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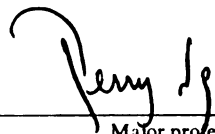
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The Impact of Twin-Screw Extrusion Processing on
Physical and Biochemical Properties of the
Major Components of Wheat Flour

presented by

Alfred Kojo Anderson

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Ph.D. degree in Food Science


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**THE IMPACT OF TWIN-SCREW EXTRUSION PROCESSING ON PHYSICAL
AND BIOCHEMICAL PROPERTIES OF THE MAJOR COMPONENTS OF
WHEAT FLOUR**

By

Alfred Kojo Anderson

A DISSERTATION

**Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of**

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

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ABSTRACT

THE IMPACT OF TWIN-SCREW EXTRUSION PROCESSING ON PHYSICAL AND BIOCHEMICAL PROPERTIES OF THE MAJOR COMPONENTS OF WHEAT FLOUR

By

Alfred Kojo Anderson

Extrusion processing technology has been successfully used commercially to produce a myriad of food products including ready-to-eat breakfast cereal and snack products. Yet, in spite of this commercial success, the chemical processes occurring among the ingredients used as raw materials in extrusion processing are not well understood. It is desirable to gain more fundamental understanding of the scientific aspects of extrusion to improve upon existing extruded food products as well as in the design of new ones. The present study looked at the effects of extrusion processing variables and feed raw material on some physical and biochemical properties of wheat flour extrudates.

Extrusion processing was achieved with a twin-screw, co-rotating experimental extruder. Extruded products were evaluated for physical characteristics such as expansion ratio, bulk density, water absorption capacity, microstructure, and specific mechanical energy inputs. Biochemically, extrudates were analyzed for changes in protein compositions, disulfide-sulphydryl interchange reactions, and hydrophobic and thermal property changes.

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The level of protein content in raw feed materials significantly affected expansion ratio, bulk density, water absorption index, and specific mechanical energy inputs of extruded products.

Higher exit die temperatures resulted in increased sulfhydryl content and slightly reduced disulfide content of extrudates. Both sulfhydryl and disulfide contents of extrudates were lower than those of the non-extruded samples. Disulfide-sulfhydryl interchange reactions were not significant in the extrusion process under the conditions studied.

Fluorescence spectroscopy data indicated changes in the conformational structure of wheat proteins consequent to extrusion processing. All extrudates showed higher emission peak wavelengths than non-extruded samples. Increasing extruder die temperatures resulted in changes in the hydrophobic environment of proteins as measured by fluorescence intensity and changes in the maximum fluorescence emission peak wavelengths.

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Finally, to my wife Adoma, and children, Kobi, Kwesi, and those yet unborn, I say many thanks for their love, support, understanding, and sacrifice throughout the course of this study.

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CHAPTER 1
INTRODUCTION

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Extrusion technology has been used extensively to produce diverse products such as modified starches, texturized vegetable proteins, snack foods, breakfast cereals, pasta products, biscuits, and pet foods (Harper 1981, Linko et al 1983). The process involves forcing a material to flow under a variety of controlled conditions and then passing it through a shaped die at a predetermined rate.

In extrusion, the material to be extruded is fed via a hopper into the hollow barrel of an extruder where it is mixed with some determined amount of liquid through a metering pump. The material is then forced towards the exit by a rotating screw (or screws). Pressure is generated through the compressed material inside the barrel of the extruder. This pressure increase is accompanied by a temperature rise as a result of both transfer of heat from the heated barrel jacket and a conversion of mechanical energy into heat energy.

The heated plasticized mass of material then passes into the die section of the extruder and into the atmosphere at lower pressure and generally lower temperature where spontaneous evaporation of water occurs (Ledward and Mitchell 1988), resulting in expansion of the product. These types of products are referred to as direct-expanded products, and are mostly made using twin-screw extruders. Indirect-expanded products in the form of pellets are commonly produced by single-screw extruders. The pellets are expanded by baking or frying prior to consumption. The major raw materials for both direct and indirect-expanded cereal products are flours and starches (Wang 1997).

Proteins in cereals can be divided into two main groups based on their biological functions. There are the biologically active enzymes and the biologically inactive storage proteins. In wheat, the storage proteins, also called gluten proteins,

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constitute about 70% of the total wheat flour proteins (Kasarda et al 1976), and are dependent on the genetic background of the plant. Thus, they are used for varietal identification.

Osborne (1907) fractionated wheat proteins into four major groups based on their solubilities in different solvents. The albumins are soluble in water, the globulins are soluble in dilute salt solutions, the gliadins are soluble in aqueous alcohol, and the glutenins are soluble in acid or alkali. Gliadins and glutenins are the two storage proteins of wheat and make up the bulk of the proteins in wheat (Lookhart 1991). The Osborne fractionation method (1907) continues to be useful for both structural and functional studies of cereal proteins in spite of cross contamination of fractionated proteins (Bushuk 1981). Chen and Bushuk (1970) modified this method to produce five fractions of wheat proteins, namely, albumins, globulins, gliadins, soluble glutenins and insoluble glutenins.

During the extrusion processing of cereals, the combination of shearing, temperature, and the generation of high pressure in the cooking zone of the extruder create many opportunities for physicochemical changes in the carbohydrates, proteins, and lipids (Wen et al 1990). For example, several studies have shown that proteins lose their native tertiary structure resulting in changes in conformation (Harper 1986, Ledward and Mitchell 1988, Camire et al 1990, Dahl and Villota 1991). Gelatinization of starch is also known to occur leading to breakdown of the starch granules and cell structure (Owusu-Ansah et al 1983, Diosady et al 1985, Mercier and Feillet 1975, Williams et al 1977, Gomez and Aguilera 1984, Wang 1997).

The engineering aspects of extrusion, rheological properties, and nutritional

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quality of extruded products have been documented (Harper 1981, Cheftel 1986, Otun et al 1986). However, the biochemical changes that take place during extrusion are poorly understood (Harper 1981) and have received little constructive attention (Ledward and Mitchell 1988). There is the need, therefore, to examine the biochemical changes that occur during the extrusion process, and how these changes impact the functional characteristics of the extruded product. These studies will lead to understanding of the physicochemical aspects of extrusion as it applies to proteins, lipids, and carbohydrates of cereals. The understanding gained will be useful in establishing a predictable extruded end-product based on some explained physicochemical behaviors of product ingredients.

In the present study, therefore, the overall objective was to investigate the physicochemical changes in the major components of wheat as a result of twin-screw extrusion processing. The study focused on the following specific objectives: (1) to assess the effects of different levels of protein content and various conditions of extrusion processing on some physical characteristics of wheat flour extrudates; (2) to evaluate the roles played by disulfide and sulfhydryl groups of wheat storage proteins during extrusion processing, and how changes in the contents of these groups affect the physical and microstructural characteristics of the extruded products; and (3) to determine the effects of extrusion processing on the biochemical structure of the major components of wheat flour.

The information obtained in these studies will contribute to a further understanding of the impact of extrusion processing on the biochemical changes in cereals in general, and wheat storage proteins in particular, as a result of the extrusion process. Such understanding will lead to improvement in quality of cereal-

based extruded foods by making possible the prediction of extruded end-products based on the known biochemical behaviors of the components of the raw feed ingredients.

This document presents a number of experiments and results that make up the study in pursuance of the above objectives, and is based on the format of *Cereal Chemistry*, an international journal published by the American Association of Cereal Chemists, St. Paul, MN. In addition to the general introduction and general literature review, the document is organized into chapters with each chapter dealing with specific questions while at the same time tying in with other chapters. The reader is, therefore, alerted to possible duplications and overlaps in issues discussed in the general literature review as well as in the various other sections of individual experiments.

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CHAPTER 2
LITERATURE REVIEW

2.1 INTRODUCTION

Extrusion technology evolved from the metallurgical industry in 1797 when it was first used to manufacture seamless lead pipes (Rhodes and Olbertz 1985). The technology was first applied to foods in the 1800's for the production of sausage and processed meats (Harper 1980), and General Mills Inc. (Minneapolis, MN) was the first to employ extrusion processing for the production of ready-to-eat (RTE) cereal products in the late 1930's (Harper 1981).

At present, extrusion processing is one of several texturization processes, which include flaking, puffing, and baking, used extensively in the food industry to produce diverse products such as ready-to-eat (RTE) breakfast cereals made from wheat, corn, rice and oats; snacks from corn and wheat, and baked goods mainly from wheat (Clark 1986). Biscuits, pasta, crackers, crisp breads, baby foods, confectionery items, chewing gum, texturized vegetable proteins, modified starches, and beverage mixes are all items which can be produced through extrusion processing (Linko et al 1983). Extrusion cooking is a versatile process that also improves organoleptic and nutritional qualities in foods (O'Connor 1987). The cost-to-benefit ratio of extrusion technology gives producers, processors, and consumers more choices by increasing the variety of ingredients used in cereal-based products (Rayas-Duarte et al 1998).

The U.S. snack food industry has a total retail value of \$16 billion of which about 5%, or \$810 million, is from extruded products. In all, about 5.7 billion pounds (2.5 million metric tons) of products are produced from extrusion annually (Purvis 1998). Extrusion can also be used for several different functions including mixing,

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cooking, forming, conveying, puffing, or drying, depending on the extruder design and the process conditions.

Food extruders are generally divided into two classifications: single-screw and twin-screw (Huber 1991). Single-screw extruders are by far the more commonly used. However, because of its improved pumping action, the co-rotating twin-screw extruder is finding increased use for producing products requiring precise process control (Hauck 1989).

Extrusion processing variables that directly control product quality attributes are called independent variables. These include the raw material formulation, raw material feed rate, configuration of the screws, the die restriction, moisture content of raw materials, screw speed, and temperature of the various zones of the extruder barrel (Huber 1991). Dependent variables change as a result of changes made to the independent variables, and they include product temperature, product moisture content, residence time in the extruder, pressure within the extruder, and moisture content of the extruded product. Responses are measured by the final product qualities that result from changes made to independent or dependent variables. These qualities include moisture content of extrudates, expansion, solubility, absorption, texture, color, and flavor (Huber 1991, Purvis 1998).

Extrusion cooking of cereals and starches requires the control of processing variables such as moisture content of the ingredients, extrusion cooking temperature, screw speed, and extruder barrel and die configurations (Owusu-Ansah et al 1983, Meuser et al 1982, Guy and Horne 1988, Chinnaswamy and Hanna 1988). Of these variables, screw design and its elements, the moisture content of the ingredients prior to extrusion cooking, and extrusion temperature are

all known to drastically affect the quality attributes of the products (Mercier and Feillet 1975, Owusu-Ansah et al 1983; 1984, Chinnaswamy and Hanna 1988).

One of the important characteristics of extruders is the possibility of high temperature short time (HTST) cooking (Harper 1978). Smith and Ben-Gera (1980) described HTST extrusion cooking as the most versatile and most economical thermal processing system due to the fact that many conversions could be carried out at lower moisture contents which lowers the drying costs for gelatinized or texturized products at shorter residence times. In HTST cooking, controlled cooking conditions are obtained by maintaining a lower processing temperature during processing of the dough and then elevating the product temperature during the last few seconds of residence time. Johnston (1979) has discussed the technical fundamentals of HTST extrusion cooking equipment, and Smith and Ben-Gera (1980) have discussed the methodologies of HTST extrusion cooking.

The following sections will review key extruder design and operating features which influence the textural and other qualities of the finished extruded products. The effects of extrusion on the biochemical components of the raw feed ingredients will also be considered.

2.2 EXTRUDER DESIGN AND COMPONENTS

There are generally three categories of extruders, namely, piston extruders, roller extruders, and screw extruders (Thorz 1986). Piston extruders are mainly used for forming, and consist of pistons which deposit precise amount of materials onto a wide conveyor. These extruders are commonly used in the confectionery

industry, for example, to deposit the center fillings of chocolates. Roller extruders consist of two counter-rotating drums that turn at similar or different speeds. The gap between the rolls can be closed to compress the material passing through the unit. Screw extruders have single, twin, or multiple screws within a stationary barrel to push the material forward and through a specially designed orifice called a die.

The single- and twin-screw extruders are the most commonly used in the extrusion processing of cereal products. The mechanical actions of both types of machines are affected by the configuration of the screw and its rotational speed, the back-pressure requirements of the die, and the characteristics of the food material being extruded (Harper 1986). Figure 2.1 shows the cross-section of a typical extruder barrel.

2.2.1 Screw Configuration

The screw is the key element of an extruder because its configuration influences the unit operation of the extruder (Harper 1978). It consists of a helical flight wound around a metal shaft enclosed within a cylindrical barrel. Individual screw elements and paddles may be arranged on a shaft in the barrel of the extruder to obtain different extruder screw configurations. Figure 2.2 shows schematic diagrams of different types of screw elements and the permissible orientation of paddles on the shaft.

Shaft: The shaft serves both as the mounting on which the slip-on agitator parts are assembled and as a means to transmit the mechanical energy from the drive to the components where it is converted to work (heat and mixing) on the feed materials.

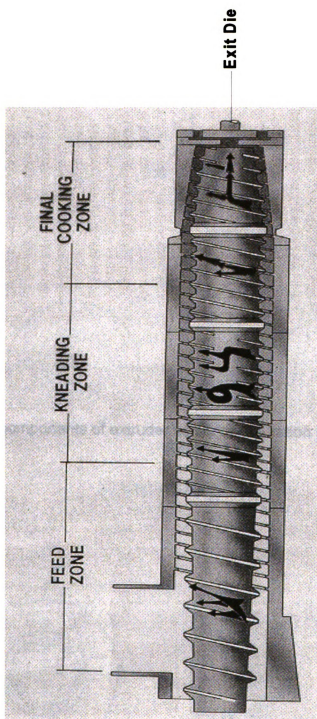
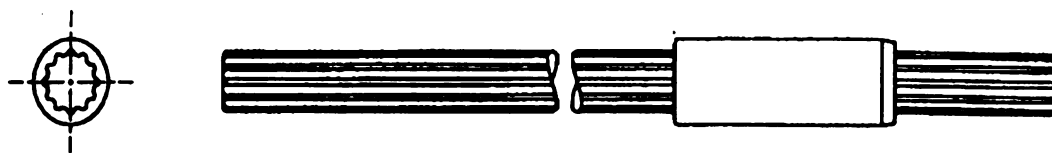


Figure 2.1

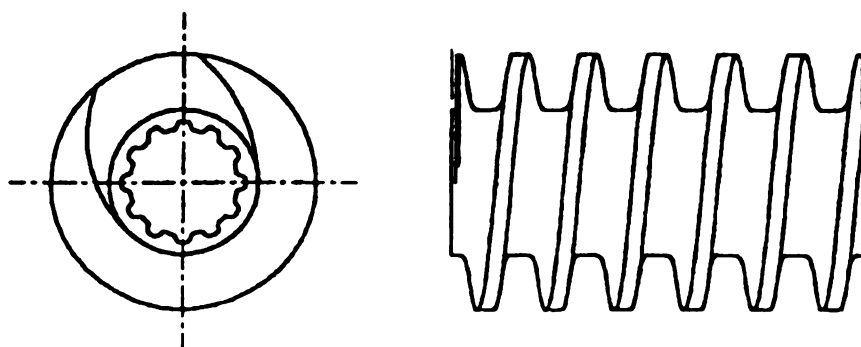
Cross-section of extruder barrel showing processing zones on the screw (from IFT 1989).

Figure 2.2

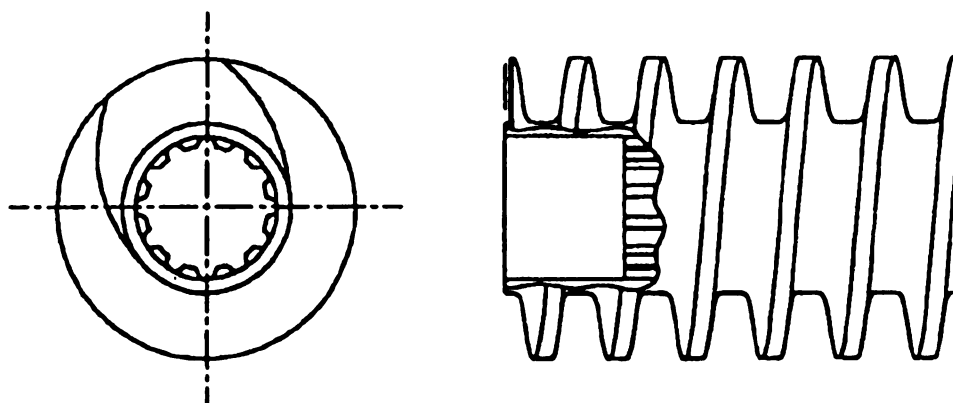
Individual components of extruder screw configuration (from APV Baker, Grand Rapids, MI).



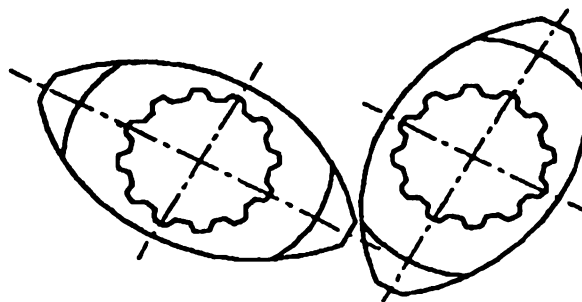
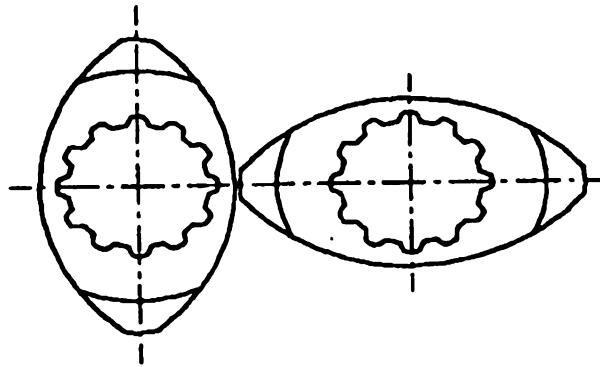
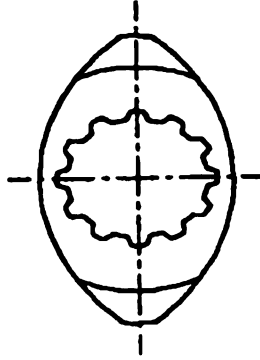
(a) Agitator Shaft



(b) Single Lead Feed Screw



(c) Single Lead Discharge Screw



(d) Paddle Pairs - Must always be at 90°

Feed Screws: The function of the feed screw components are to convey the materials downstream within the extruder. The feed screws will tend to push most materials through paddle sections.

Discharge Screws: The discharge screws have multiple functions made possible by their design. In addition to conveying the final product from the extruder against high die exit pressures, they also function as the downstream bearer of the agitator assembly.

Paddles: Paddles are the primary working components of an agitator assembly. It is the motion of the paddles relative to each other and the barrel that causes the cooking of the product and distribution of the ingredients. They provide very good mixing and generate the friction within the product which converts the mechanical energy into heat. Paddle pairs must always be oriented 90° from each other as shown in Figure 2.2(d).

The changes in molecular conformation of raw or preconditioned food ingredients, which ultimately affect the texture of extruded products, are primarily due to extrusion screw design and configuration (Harper 1986). The action of both single screw and twin-screw extruders is affected by the configuration of the screw and its rotational speed, among other variables. Mechanical energy dissipation in the screws causes the temperature of the ingredients to increase rapidly, transforming the mass from a granular state into a continuous plasticized mass. The shear in this process is very high and causes mechanical damage to food molecules. Damage to starch or protein molecules will reduce their capability of forming an elastic matrix capable of expanding and retaining texture upon passing through the die (Harper 1986).

2.2.2 Extruder Die

The die effects of extruders can be considered independently from the type of extruder used to cook materials. Die shape influences finished piece shape and texture. Harper (1986) has reviewed the effect of die shapes on extruded product shape and quality. A tapered die reduces back pressure requirements, creates a smoother product surface, and causes less mechanical damage to the extruded ingredients. On the other hand, a die insert having an abrupt cross-sectional change will cause greater mechanical damage to food ingredients and lead to a finer cell structure and softer texture.

The laminar-flow shear environment through the die can be characterized by the shear rate:

$$\dot{\gamma} = \frac{4Q_I}{\pi R^3} = \frac{4\dot{m}_I}{\pi R^3 \rho}$$

where Q_I , the volumetric rate of flow through individual die holes, is the mass rate of flow $\dot{m}$ divided by density ρ of cooked material, and R is the radius of the die hole. Dies having a high shear rate have a potentially greater effect on product texture by causing greater shear-induced damage and reduced molecular size, creating softer-textured products with smaller pores and increased solubility (Harper 1986). In high temperature extrusion, sudden release of pressure through the die allows expansion of steam with development of a puffed product (Miller 1994).

2.2.3 Single-Screw Extruders

Single-screw extruders may be regarded as friction pumps, since they rely entirely on friction between the material being processed and the barrel wall to convey materials (Purvis 1987, Starer 1996). Thus, they rely on drag flow for conveyance. The mass of material being processed must stick to the barrel wall in order to be forwarded, and the higher the frictional forces, the more efficient the conveyance is.

The operational features of single-screw food extruders were first described by Rossen and Miller (1973). The initial feed section of the screw receives and conveys the food materials into the barrel of the extruder. Decreasing flight height or other types of restriction cause the flights to become completely filled. The relationship between the reduced area for flow in later sections of the barrel to that in the feed section is defined as the compression ratio (Harper 1986).

The screw is the key element of the single-screw extruder because its geometry influences the unit operation of the extruder (Harper 1978). It consists of a helical flight wound around a metal shaft enclosed within a cylindrical barrel. A number of different screw configurations may be placed on an extruder shaft, the purpose being to convey raw materials, knead, create back-flow, input high mechanical energy turbulence, and increase or decrease residence time of materials in the extruder (Sunderland 1996). As the material is moved along the screw in extrusion cooking, it is transformed under conditions of heat, pressure and mechanical shear into a viscous mass and finally into a product with specific properties. The changes in the product correspond to specific zones of the extruder screw as shown in Figure 2.1, namely the feed zone, the kneading zone, and the

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final cooking or metering zone (Matson 1982, Hauck 1985).

The feed zone of the screw generally has deep channels which receive and mix the feed ingredients, and slightly compress the feed into a more homogenous state between the screw flights. Water may be added at this point to help develop a uniform dough and to improve the heat transfer in the extruder barrel (IFT 1989). The kneading zone applies compression, mild shear, and thermal energy to the feed, producing a visco-amorphous mass at or above 100°C (Faubion et al 1982). The final cooking zone has the shallowest channel depths and functions to compress and pump the material in the form of a plasticized mass to the die.

Throughout the extrusion process, heat input to the product comes from several sources. These include steam injection into the extruder barrel, frictional heat developed at barrel wall and in the die area during rotation of the screw, or heat transfer from a steam jacket encasing the barrel (Clark 1978). By the end of the extrusion process, the thermal processing conditions have produced changes in the physical properties, chemical properties and/or chemical composition of the feed ingredients.

2.2.4 Twin-Screw Extruders

Twin-screw extruders consist of two screws of equal length placed inside the same barrel for its entire length (Martelli 1983). An example of arrangement of screw elements in a twin-screw extruder is shown in Figure 2.3. These extruders are generally categorized according to the position of the screws in relation to one another and to the direction of screw rotation (IFT 1989). Thus, twin-screw extruders can be: (a) intermeshing, in which the flights of one screw engage or penetrate the

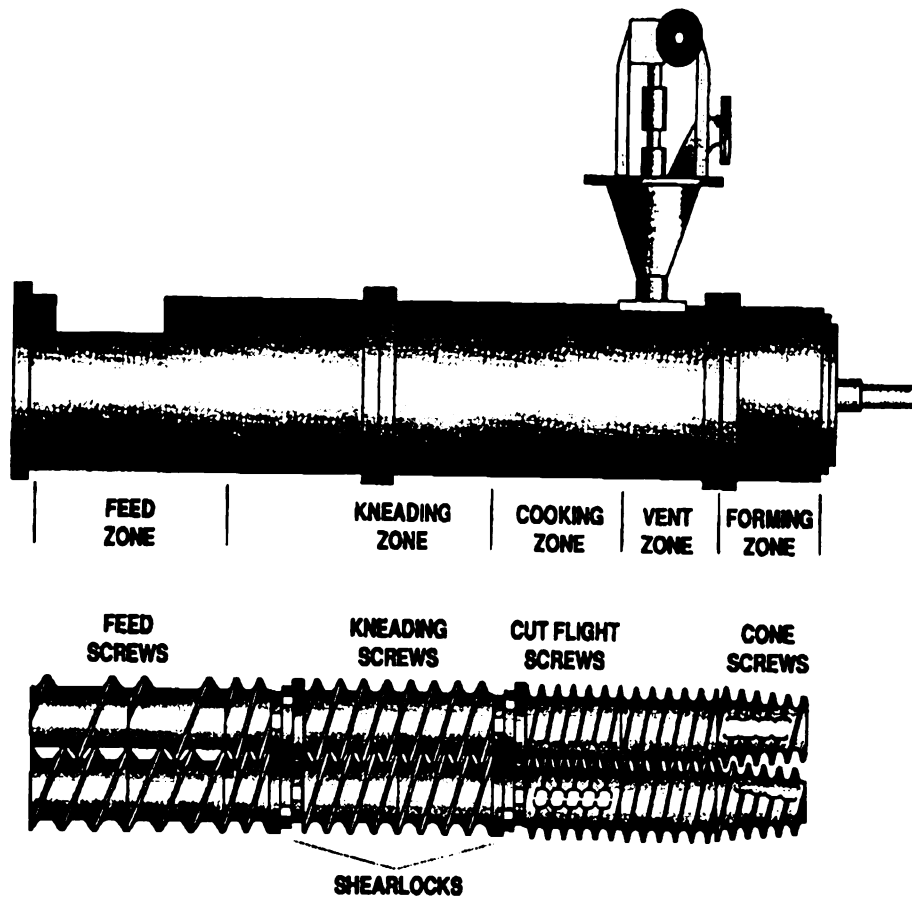


Figure 2.3

Arrangement of screw elements in extruder barrel zones (from Sunderland 1996).

channels of the other; (b) non-intermeshing, in which each screw turns without interference from the other; (c) co-rotating, in which the screws turn in the same direction with their crests matching up with opposing troughs; or (d) counter-rotating, in which the screws turn in opposite directions, as illustrated in Figure 2.4.

The fully intermeshing, co-rotating twin-screw extruders are being widely used in the food industry (Harper 1986) because of their ability to provide better process control and versatility, their flexible design permitting easy cleaning and rapid product changeover, their ability to handle difficult formulations, and their cost (IFT 1989). In twin-screw extruders, the screws can be configured to enhance conveying, kneading, shearing, pressure development, and filling of the barrel. Conveying sections are usually only filled partially with product and therefore impart relatively little energy and shear. Kneading elements create a significant shearing and mixing action and dissipate large amounts of mechanical energy (Harper 1986).

2.3 RESIDENCE TIME DISTRIBUTION

Residence time is defined as the length of time the process material spends in the barrel of the extruder during extrusion. Residence Time Distribution (RTD) reveals information about flow patterns, degree of mixing, design of equipment, processing conditions, and retention time of the material in the processing device. Twin-screw extruders are characterized as having a narrow residence time distribution.

Although the study of RTD for extrusion processes has received considerable attention, no generally satisfactory studies of residence time distribution and mixing

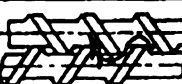
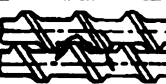
SCREW ENGAGEMENT		SYSTEM	COUNTER-ROTATING	CO-ROTATING
INTERMESHING	FULLY INTERMESHING	LENGTHWISE AND CROSSWISE CLOSED	1 	2 THEORETICALLY NOT POSSIBLE
		LENGTHWISE OPEN AND CROSSWISE CLOSED	3 THEORETICALLY NOT POSSIBLE	4 
		LENGTHWISE AND CROSSWISE OPEN	5 THEORETICALLY POSSIBLE BUT PRACTICALLY NOT REALIZED	6 
	PARTIALLY INTERMESHING	LENGTHWISE OPEN AND CROSSWISE CLOSED	7 	8 THEORETICALLY NOT POSSIBLE
		LENGTHWISE AND CROSSWISE OPEN	9A 	10A 
			9B 	10B 
	NOT INTERMESHING	LENGTHWISE AND CROSSWISE OPEN	11 	12 

Figure 2.4

Classification of twin-screw extruders (from IFT 1989).

in intermeshing, co-rotating twin-screw extruders have been done (White 1991). Jager et al (1988) and van Zuilichem et al (1988) have presented a numerical RTD model to translate the RTD into a mass flow pattern for co- and counter-rotating twin-screw extruders.

2.4 EFFECTS OF EXTRUSION VARIABLES ON EXTRUDATE CHARACTERISTICS

Extrusion variables directly control product quality attributes. Such variables may include screw speed, the configuration of the screws, temperature in the extruder barrel or at the die, moisture content of the feed ingredients, and the raw material formulation and feed rate. These variables, through interactions with themselves and/or with other variables, act to directly affect the quality characteristics of the extruded products which are observed in terms of responses in the extrudates. Such measured responses include extrudate expansion, bulk density, water absorption and solubility, texture, color, and several other measurable responses (Huber 1991). This section will review the effects of screw speed, barrel/die temperature, and raw ingredients characteristics on some measured responses which include expansion, bulk density, water absorption and solubility, and texture of extrudates.

Expansion is usually expressed as a ratio between the diameters of the extruded product and the exit die of the extruder, and is called the expansion ratio. The bulk density of extrudates is the weight of extruded material that fills a unit volume of space. It therefore follows that a well-expanded product with a high

expansion ratio will have less bulk density and vice-versa. Water absorption index (WAI) is the weight of gel obtained per gram of dry extrudate at room temperature. Because damaged starch granules absorb water at room temperature and swell, creating increased viscosity, water absorption index is found to correlate well with cold-paste viscosity (Anderson et al 1969). After reaching a maximum, related to the degree of starch damage, WAI decreases with the onset of dextrinization. Water solubility index (WSI) is the percentage of dry matter recovered from the evaporation of supernatant obtained from the WAI determination. WSI depends on the quantity of soluble molecules which is related to the degradation of starch (Jin et al 1995).

2.4.1 Effects of Screw Speed and Configuration

Extruder screw type and configuration can affect the specific mechanical energy (SME) input to the material being extruded and consequently affect expansion ratio and other physical properties of the extrudate (Sokhey et al 1994). The screw speed has been reported to have significant effects on extrudate properties. Jin et al (1995) reported that increasing the screw speed during twin-screw extrusion produced a lower overall water absorption index (WAI) and higher overall water solubility index (WSI) in corn meal extrudates. It was also observed earlier (Jin et al 1994) that increasing the screw speed from 150 to 350 rpm in a twin-screw extruder increased the product temperature by about 15°C when corn meal was extruded with soy fiber, salt and sugar. The combined effect of increased temperature and increased degradation of corn starch due to increased screw speed, enhanced the amount of soluble materials and produced increased water

solubility of the extrudates. The researchers concluded that screw speed appeared to be the most important factor influencing the extrusion process variables.

According to Diosady et al (1985), the lower WAI and higher WSI of extrudates with increasing screw speed might be related to the increased shear rate, resulting in the structural modification of starch. At low screw speed (i.e., low shear rate) more undamaged polymer chains exist and a greater availability of hydrophilic groups bind more water molecules resulting in higher values of WAI (Gomez and Aguilera 1983). In contrast, WSI depends on the quantity of soluble molecules which is related to the degradation of starch. Wen et al (1990) indicated that screw speed had a direct effect on polysaccharide size distribution. A higher screw speed resulted in more fragmentation than a lower screw speed.

Chen et al (1991) investigated the effects of extrusion conditions on sensory properties of corn meal extrudates and reported that screw speed had no significant effects on the color of extrudates. However, the surface texture of the extrudates was affected by the screw speed at low temperatures and high moisture. Decreased screw speed led to increased surface texture scores because it led to longer exposure.

The configuration of the screw elements during extrusion is as important as the speed of the screw. Sokhey et al (1994) studied the effect of screw configuration on corn starch expansion during single-screw extrusion. Three different extruder screw configurations were used: one with no mixing element; one with a single stage mixing element; and one with a dual stage mixing element. Evaluation of the bulk density, expansion, and specific mechanical energy values indicated that expansion ratios and bulk density differences were not significant. However, upon

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re-extrusion of the samples, it was observed that the different screw configurations significantly affected all variables studied, except overall expansion ratio.

The relation between extruder screw speed and mechanical energy has been established. Jin et al (1994) reported that increasing the screw speed in a twin-screw extruder decreased the extruder torque in part due to changes in the length of the filled flights in the extruder barrel. An increase in screw speed decreased the length of filled flights at a constant feed rate (Martelli 1983) and thus decreased the load on the screw shaft motor. Thus the extruder torque was reduced. Additionally, increased screw speed produced higher product temperature which indicated the temperature of the dough in the extruder barrel was greater and hence viscosity was less (Harper et al 1971, Colonna et al 1989). A reduced dough viscosity also decreased the load on the extruder motor, and therefore the torque. The specific energy might increase or decrease with increasing screw speed, depending on the relative contribution of the screw speed and dough mass viscosity (Hsieh et al 1991).

2.4.2 Effects of Temperature

Temperature plays a significant role on extrusion and product characteristics (Bhattacharya and Choudhury 1994). During extrusion processing, heat is needed to texturize the products. Although heat may need to be added, much of the final heat energy necessary to achieve high discharge temperatures comes from the dissipation of the mechanical energy input used to turn the screw (Harper 1986).

The apparent viscosity of the dough in an extruder barrel is affected by temperature or by residence time distribution (RTD). An empirical model for cooked

cereal dough has been derived (Harper et al 1971) as follows:

$$\eta = C_1 \dot{\gamma}^{C_2} e^{C_3/T} e^{C_4 M}$$

where η is the apparent viscosity, $\dot{\gamma}$ is the shear rate, T is temperature, M is moisture content, e is exponential factor, and C_1 , C_2 , C_3 , and C_4 are constants. Since C_3 is positive, at a constant shear rate and moisture content, an increase in T decreases η (Levine 1983). Changes in dough mass viscosity due to variations in extrusion temperature lead to changes in the load on the extruder motor, and therefore the torque.

The viscosity of cereal flour dough is also affected by the extent of gelatinization (Bhattacharya and Choudhury 1994). At a low temperature, gelatinization of starch is incomplete and shows lower viscosity. However, at the beginning of gelatinization, increased extrusion temperature increases apparent viscosity. Apparent viscosity drops again after the completion of starch gelatinization with further increases in temperature.

The control of temperature has a profound effect on the condition of the dough just behind the exit die and on the final expansion of the product. Harper (1986) reported that the vapor pressure of the water in the dough, which is related to temperature, provides most of the force which causes expansion once the product is released to ambient temperature and pressure. Bhattacharya and Choudhury (1994) studied the effect of extruder length-to-diameter ratio and barrel temperature on extrusion parameters and product characteristics in a twin-screw extrusion of rice flour. Increases in barrel temperature increased both the extent of gelatinization and the content of superheated steam, causing the extrudate to

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expand more and yielding a low density product. Starch gelatinization was incomplete at low temperatures. Hence, inadequate binding in the product resulted in low values for maximum breaking force. With increased barrel temperature, the extent of gelatinization increased which caused better binding, as reflected in higher values for maximum breaking force.

It has also been demonstrated by several researchers that the expansion of extruded starch-based materials depended on the degree of gelatinization, which in turn was determined by process temperature, shear rate and moisture content of the feed material (Lawton et al 1972, Chiang and Johnson 1977, Guy and Home 1988).

2.4.3 Raw Material Characteristics

The composition of the feed raw material into an extruder greatly influences the texture of the extruded food product (Harper 1986, Jin et al 1995). It is well established that starch is the predominant functional ingredient in extruded snack and ready-to-eat (RTE) cereals and other extruded products (Harper 1986, Guy and Home 1988, Colonna et al 1989). However, defatted soy protein containing about 50% protein, or protein concentrates in which a large fraction of the carbohydrate has been removed to increase protein concentration, can also be extruded to produce a layered meat-like structure (Harper 1986). Secondly, in most cases, degermed cereal grits are used as an extrusion feed material instead of a purified starch, hence, some protein, fat, and fiber are all part of the extrusion feed mixture. The following will briefly review the role of starch and protein in extruded products.

2.4.3.1 Starch

Carbohydrates are a main constituent in food products, and are generally used at a 70% level or more (Huber 1991). They are known to give functionality to extruded products by serving as binding agents, viscosity builders, suspending agents, and emulsifiers. They also influence textural and esthetic characteristics of food such as expansion, bulk density, mouth feel, gelation, clarity, and flavor (Huber 1991). Generally, carbohydrates can be grouped into four categories: fibers, starches, gums, and sugars. Starches form the bulk of the carbohydrate component in extruded cereal products in which they are responsible for adhesion, cohesion, and expansion.

Several studies have elucidated the role of starch as a feed ingredient during the extrusion process (Mercier and Feillet 1975, Gomez and Aguilera 1983; Owusu-Ansah et al 1984; Meuser and van Lengerich 1984). Native starch is reported to undergo substantial changes leading to greater molecular disorganization. In the process, starch loses its crystallinity, undergoes molecular degradation, and often forms complexes with lipids in the feed mixture. In the case of wheat starch, for example, the degree of molecular fragmentation is evidenced by decreased viscosity (Owusu-Ansah et al 1983, Diosady et al 1985), decreased water absorption index (Mercier and Feillet 1975, Williams et al 1977, Owusu-Ansah et al 1983), increased amylase susceptibility (Gomez and Aguilera 1984), and increased degree of gelatinization (Gomez and Aguilera 1984). Gel filtration chromatography studies on extruded corn and wheat starches (Colonna et al 1984, Davidson et al 1984) have also shown that starch fragmentation results in a decrease in high molecular weight materials and a corresponding increase in lower

molecular weight polysaccharides.

The roles of shear, temperature, moisture, and feed composition significantly affect the starch transformation process. Gomez and Aguilera (1984) described a process in which shear causes mechanical damage to the starch while heat and moisture are responsible for the loss in crystallinity. Lower moisture content causes increased viscosity and more mechanical damage. Davidson et al (1984) have reported that the bulkiness of the amylopectin fraction of starch prevents it from aligning itself in the direction of flow in the screw and exit die, resulting in greater mechanical damage and reduced molecular size. These damaged starches are less cohesive than gelatinized undamaged starch and therefore expand less in the longitudinal direction, creating products with smaller pores, softer textures, greater solubility, and a sticky character.

Amylose is less susceptible than amylopectin to mechanical damage in the flow environment within the extruder. Thus, high amylose products are denser, harder, and less radially expanded when extruded (Harper 1986). Mercier (1980) has also shown that amylose forms a complex with lipids during the extrusion process which helps maintain the native structure of amylose. However, the formation of amylose-lipid complexes reduces the digestibility and water solubility of cooked starches (Galloway et al 1989), and as starch in the presence of lipids is exposed to increasing heat and pressure during extrusion, the amount of amylose-lipid complexing increases.

2.4.3.2 Protein

Plant protein, particularly from soy meal protein, is the only commercially important protein material used in extrusion. However, interest has been shown in the improvement of utilization of both dairy (van de Voort 1984) and animal (Areas 1986) proteins through extrusion processing. Thermal extrusion of proteinaceous ingredients is a relatively recent industrial process. The first patent for extrusion processing of plant protein was issued in 1966 (Linko 1983).

Defatted soy protein can be extruded to create a layered meat-like structure commonly called meat analogs or extenders. It has been shown that the globular protein contained in defatted soy meal is not homogeneously distributed, but exists as nearly spherical structures known as protein bodies (Kinsella 1978, Bair and Snyder 1980, Daniels 1981). In the extrusion process, the protein molecules are restructured into a layered cross-linked mass which is further heated and sheared in the extrusion screw. The proteins unfold upon the disruption of the ionic, disulfide, and hydrogen bonds which make up the native tertiary structure of the protein molecule (Ramsen and Clark 1978). The results are denaturation, association, and coagulation involving reduction or oxidation. Extrusion screws which turn rapidly, or are configured to increase residence time, will have the most disruptive effect on the molecules' native structures. These effects include the denaturation of the protein and a loss of functionality which may lead to reduced solubility and decreased extrusion effectiveness (Harper 1981, 1986).

The quality characteristics of extruded products have been related to the structural integrity of the protein matrix. Proteins in expanded products are important for elasticity, gas retention and cellular structure, adhesiveness, stretchability,

extensibility, water absorption, binding and expansion. Kazemzadeh et al (1982) examined extrudates using differential staining light microscopy which revealed carbohydrate inclusions and steam-generated voids enclosed within a protein matrix. Frazier and Crawshaw (1984) also associated well-textured soy with a smooth, homogenous protein matrix containing small, evenly distributed, insoluble carbohydrate inclusions.

Several studies have revealed the major role of protein during extrusion texturization. Sheard et al (1985), for example, found that an extrudate from soy isolate containing 91% protein had a larger diameter and higher shear values than one from soy flour containing 50% protein, although these data are in contrast to previously reported data (Sheard et al 1984), and the data reported in the current study. Increasing protein levels significantly improved rheological properties of extruded soy, according to Rhee et al 1981. Maurice and Stanley (1978) used three levels of protein, through the addition of soy isolate, to show that protein level and second-order protein effects accounted for most of the variation in product shear values.

A number of proteinaceous materials have been used to fortify ready-to-eat breakfast cereals to improve their protein content and its quality. Cereal grains are moderately low in protein and the protein is of poor biological quality because the essential amino acid, lysine, is present in limited amounts (Harper 1981). In order to produce nutritionally improved cereal, therefore, some cereals are fortified with protein, commonly in the form of soy protein concentrate and sodium caseinate. It has been reported that the addition of 10 to 15% of these ingredients will increase the protein content of the entire blend by 12 to 20% (Harper 1981). However, the

addition of high protein ingredients tends to reduce the puffing of the extruded product and also results in the dissipation of more mechanical energy in the extrusion process and higher back pressures at the exit die of the extruder.

Vital gluten is the gluten protein of wheat flour obtained after the starch and soluble components have been removed by a washing process (Wang and Ponte 1995). It contains about 80% protein on a dry basis. The unique viscoelastic, adhesive, and cohesive properties of vital gluten improve dough strength, mixing tolerance, and handling properties, and also permit its use as an ingredient in meat, fish, and poultry products (Czuchajowska and Smolinski 1997). Its water-absorption capacity improves baked product yield, softness, and shelf-life. Even though vital gluten is commonly used in foods to improve their handling properties and to modify their texture, its use as a protein source for extrusion processing has not been explored.

The effect of gluten on texture has been mostly studied in relation to baked products of flour fortified by gluten. Dreese et al (1988a,b) tested the rheological properties of doughs made from blends of commercial gluten and starch by measuring the rheological properties as affected by moisture, starch content, and heating rate. In a recent study (Wang and Ponte 1994), the baking characteristics and freeze-thaw tolerance of frozen dough were found to be improved significantly by the addition of vital wheat gluten to relatively weak flour. The study showed that the beneficial effect provided by vital wheat gluten was due to the increase in dough strength that could overcome weakening effects caused by freezing.

In view of the success in the application of extrusion processing to produce meat analogs from soy protein sources, and given the fact that vital wheat gluten

is in abundant supply as a source of cereal protein, it would be beneficial to study the role of vital wheat gluten as a source of protein in the extrusion texturization of cereal products.

2.4.4 Effects of Moisture Content of Raw Materials

The moisture content of raw feed ingredients going into an extruder as well as any amount of liquid added to ingredients during the extrusion process directly affects the viscosity of the dough in the extruder barrel and therefore the energy requirements of the process. Bhattacharya and Hanna (1987) have concluded that any variable affecting the dough mass viscosity would also affect the extruder torque.

Water inside the extruder exists as superheated steam due to high temperature and pressure (Park et al 1993). When the extrudate exits the die, water vaporizes until the water temperature within the product reaches 100°C (Williams et al 1977). The moisture remaining in the product depends on the pressure differential between the extruder and the atmosphere (Conway 1971a). According to Park et al (1993), it is difficult to generalize the effect of feed moisture on the texture of extrudates. However, Lawton et al (1972) have shown that water in the extruder acts as a heat trap and lubricant to reduce the shear strength of extrudates. A low feed moisture can increase the power requirements of the extruder and can also result in excessive browning and stoppage of extrudate at the exit die nozzle opening (Conway 1971b).

Starch is the predominant ingredient in the extrusion of cereals, and the gelatinization of starch is a necessary condition for the expansion of cereal

extrudates. It is well documented that water content in combination with temperature has a significant effect on the conversion of starch. This is seen from the fact that the transition temperature as well as the enthalpy of transition in differential scanning calorimetry (DSC) increases with decreasing water concentration for a given starch type (Lai and Kokini 1991). In a DSC thermogram of potato starch, Donovan (1979) observed a single peak when the moisture content was 81%. When moisture content was dropped to 64%, a second endotherm was observed at a higher temperature, and became predominant when water concentration was even lower than 51%. The responses at low water concentrations have been attributed to melting of crystallites. Under excess water all the crystallites in starch might be pulled apart by swelling, leaving none to be melted at higher temperatures. However, in the limited water environment characteristic of extrusion processing, the swelling forces are much less significant, and the crystallites melt at a temperature much higher than the gelatinization temperature in excess water (Donovan 1979).

In a study to investigate the effects of raw material composition and process temperature on physical and rheological properties of high protein extrudates, Park et al (1993) found that feed moisture had a significant linear effect on extrudate shear force and water absorption, and a quadratic effect on expansion ratio and bulk density. It was concluded that an insufficient amount of water available for evaporation appeared to be the main cause of low expansion ratio and high bulk density.

2.5 BIOCHEMICAL CHANGES IN PROTEINS DURING EXTRUSION

Studies elucidating the biochemical changes that occur in proteins during extrusion are limited (Strecker et al 1995). Therefore, understanding the chemical and physical changes that proteins undergo during extrusion conditions of high temperature, pressure and shear, and low moisture is limited (Ledward and Mitchell 1988). Nevertheless, it has been established that during the extrusion process, proteins become denatured and the forces that stabilize the tertiary and quaternary structures of the proteins are weakened by a combination of increased heat and shear within the extruder (Camire 1991). The result is that the solubility of the proteins decreases as a result of extrusion processing.

Racicot et al (1981) reported a significant decrease in the solubility of corn meal protein in ethanol and alkali after extrusion. Cumming et al (1973) had earlier suggested that the exposure to heat and pressure during extrusion affects free sulfhydryl groups and disulfide bonds in soy protein, which results in lowering the solubility of the protein. Their study also observed that the solubility of water-soluble proteins, which broke into subunits of smaller molecular weights during the extrusion process, also decreased, and that the degree of insolubilization was different in different proteins according to gel electrophoresis analyses.

Wheat proteins can generally be fractionated into four groups based on the original classification by Osborne (1907). Albumins are the fractions soluble in water; globulins are soluble in salt solutions; gliadins are soluble in aqueous alcohol; and the glutenin fractions are soluble in dilute acid or alkali. Chen and Bushuk (1970) later modified this classification to include insoluble glutenins. Recently,

Ummadi et al (1995) investigated the changes in solubility and distribution of semolina proteins due to extrusion processing at two different temperatures and observed a marked decrease in the percentage of total protein present in the albumin, globulin, gliadin, and glutenin fractions with a corresponding increase in the insoluble residue fraction. It was suggested that polypeptides of albumins, globulins, and glutenins of semolina most likely aggregated after extrusion, leading to decreases in solubility of these proteins. In contrast, molecular weight distribution of gliadin proteins seemed to have been unaffected by extrusion at the experimental temperatures used in the study.

2.6 EFFECTS OF HEAT AND CHEMICAL MODIFICATIONS ON CEREAL PROTEIN COMPOSITION

Native protein structure can be described on four levels. The primary structure, which is indicated by the amino acid sequence of the protein, determines the chemical and biological properties of the protein; the secondary structure refers to the regular arrangement of the polypeptide backbone of which α -helix and β -sheet structures are examples; tertiary structure refers to the three dimensional structure of the protein molecule; and the quaternary structure is the assembly of individual protein molecules to form a functional protein aggregate (Tatham et al 1990). A combination of hydrogen bonds, hydrophobic interactions, electrostatic forces, and disulfide bonds act together to stabilize these protein structures (Tatham et al 1990).

Hydrophobic and hydrophilic interactions play an important role in the

structure-function relationships of proteins (Weegels et al 1994). Generally, the surface hydrophobicities of proteins are increased by heating (Tanford 1980, Matsudomi et al 1982, Voutsinas et al 1983), which can be explained by an increased exposure of hydrophobic core groups at the outer surface of the molecule (Tanford 1980). Surface hydrophobicity is generally determined by measuring the interaction of the protein with hydrophobic fluorescent ligands, e.g., 1-anilino-8-naphthalenesulfonic acid (ANS). Since surface hydrophobicity is related to protein structures, conformations and functionality (Aktan and Khan 1992), fluorescence may be able to reveal denatured or structurally altered proteins.

Proteins also possess native fluorescence due to the presence of the aromatic amino acids tryptophan, tyrosine and phenylalanine; however, the fluorescence contributions of tyrosine and phenylalanine are low. The fluorescence properties of these amino acid residues reflect the environment of the protein and can, therefore, be used to indicate changes in conformation and/or stability of the protein (Yeboah et al 1994).

Studies on the effects of extrusion processing on native protein conformations, especially with respect to wheat storage proteins, are limited. Weegels et al (1994) reported a study on the changes in the extractability of glutenin aggregates differing, among other things, in secondary structures of glutenins, gliadins, albumins and globulins after heating of gluten at different moisture contents. The hydrophobicity of the heated gluten was determined by interaction of the protein with ANS. The study observed that the number of binding sites of ANS decreased when gluten with a moisture content of 13% or higher was heated, indicating a decrease in hydrophobicity. Similarly, Yeboah et al (1994) used

fluorescence spectroscopy to study the conformation and dynamics of two γ -gliadin fractions.

Lakkis and Villota (1992) studied the effect of succinylation, acetylation, and reductive alkylation on substructural properties of casein, bovine serum albumin (BSA), and whey proteins. Hydrophobicities of the native and modified proteins were determined by using ANS to react with the proteins and measuring the fluorescence intensities. Data from the study revealed that surface hydrophobicity of the three proteins increased in parallel with the degree of chemical modification, indicating a change in the conformational structure of the proteins and a subsequent exposure of their hydrophobic core to the ANS probe.

It is evident from the foregoing that fluorescence spectroscopy has been extensively used to study changes in conformation of protein structure, yet such studies have not been adequately applied to the conformational changes in cereal proteins as a result of extrusion. In view of the possible applications of extrusion to both existing and future food products, it will be necessary to understand the changes in the conformation of proteins and other ingredients in order to better understand the relationship between the extrusion process and end-product characteristics.

2.7 THERMAL TRANSITIONS IN WHEAT FLOUR COMPONENTS AS MEASURED BY DIFFERENTIAL SCANNING CALORIMETRY

Wheat flour is a multi component system containing about 80% starch (by dry weight), proteins, lipids and other minor components. The protein content may vary from 9-18%, depending on the wheat variety. It has been found that the

quantity of the protein component may be too small to influence the phase transition properties of the whole system (Brent et al 1997). Thus, it may not be possible to measure the thermal properties for the protein component of the system. Secondly, on heating in water, protein denaturation reactions may occur at temperatures very close to the range associated with starch gelatinization. Wheat flour samples examined by DSC may therefore show an endothermic peak due to protein denaturation superimposed on the starch gelatinization peak (Stevens and Elton 1971). Even the major wheat proteins - gliadins and glutenins - have been reported to show little or no calorimetric response upon heating (Eliasson 1983, Ma 1990), and vital gluten containing 77.3% protein did not exhibit any obvious thermogram (Arntfield and Murray 1981). The DSC thermogram of vital gluten does not show any definite denaturation peak, indicating that there is no ordered structure in the gluten proteins (Hoseney 1994).

In view of the foregoing, therefore, the main effect of the presence of protein on wheat starch endotherms may be merely that of a diluent. It would therefore seem permissible to measure the endotherm of a flour sample and then assign T_m and ΔH values to starch (Stevens and Elton 1971).

Two thermally competing processes that can occur in starch DSC are endothermic melting of starch crystallites and exothermic formation of amylose-lipid complexes (Biliaderis 1990). The presence and disorganization of amylose-lipid complexes in starch systems have been reported to be responsible for the high temperature endotherms of 100-140°C observed in starch, which is well above the melting endotherm of starch crystallites (Kugimiya et al 1980, Donovan et al 1983, Biliaderis et al 1986). Bae and Lim (1998) have reported that transition

temperatures in the range of 85-125°C found during thermal analysis of extruded normal and high-amylose corn starch is typical for a lipid complex with amylose. Wheat starch is reported to contain about 1.12% lipid (Acker and Schmitz 1967), and Mercier (1980) has also shown that amylose forms a complex with lipids during the extrusion process which helps maintain the native structure of amylose.

2.8 ROLE OF DISULFIDE BONDS IN EXTRUSION TEXTURIZATION

Protein texturization involves a restructuring of the protein molecules into a layered cross-linked mass which is resistant to disruption (Harper 1986). Different researchers have attributed the stability of the structure of extrudates to different forces; thus the subject of structural stabilizing forces in extrudates is a subject of debate.

Cumming et al (1973) suggested that exposure to heat and pressure during extrusion texturization of soy proteins affected free sulfhydryl groups and disulfide bonds in the soy protein, which resulted in insolubilization of the protein. Recently, Li and Lee (1994) studied the effect of extrusion temperature on quantitative changes in extractable protein, disulfide bonds and sulfhydryl groups in wheat flour and a mixture of wheat flour and egg white. Three solvents [1% sodium dodecyl sulfate (SDS), 1% 2-mercaptoethanol (2-ME), and 1% SDS+2-ME] were used to investigate the types of bonding forces of protein interactions in extrudates. Sodium dodecyl sulfate can disrupt the hydrogen bonds and the hydrophobic interactions, and 2-ME can reduce the disulfide bonds to sulfhydryl groups. Their results showed a synergistic effect between SDS and 2-ME for protein solubility in all extrudates.

The disulfide and sulfhydryl group contents in the extracts of extrudates changed with the various extrusion temperatures. This indicated that the changes in solubility of protein in the extrudates were due to wheat proteins and/or egg protein aggregated by disulfide bonds and to hydrophobic interactions that occurred during the extrusion process (Li and Lee 1994). Disulfide bond formation has also been suggested as being responsible for reduced protein solubility of extrusion-texturized legume flours (Pham and Del Rosario 1984).

Burgess and Stanley (1976), in contrast, suggested that disulfide bonds played only a minor role in extrusion texturization and proposed that intermolecular amide bonds are responsible for texture formation in extruded products. However, Hager (1984) and Neumann et al (1984) later showed that the intermolecular disulfide bonding in soy is important and found no evidence for appreciable intermolecular peptide bond formation. Hager (1984) used different solvents to study the non-covalent forces that produced insolubility in extruded soy concentrate and concluded that at extrusion temperatures below 150°C, the formation of new disulfide bridges as well as changes in hydrogen bonding dominate in the protein conformation, but at higher temperatures intermolecular peptide bonds may also be formed.

2.9 EFFECTS OF EXTRUSION ON DISULFIDE AND SULFHYDRYL CONTENTS OF CEREAL PROTEINS

Sulfhydryl and disulfide groups are major contributors to the stability of native conformations of proteins (Thannhauser et al 1987) and are thus important in maintaining structure and functional properties of native proteins (Synowiecki and

Shahidi 1991). They are also thought to play an important role in the texture of cereal-based products (Chan and Wasserman 1993). The determination of the number of disulfide bonds per molecule is therefore crucial to structural studies of proteins (Thannhauser et al 1987).

Several studies have attempted to relate the disulfide and sulfhydryl contents of extrudates to some functionality of the extrudates. The exposure to heat and pressure during extrusion, for example, has been shown by Cumming et al (1973) to affect free sulfhydryl groups and disulfide bonds in soy protein, resulting in reduced solubility of the proteins. Hager (1984) and Neumann (1984) have also shown the importance of inter-molecular disulfide bonds in soy proteins to the extent that they contribute to the new and extended protein networks produced by extrusion of soy concentrate. Ummadi et al (1995) reported differences between SDS-PAGE patterns of reduced and non-reduced globulin fractions from raw and extruded semolina and attributed these differences to the role played by disulfide bonds in the extruded products.

Gluten, the storage protein of wheat which consists of gliadins and glutenins, exhibits decreased extractability when heated to 75-80°C (Booth et al 1980, Schofield et al 1983). This decreased extractability has been attributed to a decrease in extractability of the glutenin fraction (Booth et al 1980, Schofield et al 1983) which is accompanied by a disulfide-sulfhydryl interchange (Schofield et al 1983). Similarly, disulfide-sulfhydryl interchange has also resulted in the decreased extractability of heated gliadins (Booth et al 1980, Wrigley et al 1980, Feillet et al 1989).

The interchanges occurring between disulfide and sulfhydryl contents of

cereal proteins during extrusion processing have been related to thermal polymerization of gluten proteins. Strecker et al (1995) evaluated the polymerization and mechanical degradation kinetics of wheat gluten and glutenin during extrusion by analyzing protein solubility and disulfide bond content. Soluble protein and disulfide bond concentrations of gluten and glutenin extrudates were used as an index of extent of reaction. The results of the study showed the disappearance of soluble protein and the formation of disulfide bonds. The authors concluded that the reaction mechanism in gluten during extrusion was dominated by polymerization reactions contributed mainly by the formation of disulfide bonds. Schofield et al (1983), after analyzing the effects of heating on sulfhydryl-disulfide interchange reactions, concluded that the formation of protein network upon thermosetting was primarily due to interchanges between sulfhydryl-disulfide bonds. Several researchers (Beckwith and Wall 1966, Graveland et al 1978, Kaczkowski and Mielezko 1980) have supported the disulfide bond hypothesis.

Chan and Wasserman (1993) also studied the effect of twin-screw extrusion on the free sulfhydryl and disulfide contents of corn meal and reported that extrusion resulted in an increase in free sulfhydryl content and a decrease in disulfide groups, suggesting an extrusion-induced rupturing of disulfide bonds. In an experiment to study extrusion effects on sulfhydryl groups of extruded wheat flour proteins, Koh et al (1996) added cysteine to hard red winter wheat flour. After extrusion, there was a marked increase in protein sulfhydryl content and a decrease in protein disulfide content. According to Koh et al (1996), this increase in protein sulfhydryl content is a marker for sulfhydryl-disulfide interchange reactions during extrusion. Physical and chemical properties of the wheat flour were also markedly

affected by the cysteine addition. For example, the expansion ratio of samples decreased with increasing levels of cysteine. Cysteine addition also resulted in smaller cells with finer and more even structure (Koh et al 1996).

The foregoing review of the literature highlights some aspects of progress made in extrusion processing, especially as it relates to the extrusion processing of cereals. Several conclusions have been drawn by the published literature, stating that changes in physicochemical properties of cereal proteins as a result of twin-screw extrusion have not been adequately documented in spite of the commercial success of extrusion processing. Research in this area will therefore be helpful to the improvement of existing extruded products as well as in the design of new ones.

The present study was designed to seek further understanding with regard to physicochemical changes in wheat flour, with an emphasis on protein, during twin-screw extrusion.

2.10 LITERATURE CITED

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CHAPTER 3

THE INFLUENCE OF TWIN-SCREW EXTRUSION PROCESSING ON PHYSICAL AND MICROSTRUCTURAL CHARACTERISTICS OF WHEAT FLOUR EXTRUDATES

3.1 ABSTRACT

This study was conducted to assess the effects of extrusion processing parameters on the extrudate quality of wheat flour (at 9% protein content) and two wheat flour blends. Blends were made by mixing vital gluten containing 70% protein with the wheat flour (9% protein) to obtain 20 and 30% protein levels. Samples were extruded in a twin-screw extruder with full factorial combinations of die exit temperature of 120, 140, and 160°C; screw speed of 240, 320, and 400 rpm; and the three protein content levels of 9, 20, and 30%. Protein level of raw feed material significantly affected expansion ratio, bulk density, water absorption index, and specific mechanical energy. Extruder exit die temperature also affected bulk density and specific mechanical energy significantly. Overall, the linear effects of extrusion variables on expansion ratio and bulk density were not as pronounced ($R^2 = 0.15$, 0.44 , respectively) as the effect on water absorption index (WAI) ($R^2 = 0.86$), and specific mechanical energy ($R^2 = 0.94$). Correlation data showed that screw speed and specific mechanical energy were positively correlated ($r = 0.95$, $p \leq 0.001$), while die temperature was negatively correlated with bulk density ($r = -0.38$, $p \leq 0.01$). Protein content of feed negatively correlated with expansion ratio, water absorption index, and specific mechanical energy (SME), and positively with bulk density, while an inverse relationship existed between expansion ratio and bulk density. The protein content level of feed material was the only factor which significantly affected all extrusion dependent variables.

3.2 INTRODUCTION

Extrusion technology has had wide applications in the food industry for the manufacture of a wide range of snack foods, ready-to-eat cereals and other breakfast cereals using an energy-efficient, rapid and continuous system with numerous ingredients and processing conditions (Harper 1981). Texture is very important as a quality parameter in extruded products. Stanley (1986) has suggested that the texture of a food results from microstructure, which depends on the influence of physical forces on chemical components.

The texture and mouthfeel of most extruded snack foods depend on their expansion volume (Owusu-Ansah et al 1984), and several studies have reported the role of extrusion processing variables on the expansion volume of cereals (Owusu-Ansah et al 1983, Meuser et al 1982, Chinnaswamy and Hanna 1988). It has been shown that among the several extrusion variables that affect extrudate properties, the barrel temperature and moisture content of the raw feed material most prominently control expansion volume and hence texture of extrudates (Mercier and Feillet 1975, Owusu-Ansah et al 1983, 1984, Chinnaswamy and Hanna 1988).

In recent years, extrusion research has focused on how changes in extrusion variables such as extruder screw speed, feed moisture content, extrusion temperature, etc., affect the texture, microstructure, gelatinization (Case et al 1992), and other physico-chemical properties of the extruded products. Most related research has been concerned with optimization of conditions, and effect of changes on physical characteristics and microstructure of extrudate (Czarnecki et al 1993).

Chauhan and Bains (1988) investigated the effect of some extruder variables on the physico-chemical properties of extruded rice-legume blends and reported that expansion ratio of extruded products increased with increase in extruder exit temperature while bulk density decreased.

The texture of extruded food products is greatly influenced by the composition of the extrusion feed material. Jin et al (1995) studied the effects of soy fiber, salt, sugar, and screw speed on physical properties and microstructure of corn meal extrudates. The findings of the research indicated that increasing screw speed lowered water absorption index. Studies on soybean flour/oil and wheat bran effects on characteristics of cassava (*Manihot esculenta Crantz*) flour extrudates by Badrie and Mellowes (1992) showed that the crude protein content of extrudate correlated negatively with expansion and water solubility while the correlation with bulk density and water absorption was positive. Other studies have also reported that the qualities of raw materials such as contents of protein, lipid, and starch and their composition and type are also important in controlling physical properties of the extruded products (Faubion et al 1982, Chinnaswamy and Hanna 1988).

The effect of higher protein content of wheat flour on the physical characteristics of extrudates is not well documented. Proteins from different sources, varying in composition, molecular weight and structure would presumably affect the product upon texturization (Czarnecki et al 1993). This study was therefore designed to assess the effects of different levels of protein content and various conditions of extrusion processing (screw speed and exit die temperature) on some physical characteristics of wheat flour extrudates.

3.3 MATERIALS AND METHODS

Whole soft white wheat grain (cv. Harus) produced in Michigan in the 1994 crop year was milled to obtain straight grade flour with a 67% extraction rate and 9% protein content (14% moisture basis) on a Buhler MLU-202 Automatic Mill (Buhler AG, Uzwil, Switzerland). The wheat grain was tempered overnight to 14% moisture prior to milling. Vital gluten (70% protein, 14% moisture basis) was obtained from Midwest Grain Products (Atkinson, KS). Properties of wheat flour and vital gluten are given in Appendix 1.

Appropriate amounts of vital gluten were added to the wheat flour to obtain flour-gluten samples containing 20% or 30% protein. Moisture and Kjeldahl nitrogen contents of wheat flour, vital gluten and flour-gluten samples were determined according to AACC (1983) Approved Methods 44-19 and 46-11A, respectively. Nitrogen was converted to percent protein by using a factor of 5.75.

3.3.1 Extrusion Conditions

Each sample was extruded in duplicate on an APV Baker MP19TC-25 co-rotating and intermeshing twin screw extruder (APV Baker, Grand Rapids, MI), with barrel diameter of 19 mm and length-to-diameter (L/D) ratio of 25:1. The extruder barrel is divided into five zones including the die, and each zone is equipped with a temperature controller which reads the actual temperature of the barrel via a thermocouple. Discharge melt pressure developed by the screws just prior to entering the exit die is monitored with a transducer, and indicators are provided to monitor feeder speeds, screw speeds, pressure, barrel temperature of each zone,

and torque. An exit die drilled with a tapered hole of 2 mm in diameter was used in the experiment. The screw configuration used in the extrusion experiment is shown in Table 3.1. This screw configuration was shown in preliminary experiments to produce expanded cereal products.

Samples were fed into the extruder with a K-Tron K2M twin-screw volumetric feeder (K-Tron Corp., Pitman, NJ) equipped with safety hopper, variable speed drive and digital control. The feed rate was maintained constant throughout experimentation at 2 kg per hr. Deionized water was injected with an E2 Metripump positive displacement metering pump (Bran & Luebbe, Northampton, U.K.) at the rate of 0.21 kg per hr to obtain a constant feed ingredient moisture of 19%.

A full factorial arrangement with two replications was used. Extrusion variables were: (1) screw speed (240, 320, and 400 rpm), (2) extruder exit die temperature (120, 140, and 160°C), and (3) feed sample protein content (9, 20 and 30%). Temperatures at the four extruder barrel zones preceding the exit die were maintained constant at 40, 60, 80, and 100°C, respectively.

The extruder was run for at least 5 min after attaining a constant torque and pressure. Percent torque readings were taken, and the extrudates were collected for about a 5 min time period. After extrusion, samples were dried in a convection fan oven at 40°C to approximately 8% moisture and cooled to room temperature. Samples requiring grinding for subsequent analyses were ground in a Udy Cyclone Sample Mill (Udy Corp., Fort Collins, CO) through a 0.5 mm screen. Samples were then stored in airtight plastic freezer bags at 4°C till further analysis.

3.3.2 Determination of Physical Characteristics of Extrudates

3.3.2.1 Expansion ratio and bulk density

The expansion ratio of dried extrudates was determined as the cross-sectional diameter of the extrudate divided by the diameter of the die opening of the extruder (Harper 1981). The diameter of the die used in this study was 2.0 mm. Data for each sample was obtained by averaging caliper measurements from 5 random samples with five observations on each sample. Bulk density was determined according to the method of Park et al (1993). Pieces of extrudates, about 1 cm in length, were placed in a 250 ml graduated cylinder. The bottom of the cylinder was then repeatedly tapped gently on a laboratory bench until there was no further reduction in sample volume. Bulk density was calculated as the weight of sample per unit volume (g/ml).

3.3.2.2 Water absorption index and specific mechanical energy

The water absorption index (WAI) is the weight of gel obtained per gram of dry ground sample at room temperature, and was determined according to AACC method 56-20 (AACC 1983). The specific mechanical energy (SME), defined as the mechanical energy input required to obtain 1 kg of extrudate (Bhattacharya and Choudhury 1994), was calculated using the formula according to Brent et al 1997:

$$SME = \frac{\text{rpm (test)}}{\text{rpm (rated)}} \times \frac{\% \text{ torque}}{100} \times \frac{\text{horsepower}}{\text{feed rate}}$$

where rpm (test) is the screw speed for a particular test, rpm (rated) is the maximum

speed of the extruder shaft, horsepower is the extruder drive power input. The extruder drive power input and the maximum screw speed of the extruder shaft were provided by the manufacturer as being 2 kW and 500 rpm, respectively. The % torque of each experimental run was read directly from the controller unit.

3.3.2.3 Microstructure of extrudates

Microstructure of extrudates was studied using a Zeiss 10 Laser Confocal Microscope (Carl Zeiss, Inc., Thornwood, NY.) equipped with digital image acquisition capabilities. About 1 mm cross-section of a dried extrudate was cut and placed directly on the microscope slide. The slide was then placed on the viewing platform of the microscope and directly viewed through the objective lens. Images were acquired in the transmission, non-confocal mode. After acquisition, images were processed using the Adobe Photoshop Version 4.0 software.

3.3.3 Statistical Analysis

Data was analyzed using the General Linear Models (GLM) procedure of SAS Version 6.0 (SAS Institute, Cary, NC 1989). Pearson's correlation coefficient and LSMeans procedures were used to analyze relationships among variables.

3.4 RESULTS AND DISCUSSION

3.4.1 Expansion Ratio and Bulk Density

The expansion ratio of extrudate was negatively affected by increasing extruder die temperature and screw speed (Figure 3.1a), and protein content (Figure 3.1b) of raw feed material. Analysis of variance (Appendix 3) shows that the main effects as well as the interaction effects were all significant ($p \leq 0.001$).

The decreasing effect of protein content on expansion ratio has also been noted by Badrie and Mellowes (1992) who observed that increasing the crude protein content in the extrusion of cassava (*Manihot esculenta* Crantz) by adding soybean flour decreased expansion of extrudate. Park et al (1993) also noted that increasing protein level in the feed for the extrusion of soy flour, corn starch and raw beef blends decreased the expansion ratio of extrudates. In work done by Faubion et al (1982), it was shown that adding wheat gluten to wheat starch in extrusion cooking caused a reduction in expansion.

One of the possible explanations of such observations is that the addition of protein material reduced the percentage of total carbohydrate available which ultimately led to reduced water absorption (Badrie and Mellowes 1992). A reduction in the ability to absorb water inhibits the propensity to expand when the extrudate exits the die to the atmosphere. A second potential explanation is that one or more non-starch components were inhibitory to expansion (Badrie and Mellowes 1992). Eliasson (1983) had earlier observed that the lack of protein in raw extruding materials can make starch gelatinization easier because there is no material to compete with starch for water absorption. Proteins, lipids, bran and other

components in starch may also act as a heat sink, absorbing the heat that is required to gelatinize the starch as well as limiting the water available for gelatinization (Case et al 1992). These components, therefore, protect the starch and result in a lower extent of gelatinization and lower expansion.

The overall negative effect of die temperature on expansion ratio observed in this study is in contrast to some earlier reports in the literature. Bhattacharya and Choudhury (1994) observed that product expansion increased markedly with increasing barrel temperature when rice flour was extruded in a twin-screw extruder. Bhattacharya and Hanna (1987) had also noted previously that as barrel temperature was increased during extrusion of corn starch products, there was more expansion and reduced density of extrudates. In an earlier study on the effects of starch gelatinization on physical properties of extruded wheat and corn products, Case et al (1992) noted that products extruded at lower temperatures were thin and that expansion was greatest at highest extrusion temperatures which also corresponded with higher gelatinization.

However, in a single-screw extrusion of defatted soy flour, corn starch and raw beef blends, Park et al (1993) reported that increasing the process temperature increased expansion ratio up to a point and decreased thereafter with further increases in temperature. It is possible that at high temperatures, such as used in the present study, excessive starch damage occurred. Damaged starch granules could be more easily penetrated by water thereby decreasing the temperature and degree of starch gelatinization (Chang and Lii 1992). The reduced ability of starch to gelatinize reduces its ability to expand.

Increasing screw speed had overall negative effect on expansion ratio at all

protein levels. Even though the effects were not significant ($p>0.05$), the combinations of lower die temperature and lower screw speed (Figure 3.1a), and lower protein level and lower screw speed (Figure 3.1b), appeared to have produced well-expanded products with higher expansion ratios in extrudates.

The decreasing effect of screw speed on expansion ratio of extrudates indicates that increasing the screw speed increased the shear rate of raw materials and hence produced more damage to molecules. Jin et al (1995) have reported that damaged starches are less cohesive than gelatinized undamaged starches and therefore expand less and create products with smaller pores. Several researchers have demonstrated that the expansion of extruded cereals or starch-based materials depended on the degree of gelatinization, which in turn was determined by process temperature, shear rate, and moisture content of the feed material (Lawton et al 1972, Chiang and Johnson 1977, Guy and Home 1988).

Increasing temperature at the extruder exit die had an overall negative effect on bulk density, while an increasing protein content of feed ingredient positively correlated with the bulk density of extruded samples (Figure 3.2, Table 3.2). Overall, extrudates with highest bulk densities were produced by combinations of low die temperature and high protein content level within the range of extruder variables used in the present study.

A decrease in the bulk density of extrudates as a result of increasing screw speed has been noted by several researchers. Onwulata et al (1992) found that the bulk density of an extrudate varied as a function of the screw speed and was dependent on the final temperature of the dough behind the die. At a higher screw speed, there is a greater amount of mechanical work and frictional heat which

causes an increase in product temperature. As shear force and product temperature increase, more starch granules are dispersed into a polymer phase, increasing the extrudate expansion and decreasing bulk density. However, in a study of the effects of dietary fiber and screw speed on corn meal extrusion processing, Hsieh et al (1989, 1991) reported that increasing either fiber content or screw speed increased the axial expansion but decreased the radial expansion. The net result was an increase in bulk density and a decrease in specific volume of extrudate. Rayas-Duarte et al (1998) have reported a significant negative correlation between expansion ratio and bulk density of buckwheat flour extrudates, which is similar to the findings reported in Table 3.3 of the present study.

3.4.2 Water Absorption Index (WAI)

Results of correlation analyses listed in Table 3.2 indicate that extruder screw speed and feed protein content level have significant effects on WAI. Increasing protein content and extruder screw speed both decreased WAI (Figure 3.3). Overall die temperature effects were not significant ($p>0.05$). Water absorption of extruded products can be attributed to the dispersion of starch in excess water which is increased by the degree of starch damage due to gelatinization and extrusion-induced fragmentation (Rayas-Duarte et al 1998). The increasing protein levels in raw feed materials mean that increasing amounts of protein material competed with decreasing amounts of starch for water absorption. Starch gelatinization is incomplete when starch is present in relatively low amounts thereby decreasing WAI. Similar observation was made by Eliasson (1983) who, working on wheat-starch-gluten mixtures, reported that the lack of protein in raw materials made

starch gelatinization easier since there was no material which could compete with starch for water absorption.

Increasing screw speed significantly reduced WAI. This finding is in agreement with the observation of Jin et al (1995). Increasing screw speed results in an increase in the shear rate of molecules which produces structural modifications of starch (Diosady et al 1985). High screw speeds produce more damaged polymer chains and reduce the availability of hydrophilic groups; the ability of starch molecules to bind more water molecules is thus reduced, which is reflected in lower WAI values. Wen et al (1990) have reported that screw speed had a direct effect on polysaccharide size distribution. Higher screw speed resulted in more fragmentation than a lower screw speed.

In an extrusion of manioc starch, Colonna and Mercier (1983) reported that extrusion resulted in the degradation of macro-molecular amylose and amylopectin through chain splitting. Similar effect on wheat starch degradation has been observed in a single screw extrusion in which starch degradation was attributed to shear forces generated by increase in screw speed (Davidson et al 1984).

Water absorption correlated positively with expansion (Table 3.3), even though the correlation was not significant ($p \leq 0.05$). This indicates that high expansion was associated with high water absorptions, meaning that extrudates with higher expansion ratios would also be more porous, according to Rayas-Duarte et al (1998), and as indicated by the significant ($r = -0.54$, $p \leq 0.001$) negative correlation with bulk density. The amount of water absorbed by ground extrudate has been indirectly used to indicate the porosity of the material (Colonna et al 1989). Hence, as the porosity of the extrudate material increases, the water

absorption would also increase. According to Rayas-Duarte et al (1998), a slow rate of water absorption in extruded breakfast cereals is desirable to maintain crispness.

The absorption of water in extruded products can be explained on the basis of starch-water-protein interactions that govern the solid phase structure (Rayas-Duarte et al 1998). Generally, it has been attributed to the dispersion of starch in excess water. The dispersion is increased by the degree of starch damage due to gelatinization and extrusion-induced fragmentation of amylose and amylopectin molecules (Colonna et al 1989). Other factors that affect water absorption include the type of proteins, degree of denaturation, and amount of fiber present (Gujska and Khan 1990).

3.4.3 Specific Mechanical Energy

The specific mechanical energy (SME) of extrudates increased with screw speed and decreased with increasing extruder die temperature (Figure 3.4a). The protein content levels of the feed material also had a negative effect on the SME as shown in Figure 3.4b and Table 3.2. On the whole, increasing screw speed was the only factor that positively affected SME as indicated by the regression and correlation coefficients in Tables 3.2 and 3.3, respectively. It has been reported that the mechanical energy input in an extruder can be changed using the screw speed or feed rate (Blenford 1994).

In a study of extrusion cooking of corn meal with soy fiber, salt, and sugar, Jin et al (1994) found that increasing screw speed reduced extruder torque due to changes in the length of filled flights in the extruder barrel. Whalen et al (1997) also reported that increasing the barrel temperature in a twin-screw extrusion

significantly decreased the torque due to the lowering of apparent viscosity. The SME is usually calculated from the percent torque of the extruder motor and its speed (Levine 1997). In a review, Weidmann (1990) observed a decrease in SME with an increase in barrel temperature during extrusion of wheat flour which agrees with the findings in the present study.

The viscosity of cereal flour dough is affected by the extent of gelatinization (Bhattacharya and Choudhury 1994). At a low temperature and/or high screw speed, gelatinization of starch is incomplete and shows lower viscosity. However, at the beginning of gelatinization, increasing extrusion temperature or decreasing screw speed increases apparent viscosity. After the completion of starch gelatinization, apparent viscosity drops again with further increases in temperature. Consequently, the torque decreases resulting in lower SME values.

3.4.4 Relationship Between Extrusion Variables and Physical Properties

Correlation data in Table 3.3 shows that there was a significant positive correlation between screw speed and specific mechanical energy ($r = 0.95$, $p \leq 0.001$) while die temperature was negatively correlated with bulk density. Protein content of feed negatively correlated with expansion ratio and water absorption index, and positively with bulk density, while an inverse relationship existed between expansion ratio and bulk density. Park et al (1993) found a similar negative correlation between expansion ratio and bulk density and noted that the degree of puffing of an extrudate as it exits the die nozzle of an extruder is governed by the expansion ratio and bulk density. However, these two properties are not always the same, according to previous studies. Phillips et al (1984) and Falcone and Phillips

(1988) explained that the expansion ratio considers expansion only in the direction perpendicular to extrudate flow, whereas bulk density is concerned with expansion in all directions.

The relationship between and among extrusion variables and physical properties of extrudates indicates that water absorption index, protein content of raw material, and bulk density are all related to the expansion characteristics of extruded products. Thus, extrudates with high protein content tended to have low expansion and higher bulk density characteristics and reduced ability to absorb water, as indicated by correlation analysis.

3.4.5 Microstructure of Extrudates

The laser scanning micrographs revealed the microstructure of the extruded products, and are shown in Figures 3.5 and 3.6. Even though it was difficult to discern any quantitative relationship between extrusion variables and microstructure of extrudates, the most visible features of the microstructure of the extrudates observed were the number and sizes of air bubbles formed due to extrusion. Figure 3.5 shows that protein level effects were shown mainly on the size and presence of air vacuoles as well as the cell wall thickness between air vacuoles. The extrudates containing 9% protein (Figure 3.5a) had a well-defined air bubble shape (arrow) which seemed to disappear in the higher protein content sample. Physical examination of extrudates showed that the higher protein content products had reduced expansion and increased bulk density.

In the extrudates produced at higher exit die temperature (Figure 3.6b), air cells were present, but the cell wall thickness was much smaller than in samples extruded at lower exit die temperature (Figure 3.6a). Thin cell walls collapse upon evaporation of superheated water when extrudate exits the die into ambient pressure, thus producing an insufficiently expanded product. Correlation analysis showed that higher temperatures produced less expanded products.

The overall effect of screw speed on the expansion ratio and bulk density of extrudates was negative and not significant. The micrographs depicting the effects of screw speed on microstructure (data not shown) did not show any obvious differences among the various screw speed levels used in this study, except that air cell sizes and wall thickness between the vacuoles appeared to be well-defined in the higher screw speed sample.

3.5 SUMMARY

This study assessed the impact of heat processing on the functionality of wheat flour. In particular, the study focused on the effects of twin-screw extrusion processing on the physical and microstructural characteristics of wheat flour extrudates.

The role of increased protein level in extrusion raw material and how this protein augmentation, together with other extrusion variables, influenced the physical characteristics of the extruded product were investigated. Like many other studies in this area, this study has established that extrusion is a complex process which involves several variables each of which contributes differently under different sets of extrusion conditions to the overall quality characteristics of the extrudates. The interactions among variables are complex and make it challenging to differentiate among the individual variables as to changes in the characteristics of the final product.

The functional properties of extruded products result from both the structural modification and changes in physical properties resulting from the mechanical and thermal stresses applied during extrusion processing. The various interactions among constituents of the extruding feed material all have significant impact on quality attributes of extrudates.

In this study, expansion ratio correlated negatively with bulk density and had a non-significant but positive correlation with water absorption index. Similarly, bulk density correlated negatively with WAI. Protein level negatively correlated with expansion ratio and WAI, but positively with bulk density. These results suggest

that WAI, protein level, and bulk density are all related to the expansion characteristics of extrudates.

Increasing protein levels inhibit the ability of starch to absorb more water due to competition, hence the high negative correlation coefficient with WAI. The reduced ability of starch for more water absorption inhibits the expansion capability of extrudates since the flashing of water at the die of the extruder (which influences expansion), is significantly reduced. Lower expansion of extrudates corresponded with high bulk density as is seen in the negative correlation between expansion ratio and bulk density.

The results of this study provide evidence that higher protein content of raw material may not be beneficial in the extrusion processing of wheat flour.

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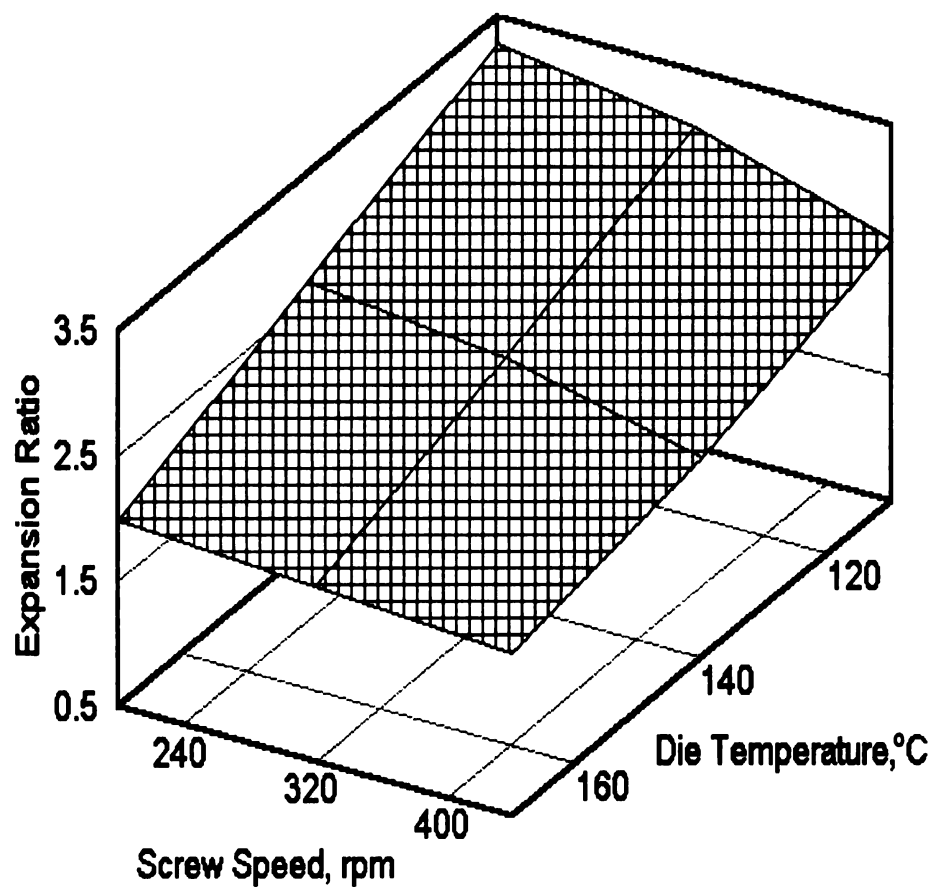


Figure 3.1a

Effects of screw speed and exit die temperature on expansion ratio of extrudates at 9% feed protein content.

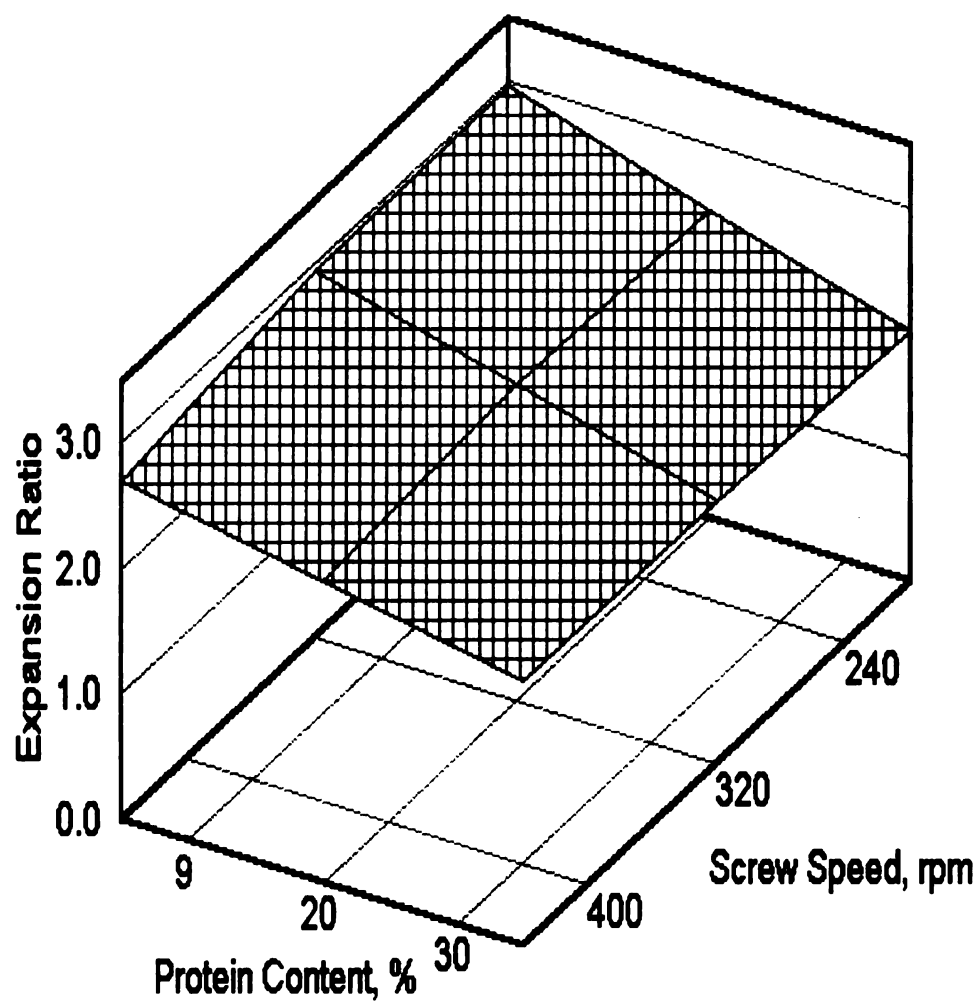


Figure 3.1b

Effects of feed protein content level and extruder screw speed on expansion ratio of extrudates at 120°C exit die temperature.

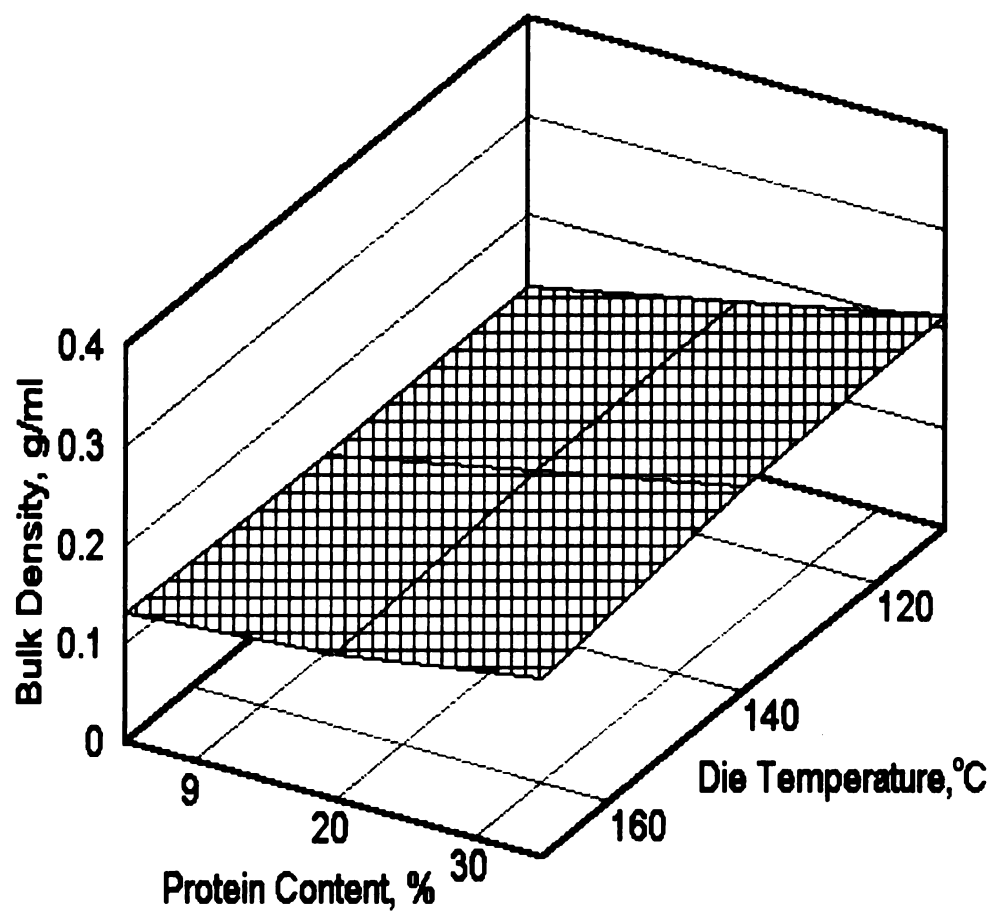


Figure 3.2

Effects of feed protein content and extruder exit die temperature on bulk density of extrudates at screw speed of 400 rpm.

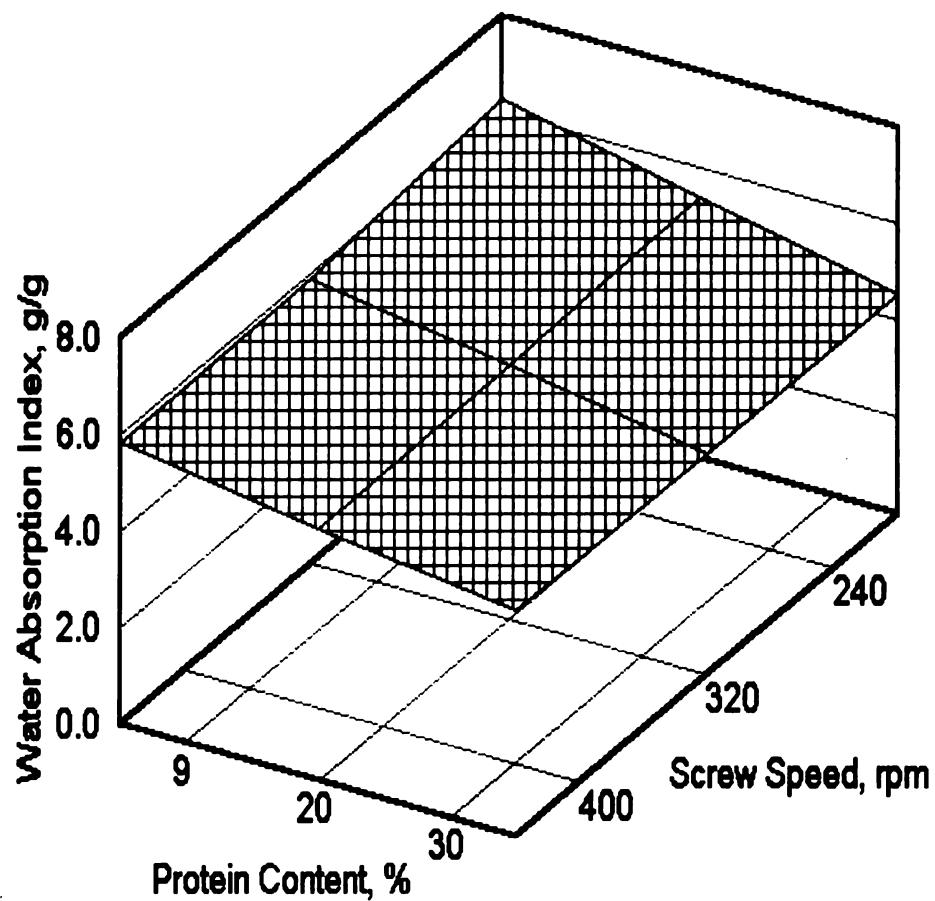


Figure 3.3

Effects of feed protein content and extruder screw speed on water absorption index of extrudates at exit die temperature of 140°C.

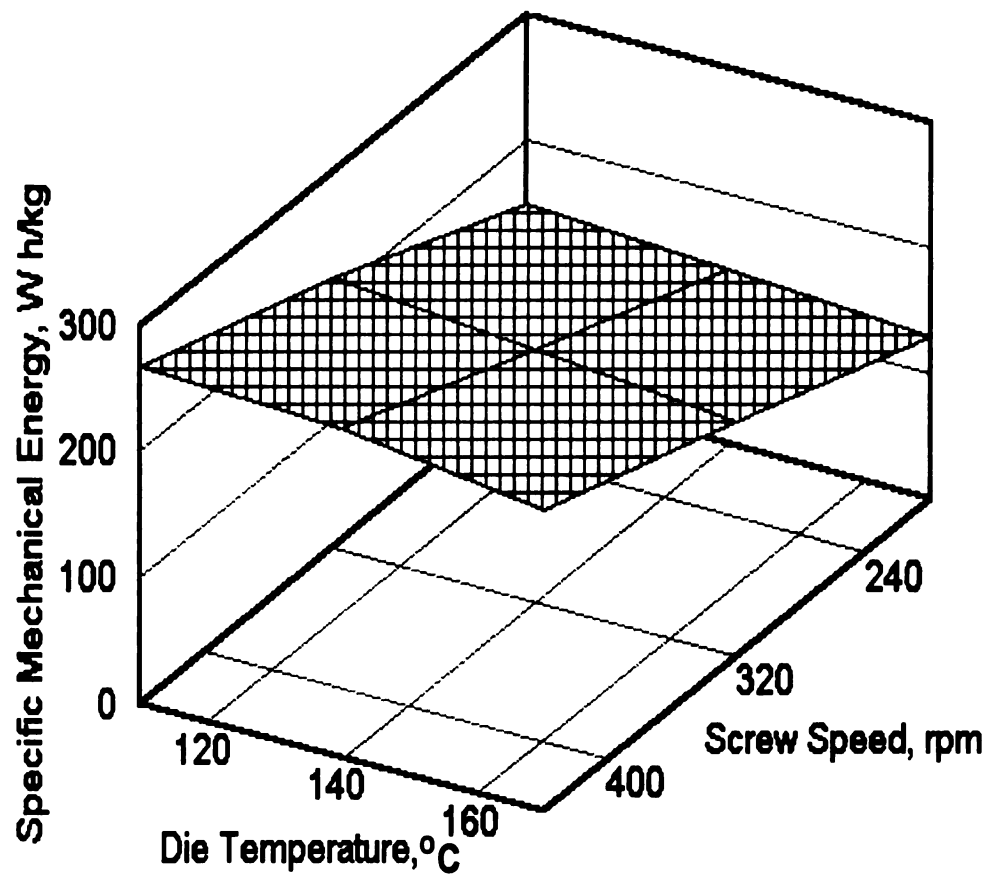


Figure 3.4a

Effects of extruder exit die temperature and screw speed on specific mechanical energy input of extrudates at feed protein content of 9%.

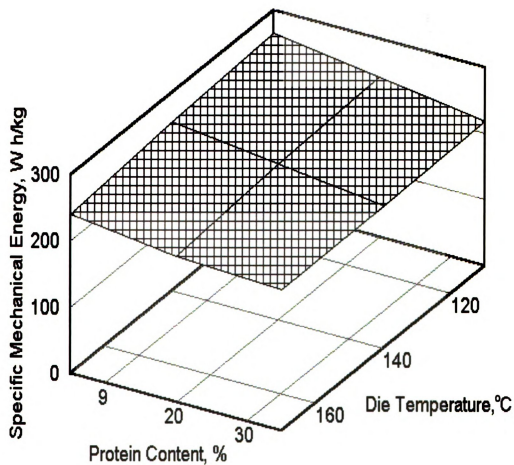
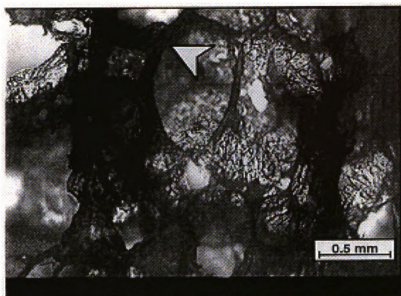
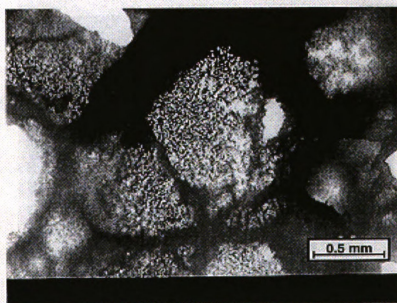


Figure 3.4b

Effects of feed protein content and exit die temperature on the specific mechanical energy input of extrudates at screw speed of 400 rpm.



(a)



(b)

Figure 3.5

Laser scanning micrographs of cross-sections of samples extruded at exit die temperature of 160°C, screw speed of 240 rpm, and feed protein content levels of: (a) 9% (b) 30%.

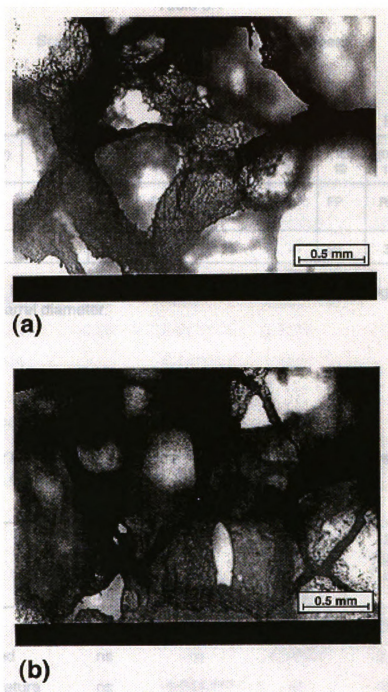


Figure 3.6

Laser scanning micrographs of cross-sections of samples extruded at screw speed of 400 rpm, feed protein content of 9%, and exit die temperature of: (a) 140°C (b) 160°C.

Table 3.1
Screw configuration in extrusion experiment

Feed Zone					Exit Die Zone				
Length (mm)	(8D) 152	(1.75D) 33.25	(8D) 152	(0.75D) 14.25	(0.75D) 14.25	(2D) 38	(1D) 19	(0.75D) 14.25	(2D) 38
Screw elements	TL	FP	TL	FP	RP	SL	FP	RP	SL
Degrees	—	30	—	60	- 30	—	60	- 30	—

TL = twin lead; FP = forward paddle; RP = reverse paddle; SL = single lead.
D = Extruder barrel diameter.

Table 3.2
Multiple Linear Regression Coefficients for Expansion Ratio, Bulk Density, Water Absorption Index, and Specific Mechanical Energy

	Expansion Ratio	Bulk Density, g/ml	Water Absorption Index, g/g	Specific Mechanical Energy, W h/kg
Intercept	3.6542	0.3250	6.8261	68.4593
Screw Speed	ns	ns	-0.0013*	0.6069***
Die Temperature	ns	-0.0013***	ns	-0.4031***
Protein Level	-0.0124*	0.0032***	-0.0718***	-0.9327**
R ²	0.15	0.44	0.86	0.94

*p≤0.05; **p≤0.01; ***p≤0.001
ns - not significant at 5% level.

Table 3.3
Multiple Correlation Coefficients for Extrusion Variables

	Expansion Ratio	Bulk Density	Water Absorption Index	Specific Mechanical Energy
Screw Speed	-0.14 ^{ns}	-0.17 ^{ns}	-0.12 ^{ns}	0.95 ^{***}
Die Temperature	-0.23 ^{ns}	-0.38 ^{**}	0.05 ^{ns}	-0.16 ^{ns}
Protein Level	-0.28 [*]	0.51 ^{***}	-0.92 ^{***}	-0.19 ^{ns}
Expansion Ratio		-0.54 ^{***}	0.18 ^{ns}	-0.02 ^{ns}
Bulk Density			-0.35 ^{**}	-0.23 ^{ns}
Water Absorption Index				-0.01 ^{ns}

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$
ns - not significant at 5% level.

CHAPTER 4

CHANGES IN DISULFIDE AND SULFHYDRYL CONTENTS AND ELECTROPHORETIC PATTERNS OF WHEAT PROTEINS UPON EXTRUSION

4.1 ABSTRACT

Vital gluten containing 70% protein (14% moisture basis) was used to increase the protein content of wheat flour from 9% to 20 and 30%. The three samples with 9, 20, and 30% protein contents, respectively, were extruded with a twin-screw extruder and analyzed for changes in disulfide and sulfhydryl contents as well as the electrophoretic patterns of the total proteins during extrusion. Increasing extruder exit die temperatures resulted in increased sulfhydryl content of 9 and 20% protein content samples, but did not appear to have any effect on the 30% protein content sample. Similarly, disulfide content decreased slightly following the same trend. Both sulfhydryl and disulfide contents of extrudates were lower than those of the non-extruded samples. Total cysteine content of extruded samples decreased by about 16% relative to non-extruded samples, but otherwise remained almost unchanged among all extruded samples. Polyacrylamide gel electrophoresis patterns of total proteins showed marked differences between reduced and non-reduced proteins with a shift in the molecular weights of certain protein bands. In all, data from this study suggest that disulfide-sulfhydryl interchange reactions were not significant in the extrusion process under the conditions studied, and that the changes in the disulfide and sulfhydryl contents of the total proteins might have predominantly resulted from the scission of disulfide bonds rather than the interchange between thiol and disulfide contents.

4.2 INTRODUCTION

Wheat flour is an important raw material in the manufacture of breakfast cereals, infant foods, snack foods, and pasta products through extrusion processing. The extrusion process has the potential to change protein structure, solubility and digestibility through a combination of shearing, heat and pressure (Phillips 1989, Wen et al 1990).

The formation of covalent bonds between polypeptide chains, or between polypeptide chains and other constituents, is considered to be the chemical basis of extrusion texturization (Jeunink and Cheftel 1979). Several studies have concluded that during the extrusion process, proteins are denatured and the forces that stabilize the tertiary and quaternary structures are weakened through a combination of increased heat and shear within the extruder, resulting in change in protein conformation (Harper 1986, Ledward and Mitchell 1988, Camire et al 1990, Camire 1991, Dahl and Villota 1991).

The cross-linking of proteins during extrusion is known to be responsible for the textural characteristics of extruded products, and disulfide bonds are one obvious source of cross-links (Koh et al 1996). Disulfide cross-links have been reported in spun soy fibers as well as in heated soy protein systems. The texture of extruded soy granules has been improved through the addition of elemental sulfur or potassium or sodium sulfites which are believed to increase disulfide cross-links (Jenkins 1970).

Sulfhydryl groups and disulfide bonds are also major contributors to the stability of the native conformations of proteins and are thus important in

maintaining structure and functional properties of native proteins (Thannhauser et al 1987, Synowiecki and Shahidi 1991). Furthermore, they are thought to play an important role in the texture of cereal-based products (Chan and Wasserman 1993).

Several studies have attempted to relate the disulfide and sulfhydryl contents of extrudates to some properties of the extrudates. Cumming et al (1973) have shown that during extrusion texturization of soy proteins, there is dissociation of water-soluble proteins followed by aggregation, and that a sulfhydryl-disulfide interchange reaction is involved in the insolubilization of the soy protein. Hager (1984) and Neumann et al (1984) have also shown that inter-molecular disulfide bonds in soy proteins contribute to the new and extended protein networks produced by extrusion of soy concentrate. Ummadi et al (1995) reported differences between sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of reduced and non-reduced fractions of albumins, globulins, and glutenins from raw and extruded semolina, and attributed these differences to the role played by disulfide linkages during extrusion.

Disulfide-sulfhydryl interchanges during extrusion processing have been related to the thermal polymerization of gluten proteins during extrusion. Strecker et al (1995) evaluated the polymerization reactions of wheat gluten proteins during extrusion through analysis of solubility and disulfide bond content and concluded that the reaction mechanism in wheat gluten during extrusion was dominated by polymerization reactions contributed mainly by disulfide bond formation. In contrast, however, Chan and Wasserman (1993) had earlier reported that extrusion of corn meal caused an increase in free sulfhydryl content and a decrease in disulfide groups relative to native samples, suggesting an extrusion-induced rupturing of

disulfide bonds.

In an experiment to study effects on sulfhydryl groups of extruded wheat flour proteins, Koh et al (1996) added cysteine to hard red winter wheat flour. After extrusion, there was a marked increase in protein sulfhydryl content and a decrease in protein disulfide content indicating a disulfide-sulfhydryl interchange as a result of the extrusion process. Physical and chemical properties of the extrudates were significantly affected by the interchange.

In order for extrusion processing to be more controllable and predictable, there is the need to gain further information on the changes in wheat proteins as a result of extrusion processing. In the present study, the effects of twin-screw extrusion processing on the sulfhydryl and disulfide contents and the electrophoretic patterns of the total wheat flour proteins were examined.

4.3 MATERIALS AND METHODS

4.3.1 Extrusion

Materials and extrusion processing were described in Chapter 3 of this document.

4.3.2 Disulfide-Sulfhydryl Analyses

Disulfide (SS) and sulfhydryl (SH) contents of non-extruded and extruded samples were determined according to a modified solid-phase colorimetric assay method of Chan and Wasserman (1993). For free sulfhydryl (free and buried SH) content determination, about 30 mg samples were suspended in 1.0 ml of reaction buffer consisting of 8 M urea, 3 mM EDTA, 1% SDS and 0.2 M Tris-HCl (pH 8.0) (Buffer A). Samples were vortexed for 30 sec and placed on a constant agitation shaker at room temperature. After 1 hr, 0.1 ml of 10 mM DTNB in 0.2 M Tris-HCl (pH 8.0) (Buffer B) was added to each sample and shaking continued for another 1 hr. Samples were then centrifuged at 13,600 x g for 10 min at room temperature, and the absorbance of the supernatant was read at 412 nm against a blank consisting of 1.0 ml of buffer A and 0.1 ml of buffer B. It was not necessary to dilute these samples before taking spectrophotometer readings.

For determination of total sulfhydryl (SH + reduced SS) content, reaction buffer consisted of Buffer A containing 0.1 M Na_2SO_3 and 0.5 mM NTSB²⁻ synthesized from DTNB according to Thannhauser et al (1987). The pH of the buffer was adjusted to 9.5. One ml of the reaction buffer was added to 30 mg of each sample and vortexed for about 30 sec at room temperature. Samples were

then shaken in the dark for 1 hr and then centrifuged at 13,600 x g for 10 min at room temperature. A 0.1 ml aliquot of the supernatant was diluted with 0.9 ml of reaction buffer and the absorbance read at 412 nm.

Free SH and total SH contents were calculated from the absorption readings using a molar absorption coefficient of 13,600 M⁻¹cm⁻¹ (Chan and Wasserman, 1993) as follows:

$$A = \epsilon bc,$$

where A is the absorbance readings, ϵ is the molar extinction coefficient, b is the cell thickness, and c is the concentration. Disulfide group content was calculated as the difference between total SH and free SH contents, using the formula:

$$SS = (TS - SH)/2,$$

where SS is disulfide content, TS is total sulfhydryl content (free SH + reduced SS), and SH is free sulfhydryl content.

4.3.3 Electrophoresis

4.3.3.1 Extraction procedure

Total reduced proteins were extracted from ground extrudates and native flour according to Pogna et al (1990). Each sample was stirred in 1 ml of extraction buffer consisting of 0.67 ml distilled water, 0.05 ml of 2-mercaptoethanol (2-ME), and 0.28 ml of stock solution containing 0.2 M Tris-HCl (pH 6.8), 7% (w/v) SDS, 30% (v/v) glycerol, and 0.04% (w/v) Pyronin Y. For non-reduced total proteins, extraction buffer did not contain 2-ME.

The extraction mixture was vortexed for 30 sec and placed on a constant agitation shaker at room temperature for 2 hr. Samples were then heated at 80°C

for 30 min and allowed to cool before aliquots of the clear solution were loaded onto gels for SDS-PAGE.

4.3.3.2 SDS-PAGE

Aliquots (25 μ l) of extracted samples were loaded onto 15% (T=15.1%, C=0.58%) SDS separating gels. For each gel, the solution mixture consisted of 15 ml of 30% acrylamide, 1.74 ml of 1.5% bis-acrylamide, 11.25 ml of 1 M Tris-HCl (pH 8.4), 0.96 ml water, 0.3 ml of 10% (w/v) SDS, 0.75 ml of 1% (w/v) ammonium persulfate and 20 μ l N,N,N',N'-tetramethylethylenediamine (TEMED). The stacking gels were 4.5% (T=4.5%, C=1.3%) and consisted of 1.575 ml of 30% acrylamide, 0.433 ml of 1.5% bis-acrylamide, 1.25 ml of 1M Tris-HCl (pH 6.8), 6.142 ml water, 0.1 ml 10% (w/v) SDS, 0.5 ml of 1% (w/v) ammonium persulfate, and 10 μ l TEMED. The gels were run at room temperature for 18 hr at a constant current of 12.5 mA per gel.

After the run, gels were stained according to the procedure of Redaelli et al (1995). Each gel was placed overnight in a staining solution consisting of 15 ml Coomassie Brilliant Blue R-250 (4 g dissolved in 1 liter of 95% ethanol), 25 ml of 60% trichloroacetic acid (TCA), and 210 ml distilled water. Gels were then washed several times with deionized water and photographed.

4.4 RESULTS AND DISCUSSION

4.4.1 Disulfide and Sulfhydryl Reactions

The relationships between the protein content and sulfhydryl, disulfide and total cysteine contents of non-extruded flour samples are shown in Figure 4.1. Increasing the total protein content of flour resulted in decreases in free sulfhydryl, disulfide and total cysteine contents of the non-extruded samples. The albumin and globulin fractions of wheat flour are known to contain higher amounts of total cysteine (6.2 and 5.4 g/16g of nitrogen, respectively) than the gluten proteins of gliadin and glutenin (2.7 and 2.2 g/16g of nitrogen, respectively) (Bushuk and Wrigley 1974). Thus, it is reasonable to expect that increasing the flour protein content from 9 to 30% using vital gluten is essentially equivalent to dilution of the total SH content per unit weight of sample. The effect of this is a declining content of free SH, disulfide content and total cysteine content as protein content is increased in the non-extruded flour.

Table 4.1 shows the results of analysis of sulfhydryl, disulfide, and total cysteine contents of non-extruded and extruded samples from flour samples with 9, 20 and 30% protein content. Analysis of variance (Table 4.2) indicates that both linear and interaction effects of variables on free sulfhydryl, disulfide, and total cysteine contents were significant ($p \leq 0.001$). At a screw speed of 240 rpm, increasing the die temperature from 120 to 160°C increased free SH content from 6.95 to 10.98 $\mu\text{M}/\text{mg}$ protein, an increase of about 58%. Similarly, increases of 40 and 67% in free SH were noted over the same temperature ranges at constant screw speeds of 320 and 400 rpm, respectively, for the extrudates containing 9%

protein. There were much higher increases in free SH for the 20% protein content extrudates, and relatively much smaller increases for the 30% protein content sample, as exit die temperature was increased. It is evident from the data that temperature was positively correlated with free SH content of all extruded samples.

When non-extruded samples are compared to extruded samples, Table 4.1 shows that at all screw speed levels, free SH contents in non-extruded samples were higher than in samples extruded at 120 and 140°C for the 9 and 20% protein content samples, and at all three temperature levels for the 30% protein samples. However, within and among all extruded samples, increasing die temperature also increased free SH at all screw speed levels, while correspondingly, SS contents decreased. This was particularly evident for the 9 and 20% protein content samples.

The decrease in free SH content of extrudates relative to non-extruded samples, and the opposite increases with increasing die temperatures among all extruded samples concomitant with decreases in SS, are in agreement with the findings reported by Chan and Wasserman (1993). These authors showed a decrease in the SH group content of extruded corn meal proteins, relative to that of non-extrudates. However, among extrudates, samples extruded at a higher temperature showed an increase in free SH content relative to those extruded at a lower temperature, and this was accompanied by slight decreases in SS group content. Burgess and Stanley (1976) had previously found slight decreases in SS and an increase in SH contents on extrusion of soy meal.

However, the results of the present study contrast the findings of Synowiecki and Shahidi (1991) who reported a decrease in the content of sulfhydryl groups and an increase in the content of disulfide bonds on heating seal meat proteins.

Together, these results suggest that the process of sulfhydryl-disulfide changes occurring in seal meat proteins, as described by Synowiecki and Shahidi (1991), may be essentially one of disulfide cross-linking of protein molecules due to oxidation of sulfhydryl to disulfide groups, while the extrusion of wheat proteins, as reported in the present study, appeared to result in the reduction of disulfide groups to sulfhydryls. Secondly, the involvement of shear force in extrusion, as in the present study, as opposed to ordinary heating alone, may be significant in the differences between the results. In general, the apparent increases in free SH group content accompanied by small decreases in disulfide group content in the present study, with increasing temperatures, may be indicative of the inter-conversion of disulfide and sulfhydryl groups during the extrusion processing of wheat proteins.

Data in Table 4.1 also show that the total cysteine contents of all extrudates were lower than that of the non-extruded flour in the 9% protein sample, and varied between 81.82 and 89.57 $\mu\text{M}/\text{mg}$ protein compared to 102.51 $\mu\text{M}/\text{mg}$ protein for the non-extruded sample. Thus, on average, extrusion reduced total cysteine content by approximately 16%.

It can also be concluded from the data in Table 4.1 that the sample containing 30% protein was virtually unaffected by extrusion in terms of changes in total cysteine content. This sample contains much higher vital gluten, as compared to the 9 and 20% protein content samples, and the protein content is composed mainly of the gluten proteins (gliadins and glutenins). Schofield et al (1983) reported that the level of total cysteine groups in gluten essentially remained constant irrespective of temperature and was unaffected by heating to 100°C.

The decrease of 16% in total cysteine content in extruded 9% protein content

samples relative to the non-extruded samples, and the negligible variations in total cysteine contents for the 20 and 30% protein samples, suggest that some cysteine was lost during extrusion. According to Koh et al (1996), this could represent cysteine loss at the extruder die as hydrogen sulfide or volatile organic compounds such as those found as flavor compounds in the extrudates. Extruded cereals are generally believed to have less flavor than those prepared by traditional baking, and this is attributed to loss of produced or added aroma volatiles when the melt leaves the extruder die and becomes an expanded solid material (Bredie et al 1997). Chan and Wasserman (1993) and Weegels et al (1994) have also suggested that conformational changes in the protein structure during extrusion might inhibit access of colorimetric reagent in the presence of SDS. Alternatively, the cysteine in the extrudates is more prone to oxidation after extrusion (Chan and Wasserman 1993), leading to the formation of more non-covalent disulfide bonds. The results of the present study did not support this theory since there were no noticeable increases in disulfide group content after extrusion.

4.4.2 Electrophoresis

Figure 4.2 shows the electrophoretic patterns of non-reduced total proteins of samples containing 9% protein. For both non-extruded and extruded samples, bands in the high molecular weight protein region (labeled i) and several bands in the low molecular weight region (labeled iii) were not visible. There was no major difference in the intensity of visible bands in the mid-region (labeled ii) among all extruded samples, and between extruded and non-extruded samples. However, a few bands which were present in the low molecular weight region of non-extruded

samples disappeared after extrusion. Similar observations were made in the electrophoretic patterns of the samples containing 20 and 30% protein (data not shown), even though these two contained more protein bands than the 9% protein content sample in view of their mixture with vital gluten. Thus, without reduction of disulfide bonds, extractability of low molecular weight proteins in sodium dodecyl sulfate (SDS) was markedly decreased after extrusion, consistent with previous studies (Koh et al 1996). Dexter and Matsuo (1977) reported a decrease in protein extractabilities of four different flours after extrusion. Wen et al (1990) have also reported previously that the size distribution of dimethyl sulfoxide-soluble proteins of corn meal extrudates was unchanged after extrusion even though significant amounts of protein became insolubilized.

Previous studies have demonstrated reductions in solubility as a function of temperature for soy protein (Hager 1984) and corn meal protein (Racicot et al 1981). The disappearance of the bands at the lower molecular weight region after extrusion may suggest that extrusion might have depolymerized the lower molecular weight proteins into even much smaller units which then eluted off the gel into the running buffer; or the proteins might have polymerized into larger aggregates making those proteins too big to enter the running gel. However, gels run under non-reduced conditions in which running was terminated when the tracking dye just reached the bottom of the running gel, showed similar patterns. This suggests that polymerization was more likely to have occurred during the extrusion process. Ummadi et al (1995) observed the aggregation of polypeptides of albumins, globulins, and glutenins of semolina under non-reduced conditions after extrusion.

Figure 4.3 shows the electrophoretic patterns of reduced 9% protein content

samples. After reduction of disulfide bonds with 2-mercaptoethanol (2-ME), strong and distinct bands appeared at the high molecular weight protein region (labeled i) in both non-extruded and extruded samples. There were no differences in intensities of bands in this region. The electrophoretic patterns of both 20 and 30% protein samples were similar. However, comparing the bands of non-extruded samples to those of the extruded samples, it was observed that a single band (arrow) in the middle region labeled "ii" of the non-extruded sample lightened and/or disappeared after extrusion. Simultaneously, there was an appearance of heavy bands (white arrows) in the lower region (labeled iii) for extrudates. There was a very light band at the same location in the non-extruded sample. It therefore appears that proteins in the middle region depolymerized into lower molecular units during the extrusion process, which then appeared in the extruded products in the lower region. However, examination of Figure 4.3 shows that samples extruded at the highest screw speeds did not show the "disappearance-appearance" pattern described, which might indicate that a combination of high shearing forces and temperatures might have provided alternate formation of new disulfide bonds through interchange reactions or by additional cystine formation from cysteine. Thus, depolymerization of proteins was followed by protein aggregation, especially at higher shear forces.

The absence of any visible differences between extruded and non-extruded samples, and among all extruded samples, in the high molecular weight protein region (glutenins) suggests that the native intermolecular disulfide cross-links remained unaltered by extrusion, and that extrusion even at the higher temperature levels used in this study did not significantly affect disulfide bonds. Alternatively, the

proteins might have aggregated through inter- and intra-molecular disulfide cross-linking after extrusion indicating that disulfide bonds still play a major role in the protein structure after extrusion under the conditions studied.

The marked differences between SDS-PAGE patterns of reduced and non-reduced samples and the decrease in protein disulfide content after extrusion are in part inconsistent with thiol-disulfide interchange reactions. According to Koh et al (1996) the characteristics of such interchange reactions include net increase in protein SH, net reduction in cross-linking, and no increase or decrease in disulfide bonds. Thus, reduction with 2-ME produces no change on SDS-PAGE patterns under such conditions. The fact that net protein disulfide content decreased and the SH content increased with extrusion in the present study indicates that extrusion might have resulted in shear-mediated scission of disulfide bonds. This observation together with changes in SDS-PAGE patterns suggest that thiol-disulfide interchange had only a minor effect under the extrusion conditions used in this study. Possible explanation of the absence of thiol-disulfide interchange reaction may be the low water contents and very short reaction times of extrusion. Studies in doughs have shown that the thiol-disulfide interchange reactions require longer periods of mixing time and also require sufficient water (Stewart and Mauritzen 1966, Jones and Carnegie 1971).

4.5 SUMMARY

Extrusion processing of wheat flour resulted in the decrease in protein disulfide content and an increase in the free sulfhydryl content while total cysteine content remained fairly unchanged. SDS-PAGE patterns of the total proteins showed marked differences between reduced and non-reduced samples indicating that there was very little or no thiol-disulfide interchange reaction during extrusion processing under the conditions of this study, and that the intramolecular scission of disulfide bonds seemed to be the predominant occurrence during the extrusion process. Intramolecular fragmentation of proteins, as evidenced by SDS-PAGE patterns, led to the formation of low molecular weight protein aggregates. This information should be helpful in determining the relationship between disulfide and free sulfhydryl contents of an extruded product and the textural characteristics of the extrudates.

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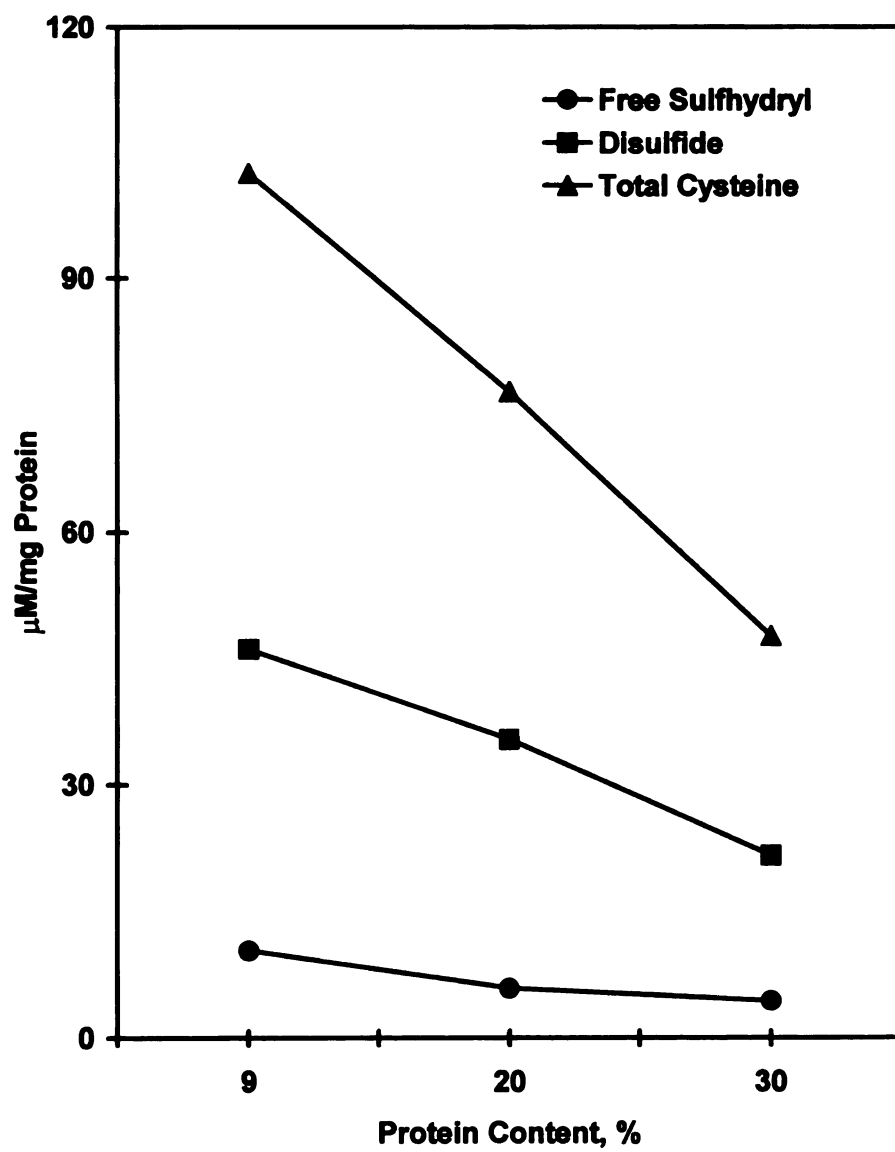


Figure 4.1

Variation in free sulfhydryl, disulfide, and total cysteine contents in non-extruded wheat flour samples of varying protein content.

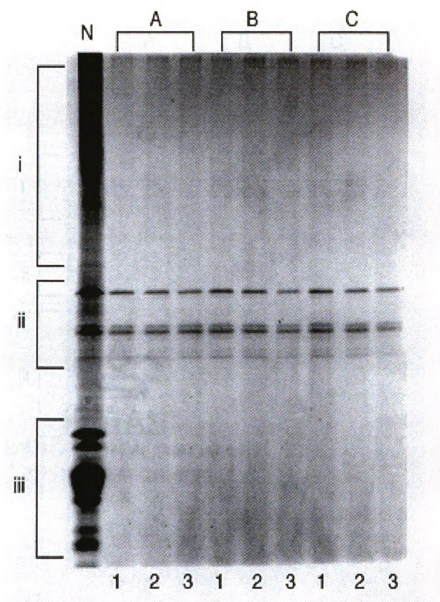


Figure 4.2

SDS-PAGE patterns of non-reduced total protein of extruded wheat flour containing 9% protein.

N = non-extruded flour sample.

Screw Speeds: A = 240 rpm; B = 320 rpm; C = 400 rpm.

Die Temperatures: 1 = 120°C; 2 = 140°C; 3 = 160°C.

i, ii, iii: see text for explanation.

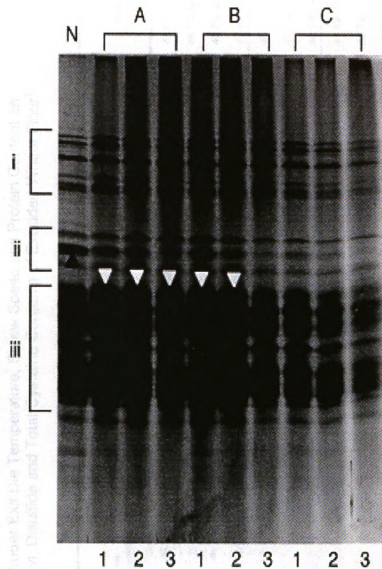


Figure 4.3

SDS-PAGE patterns of reduced total protein of extruded wheat flour containing 9% protein.

N = non-extruded flour sample.

Screw Speeds: A = 240 rpm; B = 320 rpm; C = 400 rpm.

Die Temperatures: 1 = 120°C; 2 = 140°C; 3 = 160°C.

i, ii, iii: see text for explanation.

Table 4.1

Effects of Extruder Exit Die Temperature, Screw Speed and Protein Content on Free Sulfhydryl, Disulfide and Total Cysteine Contents of Extruded Wheat Flour¹

Die Temperature (°C)	Screw Speed (rpm)	9% Protein			20% Protein			30% Protein		
		Free Sulfhydryl	Disulfide	Total Cysteine	Free Sulfhydryl	Disulfide	Total Cysteine	Free Sulfhydryl	Disulfide	Total Cysteine
Non-extruded		10.43(.31)	46.04(.11)	102.51(.69)	5.90(.18)	35.35(.40)	76.62(.14)	4.37(.12)	21.62(.07)	47.60(.17)
120		6.95(.17)	38.36(.12)	83.66(.15)	3.58(.11)	36.35(.24)	76.27(.37)	2.79(.01)	22.70(.05)	48.18(.12)
140	240	9.78(.12)	36.84(.11)	83.44(.10)	5.00(.14)	32.96(.09)	70.91(.33)	2.84(.06)	21.97(.15)	46.86(.35)
160		10.98(.14)	35.43(.05)	81.82(.14)	9.42(.04)	31.91(.02)	73.24(.01)	2.93(.03)	22.37(.08)	47.66(.20)
120		7.55(.42)	41.02(.04)	89.57(.50)	4.03(.11)	34.41(.09)	70.84(.08)	2.79(.01)	22.77(.25)	48.33(.25)
140	320	8.78(.28)	37.49(.12)	83.75(.49)	4.85(.07)	32.65(.30)	70.14(.68)	3.18(.01)	22.85(.04)	47.87(.08)
160		10.60(.21)	35.13(.22)	80.85(.23)	10.65(.21)	29.49(.27)	69.62(.33)	3.13(.04)	21.74(.26)	46.59(.35)
120		7.98(.25)	40.23(.02)	88.42(.29)	4.08(.18)	33.41(.21)	70.89(.24)	3.38(.12)	20.66(.35)	44.68(.17)
140	400	9.08(.14)	37.50(.25)	84.07(.47)	5.20(.14)	32.89(.23)	70.98(.60)	3.30(.03)	21.75(.21)	46.79(.41)
160		13.29(.27)	37.73(.28)	88.73(.23)	9.93(.11)	28.41(.10)	66.74(.07)	3.36(.08)	21.93(.28)	47.21(.48)

¹Data are mean (standard deviation) of at least two replicates, and are reported in $\mu\text{M}/\text{mg}$ protein.

Table 4.2

Statistical F-Values for Analyses of Free Sulfhydryl, Disulfide and Total Cysteine Contents in Wheat Flour Extrudates

Source	df	F-Value		
		Free Sulfhydryl	Disulfide	Total Cysteine
Screw speed	2	63.34***	29.79***	6.63***
Temperature (Temp)	2	2183.09***	955.43***	154.40***
Screw speed x Temp	4	20.27***	43.20***	39.96***
Protein (Prot)	2	6936.16***	31980.65***	47513.58***
Screw speed x Prot	4	23.58***	183.36***	202.98***
Temp x Prot	4	612.06***	265.85***	55.16***
Screw speed x Prot x Temp	8	25.64***	47.92***	76.23***
R ²		0.998	0.999	0.999

*** p≤0.001

CHAPTER 5

EFFECTS OF TWIN-SCREW EXTRUSION PROCESSING ON STRUCTURAL AND CHEMICAL PROPERTIES OF THE MAJOR WHEAT FLOUR COMPONENTS AS MEASURED BY FLUORESCENCE SPECTROSCOPY AND DIFFERENTIAL SCANNING CALORIMETRY

5.1 ABSTRACT

The effect of twin-screw extrusion processing on the structural and chemical changes in the starch and protein components of wheat flour were studied using differential scanning calorimetry (DSC) and fluorescence spectroscopy (FS). Much higher temperatures for thermal transitions were observed for starch than are normally associated with starch gelatinization in DSC. The formation of amylose-lipid complex has been suggested for such levels of transition enthalpy changes. DSC data further suggest that extrusion promoted the exothermic aggregation process between amylose and lipid. Increase in protein content of raw materials increased transition peak temperatures, T_m , and decreased transition thermal energy, ΔH , in non-extruded samples. The addition of vital gluten therefore increased the stability of the system and conferred hydrophobic properties on the system, thus reducing the net favored ΔH . Fluorescence spectroscopy data indicated changes in the conformational structure of wheat proteins consequent to extrusion processing. All extrudates showed higher peak fluorescence emission wavelengths, λ_{max} , than non-extruded samples. Increasing extruder die temperatures resulted in changes in the hydrophobic environment of proteins as measured by fluorescence intensity (FI) and changes in the maximum fluorescence emission peak wavelengths.

5.2 INTRODUCTION

Cereal starches are generally transformed into melts through gelatinization. However, Qu and Wang (1994) have reported that the transformation of starch can also be achieved under conditions of high shear rate, high temperature and high pressure such as are found during extrusion cooking. The phase transition temperatures and energies associated with such transformation are known to be affected by additives such as sodium chloride; thus information derived from thermal studies can be used to determine processing conditions and type and quantity of additives used in formulated-extruded food systems.

Unfortunately, studies conducted with the aim of elucidating information on transition phases of cereals are mostly done with pure starches or extracted protein fractions. Phase transitions in pure starches and fractionated proteins of several cereals, as they undergo heat-induced thermal events, have been studied using differential scanning calorimetry (DSC) following the pioneering work of Stevens and Elton (1971). The DSC can provide both qualitative and quantitative information in terms of transition temperatures and heat of transition, respectively, of the heating process. Eliasson (1983) studied the effect of gluten on the gelatinization of wheat starch using DSC. Biliaderis et al (1986a,b) have conducted DSC studies on the multiple melting transitions of rice starch/monoglyceride systems (a), and the thermal characterization of rice starches (b). Thermal properties of heat-moisture-treated wheat and potato starches (Donovan et al 1983), oat globulin (Harwalker and Ma 1987), thermal stability of wheat gluten (Eliasson and Hegg 1980), and the phase transitions of amylose-lipid complexes in wheat, maize, and potato starches

(Kugimiya et al 1980) have all been studied using DSC.

Information on more complex systems, however, such as wheat flour which contains both starch and proteins in addition to other components, is limited. Therefore, differential scanning calorimetry and fluorescence spectroscopy were used in the present studies to investigate phase transitions and changes in surface hydrophobicity, respectively, of wheat flour components due to extrusion processing.

5.3 MATERIALS AND METHODS

5.3.1 Extrusion

Extrusion processing conditions and materials used were described in Chapter 3 of this document.

5.3.2 Differential Scanning Calorimetry (DSC)

A Dupont 2920 DSC unit (Dupont, Wilmington, DE), calibrated with indium for temperature and enthalpy, was used to analyze phase transition peak temperatures (T_m) and enthalpy changes (ΔH) associated with the transition change for extruded and non-extruded samples. Approximately 10 mg of non-extruded flour or ground extrudate samples were weighed and sealed in an aluminum sample pan (TA Instruments, Newcastle, DE) by an encapsulating press. The DSC cell was flushed with nitrogen at 50 ml/min to maintain an inert environment during the measurements. Thermograms (plots of differential heat flow as a function of temperature) were obtained at a heating rate of 10°C/min, from 20 to 250°C. For

each thermogram, T_m and ΔH values were obtained using the General V4.1C data acquisition software program which controlled the DSC unit.

5.3.3 Fluorescence Spectroscopy

The hydrophobicity of total proteins in extruded and non-extruded samples was determined using steady-state fluorescence measurements of total proteins by interacting the proteins with the extrinsic fluorescence probe 1-anilino-8-naphthalene sulfonic acid (ANS). Flour from non-extruded and ground extruded samples were extracted with 20 ml of acetic acid-urea buffer (3mM urea in 0.1M acetic acid). Samples were varied to obtain equal protein concentrations of 10 mg/ml. The mixture was stirred for 1 hr and centrifuged at 13,000 x g for 20 min at room temperature. Ten μ l of ANS (8 mM in 0.1M phosphate buffer, pH 6.9) were then added to 2 ml of sample supernatant. Relative fluorescence intensities were measured with a SLM 4800 spectrophotometer (Urbana Champaign, IL) connected to a data acquisition and operating system from On-Line Instruments Systems (Bogart, GA). Measurements were made in duplicate in 1 cm pathlength semi-micro quartz fluorescence cuvettes held in a thermostable block maintained at 22°C. The emission spectra of supernatants were measured between 400 and 600 nm at an excitation wavelength of 390 nm (excitation wavelength of ANS). Slit width for both excitation and emission was 4 nm.

The relative fluorescence intensity (FI), measured as the height of the emission maximum, and the shift in the wavelength of maximum emission (λ_{max}), were recorded and used as indices of change in hydrophobic or polar environments (Strasburg and Ludescher 1995) of wheat total proteins.

5.4 RESULTS AND DISCUSSION

5.4.1 Thermal Transitions

The effect of protein content of raw extruder feed on the transition temperatures, T_m , and energies associated with phase change, ΔH , in non-extruded samples are shown in Figure 5.1. Increasing the protein content of raw feed material increased the transition peak temperature while, conversely, the energy associated with the phase change decreased. Increasing protein level therefore has the effect of increasing the exothermic component of the overall enthalpy. Protein aggregation is an exothermic reaction that contributes negatively to the overall transition enthalpy, ΔH (Myers 1990). The addition of vital gluten protein to wheat flour conferred more hydrophobic properties to the raw material thus decreasing the net ΔH values and increasing overall stability, as evidenced by the increases in T_m upon increasing the protein content of the raw material. Eliasson (1983) found similar decreasing effect of gluten on wheat starch gelatinization enthalpy and increasing effect on the gelatinization temperature. It was assumed that the increase in T_m on addition of gluten may be due to a delay in diffusion of water into the starch granules because of the presence of gluten on the surface of the starch granules, since higher starch gelatinization temperature in wheat flour compared to the isolated starch has been observed (Olkku and Rha 1978). Since the T_m values reported in the present study (111-125°C) are much higher than the gelatinization temperature range of starch (60-80°C), the increasing effect of protein content on T_m can be assumed to be due to decreasing amount of water available for the starch, as reported by Eliasson (1983).

Table 5.1 shows the T_m and ΔH values for extruded and non-extruded samples, containing 9% protein, with some representative thermograms shown in Figures 5.2 and 5.3, respectively. The DSC thermograms give information on both the temperature and heat required for thermal transitions. The ΔH , at the molecular level, involves the cleavage of existing hydrogen bonds between starch molecules, and the formation of new ones with water molecules. The result is a less-ordered structure with increased entropy, hence the overall process is endothermic (Stevens and Elton 1971).

For the extruded samples with 9% protein content, transition endotherm peaks were observed in the range 112.62-125.34°C. In general, increasing extruder die temperatures decreased T_m values. However, the T_m value for the non-extruded sample (111.21°C) was lower than the values for all extrudates containing 9% protein. Such increase in T_m values, according to Harwalker and Ma (1987), would indicate an increase in the heat stability of extruded samples relative to non-extrudates, possibly due to strengthening of the amylose-lipid complex. The extruded samples thus required relatively higher amounts of energy for thermal transition as evidenced by the higher T_m values. However, within the extruded samples, die temperature increases reduced the stability of samples and weakened the lipid complex formation of the extruded amylose molecules as seen by decreasing T_m values. It therefore appears that extrusion processing increased the stability of extruded samples relative to non-extrudates, but stability among extruded samples varied depending on the range of extrusion temperature used, with higher temperatures tending to reduce stabilities.

Based on the assumption that the thermal transitions occurring in starch at higher temperatures between 100-140°C are due to the presence and disorganization of amylose-lipid complexes, as stated earlier, the data for the present study would indicate that extrusion promoted the complexation of amylose with lipid, but the stability of the formed complex was subsequently weakened by increasing the exit die temperature in extrusion processing. Biliaderis (1990) reported that the formation of the amylose-lipid complex is an intermolecular aggregation process which is exothermic, which agrees with the interpretation of the T_m data for extruded and non-extruded samples in the present study.

The ΔH values generally increased with increasing die temperature at constant screw speed, while varying screw speed at constant die temperatures did not show any specific pattern of ΔH values. However, in contrast to T_m , the ΔH values for the non-extruded sample (9% protein) were higher than that of all extrudates containing 9% protein. This suggests that in converting the raw materials into an extrusion-cooked product, there was an increase in the exothermic (negative) component of the enthalpies of reaction, at least in the samples containing the lowest amount of protein, since ΔH is a combination of endothermic reactions, such as hydrogen bond disruption, and exothermic reactions including aggregation (hydrogen bond formation) and the breakup of hydrophobic interactions (Privalov and Khechinashvili 1974, Evans et al 1979). This also further supports the T_m data to the effect that the lowering of the ΔH in extrudates corresponds to the formation of amylose-lipid complexes. Biliaderis (1990) reported lower ΔH values for high lipid content starches which reflected an exothermic amylose-lipid complexation. Camire et al (1990) noted that lipid-complexed starch has a more

narrow gelatinization temperature than untreated starch, and has a lower enthalpy of gelatinization that may give it valuable functional qualities.

The data for extruded samples containing 20 and 30% protein showed some inconsistencies for both ΔH and T_m values (data not shown), perhaps due to high gluten protein, and therefore were inconclusive within the extrusion parameters studied. As reported in the literature, gluten proteins do not give good ΔH or T_m values. Nevertheless, the enthalpy value for the non-extruded 30% protein sample was higher than those of the extrudates, which is similar to the observation for the 9% protein sample.

5.4.2 Fluorescence Spectroscopy

Emission peak wavelengths (λ_{max}) and relative steady-state fluorescence intensities (FI) of extruded and non-extruded samples are given in Table 5.2. All extruded samples showed higher wavelengths of maximum emission than non-extruded samples. For the 9% protein content sample, peak wavelengths varied between 493.0 and 511.5 nm for the extrudates compared to 482.5 nm for the non-extruded sample. The 20% protein content sample showed a maximum emission variation between 490.5 and 500.0 nm for extrudates and of 489.5 for non-extruded samples, and similarly, peak emission wavelengths for the 30% protein content non-extruded samples shifted from 482.0 nm to between 491.5 and 502.0 nm after extrusion.

Data for fluorescence intensities showed opposite trend to the emission wavelength data. The relative fluorescence intensities for the non-extruded samples were all several magnitudes higher than those of extruded samples. The shift in the

emission wavelengths to higher values (i.e., red shift) and the decrease in relative fluorescence intensities in extruded samples reflect a change in the conformation of the proteins after extrusion. Yeboah et al (1994) reported an increase in wavelength of maximum emission for denatured, and reduced/alkylated wheat gliadin proteins relative to the native proteins.

The position of the maximum in the emission spectrum (λ_{max}) and the fluorescence intensity are sensitive to the polarity of the environment. An increase in fluorescence intensity occurs only when the ANS interacts with a nonpolar group on the surface of the protein (Javor et al 1991). Increases in peak wavelengths and decreases in fluorescence intensities are indicators of polar (hydrophilic) environment, and conversely, decreases in emission wavelengths and increases in fluorescence intensities reflect non-polar (hydrophobic) environment (Javor et al 1991, Lakkis and Villota 1992, Yeboah et al 1994). Thus, the present data indicate that extruded samples were in a comparatively more polar environment than the non-extruded samples. One possible explanation for this observation is that extrusion probably led to aggregation of proteins which buried the hitherto exposed hydrophobic sites on the protein surface thereby making them inaccessible to the ANS. The observed decreases in enthalpies (ΔH) of extrudates relative to non-extruded samples support the aggregation theory since aggregation is an exothermic process which contributes negative value to the overall ΔH of a reaction.

As shown in Figure 5.4, increasing extruder die temperature at constant screw speeds shifted the peak emission to lower wavelength (i.e., blue shift) for all extruded 9% protein content samples. At the same time, there appeared to be slight increases in the relative fluorescence intensities. Increases in fluorescence

intensities and blue shifts in maximum emission wavelengths are indicators of decreases in polarity (i.e., increased hydrophobicity) of the environment (Strasburg and Ludescher 1995). This suggests that within the extruded samples, temperature increases favored the unfolding of proteins thus exposing hydrophobic sites in the proteins to ANS binding. Such a change in the conformation of the proteins probably leads to an increased number of hydrophobic sites on the surface of the protein which also results in aggregation. Javor et al (1991) observed that if aggregation occurs after ANS binding and holds the ANS in a hydrophobic interaction environment, the nonpolar environment produces an extra increase in fluorescence intensity, as was reflected in the present data. In general, the surface hydrophobicities of proteins are increased by heating due to increased exposure of hydrophobic groups at the outer surface of the molecule (Tanford 1980, Matsudomi et al 1982, Voutsinas et al 1983).

5.5 SUMMARY

The effect of temperature, screw speed and protein content on the maximum emission wavelengths and relative fluorescence intensities for extruded samples were difficult to discern due to absence of any clear and definite pattern among the 20 and 30% protein content samples. Nevertheless, for the 9% protein content sample, exit die temperature had a decreasing effect on the peak emission wavelengths at all screw speed levels, while increasing screw speed at each temperature level appeared to increase the emission maximum.

The DSC and FS data reported in this study indicate that there were some

conformational changes in the starch and proteins of wheat flour as a result of twin-screw extrusion processing. DSC data suggest the formation of a complex between amylose and lipid in the wheat. This is an exothermic aggregation process that leads to a decrease in the overall thermal energy of the process. Furthermore, fluorescence data suggest that proteins in the extruded products may be in a more polar environment than those in the non-extruded products. Protein aggregation (hydrogen bond formation) prevents the exposure of hydrophobic sites on the surface of the protein and thus prevents their contact with ANS. However, for all extruded products in this study, higher temperatures seemed to unfold the proteins and expose the hydrophobic core of the proteins to ANS binding.

It seems probable that the formation of amylose-lipid complex and protein aggregation, under the present extrusion conditions, are responsible for the initial decreased access of ANS to hydrophobic sites in the protein in extruded samples. However, hydrophobicities increased (decreasing λ_{max}) as die exit temperature was increased for all extruded products containing 9% protein. Even though the same observation cannot be made for samples at higher protein levels, it appears, nevertheless, that higher extruder exit die temperatures possibly lead to the weakening of the amylose-lipid complex and disruption of hydrogen bonds which permit the unfolding of the proteins with a resultant exposure of more hydrophobic sites to ANS binding.

The information obtained from this study suggests that DSC and FS may be useful techniques for studying conformational changes in the major components of wheat after extrusion processing of wheat flour. DSC is capable of providing both qualitative and quantitative information, though limited to flour samples of

comparatively high starch and protein concentrations. Such information may include the aggregation of the proteins as well as the interaction of starch components with other minor components of the flour.

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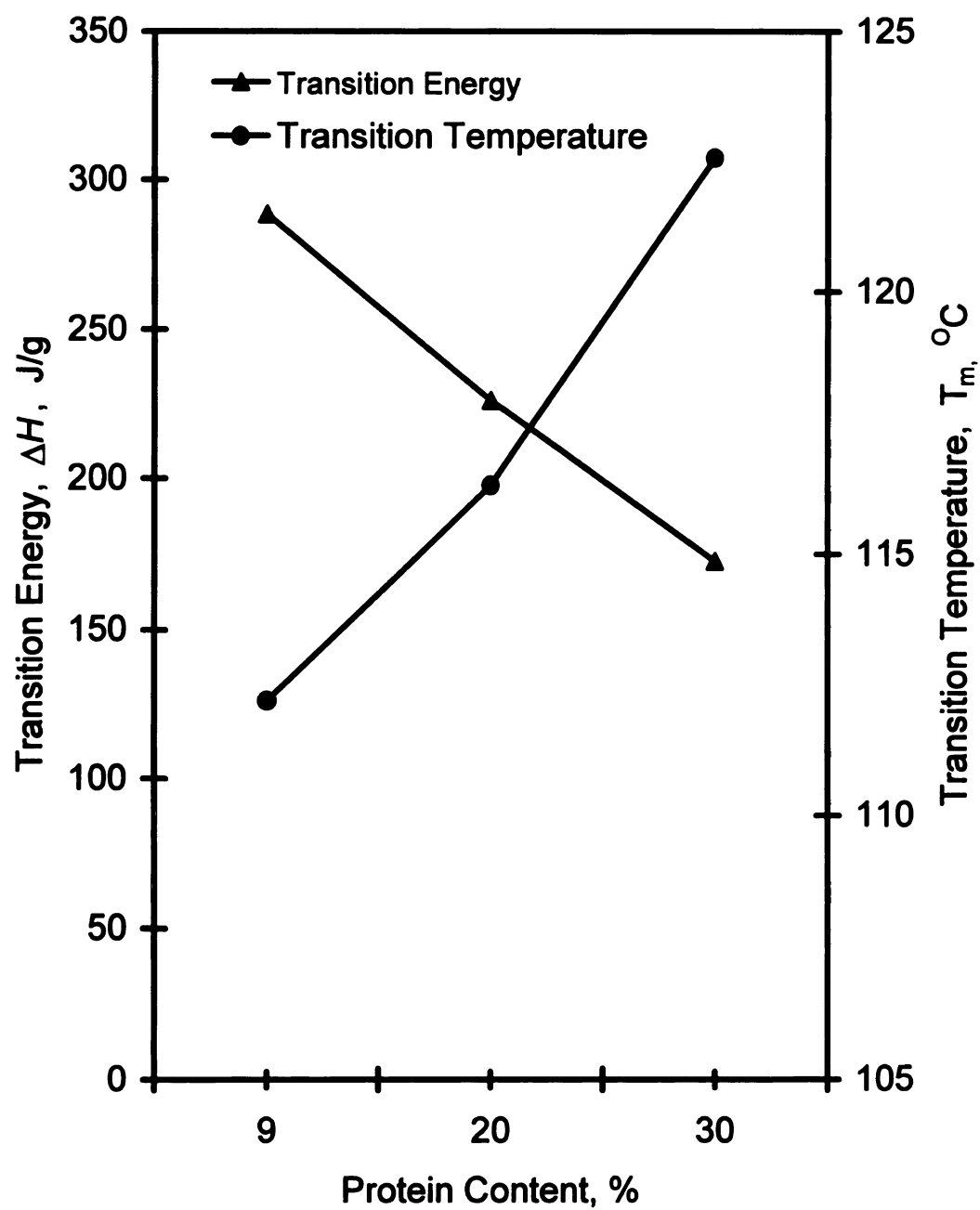


Figure 5.1

Endothermic thermal transition energy and temperature changes of non-extruded wheat flour as a function of protein content of flour.

Figure 5.2

**Representative differential scanning thermograms of non-extruded wheat flour.
(a) = 9% protein content; (b) = 20% protein content; (c) = 30% protein content.**

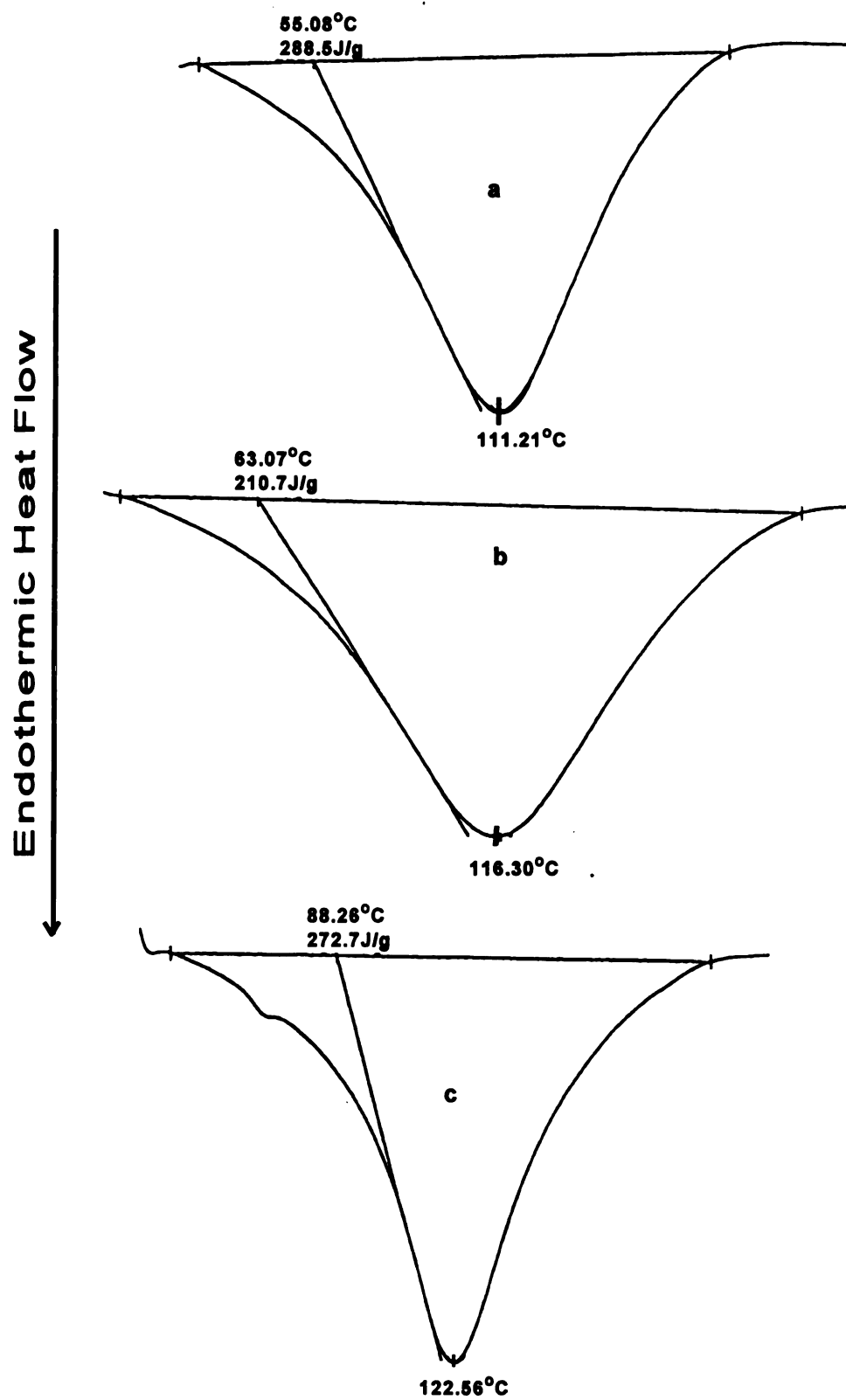
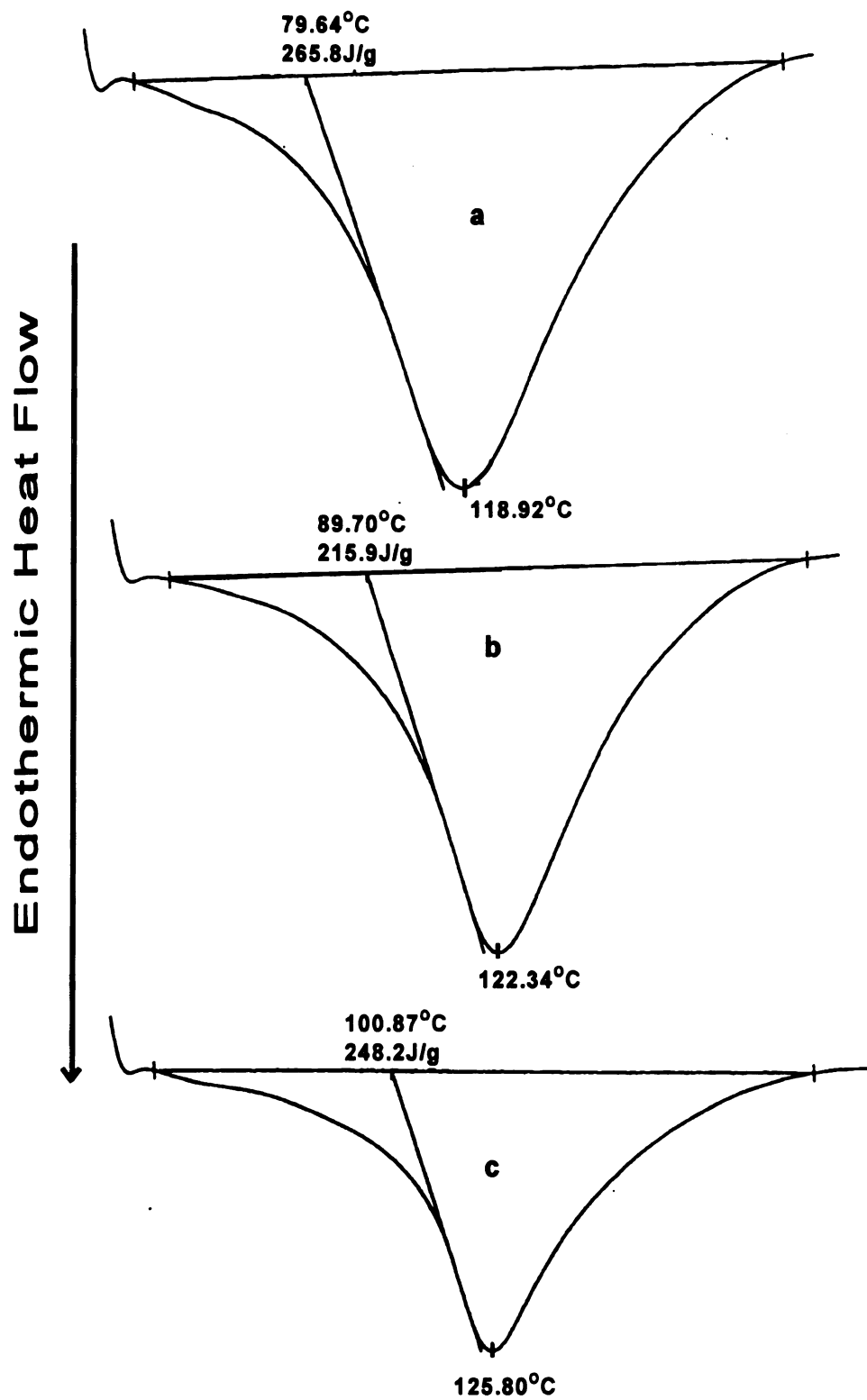


Figure 5.3

Representative differential scanning thermograms of wheat flour extruded at die temperature of 120°C and screw speed of 400 rpm.

(a) 9% protein content; (b) 20% protein content; (c) 30% protein content.



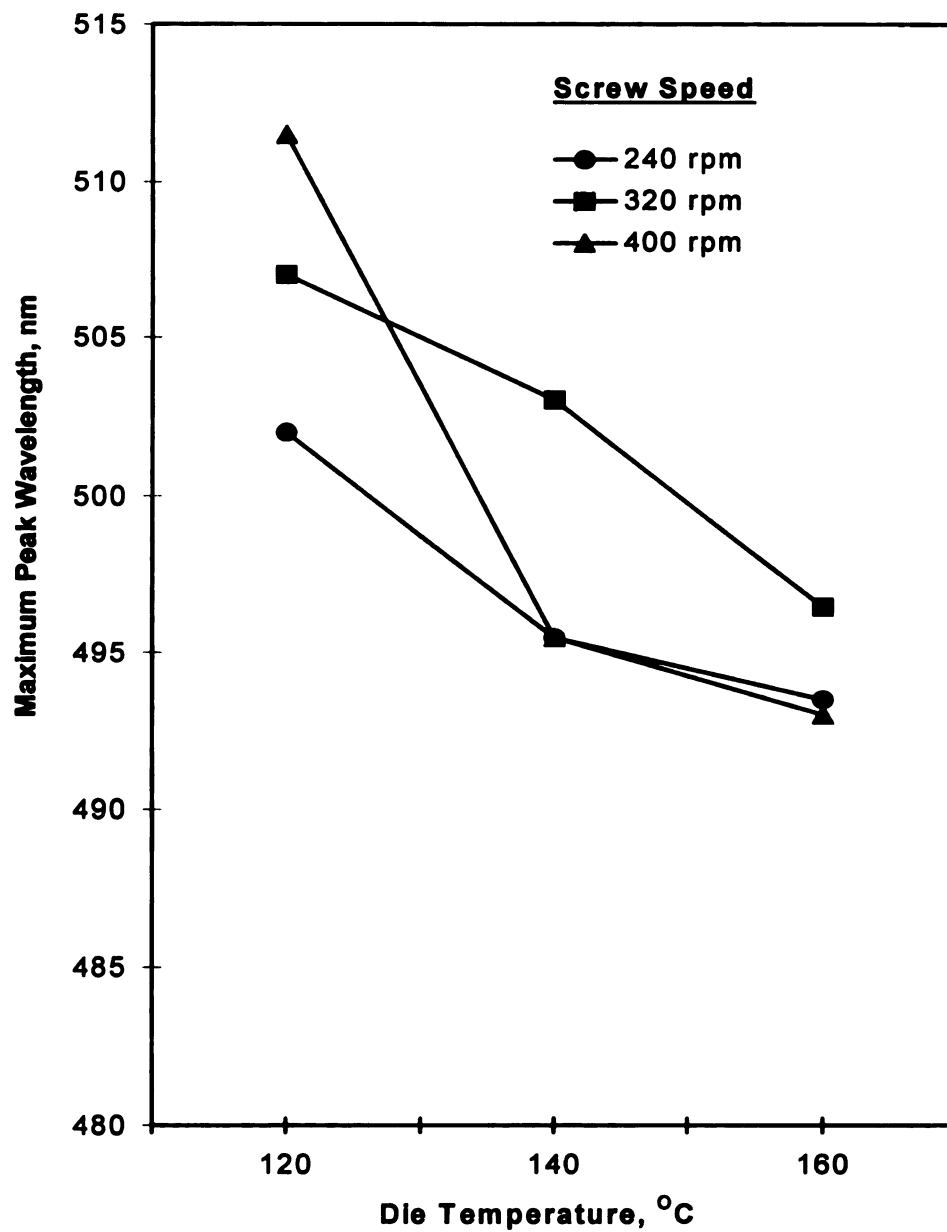


Figure 5.4

Effects of extruder die temperature on the shifts in fluorescence emission peak wavelengths of extruded wheat flour containing 9% protein.

Table 5.1

Endothermic Reaction Temperatures and Enthalpies of Extruded and Non-Extruded Wheat Flour Containing 9% Protein¹

		Protein Content 9%	
Exit Die Temperature °C	Screw Speed (rpm)	T_m (°C)	ΔH (J/g)
Non-Extruded		111.21	288.5
120	240	125.34	187.2
140		122.64	215.0
160		119.85	260.5
120	320	122.49	267.5
140		118.66	279.8
160		115.02	284.9
120	400	118.92	265.8
140		113.98	271.7
160		112.62	278.5

¹Data are mean of two replicates.

Table 5.2

Peak Wavelengths and Fluorescence Intensities of Extruded and Non-extruded Wheat Flour¹

		Protein Content					
		9%		20%		30%	
Die Temperature (°C)	Screw Speed (rpm)	Peak Wavelength (λ nm)	Fluorescence Intensity (A.U.) ²	Peak Wavelength (λ nm)	Fluorescence Intensity (A.U.) ²	Peak Wavelength (λ nm)	Fluorescence Intensity (A.U.) ²
Non-extruded		482.5	0.631	489.5	0.856	482.0	0.924
120		502.0	0.050	495.0	0.055	491.5	0.057
140	240	495.5	0.051	500.0	0.046	496.5	0.049
160		493.5	0.055	495.5	0.056	498.5	0.049
120		507.0	0.047	494.0	0.049	495.0	0.053
140	320	503.0	0.053	498.5	0.054	502.0	0.045
160		496.5	0.049	492.5	0.070	495.5	0.052
120		511.5	0.047	495.5	0.053	494.5	0.047
140	400	495.5	0.046	499.0	0.056	499.0	0.044
160		493.0	0.081	490.5	0.090	494.0	0.057

¹Data are mean of two replicates.²Arbitrary Units.

CHAPTER 6
GENERAL CONCLUSIONS

Extrusion processing has become one of the important new technologies that is assuming an increasing role in the food industry in an effort to provide an unprecedented array of novel products to satisfy consumer preferences, especially in the ready-to-eat cereal market. In spite of the apparent success of the process, fundamental understanding of the behavior of ingredients in the extrusion raw materials is lacking, and therefore needs research attention. This research project assessed the impact of extrusion processing on the functionality and physical characteristics of the major components of wheat flour in an attempt to gain some fundamental information and understanding on the physical and biochemical processes that occur during extrusion processing of cereals.

In **Chapter 3**, the role of increased protein level in extrusion raw material and how this protein augmentation, together with other extrusion variables, influenced the physical characteristics of the extruded product was investigated. The level of protein content in the feed material was found to have negative correlation with expansion ratio and water absorption index, but positive correlation with bulk density. The expansion ratio of extrudates was also found to correlate negatively with bulk density but positively with water absorption index. Similarly, bulk density correlated negatively with water absorption index. These results suggest that the protein content of raw material, water absorption index, and bulk density are all related to the expansion characteristics of extruded products.

The changes in total protein patterns as well as the possible interchanges between the disulfide and sulfhydryl contents of wheat flour proteins as a result of extrusion were examined in **Chapter 4**. Extrusion processing of wheat flour resulted in the reduction of protein disulfide bonds to form free sulfhydryl groups while total

cysteine content remained fairly unchanged. Marked differences between SDS-PAGE patterns of reduced and non-reduced total protein samples were observed. Observed data suggested that there was very little or no sulfhydryl-disulfide interchange reaction during extrusion processing, and that the intra molecular scission of disulfide bonds seemed to be the predominant occurrence during the extrusion process. This information should be helpful in determining the relationship between disulfide and free sulfhydryl contents of an extruded product and the textural characteristics of the extrudates.

Data from differential scanning calorimetry and fluorescence spectroscopy studies in **Chapter 5** indicate that there were some conformational changes in the starch and proteins of wheat flour as a result of twin-screw extrusion processing. Differential scanning calorimetry data suggest the formation of a complex between the amylose and lipid contents in the wheat flour. This reaction is an exothermic aggregation process that leads to a decrease in the overall thermal energy of the process. Furthermore, fluorescence data suggest that extruded products may be in a more polar environment than non-extruded products.

Like many other studies in extrusion processing, data obtained from this study have established that extrusion is a complex process which involves several variables, each of which contributes differently under different sets of extrusion conditions to the overall quality characteristics of the extrudates. The interactions among variables are complex, but have significant impact on quality attributes of extruded products, and make it challenging to differentiate among the individual variables in terms of changes in characteristics of the final product. Nevertheless, the results of this study add to the pool of available knowledge on the subject of

extrusion processing of cereals and further provide evidence that, continuous research in this area will be needed to fully understand the phenomena of extrusion processing as they apply to cereals, and how fundamental knowledge can be transformed into improvement of current products and the design of new ones.

The significant findings of this research can be summarized as follows:

- **The protein content in extrusion raw materials has significant effects on the water absorption and bulk density of extrudates, which in turn, are all related to the expansion characteristics of the extrudates.**
- **High protein content in wheat flour reduces expansion capability of wheat flour extrudates and their ability to absorb water. Thus, in extrusion of wheat flour, supplementation of the protein content of the flour may not be beneficial to improving the quality characteristics of the extruded products.**
- **Extrusion of wheat flour results in the reduction of disulfide bonds to form free sulfhydryl groups. The total cysteine content is not changed during extrusion processing, and intra-molecular scission of disulfide bonds appears to be the predominant occurrence. This information can be helpful in determining the relationship between and among the total cysteine, disulfide and sulfhydryl contents of wheat flour and the textural characteristics of the extrudates.**
- **Extrusion processing results in protein aggregation which increases the stability of the proteins in addition to the apparent increase in hydrophilic (polar) environment of proteins after extrusion.**

CHAPTER 7
FUTURE RESEARCH

Extrusion technology has many present and possible future applications in the food industry for cooking, forming, and expanding cereal grains, as well as for texturizing proteins. There is, however, still much to understand with extrusion processing and its impact on the components of the starting raw ingredients, and their effects on the quality of the extruded products. Certainly, extrusion processing will play a much broader role in both traditional and non-traditional products in the future. Understanding the mechanisms of ingredient interactions will be the key in this direction. Thus, future work should aim at gaining more understanding of ingredient interactions. Research will be needed to give accurate estimation of gelatinization and melting during extrusion processing.

The role of protein in extrusion technology will need much more focus in view of the current controversy about the involvement of disulfide bonds in extrusion processing. Furthermore, there is the need for more understanding of the role disulfide bonds play with respect to the texture and quality of extruded cereal products. More specifically, research will need to determine the exact relationship between disulfide and sulfhydryl contents of cereal proteins and the exact mechanism by which they are involved in the texturization process.

In view of the success of extrusion of soy protein, and considering the functional properties of vital gluten, it should be feasible to subject vital gluten to extrusion processing. Future research should therefore aim at gaining more understanding of the mechanism of vital gluten interactions with starch components and possibly the chemical and physical modifications of gluten.

Having established in the present research that extrusion operating variables have a direct impact on the quality of extrudates, future research work will need to

establish quantitatively the relationship between changes in molecular weights of proteins and starches and time, shear, and extrusion variables. Studies on modeling of extrusion parameters using response surface methodologies will be necessary to establish much more reliable relationships between dependent and independent variables. Such studies will also be useful in the upscaling of extrusion research from the laboratory into industrial processing conditions.

APPENDICES

APPENDIX 1

Composition and Farinogram Data¹ of Soft Wheat Flour (cv. Harus), Vital Gluten and Flour-Gluten Mixture Samples Used in Experiments

Component (%)	cv. Harus	Vital Gluten	Flour-Gluten Samples	
			20% Protein	30% Protein
Moisture	10.3	9.2	-	-
Protein (N x 5.7)	9.2	69.8	-	-
Ash	0.5	0.4	-	-
Fat	1.6	2.1	-	-
Carbohydrates ²	78.4	18.5	-	-
<u>Farinogram</u>				
Absorption (%)	52.2	nd	63.0	74.6
Arrival time (min)	0.5	nd	2.0	6.5
Peak time (min)	1.0	nd	7.75	9.0
Peak height (mm)	25	nd	48	69
Stability (min)	1.5	nd	29.0	> 30

¹Constant flour weight method.

²Determined by difference.

nd = not determined.

APPENDIX 2

Raw Data¹ for Extrusion Experiments

Temp	SS	Prot	Expansion Ratio		Bulk Density (g/ml)		Water Abs. Index (g/g)		Sulphydryl Content (μM/mg Prot)		Disulfide Content (μM/mg Prot)		Total Cysteine Content (μM/mg Prot)	
			1	2	1	2	1	2	1	2	1	2	1	2
120	240	9	3.15	3.20	0.132	0.130	6.16	6.14	7.00	6.90	38.28	38.43	83.55	83.76
140	240	9	2.76	2.90	0.125	0.122	6.24	6.16	9.55	10.00	36.91	36.76	83.37	83.51
160	240	9	1.80	1.95	0.163	0.164	6.91	6.70	10.95	11.00	35.39	35.46	81.72	81.92
120	320	9	3.16	3.05	0.124	0.120	5.88	5.86	7.85	7.25	41.04	40.99	89.92	89.22
140	320	9	2.66	2.55	0.129	0.132	5.72	5.82	8.60	8.95	37.40	37.57	83.40	84.09
160	320	9	1.83	1.80	0.159	0.153	6.32	6.16	10.45	10.75	35.28	34.97	81.01	80.68
120	400	9	2.55	2.63	0.116	0.119	5.45	5.55	8.15	7.80	40.24	40.21	88.62	88.21
140	400	9	2.16	2.05	0.134	0.130	5.79	5.83	9.10	9.06	37.32	37.67	83.73	84.40
160	400	9	1.80	1.75	0.132	0.129	6.07	5.93	13.10	13.47	37.90	37.55	88.89	88.56
120	240	20	1.98	2.07	0.339	0.331	5.50	5.41	3.65	3.50	36.18	36.52	76.00	76.53
140	240	20	2.50	2.52	0.164	0.165	5.57	5.65	4.90	5.10	32.89	33.02	70.67	71.14
160	240	20	2.93	2.92	0.090	0.090	5.46	5.51	9.45	9.40	31.89	31.92	73.23	73.24
120	320	20	2.33	2.42	0.242	0.237	5.92	5.43	3.95	4.10	33.47	33.34	70.89	70.77
140	320	20	2.48	2.53	0.143	0.145	5.50	5.32	4.90	4.80	32.86	32.43	70.62	69.66
160	320	20	2.89	2.98	0.074	0.074	5.28	5.07	10.80	10.50	29.30	29.68	69.39	69.85
120	400	20	2.66	2.76	0.190	0.189	5.43	5.49	3.95	4.20	33.56	33.26	71.06	70.72
140	400	20	2.40	2.37	0.149	0.147	5.31	5.11	5.10	5.30	32.73	33.05	70.55	71.40
160	400	20	2.71	2.71	0.097	0.094	5.08	4.97	9.85	10.00	28.47	28.35	66.79	66.69
120	240	30	2.15	2.30	0.184	0.181	4.23	4.32	2.78	2.80	22.66	22.73	48.09	48.26
140	240	30	2.10	2.15	0.209	0.209	4.40	4.45	2.98	2.90	22.07	21.86	47.11	46.61
160	240	30	2.15	2.20	0.218	0.214	4.50	4.39	2.95	2.91	22.43	22.31	47.80	47.52
120	320	30	2.20	2.15	0.233	0.235	4.48	4.55	2.78	2.79	22.59	22.95	47.96	48.69
140	320	30	2.15	2.25	0.183	0.183	4.44	4.52	3.18	3.17	22.87	22.82	48.92	48.81
160	320	30	2.20	2.10	0.194	0.194	4.59	4.55	3.15	3.10	21.92	21.55	46.98	46.19
120	400	30	2.10	2.13	0.202	0.204	4.76	4.63	3.46	3.29	20.41	20.90	44.27	45.08
140	400	30	2.10	2.11	0.218	0.218	4.58	4.76	3.32	3.28	21.89	21.60	47.10	46.48
160	400	30	2.26	2.10	0.185	0.174	4.64	4.66	3.30	3.41	22.13	21.73	47.55	46.87

Appendix 2, cont'd

Temp	SS	Prot	Specific Mechanical Energy (W h/kg)		Fluorescence Intensity (A.U.)		Peak Wavelength (nm)		Transition Temperature (°C)		Transition Energy (J/g)	
			1	2	1	2	1	2	1	2	1	2
120	240	9	144.00	143.80	0.05	0.05	502.00	500.00	125.34	125.32	187.20	188.90
140	240	9	153.60	150.00	0.05	0.05	495.50	497.00	122.64	121.60	215.00	214.20
160	240	9	122.40	123.80	0.06	0.05	493.50	493.50	119.85	118.99	306.50	307.10
120	320	9	208.00	209.00	0.05	0.05	507.00	510.00	122.49	124.00	267.50	267.00
140	320	9	208.00	210.20	0.05	0.06	503.00	502.50	118.66	118.45	289.80	288.40
160	320	9	179.20	180.40	0.05	0.05	496.50	496.00	115.02	114.70	284.90	284.50
120	400	9	268.00	266.00	0.05	0.04	511.50	510.00	118.92	117.82	265.80	265.20
140	400	9	260.00	263.20	0.05	0.05	495.50	494.50	113.98	113.01	288.70	289.00
160	400	9	240.00	239.00	0.08	0.08	493.00	493.50	112.62	112.44	278.50	277.40
120	240	20	144.00	143.00	0.06	0.06	495.00	496.50	121.63	120.99	181.70	182.20
140	240	20	139.20	139.00	0.05	0.05	500.00	502.00	109.20	109.21	212.10	213.00
160	240	20	132.00	135.00	0.06	0.05	485.50	484.50	124.59	123.88	222.80	221.00
120	320	20	195.20	195.00	0.05	0.05	494.00	493.00	116.62	116.32	157.00	157.50
140	320	20	192.00	192.20	0.05	0.06	498.50	498.00	115.20	115.43	207.30	207.30
160	320	20	176.00	177.80	0.07	0.07	492.50	490.50	101.59	100.47	205.40	205.60
120	400	20	240.00	239.00	0.05	0.05	495.50	494.50	122.34	123.00	215.90	215.30
140	400	20	220.00	222.20	0.06	0.06	499.00	498.50	111.68	110.98	204.10	204.90
160	400	20	240.00	239.00	0.09	0.09	490.50	490.00	125.77	124.65	257.20	258.20
120	240	30	144.00	145.80	0.06	0.06	491.50	490.00	126.18	126.77	218.50	217.90
140	240	30	139.20	139.00	0.05	0.05	496.50	495.50	127.90	127.43	222.90	223.10
160	240	30	127.20	129.00	0.05	0.05	498.50	498.00	124.48	125.04	214.40	213.80
120	320	30	192.00	192.20	0.05	0.05	495.00	494.00	140.21	141.21	201.80	200.60
140	320	30	176.00	174.00	0.05	0.04	502.00	500.00	121.77	120.94	242.20	243.10
160	320	30	176.00	177.00	0.05	0.05	495.50	495.00	124.67	125.00	239.40	239.10
120	400	30	220.00	219.20	0.05	0.05	494.50	496.00	125.80	125.32	248.20	249.00
140	400	30	220.00	222.00	0.04	0.04	499.00	498.50	129.33	129.67	224.20	223.60
160	400	30	212.00	212.20	0.06	0.06	494.00	493.50	126.66	126.00	233.20	233.00

¹Abbreviations and symbols: A.U., arbitrary units; Temp, exit die temperature (°C); SS, screw speed (rpm); Prot, protein content (%); 1,2, replicates.

APPENDIX 3

Analyses of Variance Tables for Experimental Data¹

I. Expansion Ratio

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SS	2	0.20979259	0.10489630	27.56	0.0001
TEMP	2	0.42789259	0.21394630	56.22	0.0001
SS*TEMP	4	0.13680741	0.03420185	8.99	0.0001
PROT	2	1.51922593	0.75961296	199.61	0.0001
SS*PROT	4	0.56877407	0.14219352	37.36	0.0001
TEMP*PROT	4	4.29790741	1.07447685	282.34	0.0001
SS*TEMP*PROT	8	0.63099259	0.07887407	20.73	0.0001
Error	27	0.10275000	0.00380556		
Total	53	7.89414259			

R-Square: 0.986984

C.V.: 2.586147

II. Bulk Density

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SS	2	0.00471693	0.00235846	343.28	0.0001
TEMP	2	0.02381404	0.01190702	1733.10	0.0001
SS*TEMP	4	0.00395230	0.00098807	143.82	0.0001
PROT	2	0.04184293	0.02092146	3045.17	0.0001
SS*PROT	4	0.00529074	0.00132269	192.52	0.0001
TEMP*PROT	4	0.06506430	0.01626607	2367.57	0.0001
SS*TEMP*PROT	8	0.01570137	0.00196267	285.67	0.0001
Error	27	0.00018550	0.00000687		
Total	53	0.16056809			

R-Square: 0.998845

C.V.: 1.570764

III. Water Absorption Index

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SS	2	0.37993704	0.18996852	17.31	0.0001
TEMP	2	0.09601481	0.04800741	4.37	0.0226
SS*TEMP	4	0.21068519	0.05267130	4.80	0.0047
PROT	2	20.75099259	10.37549630	945.30	0.0001
SS*PROT	4	1.31444074	0.32861019	29.94	0.0001
TEMP*PROT	4	1.07902963	0.26975741	24.58	0.0001
SS*TEMP*PROT	8	0.15873704	0.01984213	1.81	0.1194
Error	27	0.29635000	0.01097593		
Total	53	24.28618704			

R-Square: 0.987798

C.V.: 1.970178

IV. Specific Mechanical Energy

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SS	2	84921.96000	42460.9800	31018.57	0.0001
TEMP	2	2458.404444	1229.20222	897.96	0.0001
SS*TEMP	4	284.742222	71.185556	52.00	0.0001
PROT	2	3476.031111	1738.015556	1269.65	0.0001
SS*PROT	4	2008.34222	502.085556	366.78	0.0001
TEMP*PROT	4	1144.724444	286.181111	209.06	0.0001
SS*TEMP*PROT	8	636.488889	79.5611111	58.12	0.0001
Error	27	36.9600000	1.368889		
Total	53	94967.65333			

R-Square: 0.999611

C.V.: 0.622706

V. Free Sulfhydryl Content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SS	2	3.31895926	1.65947963	63.34	0.0001
TEMP	2	114.38589259	57.19294630	2183.09	0.0001
SS*TEMP	4	2.12449630	0.53112407	20.27	0.0001
PROT	2	363.42934815	181.71467407	6936.16	0.0001
SS*PROT	4	2.47087407	0.61771852	23.58	0.0001
TEMP*PROT	4	64.13894074	16.03473519	612.06	0.0001
SS*TEMP*PROT	8	5.37357037	0.67169630	25.64	0.0001
Error	27	0.70735000	0.02619815		
Total	53	555.94943148			

R-Square: 0.998728

C.V.: 2.578808

VI. Disulfide Content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SS	2	2.1254370	1.0627185	29.79	0.0001
TEMP	2	68.1644926	34.0822463	955.43	0.0001
SS*TEMP	4	6.1648296	1.541207	43.20	0.0001
PROT	2	2281.6417593	1140.82087	31980.65	0.0001
SS*PROT	4	26.1638963	6.5409741	183.36	0.0001
TEMP*PROT	4	37.9341074	9.4835269	265.85	0.0001
SS*TEMP*PROT	8	13.6748704	1.7093588	47.92	0.0001
Error	27	0.9631500	0.0356722		
Total	53	2436.8325426			

R-Square: 0.999605

C.V.: 0.614507

VII. Total Cysteine Content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SS	2	1.825378	0.912689	6.63	0.0045
TEMP	2	42.498433	21.249217	154.40	0.0001
SS*TEMP	4	21.997656	5.499414	39.96	0.0001
PROT	2	13077.673900	6538.836950	47513.58	0.0001
SS*PROT	4	111.738489	27.934622	02.98	0.0001
TEMP*PROT	4	30.365067	7.591267	55.16	0.0001
SS*TEMP*PROT	8	83.926278	10.490785	76.23	0.0001
Error	27	3.715750	0.137620		
Total	53	13373.740950			

R-Square: 0.999722

C.V.: 0.547628

VIII. Fluorescence Intensity

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SS	2	0.00045633	0.00022817	67.70	0.0001
TEMP	2	0.00175433	0.00087717	260.26	0.0001
SS*TEMP	4	0.00165933	0.00041483	123.08	0.0001
PROT	2	0.00072678	0.00036339	107.82	0.0001
SS*PROT	4	0.00042389	0.00010597	31.44	0.0001
TEMP*PROT	4	0.00068256	0.00017064	50.63	0.0001
SS*TEMP*PROT	8	0.00050111	0.00006264	18.59	0.0001
Error	27	0.00009100	0.00000337		
Total	53	0.00629533			

R-Square: 0.985545

C.V.: 3.413783

IX. Peak Wavelength

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SS	2	64.23148148	32.11574074	38.75	0.0001
TEMP	2	362.00925926	181.00462963	218.42	0.0001
SS*TEMP	4	52.04629630	13.01157407	15.70	0.0001
PROT	2	272.39814815	136.19907407	164.35	0.0001
SS*PROT	4	42.07407407	10.51851852	12.69	0.0001
TEMP*PROT	4	531.29629630	132.82407407	160.28	0.0001
SS*TEMP*PROT	8	156.98148148	19.62268519	23.68	0.0001
Error	27	22.3750000	0.8287037		
Total	53	1503.4120370			

R-Square: 0.985117

C.V.: 0.183292

X. Transition Temperature

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SS	2	66.5415593	33.2707796	124.90	0.0001
TEMP	2	361.9847259	180.9923630	679.45	0.0001
SS*TEMP	4	301.0947852	75.2736963	282.58	0.0001
PROT	2	1245.4396259	622.7198130	2337.70	0.0001
SS*PROT	4	390.8122185	97.7030546	366.78	0.0001
TEMP*PROT	4	89.8678852	22.4669713	84.34	0.0001
SS*TEMP*PROT	8	612.0284704	76.5035588	287.20	0.0001
Error	27	7.1923000	0.2663815		
Total	53	3074.9615704			

R-Square: 0.997661

C.V.: 0.427215

XI. Transition Energy

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SS	2	6115.278148	3057.639074	8853.22	0.0001
TEMP	2	9857.080370	4928.540185	14270.30	0.0001
SS*TEMP	4	4445.049630	1111.262407	3217.60	0.0001
PROT	2	30814.044815	15407.022407	44610.15	0.0001
SS*PROT	4	5845.781852	1461.445463	4231.53	0.0001
TEMP*PROT	4	3360.422963	840.105741	2432.48	0.0001
SS*TEMP*PROT	8	9898.167037	1237.270880	3582.45	0.0001
Error	27	9.325000	0.345370		
Total	53	70345.149815			

R-Square: 0.999867

C.V.: 0.252222

¹SS = Screw Speed; Temp = Exit Die Temperature; Prot = Protein Content.

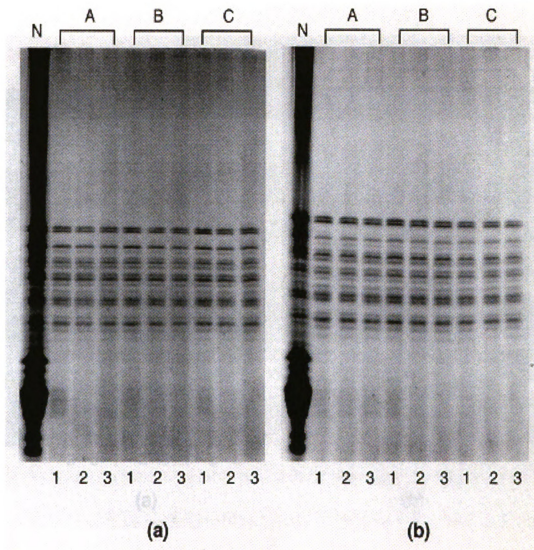
APPENDIX 4

Electrophoregrams of Non-Reduced Total Proteins in Extruded Wheat Flour

Screw Speeds: A = 240 rpm; B = 320 rpm; C = 400 rpm

Exit Die Temperatures: 1 = 120°C; 2 = 140°C; 3 = 160°C

Gels: a = 20%; b = 30%



APPENDIX 5

Electrophoregrams of Reduced Total Proteins in Extruded Wheat Flour

Screw Speeds: A = 240 rpm; B = 320 rpm; C = 400 rpm

Exit Die Temperatures: 1 = 120°C; 2 = 140°C; 3 = 160°C

Gels: a = 20%; b = 30%

