Extrusion Technology for the Development of Barley Cereal Products and Bioactive Packaging Materials

By

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

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"Many of Life's failures are people who did not realize how close they were to success when they gave up"

Thomas Edison

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α	Ratio of the volume of solution and extrudate
C_A	Mass concentration for substance A (kg m^{-3})
D (D_A)	Diffusion coefficient (for substance A) (m^2 s^{-1})
D_e	Diameter of die at extruder (m)
D_d	Diameter of extrudate (m)
E_{ab}	Apparent elastic modulus in three point bending test (Pa)
E_{ac}	Apparent elastic modulus in compression test (Pa)
F	Force (N)
l	Distance between two supports in a three point bending test (m)
L_o	Initial height of extrudate (m)
L_{se}	Specific length of extrudates (m kg^{-1})
dL	Change in length in the direction of force (m)
M_d	Moisture content of dough (%)
M_e	Moisture content of extrudate (%)
M_t	Concentration of diffusion substance in time (%, w/v)
M_∞	Concentration of diffusion substance after infinite time (%, w/v)
n	Power law index
N_A	Mass transfer rate for substance A (kg s^{-1})
P_2	Pressure reading after pressurizing cell (Pa)
P_3	Pressure reading after adding V_a (Pa)
q_n	Positive, non-zero roots of $\alpha q_n J_0(q_n) + 2J_1(q_n) = 0$ where $J_0(x)$ is the Bessel function of the first kind of order zero
r	Radius of cylindrical extrudate (m)
S	Cross sectional area of extrudate (m^2)
t	Time (sec)
T_g	Glass transition temperature (°C)
T_p	Peak temperature in endotherm (°C)
V_a	Added volume of gas (mL)
V_c	Volume of sample cell holder (mL)

V_s	Volume of solid portion of extrudate (mL)
ρ	Density (kg m^{-3})
ρ_d	Density of dough (kg m^{-3})
ρ_e	Density of extrudate (kg m^{-3})
ρ_w	Solid density of extrudate (kg m^{-3})
σ	General mechanical property (Pa)
σ_{ab}	Fracture strength in three point bending test (Pa)
σ_{ac}	Fracture strength in compression test (Pa)
x	Linear dimension (m)

ABSTRACT

This research focuses on the development of cereal products and a bioactive packaging material containing antimicrobial agents using extrusion technology. Extrusion technology has been established as a very effective cooking process, which combines thermal and shear energy input. Therefore, extrusion technology is widely used in the food industry and provides advantages such as simplification of unit process operations, high productivity, and good energy efficiency compared to some traditional processing methods. Initial research focused on the development of extruded cereal products using barley. Barley, despite its high nutrient contents and high levels of soluble and insoluble dietary fibers, has had limited success as a food source due to its coarse mouth feel and food choice. Barley meal, corn starch and a blend (50:50) were analyzed for physico-chemical properties after extrusion processing. Barley meal alone showed poor expansion, even with changes of extrusion processing variables. The extent of expansion, elastic modulus, and fracture strength of barley extrudates were significantly changed through blending with corn starch and extrusion processing variables (extrusion temperature and moisture content of dough). Also, water solubility, water absorption capacity and color of the barley extrudates were significantly affected through blending with corn starch and extrusion processing conditions. These studies demonstrated that the combination of extrusion process conditions and blending with corn starch showed potential for manufacturing barley products of desired quality.

The second study involving extrusion focused on the characterization of bioactive packaging material. Biodegradable packaging materials based on starch as an environmentally safe biopolymer have been considered as a replacement for synthetic packaging material. The objective was to develop a biodegradable starch packaging

material containing an antimicrobial agent (lysozyme) using extrusion technology. Pea starch, which inherently has good gel strength, was used as the source material for manufacturing the biodegradable packaging material. Extrudates containing 99% pea starch and 1% lysozyme were produced under various extrusion conditions (change of screw configurations, moisture content of dough, and extrusion temperature). The physico-chemical and mechanical properties of extrudates were significantly affected by extrusion processing variables. Extruded pea starch was completely gelatinized above 90°C die temperature. The intrinsic viscosity, water solubility, and paste properties of extrudates showed that there was extensive degradation of starch under severe extrusion conditions. The expansion of extrudates increased with an increase in extrusion temperature, whereas moisture content of dough had the opposite effect. The elastic modulus and fracture strengths were highly correlated in a power-law fashion to relative density, showing that the mechanical properties of extrudates were dependent on the wall solid density and the foam structure created during extrusion processing. Up to 48% of the initial lysozyme activity was recovered from the extruded pea starch and the release rate of lysozyme increased with a decrease in density of the extrudates. The antimicrobial activity of released lysozyme was confirmed through the formation of an inhibition zone for *Brochotrix thermosphacta* on agar plates. These results showed that the release profile of lysozyme and the mechanical properties of extrudates could be controlled by altering extrusion conditions. Therefore, it is concluded that extruded pea starch matrix containing lysozyme has a potential application as an antimicrobial and biodegradable packaging material.

1.0 Introduction

Food extrusion as a continuous food processing unit operation has been extensively developed in recent years. Extrusion can be used in the processing and manufacturing of a wide range of plant and animal products for human and animal consumption. It is also used extensively in manufacturing packaging films. Extrusion describes a process where plasticized materials, such as melted starch, are forced by pressure through a die or opening to create a specific shape (Harper 1992). Thermal energy generated combined with mechanical energy (shear) during extrusion quickly cause the physical-chemical changes of raw material (Mercier et al. 1989).

One of the most important characteristics of extrudates is expansion, which relates to product texture (Padmanabhan and Bhattacharya 1989). Several studies have reported the role of extrusion variables in the expansion of starch-based material. Among the extrusion variables studies, the moisture content of dough material and extrusion temperature are the most important independent variables to control expansion volume (Padmanabhan and Bhattacharya 1989). Also, expansion of extrudates based on starch has been significantly affected by other independent variables such as amylose content of starch (Chinnaswamy 1993), die nozzle configuration (Chinnaswamy and Hanna 1987), type of starch (Della Valle et al. 1997), screw configurations (Choudhury and Gautam 1998a, b) and screw speed (Chinnaswamy and Hanna 1988).

In general, extrusion cooking of starch involves gelatinization and extensive degradation of macromolecules. The changes in properties of starch have been characterized using differential scanning calorimetry (DSC) (Barron et al. 2000), intrinsic viscosity (Della Valle et al. 1989, 1997), rapid viscoanalyser (RVA)

(McPherson et al. 2000), gel permeation chromatography (GPC) (Chinnaswamy and Hanna 1990), and high performance size exclusion chromatography equipped with light scattering and refractive index detectors (RI) (McPherson and Jane 2000). The DSC results showed that the heat and shear applied during extrusion processing fully gelatinized the starch. Other study has also shown that starch under severe extrusion conditions such as low moisture content of the dough, high extrusion temperature, and high shear screw configurations was degraded more extensively than under mild conditions (McPherson et al. 2000)

Extruded starch has been characterized by dependent extruder process variables, such as water solubility and absorption, expansion, color, density, apparent elastic modulus, and breaking force (Lo et al. 1998; Lourdin et al. 1995). Usually these product properties indices are measured to assess the effect of process variables on product quality.

Barley production in North America is predominately for malt to produce beer and animal feed, rather than for human food. However, today's consumers are becoming more conscious of their diet and discriminating about eating foods with healthful benefits (Newman and Newman 1991). Berglund and coworkers (1994) reported that barley containing high levels of total dietary fiber and soluble dietary fiber had a significant effect in decreasing many kinds of human disease caused by malnutrition and unbalanced diet. Although barley can provide many health benefits, several researchers who have tried to develop new barley products as food using extrusion technology have had very limited success due to the low expansion properties of barley (Kim et al. 1989; Lee et al. 2000, 1997).

This thesis consists of first study as 'Physico-chemical properties of extruded barley/corn starch blend' and second study as 'Development and characterization of extruded pea starch containing lysozyme as an antimicrobial and biodegradable packaging material'. In first study, corn starch was used for blending with barley because corn starch was economical, readily available, and well documented in the literature for its extrusion cooking and great expansion properties. The objective of the first experiment was to determine the effect of the processing variables on the properties of the extrudates to develop a more acceptable barley product.

Key products from extrusion technology as packaging material have included cereal products. These food products can be used to make biodegradable film through extrusion technology. Native pea starch, as a C-type starch, has unique functional characteristics, such as very strong gel properties, heat and mechanical stability, low pH stability, and restriction swelling and low final solubility at 95°C. Therefore, pea starch could be used as a thin film with strong mechanical barrier property (Blenford 1994).

As a second research objective, pea starch was used as the source material to explore its potential for manufacturing a biodegradable film. The pea starch was also combined with lysozyme to develop a packaging material with antimicrobial and biodegradable properties. Lysozyme with relatively heat stability was chosen as a natural bacteriocide that can maintain antimicrobial activity after extrusion processing.

Chapter 2

2.0 Literature Review

2.1 Introduction

Food extrusion has been utilized for more than 60 years. Its initial role was limited to kneading and forming dough (Mercier et al. 1989). Now, food extrusion processing is used for manufacturing various products such as snack foods, packaging films, and meat analogs in the food and pharmaceutical industries. There is also the opportunity to utilize a wide variety of cereal crops as a raw material in extrusion processing. The value of snack foods manufactured using extrusion technology was estimated at $20 billion in the continental USA alone with the consumption rate increasing by 3% every year (Chinnaswamy 1993).

Extrusion describes a process where melted or plasticized materials are forced by pressure through a die or opening to form a desired shape (Harper 1992). Its advantage is the simplification of processing techniques for the manufacturing of final products due to a thermomechanical processing operation. The high shear stress and high temperature inside the extruder screw channel lead to a variety of fast and relatively effective chemical reactions (Kokini 1993). Extrusion is unique in that it is used with relatively dry materials, plasticizing the food mass, reducing the microbial load, denaturing enzymes, gelatinizing starch, polymerizing protein and forming the end product to a desired shape (Fichtali and van de Voort 1998). Other advantages of extrusion processing compared to traditional processing are versatility in processed products, high productivity due to continuous processing, and energy efficiency by reduction of processing time (Lee 1995). Midden (1989) reported that corn flake production using a twin-screw extruder could save 11% of production cost over traditional processing with at least equal product quality.

Food extruders can be classified thermodynamically, by pressure development, or by shear intensity (Hauck and Huber 1989). From a theoretical thermodynamic point of view, extruders are: 1) autogenous, generating their own heat by conversion of mechanical energy in the flow process; 2) isothermal, maintaining constant temperature; 3) polytropic, operating with part of energy from mechanical dissipation and part from heat transfer. These classifications are important only when modeling the behavior of the cooking process in an extruder because most extruders operate polytropically. Extruders can also be classified by their method of pressure development – positive displacement, or viscous drag. Single screw, co-rotating twin screw and non-intermeshing cooking extruders are viscous drag extruders. These different extrusion devices develop different degree of friction during extrusion processing. The most practical way to classify extruders is by the intensity with which the extrudates are sheared – low, medium, or high shear (Lee 1990). This permits easy identification and cross-reference of products to process variables and physical parameters of the extruder.

The major features of the single-screw extruder are shown in Fig. 2.1. Food material is fed into the barrel and conveyed without heating. This zone is called the *feed section*. A tapered, rotating screw moves the hydrated food dough by mixing with injected water and compresses it, causing it to heat up. This is called the *compression* (or transition) *section*. The gradual decrease in the flight depth or the pitch achieves compression in the transition section. This section occupies about half the length of the extruder. Following the compression section is the *metering section*, which is the part nearest to the discharge of the extruder and often has only very shallow flights. There is a sharp increase in the dough temperature from the conversion of mechanical work into

heat, as a result of the substantial energy dissipation by viscous flow at the high shear rate. An extruder can be analyzed or divided into a number of sections based on the type of process occurring in that section.

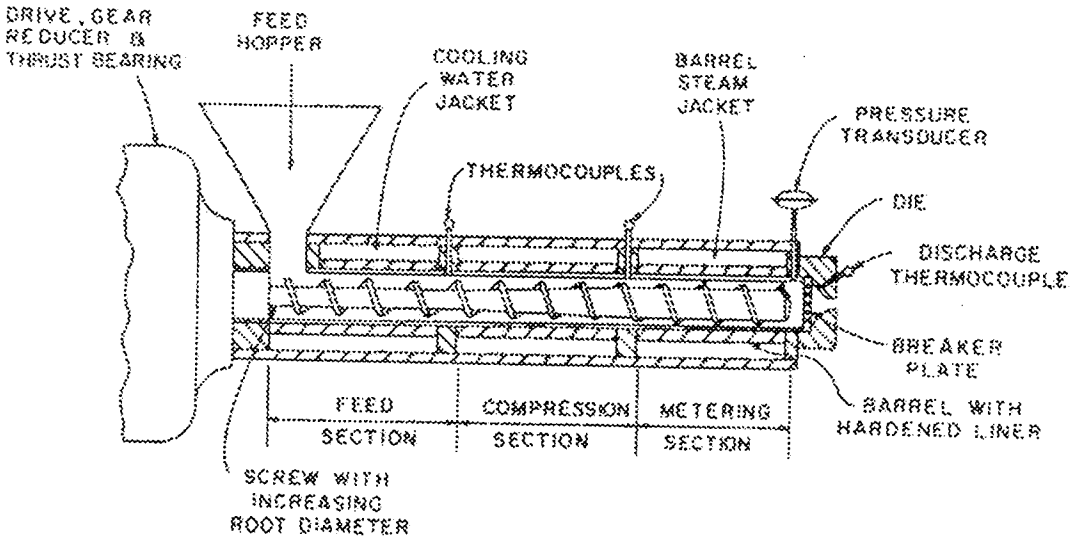

Figure 2.1. Schematic diagram of single screw extruder (Source: Harper 1981)

2.2 Applications of Extrusion Cooking

Extrusion cooking can be used in the processing and manufacturing of a wide range of plant and animal products for both human and animal consumption. Extrusion technology has found many applications in the food industry, both conventional and unconventional. The first application of food extrusion was that single screw extruders replaced the hydraulically driven traditional batch pasta or macaroni press in the 1930s (Harper 1981).

2.2.1 Barley as Extrusion Base Material

Barley meal has rarely been used as a food source, because of differences in food choice, coarse mouth-feel and poor texture (Lee et al. 2000). Barley products in North America are predominately for malt to produce beer and animal feed, rather than for human food. However, consumers are becoming more conscious of their diet and are concerned about eating foods with health benefits (Newman and Newman 1991). Some researchers have reported that dietary fiber in barley provides benefits such as cholesterol control, gastrointestinal function, cancer prevention, and general physical fitness (Berglund et al. 1994; Newman and Newman 1991). Of particular interest, β-glucan in extruded barley significantly decreased serum cholesterol levels in an animal test (Wang and Klopfenstein 1993). Therefore, barley extrudates can contribute to optimizing human health benefits, because the health benefits of barley β-glucan rely on both content and solubility. Gaosong and Vasanthan (2000) observed that the β-glucan in the extruded barley flour had higher solubility values than native barley flour; moreover, high temperature and moisture levels influenced the water solubility of β-glucan in extrusion cooking. Under optimal extrusion conditions, extruded barley increased resistant starch content compared to raw barley while the β-glucan was preserved in a macromolecular form (Huth et al. 2000).

2.2.2 Starch as Extrusion Base Material

Fennema (1985), Hoseney (1994), Kim (1995), and Manners (1989) summarized comprehensive information about starch in food chemistry. Starch found in plants in the form of granules is the second-largest biomass produced on earth. Starch consists of

amylopectin, a highly branched polymer, and amylose that is a linear polymer and tends to form helix structures that entrap other molecules such as fatty acids or properly sized hydrocarbons. Amylose is mainly in the amorphous phase, whereas amylopectin is the main component of the crystalline fraction in a starch granule. Starch granules that have tightly packed polysaccharide chains show birefringence in the form of the typical "maltese cross". The property of birefringence is attributed to the high degree of molecular order present in the starch granule. The point at which birefringence first disappears is regarded as the gelatinization point or gelatinization temperature. The term *gelatinization* describes the thermal transition of starch under excess water content; otherwise, *melting* under limited water content. During gelatinization the starch granules swell extensively. Starch gelatinization, the viscosity of starch solutions, and the characteristics of starch gels depend not only on temperature, but also on the kinds and amounts of other constituents present such as sugar, protein, fat, and water (Fennema 1985; Wang et al. 1994).

Intact starch granules give three types of X-ray patterns (designated A, B, and C). Most cereal starches give the A pattern; potato, other root starches, and retrograded starch give a B pattern; and smooth pea and bean starches give the C pattern, which is an intermediate form, probably due to mixtures of the A and B types. In general, native starch, as a semi-crystalline granule, has values of crystallinity ranging from 15 to 45% (Zobel 1992). The crystalline portion of 'C' type smooth pea starch had been shown to be comprised of a mixture of 56% 'A' type and 44% 'B' type polymorphs (Cairns et al. 1997). During heating in excess salt solution, the polymorphs in the two regions of smooth pea starch granule melted independently and showed a double endothermic peak

in the results of a differential scanning calorimetry (DSC) due to two-step swelling; 'B' polymorphs melted at a lower temperature than the 'A' polymorphs (Bogracheva et al. 1998). Therefore, the unique structure and gelatinization properties of pea starch gives different functional properties compared to A-, B-type starches.

The unique functional characterization of native pea starch is thermal and mechanical stability. Pea starch has remarkable stable hot paste viscosity at 95°C, stability in acid solution, ready gel formation, above average amylose content for natural starch, restricted swelling and low final solubility at 95°C, and higher solubility than cereal starches in the range of 60 to 90°C (Blenford 1994). The unique strong gel forming property in pea starch is responsible for its film-forming power. A hot paste applied in a thin layer dried quickly to a fine strong film (Blenford 1994).

2.2.3 Snack Foods

A few decades ago, extrusion technology replaced most conventional technologies to produce expanded cereals. Advantages in using extrusion technology include utilization of both cereal and starch ingredients, creation of highly expanded products, production of a wide variety of shapes and textures, cooking and forming in a single processing step. (Chinnaswamy 1993; Mercier et al. 1989).

Expanded shaped snack processing, such as Ready-to-Eat (RTE) cereal processing, is usually performed with great friction applied by higher mechanical energy input. To achieve high expansion snack processing uses low moisture (< 15%), high shear, and high-temperature extrusion conditions in which significant starch damage occurs (Toft 1979).

Several researchers reported development of barley RTE cereal or snack food using extrusion technology. Mixtures of barley and other cereals were used to study the effect of extrusion processing variables and to develop new products containing barley, with nutritional benefits (Fornal et al. 1987; Kim et al. 1989; Lee et al. 2000, 1997). Berglund and coworkers (1994), using extrusion cooking, developed a reconstituted grain mixture containing barley. However, they suggested that the quality of barley products still needs improvement. Their studies through blending with barley only emphasized expansion properties. Thus, a study to examine the change of various physico-chemical properties of barley extrudates related to extrusion processing variables is required.

2.2.4 Biodegradable Packaging

Extrusion technology is the most widely used in the manufacturing of various synthetic packaging materials (Richardson 1989). However, studies for biodegradable packaging material based on starch using extrusion processing are not well documented.

Krochta and De Mulder-Johnston (1997) have provided a definition of biopolymer packaging. The initial trial of 'biodegradable' plastics by mixing of natural (e.g., starch) and synthetic (e.g., LDPE) was not completely biodegradable. Therefore, some researchers requested a critical definition of what constitutes biodegradable packaging. To be called biodegradable, the package material must be degraded completely to its natural compounds by microorganisms (Krochta and De Mulder-Johnston 1997). The two steps of biodegradation are depolymerization (chain cleavage) and mineralization to carbon dioxide, water, salts, etc. The benefits of replacing

synthetic packaging with biodegradable polymers are a reduction in the use of non-renewable resources and reduced waste through biological recycling to the biosystem.

Packaging materials based on starch were summarized comprehensively by Nisperos-Carriedo (1994). High amylose starch gives stronger, tougher, and more flexible films than low amylose starch. However, the high amylose starch is difficult to disperse in water, and retrogrades very rapidly. To overcome the problems associated with these native starches, modification of the native starch by acid treatment, reduction of molecular weight and/or chemical substitution has been introduced in order to develop starch-based packaging materials with appropriate physical properties. The modified starch leads to lower gelatinization temperatures and reduces the tendency for retrogradation. Riaz (1999) found water and shear levels had to be controlled depending on type of starch to produce a less brittle starch based packaging material. Wheat starch film improved the water resistance through blending of biodegradable polyesters (Averous et al. 2001). Multilayer films based on wheat starch and various biodegradable polyesters were produced using co-extrusion or compression molding (Martin et al. 2001). The use of the intermediate layer blended with polyesters at multilayer film improved the adhesion properties of the film by up to 50%, while mechanical properties improved slightly.

2.2.5 Other Applications

Pet foods and aqueous feed represent the greatest volume of extruded products in the market (Mercier et al. 1989). The manufacture of dry pet foods using extruders was suitable for a single screw extruder with high capacity and low capital cost, nevertheless

there were some disadvantages such as a relatively high moisture product and unsophisticated shapes.

One of the most sophisticated products made by extrusion technology is texturized vegetable protein (TVP), produced mainly from defatted soybean protein flour (Harper 1981; Kitabatake and Doi 1992). Under extrusion conditions of low moisture and high temperature, cross-linked reactions between aligned protein molecules form a fibrous meat-like structure that can be hydrated and used as a meat extender in a variety of foods.

The chemical modification of food ingredients to create unique, new or improved properties as a chemical reactor is another expanding and challenging area in the application of extrusion technology. Unlu and Faller (1998) found high-amylose corn starch increased the level of resistant starch in extruded products by optimizing extruder conditions. The use of an extruder as a chemical reactor includes derivation of starch and precooking starch (Mercier et al. 1989), saccharification of starch with α- or β-amylase (Komolprasert and Ofoli 1991; Linko et al. 1983), production of sodium caseinate, preparation of fat analogues by microcoagulation of whey protein (Queguiner et al. 1992), fermentation processing of ethanol, soy sauce, and lactose (Lee 1990).

2.3 Process and System Variables

2.3.1 Definitions of Terms

Specific terms are used in reference to extrusion processing. The fluid emerging from the die is called the *extrudate*. Fichtali and van de Voort (1989) defined terms of variables for analyzing systems of extrusion. The *process variables* that one can control

during the extrusion run are commonly termed independent or control variables. They include screw speed, feed rate, water injection rate, and extrusion temperature. Extruder *design variables* include screw configurations, die size and shape, barrel length and diameter (L/D). Both the process variables and the extruder design variables can considerably affect the *system variables*, which in turn are called dependent variables or response variables for the system. They may include the product temperature profile, pressure at the die, torque, and degree of screw fill. A last set of variables, termed target variables, also require consideration, which can be sub-categorized as product characteristic variables. Depending on the process, the desired target variables must be defined and optimized.

The net mechanical energy in extrusion cooking can be defined as specific mechanical energy (SME), which provides a good characterization of the extrusion operations (Brent et al. 1997; Mercier et al. 1989).

$$SME(Wh/kg) = \frac{rpm(test)}{rpm(rated)} \times \frac{\%motorload}{100} \times \frac{motorpower(W)}{feedrate(kg/h)} \qquad (2.1)$$

2.3.2 Relationship between Extrusion Process and Product Changes

Extrusion technology covers a range of multivariate processes. In order to control such processes, it is necessary to have a good understanding of the fundamental relationship between each variable and physico-chemical changes of the products. A system diagram was set up to analyze the effect between independent process parameters and operational variables, system parameters, and product parameters (Fig. 2.2). The theoretical basis for the system analysis is based on the principle that the

chemical and physical changes of the extruded mass are dependent on the time-dependent mechanical and thermal energy input (Choudhury et al. 1997; Mercier et al 1989; Meuser et al. 1992).

The independent process variables correspond to operational variables of the extruder. These parameters govern the thermal and mechanical energy of the moving mass during extrusion processing. Both thermal and mechanical energy affect molecular structure of the starch, which in turn, causes physico-chemical changes in the final starch based product (Choudhury et al. 1997). Meuser and coworkers (1992) verified the validity of the system's analytical model based on statistical analysis in order to explain functional relationships between process variables and system variables as well as between system variables and the properties of extrudate products. They emphasized that the statistical design and analysis is an excellent method to explain trends and changes in complex relationship between extrusion variables. However, they demonstrated that the regression equations might have limitations in describing the relationship between variables on a mathematical basis.

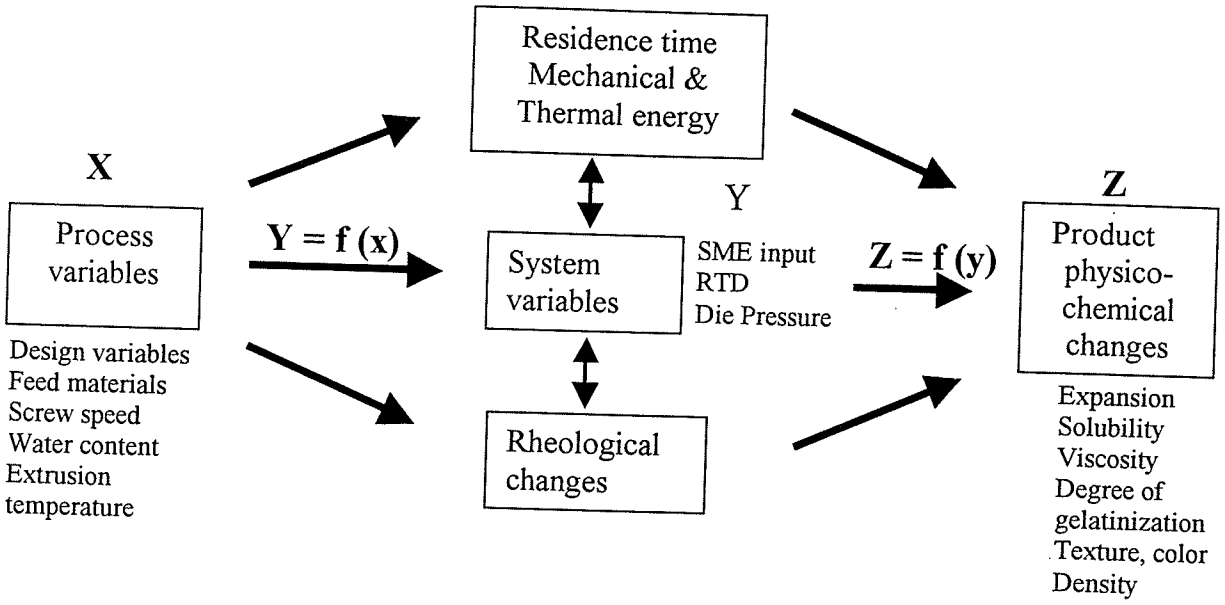

Figure 2.2. System diagram for the extrusion of starch (Adopted from Choudhury et al. 1997; Meuser et al. 1992)

2.3.3 Transformation of Starch through Extrusion Processing

Physical and chemical properties of extrudates have always been related to extruder design variables including die size and shape, ratio of nozzle length and diameter of die, ratio of barrel length and diameter (L/D), and screw configurations type of extruders.

The ratio of nozzle length and diameter of die affected the change of die pressure (Chinnaswamy 1993). High pressure increased the residence time, the degree of starch gelatinization and shear rates for starch (Chinnaswamy and Hanna 1987). The residence time, as an important intermediate process variable, was determined by measuring the distribution of cooking times for the feed material during extrusion processing. The

residence time distribution (RTD) data are used for determining the extent of mixing and cooking, process scale-up, and for optimization of processing (Choudhury and Gautam 1998b; Mercier et al. 1989). In another study, high shear rates and extended residence times induced starch degradation and resulted in reduction of expansion ratio (Colonna et al. 1983).

Kokini and coworkers (1992) investigated the difference in rheological properties between extrudates resulting from capillary and slit dies. They noted that die geometries could affect viscosity of melted starch. A smaller die size or slit die usually resulted in low shear viscosity due to a high shear rate which caused high expansion for amioca extrudates.

The effects of screw configurations have been investigated intensively (Choudhury and Gautam 1998a, 1998b; Gautam and Choudhury 1999; Kollengode et al. 1996; Lo et al. 1998; Sokhey et al. 1994; Yam et al. 1994). The type, length, and position of mixing elements, and spacing between two elements, number and position of reverse screws significantly affected molecular degradation of starch, residence time distribution (RTD), system variables (pressure, specific mechanical energy), and physical properties of extrudates including expansion, porosity, density, water solubility and absorption capacity of extrudates. In particular, reverse screw elements were highly effective for inducing conversion and degradation of starch and had the most dominant effect on residence time distribution (RTD). The RTD at screw configurations with reverse screw elements showed lower peak heights and wider distribution. The reverse screw elements also resulted in more extensive cooking due to longer residence time than forward screw elements.

The phase transition of starch and the effect of extrusion processing variables on functional properties of the final product have been extensively investigated during extrusion cooking. Camire and colleagues (1990), and Mitchell and Areas (1992) studied the proportions of gel and melt and the chemical changes of starch that occurred at various stages during the extrusion process. Increasing knowledge of the effect of shear and temperature will offer opportunities to use an understanding of phase transitions to develop a wide range of food products (Mermelstein 1997). Presently, a main area of investigation with extruder manufacturers is the study of starch behavior and flow in a barrel under shear and high temperature (Strahm 1998).

The transformations of starch during extrusion are analyzed at three levels: molecular, crystalline, and granular. The crystalline and granular modification of starch will be considered jointly because they are interrelated. The extent of macromolecular degradation during extrusion has been demonstrated for pure starch under various extrusion conditions. Many studies have been performed for verifying the effect of extrusion processing variables for final products.

Kim and Hamdy (1987) studied the effect of pressure for depolymerization of starch molecules during extrusion processing. They found that high pressure (up to 276 MPa) alone did not depolymerise starch to the desired level of fermentable sugar, but combination with heat and acid improved the depolymerization of starch during extrusion processing. Vergnes and colleagues (1987) found intrinsic viscosity decreased with increasing thermomechanical energy input in a single and twin-screw extruder. They reported that starch degradation was a direct function of the mechanical energy received during the extrusion processing. However, Politz and coworkers (1994) could

not find a correlation between the degree of fragmentation and specific mechanical energy using automated gel permeation chromatography. They observed that the fragmentation of starch was influenced mainly by the amylopectin component of the starch. The gel-permeation chromatography fractionation of extruded corn starch indicated the branched component, amylopectin, degraded to a greater extent than its linear counterpart, amylose (Chinnaswamy 1993; Chinnaswamy and Hanna 1990). High performance size exclusion chromatography (HPSEC) equipped with multiangle laser light-scattering (MALLS) and refractive index (RI) detectors indicated extruded starches degraded to different extents at various extrusion conditions (McPherson and Jane 2000). Chemical modification of corn starch and increased moisture content resulted in decreased amylopectin degradation in the extruded starches. Increasing temperature at high moisture content (40%) lowered amylopectin molecular weights of the extruded corn starches, but increasing extrusion temperature at low moisture content (30%) resulted in less degraded amylopectin molecules.

Extrusion cooking as thermomechanical processing disrupted starch granule structure, depolymerised starch components, gelatinized starch, and partly or completely destroyed the native crystalline-like structure (Arambula et al. 1998; Barron et al. 2000, 2001; McPherson and Jane 2000; McPherson et al. 2000; Vergnes et al. 1987). Barron and coworkers (2000, 2001) studied the differences between mechanical and thermal effects on degradation of starch granules and gelatinization of starch by using transmission election microscopy (TEM) and the Rheoplast® (Fig. 2.3). In a pea starch granule, darker shells may represent amorphous material whereas brighter rings may correspond to semi-crystalline zones [Fig. 2.3 (a)]. In mechanical processing, the

internal organization of the granule fragments was unmodified and it seems that starch granule fragmentation occurred without disorganization from center to periphery in a granule [Fig. 2.3 (b)]. After the thermal treatment, starch granules lost crystalline order and the granules become distorted [Fig. 2.3 (c)]. In thermomechanical processing, TEM showed a continuous mass without any visible granular structure similar to that observed for the thermally processed sample [Fig. 2.3 (d)]. Zheng and coworkers (1995) using an optical microscope coupled with an image analysis system found the mechanical energy input and material residence time in an extruder were major factors influencing the degree of starch granular size reduction. In a high shear extrusion process (T = 40°C), the weight-average granule size of extrudates was reduced from ~12.4μm to 1~2 μm, whereas in a low shear extrusion and high temperature (T = 90°C), the granular size was reduced to ~ 7μm. X-ray diffraction technique, which is the most advanced instrumental method for the estimation of crystallinity, has been used to study the effects of extrusion cooking on the organized structure of starch. The X-ray diffraction patterns showed that the crystallinity of the extruded starch decreased as the barrel temperature was increased (McPherson et al. 2000). The Rheoplast®, which is similar to an extruder, was used to simulate thermomechanical processing using A-type maize starch and C-type pea starch (Barron et al. 2000, 2001; Vergnes et al. 1987). The results showed disappearance or changes of certain diffraction peaks under thermal and/or mechanical processing. Under mild conditions (140°C, 200 rpm/min, 20 s), a new pattern corresponding to V-type amylose–lipid complexes structure appeared and under intermediate processing conditions (180°C, 200 rpm/min, 20 s), both E- and V-type structures were present.

Arambula and colleagues (1998) obtained similar results from corn extrudates under extrusion processing.

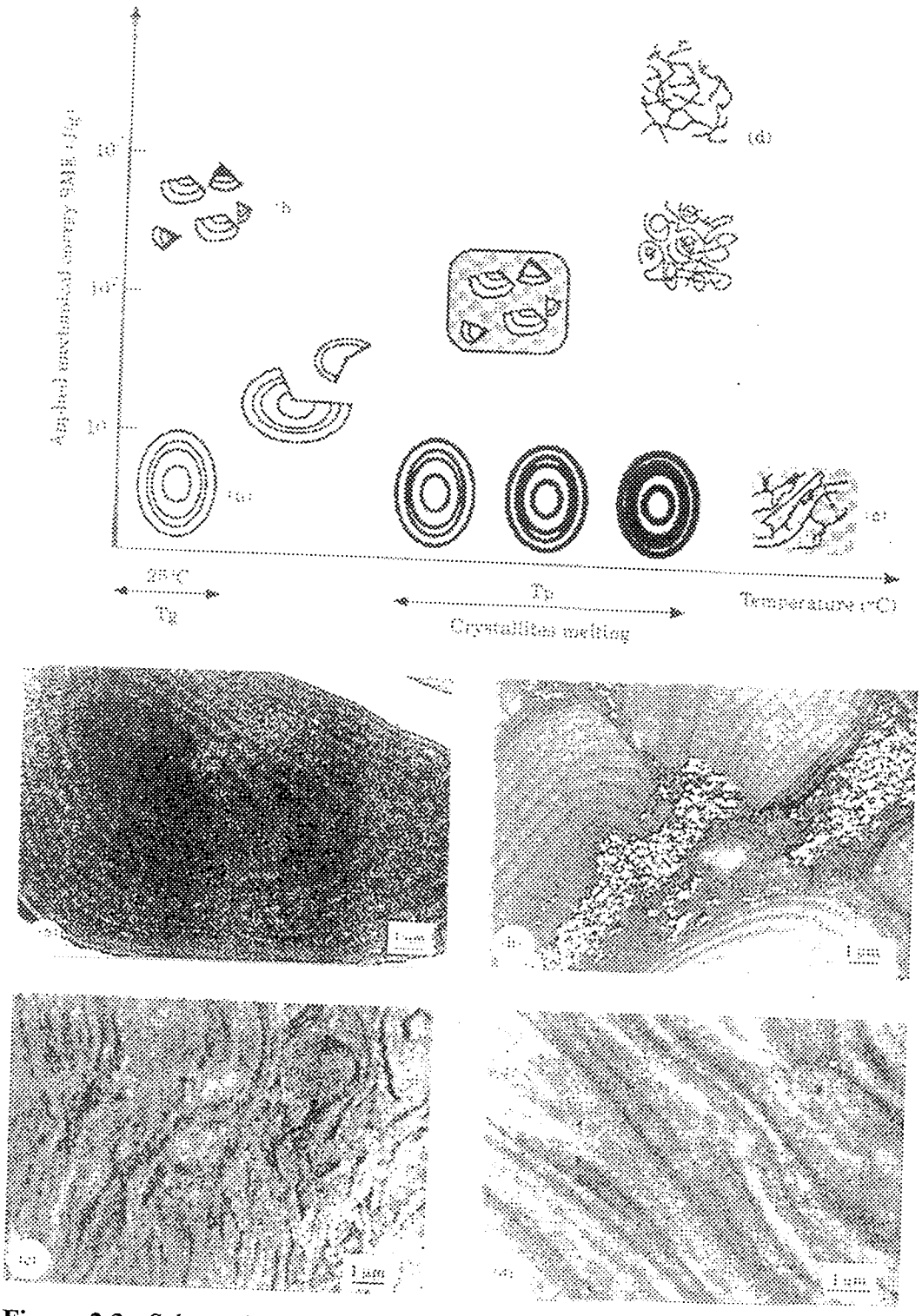

Figure 2.3. Schematic representation of smooth pea starch destruction under shear and temperature using transmission electron microscopy (TEM); native smooth pea starch (a), starch granule after mechanical processing (b), starch granule after thermal processing (c), starch granule after thermal mechanical processing (d) (Sources; Barron et al. 2000, 2001)

2.4 Product Physico-Chemical Changes

2.4.1 Differential Scanning Calorimetry (DSC)

The principle of DSC is to compare the rate of heat flow to a sample and to an inert material as a reference, which are heated or cooled at the same rate. DSC can provide quantitative data such as enthalpy of reaction or transition, heat capacity measurements, degree of crystallinity and quantitative analyses of multi-component mixtures (Biliaderis 1992). DSC has also been used to measure the enthalpy of gelatinization of native and extruded starch, and to investigate the structure and phase transitions of starch (Biliaderis 1980; Mercier et al 1989). The intensity of endothermic peak of native pea starch during thermal transition processing decreased with decreasing moisture content and the endothermic peak shifted to a higher level when moisture content decreased (Barron et al. 2000). The endothermic peak of starch was also reduced with increased severity of extrusion conditions (McPherson et al. 2000).

2.4.2 Water Solubility Index (WSI)/ Water Absorption Index (WAI)

Water solubility and absorption indices are important functional properties of the extrudates when used as a food ingredient. Water solubility increased with increased temperature and moisture content during extrusion processing (Owusu-Ansah et al. 1983). Water solubility index has been used to measure the degree of macromolecular degradation during starch extrusion (Guha et al. 1997; Meuser et al. 1992). An increase of shear energy related to specific mechanical energy (SME) input, as well as residence time affected by incorporation of different screw elements, could cause depolymerization, fragmentation, and degradation of starch (Choudhury and Gautam

1998a; Smith 1992). Water solubility index was increased with elevated specific mechanical energy (SME) input (Della Valle et al. 1989).

Water absorption capacity in non-waxy corn decreased with an increase of moisture injection during extrusion processing, but increased in the case of waxy corn with high amylopectin content (Bhattacharya and Hanna 1987). An increase in barrel temperature of the extruder caused an increase in water absorption capacity for both non-waxy and waxy corn. However, some researchers reported that increasing thermal treatment in the extruder had a negative impact on water absorption index (Guha et al. 1997; Harper 1992; Mercier et al. 1989). Gel-permeation chromatographic studies showed that low water absorption samples displayed a high degree of degradation while samples with high water absorption capacity displayed a low degree of degradation (Pan et al. 1998). WSI increased with an increase in WAI until complete gelatinization of starch granules, but subsequent macromolecular degradation caused a negative relationship between WSI and WAI (Smith 1992).

2.4.3 Viscosity

The intrinsic viscosity of a polymer suspension is used to measure a polymer molecular weight by comparing *efflux* times using capillary viscometers (Billmeyer 1984). Extrusion cooking of starch leads to granule molecular degradation. An increase of shear energy due to SME input enhanced the degradation of starch, which was reflected in a drop in intrinsic viscosity (Della Valle et al. 1989, 1997). The effect of starch degradation was consistent with a stress-related process rather than a thermal process. Vergnes and colleagues (1987) found intrinsic viscosity decreased with

increasing thermomechanical energy input in a single and twin-screw extruder. They reported that starch degradation was a direct function of mechanical energy received during extrusion processing.

The pasting characteristics of extruded starch provide useful information for the functional uses of extrudates as food ingredients. The viscosity of a paste depends to a large extent on the degree of gelatinization of starch granules and the extent of their molecular breakdown (El-Dash et al. 1983). Viscoamylogram and rapid viscosity analyses showed that increasing the severity of extrusion conditions during processing resulted in low cold paste, hot paste and low final viscosity (El-Dash et al. 1983; Harper 1992; Hoseney 1994; McPherson et al. 2000; Whalen et al. 1997).

2.4.4 Expansion

One of the most important characteristics of starch extrusion is the expansion of extrudates, which results in a cellular foam structure in extrudates and is related to product texture. Kokini and colleagues (1992) described expansion phenomena in extrudates as occurring in five stages: 1. Order-disorder transitions and chemical reaction occur. 2. Nucleation sites allow growth of bubbles. 3. Extrudates expand as a result of the release of strain energy stored within constrained feed material in a die when extrudates emerge from the die. 4. Bubbles grow in the die or outside the die. The growth rate of bubbles increases with a decrease in the melt viscosity. 5. Some bubbles collapse when bubble walls become so thin that they can no longer sustain the water vapor pressure. Fan and coworkers (1994) developed vapor bubble growth model by

taking into account time-temperature dependence and shrinkage of a bubble formed after emerging from the die.

Padmanabhan and Bhattacharya (1989) explained that there are two dominant forces that cause expansion of extrudates. One is the elastic force and the other is bubble growth force due to water vapor pressure. When the strain energy in melted dough at low barrel temperature and feed moisture is released, the elastic force was dominant. However, bubble growth force at high temperature and feed moisture was more dominant to expand extrudates than the elastic force of the molten starch dough during extrusion processing. The apparent melt viscosity and feed moisture affected radial expansion of extrudates, whereas barrel temperature had no significant effect (Iio et al. 1996; Launay and Lisch 1983). There was good evidence that bubble growth force by water vapor pressure mainly contributed to radial expansion. Also, expansion could be affected by material properties such as structure of starch, amylose and amylopectin contents, and the starch's genetic and botanical origin (Chinnaswamy 1993; Della Valle et al. 1997).

High barrel temperature, low moisture content, amylose content up to 50%, screw configuration with kneading elements rather than reverse elements, and high screw speed induced maximum expansion of starch (Bhattacharya and Hanna 1987; Chinnaswamy 1993; Choudhury and Gautam 1998a). Three dimensionless parameters of cross-sectional, longitudinal, and volumetric expansion indices have been used to describe how the extrusion process affected final product characteristics (Alvarez-Martinez et al. 1988).

2.4.5 Density

The density of extrudates had an inverse relation to volumetric expansion, but was not proportional to the cross-sectional and longitudinal expansions (Alvarez-Martinez et al. 1988). However, the radial and longitudinal expansions were dependent on elasticity and viscosity of molten dough, respectively (Launay and Lisch 1983). In addition, many studies found an inverse relationship between cross-sectional and longitudinal expansion (Alvarez-Martinez et al. 1988; Harper 1992; Launay and Lisch 1983).

The density of extrudates increased with increasing moisture content and feed rate, but decreased with increasing extrusion temperature, screw speed, and destruction of starch granules due to high SME input (Bhattacharya and Hanna 1987; Lo et al. 1998). The density was highly dependent on elasticity and viscosity of molten starch dough, and water vapor force due to superheated moisture. The elasticity and viscosity of the molten starch dough and water vapor pressure due to superheated moisture were affected by the extent of thermal and mechanical energy input during extrusion processing.

Smith (1992) found that the cells of extrudates generally changed from spherical to polyhedral shapes when the density increased. The spherical cells tended to be a rapidly set structure of bubble growth, whereas the polyhedral cells resulted from bubbles growing together and reaching an equilibrium structure, with some drawing of material from the cell faces to the cell edges before growth stopped. Shapes of cells in maize foams changed from polyhedral cells to spherical cells when the glass transition temperature, T_g, was elevated (Warburton et al. 1992).

2.4.6 Other Properties

A few theoretical models have been used to analyze the mechanical properties of extrudates such as elastic modulus and fracture strength, which are associated with the foam density and cell wall properties. Most researchers have evaluated mechanical properties of food foams from the standard procedures used for synthetic polymer foams. The standard procedures such as tensile, compression and three-point bending tests using texture testing equipment are commonly used to determine the mechanical properties, which are related to texture characteristics (Hutchinson et al. 1987).

Baer (1964) used a simple power-law relationship between mechanical properties and density for synthetic polymer foams.

$$\sigma \propto \rho^n \tag{2.2}$$

In Baer's result, the power law index was 1.5 for open cell foams of synthetic polymer. However, the power index for waxy maize extrudates was 1.16 (Lourdin et al. 1995).

The understanding of the mechanical properties of solid foams has been enhanced in recent years by the work of Gibson and Ashby (1997). They considered the idealized closed and open cell foams deforming elastically, plastically and by fracture. Theoretically, the solid foam model is given more precisely than the Baer's model:

$$\frac{\sigma_e}{\sigma_w} \propto \left(\frac{\rho_e}{\rho_w}\right)^n \tag{2.3}$$

where σ is the mechanical property, ρ is the density of the foam, subscripted quantities, e and w, indicate the property of extrudates and foam cell wall material, respectively (Gibson and Ashby 1997). The values of n in the power law depend on whether the foams are open cell or closed cell. When σ is the Young's modulus, $n = 3$ for closed cell and $n = 2$ for open cell foams in a compression test. When σ is the fracture strength, $n = 2$ for closed cell and $n = 1.5$ for open cell foams in a compression test. Gibson and Ashby (1997) found that the response of foamed polymers, ceramics and metals obeyed equation (2.3). However, Lourdin and coworkers (1995) found that when σ is the fracture strength or elastic modulus, the power law index of extruded starch was 1.27 or 1.31, respectively. The difference in results between theoretical values and experimental values for extruded starch showed that the mechanical properties of cell wall material play an important role in the behavior of solid foams. Lourdin and coworkers (1995) suggested two possibilities for the difference in the results. One possibility is that the difference between starches with various amylose-amylopectin ratios could cause the difference in the results. The other reason for the difference between predicted and experimental values was a reduction of expansion phenomena when extrudate temperature decreased below Tg, the glass transition temperature, during cooling after extrusion. Although the authors accepted that the latter explanation could only be verified by image analysis, the procedure could be performed on samples with similar density.

The power law indices in a compression and tension test of maize extrudates were close to the predicted values for open cell foams, even though foams were comprised of closed cells. Modulus values in a flexure (or bending) test were much

greater than predicted values (Hutchinson et al. 1987). Warbuton and coworkers (1990) noted that the cell wall properties of extrudates contributed to the differences between observed values and predicted values. When using a model considering the distribution of material between the faces and the edges in extrudate foams, the results for modulus values still indicated higher experimental values for the power law than predicted values.

2.5 Modeling, Control and Optimization

Automation and control of an extruder is difficult because of the strong correlation between multiprocessing variables and nonlinearities, which cause difficulty in modeling of extrusion processing. However, modeling for extrusion processing is necessary for scaling-up, prediction of product transformations, and optimization of the process and quality of products (Mercier et al. 1989).

Initial models based on solving the energy balance showed the possibility of modeling for extrusion processing (Bhattacharya and Hanna 1986; Tayeb et al. 1988a). The estimation of specific energy delivered (SED) for intrinsic viscosity and solubility of corn starch extrudates was considered as a more precise indicator than specific mechanical energy (SME) to measure the severity of extrusion processing (Della Valle et al. 1989). They determined SED, based on the energy balance of the extruder for steady state conditions, considering energy input as well as energy losses.

Modeling for transport phenomena of dough in an extruder is key for successful extruder scale-up and design. A flow model for the reverse screw element provided good predicted results compared to experimental values in maize starch extrusion processing, whereas the model using the Phan-Thien Tanner (PTT) equation did not

(Dhanasekharan and Kokini 2000; Tayeb et al. 1988b). A model for predicting changes in dough rheology under various processing conditions (Mackey and Ofoli 1990) and a model for shearing resistance provided knowledge of changed shear stress depending on each extrusion variable (Qu and Wang 1998). For optimization of process control on extrusion cooking, dynamic response analysis between system variables and processing variables, relative gain array (RGA) technology, response surface method, and regression model have been suggested (Cai et al. 1995; El-Dash et al. 1983; Gogoi and Yam 1994; Lu et al. 1992; Singh and Mulvaney 1994). Modeling of extrusion cooking of barley and other cereals using response surface methodology suggested studying effects of extrusion variables on the product properties and factors affecting the extrusion process (Vainionpää 1991).

2.6 Lysozyme

2.6.1 Properties

Lysozyme dissolves Gram-positive bacteria by cleaving the polysaccharide component of their cell walls so that the high osmotic pressure inside the cell causes bursting of the bacteria (Stryer 1981). Lysozyme, a relatively small enzyme (Mw = 14,400), consists of a single polypeptide chain of 129 amino acids. The lysozyme molecule is cross-linked in four different sites by disulfide bridges formed by the combination of sulfur-containing side chains. Lysozyme loses its activity when all the -S-S- bonds are reduced. Lysozyme showed heat stability in acidic solution at 100°C and had over 50 times more heat stability in pH 6.2 phosphate buffer than in water (Proctor and Cunningham 1988). However, lysozyme was found to rapidly lose its activity in

alkaline solution. The Gram-positive strains such as *Bacillus, Corynebacterium, Lactobacillus, Micrococcus, Sarcina, Sporosarcina, Staphylococcus, and Streptococcus* were shown to be very sensitive to lysozyme (Proctor and Cunningham 1988).

Lysozyme incorporated with EDTA in a cast corn zein film showed inhibition against *Escheria coli*, which was a Gram-negative strain (Padgett et al. 1998). Lysozyme devoid of enzyme activity at pH 6.0 over 90°C exhibited strong bacterial activity against Gram-negative and positive bacteria (Ibrahim et al. 1996a,b). These results demonstrated a relationship between structure and antimicrobial activity that was independent of its catalytic function. Ibrahim (1998) reported that the natural conformational transition of lysozyme at physiological temperatures could switch the antimicrobial specificity of lysozyme under certain conditions. Whey protein film containing lysozyme showed not only effective inhibition for the growth of *Brochothrix thermosphacta,* but also great clarity and tensile strength for the film (Han 2000).

2.6.2 Mass Transfer of Lysozyme in Food Packaging

Antimicrobial packaging as active packaging provides not only a preservative function, which means longer shelf life, but also provides barrier and protective functions. The study of diffusion profiles at low-density polyethylene (LDPE) films of various thickness containing potassium sorbate as an antimicrobial polymer showed the possibility to prolong the shelf life of food products through inhibition of yeast growth (Han and Floros 1998). Different migration models were investigated to control the release rates and migration amounts of potassium sorbate in LDPE film (Han 1997; Han and Floros 2000). Furthermore, diffusion mechanism and diffusion coefficients of

proteins that had different molecular weights were studied in lactitol-based cross-linked hydrogels to verify the factors influencing the release rate of protein delivery systems (Han et al. 2000). Diffusion rates of the proteins increased with increasing temperature from 4 to 25°C, but decreased above 25°C; shrinking of the hydrogel may be the reason.

Mass transfer operations are concerned with the transfer of materials from one stream to another. Mass transfer rate is determined by a driving force due mainly to concentration or partial pressure gradient for material being transferred, and a resistance due to properties of medium and interaction between medium and the diffusing material.

$$\text{Mass transfer rate} = \text{driving force} / \text{resistance} \tag{2.4}$$

Diffusion is the spreading-out of a material into its surrounding. When a concentration gradient for a given component exists in one direction only, its diffusion may be characterized by Fick's first law, which states that

$$N_A = -D_A \frac{dC_A}{dx} \tag{2.5}$$

Where N_A is the mass transfer rate per unit area of section for a substance A, kg· s^{-1}, C_A is the mass concentration (kg·m^{-3}), x is the distance (m), and D_A is called a diffusion coefficient or diffusivity (m^2 s^{-1}) (Lewis 1987). The resistance and driving force are $1/D_A$, (dC_A/dx), respectively.

The fundamental differential equation of diffusion in an isotropic medium is derived from equation (2.5). The migration model obeys Fick's second law as follows:

$$\frac{\partial C_A}{\partial t} = D_A \frac{\partial^2 C_A}{\partial x^2} \tag{2.6}$$

where C_A is concentration of diffusing a substance A in a matrix, t is diffusion time, s, and x is distance from the center of the polymer, m. When diffusion occurs in the x direction, the equation (2.6) states that C_A is a function of x and t in an unsteady state fashion [$C_A = C_A(x, t)$].

Studies about control of the release rates and migration amount of antimicrobial agent from packaging material are required to design and develop effective antimicrobial packaging containers or films. Han (2000) summarized comprehensively information on the release models of an antimicrobial agent from packaging materials in food systems. The mathematical model for diffusion could explain the release profile of an active substance, such as lysozyme or chemical agents from an antimicrobial packaging material into a food product. Also, the models could provide the diffusion profile of real food packaging systems, and predict the period in which the antimicrobial concentration would be maintained above the required concentration to effectively inhibit microorganisms in the food system.

Chapter 3

3.0 Physico-chemical Properties of Extruded Barley/Corn Starch Blend

3.1 Abstract

Barley meal, corn starch and a blend of the two (50:50) were analyzed for physico-chemical properties after extrusion cooking. Dough moisture contents were 25 and 34%, and die temperatures were 120 and 150°C in a co-rotating twin-screw extruder. Cross-sectional, volumetric expansion indices, and the brightness of extruded barley increased with an increase in level of corn starch, whereas the longitudinal expansion index was decreased. Extruded barley meal had a higher elastic modulus than extruded corn starch in bending and compression tests, whereas the barley extrudates had a lower fracture strength in the bending test. The elastic moduli in the bending test were highly correlated to the densities of extrudates ($R^2 = 0.92$). Water solubility and absorption indices of extrudates increased substantially after extrusion processing. These results showed that properties of barley extrudates could be controlled by blending with corn starch and also by changing extrusion processing conditions, in order to obtain a desired quality of extrudate.

Key words: extrusion, corn starch, barley meal, water solubility index, water absorption index

3.2 Introduction

Although barley is the second top-ranking cereal in Canada, it has rarely been used outside the malting and feed industry, its principal users. However, several studies have recently shown that there are many health benefits associated with certain components of this grain. Barley is known for its high content of dietary fiber, which has been shown to lower plasma cholesterol, reduce the glycemic index and reduce the risk of colon cancer.

Recently, extrusion technology has been applied to develop barley products with new functional and nutritional properties. Under an optimized extrusion condition, barley products with increased amount of resistant starch and β-glucan, in its macromolecular form, were created (Huth et al. 2000). Gaosong and Vasanthan (2000) observed that β-glucan in the extruded barley flour had higher solubility than in native flour. It appears that the high temperature and moisture levels during the extrusion cooking influenced the water solubility of β-glucan. Modeling of extrusion cooking of barley and other cereals, using a response surface methodology, illustrated the effects of extrusion variables on the product properties and factors affecting the extrusion process (Vainionpää 1991). Mixtures of barley and other cereals were used in several studies to investigate the effect of the extrusion processing variables on the properties of the final products (Fornal et al. 1987; Kim et al. 1989; Lee et al. 1997, 2000). Berglund and coworkers (1994), using extrusion cooking, developed a reconstituted grain mixture containing barley. Although the extrudate of a blend of rice and barley (50:50) reduced the extrudate densities by 50%, the appearance was similar to that of the 100% rice cereal. They suggested, however, that the quality of barley products still needed to be

improved. Besides the application of barley in breakfast cereal, barley β-amylase was used to increase the maltose content during extrusion processing for pre-gelatinized corn starch in order to replace sucrose and glucose in certain foods (Komolprasert and Ofoli 1991).

Corn starch has been frequently used to study the extrusion cooking process. Corn starch exhibits a thermoplastic behavior, which makes it easy to predict the effect of processing variables; it is also readily available since corn is the major agricultural product in North America.

The research on extruded barley meal, so far, has been limited to determination of the changes of product quality under different extrusion conditions. More thorough research is necessary to understand the relationship between process variables and various properties of extruded barley meal. The objective of this research was to investigate the effects of processing variables, such as blending of barley and corn starch, extrusion temperature, and dough moisture content, and the effect of incorporating corn starch into barley meal on the physico-chemical properties of extruded barley product.

3.3 Materials and Methods

3.3.1 Materials

Corn starch was purchased commercially (BestFoods. Englewood Cliffs. NJ) and Barley (CV. Falcon) was obtained from James Farms Ltd. (Winnipeg, MB). The components of the barley were reported as, starch 61.3% (amylose 23.8%), protein 11.6%, total β-glucans 3.64%, and soluble β-glucans 1.65% (Izydorczyk et al. 2000). Barley was milled using a 3.18 mm screen in an electric hammer mill (Horvick Manufacturing Inc., Fargo, ND). The ratios of barley meal to corn starch were 100:0, 50:50, 0:100.

3.3.2 Extrusion Process

A laboratory scale co-rotating twin-screw extruder (MP 19-25D, APV Baker Inc., England) was used for the research. The screw diameter was 19.0 mm and the length to diameter (L/D) ratio was 25:1. The diameter of the capillary die was 2.2 mm. The feed rate was 3.6 kg/h for all feeding material and screw speed was fixed at 300 rpm. Barrel temperature zone profiles were set to 30/80/110/120/120°C and 30/80/130/140/150°C from feeding zone (zone 1) to die (zone 5) (Fig. 3.1). After extrusion processing, all extrudates were dried in a forced-air injection oven (Blue M Co, Blue Island, IL) at 100°C for 18h. The final moisture contents of dried extrudates were less than 2%. Dried samples were stored in sealed plastic bags at room temperature and used for the analysis of physical properties. Some dry extrudates were ground through the 1mm screen of the Udy Cyclone Mill (Udy Co., Fort Collins, CO) for further analysis.

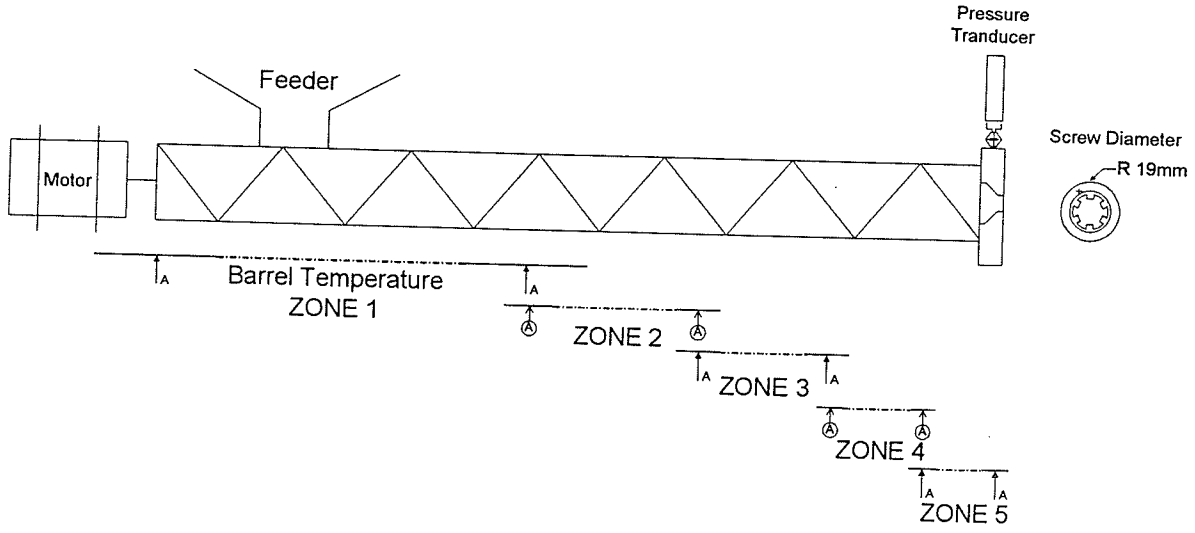

Figure 3.1. Barrel temperature zones in the twin screw extruder

3.3.3 Specific Mechanical Energy Input

Specific mechanical energy (SME) input, which represents used mechanical energy during extrusion processing, was calculated by the following equation (Brent et al. 1997).

$$SME(Wh/kg) = \frac{rpm(test)}{rpm(rated)} \times \frac{\%motorload}{100} \times \frac{motorpower(rated)}{feedrate(kg/h)} \qquad (3.1)$$

where, rpm (test) is 300 rpm, rpm (rated) is 500 rpm, motor power (rated) is 2.2 kW.

3.3.4 Expansion

Cross-section expansion index (SEI), longitudinal expansion index (LEI), and volumetric expansion index (VEI) were evaluated by the following equations described by Alvarez-Martinez and coworkers (1988);

$$SEI = (D_e/D_d)^2 \tag{3.2}$$

$$LEI = \left[\frac{\pi D_d^2}{4}\right] L_{se} \rho_d \left[\frac{1-M_d}{1-M_e}\right] \tag{3.3}$$

$$SEI \times LEI \tag{3.4}$$

where D_e is the diameter of die, which is 2.2mm, D_d is the diameter of extrudate, L_{se} is the specific length of the extrudate defined as the length of the extrudate per unit mass (m kg^{-1}), ρ_d is the density of dough behind the die which can be assumed to be constant and approximately equal to 1,200 kg m^{-3} (Alvarez-Martinez et al. 1988), M_d and M_e are the dough and extrudate moisture contents on a wet basis. Values were presented as an average of more than five readings.

3.3.5 Extrudate Density

Extrudate density was calculated by measuring the weight, diameter, and length of extrudate using a balance and calipers. Values are presented as an average of a minimum of four readings.

$$\text{Density (kg/m}^3\text{)} = \text{mass (kg)} / \text{volume (m}^3\text{)} \quad (3.5)$$

3.3.6 Mechanical Properties

Mechanical properties were determined through three-point bending and compression tests using the TA-XT2i texture analyzer (Texture Technologies Co., Scarsdale, NY). The crosshead speed was set at 1.0 $mm \cdot s^{-1}$. The apparent elastic modulus and fracture strength in compression and three point bending tests for extruded products were evaluated by the following equations (3.6 and 3.7) described by Launay and Lisch (1983). The apparent elastic modulus (E_{ac}) in axial compression of extrudates was determined by the slope (dF/dL) of the linear section of the force-distance curve.

$$E_{ac} = (dF/dL)(L_o/S) \quad (3.6)$$

where L_o is initial height of extrudate (m) and S is the cross-sectional area of the extrudate sample (m^2) Fracture strength (σ_{ac}) in axial compression was calculated as maximum peak in the force-distance curve divided by the extrudate's cross-sectional area. The apparent elastic modulus (E_{ab}) and fracture strength (σ_{ab}) in bending extrudates between two supports were determined as extrudate strand deformed in three-point bending until fracture occurred. The apparent elastic modulus (E_{ab}) in bending test was calculated as follows;

$$E_{ab} = (dF/dL)(64l^3/48\pi D_d^4) \quad (3.7)$$

Where *dF/dL* is the slope of the linear section of the force-distance curve (N/m), *l* is distance between the two supports, 66.5 mm, and D_d is the diameter of extrudate, mm.

The fracture strength (σ_{ab}) in the bending test was calculated as follows:

$$\sigma_{ab} = \frac{8Fl}{\pi D_d^3} \tag{3.8}$$

where F is the maximum force (N) in the force-distance curve. The unit of the apparent elastic modulus and fracture strength is Pa. Each value was presented as an average value of more than eight readings.

3.3.7 Microstructure

Samples were cut with a sharp knife and the cross-section was dyed with black ink. The cross-sections were photographed with SZIII Stereoscopic Microscope (Olympus, Tokyo) at various magnifications.

3.3.8 Water Absorption Index (WAI)/ Water Solubility Index (WSI)

The procedures of Guha and coworkers (1997) were used to determine the WSI and WAI of the extruded products. Dried and ground sample (1g) was suspended in 25mL deionized water in a 50mL capacity centrifuge tube, agitated in a water bath (Blue M Co., Blue Island, IL) at 30°C then centrifuged at 9000 rpm for 20 min using a RC2-B Sorvall Centrifuge (Ivan Sorvall Inc., Newtown, CT). The weight of the remaining gel in a tube was taken as WAI and was expressed as g/g. The supernatant liquid was poured into an aluminum dish and dried at 100°C in a forced air dry oven (Blue M Co.,

Blue Island, IL). The amount of dried solids in the supernatant liquid, expressed as percentage based on initial 1g dry solids, was taken as WSI.

3.3.9 Color

Color measurements were performed using the Hunterlab Colorimeter (Model D25L-2, Hunter Associate Laboratory Inc., Reston, VA). The instrument was calibrated with standard white (L = 92.4, a = -1.2, b = 0.5) and black plates and then was standardized using a standard yellow plate (L = 78.7, a = -3.1, b = 22.6), which was close to the color of extrudates. A rotation procedure (Agblor 1997) was used to determine the color values (L, a, b) of extrudates and raw powder of barley, corn starch, and blends. The native cereal powder was placed in a 2 cm thick layer at the bottom of the sample cell. The dimensions of the sample cell were 10cm x 10cm x 5cm. The extrudate was cut to approximately 5 to 7 cm length and added to the sample cell until the cell was full. The sample cell containing the native cereal product or extrudate (placed on the colorimeter) was rotated a quarter turn for each reading. The color measurements (L, a, b) were recorded after each quarter turn for three turns.

3.3.10 Statistical Analysis

A 3x2x2 full factorial experiment was designed to determine the effect of process variables (3 feed materials with barley meal and corn starch, 2 die temperatures, and 2 dough moisture contents) on expansion indices, color, mechanical properties, WSI, and WAI. All data were analyzed using a general linear model procedure (SAS Version

6.12, SAS Institute Raleigh, NC). The measured property was related to the extrusion processing variables using a general linear model:

$$Y = X_1 + X_2 + X_3 + X_1X_2 + X_1X_3 + X_2X_3 + X_1X_2X_3 \quad (3.9)$$

where Y = the extrudate properties measurement, X_1 is blend effect of barley and corn starch, X_2 is the extrusion temperature (°C), and X_3 is moisture content of the dough (%).

3.4 Results and Discussion

3.4.1 System Variables

Corn starch, barley meal and a blend of both at equal proportions (50:50) were extruded under two different moisture contents of dough and two different extrusion temperatures. Specific mechanical energy (SME) input was between 81 and 289 Wh/kg during the extrusion cooking. The lowest SME input was observed for barley meal whereas the highest was obtained for corn starch. The SME input increased as water content decreased for all feed materials (Table 3.1). The increase of SME input was caused by high dough viscosity at the lower moisture content. The SME input also increased slightly when extrusion temperature decreased. The extent to which starch polymers are melted during extrusion processing appears to be directly related to the SME input. Willett and colleagues (1995) found that melt viscosity of starch decreased with increasing extrusion temperature and moisture content of dough. Vergnes and coworkers (1987) reported that the increasing SME input was positively correlated with the extent of starch degradation during extrusion processing. Smith (1992) observed that corn grit particles remained intact at low SME input but no particle or granule structure remained at high SME input during extrusion processing. Therefore, SME input is considered as an important parameter that controls the properties of extruded products such as expansion, water solubility and absorption, and product texture (Ryu and Ng 2001).

Die pressure is also an important dependent system parameter capable of changing the physical properties of extrudates. The range of die pressure was from 13 to 64 bar during extrusion processing (Table 3.1). Barley meal showed higher die pressure

than corn starch or corn starch/barley meal blend. Die pressure decreased when moisture content of dough and extrusion temperature increased. The melt viscosity of dough affected the die pressure during extrusion processing. Increasing extrusion temperature and moisture content of dough decreased the melt viscosity of dough and consequently decreased the die pressure.

Table 3.1. System variables under the different extrusion process variables

Process variables			System variables	
Feed materials	**Dough M.C.**[a] (%)	**Die temperature** (°C)	**Die pressure** (bar)	**SME input** (Wh/kg)
Corn starch	25	120	43.5 ± 0.5	281 ± 9.2
	34	120	24.0 ± 2.0	169 ± 0.0
	25	150	28.5 ± 1.5	211 ± 1.9
	34	150	13.5 ± 0.5	138 ± 1.9
Barley meal	25	120	63.5 ± 0.5	136 ± 3.7
	34	120	25.0 ± 0.0	88 ± 0.0
	25	150	44.0 ± 1.0	112 ± 1.9
	34	150	20.5 ± 0.5	79 ± 1.8
Corn starch +	25	120	43.5 ± 0.5	218 ± 1.9
Barley meal	34	120	21.5 ± 0.5	134 ± 1.9
(1:1)	25	150	32.0 ± 1.0	184 ± 0.0
	34	150	15.5 ± 0.5	116 ± 1.8

[a] M.C. = moisture content

3.4.2 Expansion

The cross-sectional, longitudinal, and volumetric expansion indices (SEI, LEI, and VEI, respectively) of extrudates varied with the type of feed material, extrusion temperature and moisture content of the dough (Fig. 3.2). Among the three different feed materials, the highest SEI (11.1) was observed for 100% corn starch, whereas the lowest SEI (1.8) was observed for 100% barley meal. The SEI of extruded barley meal blended with corn starch generally had an intermediate value between barley meal and corn starch under all four processing conditions (Fig. 3.2). The SEI of extrudates increased as moisture content of the dough decreased. As the extrusion temperature was increased, the SEI of extrudates decreased. The LEI ranged from 0.31 to 0.82 (Fig 3.2) with the highest values for barley meal under all processing conditions. The highest VEI for barley meal, corn starch, and the blend were observed at 150°C die temperature, 25% moisture content of the dough. The effects of blending, moisture content of the dough and the extrusion temperature did not have a significant ($P < 0.05$) effect on VEI, whereas they were highly significant on SEI and LEI (Table 3.2). This may be due to the low regression coefficient for VEI ($R^2 = 0.5246$). Kim and coworkers (1989) reported that other cereals, such as corn, wheat, rye, sorghum and oat, when blended with barley, contributed to increased expansion of extrudates. The barley blended with corn grit had the greatest cross-sectional expansion index, whereas oats were the least effective in increasing the expansion indices of extrudates. The different levels of dough moisture content and extrusion temperature on expansion of extrudates affected both the vapor pressure, due to steam generation, and the elasticity of molten dough during extrusion processing. Padmanabhan and Bhattacharya (1989) noted that the elasticity of

the molten starch dough and vapor pressure due to superheated moisture mainly affected the expansion of extrudates. High viscosity of the molten dough at low extrusion temperature resulted in less expansion of extrudates because entrapped water vapor remains in the insufficiently relaxed molten dough, which does not allow the vapor to escape (Della Valle et al. 1997). However, the steam effect increased with increasing extrusion temperature. The bubble growth model of Fan and coworkers (1994) explained that high extrusion temperature was associated with a bigger bubble size and the high bubble growth rate was due to increased internal vapor pressure in extrudates. The steam effect can explain the reason for the highest VEI at the high extrusion temperature tested. Padmanabhan and Bhattacharya (1989) found that the steam effect become dominant and disrupted residual elastic effects at high extrusion temperatures. However, there are no reported theories to estimate the effect of moisture content on extrusion expansion. Launay and Lisch (1983) found the LEI correlated negatively to the SEI. They explained that this inverse relation expansion indices were possibly caused by a shear-induced alignment and compression or relaxation of the starch network during extrusion cooking. The moisture content of dough and extrusion temperature were important processing variables. However, they did not have the same effects on the longitudinal and cross-sectional expansion of extrudates.

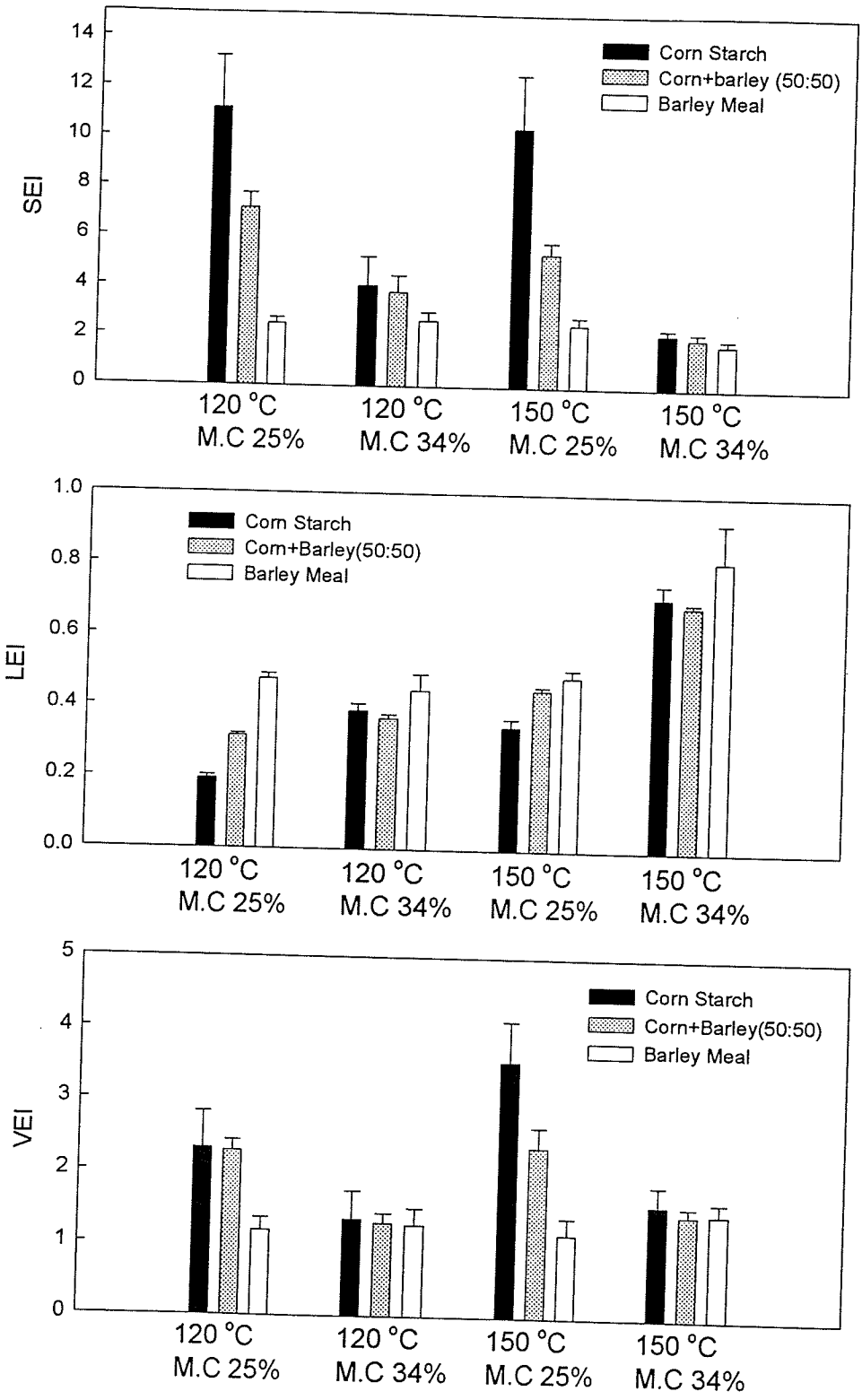

Figure 3.2. Effect of moisture content and extrusion temperature on expansion indices (SEI, LEI, and VEI) of extrudates

Table 3.2. Probability in statistical analysis of density, expansion, color, apparent elastic moduli (E_{ab}, E_{ac}), fracture strengths (σ_{ab}, σ_{ac}), water absorption and solubility indices of extrudates

Variables	SEI[a]	LEI[b]	VEI[c]	Density	Bending		Compression		Color[f]			WAI[g]	WSI[h]
					E_{ab}[d]	σ_{ab}[e]	E_{ac}	σ_{ac}	L	a	b		
F [i]	**0.0001**	**0.0001**	0.0654	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0194**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
MC [j]	**0.0001**	**0.0001**	0.5502	**0.0027**	**0.0001**	0.1954	**0.0001**	**0.0001**	**0.0001**	0.0569	**0.0001**	0.7751	0.6804
T [k]	**0.0012**	**0.0001**	0.0778	**0.0017**	0.0591	**0.0014**	**0.0091**	**0.0002**	**0.0001**	0.8047	**0.0001**	0.9539	0.9513
F * M	**0.0001**	**0.0001**	0.0502	**0.0030**	**0.0001**	**0.0007**	**0.0120**	**0.0001**	**0.0001**	0.1952	**0.0001**	0.1240	0.3350
F * T	0.4547	0.7172	0.4967	0.8662	**0.0040**	**0.0020**	0.2318	**0.0054**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	0.4390
M * T	0.7188	**0.0001**	**0.0154**	**0.0396**	**0.0001**	**0.0008**	**0.0001**	**0.0001**	**0.0248**	**0.0197**	0.2180	**0.0300**	0.3332
F * M * T	0.3862	0.1580	0.4724	0.5680	**0.0183**	**0.0002**	0.6719	**0.0003**	**0.0001**	**0.0018**	**0.0001**	0.9005	0.2066
R^2	**0.9192**	**0.9683**	**0.5246**	**0.7704**	**0.9613**	**0.9049**	**0.8140**	**0.9414**	**0.9999**	**0.9987**	**0.9959**	**0.9611**	**0.9850**

[a] Cross-sectional expansion index [b] Longitudinal expansion index

[c] Volumetric expansion index [d] Apparent elastic modulus (Pa)

[e] Fracture strength (Pa) [f] Color scales: L = brightness (0 = black, 100 = white); +a = red, -a = green; +b = yellow, -b = blue

[g] Water absorption index (g/g) [h] Water solubility index (%)

[i] Feed material [j] Dough Moisture Content (%) [k] Extrusion Temperature (°C)

3.4.3 Mechanical Properties

Mechanical properties of extrudates determined in this study included apparent elastic modulus and fracture strength. The apparent elastic modulus is indicative of the degree of stiffness of extrudates. The apparent elastic modulus values of extrudates ranged from 18 to 125 MPa in compression tests and 247 to 1,400 MPa in bending test (Fig 3.3). Launay and Lisch (1983) reported that the elastic modulus for corn semolina extrudates ranged from 12 to 480 MPa in the compression and > 48 MPa in the bending test. Smith (1992) reported that elastic modulus for wheat starch containing 0 to 67.8% glucose ranged from 50 to 5,000 MPa in the bending test.

The elastic modulus in the compression test decreased as the extrusion temperature increased at 25% moisture content of the dough, whereas there was an opposite effect at 34% moisture content (Fig. 3.3A). The extrudate of 100% barley meal had the greatest elastic modulus in the compression test, whereas corn starch extrudate exhibited the lowest. Similar trends as for compression results were observed during the bending test (Fig. 3.3B). The apparent elastic modulus in the compression test was significantly affected by blending ($P < 0.0001$), dough moisture content ($P < 0.0001$), and extrusion temperature ($P < 0.0091$) (Table 3.2). The apparent elastic modulus in the bending test was significantly affected by blending ($P < 0.0001$) and dough moisture content ($P < 0.0001$).

The fracture strength values of extrudates ranged from 1.0 to 8.4 MPa in compression and 2.3 to 13.2 MPa in bending (Fig. 3.4). Launay and Lisch (1983) reported that the fracture strength for corn semolina extrudates ranged from 1.3 to 2.5 MPa in compression. The fracture strength of extruded barley meal in the compression

test was higher than that of corn starch at 25% moisture content of dough at both extrusion temperatures (Fig. 3.4A). The opposite was observed at 34% moisture content of the dough (Fig. 3.4A). The blended extrudate of barley and corn starch exhibited, however, the lowest fracture strength (2.3 MPa) in the bending test (Fig. 3.4B). The fracture strength in the compression test was significantly affected by blending ($P < 0.0194$), dough moisture content ($P < 0.0001$), and extrusion temperature ($P < 0.0002$). The fracture strength in the bending test was significantly affected by blending ($P < 0.0001$) and dough moisture content ($P < 0.0001$).

The differences between elastic modulus and fracture strength obtained from the bending and compression tests might be related to the difference between the surface and inner cell wall properties of extrudates. Hutchinson and coworkers (1987) explained that the difference in mechanical properties measured in bending and compression tests resulted from the initial collapse of the cell structure and rapid cooling on the surface of extrudates during cooling after extrusion. Owusu-Ansah and coworkers (1983) found that the microstructure of extrudates was related to fracture strength, elastic modulus, and expansion. They reported that porosity, which indicated expansion of extrudates, increased with decreasing moisture content of the dough. The high porosity of extrudates was responsible for decreased fracture strength.

The correlation between density and elastic modulus or fracture strength provides important information about the mechanical properties of extruded foams. A power-law model, which describes the correlation between density and elastic modulus of extrudates, showed that the density was positively correlated to elastic modulus (Fig. 3.5). The correlation between mechanical properties and density of foams was reported

by Gibson and Ashby (1997). They considered idealized closed and open cell foams. Smith (1992) comprehensively summarized several theoretical models to describe the deformation of solid foams of extrudates, which related the mechanical properties to the foam density and the cell-wall properties. The mechanical properties and densities of the foam normalized by the wall value as a power-law is illustrated by the following equation:

$$\frac{\sigma_e}{\sigma_w} = C\left(\frac{\rho}{\rho_w}\right)^n \tag{3.1}$$

where σ is fracture stress or Young's modulus, ρ is density, C is a constant, subscripts e and w are extrudate and wall material, respectively, and n is index of power law. Gibson and Ashby (1997) reported that the power-law indices for synthetic polymer foams were $n = 3$ for closed cell and $n = 2$ for open cell, where σ is the Young's modulus in equation (3.1). The values of σ_w and ρ_w were not constant, neither could they be measured easily in this experiment. Therefore, the simple power law equation (3.2) introduced by Baer (1964) was considered for this study.

$$\sigma \approx \rho^n \tag{3.2}$$

where σ is Young's modulus. Values of $n = 1.43$ in compression tests, and $n = 1.66$ in bending tests were obtained from experimental results using equation (3.2) (Fig. 3.5).

The densities of extrudates ranged from 268 to 734 kg/m^3 and the values were significantly affected by feed material ($P < 0.0001$), extrusion temperature ($P < 0.0017$), and dough moisture content ($P < 0.0027$) (Table 3.2). Lourdin and coworkers (1995) studied mechanical properties of maize, potato, and pea starch extrudates. In the case of a three-point flexural bending test, mechanical properties of synthetic solid foams were obtained with $1.5 < n < 2$ using equation (3.2) (Lourdin et al. 1995). In this study, the index power values of extrudates were close to the values for foam of synthetic polymers. These results showed that the mechanical properties of cell wall material of barley or/and corn starch extrudates exhibited similar behavior to the solid foams of synthetic polymers. The extrudates followed the synthetic polymer model.

The regression coefficient (R^2) in the bending test was 0.92 between density and elastic modulus, while in the compression test it was only 0.54. The low value might be caused by the distribution of irregular air cells in extrudates of cylinder types.

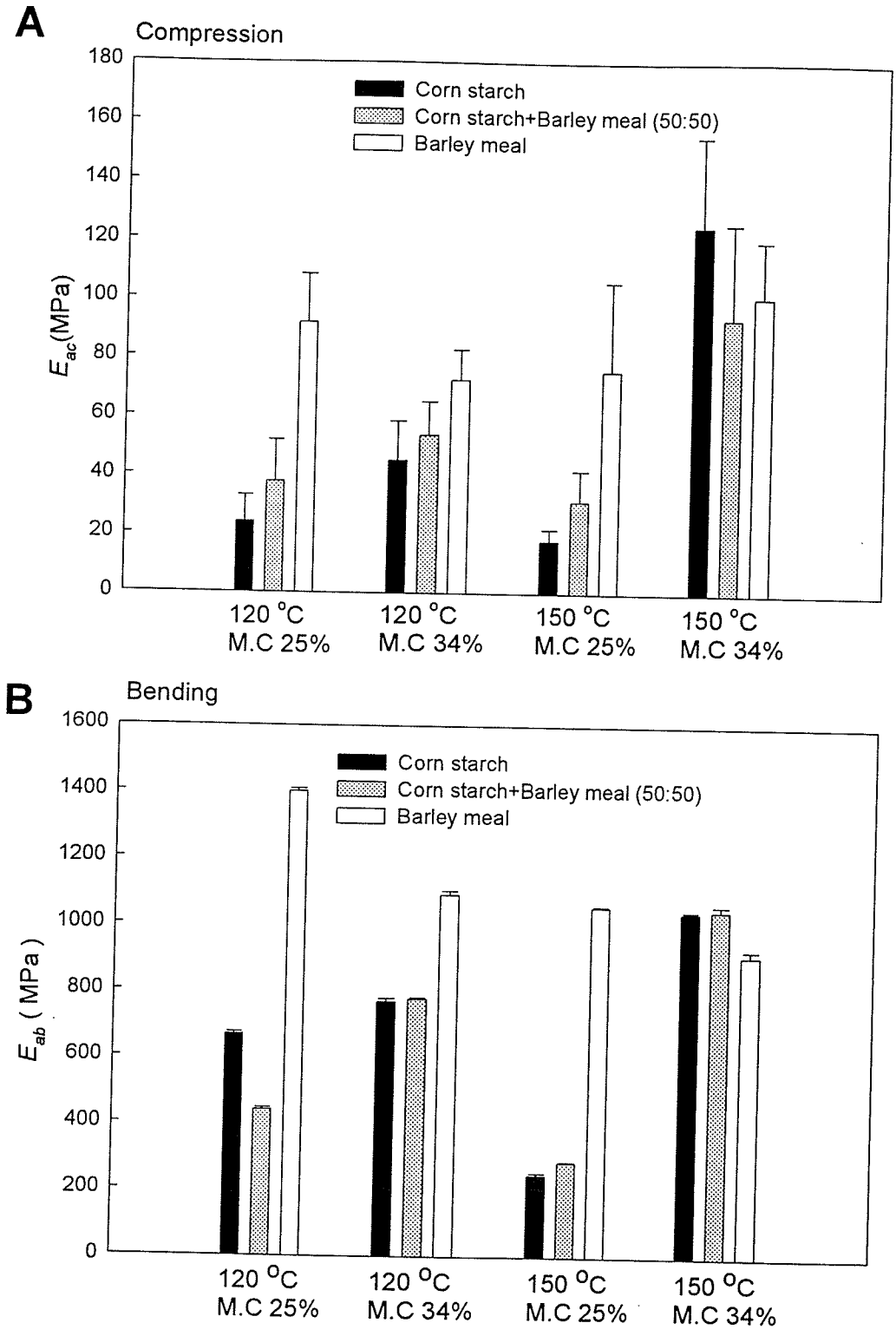

Figure 3.3. Apparent elastic modulus of extrudates at 120 and 150 °C, 25 and 34% moisture content of dough in compression (A) and bending (B) tests

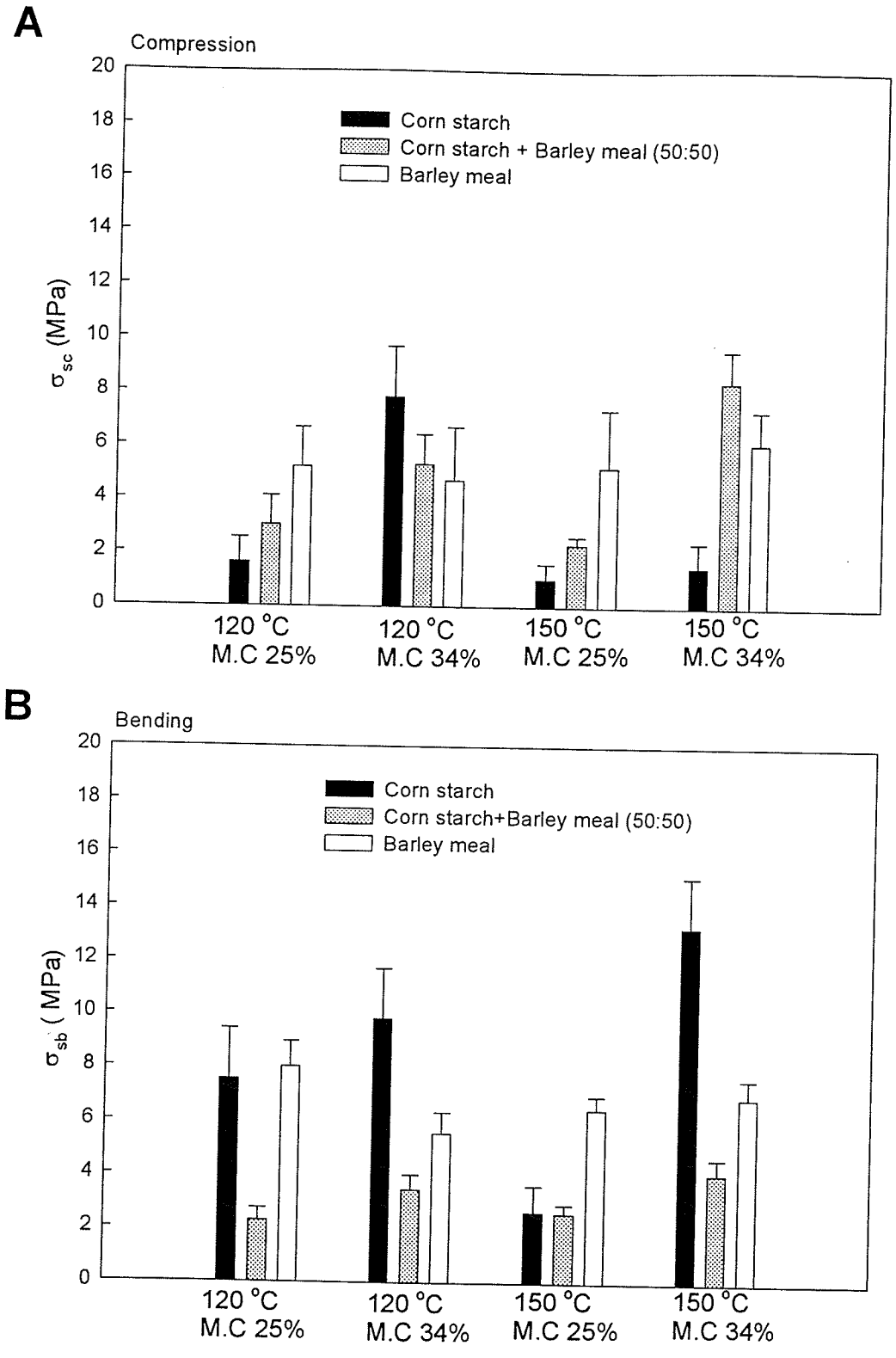

Figure 3.4. Fracture strength of extrudates at 120 and 150 °C, 25 and 34% moisture content of dough in compression (A) and bending (B) tests

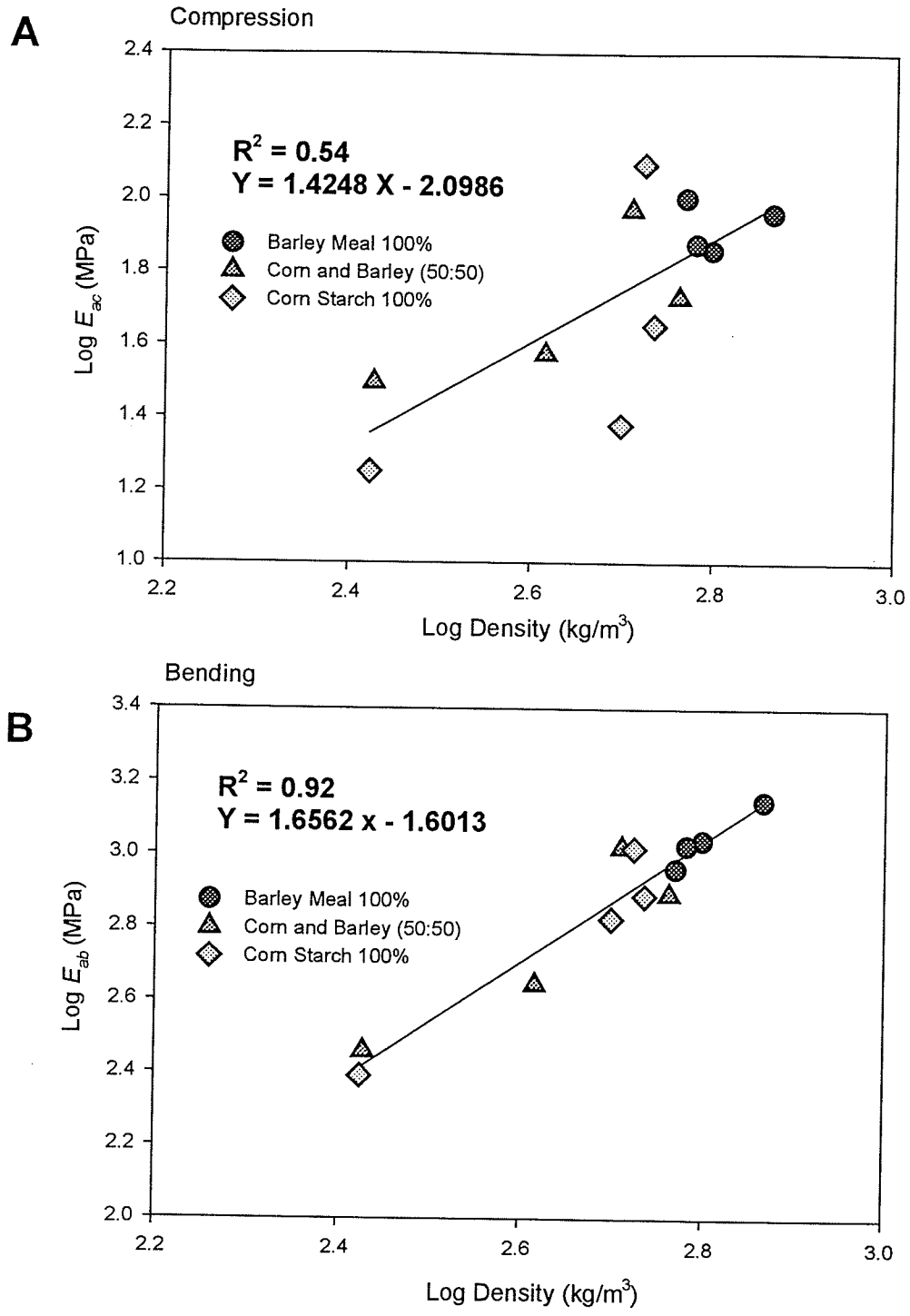

Figure 3.5. Variation of apparent elastic modulus with density for extrudates of barley meal, corn starch, and blend of barley meal and corn starch in compression (A) and bending (B) tests

3.4.4 Microstructure

Stereoscopic micrographs showed that corn starch extrudate had the largest cross-sectional area (Fig. 3.6). Microstructure of the barley extrudates indicated smaller air cells and thicker cell walls compared to that of the corn starch extrudates. Starch content was shown to be essential to develop expansion of products during extrusion processing. Increasing starch content was resulted in greater expansion of the cross-sectional surface area. Rheological properties and viscosity of feed materials might affect the size of individual air cells in the extrudate. Melted corn starch may be a more suitable polymer than barley meal to hold air bubbles, generated by vapor pressure due to superheated moisture during extrusion processing. Consequently, the molten barley meal dough would expand less in cross-sectional direction and form smaller air cells in the extrudate than that of pure corn starch. This, however, needs to be further investigated and verified.

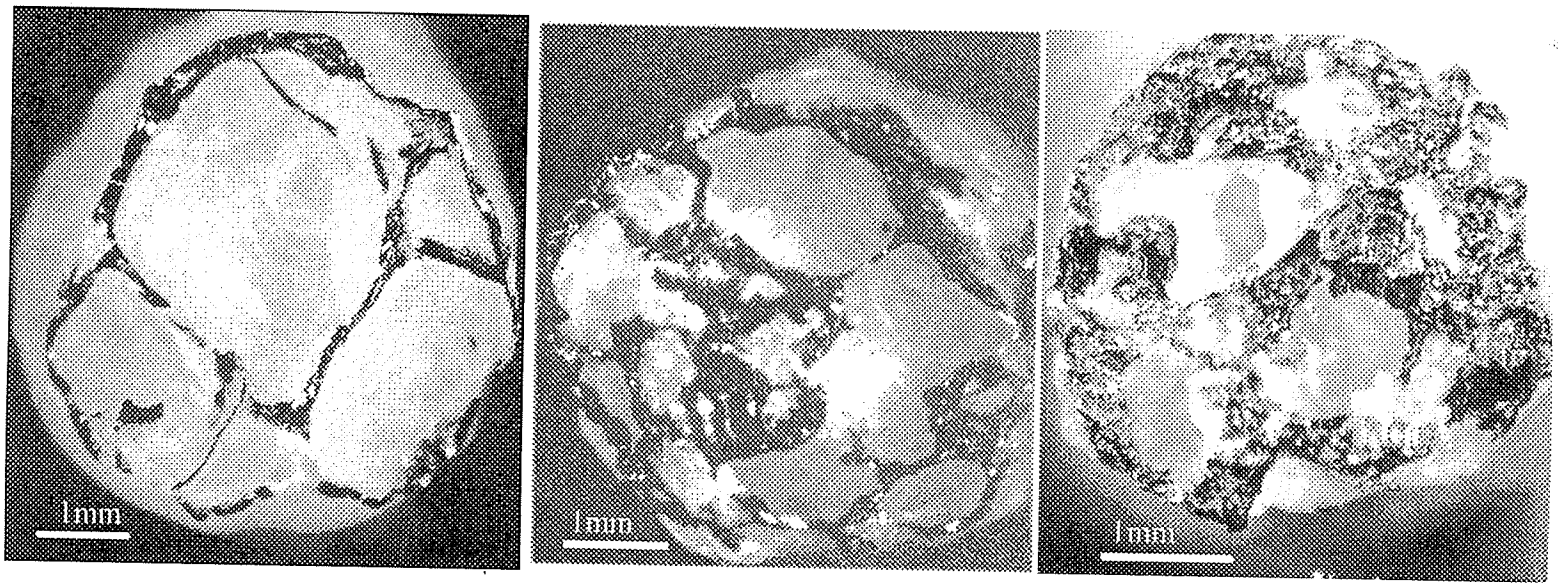

Figure 3.6. Microstructure of extruded corn starch, barley meal, and the blend of barley meal and corn starch

3.4.5 Water Solubility Index (WSI)/ Water Absorption Index (WAI)

Water solubility and absorption indices are important parameters in estimating the functional properties of extrudates as food ingredients. These parameters are important, for example, for dried pet food, which has to be rehydrated with water prior to use. WAI is related to the ability of a product to imbibe and hold water in the cross-linked extruded starch structure, whereas WSI is related to the quantity of break down of starch molecules due to their macromolecular degradation and fragmentation (Mercier et al. 1989).

Corn starch had the highest WSI (44%) after extrusion processing, even though it had the lowest WSI (0.8%) before extrusion cooking (Table 3.3). Overall, the WSI of all feed materials increased sharply after extrusion processing. The WSI of corn starch increased to a greater extent than that of the barley meal after extrusion processing (Table 3.3). The WSI of extruded barley meal blended with corn starch did not increase as much as that of extruded corn starch. Dietary fiber in barley might restrict the degradation of corn starch by affecting the shear force during extrusion processing or by holding the fragments of corn starch molecules together. WSI and WAI were significantly affected by the type of feed materials ($P < 0.0001$) (Table 3.2). In experimental trials, both the WSI and WAI increased after extrusion cooking but the difference in the extrusion processing variables, such as temperature and moisture content, did not significantly affect their values. However, Guha and coworkers (1997) reported that more severe extrusion conditions, such as very low dough moisture content, higher screw speed and higher extrusion temperature, increased the WSI, as a result of greater fragmentation of starch molecules.

Table 3.3. Effect of process variables on water absorption and solubility indices of extrudates

Feed materials	Process variables		Water Absorption Index (g/g)	Water Solubility Index (%)
	Dough M.C.[a] (%)	Die temperature (°C)		
Raw corn starch	-	-	1.00	0.82
Extruded corn starch	25	120	7.46	42.83
	34	120	7.07	41.62
	25	150	6.28	37.80
	34	150	6.44	43.92
Raw barley meal	-	-	3.53	7.27
Extruded barley meal	25	120	5.29	10.21
	34	120	5.39	10.16
	25	150	5.45	9.64
	34	150	5.91	10.09
Raw corn starch +barley meal (50:50)	-	-	2.99	4.44
Extruded corn + barley blend (50:50)	25	120	7.32	12.49
	34	120	6.80	11.98
	25	150	7.62	15.22
	34	150	7.65	12.99

[a] Moisture content

3.4.6 Color

The brightness (L) was the highest for corn starch, followed by the blend and barley meal (Fig. 3.7). The extrusion process appeared to decrease the brightness of extrudates. The highest 'a' values, which represent redness at (+) level, were observed for barley meal. The 'a' values of 100% barley and barley blended with corn starch increased after extrusion cooking. However, the extrusion cooking of 100% corn starch decreased the 'a' values of extrudates. The 'b' value (indicating yellowness) increased after extrusion processing. The blending, extrusion temperatures, and moisture contents of the dough significantly affected the L values and b values ($P < 0.01$). However, 'a' values were only significantly affected by blending ($P < 0.01$).

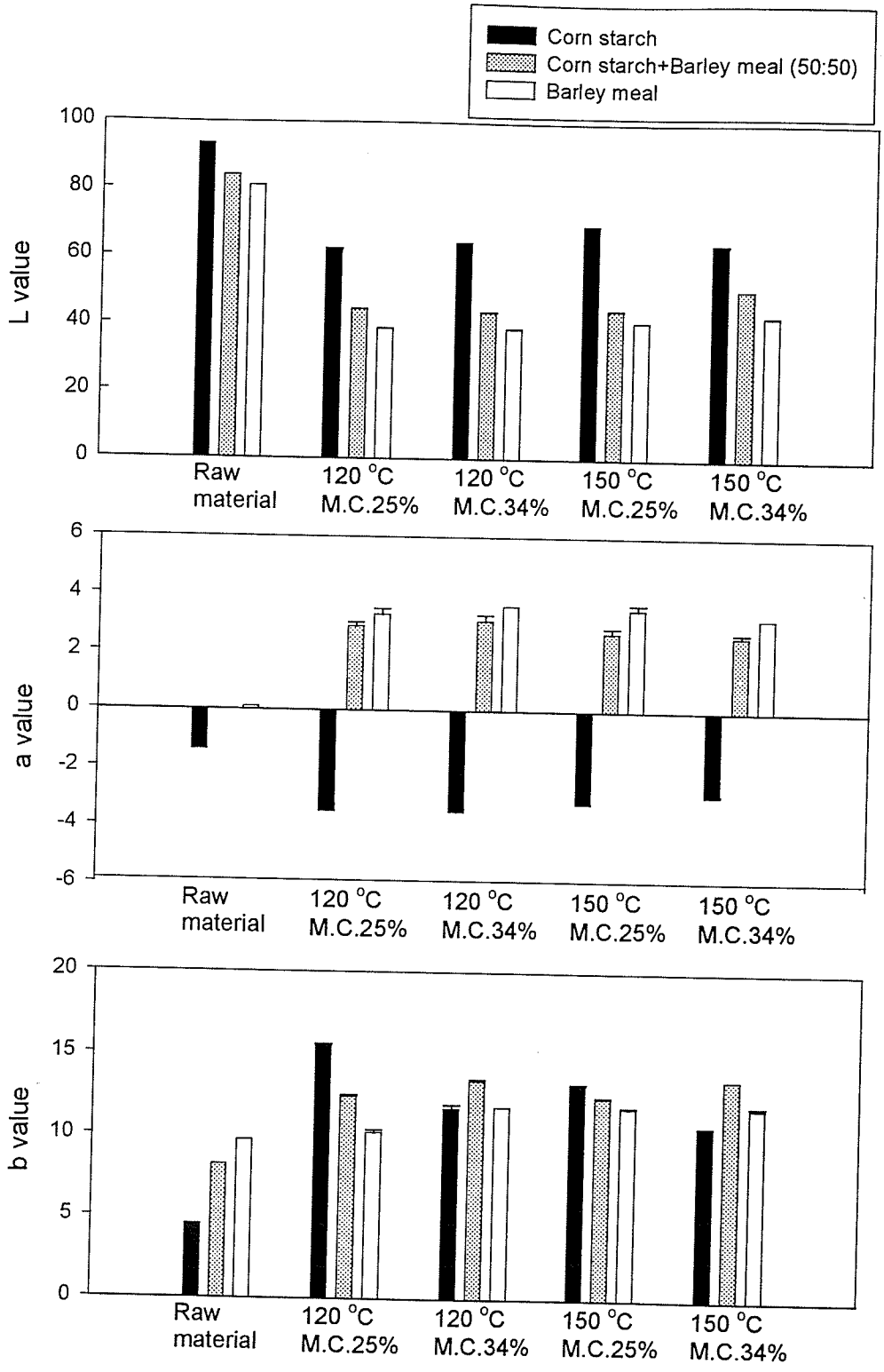

Figure 3.7. Color values (L, a, b) of extrudates affected by extrusion temperature and moisture content of dough

3.5. Conclusions

Addition of corn starch to barley meal improved expansion, color, and texture characteristic of barley extrudates. Addition of corn starch had more significant effects on such properties of barley extrudate as cross-section expansion, density, color, water solubility, and water absorption of extrudates than changes in the extrusion temperature and dough moisture content. Elevated extrusion temperature increased expansion indices, elastic modulus, and water solubility of the blend. However, it caused some darkening of the extrudates. Decrease of moisture content of the dough during extrusion cooking caused more expansion of extrudate products under both extrusion temperatures. The elastic modulus of extruded blend in the compression and bending tests significantly decreased with decreasing moisture content of the dough. This result suggests that blending barley with other cereal material and/or optimization of extrusion processing conditions can produce more acceptable barley products.

Chapter 4

4.0 Development and Characterization of Extruded Pea Starch Containing Lysozyme as an Antimicrobial and Biodegradable Packaging Material

4.1 Abstract

Extrudates containing 99% pea starch and 1% lysozyme were produced under various extrusion conditions (high and low shear screw configurations, 30-40% moisture contents, 70-150°C die temperatures). Differential scanning calorimetry (DSC) showed that extruded pea starches were completely gelatinized above 90°C die temperature. Intrinsic viscosity, paste properties, water solubility and water absorption properties of pea extrudates indicated extensive degradation of starch molecules under severe extrusion conditions (high temperature, low moisture content, and high shear). The expansion, density and mechanical properties of extrudates were significantly changed by altering processing variables. Elastic modulus and fracture strength of the extrudates were highly correlated in a power-law fashion to relative density. Up to 48% of the initial lysozyme activity was recovered from the extruded pea starch matrix. Diffusion coefficient, D, of lysozyme through high-density extruded matrix ($D = 1.23 \times 10^{-9}$ m^2/s) was less than that through an extrudate of low-density ($D = 6.45 \times 10^{-9}$ m^2/s). Release profiles of lysozyme and densities of extrudates were controllable by altering extrusion conditions. The antimicrobial activity of released lysozyme was confirmed through the formation of an inhibition zone for *Brochotrix thermosphacta* on agar plates. Extruded pea starch matrix containing lysozyme has potential application as an edible and biodegradable packaging material with antimicrobial activity.

Key words: pea starch, lysozyme, extrudates, diffusion coefficient, mechanical properties.

4.2. Introduction

In recent years, synthetic packaging materials have caused environmental pollution upon disposal. Although many governments have implemented policies regarding recycling of the waste of synthetic packaging materials, there has been very limited success in reducing the waste. Biodegradable and edible packaging materials made with biopolymers (e.g. starch, protein, and cellulose) have the potential to reduce packaging waste and increase the use of various agricultural crops.

Starch is an abundant and functional biopolymer obtained from many renewable resources, such as cereals, legumes and tubers. Starch is not only an excellent energy source in human food, but is also an important component that affects the physical properties of foods (Hoseney 1994). Intact starch granules give three types of X-ray patterns (designated A, B, and C). Pea starch displays the C pattern, which is an intermediate form arising from mixtures of the A and B type (Zobel 1992). Pea starch demonstrates strong gel forming and film forming properties, which are essential to manufacture a thin film with a strong mechanical barrier property (Blenford 1994).

One application of extrusion technology is in the manufacture of synthetic or biopolymer packaging materials. During extrusion processing of starch, the combination of shear, heat and plasticizer produces a molten thermoplastic material after disruption of the native crystalline granular structure (Averous et al. 1991; Riaz 1999; Willet et al. 1995). The molten starch can then be used for creating foam as is done with melted synthetic plastic foams. The extent of the gelatinization and degradation of starch granules depends upon the extrusion processing conditions and the source of the starch (Chinnaswamy 1993; Colonna et al. 1985; McPherson et al. 2000). Barron and coworkers (2000, 2001) studied the differences between mechanical and thermal energy

effects on pea starch degradation and gelatinization by using a Rheoplast®, which is similar to an extruder and used to simulate thermomechanical processing. They found that an increase of shear energy input caused intensive starch degradation. Some research related to the manufacture of starch films has been reported in the literature. Thin films (≈2μm) made from maize grit using extrusion technology were more homogeneous when made at high than at low temperatures (Warburton et al. 1993). High amylose native starch provided stronger and more flexible films than low amylose starch (Nisperos-Carriedo 1994). Riaz (1999) found that water and shear levels had to be controlled depending on the type of starch in order to produce a less brittle starch based packaging material. Blending of biodegradable polyesters with wheat starch improved the film's water resistance (Averous et al. 2001). Multilayer films based on wheat starch and various biodegradable polyesters were produced using co-extrusion or compression molding (Martin et al. 2001). An intermediate layer blended with polyesters and wheat starch in three-layer films improved the adhesion properties of the films by up to 50%, while mechanical properties were improved slightly compared to starch film without polyesters.

Starch-polyethylene films made by blending corn starch with polyethylene showed potential as barriers to oxygen, moisture, and mechanical force (Kim and Pometto 1994). However, the blends of natural and synthetic polymers are not completely biodegradable (Krochta and De Mulder-Johnston 1997).

Recently, antimicrobial agents, such as lysozyme or nisin, have been incorporated into packaging films. Lysozyme dissolves Gram-positive bacteria by cleaving the polysaccharide component of their cell walls and high osmotic pressure

inside the cell causes bursting of the bacteria (Stryer 1981). Ibrahim and coworkers (1996 a, b) found that the lysozyme heated either at 80°C, pH 7.0 or over 90°C and pH 6.0, and devoid of enzyme activity, exhibited strong antibacterial activity against Gram-negative and Gram-positive bacteria. Corn zein protein film containing lysozyme combined with EDTA increased the inhibitory effect of films against *E.coli* (Padgett et al. 1998). Immobilized lysozyme on an ethylmethacrylate film showed maximum adsorption efficiency on the film at pH 7.0 and immobilization efficiency on the film increased with lysozyme concentration up to 1.0 mg/mL (Kacar and Arica 2001).

The objectives of this research were to determine physicochemical properties of extruded pea starch and the activity of antimicrobial agent (lysozyme) under different extrusion conditions. The ultimate goal was to develop bioactive and biodegradable pea starch-based packaging material using the extrusion technology.

4.3 Materials and Methods

4.3.1 Materials

Yellow pea starch (*Pisum sativum L. Miranda*) was obtained from Parrheim Foods (Portage la Prairie, MB) and lysozyme (Pharmaceutical grade) was donated by Innovatech (Abbotsford, BC). The composition of the commercial yellow pea starch (amylose: amylopectin = 35: 65) was moisture 8.0±1%, protein 1.0%, fat <0.1%, ash <0.2%, total carbohydrates >98.0% (analysis provided by Parrheim Foods). The ratio of yellow pea starch blended with lysozyme was 99:1 (w/w).

4.3.2 Extrusion Process

A laboratory scale co-rotating twin-screw extruder (MP 19-25D, APV Baker Inc, Peterborough, GB) was used for the research. The screw diameter was 19.0mm and the length to diameter ratio was 25:1. The diameter of the capillary die was 2.2 mm. The two screw configurations are given as follows:

High Shear Screw Configurations	Low Shear Screw Configurations
8D Feed Screw	8D Feed Screw
1½D 30° forward paddle	1½D 30° forward paddle
6D Feed Screw	6D Feed Screw
¼D Paddle	¼D Paddle
1D Single Lead Screw	1D Single Lead Screw
½D 60° Forward Paddle	½D 60° Forward Paddle
½D 60° Reverse Paddle	½D 60° Reverse Paddle
1D Single Lead Screw	1D Single Lead Screw
¾D 60° Forward Paddle	**1¼D 60° Forward Paddle**
1D Single Lead Screw	1D Single Lead Screw
½D 60° Forward Paddle	½D 60° Forward Paddle
1D 60° Reverse Paddle	**½D 60° Reverse Paddle**
2D Single Lead Screw	**2D Feed Screw**
1D Single Lead Screw	1D Single Lead Screw

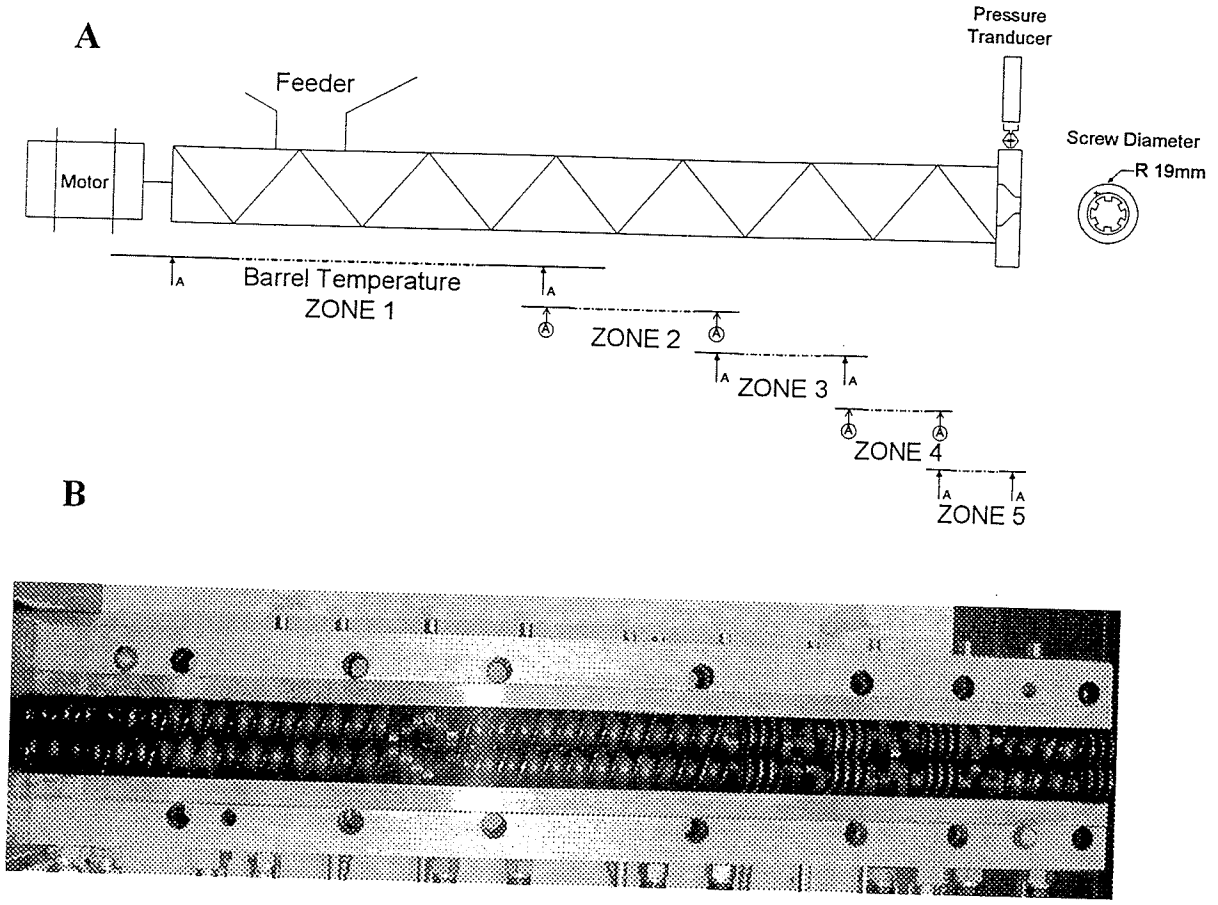

Figure 4.1. Barrel temperature zones (A) and low screw configurations (B) in the twin-screw extruder

The feed rate was 3 kg/h and screw speed was fixed at 300rpm. Barrel temperature zone profiles were set to 30/70/70/70/70°C, 30/80/80/90/90°C, 30/80/110/120/120°C, or 30/80/130/150/150°C from feeding zone (zone 1) to die (zone 5) (Fig. 4.1). Feed moisture contents during extrusion were 30, 35, or 40%. The treatment was three-factorial design with a total of 24 treatments. Three replicates of the 24 treatments were carried out but one sample (low shear, 120°C, 35% moisture content), due to an extrusion operation error, was eliminated. The research was conducted following a completely randomized design. After extrusion processing, extrudates were dried in a

Moffat forced air convection oven (Deltarex Canada Inc, Orillia, ON) at 39°C for 120 h. The final moisture contents of dried extrudates were 8±2%. Dried extrudates were stored in sealed plastic bags at room temperature and used for the analysis of physical and mechanical properties. Some dry extrudates were ground through the 100μm screen of Udy Cyclone Mill (Udy Co., Fort Collins, CO) for further analysis.

4.3.3 Specific Mechanical Energy Input

Specific mechanical energy (SME) input, which represents the mechanical energy used during extrusion processing, was calculated by the following equation (Brent et al. 1997).

$$SME(Wh/kg) = \frac{rpm(test)}{rpm(rated)} \times \frac{\%motorload}{100} \times \frac{motorpower(rated)}{feedrate(kg/h)} \qquad (4.1)$$

where rpm (test) is 300 *rpm*, *rpm* (rated) is 500 *rpm*, and motor power (rated) is 2.2 *kW*.

4.3.4 Differential Scanning Calorimetery

Differential scanning calorimetry was used to determine gelatinization enthalpy and temperature of native and extruded pea starches. DSC experiments were performed using a Dupont 9900 thermal analyzer (Wilmington, DE) equipped with a DSC cell base (Dupont 910). Indium was used as a reference standard. All samples were dried in a vacuum drying oven (Napco Scientific Co, Tualatin, OR), at 45°C for 24h. Melting of native pea starch was determined at various moisture contents (20 – 70%) between 20 and 200°C at a rate of 10°C min^{-1}. Samples with specific moisture contents were prepared directly in pans by addition of a defined quantity of water to native pea starch.

Thermal properties of extruded pea starches were determined at 60% moisture content. Peak gelatinization temperatures in endotherms of the samples were indicated as T_P.

4.3.5 Intrinsic Viscosity

All samples were dried in a vacuum drying oven (Napco Scientific Co, Tualatin, OR), at 45°C for 24h. The dried native or extruded pea starch powder (0.25g) were dissolved in 0.5N NaOH solution (100 mL) by heating and stirring for 30 min. Macromolecular degradation was evaluated by intrinsic viscosity values. Measurements were performed at 22°C in 0.5N NaOH using an Ubbelohde capillary viscometer (solvent elution time: 86.2 s). The concentration range of samples was 0.137 to 0.25% (w/v).

4.3.6 Pasting Properties of Native and Extruded Pea Starch

Pasting profiles of native and extruded starches were examined using a Rapid Visco Analyzer (RVA-4, Newport Scientific, NSW, Australia). Native or extruded starch samples (3.0 g, d.w.b.) were placed into an RVA canister equipped with a paddle. Ethanol (1.0 g) and 25 mL of deionized water was added to the canister. The following heating profile was used: hold at 25°C for 2 min, heat to 90°C at 13°C min^{-1}, hold at 90°C for 3 min, cool to 25°C at 13°C min^{-1}, and hold at 25°C for 5 min. The paddle speed was set at 960 rpm for the first 10 s and then 160 rpm for the remainder of the analysis.

The Newport Scientific information manual (1998) provide a brief description on the interpretation of pasting curves from starch-based samples tested in RVA. Peak viscosity occurs at the equilibrium point between swelling and polymer leaching, which

causes an increase in viscosity, and rupture and polymer alignment. The peak viscosity indicated the water-binding capacity of the starch or mixture. During the hold period of the RVA test, the sample is subjected to a period of constant high temperature (90 °C). This period is commonly accompanied by a breakdown in viscosity to a holding strength, hot paste viscosity or trough. Setback is sometimes measured as the difference between final viscosity and peak viscosity, rather than as the difference between final viscosity and holding strength.

4.3.7 Water Absorption Index (WAI) and Water Solubility Index (WSI)

The procedures of Guha and colleagues (1997) were used to determine WSI and WAI of the extruded products. Dried and ground sample (1g) was suspended in 25mL deionized water in a 50mL capacity centrifuge tube and agitated in a water bath (Blue M Co., Blue Island, IL) at 30°C and then centrifuged at 9000 rpm for 20 min using an RC2-B Sorvall Centrifuge (Ivan Sorvall Inc., Newtown, CT). The weight of the remaining gel in the tube was reported as WAI and was expressed as g/g. The supernatant liquid was poured into an aluminum dish and dried at 100°C in a forced air-dry oven (Blue M Co., Blue Island, IL). The amount of dried solids in the supernatant liquid, expressed as percentage based on initial 1g dry solid sample, was reported as WSI.

4.3.8 Expansion

Cross-section expansion index (SEI), longitudinal expansion index (LEI), and volumetric expansion index (VEI) were evaluated by the following equations described by Alvarez-Martinez and coworkers (1988);

$$SEI = (D_e/D_d)^2 \tag{4.2}$$

$$LEI = \left[\frac{\pi D_d^2}{4}\right] L_{se} \rho_d \left[\frac{1-M_d}{1-M_e}\right] \tag{4.3}$$

$$VEI = SEI \times LEI \tag{4.4}$$

where D_e is the diameter of the die (2.2 mm), D_d is the diameter of extrudate, m, L_{se} is the specific length of the extrudate defined as the length of the extrudate per unit mass (m kg^{-1}), ρ_d is the density of dough behind the die which can be assumed to be constant and approximately equal to 1,200 $kg \cdot m^{-3}$ (Alvarez-Martinez et al. 1988), M_d and M_e are the dough and extrudate moisture contents on a wet basis. Values are presented as an average of at least six readings.

4.3.9 Extrudate Density

Extrudate density was calculated by measuring weight, diameter, and length of extrudate using a balance and calipers. Values are presented as an average of a minimum of four readings.

$$\text{Density } (kg/m^3) = \text{mass } (kg) / \text{volume } (m^3) \tag{4.5}$$

4.3.10 Solid Density

The model 1305 multivolume gas pycnometer (Micrometritics Instrument, Norcross, GA) with helium gas was used to determine the volumes of the solid portion of extruded samples. The pycnometer operated on the principle of gas displacement to measure the volume. The extrudate volume was calculated from the equation:

$$V_s = V_c + \frac{V_a}{1 - \frac{P_2}{P_3}} \tag{4.6}$$

where V_s is the volume of the solid portion of extrudate (mL), V_c the volume of sample cell holder (mL), V_a the added volume of helium gas (mL), P_2 the pressure reading after pressurizing the cell (Pa), and P_3 the pressure reading after adding V_a (Pa). Solid density is defined as the ratio of the extrudate sample weight to the volume of the solid portion of the extrudate.

4.3.11 Mechanical Properties

Mechanical properties were determined through three-point bending using the TA-XT2i texture analyzer (Texture Technologies Co., Scarsdale, NY) and compression tests using the Lloyd instruments (Omnitronix Ltd, Fareham, GB). The crosshead speed of both instruments was set at 1.0 mm s^{-1}. The apparent elastic modulus and fracture strength in compression using the Lloyd instrument were evaluated by the equations described by Launay and Lisch (1983). The apparent elastic modulus and fracture strengths in the three point bending test were evaluated by a modified equation (Jackson and Wirtz 1983; Timoshenko and Young 1968) of the ASTM standard D5934 (ASTM 1997). The apparent elastic modulus (E_{ac}) in axial compression of extrudates was determined by the slope linear section (dF/dL) of the force-distance curve.

$$E_{ac} = (dF/dL)(L_o/S) \tag{4.7}$$

where L_o is initial height of extrudate (m) and S is the cross-sectional area of the extrudate sample (m^2). Fracture strength (σ_{ac}) in axial compression was calculated as the maximum peak in the force-distance curve divided by the extrudate's cross-sectional area of the extrudates. The apparent elastic modulus (E_{ab}), and fracture strength (σ_{ab}) in bending extrudates between two supports were determined as the extrudate strand deformed in three-point bending until fracture occurred. The apparent elastic modulus (E_{ab}) in bending was calculated as follows;

$$E_{ab} = (dF/dL)(64l^3/48\pi D_d^4) \tag{4.8}$$

where, dF/dL is the slope of the linear section of the force-distance curve (N/m), l is distance between the two supports (m) and maintained at more than ten times of ratio of longitudinal length to cross-section diameter of sample, D_d is the diameter of extrudate (m). The fracture strength (σ_{ac}) in the bending test was calculated as follows;

$$\sigma_{ab} = \frac{8Fl}{\pi D_d^3} \tag{4.9}$$

where F is the maximum force in the force-distance curve (N), l is the distance between the two supports (m). Each value is presented as an average value of more than ten readings. The unit of the apparent elastic modulus and fracture strength is Pa.

4.3.12 Color

Color measurements were performed using Hunterlab Colorimeter (Model D25L-2, Hunter Associate Laboratory Inc., Reston, VA). The instrument was calibrated with standard white (L = 92.4, a = -1.2, b = 0.5) and black plates and then was

standardized using a standard yellow plate (L = 78.7, a = -3.1, b = 22.6), which was close to the color of extrudates. A rotation procedure (Agblor 1997) was used to determine the color values (L, a, b) of native and extruded pea starch. The native pea starch powder was placed in a 2 cm thick layer at the bottom of the sample cell. The dimensions of the sample cell were 10cm x 10cm x 5cm. The extruded pea starch was cut to approximately 5 to 7cm length and placed in the sample cell until the cell was full. The sample cell containing the native or extruded pea starch (placed on the colorimeter) was rotated a quarter turn for each reading. The color measurements (L, a, b) were recorded after each quarter turn for three turns.

4.3.13 Assay of Lysozyme Activity

A modification of the method developed by Weaver and Kroger (1977) was used to assay lysozyme activity. The activity of lysozyme was determined by monitoring the decrease in turbidity of a suspension of *Micrococcus lysodeikticus* cells (Sigma Chemical Co, St. Louis, MO) at 450 nm using a spectrophotometer (Pharmacia Biotech, Cambridge). The suspension of heat-killed *Micrococcus lysodeikticus* cells was prepared in 0.066M phosphate buffer (pH 6.2) and incubated at 30°C for 30 min. Lysozyme solution (0.002%) was used as a standard. To release lysozyme in extrudates, the 2% extrudate solution containing 340 units of porcine pancreas α-amylase (Sigma Chemical Co, St. Louis, MO) was shaken on a rotary shaker (Fermentation Design Inc, Allentown, PA) at 200 rpm at 22°C for 12 h. The released lysozyme solution, 100μL, was mixed with 2.9 mL of *Micrococcus lysodeikticus* suspension in a cuvette, and the absorbance was read at 450 nm. After one minute to permit attainment of a steady state,

absorbance readings were taken every 15 s for 2min to be used for calculation of sample slope. Lysozyme activity was calculated from the following equation:

% Activity of lysozyme = (sample slope / lysozyme standard slope) x 100 (4.10)

4.3.14 Diffusion Coefficient Estimation

Pea starch extrudates with four different densities were selected to determine release rate profiles of lysozyme since the released lysozyme form extruded containers shows the antimicrobial activity. The extrudates were placed in 0.066M phosphate buffer, pH 6.2 at 22°C. The volume of the buffer solution was maintained at more than fifty times that of the extrudates to maintain infinite diffusion conditions. The extrudates in the buffer solution were shaken on a shaker (Fermentation Design Inc., Allentown, PA) at 50 rpm. The release rate of lysozyme in extrudates was determined by the lysozyme activity assay method (4.3.13) as a function of time. The equation of Crank (1975) for diffusion from a cylinder in a stirred solution was used to determine the diffusion coefficient of lysozyme in a limited volume of the solution.

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{4\alpha + 4\alpha^2}{4 + 4\alpha + \alpha^2 q_n^2} \exp(-Dq_n^2 \frac{t}{r^2}) \qquad (4.11)$$

When $\alpha \rightarrow \infty$ assumed in this experiment, the equation (4.11) was simplified as following equation:

$$\frac{M_t}{M_\infty} = 1 - \exp(-D(2.4048)^2 \frac{t}{r^2} \qquad (4.12)$$

where M_t is concentration of migrating substance (lysozyme) which has left the cylindrical extrudate in time t, M_∞ is corresponding concentration of released lysozyme through the extrudate after infinite time, α is ratio of the volume of solution and extrudate, r is the radius of a cylindrical extrudate, m, $q_{n=1} = 2.4048$ (Crank 1975), t = time , s, and D = diffusion coefficient of lysozyme, $m^2\ s^{-1}$.

4.3.15 Antimicrobial Effectiveness of Lysozyme

The indicator culture was *Brochotrix thermosphacta B2* (Department of Food Science, University of Manitoba, Winnipeg, MB), representing Gram-positive bacteria. *B. thermosphacta B2* was grown overnight on BHI (Difco Laboratories, Detroit, MI) broth media (5mL) at 22°C. The BHI broth media (200μL) was added and mixed with 15 mL of BHI agar on a plate. Cylindrical extruded samples were cut to a length of approximately 25 mm and inserted in the BHI agar plates. The plates were incubated for 48 h at 22°C in an incubation chamber (National Appliance Co, Portland, OR). After 48 h, the plates were visually examined for zones of inhibition around the extrudates, and the lengths from the extrudate to the border of zone with two different clarities were measured using a caliper. The clear and bright zone around extrudates was noted as 1st inhibition zone and the hazy zone surrounding 1st zone was noted as 2nd inhibition zone.

4.3.16 Statistical Analysis

A 2x3x4 full factorial experiment was designed to determine the effect of process variables (2 screw configurations, 3 moisture contents, and 4 die temperatures) on system variables, WSI, WAI, intrinsic viscosity, physical and mechanical properties,

and lysozyme recovery for extrudates. Data is based on three trials except expansion index, extrudate density, and mechanical properties. All data was analyzed using a general linear model (GLM), correlation test and the data was plotted using a quadratic response surface regression model (SAS Version 8.2, SAS Institute Raleigh, NC).

The measured property was related to the extrusion processing variables using the general linear model:

$$Y = X_1 + X_2 + X_3 + X_1X_2 + X_1X_3 + X_2X_3 + X_1X_2X_3 + \varepsilon \qquad (4.13)$$

where Y = the extrudate properties measurement, X_1 is the die temperature (°C), X_2 is the moisture content of dough (%), and X_3 is the shear level by screw configurations. Two regression models were generated: one for low shear and the other for high shear.

The regression model used was:

$$Y_{ij} = b_{0i} + b_{1i}X_1 + b_{2i}X_2 + b_{3i}X_1^2 + b_{4i}X_2^2 + b_{5i}X_1X_2 + \varepsilon \qquad (4.14)$$

where Y_{ij} = an observation of the j th extrudate properties measurement in the i th shear group (i = 1, 2; 1 = high, 2= low shear), X_1 is the die temperature (°C), and X_2 is the moisture content of dough (%). Non-significant parameters ($P < 0.05$) were eliminated by a stepwise method, where in each step highest order non-significant variables were removed from the model followed by rerunning and reassessment.

4.4 Results and Discussion

The experimental values for physico-chemical properties of extrudates and correlation coefficients between the properties of extrudates are stated in appendix A and B, respectively. The results of the linear regression model of individual and interaction effects between processing variables for the physical properties of extrudates are stated in Table 4.1. Significant shear effects by screw configuration elements for the properties of extrudates in a response surface regression model are stated in Table 4.2. Insignificant shear effects at the confidence level of 5% were eliminated by a step-wise method in the response surface regression model (Table 4.3). Although the response surface regression model was a curve fitting equation rather than a predictive model, it provided useful information about the individual and interaction effects between process variables.

4.4.1 System variables

Specific mechanical energy (SME) input, as a system variable, was used to present the extent of mechanical energy input, as a numerical value, during extrusion processing. The range of SME input in the experiment was 106 to 260 Wh/kg under the various extrusion conditions. In response surface regression graph of the SME input, a decrease in dough moisture content and extrusion temperature increased the extent of SME input (Fig. 4.2A). High shear screw configurations caused significantly higher SME input than low shear screw configurations ($P < 0.0001$, Table 4.1). SME input was sensitive to a change in melt viscosity of dough during extrusion cooking. Willett and coworkers (1995) have shown that the melt viscosity of corn starch, as a thermoplastic

material, decreases with increasing extrusion temperature and moisture content of dough. Vergnes and coworkers (1987) found that increased SME input positively correlated to the extent of starch degradation. Smith (1992) observed corn grit particles remained intact at low SME input but no particle or granule structure remained at high SME input during extrusion processing. SME input could be used to indicate the extent of starch degradation (Ryu and Ng 2001). Screw configurations have been shown to affect residence time distribution (RTD) and the extent of friction at the end of the screw tip. The high shear screw configurations, with more reverse screw and single lead screw zones, had longer residence time (Choudhury and Gautam 1998b) and higher consumption of energy by friction (Lo et al 1998, Tayeb et al. 1988b) than low shear configurations.

Die pressure is another system variable that affects the physical properties of extrudates. The range of die pressure was from 11 to 48 bar during pea starch extrusion processing. Decreasing the dough moisture content and extrusion temperature increased die pressure during extrusion processing (Fig.4.2B). The moisture content ($P < 0.0001$), extrusion temperature ($P < 0.0001$) and screw configurations ($P < 0.0250$) significantly affected die pressure (Table 4.1). The die pressure fluctuated under various extrusion conditions as the results of changes in melt viscosity and this was significantly affected by the extent of dough moisture content, mechanical and thermal energy input.

Table 4.1. Probability (P value) in statistical analysis of system variables and physico-chemical properties of pea starch extrudates using general linear model

Extrusion Variables	**SME** [d] (Wh/kg)	**Die Pressure** (Bar)	**SEI** [e]	**LEI** [f]	**VEI** [g]	**Density** (kg/m^3)		**Bending** (MPa)		**Compression**(MPa)	
						Extrudate	**Solid**	E_{ab} [h]	σ_{ab} [i]	E_{ac}	σ_{ac}
S [a]	**0.0001**	**0.0250**	**0.0110**	0.0939	0.1060	**0.0001**	**0.0007**	**0.0002**	**0.0055**	**0.0001**	**0.0342**
T [b]	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
MC [c]	**0.0001**	**0.0001**	**0.0002**	**0.0001**	**0.0001**	**0.0059**	**0.0001**	**0.0336**	**0.0001**	0.9495	0.2289
S*T	**0.0084**	**0.0034**	**0.0007**	0.3810	0.7295	**0.0077**	**0.0001**	**0.0140**	**0.0172**	**0.0010**	0.0817
T*MC	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	0.3762	**0.0001**	**0.0216**	**0.0001**	**0.0001**
S*MC	**0.0001**	0.9130	0.1310	**0.0330**	0.6541	0.1616	0.7836	0.0520	**0.0001**	0.1496	0.1115
S*T*MC	0.2787	0.0590	0.1105	**0.0443**	0.5864	**0.0493**	0.7690	0.2415	0.3662	0.3210	0.2017

[Continued]

Extrusion Variables	Lysozyme Activity (%)	Intrinsic viscosity (mL/g)	WAI [j] (g/g)	WSI [k] (%)	Color values [f]		
					L	a	b
S	**0.0001**	**0.0001**	0.8055	**0.0003**	**0.0001**	**0.0001**	0.8061
T	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	0.5336
MC	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0084**	**0.0001**	0.6639
S*T	**0.0008**	**0.0060**	0.8674	**0.0001**	**0.0004**	0.6437	0.4131
T*MC	**0.0001**	0.7006	**0.0246**	**0.0001**	**0.0001**	**0.0001**	0.5425
S*MC	0.5747	0.1018	**0.0233**	0.7228	0.7408	0.2796	0.6016
S*T*MC	**0.0270**	**0.0027**	0.0633	**0.0033**	0.7226	0.7894	0.6806

[a] Shear level, [b] Extrusion temperature (°C), [c] Dough moisture content (%)
[d] Specific Mechanical Energy input
[e] Cross-sectional expansion index, [f] Longitudinal expansion index
[g] Volumetric expansion index, [h] Apparent elastic modulus
[i] Fracture strength, [g] Water absorption index, [h] Water solubility index
[f] Color scales: L = brightness (0 = black, 100 = white); +a = red, -a = green; +b = yellow, -b = blue

Table 4.2. Results of regression analysis for physico-chemical properties of pea starch extrudates with effect of significant screw configurations

Intercept &	**SME** (Wh/kg)		**Intrinsic Viscosity** (mL/g)		**WSI** (%)		**WAI** (g/g)	
coefficient[a]	Low Shear	High Shear	High Shear	Low Shear	High Shear	Low Shear	High Shear	Low Shear
$\mathbf{b_o}$	1251.62 [c]	1301.1 [d]	85.52[c]	208.11[d]	-38.41[c]	-31.22[d]	1.6732[c]	4.2043[d]
$\mathbf{b_1}$	-4.37 [c]	-4.37 [c]	-0.2645[c]	-1.3357[d]	0.4476[c]	0.3582[d]	0.0627[c]	0.0627[c]
$\mathbf{b_2}$	-0.01 [c]	-41.18 [d]	3.4852[c]	-0.6214[d]	1.4179[c]	1.4179[c]	-0.0433[d]	-0.1146[d]
$\mathbf{b_3}$	0.0079 [c]	0.0079 [c]	-0.0202[c]	0.0144[d]	-0.0212[c]	-0.0212[c]	*	*
$\mathbf{b_4}$	0.0024 [c]	0.0024 [c]	0.0028[c]	0.0028[c]	0.0027[c]	0.0027[c]	-0.0002[c]	-0.0002[c]
$\mathbf{b_5}$	0.3527 [c]	0.3527 [c]	*	*	*	*	*	*
$\sqrt{MSE}$	6.4220		6.4705		3.6192		0.5224	
CV	3.9765		4.8109		29.33		11.84	
$\mathbf{R^2}$	0.9808		0.7691		0.8842		0.6502	

[Continued]

Intercept & Coefficient[a]	**Extrudate Density** (kg/m^3)		**Solid Density** (kg/m^3)		E_{ac} (MPa)		E_{ab} (MPa)		**L** values		**a** values	
	Low Shear	High Shear	Low Shear	High Shear	Low Shear	High Shear	High Shear	Low Shear	High Shear	Low Shear	High Shear	Low Shear
$\mathbf{b_0}$	3936.0[c]	5575.2[d]	957.77[c]	1519.75[d]	5006.6[c]	7361.4[d]	20745.5[c]	20345.7[d]	-81.96[c]	-94.81[d]	16.15[c]	16.92[d]
$\mathbf{b_1}$	-4.19[c]	-39.44[d]	11.69[c]	1.86[d]	-36.78[c]	-88.24[d]	-237.77[c]	-237.77[c]	-0.7553[c]	-0.6871[d]	*	*
$\mathbf{b_2}$	-126.83[c]	-126.83[c]	-6.43[c]	-6.43[c]	-88.83[c]	-88.83[c]	-244.31[c]	-244.31[c]	10.65[c]	10.65[c]	-1.0617[c]	-1.0617[c]
$\mathbf{b_3}$	1.2870[c]	1.2870[c]	*	*	0.8235[c]	0.8235[c]	2.5720[c]	2.5720[c]	-0.0279[c]	-0.0279[c]	0.0032[c]	0.0032[c]
$\mathbf{b_4}$	-0.2106[c]	-0.0539[d]	-0.0449[c]	-0.0034[d]	-0.0127[c]	0.2245[d]	0.5196[c]	0.5196[c]	0.0072[c]	0.0072[c]	-0.0005[c]	-0.0005[c]
$\mathbf{b_5}$	*	*	*	*	*	*	*	*	-0.1097[c]	-0.1097[c]	0.0109[c]	0.0109[c]
$\sqrt{MSE}$	181.39		28.64		224.77		748.32		4.2844		0.3712	
CV	15.601		1.9890		28.99		28.75		7.6307		-70.77	
$\mathbf{R^2}$	0.7350		0.8177		0.7493		0.7179		0.7732		0.8415	

[a] Model $Y_{ij} = b_{0i} + b_{1i}X_1 + b_{2i}X_2 + b_{3i}X_1^2 + b_{4i}X_2^2 + b_{5i}X_1X_2$

where Y_{ij} = an observation the j th extrudates properties measurement in the i th shear group (i = 1,2, 1 = high, 2= low shear screw configurations), X_1 is the die temperature (°C), X_2 is the moisture content of dough (%)

[b] Insignificant parameters ($P > 0.05$) were eliminated by a stepwise method , where in each step highest order non-significant variables were removed from the model followed by rerunning and reassessment (*Insignificant coefficient).

[c, d] Coefficient for the two shear groups in the same row with different superscripts are significantly different from each other ($P < 0.05$).

Table 4.3. Results of regression analysis for physico-chemical properties of pea starch extrudates with effect of insignificant screw configurations

Intercept & coefficient[a]	**Die Pressure** (bar)	**SEI**	**LEI**	**VEI**	σ_{ac} (MPa)	σ_{ab} (MPa)	**Lysozyme recovery** (%)
b_o	243.01	-87.2507	-7.4167	-4.2392	315.55	471.04	-59.32
b_1	-0.6756	1.3687	-0.0247	0.0429	-3.4210	-6.0197	0.7128
b_2	-7.9858	0.5592	0.5249	0.1778	-4.6575	-1.4696	2.7573
b_3	0.0189	*	-0.0006	-0.0021	0.0404	*	-0.0157
b_4	-0.0013	-0.0059	0.0002	0.0002	0.0073	0.0225	-0.0028
b_5	0.0652	*	-0.0067	*	*	*	*
$\sqrt{MSE}$	1.8970	7.4740	0.1378	0.2741	8.5918	19.3643	6.3572
CV	6.9859	125.05	0.3241	30.89	30.8484	35.87	31.24
$\mathbf{R^2}$	0.9731	0.3289	0.6827	0.7550	0.7432	0.7845	0.8491

[a] Model $Y_{ij} = b_{0i} + b_{1i}X_1 + b_{2i}X_2 + b_{3i}X_1^2 + b_{4i}X_2^2 + b_{5i}X_1X_2$

where Y_{ij} = an observation the j th extrudates properties measurement in the i th shear group (i = 1,2, 1 = high, 2= low shear screw configurations), X_1 is the extrusion temperature (°C), and X_2 is the moisture content of dough (%)

[b] Insignificant parameters ($P < 0.05$) were eliminated by a stepwise method, where in each step highest order non-significant variables were removed from the model followed by rerunning and reassessment (* Insignificant coefficient).

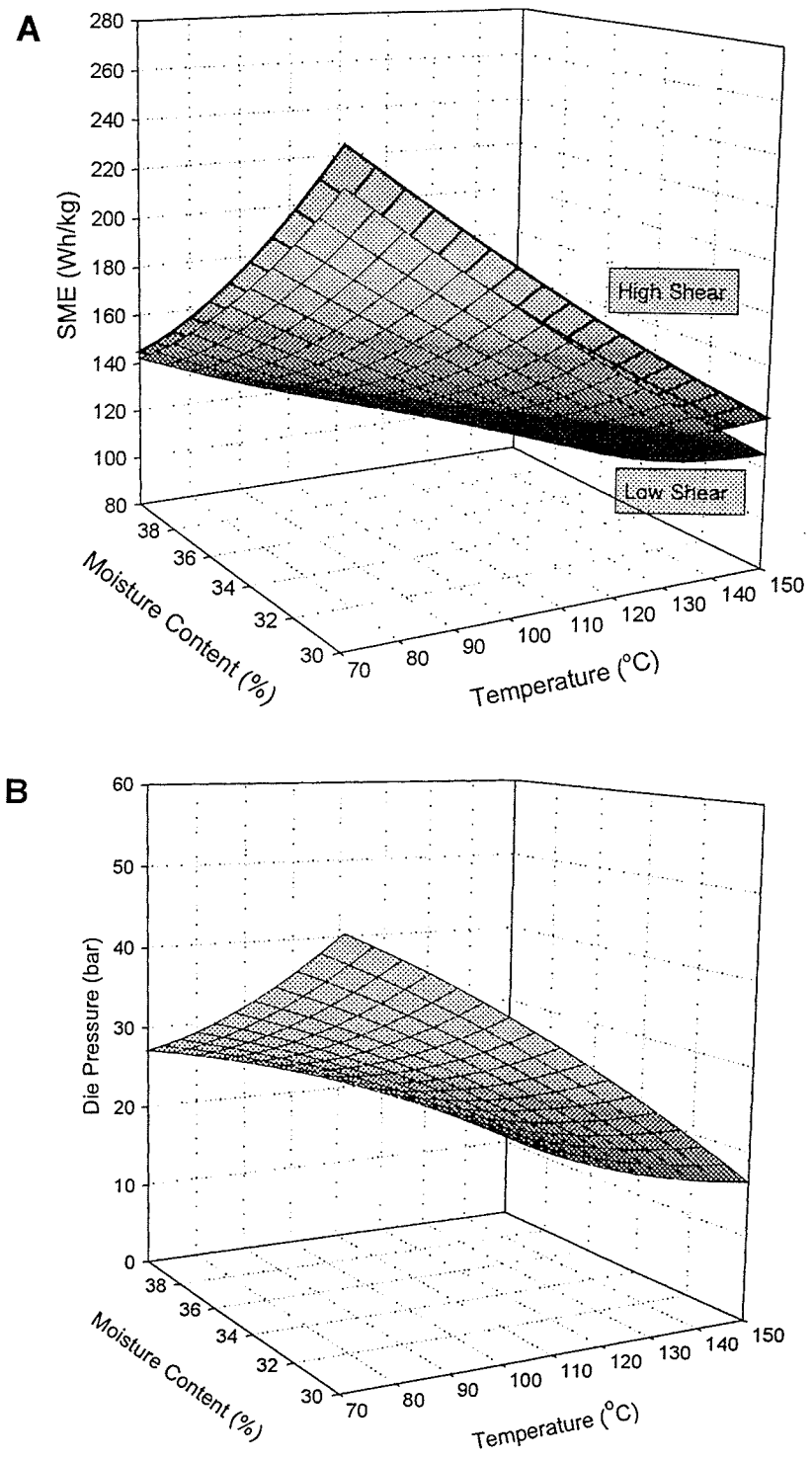

Figure 4.2. Specific mechanical energy input (A) and die pressure (B) as affected by extrusion temperature, moisture content of dough, and screw configuation elements during extrusion

4.2 Starch Gelatinization

In the experiment, thermal transitions of native pea starch were determined at various moisture contents in order to provide critical reference temperatures for thermomechanical processing. At 70% moisture content, only one endothermic peak occurred with peak gelatinization temperature (T_P), ~66°C, and the enthalpy of gelatinization (ΔH), ~10J/g (Fig. 4.3). However, a second endothermic peak was observed when moisture content was between 40 and 60%. The intensity of the gelatinization peak diminished and shifted towards a higher temperature range with decreasing moisture contents (Fig. 4.3). A number of studies have been reported to explain this behavior. Donovan (1979) suggested that when excess water is present, all starch crystallites melt cooperatively, but when water becomes a limiting factor, only part of crystallites melt, while the remaining gives rise to a second endotherm at a higher temperature. Evans and Haisman (1982) suggested that granules with the least stable crystallites are responsible for the first endothermic peak because disordered polysaccharide chains absorb more water. Therefore, the remaining ungelatinized granules melted at a higher temperature and showed the second endothermic peak. Contrary to the above study, Biliaderis and coworkers (1986), based on calorimetric data on starch and V-amylose systems, suggested that the double melting profiles may reflect a melting and reorganization process occurring simultaneously during dynamic heating in the calorimeter. Bogracheva and coworkers (1998) suggested that the first peak of the two endothermic curves of C-type pea starch represents the melting of B polymorphs and the second peak is the melting of the A polymorphs.

The thermal profiles of pea starch extrudate at 70°C showed two endothermic peaks at T_P, ~53°C and 80°C (Fig. 4.4). Starch extruded at ≥90°C showed only one

endothermic peak at T_P, ~53°C. The first peak may represent melting of a portion of retrograded pea starch, whereas the second peak melting of a portion of ungelatinized pea starch (Fig. 4.4). Fig. 4.4 shows also the effect of decreasing moisture content of the dough during extrusion on the thermal properties of extruded starch. As dough moisture content decreased, both thermal and mechanical degradation might occur cooperatively. The decrease of dough moisture content as a lubricant increased degree of mechanical degradation more than thermal degradation. Therefore, the magnitude of double endothermic peaks decreased with decrease in dough moisture content. Chinnaswamy and Hanna (1990) and McPherson and coworkers (2000) observed that the endothermic peak of corn starch after extrusion cooking disappeared. The difference between a smooth flat line of extruded corn starch and endothermic peak of extruded pea starch in DSC results might be caused by the rapid retrogradation of extruded pea starch when dried at low temperature in a dry oven. Blenford (1994) noted the rapid retrogradation of native pea starch was caused by the unique C-type structure of the granule. DSC results for retrograded corn starch showed that the endotherm of retrograded starch occurred at a lower temperature than that of native starch (Chinnaswamy et al. 1989). Davidson (1992) and Harper (1992) found that loss of crystalline structure and depolymerization of starch granules increased with increasing severity of extrusion processing, such as low moisture content and high temperature, because mechanical degradation could occur due to substantial shear stress when shear rate was high. However, most of the depolymerization of starch molecular resulted in large fragments rather than producing low molecular weight oligosaccharides. Mitchell and Areas (1992) found that mechanical energy input affects more starch degradation than thermal energy input.

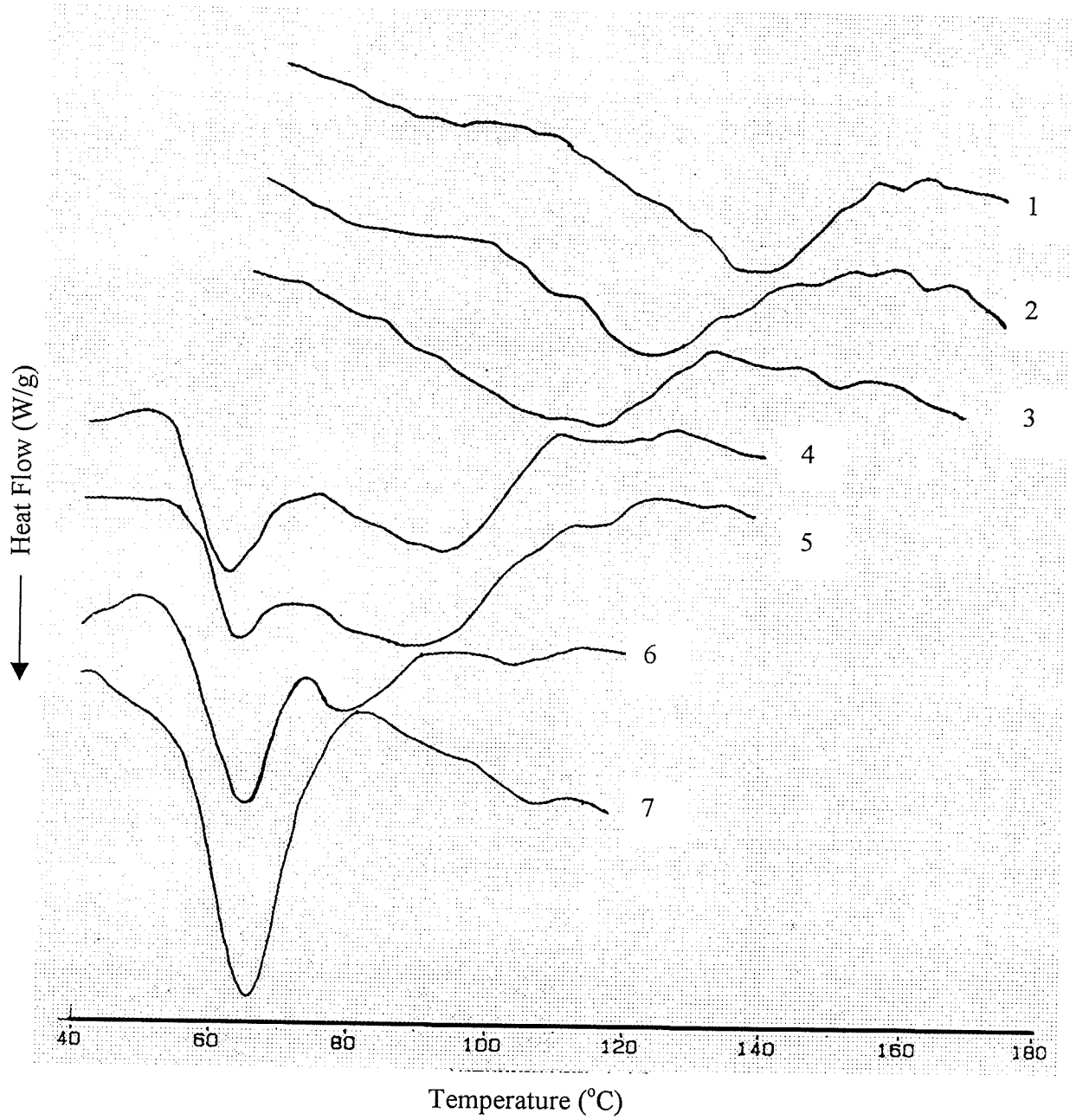

Figure 4.3. DSC-thermograms of native pea starch at various moisture contents (1. 20%; 2. 30%; 3. 35%; 4. 40%; 5. 50%; 6. 60%; 7. 70%)

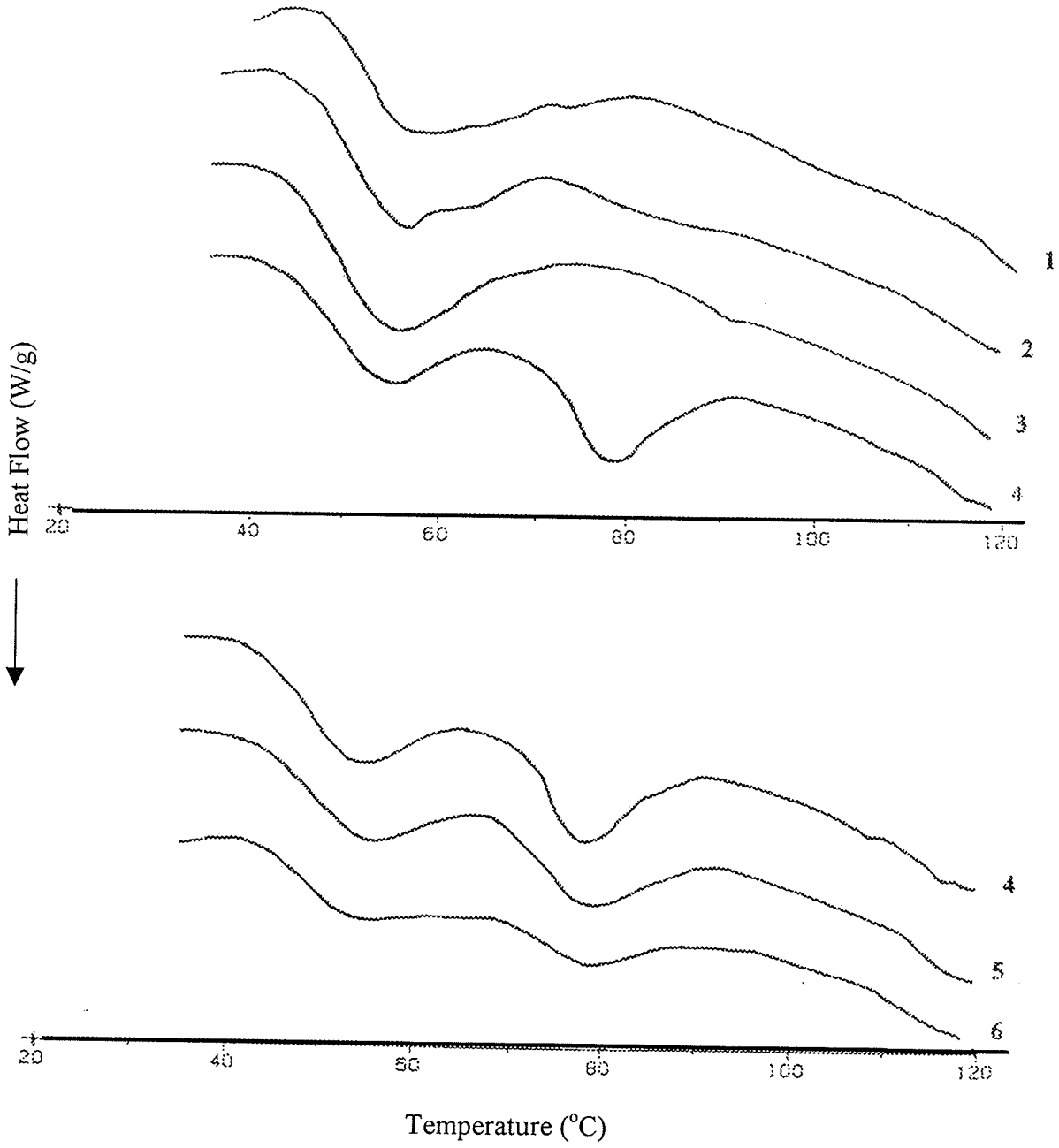

Figure 4.4. DSC-thermograms of extruded pea starch at various die temperature (°C) and moisture content (%) of dough under high shear screw configurations (1. 150°C, 40%; 2. 120°C, 40%; 3. 90°C, 40%; 4. 70°C, 40%; 5. 70°C, 35%; 6. 70°C, 30%)

4.4.3 Starch Degradation

The intrinsic viscosity of pea starch was used to measure the extent of macromolecular degradation under various extrusion processing conditions. The intrinsic viscosity of native starch was determined to be 180 mL/g and those of pea starch extrudates ranged from 112 to 158 mL/g (Appendix A). The intrinsic viscosity of extruded pea starch decreased with increasing extrusion temperature and decreasing moisture content of the dough (Fig. 4.5). The decrease of water content during extrusion processing might cause more friction between starch granules. Water is thought to act as a lubricant minimizing friction between polymer chains. As shown in Table 4.1, high shear screw configurations contributed to significantly lower intrinsic viscosity than low shear screw configurations ($P < 0.0001$) because the reverse screw zone in high screw configurations mainly contributed to more macromolecular degradation than forward screw zone. Vergnes and coworkers (1987) found that a decrease in intrinsic viscosity was caused by macromolecular degradation. Reverse screw elements caused effective starch breakdown by high shear energy input (Tayeb et al. 1988b; Yam et al. 1994) and longer residence time (Gautam and Choudhury 1999). Camire and coworkers (1990) also observed that intensive degradation of starch occurred under severe extrusion conditions such as low moisture content, high temperature, and high shear screw configurations with long reverse screw zones.

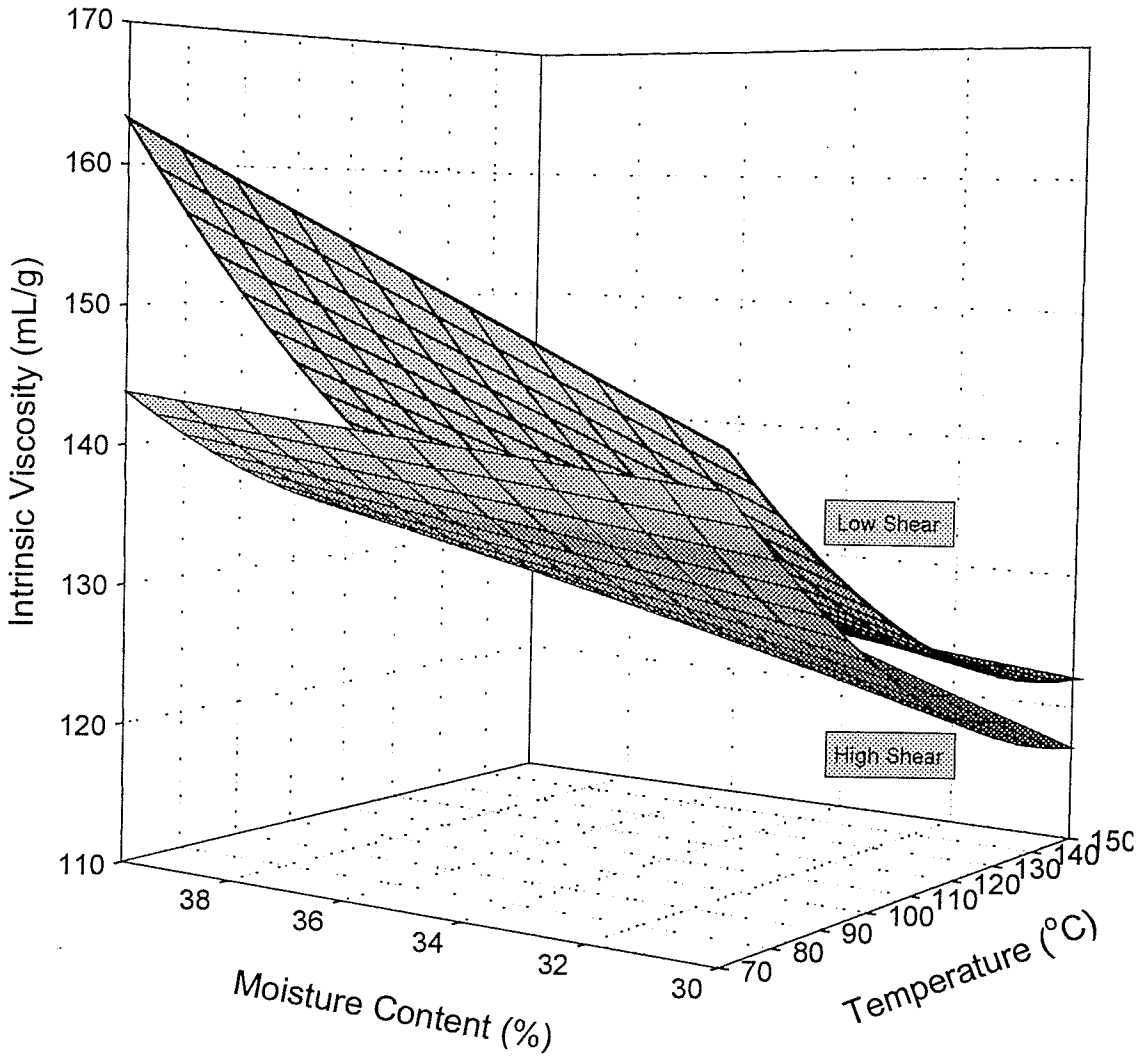

Figure 4.5. Intrinsic viscosity for suspension of pea starch under various extrusion conditions

Water solubility and absorption of extruded pea starch were also determined to study the change in properties of starch under various extrusion processing conditions. Native pea starch showed minimum water solubility index (0.6%). Maximum water solubility (37%) of extruded pea starch was shown at 150°C die temperature, 30% moisture content, and high shear screw configurations in the experiment. Water solubility of the extrudates increased with decreased moisture content of the dough and increased extrusion temperature (Fig. 4.6A). High shear screw configurations resulted in higher water solubility index (WSI) than low shear screw configurations. These results confirmed other research findings (McPherson and Jane 2000) that severe extrusion conditions can cause considerable starch degradation. The increase of fragmented soluble starch molecules due to degradation of starch resulted in the elevation of WSI. Barres and coworkers (1990) reported that increased number of reverse screw elements, which induces high degradation, increased WSI. Thus, WSI for extrudates might be used to measure the extent of starch degradation just like intrinsic viscosity. WSI was highly correlated to intrinsic viscosity ($P < 0.0001$) and had a higher regression coefficient ($R^2 = 0.8842$) than intrinsic viscosity ($R^2 = 0.7691$) in the polynomial regression model (Table 4.2). Therefore, WSI can be used as a good index with intrinsic viscosity to determine the extent of degradation of pea starch molecules after extrusion processing.

The native pea starch showed minimum water absorption index (2.5 g/g). The range of water absorption index of extruded pea starch was from 3.2 to 5.6 g/g. Water absorption of the pea starch extrudates increased with decreased moisture content of the dough and increased extrusion temperature (Fig. 4.6B). Water absorption index (WAI) values of extrudates obtained from low shear screw configurations were lower than

those from high shear screw configurations below 35% moisture content. Above 35% moisture content, however, WAI from low shear screw configurations was higher than from high shear screw configurations. However, Smith (1992) found that at mild extrusion condition (low shear and low extrusion temperature), starch, with fewer depolymerised chains, is more likely to bind with water molecules. He described that high WAI at the mild condition might cause greater availability of hydrophilic groups in the starch molecules. WAI may correlate to the degradation of starch. The evidence was that WAI was highly correlated with WSI ($P < 0.0001$) and intrinsic viscosity ($P < 0.0001$, Appendix B).

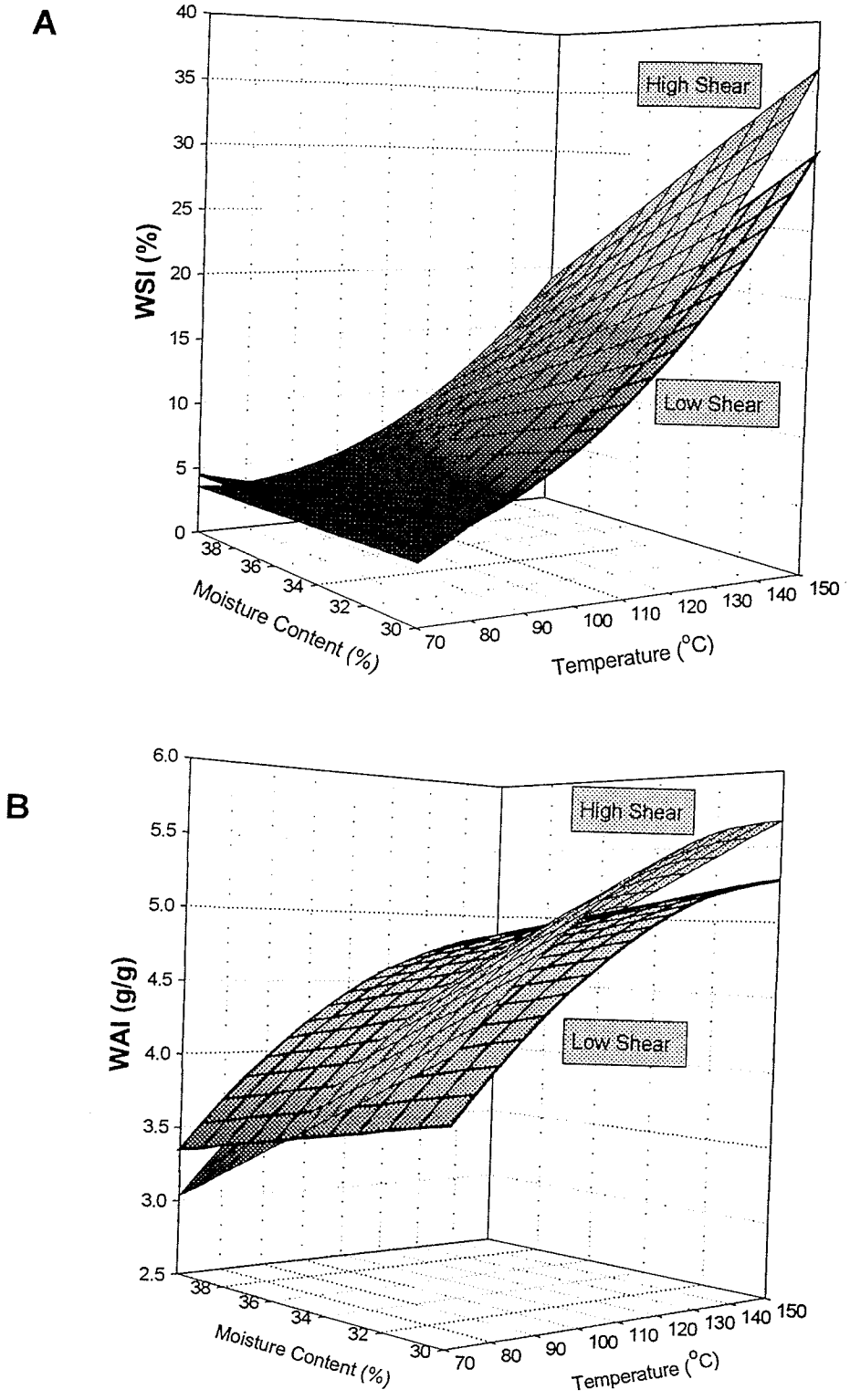

Figure 4.6. WSI (A) and WAI (B) of extrudates as a function of extrusion temperature ,moisture content of dough, and screw configurations

4.4.4 Pasting Properties

Extrusion temperature, moisture content of dough, and screw configurations affected the peak viscosity, set back, and final viscosity of pea starch as determined by a Rapid Visco Analyser (RVA). Fig. 4.7 shows the pasting properties of pea starch before and after extrusion processing at 70, 90, 120, and 150°C. Native pea starch showed a rapid rise in peak viscosity with the onset of gelatinization and maximum value in final viscosity (≪900). The exact value for the final viscosity of native pea starch could not be determined due to its extremely high viscosity (Fig. 4.7). Extruded pea starches had lower peak viscosities than native starch because they were partially or fully gelatinized. Increased extrusion processing temperature resulted in decrease of the peak viscosity, trough, and set back. When extrusion temperature was increased, the peak viscosity of pea starch was broader and associated with lower temperatures zone. Extruded samples above 120°C exhibited only cold peak viscosity instead of hot peak viscosity. The initial increase in viscosity during heating in the rapid viscoanalyser indicates swelling of starch granules and exudation of amylose. Thermal and mechanical degradations occurring during extrusion processing may be responsible for changes in the pasting properties of starches. Harper (1992) observed that native starch exhibited a rapid rise in viscosity with the onset of gelatinization, whereas extruded starches showed higher initial cold paste viscosity than peak viscosity. He demonstrated that gelatinized starch was responsible for the lack of peak viscosity in the extrudates. As evidenced from the RVA graphs, the highest peak viscosity, trough, and set back were displayed by starch extruded at 70°C (Fig. 4.7). These results confirmed the presence of some residual remaining crystalline structure in the extruded starch samples obtained at 70°C.

Similarly, McPherson and coworkers (2000) reported that fewer granules were distorted and fractured in extruded corn starch under low extrusion temperature.

Greater degradation of starch occurring at the lower moisture content of the dough during extrusion was responsible for the changes in the pasting properties of extruded starch. The RVA patterns showed that peak viscosity was associated with lower temperature and the trough and setback values decreased when the moisture content of dough during extrusion processing was reduced from 40 to 30% (Fig. 4.8). Similar results have been reported for cold viscosity, peak viscosity and setback in modified corn starch (McPherson et al. 2000) and corn meal and rice flour (Whalen et al. 1997).

Pasting properties of extruded pea starches decreased sharply as the screw configurations increased from low to high shear screw configurations (Fig. 4.9). The decrease of magnitude in peak viscosity might reflect greater degradation and gelatinization of starch. This indicated that non-gelatinized starch polymers are still present because the peak viscosity still persists when the temperature ramps up to 95°C (Fig. 4.7). Similar results have been reported for extruded corn grits (Barres et al. 1990) and hydropropylated corn starch (McPherson et al. 2000).

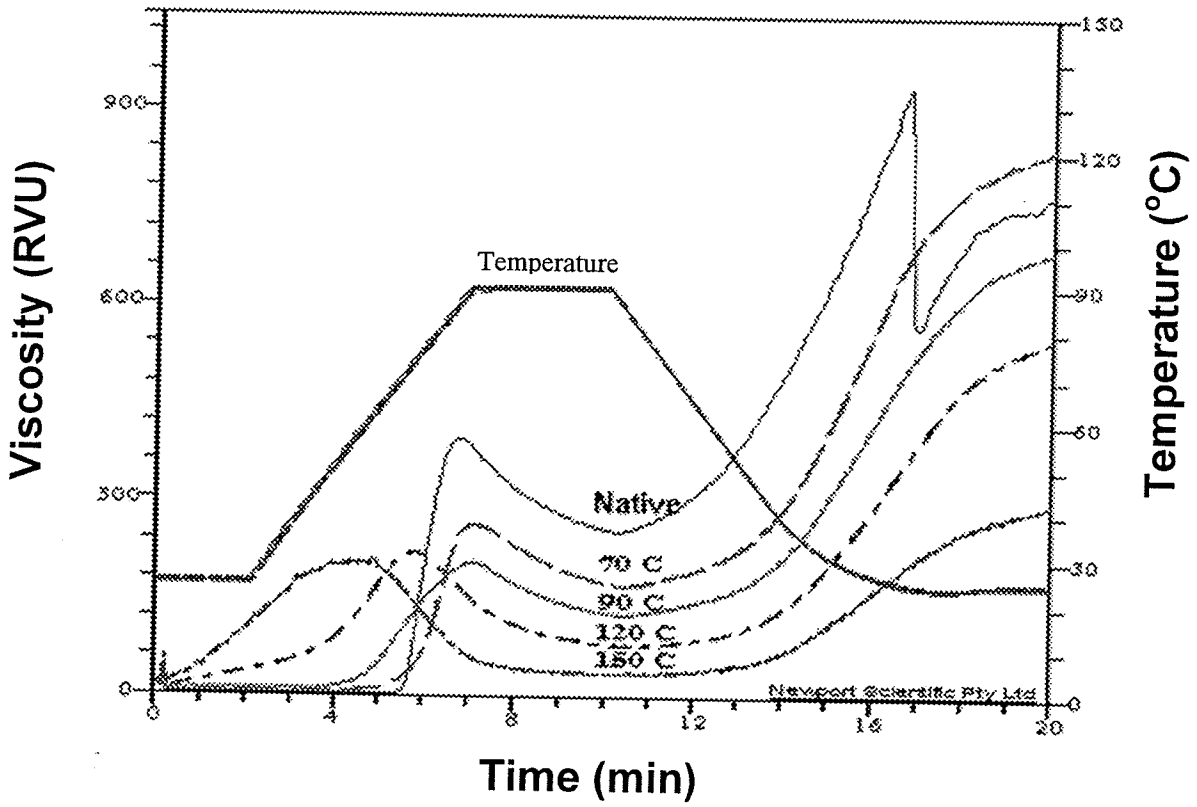

Figure 4.7. Rapid Visco Analyser pasting profiles of native and pea starch extrudates at 30% moisture content and high shear at 70, 90, 120, and 150°C temperatures

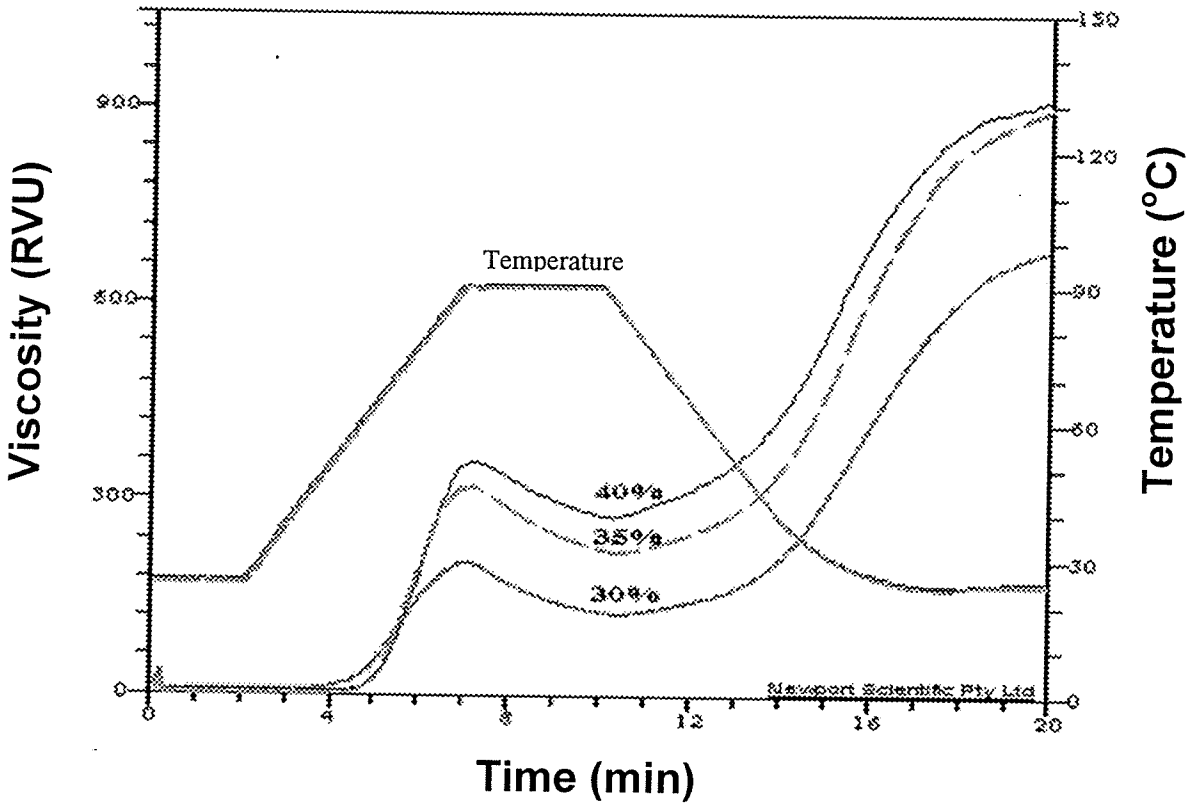

Figure 4.8. Rapid Visco Analyser pasting profiles of pea starch extrudates at 90°C temperature and high shear at 30, 35, and 40 % moisture contents

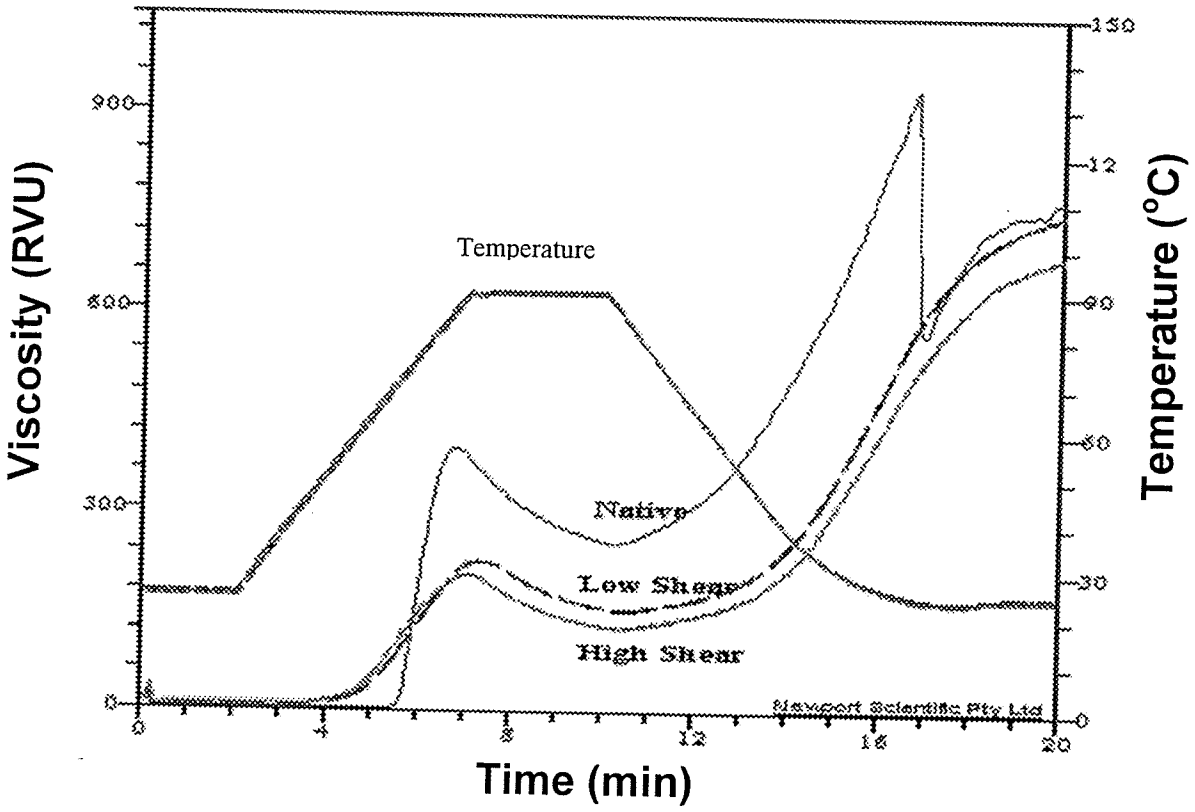

Figure 4.9. Rapid Visco Analyser pasting profiles of pea starch extrudates at 90°C and 30 % moisture injection at high and low shear screw configurations

4.4.5 Physical Properties

Expansion of pea starch extrudates was measured based on cross-sectional, longitudinal, and volumetric expansion indices to characterize the expansion process. The effect of shear by screw configuration elements was not significant except for the cross-sectional expansion index ($P < 0.0110$) (Table 4.1). In the response regression models, the effects of shear by screw configuration elements for all expansion indices were eliminated by a step-wise method ($P < 0.05$) (Table 4.3). The cross-sectional expansion index (SEI) was measured in the range of 1 to 33 in the experiment and showed a low regression coefficient ($R^2 = 0.3289$, Table 4.3). This result indicated that SEI did not fit the response surface regression model.

The maximum value (0.97) of longitudinal expansion index (LEI) was determined at 150°C, 35% moisture content, and low shear screw configurations in the experiment (Appendix A). The LEI had minimum expansion (0.03) at 120°C, 40% moisture content and high shear screw configurations in the experiment but gradually increased below or above 115°C (Fig. 4.10A). The maximum LEI obtained at 35% moisture content gradually decreased as moisture content increased or decreased. The LEI were negatively correlated to the SEI (-0.5062, $P < 0.0001$) (Table 4.4). The results were in good agreement with that of Alvarez-Martinez and coworkers (1988). The inverse relationship between LEI and SEI might be due to shear-induced alignment and compression or relaxation of the starch network in the extruder screw.

Volumetric expansion index (VEI) showed maximum expansion (2.56) at 150°C, 30% moisture content and low shear screw configurations in the experiment. The VEI declined with a decrease in extrusion temperature and an increase in dough moisture content (Fig. 4.10B). The VEI was significantly affected by the extrusion temperature

($P < 0.0001$) and the dough moisture content ($P < 0.0001$, Table 4.1). The expansion of extrudates is affected by both elastic expansion effect of the molten dough material and a vapor pressure effect due to superheated moisture when molten starch pass through a die (Padmanabhan and Bhattacharya 1989). The elastic forces were dominant factor for expansion of extrudate at low extrusion temperature and low moisture content of the dough. The moisture effects, which cause greater bubble expansion, dominated at high melt temperature and high moisture content of the dough. At low extrusion temperatures, the elastic force was dominant and the water vapor that could not generate enough to foam air bubbles in the molten dough was entrapped until the molten starch dough passed through a capillary die hole allowing the vapor to escape (Padmanabhan and Bhattacharya 1989). In fact, the pea starch extrudates showed very low expansion, which might be caused by low bubble growth due to poor vapor pressure in the experiment. An increase in dough moisture content at an extrusion temperature 70°C would lower the melt viscosity of the dough. As a result, greater expansion of extrudates occurred with high moisture content than with low moisture content at 70°C (Fig. 4.10B). Low melt viscosity of dough at high extrusion temperatures in the experiment might reduce the stored energy of starch molecules during their flow through the die and bubble formation would be maximized by high water vapor pressure (Fried 1995). Padmanabhan and Bhattacharya (1989) reported that the steam effect became dominant and disrupted any residual elastic effects at high extrusion temperatures.

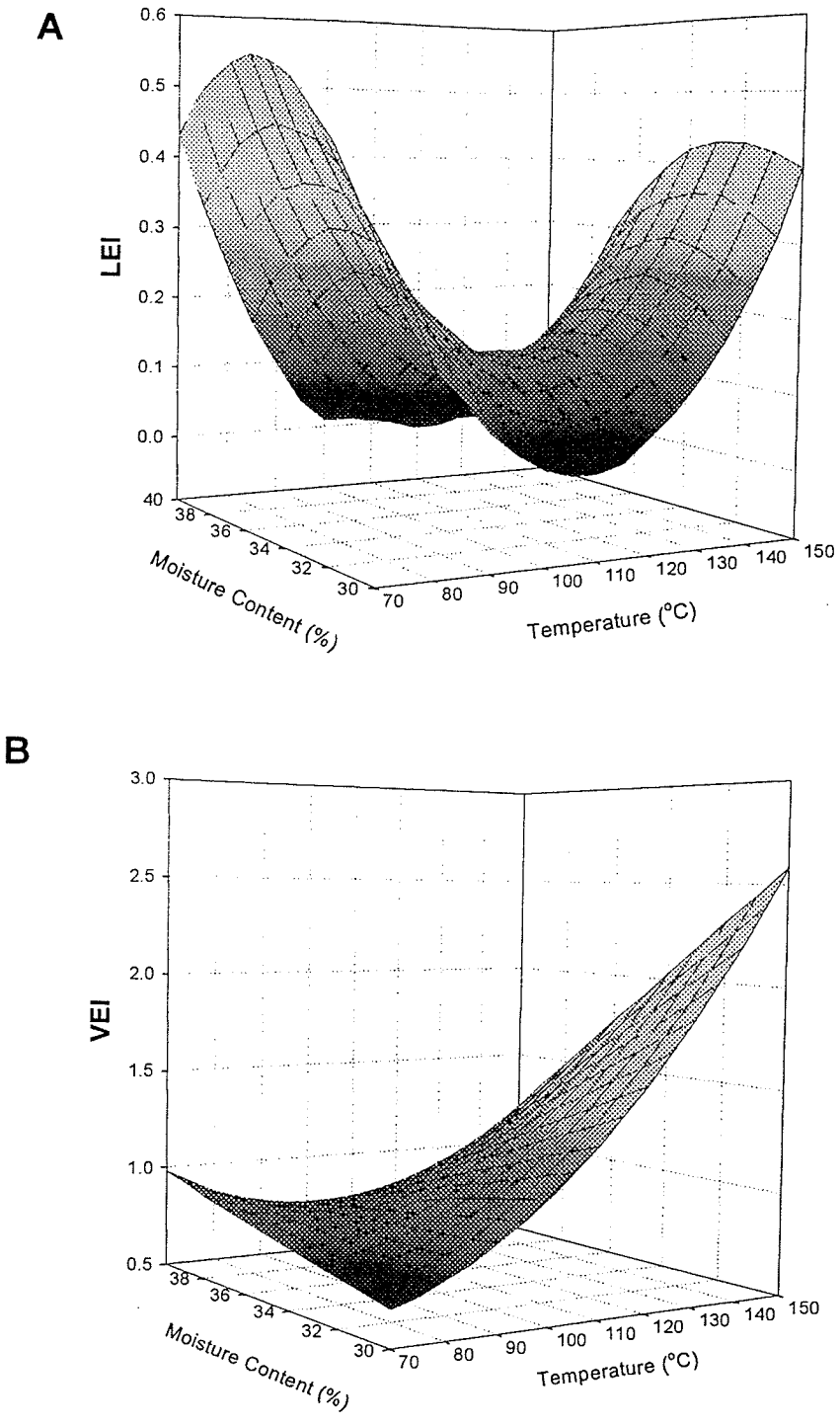

Figure 4.10. Longitudinal (A) and volumetric (B) expansion indices as a function of extrusion temperature and moisture content of dough

The densities of pea starch extrudates were measured in the range of 306 to 1,544 kg/m^3. The densities of the extrudates were inversely correlated to longitudinal and volumetric expansion (Table 4.4). The densities of some pea starch extrudates were overestimated, possibly due to irregular extrudate shapes. Therefore, although solid density must be higher than the extrudate density, a few extrudate densities had higher values than those of solid densities (Appendix A). An increase in extrusion temperature decreased the density of pea starch extrudates (Fig. 4.11). The density increased with an increase in moisture content of the dough at low extrusion temperature, whereas the opposite effect occurred at high extrusion temperature. Elastic expansion of the molten starch dough could be dominant at low temperature and low moisture content of the dough. This resulted in a high extrudate density. When the moisture content of dough was increased at low extrusion temperature, the bubble growth in molten starch increased gradually and led to a decrease in the density for extrudates. The shear effect of screw configurations significantly affected the extrudates' density ($P < 0.0001$, Table 4.1). The low shear screw configurations resulted in a higher extrudate density than high shear screw configurations. Greater starch degradation and gelatinization were induced by high shear that affects starch networks and melt viscosity.

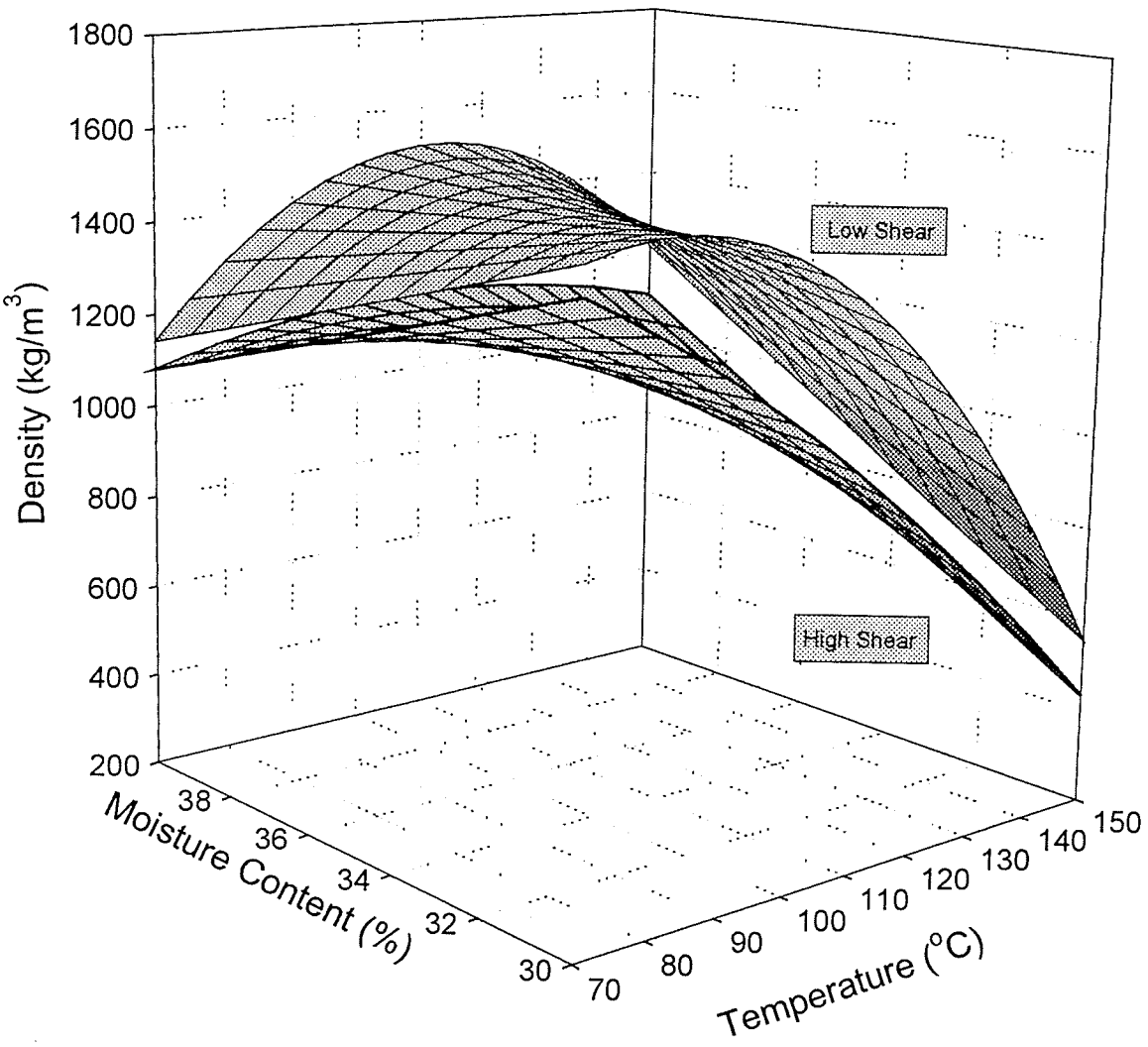

Figure 4.11. Extrudate densities as a function of extrusion temperature, moisture content of dough, and screw configurations

The solid densities of extrudates covered the range of 1,302 to 1,523 kg/m^3. When moisture content of dough increased, solid density substantially decreased (Fig. 4.12). The solid density increased with an increase in extrusion temperature. The solid densities were significantly affected by extrusion temperature ($P < 0.0001$), moisture content of the dough ($P < 0.0001$), and shear level ($P < 0.0007$) (Table 4.1). Furthermore, the solid density was negatively correlated to extrudates densities (-0.3548, $P < 0.0024$) (Table 4.4). The change in starch polymer and loss of moisture in the crumb walls of bread was related to change in the properties of the cell wall (Scanlon and Zghal 2001). Similarly, the change in solid density of extrudates during extrusion processing might be affected by a physico-chemical change in starch polymer. Therefore, the change in the cellular solid properties of the extrudates could influence the mechanical properties of extrudates, which is described by a power-law model that has linked cellular structural properties to mechanical properties. Future studies on solid structure using image analysis and microscopic technology would be useful to explain the effect of processing variable for the solid densities of extrudates.

Table 4.4. Correlation coefficients between expansion indices and densities (kg/m^3)

	Extrudate Density	SEI	LEI	VEI
SEI	-0.1267			
LEI	-0.4565**	-0.5062**		
VEI	-0.8735**	0.0249	0.3552**	
Solid Density	-0.3548**	0.1161	-0.0548	0.4592**

* Significant at 95%, **Significant at 99%

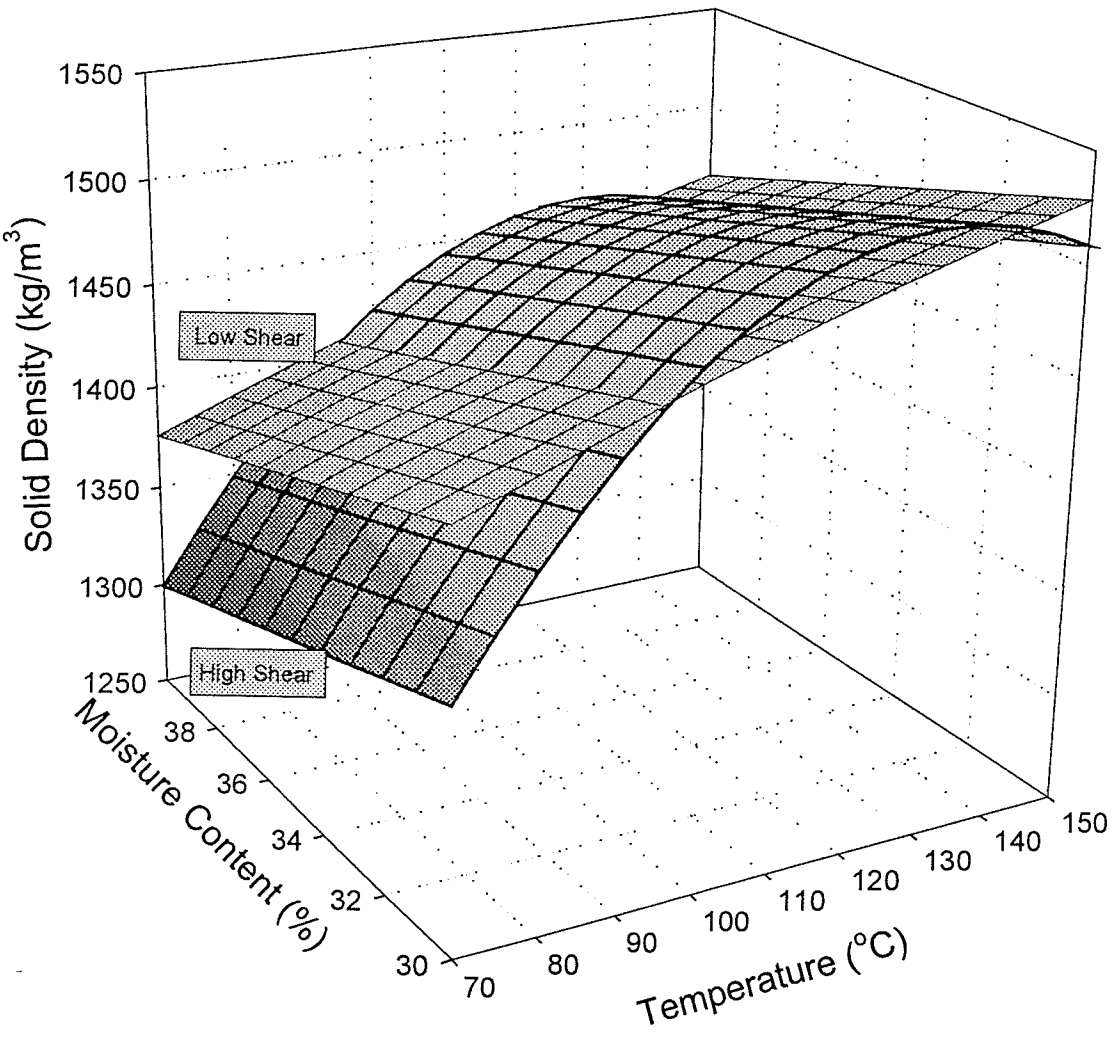

Figure 4.12. Solid densities as a function of extrusion temperature, moisture content of dough, and shear level of screw configuration elements

4.4.6 Mechanical Properties

The elastic modulus values were in the range of 54 to 1,436 MPa in compression and 225 to 4,755 MPa in bending test. Elastic modulus of the extrudates in the compression and bending tests decreased with an increase in extrusion temperature (Figs. 4.13A, B). Increases in dough moisture content slightly increased elastic modulus but high screw configurations, which increase the shearing effect, decreased elastic modulus. The elastic modulus in the bending test showed a much higher value than that in the compression test (Figs 4.13A, B). The elastic modulus would be affected by the degree of starch degradation, which occurs in an extruder due to shear and thermal energy, and the alignment of the molecules during their laminar flow through the extruder screw and die. With more intensive degradation of starch under severe extrusion conditions, the elastic modulus was even lower (Figs. 4.13A, B). In general, the severe extrusion conditions include low moisture content of the dough, high extrusion temperature, and high shear energy input. Hutchinson and coworkers (1987) found the density of cylindrical maize extrudates was directly proportional to compressive strengths and bending strengths. Riaz (1999) observed that inadequate moisture levels produced an extrudate with very low density and crispiness, but the addition of too much water resulted in a heavy and brittle product. From the DSC results (Fig. 4.4), the degree of gelatinization of extruded pea starch at low temperature increased with a decrease of moisture content. The degree of gelatinization affected elastic modulus at 70°C. A decrease in the dough moisture content at 70°C caused considerable gelatinization and resulted in an increase of elastic modulus (Figs. 4.13A, B). The difference between the elastic modulus in the compression and bending tests could be caused by the cell wall properties of extrudates. Hutchinson and coworkers (1987) found the force to break the

walls at the surface of the extrudate to be greater than that required for breakage of an internal pore wall. They attributed the difference in mechanical properties between compression and bending to the initial collapse of the cell structure at the surface during cooling after extrusion processing. The alignment of starch molecules and the different cell wall properties of the extrudate accounted for the higher elastic modulus in bending versus compression. Another possible contribution for the difference in mechanical properties between compression and bending was the anisotropy of the foam cells, which is known to affect mechanical properties. The anisotropy of the foam cells between the axial and radial direction indicated that the polyhedral cells are more elongated in the extrusion direction than spherical cells (Warburton et al. 1992). In fact, the restriction of cell growth near the surface of the foam tended to squeeze cells in the radial direction and elongate them in the tangential direction.

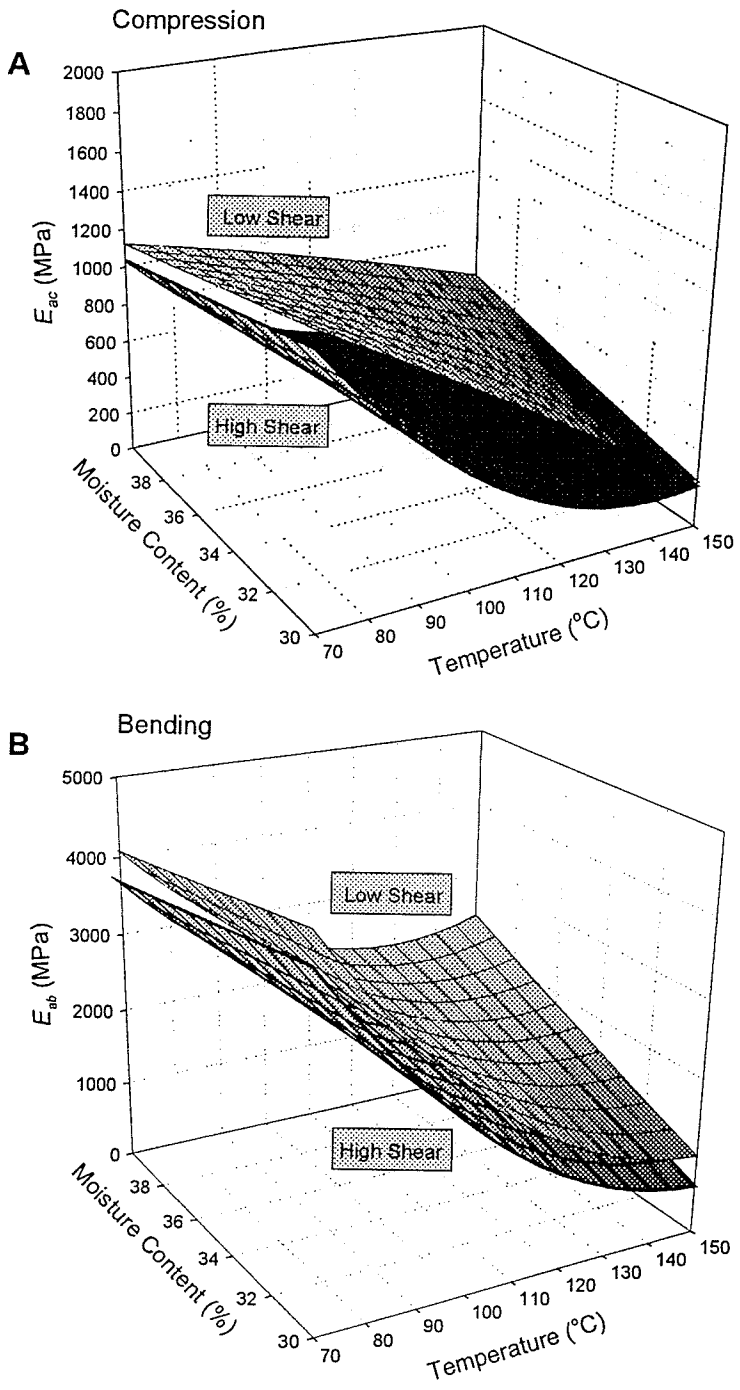

Figure 4.13. Elastic moduli of extrudates in compression (A) and bending (B) test as a function of extrusion temperature, moisture content of dough, and screw configurations

Fracture strengths of pea starch extrudates were in the range of 2 to 53 MPa in compression tests and 5 to 120 MPa in bending tests. The fracture strengths in the compression and bending tests had trends similar to the elastic moduli (Figs. 4.14A, B). Like the elastic modulus, the fracture strengths were significantly affected by extrusion temperature and shear level in both tests (Table 4.1). Also, the fracture strengths were highly correlated to the elastic moduli in the compression ($P < 0.0001$) and bending ($P < 0.0001$) tests (Appendix B). Dough moisture content affected the fracture strength of extrudates in the bending test ($P < 0.0001$), whereas it did not affect fracture strength in the compression test ($P < 0.2289$) (Table 4.1). The fracture strength in the bending test was higher than that in the compression test for the high-density samples, presumably because the force required to break the surface of the extrudate foam was much larger than that to break an internal pore wall in the high-density samples. Smith (1992) stated that the variation in results between bending and compression test might be caused by the difference in physical properties between surface and internal structure as a result of rapid cooling of surface of extrudates after extrusion process. The compressive strengths for other biodegradable packaging material as reported in the literature (Richardson 1989) ranged from 186 to 344 MPa for casein plastic and 69 to 241 MPa for cellulose acetate plastic. In addition, thermosetting polyester as a synthetic polymer was in the range of 90 to 345 MPa (Richardson 1989).

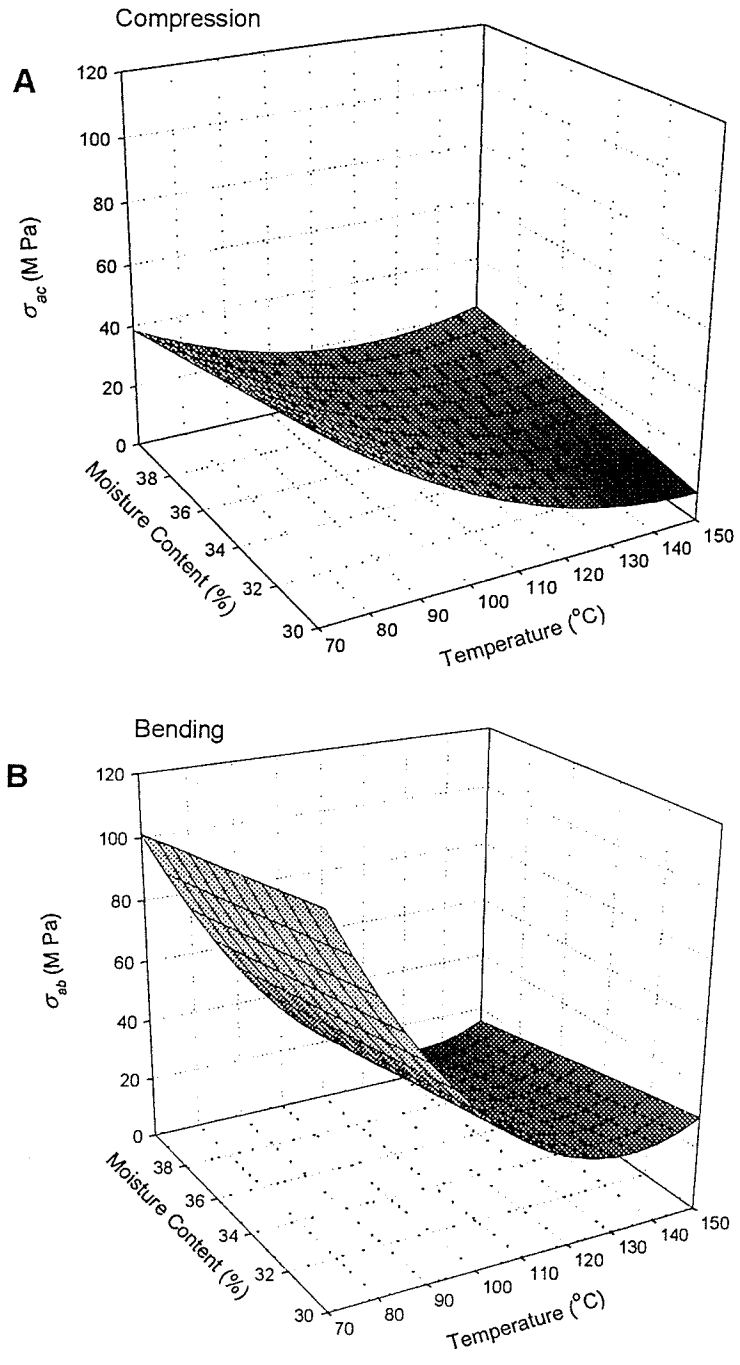

Figure 4.14. Fracture strengths of extrudates in compression (A) and bending (B) tests as a function of extrusion temperature and moisture content of dough

The dependence of the mechanical properties of extruded foams in the compression and bending tests on their relative density is shown in figs. 4.15 and 4.16. Relative density is obtained by dividing extrudate density by solid density. There is a theoretical model to describe the deformation of solid foams of extrudates, which relates the mechanical properties to the foam density and the cell-wall properties (Smith 1992). The solid foam theoretical relationship (Ashby's equation) for synthetic polymers was used for pea starch extrudates and is given as follows:

$$\frac{E_e}{E_w} \approx \left(\frac{\rho_e}{\rho_w}\right)^n$$

where E is elastic modulus (MPa), ρ is density (kg/m^3) , subscript e and w indicate extrudates and cell wall of extrudates, respectively, and the index n indicates the properties of cell wall material. The value of E_w was not determined in the experiment because of experimental difficulty. The material behavior of the extrudates as determined by compression and bending gave a similar response, even though the actual mechanical test differs. Linear regression was performed on the data for elastic modulus following the power-law fashion. The results of linear regression were n = 1.8173 (R^2 = 0.80) in the compression test and n = 1.6386 (R^2 = 0.81) in the bending test. The power law index was lower than the theoretical values predicted by Ashby's equation for open cell foams (n = 2) or closed cell foams (n = 3) (Smith 1992). The starch blends with various amylose content had n = 1.31 (R^2 = 0.69) for relative Young's moduli (Lourdin et al. 1995).

The solid relationship for fracture strength was as follows (Hutchinson et al.1987):

$$\frac{\sigma_e}{\sigma_w} \approx \left(\frac{\rho_e}{\rho_w}\right)^n$$

where σ is fracture strength (MPa), and subscript e and w indicate extrudates and cell wall of extrudates, respectively. The results of linear regression for fracture strength were n = 1.5330 (R^2 = 0.71) in the compression test and n = 1.5075 (R^2 = 0.37) in the bending test. The results for fracture strengths were in good agreement with the value predicted by Ashby's equation for open cell foams (n = 1.5) but not for closed cell foam (n = 2). However, the starch blends with various amylose content had n = 1.27 (R^2 = 0.78) for fracture strengths (Lourdin et al. 1995). These results showed that different cell wall material could change behavior of solid foams and cause changes in mechanical properties. Warburton and coworkers (1990) reported the fracture strength in maize grit extrudates was in excellent agreement with predicted values (n = 2) (Ashby's equation for closed cells). They stated that closed cell foams behave as open cells due to the draining of material under surface tension, which causes the cell faces to be much thinner than the cell edges. This also provides a good explanation why the elastic moduli of pea starch extrudates follow the open cell foam model rather than the closed cell foam model. This contradicted observation of pea starch extrudates, which showed that they possessed closed cell structure (results not shown).

It would have been more satisfactory to perform experiments using a wider range of foam densities, as the densities for pea extrudates were not distributed well in these experiments.

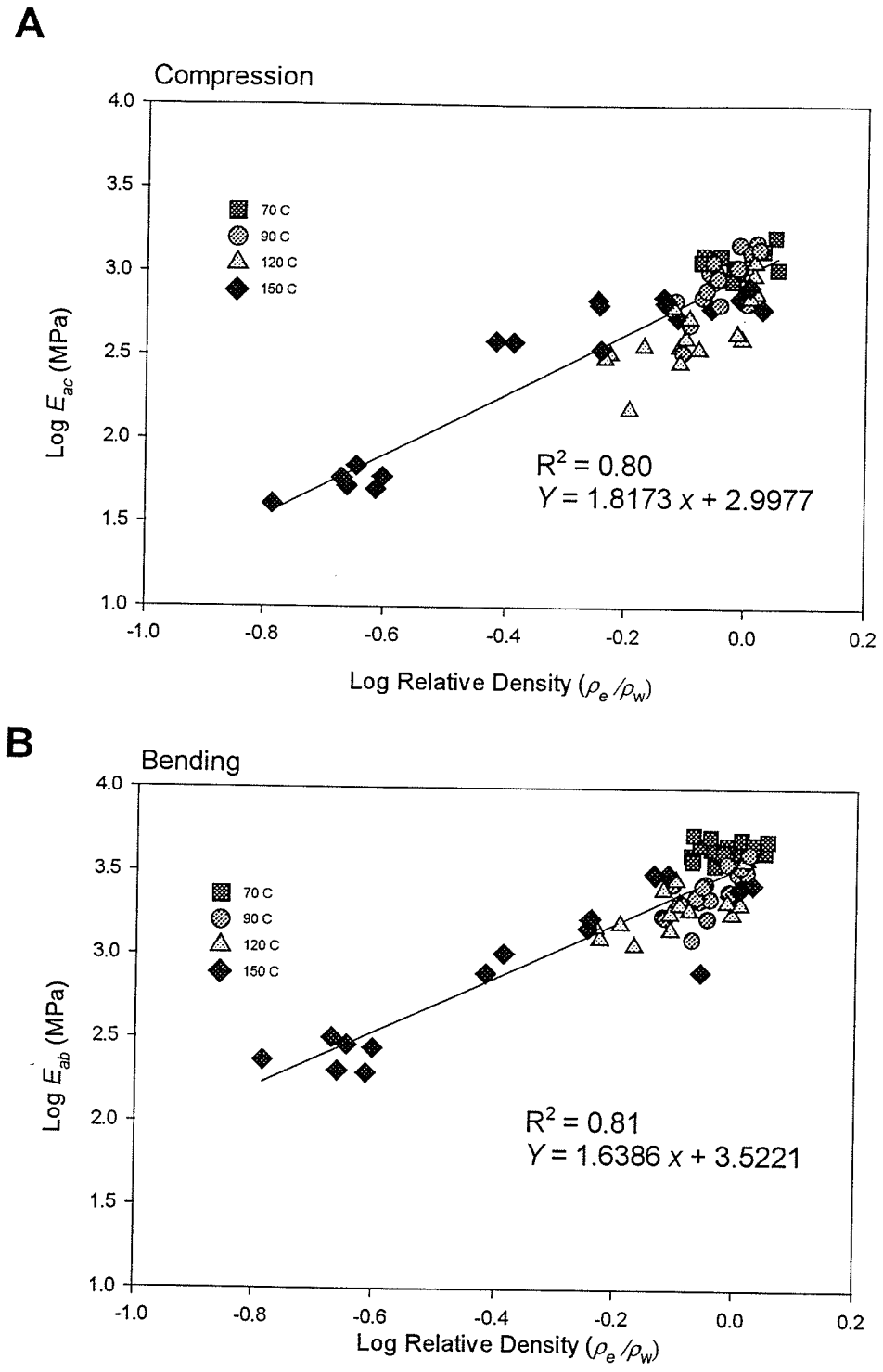

Figure 4.15. Variation of apparent elastic modulus with relative density for pea starch extrudates in compression (A) and bending (B) tests

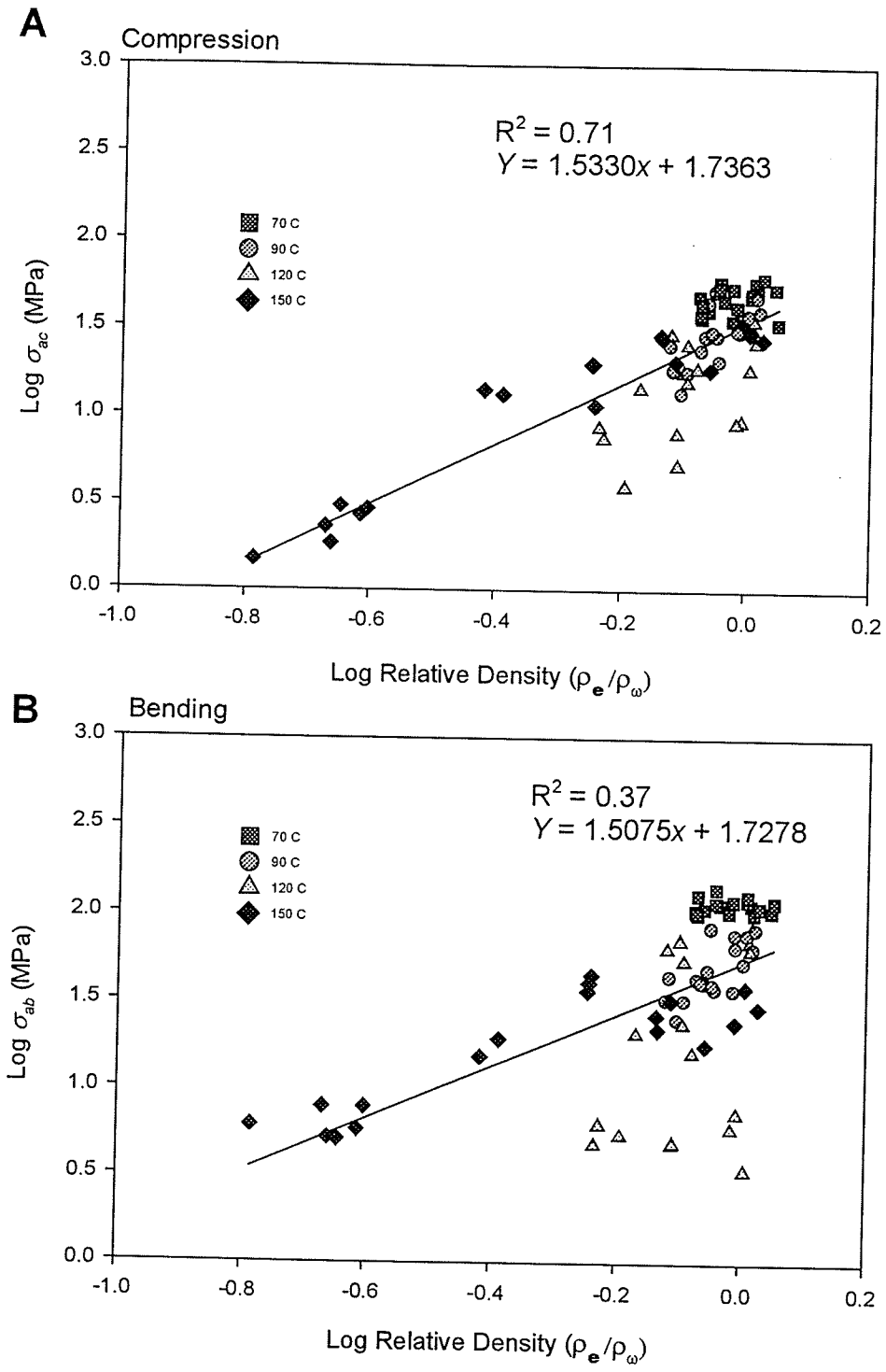

Figure 4.16. Variation of fracture strengths with relative density for pea starch extrudates in compression (A) and bending (B) tests

4.4.7 Color

When pea starch extrudates are used for packaging material, the color of the extrudates will be an important issue for manufacturing desired packaging material. A light color has more flexibility for creation of a range of final colors and so will be more acceptable than dark color of extrudates. The method to color packaging material is to blend the least costly pigments into the base resin (Richardson 1989). Extrudates with light color can decrease the cost for the dye. The 'L' values from a Hunterlab colorimeter represent lightness between 0 and 100. The 'a', and 'b' values from the colorimeter represent redness and yellowness at positive values and greenness and blueness at negative values, respectively. The 'L', 'a', and 'b' values for native pea starch were 92.6, -2.2, and 3.7, respectively. The 'L' values of pea starch extrudates ranged from 44 to 73 in the experiment. The 'L' values decreased with an increase in extrusion temperature up to 100°C (Fig. 4.17A). The 'L' value decreased with an increase in moisture content of the dough at low extrusion temperature, but increased with an increase in moisture content of the dough above 100°C. Low shear screw configurations produced lighter colored extrudates than high shear screw configurations. The range of 'a' values was from –1.9 to 1.1 in the experiment. The 'a' value showed opposite trends from 'L' values (Fig. 4.17B).

The pea starch extrudates at 70°C showed maximum lightness because the starch was partly gelatinized (Fig. 4.17A). The 'L' value decreased until 100°C at which point pea starch would be completely gelatinized (Barron et al. 2000; Biliaderis et al. 1980). In addition, heating during extrusion processing would cause the Maillard reaction, which develops dark color. Bates and coworkers (1998) using a quadratic response surface regression model found that the color development of wheat starch extrudates

was related to pH, moisture content, and temperature. They reported that the rate of color development due to the Maillard reaction was temperature dependent.

The completely gelatinized pea starch showed great expansion above 100°C. The lightness of extrudates increased with a decrease in solid density. In fact, 'L' values were correlated to solid densities more than density of extrudates ($P < 0.0001$, Appendix B) and increased with increase of extrusion temperature (Fig. 4.17A). It might be related to the increase of light scattering due to increase of air bubble in cell walls of extrudates. Lo and coworkers (1998) found that the increase of extrusion temperature from 135 to 172°C caused great expansion of corn meal extrudates and it resulted in light color of extrudates. The 'a' value showed that redness of extrudates increased with an increase in the degree of gelatinization of starch (Fig. 4.17B).

Decrease in water content as a lubricant increased the degree of shear at low extrusion temperature and resulted in a decrease in the 'L' value of extrudates. However, the decrease of water content at high extrusion temperature caused greater expansion of extrudates. The lower density of extrudates increased the 'L' value and decreased the 'a' value. It is concluded that the degree of gelatinization and heating of pea starch controlled the 'L' and 'a' value at low extrusion temperature below 100°C, whereas expansion phenomena had greater effects at high extrusion temperature. The great expansion resulted in increase of lightness of the extrudates. The 'b' values of pea starch extrudates were insignificant for extrusion temperature, moisture content of the dough, and shear effect (Table 4.1).

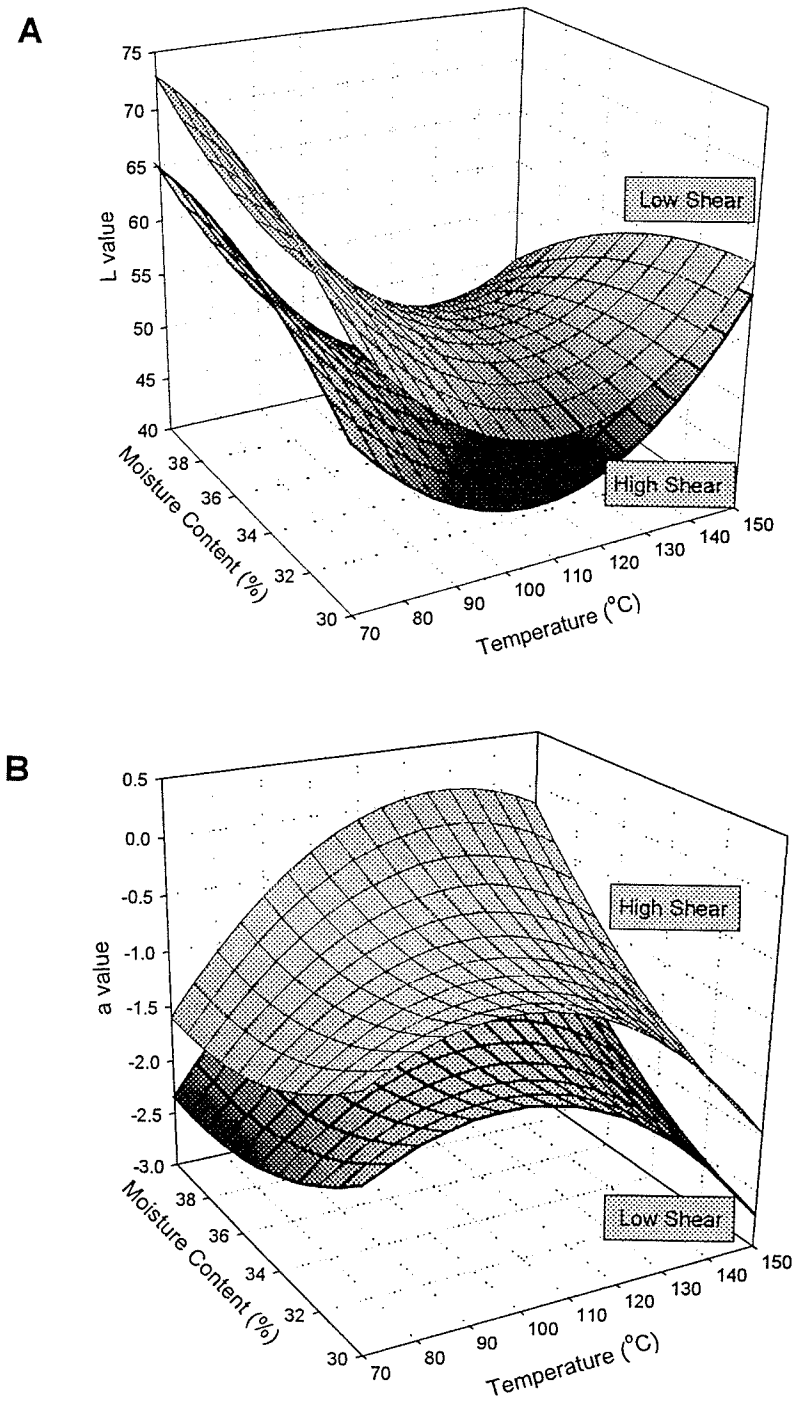

Figure 4.17. Color values (L, a) of extrudates as a function of extrusion temperature moisture content of dough, and screw configuations

4.4.8 Lysozyme Activity and Diffusion

Lysozyme activity was determined in pea starch blended with 1% lysozyme after extrusion processing. Up to 48% lysozyme recovery was recorded in the experiment depending on extruder operating conditions. Lysozyme recovery sharply decreased with an increase of extrusion temperature, whereas moisture content of the dough had the opposite effect (Fig. 4.18). The decrease of the amount of water as a lubricant may have accelerated inactivation of lysozyme due to high shear input during extrusion processing, even though below 70°C extrusion temperature was not high enough to inactivate the lysozyme (Proctor and Cunningham 1988). Shear effect by screw configurations was significant to lysozyme recovery ($P < 0.0001$). Specific mechanical energy input (SME) was highly correlated to lysozyme recovery ($P < 0.0012$, Table 4.1). Thus, both thermal and shear energy might induce inactivation of lysozyme by destroying disulfide bonds in lysozyme.

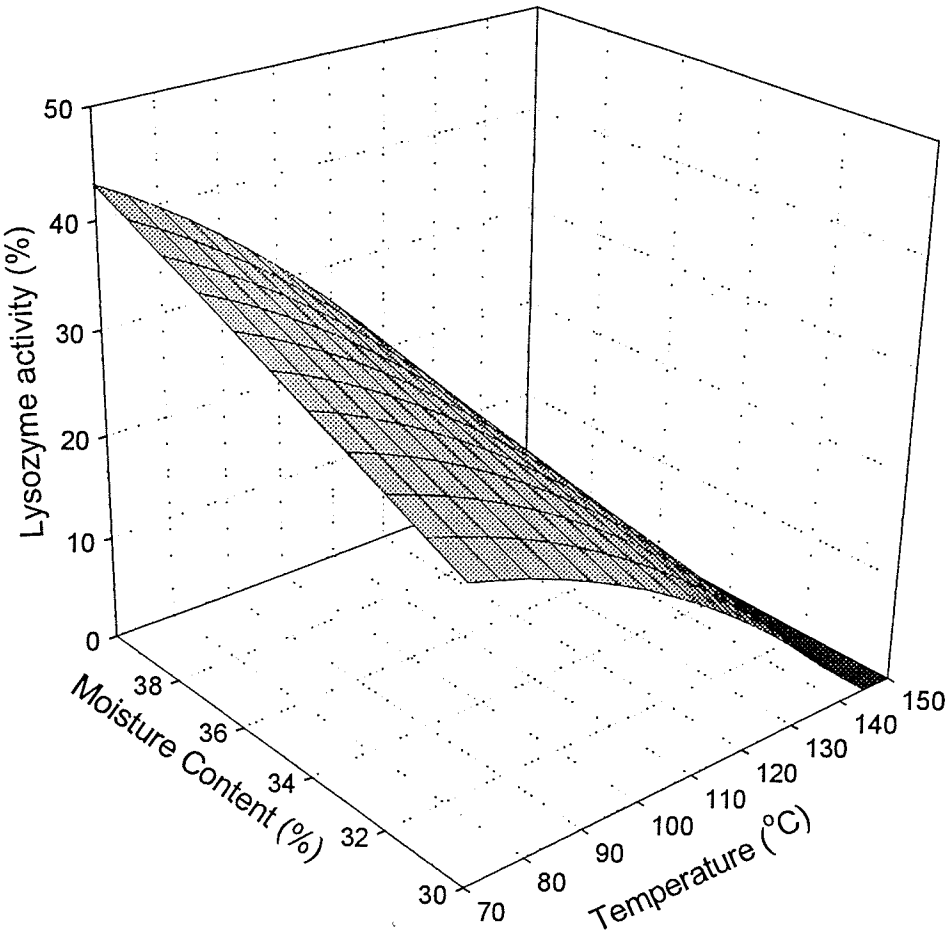

Figure 4.18. Lysozyme recovery after extrusion as a function of extrusion temperature and moisture content of dough

The lysozyme diffusion coefficients were measured for extrudates with four different extrudate densities (Fig. 4.19) (Table 4.5). In the experiment, it was assumed that complete release of lysozyme could be achieved at infinite time. The density and extent of gelatinization of pea starch extrudates affected the release rate of lysozyme at 22°C. The lysozyme diffusion coefficients (D) were obtained by Crank's equation (1975) and ranged from 1.23×10^{-9} m^2/s to 6.45×10^{-9} m^2/s (Table 4.5). The fastest diffusion coefficient of lysozyme was obtained for pea starch extrudates with the lowest density. The release rate of lysozyme might be hindered by partly gelatinized starch granules in the extrudate network. According to DSC results, the 1,181 kg/m^3 sample (Table 4.5) was partly gelatinized (Fig. 4.4). The partly gelatinized extrudate would swell less than more fully gelatinized extrudates and tightly packed starch granules in the partly gelatinized extrudate could cause hindrance for the release of lysozyme from the extrudate network. Therefore, the 1,181 kg/m^3 samples with a low density had a much lower diffusion coefficient than other samples with a higher density. Fig. 4.19 shows the linear relationship between the logarithmic-transformed fractional release of the lysozyme and the time divided by the square of diameter of extrudates. The regression coefficient (R^2) for the diffusion of lysozyme was in the range of 0.7117 to 0.9629 (Table 4.5). The result of this experiment showed that the extrudate densities could affect the release rate of lysozyme.

Table 4.5. Diffusion coefficients (D) of lysozyme in pea starch extrudates with different densities

Extrusion Conditions	Extrudate Density (kg/m^3)	Diffusion Coefficient (10^{-9} m^2/s)	R^2
LS - 90°C – 30%[a]	1576	1.23	0.7117
HS - 70°C – 30%	1181	1.63	0.9629
LS - 70°C – 40%	1352	4.94	0.9487
HS - 90°C – 40%	1073	6.45	0.8797

[a] LS and HS are low and high shear screw configuration, respectively. die temperature (°C), moisture content of the dough (%).

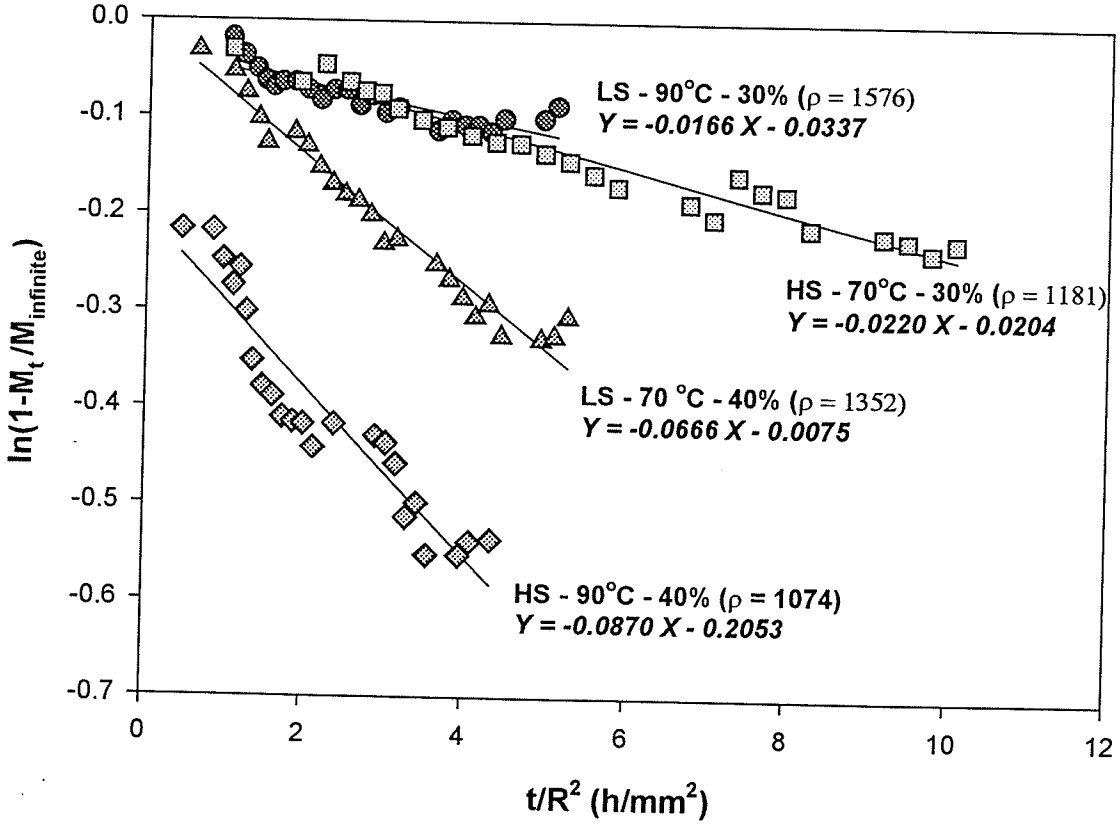

Figure 4.19. Release profiles of lysozyme from extrudates at 22°C.

Lysozyme (1%) and pea starch (99%) were incorporated into extruded pea starch. The lysozyme in extrudates was evaluated for inhibition against *Brochotrix thermosphacta B2*. Lysozyme splits the gram-positive bacteria cell wall linkage (Proctor and Cunningham 1988). Lysozyme as a bacteriocide may damage the bacterial cells that may subsequently recover resulting in an inhibition zone. The 1st inhibition zone showed very narrow zone (Fig. 4.20). The difference in the clarity may be caused by the difference in concentration of released lysozyme from extrudates. Longitudinal inhibition zone from extrudates was less than the cross-sectional inhibition zone (Table 4.6). The distance of the 1st inhibition zone in the longitudinal side of extrudates was double that of the cross-sectional side. This suggests that the release rate of lysozyme in the extrudate network might be differ depending on the surface area of extrudates.

Table 4.6. Effect of lysozyme in extruded pea starch on inhibitory zone distance against *B. thermosphacta*

Extrusion conditions	[b]**Longitudinal distance** (mm)		[c]**Cross-sectional distance** (mm)	
	1st zone	**2nd zone**	**1st zone**	**2nd zone**
LS–70°C–40%[a]	1.0 ± 0.31	12.8 ± 0.68	2.0 ± 0.19	14.9 ± 0.53
LS–90°C–40%	1.6 ± 0.41	15.1 ± 0.20	3.2 ± 0.12	16.5 ± 1.21

[a] L and H are low and high shear screw configuration, respectively.
Extrusion temperature (°C), moisture content of the dough (%)
[b] Plate geometry
[c] Cylindrical geometry

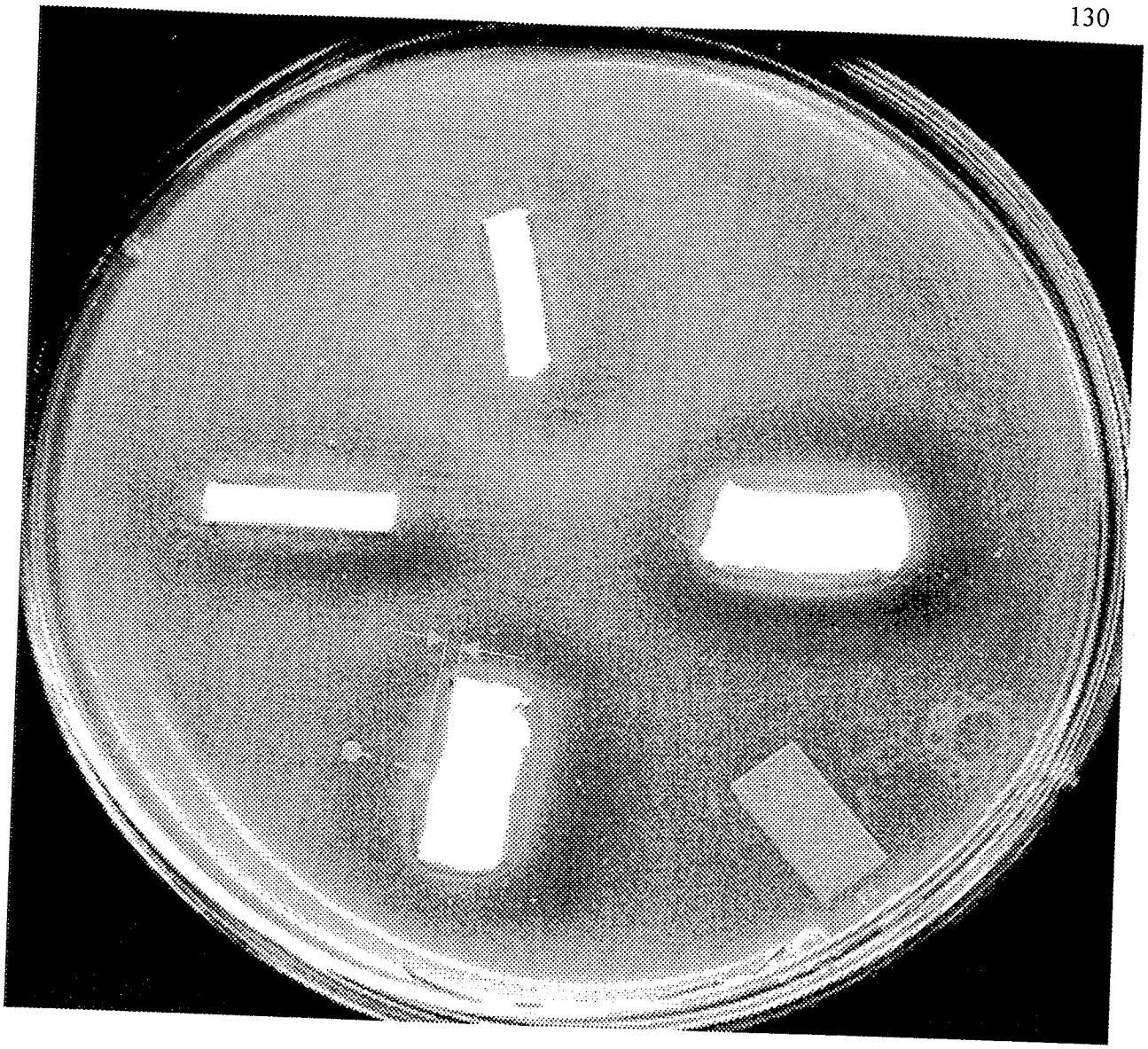

Figure 4.20. Inhibition zone against *B. thermosphacta* from pea starch extrudates containing 1% lysozyme (◄—► 1st inhibition zone; ●– · –● 2nd inhibition zone)

4.5 Conclusions

Although pea starch has excellent properties as a biodegradable packaging material, it has not been utilized in the packaging industry. This has largely resulted from insufficient research on pea starch. In this study, extrusion technology was shown as a powerful tool for manufacturing packaging material based on pea starch. Lysozyme as a natural bacteriocide was incorporated into pea starch extrudates for antimicrobial packaging aspects.

Physico-chemical and mechanical properties of extrudates were significantly altered by various extrusion conditions. An increase in thermal and mechanical energy input caused greater starch gelatinization, melting and degradation. The moisture content of the dough, which is one of most important extrusion processing variables, significantly affected the extent of thermal and mechanical energy input during extrusion processing. Thermal and mechanical degradation of starch granule and molecules affected the physico-chemical properties, such as magnitude of endothermic peak, intrinsic viscosity, pasting properties, water solubility, the extent of expansion, color, bulk and cell wall solid density of pea starch extrudates. Furthermore, apparent elastic modulus and fracture strengths were a function of relative density, indicating that starch extrudates had similar mechanical behavior to synthetic polymers. Lysozyme release rate was affected by density of extrudates. After extrusion, lysozyme in extrudates showed clear inhibition zones as a bacteriocide for *Brochotrix thermosphacta B2*. The extrudate with a wide surface area had bigger inhibition zones than the extrudate with a narrow surface area.

These results indicated that extrusion technology using pea starch could provide the potential for a bioactive packaging material. The pea starch extrudates with strong

mechanical properties showed potential application for manufacturing biodegradable and rigid containers. For manufacturing pea starch film, study would be required to measure mechanical, moisture and gas barrier properties of pea starch film containing a plasticizer will be required.

5.0 General Discussions

Extrusion technology has been shown in this study to be a versatile unit operation in food process technology. Extrusion technology, through changes in physico-chemical properties of feed materials showed great potential for developing functional snack food and biodegradable packaging materials. The choice of various feed materials as a processing variable significantly affected physico-chemical properties of extrudates. Starch as a feed material was shown to be the important component in both experiments. Extrusion technology can be used to modify properties of feed material to create extrudates with snack food potential. Important properties for snack foods include expansion, mechanical properties, and color while in the manufacture of packaging material, important extrudate properties include mechanical strength and color. The physico-chemical changes of starch molecules during extrusion processing significantly correlated to physical and mechanical properties of the final product. Extrusion processing combining thermal and mechanical energy under various processing variables caused macromolecular degradation and transformation, changes in physico-chemical properties of extrudates and inactivation of the added enzyme - lysozyme. The relationship between the extrusion processing variables and physico-chemical properties of extrudates were compared based on statistical analysis. The water holding capacity and solubility of extrudates in both studies showed that the extent of macromolecular degradation of feed materials significantly increased under more severe conditions than mild extrusion processing conditions as could be predicted. The macromolecular degradation of starch as a major component of the feed material under various extrusion processing conditions was in good agreement with other studies as referenced in the literature review.

Extrusion temperature was an important processing variable during extrusion cooking. In the snack food study, the highest extrusion temperature was required for maximum expansion while in the packaging study temperature control was more sensitive and was controlled slightly above the gelatinization temperature of the pea starch to obtain desired properties.

The physical and mechanical properties of extrudates were directly affected by the expansion of extrudates. Starch that has a thermoplastic behavior showed high expansion properties, whereas barley that contains multi-components such as starch, protein, fiber, showed relatively low expansion properties. Camire and coworkers (1990) also reported that fiber-enriched extrudates show reduced expansion during extrusion processing. Therefore, the addition of corn starch in barley meal resulted in an increase of the expansion of extrudates in the snack food experiment due to the reduction of non-starch contents. The addition of lysozyme to pea starch provided multi-functional benefits such as antimicrobial and biodegradable packaging materials. The proper selection of feed materials under various extrusion conditions is necessary to obtain the desired final product characteristics.

Mechanical properties, such as elastic modulus and fracture strength of extrudates followed a power-law fashion for synthetic polymers. The power-law fashion model, which relates the mechanical properties to the foam density and cell-wall density, was used to characterize the foam behavior of extrudates in this study. Both the snack food and packaging experiments identified the behavior of the cellular solid structure of the extrudate. The cellular solid structure, which affects mechanical behavior of the extrudate, followed the theoretical power-law model. The model provided useful information for optimization of extrusion processing conditions to produce the desired

final products. Expansion properties are important for snack foods while packaging materials require mechanical strength measured by bending and compression tests.

The color of snack food and packaging materials is an important quality factor for acceptability. Extrudate color in both experiments was affected by both feed material and extrusion processing conditions. Corn starch, as industrial standard feed material, showed less color change than barley meal after extrusion processing. The brightness of pea starch extrudates was significantly decreased after extrusion processing. However, the extrudate above gelatinization temperature showed semitransparent properties providing beneficial properties to the packaging material. The color values of extrudates were significantly correlated to the mechanical properties and degree of the expansion. Therefore, understanding the relationship between physico-chemical properties and mechanical properties must be considered in optimizing extrusion processing conditions.

6.0 Overall Conclusions

Cereal and legume crops offer potential for value added products through extrusion technology. Barley is underutilized as a food source, however, is of high nutritional value. Pea starch, resulting from fractionation of field peas, demonstrates the functional property of strong gel strength.

Barley as a traditional crop in Manitoba occupied 19.0 percent of harvested area (481,600 hectares) with production of 1.62 million tones in 2000. Extruded barley in this study was shown to have potential in the lucrative snack and ready to eat breakfast cereal industries. In combination with other starch sources such as maize, favorable extrudates were obtained by great expansion, which can provide more acceptable mouth-feel.

Pea starch extrudates were used as a base material for packaging films or containers to create biodegradable materials. Lysozyme was successfully incorporated into the extrudates through extrusion technology offering the potential of a biodegradable and antimicrobial packaging material. A new generation of active packaging, including a natural antimicrobial agent, from biodegradable material has advantages such as enhanced food safety and reduction in pollution.

Extrusion process variables affected the properties of the end product and the extrusion process must be optimized for manufacturing each desired product. Extrusion technology using various cereal and legume materials proved its important role as a powerful tool to develop various food products in food technology. Lysozyme incorporation in extrudates will allow wide application of extrusion technology to develop new functional food products.

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Appendix A. Data for Physico-chemical Analysis of Extruded Pea Starch

[a]Extrusion	[b]SME (Wh/kg)		Die Pressure (Bar)		Intrisic Viscosity(mL/g)		[c]WAI (g/g)		[d]WSI (%)		Lysozyme Activity(%)	
Condition	Average	[e]S.D.	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.
H-70-30	259.6	0.00	46.0	1.00	131.7	1.3584	3.83	0.1278	5.39	0.2599	20.95	1.8753
H-70-35	192.1	2.54	33.3	0.58	133.5	1.3969	3.32	0.1053	3.50	0.3053	29.46	0.9643
H-70-40	154.0	0.00	26.3	0.58	150.6	5.7500	3.22	0.0481	2.64	0.1228	36.66	2.1278
H-90-30	240.5	2.54	45.0	1.00	124.6	0.2544	5.41	0.8065	10.83	1.9062	28.34	3.4053
H-90-35	183.3	2.54	34.3	1.53	133.4	1.7415	4.57	0.6899	7.78	2.2896	38.88	1.4690
H-90-40	140.8	4.40	25.0	1.00	134.2	0.2017	3.77	0.2507	3.95	0.5489	43.52	2.9315
H-120-30	178.9	2.54	28.3	1.53	121.3	2.0465	5.29	0.2966	17.33	2.3283	4.29	0.5967
H-120-35	137.9	2.54	21.7	1.16	129.8	1.3155	4.59	1.0031	10.87	4.2474	9.54	1.6866
H-120-40	117.3	5.08	17.7	0.58	130.1	2.4108	3.72	0.1535	5.93	0.5008	13.11	1.6012
H-150-30	151.1	5.08	17.7	0.58	112.2	3.9300	5.37	0.3426	27.92	3.3005	0.21	N/A
H-150-35	121.7	6.72	13.3	0.58	124.0	1.0000	5.55	0.4587	24.64	4.2941	0.92	N/A
H-150-40	107.1	2.54	12.7	0.58	132.1	0.3123	4.61	0.6035	13.24	1.5833	2.85	0.6253
L-70-30	242.0	0.00	48.3	0.58	138.1	1.9868	3.77	0.1099	4.95	0.2551	26.93	1.3707
L-70-35	181.9	2.54	36.7	2.08	146.2	9.9078	3.25	0.0454	3.04	0.3664	34.76	1.3441
L-70-40	145.2	4.40	27.3	1.53	158.1	1.2340	3.27	0.1053	2.51	0.1711	39.69	0.3037
L-90-30	222.9	6.72	43.7	1.53	135.3	2.7576	4.40	0.0565	8.85	0.4019	30.46	2.0290
L-90-35	171.6	0.00	34.0	1.00	145.1	2.9292	4.21	0.373	6.01	0.9929	37.09	1.1150
L-90-40	139.3	2.54	26.7	0.58	152.9	0.5518	4.17	0.8433	11.36	7.7662	47.70	1.3391
L-120-30	164.3	5.08	29.7	2.08	124.6	1.7795	4.97	0.1441	17.46	1.2263	5.79	1.3368
L-120-35	138.6	N/A	21.5	N/A	133.3	1.3360	5.23	N/A	14.00	N/A	13.30	N/A
L-120-40	115.9	2.54	18.3	0.58	134.9	2.0793	3.73	0.1846	6.03	1.1614	15.84	0.9501
L-150-30	140.1	0.00	18.3	0.58	113.5	6.8707	5.24	0.1776	37.25	3.1657	0.74	N/A
L-150-35	115.9	2.54	12.7	0.58	123.9	2.2003	5.20	0.2752	35.62	2.1022	1.15	N/A
L-150-40	105.6	0.00	11.3	0.58	125.6	1.6581	4.95	0.5692	15.63	3.2055	3.85	0.5473

[a]Extrusion processing condition: H, L-screw configuration, extrusionj temperature (°C), and moisture content (%)

[b]Specific mechanial energy input [c]Water absorption index [d] Water solubility index [e]Standard deviation

Extrusion Condition	[a]SEI		[b]LEI		[c]VEI		Extrudate Density (kg/m^3)		Solid Density (kg/m^3)	
	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.
H-70-30	2.09	0.3389	0.332	0.0282	0.692	0.1090	1337.5	57.22	1443.7	33.46
H-70-35	1.45	0.0997	0.449	0.0503	0.648	0.0363	1314.5	106.48	1406.4	21.95
H-70-40	1.38	0.1744	0.444	0.0266	0.612	0.0750	1167.4	25.79	1380.5	23.59
H-90-30	3.23	0.4429	0.227	0.0175	0.729	0.0744	1241.1	104.48	1461.7	38.90
H-90-35	3.05	0.2193	0.238	0.0111	0.726	0.0519	1178.6	101.58	1434.5	31.65
H-90-40	2.52	0.1148	0.258	0.0128	0.651	0.0298	1144.8	66.11	1391.1	21.26
H-120-30	4.41	0.4158	0.182	0.0080	0.801	0.0426	1183.6	44.18	1505.2	8.30
H-120-35	25.04	17.5845	0.075	0.0856	0.875	0.1507	1003.8	130.53	1472.0	24.01
H-120-40	33.31	10.9047	0.027	0.0069	0.837	0.1154	958.3	134.37	1439.7	21.32
H-150-30	4.62	0.5938	0.550	0.0243	2.532	0.2456	361.7	26.67	1523.3	7.27
H-150-35	1.37	0.2672	0.823	0.2040	1.161	0.4641	830.5	424.72	1489.1	16.70
H-150-40	5.50	0.9477	0.134	0.0245	0.719	0.0182	1096.6	33.18	1472.6	8.96
L-70-30	1.84	0.0235	0.367	0.0094	0.676	0.0244	1428.3	48.30	1331.2	19.38
L-70-35	1.30	0.0525	0.478	0.0056	0.622	0.0299	1344.9	55.81	1323.5	15.87
L-70-40	1.26	0.1410	0.502	0.0079	0.630	0.0640	1267.5	191.13	1302.1	52.64
L-90-30	2.77	0.1408	0.242	0.0149	0.669	0.0235	1480.3	44.31	1496.1	3.49
L-90-35	2.84	0.5667	0.214	0.0165	0.605	0.1171	1508.7	77.71	1459.7	41.45
L-90-40	2.68	0.3330	0.239	0.0310	0.633	0.0327	1275.3	113.50	1391.8	55.60
L-120-30	4.23	0.4519	0.186	0.0039	0.784	0.0741	1544.3	56.71	1494.6	6.94
L-120-35	3.73	N/A	0.203	N/A	0.977	N/A	1215.4	N/A	1480.7	N/A
L-120-40	20.87	3.0034	0.032	0.0061	0.652	0.0390	1408.2	68.26	1423.3	12.76
L-150-30	5.51	1.0656	0.470	0.0564	2.555	0.2231	306.0	51.35	1518.3	6.57
L-150-35	1.03	0.1214	0.974	0.0814	0.996	0.0581	837.0	8.25	1472.3	4.64
L-150-40	5.92	1.4472	0.093	0.0125	0.541	0.0898	1487.2	71.45	1459.0	24.44

[a] Cross-sectional expansion index [b] Longitudinal expansion index [c] Volumetric expansion index

Extrusion Condition	[a]E_{ac}(MPa)		[b]σ_{ac}(MPa)		E_{ab}(MPa)		σ_{ab}(MPa)	
	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.
H-70-30	1104.1	171.62	53.0	6.43	3953.4	448.25	111.88	6.35
H-70-35	1306.1	84.44	51.0	9.51	4645.1	531.18	120.28	17.41
H-70-40	1203.3	67.41	43.1	6.13	4395.0	808.24	111.11	15.48
H-90-30	784.9	390.35	36.9	20.57	2498.2	310.72	42.54	1.63
H-90-35	587.2	81.65	21.3	3.80	1998.0	244.52	34.07	2.89
H-90-40	732.4	55.38	23.7	5.14	1726.7	486.89	42.54	1.63
H-120-30	522.3	110.68	24.1	5.81	2458.3	381.06	62.13	8.13
H-120-35	326.8	37.92	9.0	4.73	1277.9	136.33	10.58	8.74
H-120-40	273.6	108.56	6.8	2.53	1601.7	156.93	5.00	0.32
H-150-30	54.3	4.93	2.5	0.56	225.4	44.84	6.27	1.35
H-150-35	462.1	135.17	15.2	2.85	869.2	135.75	17.36	1.99
H-150-40	641.9	90.91	25.8	4.57	3075.9	53.63	26.75	5.11
L-70-30	1435.5	200.97	51.6	2.87	4252.7	78.43	107.97	10.50
L-70-35	1214.4	183.04	51.7	9.72	4754.7	212.91	118.16	8.90
L-70-40	1041.9	136.26	35.8	1.96	4256.7	592.00	107.42	8.43
L-90-30	1298.2	203.42	32.1	3.33	2754.8	590.60	72.34	6.79
L-90-35	1192.2	461.72	41.9	5.52	3457.9	539.69	66.00	14.93
L-90-40	1064.7	117.74	30.0	1.17	2625.6	948.88	41.54	6.06
L-120-30	961.5	204.62	30.4	4.40	3145.6	565.66	71.00	10.42
L-120-35	442.6	N/A	17.1	N/A	1934.1	N/A	23.36	N/A
L-120-40	515.2	170.22	12.3	5.29	1959.8	162.78	5.51	1.92
L-150-30	56.5	14.44	2.3	0.77	281.0	44.39	6.34	1.35
L-150-35	559.0	184.68	17.0	4.78	1593.7	115.95	40.42	4.23
L-150-40	720.1	111.20	30.2	2.74	2501.6	175.12	30.38	7.16

[a] Apparent elastic modulus
[b] Fracture strength

Extrusion Condition	L values		a values		b values	
	Average	S.D.	Average	S.D.	Average	S.D.
H-70-30	52.83	4.928	-0.711	0.150	11.37	0.736
H-70-35	60.04	2.237	-1.000	0.361	11.84	0.869
H-70-40	62.37	1.785	-1.000	0.267	11.31	1.050
H-90-30	52.44	5.848	-0.167	0.120	12.83	1.278
H-90-35	54.64	5.839	-0.522	0.126	12.56	0.601
H-90-40	57.89	4.801	-0.522	0.227	13.10	0.137
H-120-30	45.09	2.552	0.400	0.067	12.43	0.433
H-120-35	43.98	5.372	0.756	0.435	11.70	1.074
H-120-40	44.72	0.923	1.067	0.088	11.69	0.593
H-150-30	60.98	1.435	-0.867	0.088	11.74	0.291
H-150-35	60.21	5.188	-0.189	0.150	12.63	0.723
H-150-40	44.93	1.102	1.100	0.120	12.97	0.367
L-70-30	65.97	4.652	-1.556	0.724	12.76	0.356
L-70-35	70.69	2.5	-1.889	0.423	11.70	0.617
L-70-40	72.61	3.825	-1.867	0.273	11.48	0.222
L-90-30	54.88	1.723	-0.989	0.499	12.39	0.826
L-90-35	55.48	1.797	-1.300	0.498	12.57	1.889
L-90-40	60.34	2.848	-1.311	0.201	13.07	1.212
L-120-30	48.54	0.963	-0.189	0.164	12.91	0.019
L-120-35	59.62	N/A	-1.056	N/A	12.13	N/A
L-120-40	49.70	1.117	0.456	0.150	12.98	0.402
L-150-30	61.67	0.987	-1.244	0.150	12.11	0.495
L-150-35	66.18	1.862	-1.033	0.145	13.00	0.167
L-150-40	48.07	3.205	0.456	0.222	13.64	0.534

Appendix B. Probabilities of Correlation Coefficients for Physico-chemical Properties of Extruded Pea Starch

Variables	SME	Pressure	SEI	LEI	VEI	Density	Solid density	E_{ac}	E_{ab}
SME	**1.0000**								
Pressure	0.0001	**1.0000**							
SEI	0.0031	0.0113	**1.0000**						
LEI	0.8630	0.2898	0.0001	**1.0000**					
VEI	0.1515	0.0046	0.8360	0.0024	**1.0000**				
Density	0.0045	0.0001	0.2924	0.0001	0.0001	**1.0000**			
Solid Density	0.2207	0.0060	0.3350	0.6499	0.0001	0.0024	**1.0000**		
E_{ac}	0.0001	0.0001	0.0002	0.9956	0.0001	0.0001	0.0001	**1.0000**	
E_{ab}	0.0001	0.0001	0.0063	0.6207	0.0001	0.0001	0.0001	0.0001	**1.0000**
σ_{ac}	0.0001	0.0001	0.0001	0.8302	0.0001	0.0001	0.0001	0.0001	0.0001
σ_{ab}	0.0001	0.0001	0.0001	0.1007	0.0001	0.0001	0.0001	0.0001	0.0001
Lysozyme Activity	0.0012	0.0001	0.0341	0.2971	0.0001	0.0001	0.0001	0.0001	0.0001
Intrinsic viscosity	0.1055	0.0020	0.1421	0.8472	0.0001	0.0001	0.0001	0.0001	0.0001
WAI	0.1967	0.0097	0.1941	0.2174	0.0001	0.0004	0.0001	0.0001	0.0001
WSI	0.0035	0.0001	0.3034	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
L	0.2549	0.1243	0.0001	0.0001	0.2525	0.2872	0.0001	0.0054	0.0244
a	0.0010	0.0001	0.0001	0.0001	0.4541	0.6992	0.0002	0.0001	0.0009
b	0.3975	0.3739	0.2556	0.5320	0.4623	0.7895	0.5327	0.7693	0.1949

Variables	σ_{ac}	σ_{ab}	Lysozyme Activity	Intrinsic viscosity	WAI	WSI	L	a	b
σ_{ac}	**1.0000**								
σ_{ab}	0.0001	**1.0000**							
Lysozyme activity	0.0001	0.0001	**1.0000**						
Intrinsic viscosity	0.0001	0.0001	0.0001	**1.0000**					
WAI	0.0001	0.0001	0.0001	0.0001	**1.0000**				
WSI	0.0001	0.0001	0.0001	0.0001	0.0001	**1.0000**			
L	0.0118	0.0002	0.0007	0.0002	0.0313	0.8253	**1.0000**		
a	0.0001	0.0001	0.0001	0.0001	0.0275	0.6375	0.0001	**1.0000**	
b	0.4609	0.2087	0.4475	0.2199	0.7079	0.8810	0.1852	0.1469	**1.0000**