muscle fiber and alters the protein and lipid composition of the meat which may result in flavor instability and formation of undesirable functional characteristics, protein denaturation and lipid oxidation (Johnson et al., 1974; Dawson and Gartner, 1983). These undesirable changes are the result of the process conditions, which incorporate air, the contact with metals and uses high temperatures in some cases. The changes are also enhanced by the final characteristics of MDPM such as particle size, heme pigments and unsaturated fatty acids from bone marrow (Newman, 1981). Antioxidants such as ascorbic and citric acids, and a combination of chelating agents and polyphosphates were effective in maintaining a desirable flavor in MDPM during storage (Froning, 1981, Smith, 1987).

Mechanical deboning may also enhance microbial growth. Studies have indicated that microbial quality of MDPM is variable and depends upon factors such as meat source, temperature of deboning, handling and storage. These conditions cause variation in microbial counts from 100,000 to 1000,000 microorganisms/g and counts from 10 to 1000 coliforms/g (Froning, 1976). Total aerobic counts of delayed-processed (held 5 days at 3-5^{o}C before deboning) MDCM were higher than in conventionally processed meat and remained the same throughout storage (Maxcy et al., 1973).

Ostovar et al. (1971) reported that total aerobic counts and Most Probable Number (MPN) fecal coliforms may increase during storage at 3^{o}C. *Salmonella, Clostridium perfringens. Pseudomonas, Achromobacter, and Flavobacterium* are the dominant psychrotolerant genera. Freezing significantly reduced fecal coliforms.

The microbial characteristics of MDPM have been reviewed (Ostovar et al., 1971). It has been suggested that the same safety and good manufacture conditions used for hand-deboned meats apply to MDPM (Froning, 1981; Newman, 1981). To insure low initial bacteria populations and to reduce access to pathogens, strict sanitary conditions should be taken during production, storage and use. To inhibit the growth of bacteria, MDPM should be quick-frozen immediately in small volumes. Frozen products should be thawed rapidly and used immediately (Ray et al., 1984).

FUNCTIONAL PROPERTIES OF MDPM

Proteins

The functional properties of MDPM are related to the major components in the meat system which include myofibrillar and connective tissue proteins and fats. Amount of protein, extent of protein denaturation, overall composition and storage conditions may also affect the functional properties of MDPM. These characteristics are influenced by the mechanical deboning process. The functional and compositional interrelationships have been reviewed by Froning (1976, 1981) and Field (1988).

Emulsion Properties

Salt-soluble proteins play the most important role in meat emulsions since they participate in forming the interfacial films (Swift and Sulzbacher, 1963). In general, the high pH value above 6.5 of MDPM increases extraction of the muscle proteins. This pH improves the emulsifying characteristics of the meat if other factors that influence emulsification can be controlled (Field, 1988).

Investigations of the emulsifying capacity of chicken muscle proteins indicated that myosin by itself participates more rapidly in the formation of an emulsion than actomyosin. The presence and interaction with actin may interfere in the reaction since actin was the last protein being taken up into the internal phase of the emulsion (Galluzzo and Regenstein, 1978a; 1978b).

The functional properties of products made from MDT containing different levels of protein and fat were evaluated. Higher protein levels in the products increased shear values and emulsion viscosity, but reduced the sensory scores for tenderness and juiciness. Higher fat levels produced firmer products with more viscous emulsions (Baker and Darfler, 1975).

Meat batters prepared from MDPM containing 11 and 12% protein were studied using a temperature controlled capillary extrusion viscometer (Froning, 1970). Batters with 12% protein were more viscous and exhibited less water and fat release during emulsion stability tests than batters containing 11% protein. The batters were less stable at higher fat concentrations. It was observed that MDPM had less protein matrix available for emulsion formation. The lack of protein matrix was caused by protein loss due to heat denaturation during the deboning process (Froning, 1970).

Water-holding capacity and emulsifying properties can influence the cooking loss values in processed meat products containing MDM. Cooking losses can vary from 17.8 to 9.9% in products containing 0 to 20% of MDM respectively (Pisula and Rejt, 1979). Products which contained MDM showed slightly lower cooking losses as compared to similar products made with hand-deboned meat. These results and other similar studies suggested that cooking losses of most products do not change when MDM is added at levels which range from 10 to 20% (Field et al., 1977).

The amount of skin in MDPM affects emulsifying characteristics. Skin may reduce emulsion stability and emulsion capacity due to the increase in fat content in MDPM (McMahon and Dawson, 1976). Froning (1981) found no significant change in emulsifying capacity between MDPM produced with and without skin.

Using timed emulsification, low-salt soluble proteins showed little emulsifying effect in the presence or absence of high-salt soluble proteins of chicken breast muscle. Therefore, the results indicated that low-salt soluble proteins have a limited role in emulsions (Gaska and Regenstein, 1982a). Exhaustively washed chicken breast muscle fractions (EW) and purified myosin produced emulsion stabilities which suggested that these two different protein solutions emulsified oil in a similar manner.

Greater amounts of protein for EW muscle emulsions was needed to achieve the same stability of emulsions made with myosin. The difference was attributed to the insolubility of EW which had inferior emulsifying properties (Huber and Regenstein, 1988). Low concentrations of myosin heated to high temperatures before emulsification or after centrifugation did not change the stability. Heating between 40 and 60^{o}C after mixing but before centrifugation caused great improvement in emulsion stability. Pre-centrifugation cooking produced greater weight emulsions than emulsions prepared by any other heating regime which indicated an initially larger network of protein, oil and water. Post-centrifugation slightly improved emulsion stability and removed some of the water from the emulsions, resulting in a reduction in the original weight (Huber and Regenstein, 1988).

Cooking temperatures of model chicken breast muscle emulsions before mixing or after centrifugation produce different results in timed emulsification and emulsion stability studies. All emulsions cooked after centrifugation were stable, but emulsions of actomyosin showed a large decrease in emulsion stability when heated above 40^{o}C prior to mixing. Timed emulsification did not decrease significantly until proteins were heated between 60 and 75^{o}C (Perchonok and Regenstein, 1986).

The amount of red marrow which increases the amount of lipids in MDPM has a significant effect on the emulsion properties by decreasing the emulsion stability. Marrow is high in albumin and hemoglobin but has no myosin, which has superior binding strength of meat pieces compared to other muscle proteins (Ford et al., 1978).

Gelation

Several researchers have recognized that the gelling properties of muscle components are very important in restructured meat products. The need to develop an understanding of the rheological changes in the proteins during processing and the physical attributes of the final heat-set gel products and their relationship to sensory texture was pointed out by Montejano et al. (1985). Although information on changes of proteins during heating is still limited, changes such as shrinkage, toughening and release of juice are attributed to alterations in muscle proteins. Modifications in proteins affected appearance, flavor and texture of chicken meat proteins (Kijowski and Mast, 1988a, 1988b).

The gel-forming proteins may determine the rheological characteristics in the product since they act as texture and structure building components (Acton et al., 1983; Asghar et al., 1985). Heat induced gelation of myofibrillar proteins, especially myosin, is responsible for the texture of comminuted meat products (Webb, 1974; Schut, 1978; Comer, 1979).

Different gelation properties between salt-soluble proteins from breast and thigh have been associated with interactions among myofibrillar proteins, especially myosin and actomyosin ratio. Other possible reasons are differences in thermal transitions and association properties of protein isoforms. (Dudziak et al., 1988).

Ishioroshi et al. (1980) observed that the rigidity of myosin gels was enhanced at a myosin/actin weight ratio of 15:1.

Protein gelation takes place by intermolecular interactions which result in forming a three-dimensional network of protein fibers. This protein system holds water molecules by capillary forces (Asghar et al., 1985). Different proteins may have different gel forming mechanisms since gelation is influenced by the type of molecular interactions that stabilize the gel of each protein system. The interactions include hydrogen bonds, disulfide linkages, peptide bonds, electrostatic and hydrophobic interactions (Stainsby, 1977).

The source of myosin will affect gelation. The gelation of chicken myosin occurred at 49^{o}C. The rigidity of myosin gels obtained from chicken breast was greater than myosin gels prepared from chicken legs (Asghar et al., 1984). Using differential scanning calorimetry (DSC), Kijowski and Mast (1988a) identified the thermal transitions of muscles and isolated fractions of chicken broilers. They found five endothermic transitions at 57.1, 62.5, 67.3, 72.8 and 77.7 ^{o}C in the case of breast muscle. The thermal curves of isolated protein fractions showed two major transformations at 57.1 ^{o}C and 77.7 ^{o}C which were related to myosin and actin, respectively. These thermal transitions changed when muscles and water-washed myofibrills were treated with NaCl and either pyrophosphate or tripolyphosphate.

Salts decreased the thermal stability of muscle systems producing only one transition temperature at 66.6 ^{o}C as compared with five transition temperatures of the control muscle. The presence of phosphate at concentrations of 0.25 and 0.5% enhanced the thermal stability of myosin (Kijowski and Mast, 1988b). Three major thermal transitions were also observed in chicken breast salt-soluble proteins at holding temperatures of 55-85 ^{0}C (Wang et al., 1989).

Water Holding Capacity

MDPM has higher pH as compared to the hand-deboned meat. The increase in pH is due to the incorporation of marrow which has a pH range of 6.8-7.4 (Field, 1981). It has been found that there is a linear increase in pH with an increase in red marrow:muscle ratio. The increase of pH is also accompanied by a linear increase in water-holding capacity (WHC) (Arasu et al., 1981).

Water-holding capacity of MDPM may be affected by the presence of calcium and magnesium from bone particles. Investigations have reported the decrease of WHC by divalent cations as Ca^{2+} and Mg^{2+}. When these divalent cations are present, the pI of myosin is shifted to a more alkaline pH range (>5.76) and affects the water holding capacity (WHC) (Asghar et al., 1985). Studies suggested that there is a negative correlation between WHC and cations content. This effect could offset the increased extraction of the muscle proteins due to the increased pH in MDPM (Gola et al., 1977).

The presence of Ca^{2+} ions caused the formation of ordered nonfilamentous aggregates from myosin (Koretz et al., 1982). These divalent calcium cations may reduce the muscle structure spaces by forming cross-bridges between peptide chains (Winegrad, 1965). These positively charged elements can also bind with negatively charged groups such as phosphate, hydroxyl, sulfhydryl and carboxyl which are associated with proteins (Schut, 1976).

Effect of Processing on Protein Solubility

Heat processing of meats causes both chemical and physical changes in sarcoplasmic and myofibrillar proteins. This effect has been studied by the loss of protein solubility and changes in protein electrophoretic mobilities (Crespo and Ockerman, 1977; Davis et al., 1987; Steele and Lambe, 1982). Solubility loss of sarcoplasmic proteins extracted from muscle with water or low ionic strength solutions (0.9% NaCl) has been investigated as an indication of the degree of heat denaturation (Hamm, 1977; Lee et al., 1974; Crespo, 1977).

Food Safety and Inspection Service (FSIS) is using a protein "Coagulation Test" for monitoring the maximum internal temperature (MIT) of meat products heat processed at temperatures lower than $65^{0}C$ (Townsend and Blankenship, 1989). In the coagulation test, soluble muscle proteins are extracted with 0.9% NaCl solution, the extract is filtered and heat is applied to the filtrate. The loss of protein solubility is measured as temperature of the product is increased. The temperature at which the first signs of cloudiness or turbidity is recorded as the MIT of the product (USDA-FSIS, 1987). The test is considered empirical because the results are based on visible observations (Townsend et al., 1984; Townsend et al., 1985).

In the early seventies, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of water-soluble proteins was tested for assessing the maximum internal processing temperature (MIT) of bovine muscle. Six distinctive bands were identified at 65°C. Increasing temperature up to 70°C one band disappeared and the intensity of the other 5 decreased. It was possible to determine the cooked temperature of the muscles within a range of ±5 °C. The presence of 4 or less distinct proteins bands in the electrophoretic method was an indication that the meat had been cooked above 70 °C. The intensity of myoglobin did not change significantly between 65°C and 75°C which indicated that there was not extensive denaturation (Lee et al., 1974).

Caldironi and Bazan (1980) also utilized SDS-PAGE for quantitative determination of low-salt soluble protein patterns of bovine muscle. They concluded that low-salt soluble protein gradually disappeared when meat is cooked between 60-80^{o}C. Similar investigation used gradient SDS-PAGE to separate and characterize the protein changes which occurred as a result of heating muscle extracts to temperatures from 65-90^{o}C (Steele and Lambe, 1982).

Investigations on different meat sources have reported that protein extractability decreased greatly as temperature increased. Using SDS-PAGE it was observed that as temperature increased from 65°C to 90°C, the intensity of six bands from proteins in supernatant extracted with distilled water from bovine muscle, decreased and another disappeared. At 85°C , one weak and two distinct bands were still detectable. At 90°C only one band was identified at the bottom of the gel. Soluble protein content of extracts (0.9% salt) of semitendinosus roast cooked to 60°, 68° and 75°C internal temperature was evaluated (Lee et al., 1974).

The densitometric scans of isoelectrofocusing gels measured with a laser densitometer showed some water-extractable proteins which remained at 75°C (Lyon et al., 1986). Water soluble extracts of samples from porcine longissimus muscles were composed of 14 proteins which were quantified and identified by reverse phase high performance liquid chromatography and SDS-PAGE. After heating to 75°C, the remaining soluble portion contained 3 proteins (McCormick, 1987). These results on insolubilization of sarcoplasmic proteins have shown some differences which have been related to variations in different species (Scopes, 1970) and environmental conditions such as pH and temperature (Greaser, 1986). The extraction procedure and identification method may also produce differences in solubility.

The results of these investigations suggest that SDS-PAGE technique could be used to characterize and differentiate protein related changes in meat products during heat processing.

NON-MEAT PROTEINS AND BINDERS

General Characteristics

Non-meat proteins such as starch and other polysaccharides, are added to foods to enhance functional properties such as texture, fat and water binding capacity, foaming and emulsion stability. A specific product can be developed using a variety of ingredients and processes. The characteristics of the final product will depend on the functional ingredient used (Hermansson, 1983).

Non-meat proteins are described as fillers when they are used in processed meat products. Comminuted meat products are the largest and most complex class of such products. The other type of meat products where non-meat proteins are used are the coarsely chopped sausage and patty products (Comer and Allan-Woytas, 1988). Methods developed for the texturization of vegetable proteins or minced meat have been applied to restructure MDM. Studies reviewed by Maurer (1979) reported the need of using non-meat proteins to improve the binding of pieces MDPM. It has not been possible to restructure MDPM without the addition of binders.

Non-meat binders are usually milk-derived and plant-derived products. The last group includes soy isolates, wheat glutens, starches, soy concentrates, textured soy flours, legume flours, baked cereals and modified starches. Proteins and carbohydrates provide functionality when incorporated into comminuted meat products (Comer et al., 1986). The functional effects of adding non-meat proteins and starch-based products to meat systems may include increased yield, textural firmness and stability.

The effect of specific non-meat proteins has been investigated and described in a recent review by Mittal and Usborne (1985). Attempts to replace myofibrillar proteins with non-meat proteins resulted in softer and less chewy products (Comer and Dempster, 1981; Decker et al., 1986). Other studies concluded that although replacement of lean meat proteins with non-meat proteins results in loss of textural firmness, chemical stability may be maintained or even enhanced (Comer, 1979; Comer and Dempster, 1981).

Starch

Starch granules generally are a mixture of amylose and amylopectin with amylose content of 15-30% (Sterling, 1978). Amylose is a linear polymer of α-D-glucopyranosyl units linked by α-1, 4 glucosidic bonds. Average molecular weight

(MW) of corn starch amylose is about 250,000. Amylopectin has short branches on about 4% of the D-glucosyl residues. The branches have many short (26-30 glucose units) α-1,-4 linked chains attached through α-1, 6 branch points. The MW of amylopectin ranges from 50 million to over 100 million (Sterling, 1978).

Starch Gelatinization

Gelatinization describes the swelling and hydration of granular starches. If enough water is present, starch grains will swell markedly upon heating to a sufficiently high temperature (Schoch, 1965). Starch is gelatinized when the granule has swollen to a point where the amylose begins to diffuse out of the starch granule. At this stage and temperatures below 115°C the hydration process is not reversible and the starch granule can not be returned to its original raw state (Remsen and Clark, 1978). The amylopectin fraction of starch is responsible for the bulk structure of starch granules. When most of the amylose is removed from starch granules by leaching in hot water, the granule retains its basic structure that is primarily amylopectin (Banks and Greenwood, 1975).

Gelation of amylopectin takes place at a much slower rate than does amylose and requires higher concentrations. Amylose gels are very firm and temperatures as high as 115-120 ^{0}C are necessary to reverse gelation. Amylopectin gels are soft and may be thermally reversed at temperatures of 50 ^{o}C to 80 ^{o}C (Zobel, 1984).

The rheological behavior of starch is influenced by the movement of amylose from within the granule structure into an aqueous phase. In hot systems, amylose affects the viscosity of the aqueous phase in which swollen amylopectin rich particles may be dispersed (Inglett, 1970).
During gelatinization there is an increase in viscosity, translucency, solubility and loss in birefringence when granules are viewed under the microscope. The greater the amylose content, the higher the temperature of gelatinization. Starches from different sources will gelatinize at different temperatures such as 60 ^{o}C in the case of potato starch and 70 ^{o}C or higher for corn starch (Sterling, 1978).

On cooling, amylose chains, originally dispersed by gelatinization, tend to associate by hydrogen bonding which causes retrogradation. The chains being brought closer together, are more likely to come in contact with one another after the first bonds are established (Schoch, 1965).

There have been many tests developed over the years to study and characterize starch gelatinization. No single test adequately described the condition and functionality of starch granules after extrusion; therefore, Chiang and Johnson (1977) used two or more tests to describe the effect of extrusion variables on starch gelatinization. Most traditional methods to determine total available glucose involve the hydrolysis of starch, containing no appreciable quantities of low molecular weight oligosaccharides such as α-D-glucose polymers, with acid or amylolytic enzymes.

The determination of glucose is conducted by chemical or enzymatic procedures (Shetty et al., 1974). A modification of the glucoamylase methods (AOAC, 1984, methods 7.808 and 14.075) are considered the most useful enzymatic methods for determining the degree of gelatinized starch in extruded foodstuffs. Budke (1984) reported a method for the determination of total available glucose in corn based materials. The method is based on the principle that gelatinized starch is easily digested by glucoamylase to form glucose. Glucose content was determined using a Yellow Spring Instrument Analyzer (Budke, 1984).

Use of Starch in Food Products

Starch can be modified chemically and physically to fulfill specific requirements in foods. The modifications can be carried out in drum dryers, stirred tank reactors and recently in extrusion-cookers which are used as reactors (Wiedmann, 1987). Complete reviews on starch characteristics, their role in different foods and applications in the food industries have been reported (Zobel 1984; 1988).

Food starches are used as a nutritive stabilizer to provide the characteristic viscosity, texture, mouth-feel and consistency of many foods such as sauces, puddings, gumdrops, and tableted products (Moore et al., 1984). The second use of starch is as a processing aid to facilitate manufacturing and enhance functional properties which include texture and freeze-thaw stability of bread and cakes (Michael and Brown, 1968).

Protein-Starch Interactions

There is limited information on functional properties of extruded muscle-based foods containing starch and protein-starch interactions. Texture, particle cohesion, binding of water and fat of starch-fish proteins systems, such as surimi, have been studied (Lee, 1984). Surimi is a wet frozen concentrate of myofibrillar proteins of fish muscle that may contain up to 10% starch. The texture of meat and fish products which contain starch is influenced by changes in its viscosity and solubility in the gelatinized state, amylopectin content and the nature of modification (Lanier, 1986).

Protein gel structure and integrity has been closely associated with textural strength, elasticity and entrapment of water and fat in the finished product (Lanier, 1986). The increase in cohesiveness and rigidity of fish protein-starch products has been correlated to an increase in the viscosity and water up take of the gelatinized starch as well as high amylopectin content of starch granules which were dispersed in the matrix (Wu et al., 1985; Lee, 1986).

The results indicated that the addition of starch reinforces the composite matrix upon gelatinization due to its structural continuity which fills the matrix (Lee, 1986).

The gel structure may be modified by the presence of water-soluble proteins or addition of water-imbibing ingredients such as pregelatinized starch. Lanier (1986) reported that the total quantity of protein or other solids may alter gel rigidity, while factors which affect the gel-forming ability of proteins would alter the cohesiveness of the gel.

The strengthening effect of native starch granules in a muscle protein matrix may be reduced by the presence of water-soluble proteins. This group of proteins possess very poor gelling properties. Water-soluble proteins retard gel network formation by interfering with the actomyosin cross-linking process (Lee, 1984). These results suggested that product functionality was influenced by type, purity and total protein content. The functionality of surimi was dependent on the myofibrillar protein fraction and the concentration of these proteins was increased by washing of minced fish to prepare surimi (Lanier, 1986).

Different types of starches also affect the texture of the product. Pregelatinized starch has excellent water-holding properties but no granule structure. It produced a loose coagulum in the muscle gel which retained water but did not have structural integrity. Pregelatinized starch interfered with gel formation of muscle proteins (Wu et al., 1985).

Changes in textural properties of fish and meat protein gels have been related to protein thermal transitions during heat processing (Liu et al., 1982; Montejano et al., 1985) Previous studies, using a Thermal Scanning Rigidity Monitor cell in an Instron Universal Testing Machine to scan muscle sol-to-gel transformations, have suggested that proteins in complex foods have different transition temperatures than the same purified proteins due to interactions with other molecules (Montejano et al., 1983). The thermal transitions of fish-protein systems determined using thermal scanning rigidity monitor and differential scanning calorimetry, did not shift in temperature with the addition of starch. This suggested that thermal transitions of starch and fish proteins occurred independently since temperatures were similar to surimi systems without starch. A marked effect on rigidity was observed after starch began to gelatinize (Wu et al., 1985).

Although extruded products are processed differently, the results of these studies might be useful for understanding the possible interactions between MDC myofibrillar proteins and starch upon extrusion, since water, fat, particle binding and texture have been linked to the development and formation of a gel network structure in most food systems (Acton et al., 1983; Montejano et al., 1984).

Soy Proteins

Soy proteins are in the group of non-meat proteins that are used to replace or extend animal proteins in meat, poultry and seafood. The soy proteins include flour, grits concentrates, isolates and chemically texturized flours, (Roberts, 1979; Wilcke et al., 1979).

Soy protein isolate contains no less than 90% protein (N x 6.25) on a moisture-free basis. Defatted soy bean flakes are used to manufacture soy protein isolates (Smith and Circle, 1972). The storage proteins which include 7S (conglycinin) and 11S (glycinin) represent about 90% of the proteins and are the principal components of soy protein. Estimates indicate that glycinin accounts for 60-70% of the soybean globulins (Peng et. al., 1984).

The major functional characteristics of soy protein isolates are water absorption and retention, emulsion formation and stabilization, fat absorption, gelation, film formation, adhesion, cohesion and elasticity (Wolf, 1970; Hutton and Campbell, 1977; Kinsella, 1979). Low-heat treated flakes are used to enhance extractability of proteins to produce an isolate with high water solubility and functionality (Meyer, 1971). The isolated proteins can be modified with various chemicals, enzymes or heat to improve protein binding, water absorption, gelation and fat emulsification (Cogan et al., 1967).

Low temperature produces dissociation of 11S into 4S subunits which slowly aggregate as temperature increases up to 70^{0} C and precipitate at 90 ^{0}C. High ionic strength solutions stabilize 11S globulins against thermal aggregation up to 80 ^{0}C. Aggregation is favored at low ionic strength. The opposite reaction was observed in 7S globulins at high and low ionic strength, respectively (Kinsella, 1979).

Solubility, turbidity and titration assays were used to determine that interactions between purified soy 11S proteins and myosin occurred at 85-100^{o}C (Peng et al., 1982b). Interactions between glycinin and myosin occur at temperatures of 85^{0}C and higher. Basic subunits of the 11S protein were involved in the interaction with myosin heavy chain. No interactions between glycinin and myosin were observed at 34.5^{o}C or 72^{o}C. This indicated that effects due to soy protein in meat products heated at temperatures lower than 70^{o}C are not due to a direct interaction of the 11S fraction (Peng et al., 1982a). The acidic components of glycinin and myosin heavy chain were not involved (Peng et al., 1982a, 1982b). Soy 11S protein may associate with unheated actomyosin but not myosin (Foegeding and Lanier, 1987)

Soy proteins are utilized in processed meats to develop products with desirable functional qualities and less expensive (Peng et al., 1982a). Investigations using soy proteins as binders in frankfurters or similar foods reported that the final products were compatible with those products made exclusively with meat (Smith et al., 1973; Terrel and Staniec, 1975; Sofos et al., 1977). The use of 8% soy protein isolate in a brine increased the yield of meat loaf products by 25% after cooking (Desmyter and Wagner, 1979). Mixed and flake-cut MDPM was structured into patties using soy proteins (Lyon et al., 1978; Lyon et al., 1980).

Wheat Gluten

Vital wheat gluten (VWG) is the principal commercially available wheat protein. Wheat flour contains about 10-15% gluten. There are two processes, air classification or water-washing, that may be used to separate gluten from wheat flour (Wookey, 1979). The functional properties of the separated gluten are influenced greatly by the wheat source, separation and drying processes. In general, VWG is about 70-80% protein and is 85% insoluble in 0.5 M NaCl solution (Wookey, 1979).

Glutenin and gliadin are the two major proteins in gluten. When gluten is hydrated and mixed it develops into a firm, resilient and cohesive substance that is insoluble in water and has both elastic and cohesive properties. The glutenin fraction, forming about 50% of the total, is tougher and less easily stretched, whereas the gliadin fraction has less cohesiveness and elasticity. In dough, gluten is a continuous film which may trap solid particles and gas. Heating gluten at temperatures higher than 85^{o}C causes irreversible coagulation and yields a firm, non-sticky, moist and resilient gel (Kalin, 1970).

The compositional factors responsible for molecular associations involved in the adhesion and cohesion of proteins and other polymers have been summarized by Wall and Heubner (1981). They concluded that molecular size, shape, and interpolypeptide S-S linking, all play a major role in the adhesion, cohesion, and film properties of gluten.

Differential scanning calorimetry (DSC) results of glass transition temperature (Tg) measurements of commercial VWG indicated that the functional properties of gluten are affected by interactions with other components, such as water and lipids. Gluten with 6% water had lower Tg (66^0C) than VWG and lipids (79^0C). The different Tg values suggested that once heated through its Tg, gluten has enough mobility, due to thermal and water plasticization, to form a thermo-set network via disulfide crosslinking (Slade et al., 1989). Tg decreased as a function of moisture content of gluten from 160^0C at 1% water to 20^0C at 16% water.

Denaturation of gluten, measured as a change in viscosity and optical rotation of the dispersion, occurred at various temperatures. Little denaturation was observed at 70 ^0C after heating for 20 min. Denaturation increased at 85 ^0C and was more pronounced and rapid at 90 ^0C if the moisture content was above 35% (Dalek et al., 1970).

Vital wheat gluten hydrates rapidly and can bind 1.5 to 2 times its own weight of water. Viscosity increased as a result of hydration and swelling. The use of wheat gluten is important in the meat industry because of its binding capacity and film-forming properties. It has been reported that the ability of wheat gluten to bind pieces of meat is similar to that of other non-meat proteins because wheat gluten has the capability to interact with myosin (Siegel and Schmidt, 1979; Siegel et al., 1979a; 1979b).

The use of wheat gluten in different meat products has been reported. Keeton et al. (1984) compared the effect of vital wheat gluten and other non-meat proteins on physical and sensory properties of frankfurters. They found that vital wheat gluten was a satisfactory binder for meat pieces because of its ability to produce intact turkey loaves. Hand et al. (1981) observed that restructured beef steaks containing 3.6% VWG were similar in overall desirability to all-meat controls. Cooking losses in meat emulsion systems were decreased using up to 40% devitalized wheat gluten (Randall et al., 1976). Exchanging VWG for 3.5% of the composite meat portion did not affect the texture of frankfurters cooked to 70°C. Patana-Anake and Foegeding (1985) observed that VWG decreased the viscosity of raw meat batters, facilitating conveying during extrusion.

TEXTURE AND MICROSTRUCTURE IN RESTRUCTURED MEATS

Measurement of Textural Properties

Different instrumental procedures have been developed to evaluate texture in restructured products. Empirical procedures for determining adhesion and apparent tensile strength were designed to measure the amount of force required to pull a single piece of restructured meat into two pieces (Booren et al., 1981). A slightly different device was developed for measuring apparent tensile strength in processed meats and can be used in certain types of restructured meats. A series of pins hold the sample while it is pulled apart by an Instron (Gillett et al., 1978).

The Warner-Bratzler shear has been widely used to measure the force required to pass a V-notched blade completely through the sample (Voisey and Larmond, 1974). Kramer shear technique is a variation of the Warner-Bratzler system in which one or more flat-edged blades are forced through the material (deMan et al., 1976). Using these methods meat samples may fail in tension, compression shear or a combination of these stresses.

A torsional failure and Instron texture profile compression analysis (TPA) were related to sensory notes from a trained texture profile panel (Montejano et al., 1985). For the torsion test, cylindrical specimens were milled to dumbbell shapes with a minimum diameter of 1.0 cm at the mid section. The torsion fixture was mounted on an

Instron universal testing machine . Shear stress and true shear strain at failure and initial shear modulus were calculated according to Hamann (1983).

Measurements of strain were highly correlated to sensory and texture profile analysis (TPA) cohesiveness, while stress, measured as compressive force to failure, was correlated to TPA hardness and sensory firmness (Montejano et al., 1985). Stress and strain to failure were related to textural strength and textural elasticity, respectively (Lanier, 1986).

During compression, true stress and strain can not be calculated because a uniform cylinder is not maintained. Diehl et al. (1979), reported a method to calculate apparent stress and strain at failure. A recent study reported that apparent shear stress and strain at failure was suitable for measuring some textural properties in meat by-product model systems (Nuckles et al., 1990).

Microstructure of Meat Products

Electron microscopy has been used as a tool to study microstructures in meat protein gels, meat emulsions and their changes due to ingredients and processing (Lee, 1985; Barbut, 1988). The greatest advantage of scanning electron microscopy (SEM) is the large depth of focus which is about 300 times that of the light microscope (Lewis, 1979). The scanning principle, the basic functions of the SEM and its use to study meat products have been previously reviewed

(Voyle, 1981). A drawback to using SEM has been how to clearly distinguish fat from protein components (Theno and Schmidt, 1978). The authors showed that commercial frankfurters vary widely in microstructure from fine protein structure to a very coarse matrix structure containing large fat globules and intact muscle pieces.

The effect of ionic strength, pH, and temperature on myosin gels was studied by Siegel and Schmidt (1979) using SEM. They observed that in the absence of salt, myosin forms a network structure gel. Turkey myofibril gel microstructure changed from a continuous filamentous matrix to a globular matrix with decreased water holding capacity during frozen storage. Microstructures of hand deboned and mechanically deboned turkey gels did not show differences between myofibril gels made from the same meat type stored with and without antioxidant during frozen storage (Smith, 1987).

Siegel et al. (1979a, 1979b) used SEM to study the gel structure of soy isolates and other non-meat proteins in model systems alone and in the presence of myosin. Different structures and interactions were identified between non-meat proteins and myosin gels. The dispersability of wheat gluten and soy proteins in comminuted meat products were observed using SEM and TEM (Comer et al., 1986). The same study reported the features of corn starch and wheat starch granules in wieners. These studies indicated that by studying the microstructure of comminuted meat products, it could be possible to establish a relationship between texture and stability due to processing conditions.

Studies have suggested that protein material surrounding fat globules stabilized the meat batter by the true emulsion theory (Borchert et al., 1967; Theno and Schmidt, 1978; Jones and Mandigo, 1982). Changes in the microstructure of meat emulsion as influenced by the type of meat and quantity of fat, moisture and salt were studied using light microscopy, SEM and transmission electron microscopy (TEM). Scanning electron microscopy and TEM were best suited for assessing fat globule morphology, especially the interface between the fat and protein matrix. The results suggested that the nonemulsion theory (physical entrapment of fat in protein matrix) should be considered to explain the matrix structure of these meat products (Lee, 1985).

The effect of meat massaging, salt and phosphate concentration in commercial frankfurters were studied using SEM (Theno et al., 1977; 1978). Salt reduction from 2.5 to 1.5% combined with 0.4% tripolyphosphate, hexametaphosphate or sodium acid pyrophosphate and chopping time were studied in poultry meat batters.

Scanning electron micrographs of reduced salt batters showed fat globule membranes which lost fat during cooking. The average fat globule size decreased from 840 to 360 μm^2 as chopping time increased from 40 to 100 revolutions, respectively (Barbut, 1988). Using SEM, Hermansson (1983) found that salt caused a disintegration of the myofibrils and formation of an aggregated protein network.

It was possible to evaluate meat microstructures from commercial sausages using both light and electron microscopy. Fat globules as small as 0.1 cm in diameter surrounded by distinct protein membranes were identified (Borchert et al., 1967).

TWIN-SCREW EXTRUSION

Principles of Food Extrusion

An extruder is simultaneously a pump, a forming device, a mixer, heat exchanger and in some cases a chemical reactor. Food extrusion was developed based on technology used for thermoplastics. The apparent viscosity of food materials increases with cooking, whereas in most polymers the apparent viscosity decreases as the temperature increases (Clark, 1978).

When a food material is extruded several different processes occur. The material is mixed and subjected to considerable shear forces. Heat generated by viscous dissipation of mechanical energy and/or heat added through the barrel leads to gelatinization of starches, denaturation of proteins, cooking or even sterilization. At the die, the material is formed while the sudden release in pressure at the exit of the die can lead to puffing or (flash) drying of the food material (Bruin et al., 1978).

Design and Performance

The major characteristics that may be used to define an extruder are the number of screws, single or twin, and geometry of the screw(s), temperature control of the barrel, and the number and design of die(s). Several studies have reported comprehensive reviews on the various types and characteristics of extruders used in the food industry (Clark, 1978; Harper, 1980; 1985; Martelli, 1983; Janssen, 1985, Dziezak, 1989). Twin-screw extruders (TSE) have improved conveying and mixing capabilities using interchangeable screw profiles created by slipping various screw sections onto parallel shafts, making them very flexible and amenable to a variety of process applications (Harper, 1985). TSE can be characterized in a number of ways. The relative direction of rotation of the screws is the first method of differentiation: co- or counter-rotating. Co-rotating machines have the screws turning in the same direction. This type of machine has been the most popular for food processing because of their higher capacity (Ell, 1984).

The advantages of twin-screw extrusion, a continuous one-step process that concurrently accomplishes multiple process operations, include versatility, increased process control over wide variations in ingredient quality, improved control of shear and temperature during processing, improved ability to handle sticky non-flowable materials and improved convective heat transfer coefficients (Morgan, 1988).

The co-rotating twin-screw extruder has various sections which perform different functions in the extrusion process. Screw sections can have either rounded or square channels, fully intermeshing and self-wiping. Self-wiping screws prevents build-up of ingredients along the shank of the screw, known as screw wrap, which can cause an interruption of the conveying action and surging. From the various reviews, the operating principles can be summarized as follows:

Initial sections of the screw profile on a co-rotating twin-screw extruder are designed to convey the ingredients into the machine. The conveying capability of twin-screw extruders allows them to handle sticky and/or otherwise difficult to convey feed ingredients. The conveying action is from one screw to the next, increasing intermixing of the materials in the channel (Martelli, 1983).

To increase mechanical energy dissipation and enhance mixing, kneading disks are employed. The two-lobed element is most commonly used in food applications. Food materials are passed from one disk to another, changing direction to accomplish the kneading and mixing actions. The relative position of the lobes on the shaft can be arranged so that the tips form a helical pattern which also imparts a conveying action (Schuler, 1986).

Mixing, heat transfer, and viscous dissipation of mechanical energy input occurs in sections of the twin-screw extruder which are completely filled. To create filled sections, some sort of restriction is placed in the screw configuration. The restrictive devices used to back-fill twin-screw extruders include: dies, reverse pitch screws, reduced pitch screws, cut flight screws and dams (Harper, 1985).

The mixing capabilities of twin-screw extruders make them well suited as heat exchangers for viscous food materials. Contact with the barrel wall is essential for efficient heat transfer and requires the heat exchange section to be completely filled with the food ingredients. The pressure down the length of the TSCE barrel can be varied with the reverse pitch elements and venting, with partial cooling of ingredients and/or the incorporation of additional components. Subsequent sections convey, pressurize, and force the materials through the die opening (Schuler, 1986).

Use of TSE in Food Processing

Extrusion has become one of the most popular new processes used by the food and feed industries. Utilization of extruders in food processing has been reported since about 1935 when a single-screw extruder was first used for continuous extrusion of paste products (Rossen and Miller, 1973).

Extrusion is used to prepare different food products which include snacks, cereals, pasta foods, confectionery products, pet foods, animal feed, sausage products, protein supplements, meat analogs, beverage powders, and breadings. (Harper and Harmann, 1973; Rossen and Miller, 1973). There is a continuous increase in the number of foods being produced by extrusion. Besides the traditional applications of forming, cooking, expansion and texturizing of vegetable protein ingredients, TSE are used to produce candies, due to their ability to convey and continuously heat materials (Harper, 1983).

Co-extrusion of food products is an area of increased interest. Examples of these products are filled snacks, ready to eat (RTE) cereals with a fruit gel and pet foods containing multicolored textured pieces to simulate bones or meat products (Cheftel et al., 1981). Other applications are extrusion of oil seeds, production of flat breads and crackers, stabilization of rice bran and starch preconditioning for ethanol fermentation process (Mercier and Feillet, 1975).

Extrusion of Starch

Starch is perhaps the most convenient substrate for the study of extrusion because of its versatility and relatively low cost. During extrusion, starch is subjected to high temperatures, pressures and shear stresses which determine the final extruded product (Chiang and Johnson, 1977).

Starch plays an important role in the texture of expanded snack foods, ready to eat cereals and pet foods that are processed by high temperature short time (HTST) extrusion cooking. The typical characteristic of most of these products, crispness, is strongly related to product expansion which is influenced by starch and extrusion parameters (Chinnaswamy and Hanna, 1988a). A wide variety of textures and finished products are produced utilizing proper blends of modified starches. The type of starches and cooking conditions to produce snack products were described by Feldberg (1969).

The relationship between extrusion processing conditions and product quality for starch have been investigated and in some cases, mathematical models have been reported (Owusu-Ansah et al., 1984; Bhattacharya and Hanna, 1987). The effect of extrusion on functional properties, macromolecular composition and degradation of starch were investigated. The intrinsic viscosity of starch and its amylose fraction were lowered and starch polymers were degraded during twin-screw extrusion (Diosady, 1985). Molecular changes in starch were found due to the extrusion process. Intrinsic viscosity decreased, indicating degradation of starch molecules (Colonna et al., 1984).

Mestres et al. (1988) found that extrusion cooked gels have properties very similar to those of amylopectin gels. They concluded that the high shear treatment during extrusion dissociates amylose and amylopectin, with homogeneous mixing of the macromolecules. Thus, it is more likely that co-crystallization of amylose and amylopectin occurs during extrusion cooking of starch gel.

Different studies have reported effects of extrusion-cooking on texture, microstructure, interaction of macromolecules and rheology of starch (Gomez and Aguilera, 1984; Diosady et al., 1985; Chinnaswamy et al., 1989). Information on the extrusion of starch in combination with other food ingredients and changes in physical, chemical and structural characteristics during and after extrusion, is still very limited or not available (Chinnaswamy et al., 1989).

Restructuring of MDPM by Extrusion

Continuous restructuring of high moisture protein-based material, such as MDC, has been accomplished only on a limited basis. Lingle (1987) pointed out that TSCE may have application in a variety of traditional and non-traditional meat products. Twin-screw extrusion performance of meat proteins and interactions with starch were studied by Chakraborty et al. (1987). They concluded that protein-protein and protein-carbohydrate interactions are affected by composition, and process parameters highly influenced the performance of protein and quality of the texturized products.

Maurer (1979) reviewed studies on extrusion and texturization of poultry products. Acceptable products were obtained using single screw extruders or by addition of non-meat proteins, although further improvement in the meat pieces binding of restructured MDC was needed (Lampila et al., 1985). Vegetable proteins are used as extenders in many extruded poultry products. Minced textured vegetable proteins and other isolates provide specific protein functions when combined to improve meat characteristics (Maurer, 1979).

Few studies have been reported on the twin-screw extrusion of mechanically deboned meat. Mechanically deboned chicken meat containing 60-65% water, 20-22% lipids and 15-17% protein was restructured by HTST extrusion-cooking with or without addition of various binding agents such as soy protein isolate, wheat flour, corn starch and carrageenan (Megard et al., 1985). Temperatures higher than 150^{0}C, residence times from 30-240 sec and feed rate 20 Kg/hr were used in extrusion processing. Extruded products containing 10-20% wheat flour or pregelatinized corn starch resembled those of meat-loaf with hardness values of 2.82-7.49 N; cohesiveness 49-67.6% and water holding capacity 78.6-97.9%. Addition of 25% SPI produced a gel matrix containing dispersed meat particles (Megard et al., 1985). They concluded that it is possible to continuously compress and texturize MDCM in a twin-screw extruder. It was suggested that the use of adequate gear pumps, a longer barrel extruder equipped with multiple inlets and a final cooling section, would permit operation in a wider range of moisture contents to improve the restructuring of MDCM in a wider variety of products. Clarke et al., (1989) studied the processing of mechanically deboned turkey and corn flour by TSCE at 111 and 156 ^{o}C. They found that higher levels of corn flour (60%) reduced the moisture content and lipid oxidation. Higher temperatures produced higher shear forces values in the extruded products.

Studies on twin-screw cooking extrusion have used lean pork, beef heart and pigskin at 40% and 70% (w/w) moisture content (van Zuilichem and Jager, 1990). It was possible to establish stable extrusion cooking processes between 60 and 200°C when lean pork and corn starch were mixed at ratios of 1:4 to 4:1. Extrudates were evaluated by water absorption index, water solubility, protein dispersability as an indication of protein denaturation, viscosity and textural properties. Feed problems during extrusion were related to high moisture content in the meat, thus the authors recommended lowering the moisture content below 40% to solve the feed problems. It was observed that barrel temperature did not affect protein denaturation as expected. The formation of a structure consisting of meat particles enveloped in a starch matrix was the possible explanation for the protection of meat proteins against denaturation.

III. MATERIALS AND METHODS

I. Study I. Restructuring of Mechanically Deboned Chicken and Non-Meat Binders in a Twin-screw extruder

Materials

Fresh MDC was obtained from Nottawa Gardens Co. (Athens, MI) and held at -25 ^{0}C until used within two months. Thawed MDC was preblended with 1.5% salt and 0.5% phosphate for 15 min in a Butcher Boy mixer (Model 250F, Lasar Mfg. Co., Inc. Los Angeles, CA) 24 hr prior to use.

Corn starch (CS, Argo, CPC International Inc., Englewood Cliffs, NJ), vital wheat gluten (WG, Manildra Corp, Shawnee Mission, KS), and soy protein isolate (SPI, 500 E, Ralston Purina, ST. Louis, MO) were used as binders during extrusion. In the first experiment MDC was extruded with the three binders at concentrations of 10-30%. In the second experiment to evaluate the influence of temperature, MDC was mixed with 10 and 15% corn starch while preblending and then extruded.

Extrusion Processing Conditions.

First experiment. A pilot scale Baker-Perkins MPF 50 D/25 twin-screw cooking extruder (APV Baker Inc., Grand Rapids, MI) with a die composed of 3 parallel slits each measuring 1.8 x 0.3 cm, was used to restructure MDC at 350 rpm, die temperature 96^{0}C, barrel temperatures (40-158 ^{0}C) and product feed rates (0.30-1.0 Kg/min)(Table 1). The effective length:diameter ratio of the extruder was 25. Binders were metered into the extruder at the first feed section port using a double-screw feeder (K-tron Corp., Glassborn, NJ). MDC was fed into the second feed section port of the extruder at a constant rate using a Moyno pump (Model IFF J4, Robbins and Meyers, Inc., Springfield, OH). Extrudates from each of three extrusion runs were collected in duplicate in 14x14x5 cm plastic containers and 2.8 cm (diameter) x 15 cm (length) plastic tubes and held at 4 ^{0}C until analyzed within 5 days.

Second experiment. The extrusion system and processing conditions used were the same as described in (1) with the following modifications: Mixtures of MDC and CS were fed into the first feed port using the Moyno pump. The influence of temperature on MDC extrudates containing 10 and 15% starch was determined by adjusting barrel temperatures to obtain die temperatures of 71, 82, 93, 104, and 115 ^{0}C (Table 2). Extrudates were fluid like at the die and solidified in the molds within 3 min after extrusion.

Table 1. Extrusion parameters established to restructure mechanically deboned chicken with different concentrations of non-meat binders[a]

Parameter	Conditions	Standard Deviation
Screw speed	350 rpm	
Torque	7.8 %	0.93
Product feed rate	0.488 Kg	0.06
Zone Number	Temperature (°C)	
1	42	2.3
2	89	1.8
3	89	1.8
4	94	1.9
5	113	1.7
6	158	7.1
7	123	1.0
8	109	1.22
9	99	9.3
Die temperature (°C)	96	3.31
Die pressure (psig)	41.1	12.44
Die type	3 slit (1.8 x 0.3 cm each)	

[a]Values are the means of duplicate determinations from triplicate extrusion runs.

Table 2. Extrusion parameters used to evaluate die temperature effects on mechanically deboned chicken containing 10% and 15% corn starch[a]

Die pressure (psig)	Die type	Screw speed (rpm)	Torque %	Product Feed Rate (kg/min)
17.5	3 slit (1.8 x 0.3cm)	350	7.21	0.485

Die Temperature (°C)	Zone Temperatures (°C)								
	1	2	3	4	5	6	7	8	9
71	59.4	87.2	93.3	72.0	70.7	70.9	70.3	70.9	70.9
82	59.2	87.0	93.3	81.3	82.7	83.1	82.3	82.7	82.5
93	59.5	87.0	93.3	97.4	97.4	98.5	97.2	98.3	96.6
104	62.2	87.0	92.2	109	109	109	109	110	108
115	57.5	87.9	95.3	112	112	113	111	112	112

[a]Values are the means of duplicate determinations from triplicate extrusion runs.

Product Analysis

Proximate analysis (moisture, fat and protein content) was performed in duplicate on raw MDC and extrudates following AOAC (1984) procedures after each extrusion run.

Lipid oxidation was measured in duplicate by the distillation thiobarbituric acid (TBA) method (Tarladgis et al., 1964). Extrudates were collected in plastic bags, sealed, placed immediately in ice and refrigerated. Samples were analyzed within 2 hr after extrusion. Results were expressed in TBA number (mg malonaldehyde/kg extrudate).

Severe reheat yield was determined as described by Smith (1987) with some modifications. Approximately 20 g of extruded meat was boiled for 15 min in 150 ml water. After boiling, the samples were drained at room temperature for 10 min and weighed. Percentage reheat yield was calculated by dividing the weight of reheated sample by its initial weight then multiplying by 100.

Apparent tensile strength (ATS) was determined in triplicate using 14x14x0.8 cm thick slices with the Thin Slice Tensile Cell (Model ST) attached to an Allo-Kramer Shear Press (FTC Model TRS, Texture Press, Rockville, MD) (Gillett et al., 1978). Apparent tensile strength was calculated by dividing the force needed to break the meat slice by the cross-sectional area (1.1×10^{-3} M^2). Samples were equilibrated at 4°C prior to testing. Tensile strength was described as apparent because the meat fibers in the extrudates were not in parallel alignment. ATS was expressed in units of Pascals.

Warner Bratzler shear stress (kPa) was measured using a Warner Bratzler meat shear (G.R. Elec. Mfg. Co. Manhattan, KS) on triplicate 2.8 cm (diameter) x 5.0 cm (length) extruded meat cores and calculated by dividing the rupture force by the cross-sectional area (6.15×10^{-4} M^2) of the extrudate cores. The extruded MDC samples were equilibrated to 4^0C before testing. WBSS was expressed in units of Pascals. Extrudates tested by this method may fail in tension, shear, compression or a combination of stresses.

Statistical design and analysis.

Triplicate extrusion runs were used to evaluate each treatment. Statistical significance was determined using a two-way analysis of variance and a completely randomized design. Tukey's honestly significant difference test was used to evaluate the significant differences between the means at the 5% level of probability (MSTAT, 1989).

II. Study II. Effect of Extruder Die Temperature on Texture and Microstructure of Restructured Mechanically Deboned Chicken and Corn Starch

Materials

MDC used in this study was from the same source as described in Study 1. Corn starch (Argo, CPC International

Inc., Englewood Cliffs, NJ) was used as a binder during extrusion. Thawed MDC was mixed with 10% and 15% corn starch while preblending with 1.5% salt and 0.5% phosphate for 15 min in a Butcher Boy mixer (Model 250F, Lasar Mfg. Co., Inc., Los Angeles, CA) 24 hr prior to use.

Extrusion Processing Conditions.

Extrusion system and processing conditions were the same as described in Study 1.

Product Analysis

Moisture, fat, and protein were performed as described in Study 1. Textural properties of the extrudates were evaluated following the method reported by Diehl et al., (1979). Apparent shear stress (kPa) and apparent strain at failure (dimensionless) were calculated from duplicate 1.5 x 1.5 cm extrudate cores using an Instron universal testing machine (Model 4202, Instron Engineering Corporation, Canton, MA) at a crosshead speed of 10 mm/min with a 50 N compression cell. The cores were removed with a 1.5 cm diameter cork borer from extrudates collected from three extrusion runs in 2.8 cm (diameter) x 15 cm (length) plastic tubes and equilibrated at 4^{o}C before testing. The cores were compressed between two parallel plates at a constant deformation rate. The force that developed was recorded continuously and used to calculate the apparent shear stress and strain at failure. Strain is calculated from the compression peaks by measuring the millimeters (X-direction on recorder paper) the cross head travels before the sample fractures and dividing by the original sample length. Stress is measured by determining the height in Newtons force (N) (Y-direction on the recorder paper) the crosshead achieves before the sample fractures. The parameters were calculated according to Diehl et al. (1979) using the following expressions:

Apparent strain at failure (E_H)

$$E_H = -\ln (1.0 - d_x)$$

d_x = strain calculated from recorder paper

Apparent stress at failure (σ)

$$\sigma = \frac{F}{\pi R^2 (1.0 + V E_x)}$$

F = Force (N) calculated from the recorder paper

R = Sample radius (m)

V = Poisson's ratio = 0.48 (a constant)

Scanning Electron Microscopy.

Two samples from triplicate extrusion runs were prepared for SEM observations following the procedure described by Klomparens et al. (1986). Samples were equilibrated at 4^{o}C and 1.5 mm x 1.5 mm cubes were cut with a chilled razor blade.

Cubes were placed in vials with fixative (5% gluteraldehyde in 0.1 M Na phosphate buffer, pH 7.2) and fixed for 2 hr. The fixative was removed from the vial and replaced with buffer (0.1 M Na Phosphate buffer, pH 7.2)

Samples were washed three times for 20 min each with 0.1 M Na Phosphate buffer. Buffer was removed and samples were post-fixed by placing them in 1% sodium buffered OsO_4 for 2 hr at room temperature and rinsed two times in buffer for 15 min each

Buffer was removed and 25% ethanol was added. Extrudate cubes were dehydrated in a graded ethanol series of 25%, 50%, 75%, 95% to 100% (v/v) using 15 min at each step. Samples were rinsed 3 times for 15 min each in 100% ethanol. Samples were placed in critical point drying baskets in 100% ethanol and then transferred very quickly from the 100% ethanol to the critical point drying chamber.

Fixed samples were dried for 3 min using the carbon dioxide method in which CO_2 was the transitional fluid. Pressure in the drying chamber was about 1400 to 1500 lb/in^2 at 40-45^oC.

Dry samples were mounted on 10 mm diameter x 5 mm high stubs (75350, Electron Microscopy Sci. Washington, PA) Adhesive mounting tabs (M. E. Taylor Engineering) were used to fasten the sample to the stub. The samples were pressed onto the tabs using a sharpened applicator stick. A fine line of Conducting Graphite Paint (Ladd 60780) was applied from the sample over the edge of the metal stub prior to coating.

An Emscope Sputter Coater (Model SC500, Kent, England) was used to coat the samples with gold under a vacuum of 0.06 Torr. Dried and coated samples were stored in a vacuum desiccator jar with silica-gel in the bottom.

SEM micrographs were made using Polaroid film (665P/N) and a JEOL scanning electron microscope (Model JMS-35CF, Osaka, Japan) equipped with a tungsten electron gun at 15 kV accelerating voltage. Working distance was 15 mm and condenser lens 400.

Statistical Analysis

Triplicate extrusion runs were used to evaluate each treatment. Statistical significance was determined using 2 x 5 factor factorial (10 and 15% corn starch concentrations and temperatures of 71, 82, 93, 104 and 115 ^{o}C). Tukey's honestly significant difference test and standard error of the means were used to evaluate the significant differences between the means at the 5% level of probability (MSTAT, 1989).

III. Study III. Protein Insolubility and Corn Starch Gelatinization of Mechanically Deboned Chicken and Corn Starch During Twin-Screw Extrusion

Materials

MDC used in this study was from the same source but a different batch as described in Study I. Corn starch used in this study was the same as described in study 2.

Extrusion Processing Conditions.

The extrusion system was the same as described in Study 1. Processing conditions were modified as follows: Mixtures of MDC and 15% CS were fed into the first feed port using a Moyno pump. The extrusion process was stabilized and held constant once temperatures were obtained. Barrel temperatures in the extruder were adjusted to obtain a die temperature of 115 ^{o}C (Table 4).

Residence time of the MDC:CS mixture in the extruder barrel was determined by the tracer method using 3% erythrosin-ß (red) (Sigma Laboratories, St. Louis, MO) in water as reported by Ofoli et al. (1990) with some modifications. One milliliter of dye was applied at the first feed port. Extrudates were collected at steady state extrusion conditions at 1 min intervals after a slight change in color was observed. Samples were ground two times through the 4 mm plate of a Kitchen Aid Grinder (Model k5-A, Hobart, Troy, OH) and mixed. Ground samples were pressed

Table 3. Extrusion parameters used to evaluate die temperature effects on mechanically deboned chicken containing 15% corn starch[a]

Die pressure (psig)	Die type	Screw speed (rpm)	Torque %	Product Feed Rate (kg/min)
17.5	3 slit (1.8 x 0.3cm)	350	7.21	0.485

Die Temperature (°C)	Zone Temperatures (°C)								
	1	2	3	4	5	6	7	8	9
115	57.5	87.9	95.3	112	112	113	111	112	112

[a]Values are the means of duplicate determinations from triplicate extrusion runs.

into 9 cm (diameter) by 8 mm (height) plastic containers. The color was measured using the Hunter Color Difference Meter (Model D25-2 Fairfax, VA) standardized with the pink standard plate, No. C2-6005; L=67.6, a_L=21.4, b_L=11.9. The approximate average residence time, *t*, was calculated by using the expression reported by Ofoli et al. (1990).

$$\bar{t} = \frac{\Sigma\, t_i\, (a_i - a_o)\ \ t_i}{\Sigma\ (a_i - a_o)\ \ t_i}$$

where: a_i is the redness value for the colored extrudate at discrete time values t_i.

a_o is the redness value for MDC:CS without added dye.

To monitor changes in protein solubility and starch gelatinization due to temperature of the barrel, samples were taken from 5 different positions in the extruder. The extruder barrel was divided in 4 sections for sampling. The length (cm) of the sections starting at the end of the barrel opposite the die were: section 1, feeding port; section 2, 0-42.1cm; section 3, 42.1-84.5cm; section 4, 84.5-1270cm; section five, product exiting the die. Once extrusion conditions were in the steady state, the system was turned off, the barrel was quickly opened (2 min) and samples were taken from the material attached to the screw. Samples were collected in plastic bags and placed immediately in ice and kept at 4 ^{o}C. For changes in protein solubility and starch gelatinization of the MDC extrudates, analyses were performed within 24 hr of sampling.

Proximate Analysis

Proximate analysis (moisture, fat and protein) was performed in duplicate on raw MDC and extrudates following AOAC (1984) procedures.

Protein Extractability

Protein extractability of the extruded material from the 5 extruder barrel sections was determined. Proteins were extracted from the extrudates according to the procedure of Lyon et al. (1986) with some modifications. Samples were ground three times through a 4 mm plate of a Kitchen Aid Grinder (Model K5-A, Hobart, Troy, OH). Proteins were extracted from a 10 g sample with 3 volumes of 0.9% saline solution for 30 min with continuous agitation at 4 ^{o}C. Samples were filtered through glass wool and the filtrate was centrifuged at 23,000 x g for 15 min. The supernatant was stored at 4°C and used for protein evaluations.

Protein content (mg protein/mL supernatant) of extrudates extracts from section 1 was measured using Kjeldahl AOAC Method 24.038-24.040 (AOAC, 1984), except that H_2O_2 was not used for sample digestion. Protein contents of the same extrudate determined previously and without H_2O_2 during digestion had the same values. Protein content was determined in duplicate. The protein in the extract from barrel section 1 was considered 100% extractable. Protein content of extrudate extracts represented only the fraction corresponding to water soluble proteins, mainly sarcoplasmic proteins, from the total protein determined by proximate analysis.

Electrophoresis

Extracts were prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by mixing 4.5 ml of supernatant with 0.5 ml 10% (w/v) SDS. The mixtures were boiled for 5 min and three drops of ß-mercaptoethanol, 1 ml glycerol and a trace of bromophenol blue were added. Prepared extracts were stored at -20 ^{o}C.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the 0.9% NaCl extracted protein was performed using 10% polyacrylamide gel according to Smith and Brekke (1985). Electrophoresis was performed in a Hoeffer Vertical Electrophoresis Unit (Model SE 600; Hoeffer Scientific Instruments, San Francisco, CA) and constant voltage power supply (Model 1P-17 Heathkit, Benton Harbor, MI) using a modification of the method described by Laemmli (1970). The gel consisted of two portions:(1) stacking gel (upper gel) consisting of 1.25 M Tris, pH 6.8 and 4% polyacrylamide and (2) resolving gel (lower gel) consisting of 0.75 M Tris, pH 8.8 and 10% polyacrylamide. Both gels were polymerized using tetramethylenediamide

(TEMED), ammonium persulfate 1% solution, and 0.1% and 0.26% N,N'-methylene-bis-acrylamide for 4% and 10% polyacrylamide gels, respectively. The electrode buffer consisted of 0.025 M Tris, 0.192 M glycine pH 8.3

Extracts containing 164 μg (15 μl) of protein from extruder section 1 were applied to the stacking gel. To measure changes in extractability in the other extruder barrel sections, the volume of protein extract applied to the stacking gel was adjusted as follows: section 2 : 15 μl, sections 3 through 5: 75 μl. A constant current of 25 mA was maintained until the protein migrated into the resolving gel. The current was then increased to 60 mA. Electrophoresis was stopped when the tracking dye was about 1.0 cm from the bottom of the gel. Gels were stained for 6 hr in 0.4% Coomassie blue 9/45/45 (v/v/v) acetic acid-methanol-water. Gels were destained in 7.5/25/67.5 (v/v/v) acetic acid-methanol-water until the gel was clear. Gels were preserved in 7.5% acetic acid solution.

Molecular weights of protein bands were determined by comparing relative retention times to molecular weight standards SDS-6H and SDS-7 (Sigma Chemical Company, St. Louis, MO). Purified protein standard No. SDS-6H contained Carbonic anhydrase, egg albumin, bovine albumin, phosphorylase B, ß-galactosidase and myosin. Standard No. SDS-7 included bovine albumin, egg albumin, glyceraldehyde-3-phosphate dehydrogenase, carbonic anhydrase, trypsinogen, trypsin inhibitor and α-lactoalbumin.

The identification of major bands was based on studies that reported subunit molecular weights from SDS-PAGE electrophoregrams of unheated water-soluble muscle protein extracts (Scopes, 1970; Scopes and Penny, 1971; McCormick et al., 1988). The linear regression equations used to calculate the apparent molecular weights were:

(a) $Y = 5.38 + (-1.52) * X$, ($r=0.98$), for high MW

(b) $Y = 5.07 + (-0.97) * X$, ($r=0.99$), for low MW

The protein bands were quantified by densitometric scans at 580 nm (Shimadzu Dual-Wave Length Thin-Layer Chromoto Scanner (Model CS-930, Kyoto, Japan). The amount of protein in each peak as determined by densitometry was expressed as a percentage of the total extractable protein. Relative protein extractability was calculated by correcting for original sample volume and dividing the total area of the tracing of extrudates from each section by the total area of the tracing for section 1, then multiplying by 100.

Starch Gelatinization

The percentage of gelatinized starch was determined by the glucoamylase method (Budke, 1984) with some modifications. Samples were ground but not sieved through a 40 mesh screen and were not diluted to 200 mL volume. Chemical gelatinization with 2N NaOH was used instead of gelatinizing with water at 122°C. Conversion of gelatinized starch to glucose with amyloglucosidase was performed at 40°C rather than 55°C.

Amyloglucosidase (Diazyme L-200, activity= 200 Diazyme units/ml) was obtained from Miles Laboratories, Inc., Elkhart, IN. The enzyme was stored at $4^{0}C$. Extrudates were ground 3 times through a 4 mm plate and mixed using a Kitchen Aid Grinder (Model K5-A, Hobart, Troy, OH). Two samples of 0.5 g each were taken from each extrudate. One sample was chemically gelatinized and the other was digested enzymatically to determine percentage gelatinized starch.

Gelatinization procedure

Ten milliliters of water and 0.5 g extrudate were stirred until the sample was evenly suspended. Ten milliliters of 2N NaOH were added to the suspension and the flasks incubated at room temperature for 20 min with occasional stirring. Gelatinization was stopped by the addition of 10 mL 2N HCl and the total volume was adjusted to 100 mL with distilled water. Glucose concentration in the gelatinized samples was determined using a YSI Model 27 Analyzer (Yellow Spring Instrument Co., Yellow Springs, OH) equipped with glucose oxidase membrane (YSI No. 2365) and a carrier buffer (0.47 M Na phosphate buffer, YSI No. 2357). A sample solution of 25 μl was injected into the glucose analyzer. The glucose concentration in the gelatinized samples (G_{GS}) was measured as mg/dL and used to calculate the percentage of starch on a dry basis (%S) (Budke, 1984).

Enzymatic digestion

The sample (0.5 g), 25 mL of water, 10 ml of 1N Na Acetate buffer (pH 4.2) and 5 mL of Diazyme stock solution (30 units/ml) were combined, stirred and held in a 40 ^{o}C water bath for 60 min. An enzyme blank solution was prepared as described above but without the sample. Five milliliters of trichloroacetic acid (25% (w/v)) were added and flasks were cooled to room temperature. The volume was adjusted to 100 mL with distilled water. Glucose concentration for sample (G_E) and blank (G_{EBS}) solutions subjected to enzymatic digestion were determined using the YSI Analyzer as described above.

Starch gelatinization was calculated with the following expressions reported by Budke (1984).

$$\%S = \frac{(G_C - G_{EBS})\ (0.9)\ (100)(100\%)}{(1000)\ (W_a)\ (\%DS)}$$

$$\%SG = \frac{(G_E - G_{EBS})\ (0.9)\ (100)\ (100\%)}{(1000)\ (W)\ (\%DS)\ (\%S)}$$

where: %S= Percent starch on a dry solid basis

%SG= Percent of gelatinized starch

%GT= Degree of gelatinization starch (%)

G_E= YSI reading for chemically gelatinized sample

Gs= YSI reading for enzymatically digested sample.

G_{EBS}= YSI reading for enzyme blank solution

(mg glucose/dL)

100= Total volume of sample

100%= Conversion to percentage

1000= Conversion factor for YSI reading (mg/dl to g/100 ml)

W_a= Weight in g of chemically gelatinized sample

W= Weight in g of enzyme digested sample

%DS= Percent dry solids in sample (100 - % MC)

0.9= Ratio of % starch to % glucose

Statistical Design and Analysis

Triplicate extrusion runs were use to evaluate each treatment. Statistical significance to study the effect of temperature in the 5 extruder sections on protein extractability and starch gelatinization was determined using a one-way analysis of variance and a complete randomized design with MSTAT software (version C, East Lansing, MI). Tukey's honestly significant difference test ($P<0.05$) and standard error of the means were used to evaluate the significant differences between the means.

IV. STUDY 1

Restructuring of Mechanically Deboned Chicken and Non-Meat Binders in a Twin-Screw Extruder

ABSTRACT

A twin-screw cooking extruder was used to restructure mechanically deboned chicken (MDC) in combination with three non-meat binders, corn starch (CS), soy protein isolate (SPI) and wheat gluten (WG), at concentrations of 10-30%. Extrudates were evaluated by apparent tensile strength (ATS), Warner Bratzler shear stress (WBSS), proximate composition and reheat yield. SPI and WG were less effective than starch for increasing the ATS and WBSS of extruded MDC. The effect of die temperatures between 71-115°C on chemical and textural properties of extrudates containing 10-15% starch were investigated. The ATS and WBSS increased as a function of temperature up to 104°C. Fat content and lipid oxidation decreased as extrusion temperatures increased from 71 to 104 °C.

INTRODUCTION

Mechanical deboning has become a widely accepted and economical means of removing meat from poultry carcasses (Lampila et al., 1985). Mechanically deboned poultry meat (MDPM) has been used in the United States since about 1965. The use of MDPM in further processed products reached 1.82 x 108 kg in 1984 (Schuler, 1985). Restructuring into value-added products is of considerable interest to the poultry meat industry as a means of upgrading the value of MDPM, which is currently sold for about 46-53 cents/kg (Urner Barry's, 1989).

Mechanical deboning disrupts muscle fibers and alters the protein and lipid composition of meat, which may result in flavor instability, formation of undesirable characteristics, protein denaturation and lipid oxidation (Johnson et al., 1974; Dawson and Gartner, 1983). The use of mechanically deboned chicken (MDC) may cause a mealy, coarse texture in finished processed products. Other problems which occur when using MDC in some processed products are poor water holding capacity and poor binding between meat pieces (Lampila et al., 1985).

Maurer (1979) reviewed studies on the texturization and extrusion of poultry products. Acceptable products were obtained using single screw extruders or by addition of non-meat proteins, although further improvement in the binding of restructured MDC was needed (Lampila et al., 1985). Non-meat proteins are used to improve the water holding capacity and textural properties of processed meat products (Maurer, 1979). Continuous restructuring of high moisture protein-based material, such as MDC, has been accomplished only on a limited basis.

In the last decade, twin-screw extruders (TSE) have received significant attention in the food industry. The advantages of twin-screw extrusion (a continuous one-step process that concurrently accomplishes multiple process operations) include versatility, increased process control over wide variations in ingredient quality, improved control of shear and temperature during processing, improved ability to handle sticky non-flowable materials and improved convective heat transfer coefficients (Morgan, 1988). Twin-screw extruders reduce processing time and may eliminate many of the multiple processing operations used in the meat industry.

While twin-screw extruder systems may have application in a variety of traditional and non-traditional meat products (Lingle, 1987), few studies have been reported on the twin-screw extrusion processing of mechanically deboned meat (MDM). Megard et al. (1985) investigated the restructuring of mechanically deboned chicken meat by high temperature short time extrusion cooking. They concluded that it is possible to continuously compress and texturize MDM in TSE. Current research needs include the development of an adequate knowledge base describing the effects of ingredients and TSE process conditions on system performance and final product quality.

The objective of this study was to assess the effects of TSE process conditions and non-meat binders on restructured MDC product quality, specifically texture, reheat yield and lipid oxidation.

MATERIALS AND METHODS

Materials

Fresh MDC was obtained from Nottawa Gardens Co. (Athens, MI) and held at -25°C until used within two months. Thawed MDC was preblended with 1.5% salt and 0.5% phosphate for 15 min in a Butcher Boy mixer (Model 250F, Lasar Mfg. Co., Inc. Los Angeles, CA) 24 hr prior to use. Corn starch (CS, Argo, CPC International Inc., Englewood Cliffs, NJ), vital wheat gluten (WG, Manildra Corp, Shawnee Mission, KS), and soy protein isolate (SPI, Ralston Purina, ST. Louis, MO) were used as non-meat binders during extrusion. In the first experiment MDC was extruded with the three binders at concentrations of 10-30%. In the second experiment to evaluate the influence of temperature, MDC was mixed with 10 and 15% corn starch while preblending and then extruded to evaluate the influence of die temperature.

Extrusion Processing Conditions

A pilot scale Baker-Perkins MPF 50 D/25 TSE with a three slit die, composed of 3 parallel slits each measuring 1.8 x 0.3 cm, was used to restructure MDC at 350 rpm under a range of die temperatures (71 to 115°C), barrel temperatures (40-158°C) and product feed rates (0.30-1.0 Kg/min). The effective L/D ratio of the extruder was 25.

In the first experiment, non-meat binders were metered into the extruder at the first feed port using a double-screw feeder (K-tron Corp., Glassborn, NJ). MDC was fed into the second feed port of the extruder at a constant rate using a Moyno pump (Model IFF J4, Robbins and Meyers, Inc., Springfield, OH). In the second experiment, mixtures of MDC and CS were fed into the first feed port using the Moyno pump.

The influence of temperature on restructured MDC extrudates containing 10 and 15% starch was determined by adjusting barrel temperatures to obtain die temperatures of 71°C, 82°C, 93°C, 104°C, and 115°C. Extrudates were fluid-like at the die and solidified in the molds within 3 min after extrusion. Extrudates from each of three extrusion runs from each experiment were collected in duplicate in 14x14x5 cm plastic containers and 2.8 cm (diameter) x 15 cm (length) plastic molds and held at 4°C until analyzed within 5 days.

Product Analysis

Proximate analyses (moisture, fat, protein) were performed in duplicate on raw MDC and extrudates following AOAC (1984) procedures after each extrusion run. Apparent tensile strength (ATS) was determined in triplicate using 14x14x0.8 cm thick slices with the Thin Slice Tensile Cell (Model ST) attached to an Allo-Kramer Shear Press (Model TRS, Food Technology Corporation, Rockville, MD) (Gillett et al. 1978). Apparent tensile strength was calculated by dividing the force to break the meat slice divided by the cross-sectional area (1.1×10^{-3} m^2). Tensile strength was described as apparent because the meat fibers in the extrudates were not in parallel alignment.

Warner-Bratzler shear stress (WBSS) was measured with a Warner Bratzler meat shear (G.R. Elec. Mfg. Co. Manhattan, KS) on triplicate 2.8 cm (diameter) x 5.0 cm (length) extruded meat cores and calculated by dividing the rupture force by the cross-sectional area (6.15×10^{-4} m^2) of the extrudate cores. Extrudates tested by this method may fail in tension, shear, compression or a combination of stresses. Both ATS and WBSS were expressed in units of Pascals.

Severe reheat yield was determined as described by Smith (1987) with some modifications. Approximately 20 g of extrudate was boiled for 15 min in 150 ml water. After boiling, the samples were drained at room temperature for 10 min and weighed. Percentage reheat yield was calculated by dividing the weight of reheated sample by its initial weight then multiplying by 100. Lipid oxidation was measured in duplicate by the distillation thiobarbituric acid (TBA) method (Tarladgis et al., 1964). Results were expressed in TBA number (mg malonaldehyde/kg extrudate).

Statistical Design and Analysis

Triplicate extrusion runs were made to evaluate each treatment. Statistical significance was determined using a two-way analysis of variance and a completely randomized design. Tukey 's honestly significance difference test and standard error of the means were used to evaluate the significant differences between the means at the 5% level of probability (MSTAT, 1989).

RESULTS AND DISCUSSION

Twin-Screw Processing Conditions

Mechanically deboned chicken could not be extruded without non-meat binders because the extruder barrel clogged and the extrudate did not bind or hold water. The free water and fat released in the extruder disrupted flow patterns and led to plugging of the die. Extrusion parameters for restructuring MDC with non-meat binders were established in preliminary experiments (Table 1). Extrusion parameters were selected to produce low pressures (41 psig) and temperatures (96°C) at the die. Preliminary experiments also indicated that MDC must contain less than 19% fat to prevent fat separation under the extrusion conditions used in this study. Fat content was standardized at 16.4% for all extrusion runs.

The highest temperature (158°C) was applied in zone 6 of the barrel to promote starch gelatinization and protein gelation in a short time, as residence time in the extruder was about 2 min. Extrusion at the relatively low die temperatures of 96°C contrasts with the work by Megard et al. (1985) who used temperatures above 150°C. Lower extrusion temperatures should improve nutrient retention (Asp and Bjorck, 1982; Bjorck and Asp, 1983).

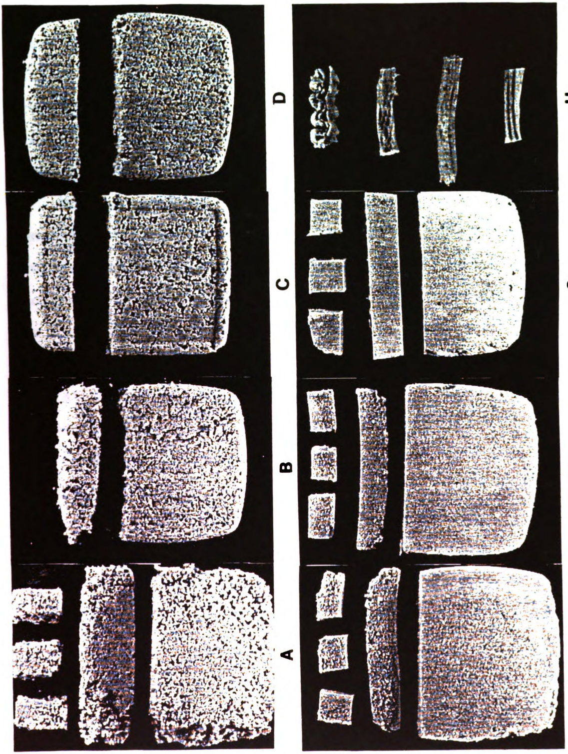
Temperatures used in the second series of tests (Table 2) to evaluate heating effects were similar to other studies on extrusion of corn starch by Chinnaswamy and Hanna (1988b). They reported optimum extrusion temperatures of 140-150°C to prevent degradation of corn starch.

Effect of Non-Meat Binders

Corn Starch, WG and SPI were extruded at 10, 15, 20, 25 and 30% concentrations with MDC. Preliminary results showed that MDC and different concentrations of non-meat binders had a significant effect on texture and proximate composition of samples when extruded under the same conditions.

Photographs of some of the extruded products were made depending on the die and mold used to collect the extrudates (Fig. 1). Extrudates made with SPI and WG had a uniform

Fig. 1 - Effect of different types and concentrations of non-meat binders on the appearance of mechanically deboned chicken extrudates. (a) 10% soy protein isolate, (b) 10% gluten, (c) 25% gluten, (d) 30% gluten, (e) 10% starch, (f) 15% starch, (g) 30% starch, (h) from top to bottom: starch at 10%, 15%, 20% and 30%.



pinkish color but were grainy and crumbled easily. Extrudates made with starch were very cohesive, did not crumble and could be cut into thin slices, resembling luncheon meats. Fig. 1H illustrates snack type products forms made from MDC:CS extrudates collected at the die without the use of molds. A wide variety of products were produced in preliminary experiments when using different dies, however, molds were used in this experiment to collect extrudates of uniform size for texture testing.

Moisture content of the extrudates decreased as the concentration of non-meat binders increased (Table 4) Protein content of extrudates containing WG and SPI increased as non-meat binder concentration increased, due to the high protein content of the binders. The addition of corn starch decreased the protein content of the extrudates. The fat content of the extrudates was lower than that of raw MDC and was lower than expected due to dilution with non-meat binders. Fat may be melting and separating from the MDC:non-meat binder matrix during extrusion. A small amount of free fluid was observed at the die. Other researchers have observed a decrease in extractable fat which they attributed to protein-lipid and starch-lipid interactions (Fabriani et al., 1968; Asp and Bjorck, 1982; Izzo and Ho, 1989).

The ATS and WBSS of extrudates made with SPI and WG were significantly lower than those for CS at the same concentration (Table 5). Increasing CS from 10% to 30%

Table 4. Proximate analysis of mechanically deboned chicken extrudates containing different concentrations of non-meat binders[a]

Treatment	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Raw	64.0 +3.6	14.7 +1.1	16.4 +1.9	3.8 +0.4
Corn Starch				
10%	60.5 +3.4	14.3 +0.8	10.8 +2.2	2.5 +0.1
15%	58.7 +0.4	13.8 +1.3	9.8 +0.6	2.5 +0.1
30%	44.0 +1.6	12.9 +1.4	10.4 +1.0	2.7 +0.2
Vital Wheat Gluten				
20%	52.2 +1.7	33.4 +2.0	8.4 +0.7	1.9 +0.2
25%	49.8 +6.2	37.0 +5.7	7.5 +1.4	2.1 +0.3
30%	42.8 +9.1	41.2 +8.8	6.1 +2.3	1.7 +0.5
Soy Protein Isolate				
10%	54.7 +0.2	22.7 +1.3	16.0 +0.6	2.9 +0.2

[a] Means ± standard deviation are the average of duplicate determinations from triplicate extrusion runs

Table 5. Textural characteristics and reheat yield of mechanically deboned chicken:non-meat binder extrudates[a]

Treatment	Apparent tensile Strength (kPa)	Warner Bratzler Shear Stress (kPa)	Reheat Yield (%)
Corn Starch			
10%	11.8 ±2.4[b]	3.7 ±0.8[b,c]	97.0 ±0.3[b,c]
15%	55.0 ±3.5[c]	6.2 ±0.3[d]	97.3 ±0.5[b]
30%	145.0 ±5.3[d]	12.8 ±1.7[e]	99.7 ±0.3[d]
Vital Wheat Gluten			
20%	< 0.4[e]	3.3 ±0.02[b,e]	95.0 ±0.6[c]
25%	< 0.4[e]	4.1 ±0.60[c]	95.9 ±0.2[b,c]
30%	< 0.4[e]	6.5 ±0.70[d]	96.1 ±2.5[b,c]
Soy Protein Isolate			
10%	< 0.4[e]	3.0 ±0.40[e]	95.0 ±1.9[c]

[a] Means + standard deviation are the average of duplicate determinations from triplicate extrusion runs

[b-e] Means in the same column bearing a common superscript are not significantly different ($p < 0.05$)

significantly increased ATS, WBSS and reheat yield. The ATS and reheat yield of extruded MDC containing 10 and 15% corn starch were similar to published values for traditional poultry rolls which had ATS values of about 22.5 kPa (Smith and Alvarez, 1988).

Concentrations of WG above 20% were necessary to prevent clogging in the extruder barrel. The WBSS increased in extrudates as WG concentration increased from 20% to 30%. In contrast with WG, only those products containing 10% SPI held together and the ATS and WBSS were lower than those for CS and WG. It was not possible to measure textural parameters at concentrations above 10% SPI because the products did not hold together. Other binder concentrations and different extrusion conditions may be necessary to increase the ATS and WBSS of MDC extrudates containing WG and SPI.

Both starch gelatinization and protein denaturation were probably responsible for the texture, appearance and water holding capacity of the extrudates. Extruder barrel temperatures were greater than those needed for the gelatinization of CS and denaturation of the salt soluble muscle proteins, WG proteins and SPI. Corn starch begins to gelatinize at 70°C (Banks and Greenwood, 1975). Lawton et al. (1972) reported that the maximum gelatinization of CS occurred at 90°C to 150°C, depending on the moisture content.

Salt-soluble chicken muscle proteins begin to denature at approximately 55°C to 60°C (Xiong et al., 1987) and are the muscle proteins primarily responsible for meat texture. Soy protein isolate and WG denature at temperatures above those of the salt soluble muscle proteins. Peng et al. (1982a, 1982b) reported that interactions between soy protein and myosin occurred at 85°C to 100°C and suggested that soy protein would have minimal effects on texture in meat products heated below these temperatures.

Foegeding and Lanier (1987) reported that soy protein decreased the cohesiveness and hardness of frankfurters and other meat products. When WG or SPI were added to replace the beef component of a meat emulsion, cooking yield increased at substitution levels above 40% (Randall et al., 1976).

Comer (1979) found that most non-meat binders improved emulsion stability and cooked yield, but decreased product firmness. The contribution of non-meat binders to meat product texture and yield depend largely on the processing conditions used (Foegeding and Lanier, 1987). If meat products containing non-meat binders are processed to temperatures at which the binders denature and interact with the salt soluble muscle proteins then the textural properties of the products may be improved. However, if the processing temperatures are low then the non-meat binders generally act only as fat and water binders.

Reheat yield of the extrudates was very similar and ranged from 95-99.7% (Table 5). Results in the present study suggest that WG and SPI were only partially denatured due to the short residence time in the extruder (less than 2 min) and thus did not improve the WBSS or ATS of extrudates. Corn starch was probably gelatinized and therefore improved the measured textural attributes of the MDC extrudates.

Effect of Temperature

Changing the temperature of the extruder barrel and die had a significant influence on the textural properties of the MDC:CS extrudates. The WBSS and ATS increased as a function of starch concentration and extrusion temperature (Fig. 2).

The ATS and WBSS of extrudates containing 15% CS were at least twice that of extrudates containing 10% CS at die temperatures of 93°C and above. These results suggest that CS had a larger influence on the extrudate textural properties than MDC proteins. The ATS of the extrudates could not be measured until a die temperature of 93°C was used, indicating that CS gelatinization occurred in the extruder under these conditions. CS was completely gelatinized at barrel and die temperatures of 104°C as increases in temperature did not significantly increase ATS of the extrudates. The WBSS increased as extruder die temperature increased from 71°C to 93°C. Further increases in barrel and die temperatures did not improve the WBSS.

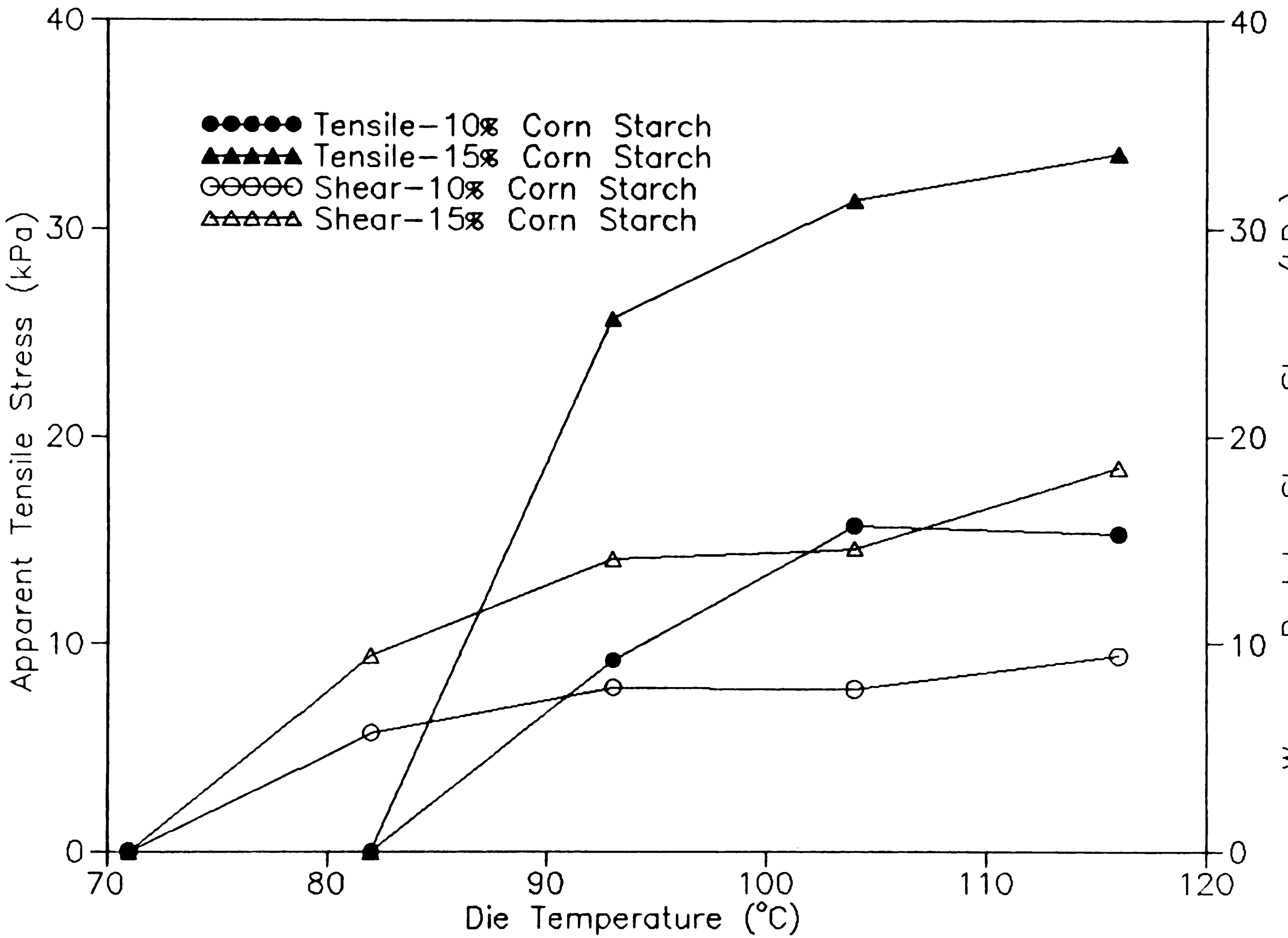


Fig. 2. Effect of die temperature on apparent tensile strength and Warner Bratzler shear stress of extruded mechanically deboned chicken containing 10% and 15% corn starch (Standard error of the means are: 0.38 and 0.69 for shear stress and 1.19 and 2.58 for tensile strength, at 10 and 15% corn starch, respectively)

MDC extruded with 15% CS using die temperatures above 90°C resulted in meat-like products. Complete gelatinization of CS has been reported to occur at 90°C to 150°C depending on the moisture content (Lawton et al., 1972; Banks and Greenwood, 1975), although barrel temperatures above 110°C caused starch degradation and textural problems in the extrudates (Chinnaswamy and Hanna, 1988b). Results suggest that a range of textural properties can be produced in MDC extrudates by changing extruder barrel and die temperatures.

Protein content averaged 15.2% and 14.6% in the 10% and 15% CS extrudates, respectively, and did not change significantly as barrel and die temperatures were increased. Fat content decreased as extrusion temperatures were increased from 71°C to 82°C (Fig. 3). Lipid oxidation as measured by TBA numbers was observed at all die temperatures, although the lowest temperature (71°C) resulted in the highest TBA number. The initial TBA number for the raw mixtures of MDC and CS was 0.28.

TBA values decreased to 5.4 when die temperature was increased to 115.5°C, suggesting that antioxidative compounds were produced as extruder barrel and die temperatures increased. Catalysis of lipid oxidation occurs at about 70-80°C, while higher temperatures may produce an antioxidative effect due to the formation of Maillard reaction products (Zipser and Watts, 1961; Jantawat and Dawson, 1980; Smith et al., 1987). Heating meat to 70°C

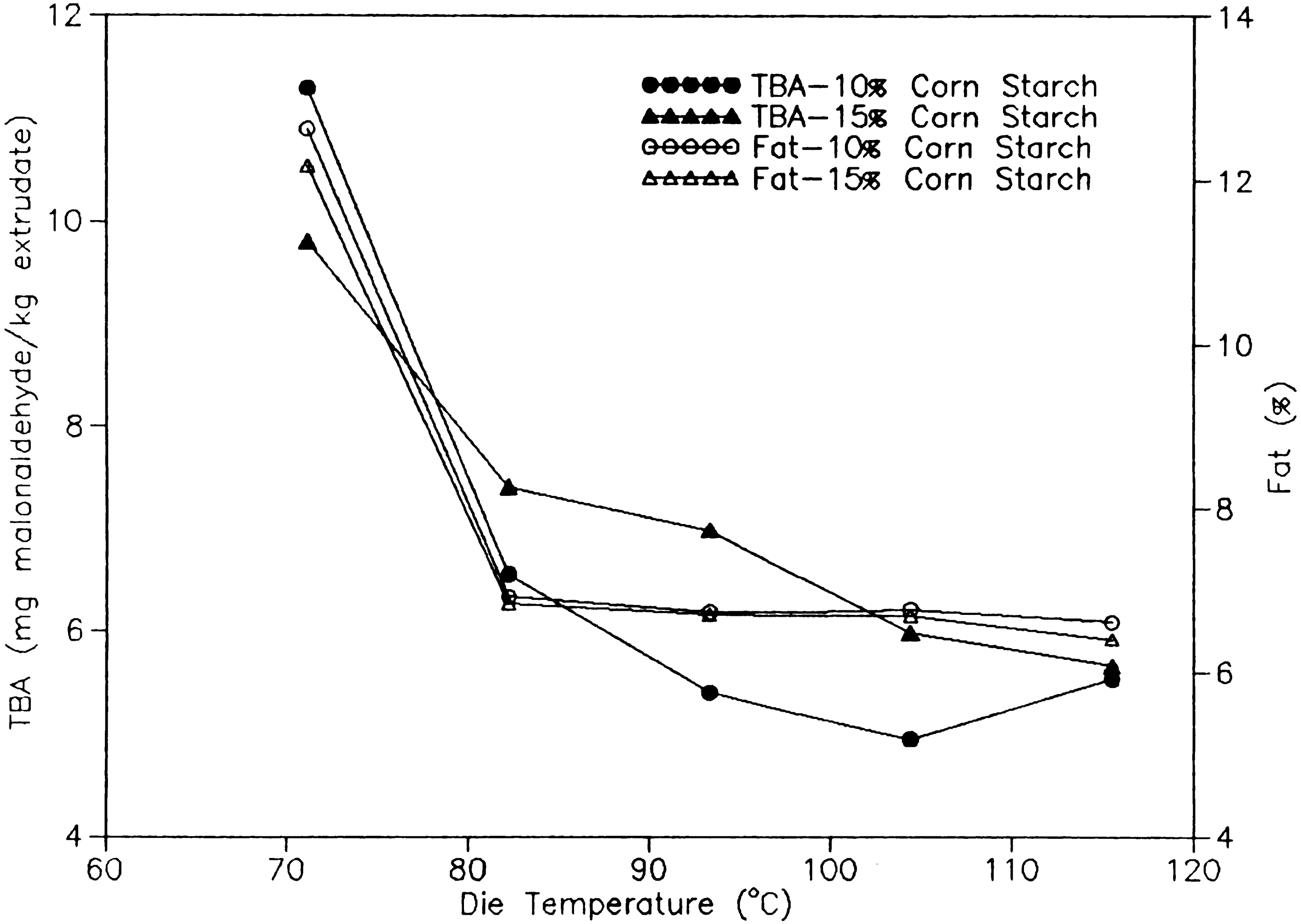


Fig. 3. Effect of constant barrel and die temperature on 2 thiobarbituric acid and fat composition of mechanically deboned chicken extruded with 10 and 15% corn starch (Standard error of the means are 0.431, 0.431 for fat and 0.555, 0.489 for TBA at 10% and 15%, respectively).

increased lipid oxidation, but heating to 120°C produced substances with antioxidant activity which were attributed to browning products (Sato and Hegarty, 1971; Yamauchi, 1972). The reduction of TBA values from 70 to 82°C may also be caused by a decrease in extractable fat content of extruded products (Delort-Laval and Mercier, 1976).

CONCLUSIONS

The results indicated that restructuring MDC into a variety of product forms was possible using the TSE processing conditions established here. Corn starch was the best binder for the product forms evaluated; however, SPI and WG might be useful for other applications using different processing conditions. Careful control of the screw configuration, die design and barrel temperatures could result in a wide variety of desirable products and could overcome many of the limitations previously reported.

V. STUDY 2

Effect of Extruder Die Temperature on Texture and Microstructure of Restructured Mechanically Deboned Chicken and Corn Starch

ABSTRACT

Proximate composition, textural properties and microstructural changes of mechanically deboned chicken/corn starch extrudates were evaluated as a function of die and barrel temperature in a twin-screw extruder. Moisture and protein content did not change significantly, but fat content decreased as die temperature increased. When die temperature increased from 71°C to 104°C, apparent stress at failure of extrudates containing 10% corn starch increased by 12 kPa while those containing 15% corn starch increased by 44 kPa. Textural changes in extrudates were correlated to changes in the protein matrix, fat globules and starch granules observed by scanning electron microscopy.

INTRODUCTION

Twin-screw extrusion (TSE) is one of the most popular new processes used by the food industry to produce expanded snack foods, ready to eat cereals and pet foods (Chinnaswamy and Hanna, 1988a Chinnaswamy et al., 1989). Extrusion technology is used because of its multiple controls and ability to accomplish a variety of food processing operations (Morgan, 1988). Twin-screw technology has been used extensively to prepare meat analogs from defatted vegetable proteins in the United States and to texturize cereals to produce bread analogs in Europe. This technology has also been extensively utilized in Japan (Gwiazda et al., 1987).

Mechanically deboned chicken (MDC) is used in a variety of comminuted products such as bologna and frankfurters due to its lack of whole muscle texture caused by the mechanically deboning process (Froning et al., 1971; Gruden et al., 1972). Studies on extrusion and texturization of mechanically deboned poultry indicated the need for further improvement in the binding of restructured products (Lampila et al., 1985). The use of TSE was suggested to improve the texture of MDC (Lingle, 1987). Restructuring of MDC using TSE has been accomplished with the addition of various binding agents such as soy protein isolate, vital wheat gluten, wheat flour, corn starch and carrageenan (Megard et al., 1985; Alvarez et al., 1990).

Microstructural studies have been shown to be a useful tool for observing textural changes in processed meat products. Scanning electron microscopy (SEM) has been used to study microstructures of meat gels, emulsions and batters and their changes due to ingredients and processing (Lee, 1985; Barbut, 1988). Studies demonstrating the effect of extrusion-cooking on the textural and microstructural properties of starch have been reported (Colonna and Mercier, 1983; Gomez and Aguilera, 1984; Diosady, 1985). Using SEM, several studies have identified different structures and interactions between wheat gluten, soy protein isolate, corn starch and meat protein in different processed products (Siegel et al., 1979a; 1979b; Comer et al., 1986).

Information on the microstructure and textural properties of mixtures of food ingredients such as MDC and corn starch during and after extrusion is very limited (Chinnaswamy et al., 1989). Little information is available concerning the effect of specific extrusion conditions, such as barrel and die temperatures, on extrudate microstructure and texture. The objectives of this study were to investigate changes in the texture and microstructure of restructured MDC/corn starch mixtures as a function of extruder barrel and die temperature.

MATERIALS AND METHODS

Materials

Fresh MDC was obtained from Nottawa Gardens Co. (Athens, MI) and held at -25°C until used within two months. Corn starch (Argo, CPC International Inc., Englewood Cliffs, NJ) was used as a binder during extrusion. Thawed MDC was mixed with 10% or 15% corn starch, 1.5% salt and 0.5% sodium tripolyphosphate for 15 min in a Butcher Boy mixer (Model 250F, Lasar Mfg. Co., Inc., Los Angeles, CA) 24 hr prior to use.

Cooking extrusion conditions

A pilot scale Baker-Perkins MPF 50 D/25 twin-screw extruder (APV Baker Inc., Grand Rapids, MI) with a die composed of 3 parallel slits, each measuring 1.8 x 0.3 cm, was used to restructure MDC at 350 rpm, barrel temperatures of 40 to 158°C and product feed rates of 0.30-1.0 Kg/min. The effective length:diameter ratio of the extruder was 25. Mixtures of MDC and CS were fed into the first feeding section port using a Moyno pump (Model IFF J4, Robbins and Meyer, Inc., Springfield, OH). The influence of temperature on extrudates was determined by adjusting barrel temperatures to obtain die temperatures of 71, 82, 93, 104, and 115°C. Extrudates from each of three extrusion runs were collected in duplicate at the die in 2.8 cm (diameter) x 15 cm (length) plastic tubes and held at 4 °C until analyzed within 5 days.

Product Analysis

Proximate analysis (moisture, fat and protein) was performed in duplicate on raw MDC and extrudates following AOAC (1984) procedures after each extrusion run. Textural properties of the extrudates were evaluated as reported by Diehl et al. (1979). Apparent shear stress (kPa) and apparent strain at failure (dimensionless) were calculated from duplicate 1.5 x 1.5 cm extrudate cores using an Instron universal testing machine (Model 4202, Instron Engineering Corporation, Canton, MA) at a crosshead speed of 10 mm/min with a 50 N compression cell. Core samples were removed from the extrudates with a 1.5 cm diameter cork borer. Cores were cut to 1.5 cm lengths using a template and razor blade.

Scanning electron microscopy

Specimen preparation. Extrudates were prepared for SEM observations following the procedure described by Klomparens et al. (1986). Extrudates were equilibrated at 4 °C and 1.5 mm x 1.5 mm cubes were cut with a chilled razor blade. Samples were fixed with 3% (v/v) gluteraldehyde, post-fixed in 1% (w/v) sodium buffered OsO_4, dehydrated in a graded ethanol series and critical point dried using carbon dioxide. To compare the effect of fixation, samples extruded at 72°C and 115°C were also prepared without post-fixing with 1% (w/v) sodium buffered OsO_4. Dry samples were mounted on 10 mm diameter x 5 mm high stubs (75350, Electron Microscopy Sci. Washington, PA) Adhesive mounting tabs (M. E. Taylor Engineering) were used to fasten the sample to the stub. An Emscope Sputter Coater (Model SC500, Kent, England) was used to coat the samples with gold under a vacuum of 0.06 Torr. Observations were made using a JEOL scanning electron microscope (Model JMS-35CF, Osaka, Japan) equipped with a tungsten electron gun at 15 kV accelerating voltage.

Statistical Analysis

Triplicate extrusion runs were used to evaluate each treatment. Statistical significance was determined using 2 x 5 factor factorial (10 and 15% corn starch concentrations and temperatures of 71, 82, 93, 104 and 115°C). Tukey's Honestly significance difference test and standard error of the means were used to evaluate the significant differences between the means at the 5% level of probability (MSTAT, 1989).

RESULTS AND DISCUSSION

Proximate Composition

Increasing barrel and die temperatures from 71°C to 115°C during twin-screw extrusion changed the proximate composition of the extrudates (Tables 6,7). Protein content did not change significantly as barrel and die temperature were increased. There were some significant differences observed in moisture content of extrudates containing 10% and 15% corn starch. The increase in moisture content as die temperature increased could be associated with an increase in the water binding capacity of the MDC/CS mixtures due to the formation of a protein-starch matrix. The decrease in fat content of extrudates may also contribute to the proportional increase in moisture content. Alvarez et al. (1990) reported a moisture content increase of 5% in extrudates when die temperature was increased from 71°C to 82°C. Van Zuilichem and Jager (1990) found that moisture content of mixtures of lean pork/corn starch (80/20) decreased 7% and 17% after extrusion at 160°C and 200°C, respectively. Fat content in extrudates containing 10% and 15% CS decreased significantly as extrusion temperatures increased. Cooking temperature and shear during extrusion may have caused melting of fat which could not be retained by the protein-starch matrix. This is in agreement with studies on restructuring of MDC which reported that

Table 6. Moisture, protein and fat content of mechanically deboned chicken containing 10% corn starch extruded at different die temperatures.

Die Temperature °C	Moisture %	Protein %	Fat %
25	66.85[a]	14.12[a]	9.08[a]
71	66.26[a]	14.97[a]	8.75[a,b]
82	66.25[b]	15.15[a]	8.12[a,b,c]
93	67.95[b,c]	15.18[a]	7.16[a,b,c]
104	67.76[b,c]	15.28[a]	6.84[b,c]
115	68.28[c]	15.35[a]	6.51[c]

[a,b,c] Means within columns followed by the same letter do not differ significantly ($P<0.05$).

Standard error of the means are: ±0.381 for moisture, ±0.115 for protein and ±0.096 for fat.

Table 7. Moisture, protein and fat content of mechanically deboned chicken containing 15% corn starch extruded at different die temperatures.

Die Temperature °C	Moisture %	Protein %	Fat %
25	60.71[b]	15.12[a]	9.71[a]
71	60.43[a]	14.79[a]	7.93[a,b]
82	63.25[a]	14.63[a]	7.48[b]
93	63.60[a]	14.56[a]	6.62[b]
104	63.69[a,b]	14.78[a]	6.62[b]
115	64.28[a]	14.41[a]	6.31[b]

[a,b,c] Means within columns followed by the same letter do not differ significantly ($P<0.05$).

Standard error of the means are: ±0.298 for moisture, ±0.136 for protein and ±0.116 for fat.

increasing die temperature from 71-115 °C decreased the fat content of extrudates (Alvarez et al, 1990).

Textural Properties

Apparent stress and strain at failure of extrudates were affected by die temperature (Table 8). Apparent stress at failure has been related to textural properties such as hardness and sensory firmness, while apparent strain at failure was related to textural elasticity and cohesiveness (Montejano et al., 1985; Lanier, 1986).

Apparent stress at failure of extrudates increased significantly from 6.45 kPa to 18.6 kPa as die temperature increased from 72°C to 104°C in extrudates containing 10 % corn starch. In samples with 15% CS, an increase in die temperature from 72°C to 104°C, caused an increase in apparent stress at failure from 8.34 KPa to 52.54 KPa. A die temperature of 115°C resulted in a decrease in the apparent stress of extrudates to 17.6 KPa and 31.61 KPa at 10% and 15% CS, respectively.

The concentration of corn starch also influenced the textural properties of the extrudates. Apparent stress at failure of extrudates containing 15% corn starch were higher than those with 10% corn starch at die temperatures of 93 °C and 104 °C, as stress at failure was approximately 2.7 times larger for extrudates with 15 % corn starch than those with 10 % corn starch. Starch imparts structure to extruded products through gelatinization, which occurs during heat

Table 8. Apparent stress and strain at failure of mechanically deboned chicken mixed with 10% and 15% corn starch extruded at different die temperatures.

Die Temperature (°C)	Apparent Stress at Failure (kPa)		Apparent Strain at Failure	
	Corn Starch		Corn Starch	
	10%	15%	10%	15%
71	6.45[d]	8.34[d]	0.31[d]	0.40[b]
82	14.50[c]	31.20[c]	0.52[a]	0.38[b]
93	16.80[b]	45.39[b]	0.38[c]	0.44[a]
104	18.60[a]	52.54[a]	0.44[b]	0.32[c]
115	17.60[b]	31.61[c]	0.30[d]	0.47[a]

a,b,c,d Means within columns followed by the same letter do not differ significantly ($P<0.05$).

Standard error of the means are: ±0.227 and ±0.679 for apparent stress, and ±0.127 and ±0.154 for apparent strain at failure, at 10% and 15% corn starch, respectively processing and shearing in the presence of water (Owusu-Ansah et al., 1984).

The apparent strain at failure of extrudates containing 10% and 15% corn starch were also influenced by the die temperature (Table 8). Apparent strain at failure increased to a die temperature of 82 °C and reached maximum values of 0.52 and 0.44 at 10% and 15% CS, respectively. At temperatures higher than 104 °C, the apparent strain decreased in extrudates containing 10% and 15% CS. Differences in fat content of the extrudates might be one possible reason for variations in apparent stress and strain. Starch gelatinization which alters the density of the extrudates and protein denaturation may have also affected the rigidity of extrudates. Denaturation of salt soluble proteins and the presence of non-meat binders were the cause of changes in texture of meat batters (Foegeding and Ramsey, 1987).

Nuckles et al. (1990) reported values of 42.8 kPa and 0.76 for apparent stress and strain at failure, respectively, in frankfurter model systems containing MDC. The results in this study indicated that 15% corn starch produced similar strength between temperatures of 82° to 93°C. However, apparent strain at failure of MDC extrudates was lower as compared to the model system frankfurters. Apparent stain at failure increased to 0.52 and 0.38 at 10% and 15% CS, respectively, when temperature increased up to 83 °C.

Other studies reported that the force to fracture and true shear stress in frankfurters containing different gums increased from 40-60 kPa as a function of temperature up to 60°C and then declined. Hardness of the same products showed a continuous increase when temperature changed from 40°C to 70°C (Foegeding and Ramsey, 1986). Later studies compared the rigidity at failure of gelled meat batters heated from 35°C to 75°C. Rigidity increased in meat batters containing different gums increased when temperature was above 60°C (Foegeding and Ramsey, 1987). Since protein content was held constant, the changes in the texture were attributed to variation in fat composition. The gums selectively affected the textural properties and water holding capacity.

Extrudate Fixation and Microstructure

Extrudates containing 15% corn starch were observed by SEM. The SEM micrographs of MDC-corn starch extruded at die temperatures of 71°C and 115 °C revealed structural differences in specimens fixed with 3% gluteraldehyde (Fig. 4A, 5A) as compared with those fixed with 3% gluteraldehyde and post-fixed in 1% OsO_4 (Fig. 4B, 5B). Specimens of low temperature extrudates prepared with a post-fixative revealed protein structures (p), starch granules (s), lipid globules (f) and a few voids (v). Specimens prepared without a post-fixation step exhibited an absence of lipid globules and more voids (v). This observation is in

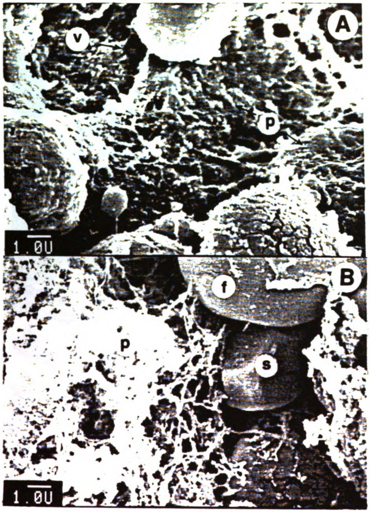


Fig. **4** - Scanning electron micrographs of mechanically deboned chicken and 15 % corn starch mixtures extruded by twin-screw extrusion at a die temperature of 71 ^{o}C. (A) Sample fixed with 3% gluteraldehyde. (B) Sample fixed with 3% gluteraldehyde and post-fixed in 1% OsO_4. (v) voids, (f) lipid globules, (s) starch granules.

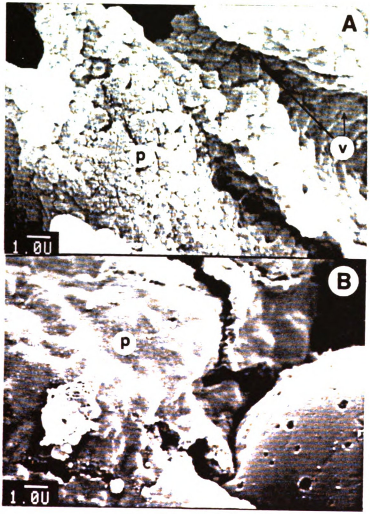


Fig. 5 - Scanning electron micrographs of mechanically deboned chicken and 15 % corn starch mixtures extruded by twin-screw extrusion at a die temperature of 115 ^{o}C. (A) Sample fixed with 3% gluteraldehyde. (B) Sample fixed with 3% gluteraldehyde and post-fixed in 1% OsO_4. (p) protein-starch matrix, (v) voids.

agreement with Basgall et al. (1983) who reported that lipids containing unsaturated fatty acids can be fixed with OsO_4. The presence of lipid droplets is an indication that the original structure has not been altered significantly during specimen preparation. Fixation with osmium tetraoxide helps harden surface features so that disruption of cell surfaces and loss of structures are minimized. Osmium crosslinks the unsaturated fatty acids thereby fixing the lipid containing membranes in the specimen (Klomparens et al., 1986).

The protein matrix observed at 72 ^{o}C (Fig 4 A,B) is similar to the microstructure of gels prepared from bovine myosin at different pH and ionic strength (Hermansson et al., 1986). The gel structure of bovine myosin changed from fine stranded to coarsely aggregated during heating at 60 °C and when ionic strength changed from 0.25 to 0.6M KCl. Similar observations were reported in SEM studies of chicken breast salt soluble protein gels (Wang, 1989). Gel microstructure was described as coarsely aggregated in 0.6 M NaCl at temperatures above 65 °C (Wang, 1989).

Post-fixation was also advantageous for preparing extrudates obtained at die temperatures of 115°C. Figure 5A shows structural differences as compared to Figure 5B. In Figure 5A a discontinuous protein matrix with empty voids was observed which may have contained fat droplets. Although fat globules could not be identified, the structure in Figure 5B shows that the starch-protein matrix is well preserved, continuous and has fewer empty voids.

Effect of Temperature on Microstructure

Extrusion at a die temperature of 71°C produced extrudates with microstructures exhibiting large areas of discontinuous, spongy gel type proteins (p), starch granules (s) and fat particles (f) of different sizes distributed throughout the matrix (Fig 6). Similar structures have been identified in restructured and comminuted meat products (Comer et al., 1986; Barbut, 1988; Comer and Allan-Wojas, 1988). Incomplete protein gelation and starch gelatinization in samples extruded at 71°C may have caused the low apparent stress values.

Microstructures of extrudates obtained at 82°C die temperature (Fig 7) did not differ markedly from samples extruded at 71 °C. Large regions of a discontinuous protein matrix (p), intact starch granules which have an irregular shape (s) and spheric fat globules (f) of various sizes were observed. The discontinuous protein matrix and intact starch granules may indicate that extrusion die temperatures of 71°C and 82°C resulted in protein denaturation and gelation. However, these die temperatures were not high enough and the residence time was too short for starch gelatinization which may be necessary to form a homogeneous protein-starch matrix. The gelation of myofibrillar proteins during heating is due to the association of

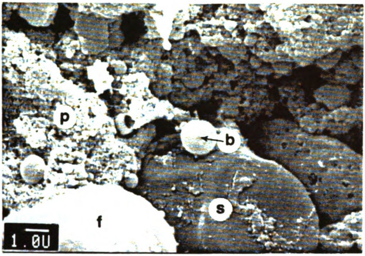


Fig. 6 - Scanning electron micrographs of mechanically deboned chicken and corn starch mixtures extruded by twin-screw extrusion at a die temperature of 71 ^{o}C, fixed with 3% gluteraldehyde and post-fixed in 1% OsO_4. (p) protein matrix, (b) bacteria or lipid globules, (s) starch granules, (f) lipid globules.

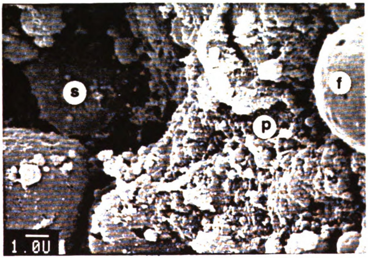


Fig. 7 - Scanning electron micrographs of mechanically deboned chicken and 15 % corn starch mixtures extruded by twin-screw extrusion at a die temperature of 82 ^{o}C, fixed with 3% gluteraldehyde and post-fixed in 1% OsO_4. (s) starch granules, (p) protein matrix, (f) lipid globules.

denatured proteins. A combination of mechanical action, ionic strength, pH and temperature causes tissue disruption, protein extraction and gelation which forms a heat set matrix to entrap components such as fat, starch and water (Siegel and Schmidt 1979a; Samejima et al., 1981; Trout and Schmidt, 1984). Chicken myofibrillar proteins begin to denature at 57° to 60°C (Kijowski and Mast, 1988a). Thermally induced unfolding and aggregation of muscle proteins from a matrix that provides processed meats their functional properties such as texture, water-holding and fat holding (Foegeding, 1988b). The presence of ungelatinized starch may explain the low apparent shear and strain to failure measured in the extrudates.

The scanning electron micrograph in Fig. 8 shows that the protein in samples extruded at a die temperature of 93°C changed from a discontinuous protein matrix to a partially continuous protein-starch structure. A discontinuous protein matrix (p) was observed which was similar to the structure observed at 71 and 82°C. Also, a continuous protein-starch matrix (cp) was identified. These observations suggest that the physical effect of cooking extrusion converted the denatured protein and gelatinized starch into a homogeneous matrix, which trapped water, fat, and other ingredients. Starch gelatinization occurs between 90 and 150°C, depending on the moisture content (Lawton et al., 1972). These microstructural changes resulted in an increase in apparent stress at failure at both 10 % and 15 %

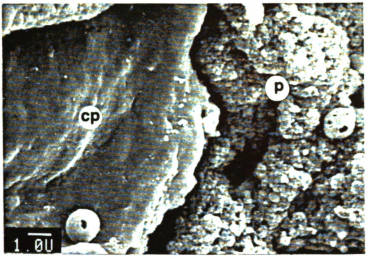


Fig. 8 - Scanning electron micrographs of mechanically deboned chicken and corn starch mixtures extruded by twin-screw extrusion at a die temperature of 93 ^{o}C, fixed with 3% gluteraldehyde and post-fixed in 1% OsO_4. (cp) continuous protein-starch matrix, (p) protein matrix.

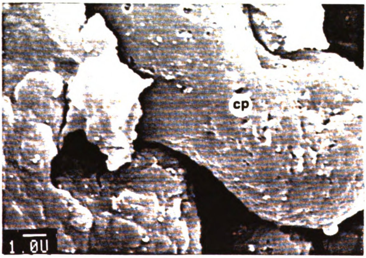


Fig. 9 - Scanning electron micrographs of mechanically deboned chicken and 15 % corn starch mixtures extruded by twin-screw extrusion at a die temperature of 104 ^{o}C, fixed with 3% gluteraldehyde and post-fixed in 1% OsO_4. (cp) continuous protein-starch matrix.

CS. Apparent strain at failure decreased in extrudates processed at 93 °C and may be due to starch gelatinization which decreased extrudate elasticity.

Spheric structures (b) with a diameter of approximately 1 μm were visible in micrographs (Fig. 6 and Fig. 10) and may be fat globules or bacteria. Fat globules may vary widely in size and shape when dispersed mechanically in a protein matrix. Fat particle sizes found in meat emulsions were in the range size of 1-100 μm (Lee, 1985). Fat globules of 10-50 μm sizes were identified in SEM micrographs of poultry meat batters (Barbut, 1988). Bacteria were visible as 1 μm spheres in SEM micrographs of commercially prepared frankfurters (Schmidt, 1984). Although samples were held under refrigeration and studied within five days, the possibility of microbial contamination existed.

Extrudate microstructures also contained numerous voids which could be either air or aqueous regions. Air may have been trapped in the extrudates when collecting the molten samples and may have led to the formation of voids. Air pockets have been reported to influence the texture of restructured beef products (Bernal and Stanley, 1986). Products restructured under vacuum were more dense with different textural and structural characteristics than those in which air was included (Wiebe and Schmidt, 1982). In micrographs of extrudates obtained at die temperatures of 104°C (Fig 9) a continuous protein-starch matrix (cp) and a

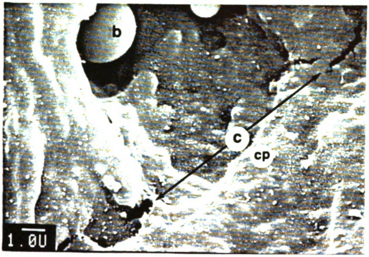


Fig. 10 - Scanning Electron Micrographs of mechanically deboned chicken and 15 % corn starch mixtures extruded by twin-screw extrusion at die temperature of 115 ^{o}C, fixed with 3% gluteraldehyde and post-fixed in 1% OsO_4. (b) bacteria or lipid globules, (c) cracks, (cp) continuous protein-starch matrix

more compact structure was observed. This indicated that extrusion conditions and die temperatures of 104°C caused protein denaturation and starch gelatinization. Protein-starch matrix formation during extrusion and the increase in the apparent force at failure may indicate that as temperature increased the matrix became more cohesive due to protein gelation and starch gelatinization.

Increasing the die temperature to 115°C altered the continuous structure of the extrudates (Fig. 10). Small particles (sp) were observed on the surface of the protein matrix. Examination of the same figure showed that the protein matrix contained cracks (c). Temperatures of 115°C and higher may negatively affect the protein-starch matrix formed during extrusion. The decrease of apparent stress in samples extruded at 115°C may be caused by the partial breakdown of the starch-protein matrix, which may be the result of excessive protein aggregation and starch degradation. The highest temperature for one of the major thermal transitions for the gelation of chicken muscle proteins is between 80-83°C depending on the muscle and testing conditions. Above these temperatures, muscle proteins may be completely denatured, aggregate and negatively affect the gel properties (Kijowski and Mast, 1988a, b).

The possibility of excessive protein aggregation and starch degradation is supported by studies which indicated that severe thermal treatment of proteins may result in structural changes due to hydrolysis of peptide bonds, formation of new covalent cross-links and modification of amino side chains (Cheftel et al., 1985). Heating plasma protein gels above gelation temperatures caused shrinkage and partial disruption of the gel network which became more aggregated and denser (Hermansson, 1983). Extrusion at 115°C may also have negatively affected the gelatinized starch which imparts structure to the final product (Owusu-Ansah et al., 1984). Chinnaswamy and Hanna (1988b) observed that increasing barrel temperatures above 110°C initiated starch degradation and textural problems in corn starch extrudates, evaluated by shear strength and expansion ratio.

CONCLUSIONS

The results indicate that die temperature had significant effects on the textural properties of extruded MDC and corn starch mixtures. It was possible to obtain different textural attributes in the finished product by changing the die temperature. Scanning electron microscopy was useful for observing protein-matrix formation, lipids and starch granules within extruded products. Since little information is available in this field, this work should be regarded as an initial step towards identifying microstructural changes in restructured MDC and corn starch by twin-screw extrusion.

VI. Study 3

Protein Insolubilization and Starch Gelatinization of Mechanically Deboned Chicken and Corn Starch During Twin-Screw Extrusion

ABSTRACT

Using high-temperature short-time twin-screw extrusion, 0.9% NaCl soluble protein extractability and starch gelatinization of mechanically deboned chicken/15% corn starch extrudates were determined as a function of position and temperature in the barrel. Protein extractability decreased and starch gelatinization increased as product passed through the barrel. Minimum extractability occurred in section 5 at 104°C and maximum starch gelatinization occurred in section 3 at 112 °C. The number of protein bands decreased from 15 in the first barrel section at 48 °C to 5 in the last barrel section at 104 °C. The relative protein molecular weight ranged from 13000-201000.

INTRODUCTION

The use of twin-screw extruders (TSE) to restructure high-moisture protein products such as mechanically deboned poultry meat (MDPM) and non-meat proteins has been reported in recent years. Structure formation was obtained in these materials using high temperature-short time (HTST) extrusion systems which have sophisticated temperature controls, barrel designs, screw configurations and dies (Kitabatake et al., 1985; Clarke et al., 1989; Alvarez et al., 1990). The restructuring of MDPM and other meats has been accomplished only with the addition of non-meat binders (Megard et al., 1985; van Zuilichem and Juger, 1990). Mixtures of mechanically deboned chicken (MDC)/15% corn starch (CS) produced extrudates with a wide range of textural properties when die temperature increased from 71 to 115°C (Alvarez et al., 1990). Researchers reported some limitations in the extrusion system utilized such as length of barrel and control of temperatures, (Megard et al., 1985) or variations in finished product quality due to low water binding properties of the extrudates (van Zuilichem and Juger, 1990).

During extrusion processing, proteins and non-protein materials such as corn starch are transformed due to exposure to shear stresses, high temperatures and high pressure. Proteins are converted into a continuous molten material which flows and travels down the extruder barrel (Harper, 1980). Corn starch is gelatinized and has been found to influence the textural properties of the final extruded product (Chiang and Johnson, 1977; Chinnaswamy and Hanna, 1988c; Alvarez et al., 1990). Most studies have examined the effect of extrusion variables on the finished product quality. There is little information available on the changes that may occur in proteins and starch along the barrel during extrusion.

Changes in protein extractability might be used as an indication of protein denaturation and changes in functionality of proteins during extrusion. Salt-soluble chicken muscle proteins are responsible for the texture of meat products (Acton et al, 1983; Asghar et al., 1985; Hamann, 1988). It was reported that starch gelatinization and protein denaturation were responsible for changes in texture of twin-screw restructured mechanically deboned chicken mixed with corn starch when die temperature increased from 71°C to 115°C (Alvarez et al., 1990).

Decreases in solubility of proteins extracted from meat products with low ionic strength solutions (0.9% NaCl) has been investigated as an indication of the degree of heat denaturation (Crespo and Ockerman, 1977; Lee et al., 1974; Lyon et al., 1986). Using sodium dodecyl sulfate polyacryamide gel electrophoresis, it was observed that as temperature increased from 65°C to 90°C, the number and intensity of six protein bands extracted with distilled water from bovine muscle was reduced to one (Lee et al., 1974). In studies using high performance liquid chromatography and SDS-PAGE with protein extracts from porcine longissimus muscles, the number of proteins was reduced from 14 to 3 after heating to 75°C (McCormick et al., 1987). Similar effects of cooking on protein extractability from roast beef semitendinosus were reported using densitometric scans of isoelectrofocusing gels (Lyon et al., 1986).

The objective of this study was to determine changes in extractability of 0.9% NaCl soluble proteins and starch gelatinization during extrusion of MDC/CS mixtures as a function of barrel position and temperature at constant extrusion conditions.

MATERIALS AND METHODS

Materials

Fresh mechanically deboned chicken (MDC) was obtained from Nottawa Gardens Co. (Athens, MI) and held at -25 °C until used within two months. Corn starch (CS) (Argo, CPC International Inc., Englewood Cliffs, NJ) was used as a binder during extrusion. Thawed MDC was mixed with 15% CS, 1.5% salt and 0.5% Na tripolyphosphate for 15 min in a Butcher Boy mixer (Model 250F, Lasar Mfg. Co., Inc., Los Angeles, CA) 24 hr prior to use.

Extrusion conditions

A pilot scale Baker-Perkins MPF 50 D/25 twin-screw cooking extruder (APV Baker Inc., Grand Rapids, MI) with a die composed of 3 parallel slits each measuring 1.8 x 0.3 cm was used to restructure MDC at 350 rpm and a product feed rate of 0.45 Kg/min. The effective L/D ratio of the extruder was 25. Barrel temperatures in zones 1-3, 4-5, 6-7, and 8-9 in the extruder were adjusted to 48.8, 103, 112.5 and 111.8 °C, respectively, to obtain a die temperature of 104.4 °C. The extrusion process was stabilized and held constant once temperatures were obtained. Mixtures of MDC and CS were fed into the first feeding section port using a Moyno pump (Model IFF J4, Robins and Meyers, Inc., Springfield, OH).

Residence time of the MDC:CS mixture in the extruder barrel was determined by the tracer method using 3% (w/v) erythrosin-ß dye (Sigma Laboratories, St. Louis, MO) in water as reported by Ofoli et al. (1990) with some modifications. One milliliter of dye was applied at the first feed port. Two hundred grams of extrudate were collected under steady state extrusion conditions at 1 min intervals, ground two times through a 4 mm plate of a Kitchen Aid Grinder (Model k5-A, Hobart, Troy, OH) and mixed. Ground samples were pressed into 9 cm (diameter) by 8 mm (height) plastic containers. The color was measured using the Hunter Color Difference Meter (Model D25-2 Fairfax, VA) standardized with the pink standard plate, No.

C2-6005; L=67.6, a_L=21.4, b_L=11.9. The a_L values were plotted against the elapsed extrusion time.

To monitor changes in protein extractability and starch gelatinization due to barrel location, samples were taken from 5 different positions in the extruder. The length of the extruder barrel was divided in 4 sections for sampling. The length of the sections starting at the end of the barrel opposite the die were: section 1, feeding port; section 2, 0-42.1 cm; section 3, 42.1-84.5 cm; section 4, 84.5-127.0 cm; section 5, product exiting the die. Once extrusion conditions were stabilized, the extruder was stopped, the barrel quickly opened and samples taken from the material attached to the screw. Samples were collected in plastic bags and placed immediately in ice and kept at 4 ^{o}C. Analyses were performed within 24 hr of sampling.

Proximate Analysis.

Proximate analysis (moisture, fat and protein) was performed in duplicate on raw MDC and extrudates following AOAC (1984) procedures.

Protein Extractability

Protein extractability of the extruded material from the 5 extruder barrel sections was determined. Proteins were extracted from the extrudates according to the procedure of Lyon et al. (1986) with some modifications. Samples were ground three times through a 4 mm plate of a

Kitchen Aid Grinder (Model K5-A, Hobart, Troy, OH). Proteins were extracted from a 10 g sample with 3 volumes of 0.9% saline solution for 30 min with continuous agitation at 4 °C. Samples were filtered through glass wool and the filtrate was centrifuged at 23,000 x g for 15 min. The supernatant was stored at 4°C and used for protein evaluations.

Protein content (mg protein/mL supernatant) of extrudates extracts from section 1 was measured using Kjeldahl AOAC Method 24.038-24.040 (AOAC, 1984), except that H_2O_2 was not used for sample digestion. Protein contents of the meat samples determined in preliminary experiments with and without H_2O_2 during digestion had the same values. Protein content was determined in duplicate. The protein in the extract from barrel section 1 was considered 100% extractable. Protein content of extrudate extracts represented only the fraction corresponding to water soluble proteins, mainly sarcoplasmic proteins, from the total protein determined by proximate analysis.

Electrophoresis

Extracts were prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by mixing 4.5 ml of supernatant with 0.5 ml 10% (w/v) SDS. The mixtures were boiled for 5 min and three drops of ß-mercaptoethanol, 1 ml glycerol and a trace of bromophenol blue were added. Prepared extracts were stored at -20 °C.

Electrophoresis of 0.9% NaCl extracted protein was performed using 10% polyacrylamide gels in a Hoeffer Vertical Electrophoresis Unit (Model SE 600; Hoeffer Scientific Instruments, San Francisco, CA) and constant voltage power supply (Model 1P-17 Heathkit, Benton Harbor, MI) using a modification of the method described by Laemmli (1970).

Extracts containing 164 μg (15 μl) of protein from extruder section 1 were applied to the stacking gel. To measure changes in extractability in the other extruder barrel sections, the volume of protein extract applied to the stacking gel was adjusted as follows: section 2 : 15 μl, sections 3 through 5: 75 μl.

Molecular weights of protein bands were determined by comparing relative retention times to molecular weight standards (SDS-6H and No. SDS-7, Sigma Chemical Company, St. Louis, MO). The identification of the major bands was based on studies that reported subunit molecular weights from SDS-PAGE electrophoregrams of unheated water-soluble muscle protein extracts (Scopes, 1970; Scopes and Penny, 1971; McCormick et al., 1988). The linear regression equations used to calculate the apparent molecular weights were:

(a) $y = 5.38 + (-1.52) * x$; $(r=0.98)$ for high MW

(b) $y = 5.07 + (-0.97) * x$; $(r=0.99)$ for low MW.

The protein bands were quantitated by densitometric scans at 580 nm (Shimadzu Dual-Wave Length Thin-Layer Chromoto Scanner (Model CS-930, Kyoto, Japan). The amount of protein in each peak as determined by densitometry was expressed as a percentage of the total extractable protein. Relative protein extractability was calculated by correcting for original sample volume and dividing the total area of the tracing of extrudates from each section by the total area of the tracing for section 1, then multiplying by 100.

Starch Gelatinization

The percentage of gelatinized starch was determined by the glucoamylase method (Budke, 1984) with some modifications. Amyloglucosidase (Diazyme L-200, activity= 200 Diazyme units/ml) was obtained from Miles Laboratories, Inc., Elkhart, IN. The enzyme was stored at 4^{0}C. Extrudates were ground 3 times through a 4 mm plate and mixed using a Kitchen Aid Grinder (Model K5-A, Hobart, Troy, OH). Two samples of 0.5 g each were taken from each extrudate. One sample was chemically gelatinized and the other was digested enzymatically to determine the percentage of gelatinized starch.

Gelatinization Procedure

Ten milliliters of water and 0.5 g extrudate were stirred until the sample was uniformly and suspended. Ten milliliters of 2N NaOH were added to the suspension and the flasks incubated at room temperature for 20 min with occasional stirring. Gelatinization was stopped by the addition of 10 mL 2N HCl and the total volume was adjusted to 100 mL with distilled water. Glucose concentration in the gelatinized samples was determined using a YSI Model 27 Analyzer (Yellow Spring Instrument Co., Yellow Springs, OH) equipped with glucose oxidase membrane (YSI No. 2365) and a carrier buffer (0.47 M Na phosphate buffer, pH 8.3 YSI No. 2357). A sample solution of 25 μl was injected into the glucose analyzer. The glucose concentration in the gelatinized samples (G_{GS}) was measured as mg/dL and used to calculate the percentage of starch on a dry basis (%S) (Budke, 1984).

Enzymatic Digestion

The sample (0.5 g), 25 mL of water, 10 mL of 1N Na Acetate buffer (pH 4.2) and 5 mL of Diazyme stock solution (30 units/mL) were combined, stirred and held in a 40 ^{o}C water bath for 60 min. An enzyme blank solution was prepared as described above but without the sample. Five milliliters of trichloroacetic acid (25% (w/v)) were added and flasks were cooled to room temperature. The volume was adjusted to 100 mL with distilled water. Glucose concentration for sample (G_E) and blank (G_{EBS}) solutions subjected to enzymatic digestion were determined using the YSI Analyzer as described above.

Calculations

Starch gelatinization (%SG) was calculated using the following expression:

$$\%SG = \frac{(G_E - G_{EBS})\ (0.9)\ (100)\ (100\%)}{(1000)\ (W)\ (\%DS)\ (\%S)}$$

Where: %SG= Percentage of gelatinized starch, G_E= YSI reading for enzymatically digested sample (mg glucose/dL); G_{EBS}= YSI reading for enzyme blank solution (mg glucose/dl), 100= Total volume of sample, 100%= Conversion to percentage; 1000= Conversion factor for YSI reading (mg/dl to g/100 ml) W= Weight (g) of enzyme digested sample, %DS= Percent solids in sample (AOAC, 1984) and 0.9= Theoretical factor for conversion of glucose to starch (Budke, 1984).

Statistical Design and Analysis

Triplicate extrusion runs were used to evaluate each treatment. Statistical significance, to study the effect of temperature in the 5 extruder sections on protein extractability and starch gelatinization, was determined using a one-way analysis of variance and a complete randomized design with MSTAT software (Version C, East Lansing, MI). Tukey's honestly significant difference test and standard error of the means ($P<0.05$) were used to evaluate the significant differences between the means.

RESULTS AND DISCUSSIONS

Residence Time

Mean estimated residence time was 156 sec. Determination of this parameter has been considered very useful for scale up and for determining optimal process conditions during twin-screw extrusion (Eerikainen and Linko, 1989). In this study, it was not possible to relate the residence time to sample position in the different barrel sections. The complex geometry of the screw made it difficult to predict the flow pattern of the material due to the conveying, mixing and kneading that occur during extrusion. Material moves faster or slower in each section depending on the screw configuration at that specific position. A detailed discussion of all the factors involved in flow patterns in the screw has been reported (Janssen, 1985). However, it can be assumed that residence time increased as material moved from section 1 through section 5.

Protein and Moisture Content

There were significant changes in protein and moisture in extrudates sampled at different sections of the barrel due to different conditions such as temperature and time (Table 9). Earlier studies in our laboratory (Alvarez et al., 1990) indicated that protein and moisture content of twin-screw restructured MDC:corn starch mixtures sampled at the die did not change significantly as a function of barrel and die temperatures between 72°C and 115°C. Monitoring the changes of these two components was important during extrusion since myofibrillar proteins were responsible for the development of texture (Acton et al., 1983; Alvarez et al, 1990) and moisture content influenced temperature of

Table 9. Protein and moisture content of mechanically deboned chicken-15% corn starch extrudates sampled in different zones of twin-screw extruder barrel.

Extruder Section	Section Temperature (^{o}C)	Protein (%)	Moisture (%)
1	48.8	$14.4^{b,c}$	43.8^{a}
2	103.0	$14.7^{b,c}$	44.9^{a}
3	112.5	$15.1^{a,b}$	48.1^{b}
4	111.8	15.7^{a}	47.4^{b}
5	104.4	14.0^{c}	45.4^{a}

[abc]Means within columns followed by the same letter do not differ significantly ($P<0.05$).

Standard error of the means are ±0.152 for protein and ±0.433 for moisture.

starch gelatinization (Chiang and Johnson, 1977; Owusu-Ansah et al., 1984).

Gelatinization

Starch gelatinization in extruded samples occurred as the materials passed through the different sections in the barrel (Table 10). Extrudates from section 1 at 48.8 ^{o}C and shortest residence time had the lowest quantity of gelatinized starch. Corn starch begins to gelatinize at approximately 70^{o}C (Zobel, 1984), although this temperature may vary during twin-screw cooking extrusion due to the water content of the extruded materials (Chiang and Johnson, 1977). Higher water content (> 30-40%) in corn starch resulted in lower gelatinization temperatures during extrusion (Lawton et al., 1972).

As temperature and residence time increased, higher quantities of gelatinized starch were measured in the extrudates with a maximum of 75.4% gelatinization in section 3 at 112 °C. Complete corn starch gelatinization was observed at extrusion temperatures of 110-135 °C (Chiang and Johnson, 1977).

Starch gelatinization decreased significantly in extrudates from sections 4 and 5 at 112 °C and 104 °C, respectively. The low values might be associated with changes in gelatinized starch which may have reduced the apparent activity of glucoamylase. Starch-lipid (Owusu-Ansah et al., 1982; Colona and Mercier, 1983; Schweizer et

Table 10. Percentage starch gelatinization and relative 0.9% NaCl protein extractability of mechanically deboned chicken-15% corn starch extrudates sampled in different sections of twin-screw extruder barrel.

Extruder Section	Section Temperature (°C)	Extractability (%)	Starch gelatinization (%)
1	48.8	100.0[a]	5.4[d]
2	103.0	58.1[b]	17.5[c]
3	112.5	6.6[c]	75.4[a]
4	111.8	6.0[c]	62.3[b]
5	104.4	5.6[c]	58.9[b]

[abcd] Means within columns followed by the same letter do not differ significantly ($P<0.05$).

Standard error of the means are: ±6.074 for protein extractability and ±5.108 for starch gelatinization.

al., 1986) and starch-protein interactions (Asp and Bjork, 1982) due to long exposure to high temperature may have reduced the availability of starch to glucoamylase and thus decreased the percentage starch gelatinization measured by this method. Starch degradation which occurs at temperatures above 110°C (Chinnaswamy and Hanna, 1988b) may have been another factor that interfered with the enzyme activity (Budke, 1984).

Protein Extractability

Relative extractability of 0.9% NaCl soluble extrudate proteins obtained from 5 sections of the barrel are shown in Table 10. Protein extractability decreased as material moved through the barrel. Extractability decreased significantly to 58.1% in section 2 at 103°C. Another significant change occurred in section 3 at 112°C where relative protein extractability decreased to 6.6 %. Changes in extractability with a lesser degree occurred between section 4 and 5. These results indicated that during cooking extrusion, insolubilization and/or coagulation of 0.9% Na Cl extractable proteins was temperature and time dependent.

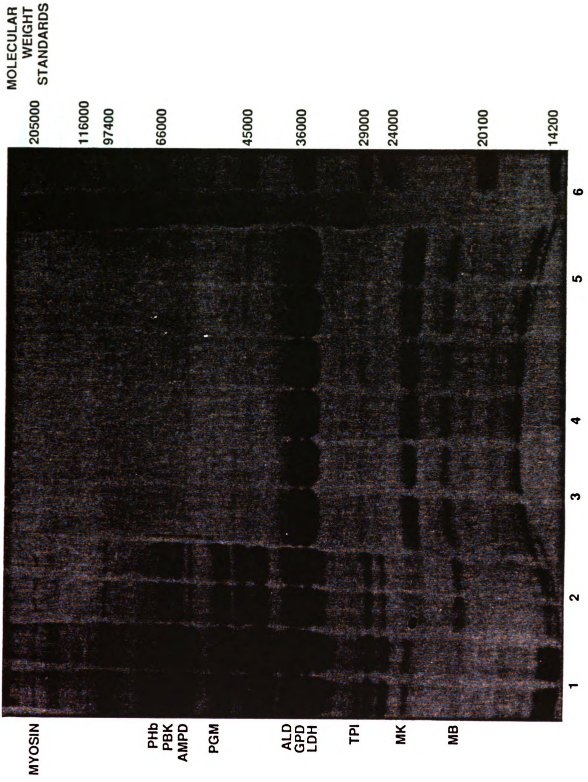
The changes in water extractable proteins of extrudates from different extruder sections may be used to monitor and indicate at what position of the barrel, protein insolubilization is achieved during extrusion. Monitoring changes in protein extractability has been useful in evaluating the effect of temperature on cooked meat products (Lee et al., 1974; Caldironi and Bazan, 1980).

Extrudate Protein Composition

The electrophoregram of 0.9% NaCl extractable proteins from extrudates obtained in the 5 extruder barrel positions is shown in Fig. 11. Subunit molecular weights and band intensity of proteins extracted from all extruder sections are shown in Table 11. A total of 19 protein bands were observed in extrudate extracts from section 1. Any band with a relative intensity below 0.5% could not be accurately measured by the densitometer. The observed migration and subunit molecular weights of 11 bands corresponded to published values for muscle sarcoplasmic proteins (Scopes, 1970; Scopes and Penny, 1971). There were differences between the published values and the molecular weights estimated in this study (Appendix A). The MW of enolase (EN) determined in this study was approximately 9.7 % larger than the MW reported by Scopes and Penny (1971). The rest of the molecular weights identified differed by 1.0 to 5.5 % from published values.

In terms of quantity, the predominant proteins included enolase and aldolase (ALD). Other proteins that may have been included in the same band due to their similarities in MW of about 34,000 are glyceraldehyde-3-phosphate dehydrogenase (GAPDH, phosphoglycerate mutase (PGAM) and lactate dehydrogenase (LDH). All these proteins and an

Fig. 11 - Electrophoregram of 0.9% NaCl extractable proteins from different sections of the extruder barrel and protein markers resolved on 10% polyacrylamide gels Bands (1) through (5) are proteins from the corresponding extruder sections, (6) protein markers



MOLECULAR WEIGHT STANDARDS
205000
116000
97400
66000
45000
36000
29000
24000
20100
14200
MYOSIN
PHb
PBK
AMPD
PGM
ALD
GPD
LDH
TPI
MK
MB
1
2
3
4
5
6

Table 11. Apparent molecular weight (MW) and relative intensity of 0.9 % NaCl extractable proteins from mechanically deboned chicken-15% corn starch extrudates sampled from 5 different sections of twin-screw extruder.

Rf	MWx10^3 (a)	Difference % (b)	Protein		Relative Intensity (%) Section 1	Section 2	Section 3	Section 4	Section 5
0.05	201.000±0.0	0.0	MYO	Myosin	2.9 ±0.13	1.4 ±0.3			
0.08	181.000±1.7	---	----		2.5 ±0.17	<0.5			
0.19	123.000±1.3	---	----		5.4 ±0.29	1.5 ±0.2			
0.29	87.000±0.0	5.5	PHb	Phosphorylase B	2.6 ±0.26	7.4 ±0.7			
0.32	78.000±0.9	2.1	PBk	Phosphorylase B kinase	4.6 ±0.21	3.8 ±0.8			
0.35	70.000±1.5	4.9	AMPD	AMP deaminase	7.3 ±0.42	<0.5			
0.40	59.000±1.9	3.3	PK	Pyruvate kinase	12.1 ±0.67	8.8 ±0.9			
0.44	51 000±1.2	---	----		---------	15.3 ±1.5			
0.47	46.000±1.0	9.7	EN	Enolase	18.0 ±0.99	10.6 ±1.3			
0.52	39.000±0.5	2.5	ALD	Aldolase	12.3 ±0.53	19.7 ±2.6	9.8 ±0.6	12.8 ±0.1	11.2 ±1.0
0.56	34.000±1.2	5.5	GAPDH	Glyceraldehyde phosph dehydr	5.8 ±0.27	10.9 ±1.3			
0.59	31.000±1.2	3.0	F-P	F-protein	6.2 ±0.34	8.2 ±0.5			
0.66	27.000±0.7		TPI	Triose phosphate isomerase	<0.5				
0.68	26.000±0.2	---	----		5.2 ±0.13	4.0 ±0.2			
0.70	25.000±0.7	---	----		2.9 ±0.16	2.3 ±0.2			
0.81	19.000±0.8	4.3	MK	Myokinase	<0.5	<0.5	3.4 ±0.7	4.0 ±0.0	4.5 ±0.1
0.86	17.000±0.7	4.3	MB	Myoglobin	<0.5	3.9 ±0.1	2.8 ±0.7	1.4 ±0.1	1.1 ±0.1
0.89	16.000±0.3	---	MLC	Myosin light chain	<0.5	1.8 ±0.4			
0.92	15.000±0.8	---	----		<0.5	<0.5	0.8 ±0.1		
0.96	13.000±0.2	---	----		9.9 ±0.14	3.3 ±0.7	2.0 ±0.3	2.32 ±0.1	3.2 ±0.1

[a]Standard deviation $\pm 10^3$. [b] Difference in MW from published data, (Scopes and Penny,1971; McCormick et al., 1988)

unidentified protein band with a subunit molecular weight of 14000, were the predominant 0.9% NaCl extractable proteins constituting 51.3% of the total. Other proteins identified in section 1 extracts included phosphorylase B (PHb), phosphorylase B kinase (PBK), AMP deaminase (AMPD) and glycerophosphate dehydrogenase (GDP). Proteins present at very low concentration included triose phosphate isomerase (TPI), myokinase (MK) and myoglobin (MB). Other proteins observed but not identified as sarcoplasmic proteins included bands with molecular weights of 201000, 181000, 123000, 25000, 24000, 16000 and 15000.

The subunit molecular weights of 201000 and 16000 suggested the presence of myofibrillar proteins in the extracts (Scopes and Penny, 1971; Goll et al., 1977). The molecular weights estimated corresponded to myosin heavy chain (MYO 201000) and myosin light chains (16000). It is possible that protein bands previously identified as sarcoplasmic proteins MK and MB may include small amounts of myosin light chain (MCL) and troponin-C (TN-C) which have a molecular weight of 19000 and 17000, respectively (Goll et al., 1977). Many myofibrillar proteins are water soluble once they have been extracted from the myofibril (Goll et al., 1977). After complete disruption of the myofibril, extracted myosin remained soluble at ionic strengths below 0.001 (Goll et al., 1977).

The sarcoplasmic proteins identified from section 1 at 48 °C resulted in protein band patterns similar to previous studies of unheated water-soluble protein extracts of pig and rabbit (Scopes, 1970; McCormick et al., 1988). Some differences in the migration and band intensity from published data were expected due to conditions during extraction such as speed and extent of homogenization, pH, temperature, proteolysis, protein interaction or a combination of these changes that occur prior to extraction (Greaser, 1986).

As MDC/corn starch moved through the barrel, the intensity of many bands decreased and fewer bands were observed. Protein extracts from section 2 contained 13 distinct bands. Two bands of molecular weight 181000 and 31000 disappeared and the others decreased in intensity relative to section 1. The appearance of a new band of molecular weight 51000 was evident. The greatest change was observed in protein bands of extrudates from section 3, 4 and 5 in which all proteins with relative molecular weight higher than 39000 disappeared completely. These results indicated that at this position in the barrel, most proteins were denatured and had lost their extractability at low ionic strength.

A high intensity band with a molecular weight between 33000-39000 was the predominant fraction in extracts from sections 3-5. Other bands observed corresponded to the molecular weight of MK, MB, myosin light chain MLC (16000) and two bands with molecular weights of 25000 and 14000. This study showed that the apparent molecular weight of two of the remaining protein bands identified as ALD and MB are similar to those reported by McCormick, et al., (1987). The authors suggested that the protein with an apparent molecular weight of 40000 could have been LDH, which according to Scopes and Penny (1971), has an apparent molecular weight of 35000. The molecular weight of 39000 of the protein identified in this study could ok be ALD or LDH according to the published molecular weight of 35000 for LDH or 40000 for ALD (Scopes, 1970; Scopes and Penny, 1971).

Although there were some differences in the molecular weights, the number of bands identified in section 3 was similar to studies on extraction of heat treated water soluble muscle proteins of different animals. Extracts from bovine muscles cooked at 68°C showed 6 protein bands. These bands gradually disappeared when the temperature increased and could not be detected above 80°C (Caldironi and Bazan, 1980). Studies of maximum processing temperature (65°-95°C) in meat from ox, horse, pig, rabbit, duck and chicken by SDS-polyacrylamide gradient gel electrophoresis, identified a total of 35 distinct protein bands in the 6 different species. There were 6 bands which corresponded to chicken meat. McCormick et al. (1987) reported that remaining water soluble proteins included LDH, PK and myoglobin in nearly equal amounts when porcine longissimus muscles were heated at 70°C and 75°C.

Observations on the remaining proteins in this study were different from works on loss of extractable proteins from heat treated meat products. There were some distinct bands present in extracts from sections 2, 3, 4 and 5 at temperatures above 100°C. Most studies on water extractable proteins have reported the disappearance of sarcoplasmic proteins at temperatures between 75°C and 90°C. However, it was suggested that monomeric proteins containing one chain or subunit per molecule are expected to be resistant to heat (McCormick et al., 1987). A recent study on extrusion at barrel temperatures from 60° to 200°C of lean pork and corn starch mixtures at different concentrations reported that the temperatures of the barrel did not affect protein dispersibility of the soluble fraction as expected (van Zuilichem and Jager, 1990). The authors suggested the possibility of meat proteins being protected against denaturation by a starch matrix formed during extrusion. These observations may provide a possible explanation for the presence of extractable proteins at barrel temperatures above 100°C in this study.

The residual proteins identified could be the result of the disappearance of high molecular weight proteins observed in lanes 5 through 7 of Figure 11. As high MW proteins disappeared, proteins with low molecular weights of 19000, 17000 and 14000, increased in relative intensity. These observations may be supported by studies that investigated changes of water extractable proteins from vegetable proteins extruded at various temperatures (Cumming et al., 1973). The authors reported loss of water solubility and the breakdown of proteins into sub-units were the result of their exposure to several processes which occurred during extrusion. Data in this study do not provide enough evidence to support these assumptions, thus further research is suggested.

CONCLUSIONS

The results of this study indicated that it is possible to identify changes in protein extractability and corn starch gelatinization as they pass through the extruder barrel. Although there were some limitations, the glucoamylase method was useful for measuring starch gelatinization at extrudate temperatures up to 112°C. The observations made in this study may be helpful in establishing extrusion temperatures to promote optimum protein denaturation and starch gelatinization for desired product texture and quality. These two changes have been found to be important for protein-starch matrix formation in extruded products.

VII. SUMMARY AND CONCLUSIONS

Study 1

Restructuring of mechanically deboned chicken into a variety of products forms was possible using the twin-screw extruder processing conditions established in this study. Under these extrusion conditions corn starch was the best binder for the product forms evaluated. However, soy protein isolate and wheat gluten may be useful for other applications using different processing conditions.

Study 2

The results indicate that die temperature has a significant effect on the textural properties of extruded MDC and corn starch mixtures. It was possible to obtain different textural attributes in the finished product by changing die temperature and holding the other extrusion conditions constant. Scanning electron microscopy was useful for observing protein-starch matrix formation and lipids within extruded products. The microstructural changes correlated with the textural properties measured.

These results suggest that new products with specific textural characteristics can be developed by adjusting extrusion temperatures, without the need for significant changes in the formulation and/or other extrusion conditions.

Study 3

It was possible to identify changes in protein extractability and corn starch gelatinization as MDC/CS passed through the extruder barrel. The changes were correlated to the temperature and position of the product in the barrel. Identifying barrel position and temperatures at which proteins denature and starch gelatinizes may be useful if specific reactions of proteins and starch with other additives are desired.

The results suggest that it might be possible to establish extrusion temperatures to promote optimum protein denaturation and starch gelatinization for desired product texture and quality.

VIII. RECOMMENDATIONS FOR FUTURE RESEARCH

1. Develop a specific extruded product and define the finished product quality and stability using the same combination of MDC/15% CS.

    a. Sensory attributes in the product may require addition of additives or spices to provide good taste and aroma.

    b. Further research on textural parameters is necessary so that the extruded product could be comparable to existing food products.

    c. Appearance, which includes color and shape form, is influenced by the process and formulation. The use of additives to make the product more attractive needs to be studied.

    d. Investigate the effect of extrusion processing on the protein quality of MDC to determine the nutritional value of the extrudate.

    e. Chemical and microbiological safety need to be evaluated. The high temperature short time extrusion process may not be sufficient to eliminate microbial contamination.

2. Research to better understand the textural and chemical changes that occur in extruded products. The type of binding that is developed between meat proteins and corn

starch and holds the matrix together needs to be investigated.

3. Investigate the individual effect of other extrusion conditions such as screw geometry, screw speed, feed rate and die size on finished product characteristics. Using the same corn starch concentrations, different extrudates from those obtained in this study may be developed.

4. Study the combined effect of various extrusion conditions on finished product quality. This might require the use of mathematical models and computer software due to the complexity of the twin-screw system. Such studies could be useful in better understanding and controlling the restructuring of MDC by twin-screw extrusion.

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APPENDIX A

APPENDIX A

Table A1

Subunit Molecular Weights (MW) from sodium dodecyl sulfate polyacrylamide gel electrophoresis of unheated water soluble muscle protein extracts. [a]

Enzymes	Subunit size mol. wt.X 10^{-3}	Calculated molecular weight native protein X 10^{-3}
Phosphorylase B (PHb)	92.5	185
Phosphoglucomutase (PGM)	63	63
Phosphoglucose isomerase (PGI)	54	108
Phosphofructokinase (PKF)	74	295
Aldolase (ALD)	40	160
Triose phosphate isomerase (TPI)	27	54
α-Glycerophosphate dehydrogenase (GPD)	33.5	67
Glyceraldehyde phosphate dehydrogenase (GAPDH)	36	144
Phosphoglycerate kinase (PGK)	48.5	48.5
Phosphoglycerate mutase (PGAM)	33	66
Enolase (EN)	41.5	83
Pyruvate kinase (PK)	57	228
Lactate dehydrogenase (LDH)	35	140
Creatine kinase (CK)	41	82
Myokinase (MK)	21.5	21.5
AMP deaminase (AMPDA)	67	270
Phosphorylase B kinase (PBK)	80	>1000
F-protein^{32} (F-P)	30.5	30.5
Actin (ACT)		

[a]Scopes and Penny (1971)