2017

Understanding the Impact of Extrusion Processing on Rheological, Textural and Functional Properties of High-protein, High-fiber Extrudates

Poonam Singha
South Dakota State University

UNDERSTANDING THE IMPACT OF EXTRUSION PROCESSING ON RHEOLOGICAL, TEXTURAL AND FUNCTIONAL PROPERTIES OF HIGH-PROTEIN, HIGH-FIBER EXTRUDATES

BY

POONAM SINGHA

A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy

Major in Agricultural, Biosystems and Mechanical Engineering

South Dakota State University

2017

UNDERSTANDING THE IMPACT OF EXTRUSION PROCESSING ON RHEOLOGICAL, TEXTURAL AND FUNCTIONAL PROPERTIES OF HIGH-PROTEIN, HIGH-FIBER EXTRUDATES

POONAM SINGHA

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this dissertation does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

K. Muthukumarappan, Ph.D. Date
Major and Dissertation Adv

Van Kelley, Ph.D. Date
Head, Department of Agricultural and Biosystems Engineering

Dean, Graduate School Date

I dedicate this dissertation to my beloved pet Bobby.

ACKNOWLEDGEMENTS

I would like to sincerely thank my supervisor Dr. Kasiviswanathan Muthukumarappan (Dr. Muthu) for his guidance, support and encouragements during my Ph.D. program. Thanks to his help I could deepen my knowledge in extrusion and food process engineering. Dr. Muthu's door was always open; any time of the day no matter how big or small a problem was.

I would like to extend my appreciation to all my advisory committee members--Dr. Padmanaban Krishnan, Dr. ZhengRong Gu, and Dr. Hemachand Tummala for their suggestions regarding my research project, their valuable time, and serving on my committee.

Special thanks to my committee member and mentor, Dr. Krishnan, who broadened my background in cereal science and guided me in my experiments. Throughout the four years Dr. Krishnan was always available for advice and to discuss any problems I encountered. I am grateful to Dr. Krishnan's group in Department of Dairy and Food Science especially Mr. Bipin for helping me in analyzing the dietary fiber content of my samples, Ms. Jigyasa and Ms. Ashley for helping me to process DDGS for food application and Ms. Hadeel for her friendship and support.

I am extremely grateful to extremely talented researchers of my group for their valuable cooperation, support and friendship. I owe special thanks to Dr. Bishnu, Dr. Umesh, Dr. Sushil, Dr. Arash, Dr. Shalini, Dr. Vijay and Jasmeen who assisted me during the extrusion projects. Thanks to their friendship and making my Ph.D. program an enjoyable experience.

I would specially thank to Dr. Jeyam Subbiah, for providing me the facility to use the extruder in University of Nebraska-Lincoln and his student Mr. Tushar in helping me during the one of the extrusion trials.

I am thankful to Dr. Ananya Biswas of Department of Dairy and Food Science in providing me the facility to use Kjeldahl apparatus whenever I requested for. I express my sincere gratitude to Ms. Simon Deon of Department of Animal Science for her kind support in analyzing fat and protein content of my samples. I really appreciate the gracious aid by Mr. Reza, Mr. Nirmal and Ms. Sally of Department of Electrical Engineering in running my samples for electron microscopy and FTIR.

No words can be enough to thank my parents, Mrs. Rekha Singha and Mr. Siben, Singha who have always supported my decisions and encouraged me to move forward. Their love and prayers made this work possible.

Throughout my PhD I had an amazing and supportive husband, Sushil. He listened to all my presentations and complications; he also helped me in the tough task of formatting my thesis, thanks Sushil!

TABLE OF CONTENTS

ABBREVIATIONS

°C	-	Degree Celsius
a*	-	Redness/Greenness
AA	-	Antioxidant Activity
ANOVA	-	Analysis of Variance
AP	-	Apple Pomace
b*	-	Yellowness/Blueness
BD	-	Bulk density
CAGR	-	Compound Annual Growth Rate
CCRD	-	Central Composite Rotatable Design
CG	-	Corn Grits
C_{sr}	-	Correction factor for shear rate
C_{ss}	-	Correction factor for shear stress
CV	-	Coefficient of Variance
db	-	dry basis
DDG	-	Distiller's Dried Grain
DDGS	-	Distiller's Dried Grain with Solubles
DDS	-	Distiller's Dried Solubles
df	-	degree of freedom
DSF	-	Defatted Soy Flour
DW	-	Dry Weight
ER	-	Expansion Ratio

FDDG	-	Distiller's Dried Grain processed for Food applications
FTIR	-	Fourier Transform Infrared Spectroscopy
GAE	-	Gallic Acid Equivalent
GF	-	Garbanzo Flour
HP	-	Horse Power
IDF	-	Insoluble Dietary Fiber
L*	-	Brightness/Darkness
*L*s	-	Screw length
MFR	-	Mass Flow Rate
MS	-	Mean Squares
NFE	-	Nitrogen Free Extract
R^2	-	Coefficient of Determination
r_b	-	Inner barrel radius
r_{corr}	-	Radius correction
r_{eff}	-	Effective radius
rpm	-	revolution per minute
RSM	-	Response Surface Methodology
SDF	-	Soluble Dietary Fiber
SEM	-	Scanning Electron Microscopy
SME	-	Specific Mechanical Energy
SS	-	Sum of Squares
TDF	-	Total Dietary Fiber
TPC	-	Total Phenolic Content

UD	-	Unit density
WAI	-	Water Absorption Index
wb	-	wet basis
WSI	-	Water Solubility Index
ΔE	-	Color change
η_{app}	-	Apparent viscosity
τ_s	-	Shear stress
Ω	-	Net torque
ω	-	Angular velocity
$\dot{\gamma}_t$	-	Shear rate

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

UNDERSTANDING THE IMPACT OF EXTRUSION PROCESSING ON RHEOLOGICAL, TEXTURAL AND FUNCTIONAL PROPERTIES OF HIGH-PROTEIN, HIGH-FIBER EXTRUDATES

POONAM SINGHA

2017

Extrusion processing is a technology widely used to make ready-to-eat snack and breakfast cereal products. Expanded products mainly consists of high levels of starch resulting in optimal texture and consumer acceptance. However, these products are usually low in nutritional value. One of the many alternatives are to combine legumes and cereals to improve the protein quality of the product. Another possibility is to enhance the nutritional value by incorporating fruits and vegetables. Fruits and vegetables are consistently under-consumed by the American population and incorporation into extruded products may help increase the intake of important nutrients, such as dietary fiber.

The study was divided in to three parts. In the first part, a lab-scale single screw extruder was used for processing directly expanded corn based products containing varying proportion of apple pomace and defatted soy flour. Experimental design with apple pomace level (0 to 20%), barrel and die temperature (100 to 140^{o}C), screw speed (100 to 200 rpm), and moisture content of blend (14 to 20% wet basis) as independent variables produced 27 different combinations that were studied using response surface

methodology to investigate the effect of these variables on system parameters (apparent viscosity, mass flow rate, torque, die pressure, dough temperature, and SME). As the temperature profile increased, apparent viscosity, die pressure and specific mechanical energy decreased. Increasing AP content in the blends significantly (P<0.05) increased the bulk density, total phenolic content and the antioxidant activity of the extrudates. The expansion ratio increased with 5% pomace level of inclusion compared with control (0% AP) but decreased significantly (P<0.05) at higher levels of inclusion (10%-20%). Moisture content had quadratic influence on water absorption and solubility indices. Optimal extrusion cooking conditions most likely to produce AP enriched extruded snack food were at 140^{o}C, 20% moisture content and 200 rpm. Extruded snacks with low hardness value exhibited high crispness and brittleness. The surface morphology was examined through scanning electron microscope. The increase in extrusion temperature and shear resulted in more damaged cellular structure of the extrudates. Presence of air cells indicated crisper extruded snacks. The changes in the molecular distribution in the extruded snacks were determined using Fourier Transform Infrared Spectroscopy (FTIR). FTIR showed that there were significant changes in the carbohydrate components and the structure of the proteins on extrusion, with consequent effects on the expansion and density of the extruded product. This study showed the variation in internal structure and molecular bonds and their effect on physical property such as texture of the extruded snacks. It also indicated active interaction between apple pomace and starch during expansion process.

In the second part, single screw extrusion of distillers dried grains processed for food application (FDDG), garbanzo flour and corn grits were investigated to produce

high-protein and high-fiber extrudates. A four-factor central composite rotatable design was adopted to study the effect of FDDG level, moisture content of blends, extrusion temperature, and screw speed on the apparent viscosity, mass flow rate or MFR, torque, and specific mechanical energy or SME during extrusion and on the physical properties (expansion ratio, bulk density, color parameters), functional properties (water absorption and solubility indices) and nutritional properties (total dietary fiber, soluble and insoluble dietary fiber) of the extrudates. With increase in the extrusion temperature from 100 to 140^{o}C, apparent viscosity, specific mechanical energy and torque value decreased. Increase in FDDG level resulted in increase in apparent viscosity, SME and torque. FDDG had no significant effect (P>0.05) on mass flow rate. SME also increased with increase in the screw speed which could be due to the higher shear rates at higher screw speeds. Screw speed and moisture content had significant negative effect (P<0.05) on the torque. The apparent viscosity of dough inside the extruder and the system parameters were affected by the processing conditions. FDDG incorporation had a significant effect on the total dietary fiber, color parameters and the functional properties of the extrudate snacks. Desirable expanded extrudates with high level of total dietary fiber were obtained with blends containing 20 % FDDG at 140^{o}C extrusion temperature, 167 rpm screw speed and 19% feed moisture content.

In the third part, twin screw extrusion of defatted soy flour and corn grits blends enriched with apple and grape pomace were conducted in two separate studies. Apple pomace-defatted soy flour-corn grits blends were extruded in a conical counter rotating twin-screw extruder. Response surface methodology using a central composite design was used to evaluate the effects of independent variables, namely apple pomace level (0-

20%), die and barrel temperature (100-140^{o}C), screw speed (100–200 rpm) and moisture content (14-20 % wb) on the product responses (expansion ratio, bulk density, water absorption index, water solubility index, texture and color). The product responses were most affected by changes in moisture content, pomace level and to a lesser extent by screw speed. Numerical optimization studies resulted in 15% pomace level, 110^{o}C temperature, 175 rpm screw speed and 15.5 % moisture content as optimum variables to produce acceptable extrudates. Structural observation of the extruded snacks was also conducted. Pomace level and processing conditions significantly affected the internal structure of the extrudates. Porous structure exhibited crispness and compact structure indicated dense product. FTIR analysis was carried out to understand the changes in the polysaccharide and amide bonds in the extrudate snacks.

Different ratio of grape pomace was mixed with defatted soy flour mixed and corn grits for the development of extrudates using conical twin screw extruder. Response surface methodology was used to investigate the effect of grape pomace level and extrusion processing conditions on the product properties. Five different blends at a level of 0-15 % w/w grape pomace (GP) were extrusion cooked with varied barrel and die temperature (100-160 ^{o}C), screw speed (100-250 rpm) and feed moisture (15-25 % wet basis). Increasing GP content in the blends significantly (P<0.05) increased the bulk density and water absorption index and decreased the expansion ratio, lightness, water solubility index of the extruded snacks. Moisture content had quadratic influence on water absorption and solubility indices. Increase in GP level in the blends significantly increased the antioxidant properties of the extrudates. The findings showed that extrusion processing determines the final extrudates properties and AP, GP and FFDG addition in

cereal and legume flours significantly improves the functional and nutritive properties of the extrudates.

CHAPTER 1

Introduction and Background

1.1 Introduction

1.1.1 Background

Modern life is characterized by limited free time and long working hours, making it difficult for most people to have proper meals resulting in an increase in consumer preference for ready-to-eat products. This change is now growing the demand for single-portion, portable, and healthier snack products (Euromonitor International, 2015). Forecast of sales of snacks in USA is shown in Table 1.1. Growing number of young population are attracted to snack products which are particularly tasty and easy to be consumed. According to a report published by Mintel (2015), in USA, 94% of consumers snack at least once a day, while more than 50% are likely to snack 2 to 3 times a day. As snacking is replacing traditional meals, 33% of consumers are snacking on healthier foods more than ever before. Therefore, food industries have increased the production of ready-to-eat products using several processes. One such well-established food processing technique in industries is extrusion. It is a high temperature-short time which is characterized by continuous cooking, mixing and forming processing (Singh and Muthukumarappan, 2017b) and produces direct expanded materials with high quality. Extrusion is flexible in the production of new products, such as cereal baby foods, breakfast cereals, snack foods, bakery products, pastas, etc. The extrusion process offers the ability to choose the ingredients and the ways of processing them. Manufacturers use extrusion to produce healthier snacks with varied ingredients. Moreover, the extrusion process eliminates some of the naturally occurring toxins and reduces the micro-

organisms present in the final product, thereby making them safer for consumption. According to IMARC (2017), the global extruded snack food market reached a value of around US$ 50 Billion in 2016, growing at a CAGR of around 3% during 2009-2016.

During the extrusion process, the screw exerts shearing action on starch and protein- based materials and along with high-temperature transforms the material to a viscoelastic mass. At the exit of the die, the pressure drops suddenly and the emerging material expands. The physical properties of the product (density, expansion, texture, etc.) relies on the material composition (such as presence of protein, starch, fiber and moisture), and the processing conditions during extrusion.

The fruit processing industry generates tons of fruit “waste” every year. Depending on the processing method e.g. juice pressing to develop final products; some create more “waste” or by-product than others. This “waste” or by-product can be described as the pips, kernel, skin or peel of the fruit. Presently, there are no functional applications for these by-products. Processors can offer some types of by-products e.g. apple pomace or grape pomace, to farmers for animal food but the remaining fruit waste streams are incinerated or dumped in landfills, at a cost to the processor. Fruit by-products still contain an abundant quantity of macro and minor nutrients e.g. dietary fiber and minerals. As these fruit by-products are derived from the exterior of the fruit, they tend to contain a substantial quantity of fiber e.g. cellulose, hemicelluloses and pectin. Not only does fiber offer significant health benefits, but it has also been demonstrated to contain a high functionality which can be utilized as an ingredient in food products. It has such functional properties as water holding, binding, swelling and the ability to form viscous solutions. In recent times, consumers have become more conscious of the source

of the ingredients which make up the products they consume. Preference for natural ingredients in food products has increased. As fruit byproduct flours are from a natural source and have not undergone any chemical changes they may be considered to be natural ingredients. They are also free of gluten and lactose, which makes them ideal candidates for gluten and lactose-free products.

Maga and Kim (1989) were the first to report addition of fruits to extruded snacks. Researchers have since then studied the addition of fruit juice, paste, powders, pomace, peels and seeds into extruded products. One motivation is the addition of value to food processing residues and reduction of waste (Yagci and Gogus, 2010). Other drivers are the concentrated nutrient content of the byproducts (especially in terms of bioactive compounds) and growing interest in increasing the dietary fiber content of foods.

The most widely consumed extruded snacks are made primarily with cereals/grains due to their good expansion characteristics. Cereals such as corn are gluten free, easily accessible and have a high starch content, which can give excellent expansion characteristics. For this reason, corn in different form has been widely used as raw material for extrusion (Singha and Muthukumarappan, 2017a). Most cereal-based snack products consumed by children are made from corn and hence there is a need to improve the nutritional value of this kind of food. Combining corn with legumes increases both the amount and quality of the protein (Young, 1991). Legumes are an excellent source of protein, fiber and many nutrients.

Legumes such as soybean are rich source of protein that can be used to improve the diet of millions of people. Typically, on a wet basis, stored mature soybeans contains

13 % water and hence it contains about 35% protein, 17% oil, 31% carbohydrate, and 4.4% ash (Liu, 1997). Soybean in different forms (Bookwalter et al., 1971; Chiang, 2007; Iwe et al., 2001a; Lobato et al., 2011; Singh and Muthukumarappan, 2014a; Singh and Muthukumarappan, 2016; Yu et al., 2014) have been extensively used as a major ingredient during food and feed extrusion. Addition of soybean can act as a good source of protein in formulated food products besides offering other functional, nutritional and health benefits (Friedman and Brandon, 2001). The defatted soybean flour, a by-product of oil processing industry, has been used to increase the protein content of the extruded snacks (Alam et al., 2016).

To cater to the millennial's demand of vegan protein, food industries are developing protein isolates from unconventional legume sources. Garbanzo have a high (40–50%) starch content, which may favor an extrusion process to produce directly expanded snack foods. It has good nutritional value with almost 20-28% protein and protein efficiency ratio or PER of 2.64. However, processing of garbanzo into extruded snacks is limited (Batistuti et al., 1991). Previous studies on extrusion of blends containing garbanzo dealt with relatively simple raw material compositions. In those studies, effects of extrusion processing on the important aspect of the nutritional properties (protein digestibility, functionality, and antioxidant) and physical attributes (sensory characteristics) of extrudates of pulse-based flours are not investigated. Furthermore, garbanzo is high in amino acid lysine but low in sulfur containing amino acids.

Dry-milling process involved in corn ethanol production, produces distillers dried grains (DDG) and distillers dried solubles (DDS). Distillers dried grains with solubles

(DDGS) is produced after mixing and drying these two co-products (Singh, 2016). DDGS contain high levels of protein since most of the starch is removed (Rosentrater and Krishnan, 2006). It is usually used as cattle feed. However, few studies have been reported on its application in human food (Rasco and McBurney, 1989; Rosentrater and Krishnan, 2006; Wu et al., 1987). The growing interest in the health benefit of protein and fiber justifies exploring the use of DDGS as a protein and fiber supplement in food products. DDGS supplementation will improve the nutritive value of food products by enriching their protein and fiber content, and expand the use of the co-product from alcohol fermentation (Tsen et al., 1982).

1.1.2 Purpose of the study

The overall objective of this research is to determine the feasibility of using apple pomace, grape pomace, distiller's dried grains and garbanzo flour as an ingredient in the manufacture of high- protein high-fiber expanded extruded snacks.

1.1.3 Significance and rationale of the research

The studies are expected to provide new knowledge on the behavior of flow of feed materials within the extruder, and effects of extrusion process on the physical, functional and structural characteristics of the products. Successful demonstration of acceptable product will also provide new opportunities for the snack food industry and increase market for apple pomace, grape pomace, distiller's dried grains, defatted soy flour and garbanzo flour products.

1.1.4 Objectives

The primary objective of this research was to utilize fruit and ethanol industry by-products and co-products in the development of expanded extruded snacks. The

hypothesis of the research was inclusion of apple pomace will improve the total phenolic content (TPC) and antioxidant activity (AA), inclusion of grape pomace will improve the anthocyanin content and inclusion of DDGS processed for food application (FDDG) will improve the dietary fiber and protein content of the extruded snacks.

The specific objectives of the present work were therefore as follows:

1. Evaluate the influence of extrusion process variables on the apparent viscosity and system parameters during single screw extrusion of (i) blends of apple pomace, defatted soy flour and corn grits and (ii) blends of food grade distillers dried grains, garbanzo flour and corn grits, using RSM to understand the nature of the process and identify system variables. (CHAPTER 2 and CHAPTER 5)
2. Evaluate the influence of extrusion process variables on the physico-chemical properties of extruded apple pomace, defatted soy flour and corn grits blends obtained using a single and twin-screw extrusion process, using RSM to understand the nature of the process, identify system variables and obtain an optimized extrusion condition. (CHAPTER 3 and CHAPTER 7)
3. Understand the influence of the extrusion processing on the textural properties, microstructure and molecular distribution of the extruded snacks prepared from blends of apple pomace, defatted soy flour and corn grits blends. (CHAPTER 4)
4. Evaluate the influence of extrusion process variables on the physico-chemical properties of extruded food grade distillers dried grains, garbanzo flour and corn grits blends, obtained using a single-screw extrusion process, using RSM to understand the nature of the process, identify system variables and obtain an optimized extrusion condition. (CHAPTER 6)

5. Evaluate the influence of extrusion process variables on the physico-chemical properties of extruded grape pomace, defatted soy flour and corn grits blends obtained using a twin-screw extrusion process, using RSM to understand the nature of the process and identify system variables. (CHAPTER 8)

1.2 Literature review

1.2.1 Food extrusion

Extrusion is defined as "shaping by force through a specially designed opening often after previous heating of the material" (Harper, 1981). Extrusion is the continuous formation of semi-solid materials through a die. Extrusion combines several unit operations including mixing, cooking, kneading, shearing, shaping and forming (Riaz, 2000b, 2007). Extrusion cooking combines the heating of food products with the act of extrusion to create a cooked and shaped food product. It is a process in which moistened, starchy, proteinaceous foods are cooked and worked into viscous, plastic-like dough. The results of cooking the food ingredients during extrusion are: gelatinization of starch, denaturation of protein, inactivation of raw food enzymes, destruction of naturally-occurring toxic substances, diminishing of microbial counts in the final product, etc. Upon discharge through the die, the hot, plastic extruded product expands rapidly with loss of moisture and heat because of the sudden decrease in pressure. After expansion cooling, and drying, the extruded product develops a rigid structure and maintains a porous texture. Advantages of food extrusion are adaptability, wide variety of product characteristics, energy efficiency, versatility, high productivity, low cost, ability to shape the product, high product quality, production of new foods, and no effluents or waste (Riaz, 2000b). Extrusion processing is widely used in food and feed applications.

1.2.2 Food extruders

The screw system is the central portion of the extruder that accepts the feed ingredients at the feed port, conveys, works and forces them through the die restriction at the discharge point. The helical metal ribs wrapped around the screw shaft called "flights" convey the material mechanically towards the discharge end. The flights are of different height and shape. At the feed section, the flights are deeper or with greater pitch to ensure easy filling for conveying along the barrel. In the central part of the screw, called the "compression or transition section", the feed material is compacted and converted from a flowing granular or sticky mass to a relatively uniform plasticized dough. This section of the screw is followed by the metering section that has relatively shallow flights of reduced pitch to thoroughly mix, and/or increase the temperature of the material and the shear rate in the channel.

The continuous central shaft, known as the root of the screw, may sometimes be hollow to pass heating or cooling medium. The ratio of the distance between root surface of the screw and internal surface of the barrel, at the beginning of the barrel and at the end, is called "compression ratio", which is an important factor that characterizes the extruder. The extrusion drive system, which drives the screw, consists of a drive motor and a stand. Continuous variability of the speed is possible through magnetic, electrical or mechanical controls. The motor speed is normally less than 500 rpm. A transmission is used to reduce the speed with a proportional increase in the torque of the motor. The whole extruder assembly is mounted on a frame or a stand that is bolted to the floor. Often it is equipped with special disassembly devices for ease of functioning and

maintenance. The food material leaving the extrusion screw enters the discharge section that normally holds the extruder die, cutters and takes away devices.

The dies have small openings that shape the food material as it flows out of the extruder. The shape of the die varies. The simple one being a hole. Expansion of the extrusion material occurs as the product under high temperature and pressure leaves the die with a rapid release of pressure to ambient conditions. Cutters are used to cut the extruded material coming out from the die. They are employed in combination with the take away devices, and/or the drying or the cooling systems. Drying and cooling section ensures careful decrease in the moisture or temperature of the product while maintaining the textural quality.

1.2.3 Types of extruders

In any other extruder, the screw has three sections: the feeding section conveys the material downstream; the compression section shears, heats and kneads the mass into a continuous dough; and the metering section shears and heats the dough further, as it delivers it to the die exit. Throughout the barrel there are grooves that prevent slippage at the walls. The screw has shallow flights of decreasing pitch as it advances forward, so the shear is gradually increased (Harper, 1981). Extruders used in food applications are usually single-screw extruder or twin-screw extruder.

1.2.3.1 Single screw extruder

Single screw extruders are available in a number of sizes and shapes, and their barrel, screw configuration, and screw could be usually varied to get the desired product characteristics (Harper, 1978). The mechanism of conveying is due to friction between

the screw and product and the friction between barrel and product (van Zuilichem et al., 1983). A schematic of typical single screw is represented in Figure 1.1.

The movement and transformation of material in the extruder can be divided into three sections: feeding, kneading or transition and final cooking zones. The feeding zone receives preconditioned material and is conveyed to processing zone where free-flowing amorphous material is worked into dough. The compression ratio is increased to assist in blending in kneading zone. In the cooking zone, the thermal and mechanical energy input plasticizes the material above its melting point. The final screw element reduces the volumetric displacement and adds compression (Riaz, 2000b).

1.2.3.2 Twin screw extruders

Twin-screw extruders appeared in Europe over 30 years ago. Their wider adoption in North America began in the 1980s. Nowadays, twin-screw extruders have been designed to function in a wide range of applications. The advantages of twin- *vs.* single-screw extruders have led to them replacing single-screw extruder in many applications. The term 'twin-screw' applies to extruders with two screws of equal length placed inside the same barrel (Guy, 2001). Twin-screw extruders are more complicated than single-screw extruders, but at the same time provide much more flexibility and better control. Twin screw extruders are available with different screw configurations and direction of screw rotation (co-rotating and counter-rotating). On the basis of the position of the screws in relation to one another into the following: intermeshing and non-intermeshing (Janssen, 1989; Riaz, 2007). Figure 1.2 shows the common types of twin screw used in extruders. The twin-screw extruder was originally developed for processing plastics. In the food industry, twin-screw extruders were widely used from the mid-1980s

to the mid-1990s. Food companies began using twin-screw extruders for producing products like sticky caramels and candies that could not be made with single-screw machines. Very soon, twin-screw extruders became popular with the food manufacturers for specialized food items.

In twin screw extruders, the transport mechanism is due to the friction between the screw and the product. Twin screw extruders are capital intensive and have high throughput. (Riaz, 2000b).The advantages of twin-screw extruders over single screw extruders are:

a) They handle viscous, oil, sticky or very wet materials and some other products which will slip in single extruder.
b) They have positive pumping action and reduced pulsation at the die.
c) There is less wear in smaller parts of the machine than in the single-screw extruder.
d) A wide range of particle size may be used.
e) Clean-up is relatively easy because of self-wiping mechanism.

1.2.3.3 Comparison of single-screw extrusion and twin-screw extrusion

Compared to single-screw extruders, twin- screw extruders are more flexible in controlling both product and process parameters. They have a flexible design permitting easy cleaning and rapid product changeover (Hauck, 1988).

Single-screw extruders are limited to a maximum fat level in the formula of 12–17%. Greater fat levels reduce friction because of their lubrication effects, thus not allowing the hardware to transform mechanical energy into heat for cooking purposes. Comparatively, fat levels in recipes for twin-screw extruders can be as high as 18–22%

while still maintaining the required mechanical energy (Guy, 2001). Moisture is another key factor. A twin-screw extruder can process a much wider range of moisture content in the feed stock than a single-screw extruder.

Processors should consider twin-screw extruders in the following situations (Riaz, 2000a):

- Frequent product changeovers
- Products with high internal fat content (above 17%)
- Addition of a high level of fresh meat in the product (up to 35%)
- Uniform size and shapes
- Ultra-small product sizes (less than 1.5 mm)
- Products made with low density powder
- Special formulations

The variables and parameters involved in extrusion processing are represented in Figure 1.3.

1.2.4 Classification of extrusion cooking ingredients by components

Wheat and corn flours are the most commonly used materials for food extrusion. Other materials like rice flour, soy, potato, rye, barley, oats, sorghum, cassava, tapioca, buckwheat, pea flour and other related materials can also be used. Corn meal is the most common ingredient of expanded snacks in the food market. Because of its composition, ratio of vitreous to floury endosperm, and particle size, under optimal extruding conditions corn meal makes for a light, highly expanded, crunchy and soft product. The basic recipe may vary widely; however, the main ingredient components which affect the

characteristics of the final product are starch, water, protein, fiber, oil, additives and particle size.

1.2.4.1 Water

Water is an important medium in extrusion. It is needed for starch gelatinization and ingredient dispersion. In the formation of a viscous fluid, it is conveyed and cooked. Air cell creation and expansion by evaporation at the die exit also depends on the optimum moisture content of raw materials. Janssen (1978) in summarizing the principal differences between twin- and single-screw extruders concluded that the former may be more suitable for handling wet material. Moisture is always listed as a separated variable in addition to feed ingredients because it is often controlled separately in the extruder. Moisture can be added directly to the feed, injected into the barrel, or added in the form of steam to the pre-conditioner or barrel; it will also affect the temperature of the feed material (Harper, 1981).

1.2.4.2 Starch

Starch is the main component of the final product; it provides the underlying structure (Guy, 2001). Starch is present in a large variety of plant crops, such as cereals (50-80% starch), legumes (25-50% starch) and tubers (60-90% starch) (Harper et al., 1989). During extrusion, starch granules are gelatinized and dispersed, resulting in the formation of a continuous phase of the melt inside the extruder. Average molecular weight is decreased, which allows for optimum formation and stability of air cells at the die exit. Both amylose and amylopectin are needed to give the best expansion characteristics (Huber, 2001).

1.2.4.3 Protein

Single- and twin-screw extrusion of protein has been the subject of several studies. Textured products manufactured by this process were sold as meat analogues in Japan (Harper et al., 1989) and China. Given the nutritional factor content of plant proteins, a range of different products high in plant protein are preferred by consumers.

1.2.4.4 Fiber

Fiber has the nutritive value in food products and it has been connected with a 14 healthy modern diet. Fibrous materials such as bran can be part of the dispersed phase of extruded products, included in the starchy continuous phase (Guy, 2001). Fiber is chemically unchanged by the extrusion process, and influences the expansion of the product (Huber, 2001). Fibrous fragments disrupt the starchy film of air cell walls, reducing their formation and swelling, and altering air cell size.

1.2.4.5 Lipid

Lipids have two functions in extrusion process; they can influence the quality of the product and act as a lubricant during the process. Most of the lipids will melt at 40ºC, and the shear process will break them down into 10 µm parcels and then disperse them throughout the system. When the moisture of the material is lower than 25%, the addition of 0.5-1% lipids will greatly reduce the energy input needed for extrusion. When oil content rises up to 2-3%, it can have undesirable effects, such as reducing the extrudate expansion (Zhang, 1998).

1.2.4.6 Additives

Additives are also important in the makeup of the final product, as their reactions during the extrusion process can greatly affect the flavor or color of the product. Flavoring or

coloring agents are mixed into the product and confer a different appearance to the product during the process. Additives used for increasing expansion and cell wall formation/swelling are termed nucleating agents and include sodium bicarbonate and calcium carbonate. Monoglycerides are commonly used in commercial operations (Guy, 2001) as surfactants (lubricants). Compared to regular corn puffs, the emulsifiers from rice bran have been shown to produce corn meal extruded products of lower bulk density, lighter structure, softer texture and more evenly distributed air cells (Barron et al., 2002).

1.2.5 Raw materials for extrusion cooking

Common food ingredients that have been used in food extrusion are cereal based ingredients such as maize, wheat, rice, oat and barley as the major ingredient, while non-major ingredients include tuber sources, potato and tapioca. Food industry by-products such as apple and grape pomace and also ethanol industry co-product i.e. DDGS are rare in snack processing. All ingredients provide different functional roles in terms of formation, stabilization, color, flavor, texture and nutritional qualities of the extruded products (Guy, 2001). This section will briefly outline three main ingredients that have been used in this study which were pumpkin and corn. Chemical and nutritional changes of the raw material components also will be covered.

1.2.5.1 Corn grits

Maize or corn is the third most important crop worldwide and commonly has been used as a base ingredient in the snack industry. In general, there are several uses of corn in food as well as feed industry such as the production of flour, corn meal, grits, sweeteners, starches, alcoholic beverages, cooking oil, tortilla, snacks, breakfast foods and other products. Dry-milled corn meal is the most common primary ingredient used in

corn-based extruded snacks. Corn is processed into a wide variety of products. Basically, most corn processed for the food industry either by wet or dry milling. Dry milling of corn produces the maximum output of clean grits, with less fat, fiber and speck from the hilum. The unique characteristics of corn resulted in it becoming the choice of most snack processors for use in corn-based extruded snacks especially in terms of starch content which has good expansion characteristics, it also has a definite flavor with a natural yellow color.

1.2.5.2 Defatted soy flour

Soybeans contain all three macro-nutrients required for good nutrition: complete protein, carbohydrate and fat, as well as vitamins and minerals, including calcium, folic acid and iron. Soybeans are the only plant food that contains complete protein. Soybean protein provides all the essential amino acids in the amounts needed for human health. The amino acid profile of soy protein is nearly equivalent in quality to meat, milk and egg protein. The possible health benefits associated with eating soy foods is concentrated in three main areas - cardiovascular disease (Dotzel, 1999), cancer (Wiseman, 1997) and postmenopausal symptoms (Eden, 2001) which includes hot flashes, osteoporosis, estrogen replacement therapy, and cardiovascular function. These benefits are associated with soy isoflavones.

Defatted soy flour (DSF) is the residue produced after the nearly complete removal of the oil from soybean. DSF occupied about 80% of dry soybean flour (Liu, 1997). DSF is the most commonly used type of soybean flour. It has been valued by the food industry for its functionality in the areas of water and fat absorption capacity and adhesiveness (Erickson, 1995; Liu, 2004). DSF contains most soy protein and

carbohydrates and is usually used for animal feed. Only a small portion of it is further processed into different types of soy protein products for human consumption (Liu, 1997). DSF contains about 38% total carbohydrates, including 15% soluble mono- and oligosaccharides, and 13% polysaccharides that are later removed if soy protein concentrates or isolates are made (Lusas and Rhee, 1995). The protein content of DSF ranges from 56-59% (moisture free basis) (Endres, 2001). A protein dispersibility index (PDI) value is assigned to indicate degree of solubility potential of the protein product. This value may serve as an indirect measurement of the level of heat treatment by which the product has been exposed. DSF is commonly available in 20 (low), 70 (medium), and 90 (high) PDI forms. A PDI of 90 designates that the particular flour has undergone a low degree of heat treatment and thus should demonstrate high solubility and functional characteristics. Low and medium PDI defatted soy flour varieties are mainly used in the baking industry. For bread specifically, these flours have been able to improve moisture retention during processing (Liu, 2004).

1.2.5.3 Garbanzo flour

Garbanzo, commonly known as chickpeas, are the third most important legume in the world after dry beans and dry peas (Singh et al., 1991). Chickpeas have one of the highest nutritional compositions of any dry edible pulse and do not contain any specific major antinutritional factors (Chavan et al., 1987). Research has shown that chickpeas are an excellent source of protein (24.4%), dietary fiber (9.0%), complex carbohydrates (60.0%), folate, and trace minerals such as iron, molybdenum, manganese (Poltronieri et al., 2000). In addition, chickpeas have been reported to reduce the levels of cholesterol and blood glucose (Singh and Singh, 1992). Hence, Chickpeas are increasingly being

used in healthy diets to promote general well-being and to reduce the risk of cardiovascular diseases and diabetes. Chickpeas have a high (40–50%) starch content (Huang et al., 2007), which may favor an extrusion process to produce directly expanded snack foods. Using chickpeas instead of cereals in extruded snacks would improve the nutrient density of these foods because chickpeas have more protein. Hence, development of chickpea-based snacks could provide an attractive outlet for chickpea utilization. However, processing of chickpeas into extruded snacks is limited. Few reports (Bhattacharya and Prakash, 1994; Shirani and Ganesharanee, 2009) indicated that incorporation of chickpeas into rice flour decreased product expansion. The extrudates exhibited increased density and breaking strength, indicating poor textural effects of chickpea flour inclusion. The above studies dealt with relatively simple raw material compositions. A more complex mixture, involving starch and protein fortification, may promote expansion and nutritional quality of a chickpea based snacks.

1.2.5.4 Distillers' Dried Grains with Solubles

Distillers' dried grain with solubles (DDGS) is a co-product of bio-fuel ethanol and potable ethanol production using mostly corn as the biomass for fermentation. In a typical dry grind corn-based ethanol production, corn is thermally processed at 90^{o}C with enzymes to break down corn starch into sugar. The sugar is fermented into ethanol solution at 60^{o}C that is later distilled into 95% pure ethanol solution and dehydrated and denatured to 100% ethanol as automotive fuel. The underflow from the distillation column, called whole stillage, is centrifuged to obtain distiller's wet grains (DWG) that contain 30-35% (w/w) solids. The DWG is dried to obtain Distillers' Dried Grain (DDG). Corn is an abundant source of phenolic acids (Adom and Liu, 2002), and yeasts used

during the fermentation process do not utilize these components (Baranowski et al., 1980). Indeed, residues remaining after fermentation of other grains are high in these beneficial phenolic acids (Mussatto et al., 2006).

DDGS has many potential applications ranging from animal feed to charcoal production (Mussatto et al., 2006); currently only animal feed garners the significant use of this biomaterial. However, DDGS has the unique potential for commercial food uses, particularly in baked goods. The DDGS consists of mainly resistant starch, fiber, protein, and unsaturated lipids (Wu, 1994). Inclusion of DDGS could expand markets of traditional baked foods to health-conscious consumers in terms of favorable nutritional profile and lower glycemic effect.

Food applications of DDGS from liquor and ethanol production known as brewer's spent grain have been intermittently studied primarily in late 1970s and early 1980s against a backdrop of an oil. crisis in the world. The general conclusion from that limited research on food applications is that DDGS can be used as supplemental ingredients for certain baked foods (Abbott et al., 1991; Bookwalter et al., 1971; Maga and Van Everen, 1989). The majority of baked foods were wheat breads and cookies (for example, Tsen et al. (1982)) with few studies on pasta (Maga and Van Everen, 1989; Wu et al., 1987) and muffins (Abbott et al., 1991). Concentration for most nutrients in DDGS is higher than in original feedstock due to starch removal (Weigel et al., 1997). Reduced starch intake increases the consumption of digestible fiber, and helps to reduce or prevent the occurrence. Besides being a good protein source in growing and finishing diets, some studies suggest that corn DDGS has also proven to have greater energy for growth than

dry rolled corn (Firkins et al., 1985; Ham et al., 1994; Klopfenstein, 1996; Waller et al., 1980).

1.2.5.5 Apple and grape pomace

Apple pomace (AP) is a by-product of the apple processing industry and consists of peels and the core, which are dried and ground. The typical composition is 1.2-10.8% moisture, 0.5-1.9% ash, 2.4-7.3% protein, 1.6-4.5% fat, and 51.1-89.8% total dietary fiber (36.5-81.6% insoluble and 4.14-14.6% soluble) (Chen et al., 1988; Sudha et al., 2007). AP has high total phenolic content and antioxidant activity, which along with the high fiber content, is the main reason it has been extensively studied in the past few years as a potential food ingredient.

Grape (except for orange) is the world's largest fruit crop, with more than 61 million metric tons, cultivated mainly as *Vitis vinifera* for wine production (STAT). The main by-products are collected during de-stemming (stems), grape crushing and pressing (skins, seeds and lees). At present, only minimum amounts of these wastes are up-graded or recycled and this is particularly true in Europe, where vegetable wastes are generally dumped or used for animal feed or compost, without any pre-treatment. Part of the grape pomace is destined for distillation but this allows recovery of a minimum amount of the material as volatile constituents of alcoholic beverages. With respect to the chemical studies carried out on wine constituents, grape pomace has scarcely been investigated, but it is undoubtedly rich in polyphenols (Torres et al., 2002). The high level of polyphenols in this waste, a disadvantage for their possible use as animal feed, composting or discharge, may be turned to an advantage through the extraction of polyphenols before further utilization or treatment. In fact, many recent studies have highlighted the

beneficial effects of grape or wine polyphenols for human health (Rice-Evans et al., 1997; Simic and Jovanovic, 1994). More specifically, the antioxidative properties of many natural polyphenols may exert a chemopreventive role toward cardiovascular and degenerative diseases, (Halpern et al., 1998), including neurodegenerative pathologies (Esposito et al., 2002). Grape's skins are rich in anthocyanins, a group of polyphenols well-known for their beneficial properties (Ghiselli et al., 1998; Katsube et al., 2003; Kong et al., 2003; Wang et al., 1997).

(Poly) phenols vary structurally from simple molecules such as phenolic acids with a C6 ring structure to highly polymerized compounds such as tannins. The majority of (poly)phenols have a sugar residue linked to the carbon skeleton. Glucose is a common sugar residue including different monosaccharides, disaccharides, or oligosaccharides. Other compounds including amines, organic acids, carboxylic acids, lipids, and other (poly)phenols may also be linked to the basic (poly)phenolic structure (Bravo, 2007). Phenolic compounds can be classified by their sources of origin, biological function and chemical structure.

O'Shea et al. (2014) reported the optimal extrusion conditions and AP inclusion to produce a high-quality snack were die head temperature of 150°C, screw speed approximately 69 rpm and AP addition of 7.7 % into corn flour. Increased level of pomace decreased expansion ratio and hardness of final extrudates. Moreover, Reis et al. (2014) observed that 20% of pomace inclusion with rice flour and wheat semolina improved fiber content, phenolic content and antioxidant capacity of extrudates by 1.8, 4 and 2.8 times, respectively. Contrarily, Altan et al. (2009a) reported decrease in AA and TPC of barley, barley–tomato pomace and barley–grape pomace extrudates after

extrusion cooking. However, increasing pomace levels increased the TPC, AA and WSI and decreased WAI due to competition of absorption of water between pomace and available starch. Altan et al. (2008c) found the extrusion conditions of 155-160°C, 4.47-6.57% pomace level and 150-187 rpm produced acceptable extrudates. Increasing grape pomace level decreased sectional expansion index, crispness, whereas increased hardness and brittleness of extrudates.

Table 1.1 Forecast volume sales of snack products in the United States, in'000 tons (Euromonitor International, 2015).

Category	2016	2017	2018	2019	2020	2016-2020 CAGR(%)
Snacks	3940.5	4009.7	4081.3	4155.2	4227.9	1.8
Tortilla chips	824.1	842.6	860.5	879.7	898.6	2.2
Crisps	800.9	815.7	832.4	850.1	868	2
Extruded snacks	492.7	500.8	508.1	515	521.6	1.4
Popcorn	407.2	416.5	423.3	428	430.2	1.4
Nuts	356.5	363.6	371.3	379.2	387.1	2.1
Other snacks **	337.3	342.5	348	353.8	359.8	1.6
Snack bars	302.3	307.2	313.1	319.6	326.3	1.9
Pretzels	300.1	302.5	306.1	310.4	315.4	1.2
Fruit snacks	119.4	118.3	118.5	119.5	121	0.3

** Other snacks include jerky, snack mixes/combo packs, trail mixes, seeds, pita chips, and pork scratchings

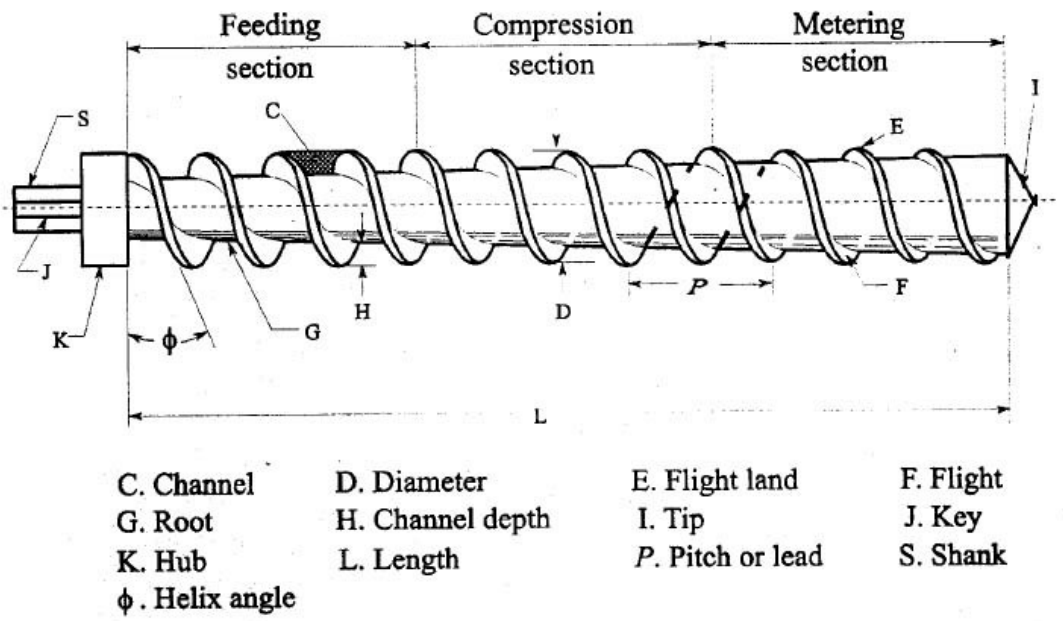

Figure 1.1 Geometric features of a single screw (Vlachopoulos and Strutt, 2013)

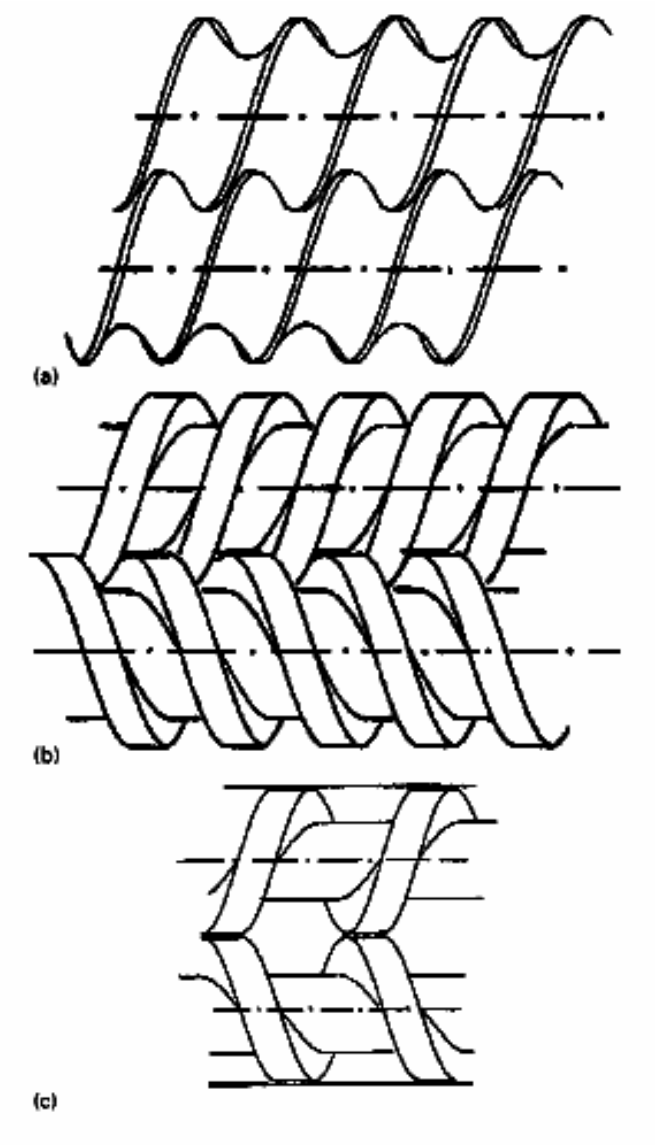

Figure 1.2 Three common types of twin-screw extruders (Vlachopoulos and Strutt, 2013) (a) Intermeshing co-rotating; (b) Intermeshing counter-rotating; (c) Non- intermeshing counter-rotating

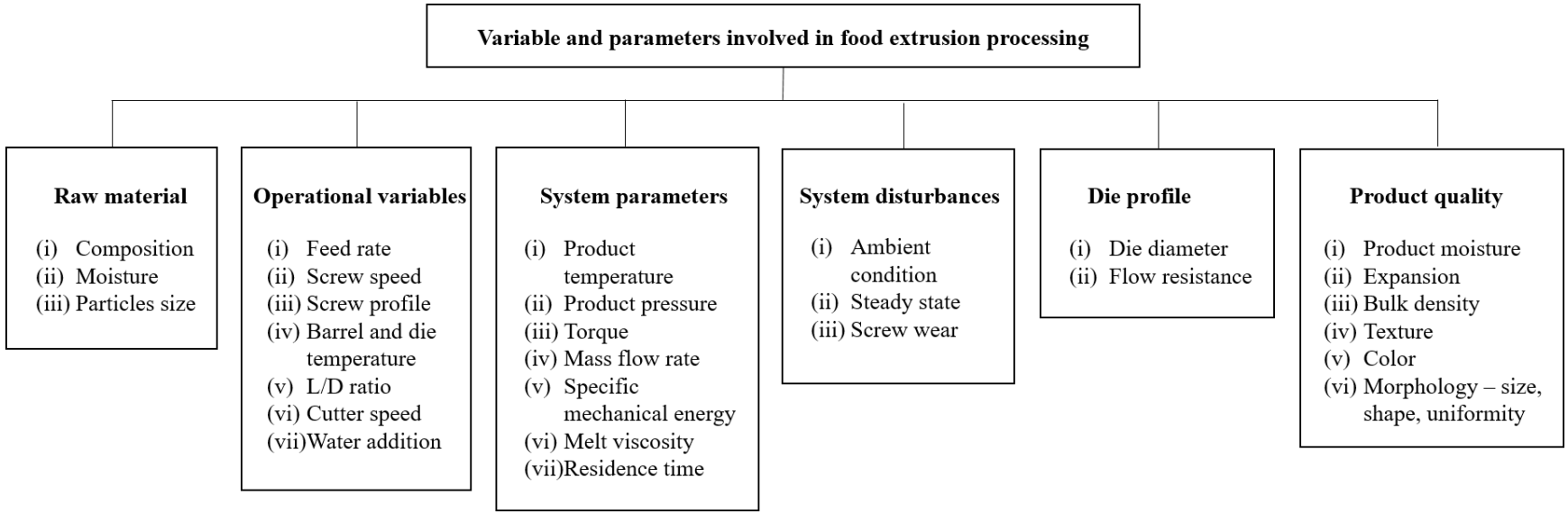

Figure 1.3 Schematic of variable and parameters involved in extrusion processing

CHAPTER 2 †

Effect of Processing Conditions on the System Parameters During Single Screw Extrusion of Blend Containing Apple Pomace

2.1 Abstract

The nutritional profile of apple pomace and soy makes them ideal for development of a new extruded snack food with health benefits. Understanding how blend of apple pomace, defatted soy flour and corn grits behave under different process conditions during extrusion will help in formulating the extrusion process. Experimental design with apple pomace level (0 to 20%), barrel and die temperature (100 to 140^{o}C), screw speed (100 to 200 rpm), and moisture content of blend (14 to 20% wet basis) as independent variables produced 27 different combinations that were studied using response surface methodology to investigate the effect of these variables on system parameters (apparent viscosity, mass flow rate, torque, die pressure, dough temperature, and SME). As the temperature profile increased, apparent viscosity, die pressure and specific mechanical energy decreased. Increase in apple pomace level resulted in significant ($P<0.05$) decrease in apparent viscosity, SME and torque, and significant ($P<0.05$) increase in the mass flow rate. Response surface regression models were established to correlate the system parameters to the process variables. The data obtained from the study could be used for control of extrusion process of blends containing the aforementioned ingredients.

---

† Singha, P., Muthukumarappan, K., (2017). Effects of processing conditions on the system parameters during single screw extrusion of blend containing apple pomace. Journal of Food Process Engineering 40(4), 1-11.

2.2 Introduction

With the rise in global population, food researchers' focus is to directly and efficiently transform raw agricultural products consisting of starch, plant protein, and fat into foods of high acceptability (Iwe et al., 2001b). Extruders has the ability to continuously combine, cook and texturize food components quickly and efficiently, thus making it ideally suited for the development of puffed snack foods, breakfast cereals, etc.

The food material is sheared, mixed and compressed during extrusion cooking and then texturized and shaped in the die (Alvarez-Martinez et al., 1988). The thermomechanical action during extrusion brings about gelatinization of starch, denaturation of protein and inactivation of enzymes, microbes and many anti-nutritional factors; all this occurs in a shear environment, resulting in a plasticized continuous mass (Bhattacharya and Prakash, 1994). The rate and extent of heating, mixing, shearing, and compressing of the materials inside the barrel, and subsequently the die, is strongly related to the properties of the raw materials and process conditions used.

Since all materials have distinct rheological properties, an understanding of rheology and momentum transfer in an extruder can optimize development of food products, processing methods, scale up of new processes, process control, and product quality (Lam and Flores, 2003). Apparent viscosity is one of the most important rheological properties of non-Newtonian biological materials. It is often used for continuous online monitoring of the extrusion process and control of subsequent product characteristics (Chen et al., 1978; Lam and Flores, 2003) because it can be an indicator of a dough's behavior, and the changes therein, during processing. Meuser et al. (1987) suggested that viscosity (or shear stress) can be used as a variable for continuous on-line

control. Once viscosity is found, momentum and heat transfer analysis for extrusion can be studied further. Two important reactions, namely protein denaturation and polysaccharide gel formation can affect viscosity during extrusion (Bhattacharya and Hanna, 1986). The viscosity of dough inside extruders has been studied by many authors. For example, the viscosity of doughs containing wheat, corn, and soybeans were measured using straight tube viscometers (Harper et al., 1971), cylindrical dies of different lengths (Harmann and Harper, 1974), capillary die rheometers (Singh and Muthukumarappan, 2017a, b) and viscoamylographs (Remsen and Clark, 1978) attached to food extruders. All of these methods require separate attachments with additional instrumentation to measure the viscosity of the dough during processing.

Even after extensive use of extrusion processing, it is still a complicated process that has yet to be mastered. Small variations in processing conditions not only affects process variables but also the product quality. The extrudates' quality can vary considerably depending on the following extrusion parameters, such as the extruder type, screw configuration, feed moisture, temperature profile in the barrel and die, screw speed and the feed compositions. Hence, understanding the physical, rheological, and chemical properties of an ingredient melt inside the barrel is very important for product development, process control, final product quality, and scaling up operations (Ludewig, 1989). Each material exhibits distinct behavior during processing and is often quantified by determining temperature and pressure responses, mass flow, and energy consumption.

Cereals such as corn are ideal for extrusion; they are gluten free, easily accessible and have a high starch content, which can give excellent expansion characteristics. The only disadvantage of using corn is that it is likely to be relatively low in nutrients such as

dietary fiber and minerals (Pastor-Cavada et al., 2011). Legumes such as soybean are rich source of protein. Soybean in different forms (Bookwalter et al., 1971; Chiang, 2007; Iwe et al., 2001b; Lobato et al., 2011; Singh and Muthukumarappan, 2014a; Singh and Muthukumarappan, 2016; Yu et al., 2014) have been extensively used as a major ingredient during extrusion.

Many researchers (Altan et al., 2008a; Altan et al., 2008c; Kaisangsri et al., 2016; O'Shea et al., 2013) have been focusing on incorporating by-products after fruit processing as a solution to enhance the nutrition of an extruded puffed snack without compromising the snacks structure. During fruit processing, e.g. the pressing of apples, to produce apple juice, up to one third of the fruit is discarded (Shalini and Gupta, 2010). Wineries and juice industries usually sell the pressed residue to silage processor who uses the pomace for cattle feed supplement or recycle as compost. As a common application, apple pomace is used for pectin recovery usage (gelling agent, stabilizer and source of dietary fiber). When the above options are not feasible the pomace is directly disposed to soil in a landfill. These applications are not sufficient to utilize the 1.3 million metric tons of apple pomace produced in the United Sates every year (Jung et al., 2014); therefore, studies have got momentum to valorize the apple pomace for other purposes also. Researchers have shown that by-products such as apple pomace contain a high level of dietary fiber and bioactive, e.g. vitamins, minerals and phytochemicals (Gorinstein et al., 2001; Wijngaard et al., 2009). Few studies (O'Shea et al., 2013) have been conducted on its effect on the process parameters during extrusion. This is particularly relevant now, as apple pomace has been described as a potential ingredient due to its nutrition and physiochemical attributes.

The objective of this research was to study the effects of extruding blends of apple pomace, defatted soy flour and corn grits and other process conditions namely moisture content of blend, barrel and die head temperature (hereafter referred to as temperature) and screw speed in a single screw extruder with particular reference to how specific mechanical energy, apparent viscosity, mass flow rate, pressure, product temperature and torque are affected during the extrusion process, by using response surface analysis.

2.3 Materials and Methods

2.3.1 Raw materials and blend preparation

Apple pomace (AP) powder provided by Tree Top, Inc. (Selah, WA) was stored at -18 °C. The initial moisture content of AP powder was 10.08% (wet basis). The proximate composition of the AP powder was: 4.14% protein, 2.79% fat, 2.00% ash, 24.73% total fiber and 0.20% nitrogen free extract (NFE, dry basis). Corn grits (CG) was obtained from Bob's Red Mill (Milwaukie, OR). The initial moisture content of CG was 11.46% (wet basis). The proximate composition of the CG was: 6.78% protein, 1.69% fat, 2.26% ash, 1.02% total fiber and 81.80% NFE (dry basis). Defatted soy flour (DSF) was obtained from Hodgson Mill, Inc. (Effingham, Il). The initial moisture content of DSF was 7.75% (wet basis). The proximate composition of the DSF powder was: 53.94% protein, 0.77% fat, 6.87% ash, 2.93% total fiber and 26.09% NFE (dry basis). The different ingredients i.e. DSF, CG and AP were mixed in to five different compositions (Blend I to V) as shown in Table 2.1. The ingredients were mixed in a laboratory scale mixer (KitchenAid Professional 5 Plus, Troy, Ohio, USA) for 10 minutes and stored overnight at ambient temperature for moisture stabilization. The moisture content of the

prepared blends was determined by using the method 44-19 (AACC, 2000). The proximate compositions of blends are shown in Table 2.1.

2.3.2 Extrusion processing

After mixing and conditioning, the blends were randomly extruded using a single-screw laboratory extruder (Brabender Intelli-Torque Plasti-Corder®, South Hackensack, NJ) at Food Innovation Center, University of Nebraska, Lincoln, NE which was powered by a 7.5-HP motor with an operating range of screw speeds from 0 to 225 rpm. The extruder had a barrel with length to diameter ratio of 20:1 and barrel diameter of 19.18 mm. A uniform 19.05 mm pitch screw having 381 mm (15.0 in.) screw length, a 19.05 mm (0.75 in.) constant outside (top of flight) diameter, a 3.81 mm (0.15 in.) initial screw feed depth, an 11.43 mm (0.45 in.) initial screw root diameter, and a screw compression ratio (feed channel depth to metering channel depth) of 1.5:1 was used in the experiments. A schematic representation of single screw extruder is shown in Figure 2.1.

Processing conditions were monitored by measuring dough temperature at the metering zone and the die, pressure at the die, and net torque exerted on the extruder drive (N-m) (net drive torque accounts for the amount of torque required under no-load conditions compared with the amount required during processing). Dough temperature measured at the end of the metering zone and die were determined using stock thermocouples (model 05-00-317, C. W. Brabender) inserted into the barrel. Torque readings were recorded by the extruder's computer control system. During experiments, extrudate samples were collected every 30s, and the mass flow rate was then determined (g/s) by the method mentioned by Rosentrater et al. (2005). Based on the torque and the

mass flow rate data, SME (W-h/kg) consumption was calculated (Lam and Flores, 2003) as:

$$SME = \frac{\Omega.\omega}{MFR\left[\frac{3600}{1000}\right]} \tag{2.1}$$

where Ω is the net torque exerted on the extruder drive (N-m), ω is the angular velocity of the screw (rad/s) and *MFR* is the mass flow rate of dough (mass throughput, g/s).

The apparent viscosity of the dough in the extruder was calculated by approximating extruder behavior as that of a coaxial viscometer but corrected for the tapered screw geometry (Figure 2.2) of the extruder barrel (Konkoly, 1997; Lam, 1996; Rogers, 1970). As discussed by Lam and Flores (2003), the shear stress (τ_s) at the screw surface (N/m^2) and the shear rate ($\dot{\gamma}_s$, 1/s) were calculated from the following equations:

$$\tau_s = \Omega / (2.\pi.(r_{coor})^2.L_s) = C_{ss}\Omega \tag{2.2}$$

$$\dot{\gamma}_s = (2.\omega.r_b^2) / (r_b^2 - (r_{coor}^2)) = C_{sr}\omega \tag{2.3}$$

where r_{corr} is the radius correction due to the screw's frustum geometry

$$r_{coor} = \left(\sqrt{(r_{eff\,1}^2 + r_{eff\,1}r_{eff\,2} + r_{eff\,2}^2)/3}\right) \tag{2.4}$$

r_{eff} is the effective radius, including the screw root radius and half of the flight height (m), Ω is the net torque exerted on the screw (N m), *L*s is the screw length in the axial direction (m), ω is the angular velocity of the screw (rad/s), C_{ss} is an empirical correction factor for shear stress (10321.5 for the geometry used in the experiments), $\dot{\gamma}_s$ is the shear rate at the screw surface (1/s), r_b is the inner barrel radius (m), and C_{sr} is the empirical correction factor for shear rate (3.48 for the geometry used in this study). This calibration

value for this extruder have been calculated from the calculation reported elsewhere (Lam and Flores, 2003). The apparent viscosity of the dough in the extruder was then calculated by taking the ratio of Eq. (2.2) to Eq. (2.3) according to (Konkoly, 1997; Lam, 1996):

$$\eta_{app} = \frac{\tau_s}{\dot{\gamma}_s} = \left(\frac{C_{ss}}{C_{sr}}\right)\left(\frac{\Omega}{\omega}\right) \tag{2.5}$$

where η_{app} is the apparent viscosity of the dough in the extruder (Pa.s).

2.3.3 Experimental design and statistical analysis

Experiments were conducted using the central composite rotatable design (CCRD) which was developed using Design-Expert 8.0.7.1 (Statease, Minneapolis, MN, USA), consisting four numerical independent variables namely apple pomace (X_1), temperature (X_2), screw speed (X_3) and moisture content (X_4) each at five levels as shown in Table 2.2. Three replicates were taken (optional) at the design center (0, 0, 0) and the total number of observations were 27 [24 (axial points) and 3 (center points)]. Using Eq. (2.6), the numerical independent variables in actual form (X_1, X_2) were converted to their coded form (x_1, x_2):

$$x_i = \frac{X_i - X_0}{\Delta X} \tag{2.6}$$

where x_i is the dimensionless coded value of the i-th independent variable, and X_i, X_0, and ΔX correspond to the actual value, actual value at the center point, and the step change of the i-th variable, respectively. The experimental design and the codes for the processing variables have been reported in Table 2.3.

Second-order polynomial regression models were established for the dependent variables to fit experimental data for each response using statistical software Design-

Expert 8 (Stat-Ease Inc., Minneapolis, MN). Mass flow rate (Y_{MFR}), specific mechanical energy (Y_{SME}), apparent viscosity of dough (Y_{AV}), torque (Y_{Tor}), die pressure (Y_P), temperature of dough in the metering zone (Y_{TMZ}) and die (Y_{TD}) were taken as the responses of the designed experiments. A second-order polynomial regression models were established for the dependent variables to fit experimental data for each response.

$$y_i = b_0 + \sum_{i=1}^{a} b_i x_i + \sum_{i=1}^{a} b_{ii} x_{i^2} + \sum_{i=1}^{a} \sum_{j=1}^{a} b_{ij} x_i x_j \qquad (2.7)$$

where y_i is the predicted response; b_0 is the interception coefficient; b_i, b_{ii}, and b_{ij} are coefficients of the linear, quadratic, and interaction terms; and x_i is the independent variables studied. Experimental data were fitted to the selected models and regression coefficients were obtained. Statistical significance of the terms in the regression equation was examined by analysis of variance (ANOVA) for each response. To evaluate the goodness of the models, coefficient of determination (R^2), *F*-values, the derived *P* values, and coefficient of variance (CV) were determined. The lack-of-fit term was also used to judge adequacy of model fit. Three-dimensional response surfaces were used to visualize the interactive effects of the independent variables.

2.4 Results and Discussion

2.4.1 Effect of processing conditions on apparent viscosity

Multiple linear regression analysis of the experimental data yielded second order polynomial model for apparent viscosity. The regression model shown in Table 2.4 allowed prediction of the independent variables on apparent viscosity (Y_{AV}).

An ANOVA was conducted to assess the significant effects of the independent variables on responses and which of the responses were significantly affected by the varying processing conditions. Regression analysis indicated that the fitted model had a

significant coefficient of determination (R^2) of 0.96 in the experimental data. Table 2.5 shows that the model for apparent viscosity was significant (P<0.05), with significant lack-of-fit variation. Even though lack of fit was significant, the coefficients of variation was found to be at the level of 10.86%. Based on the analysis of variance (Altan et al., 2008a), the selected model adequately represented the data for apparent viscosity. Apple pomace (X_1), temperature (X_2), screw speed (X_3) and moisture content (X_4) had significant negative linear effects (P<0.001), whereas screw speed had significant positive quadratic effect (P<0.05).

The obtained values of apparent viscosity during extrusion of the blends varied between 2540 to 4751 Pa.s The highest apparent viscosity was observed at lowest screw speed and a moisture content of 17%. Figure 2.3(A-D) shows the response surface plots of viscosity changes at different apple pomace level, moisture content, temperature and screw speed. Viscosity was highest at low moisture and apple pomace content and it was lowest at high moisture and apple pomace content. This could have happened due to the presence of sugar in the pomace, since low molecular weight carbohydrates acts as plasticizers and can reduce the melt viscosity (Karkle et al., 2012). Low apple pomace content also means high levels of defatted soy flour in the blend. In another study, Bhattacharya and Hanna (1986) found that with increase in percentage of soy, the pseudoplastic behavior increased and therefore the apparent viscosity also increased.

The feed moisture content was found to have a highly significant effect (P<0.05) on the apparent melt viscosity. The water acts as a plasticizer on the starch-based material, reducing the viscosity and the mechanical energy dissipation during extrusion. When the starch granules swell the viscosity increases, but the shear forces in extruder

can break these granules into smaller fragments causing a reduction in the melt viscosity (Ilo et al., 1996).

Temperature had significant effect (P<0.05) on the apparent viscosity (Table 2.5). With increase in the level of extrusion temperature the viscosity of the apple pomace blend within the extruder decreased. In general, viscosity of fluid materials decreases with increasing temperature in an Arrhenius fashion, so results of this type would be expected in the fully melted feed material and the resulting dough. The apparent viscosity decreased on increasing the screw speed from 100 rpm to 200 rpm (Figure 2.3), indicating that the molten dough inside the barrel exhibited shear thinning behavior. Increasing screw speed reduces viscosity due to increased shear rates and molecular degradation (Singh and Muthukumarappan, 2017b).

2.4.2 Effect of processing conditions on mass flow rate

Mass flow rate in a single screw extruder depends on the drag flow developed by screw rotation and the pressure developed due to constriction at the die (Ludewig, 1989). Multiple regression equation for MFR (Y_{MFR}) is shown in Table 2.4.

Regression analyses showed that MFR was significantly (P<0.05) affected by linear and quadratic effects of apple pomace (X_1) and screw speed (X_3). The response was analyzed using ANOVA and the data are presented in Table 2.5. The regression model for the influence of apple pomace and screw speed on MFR of extrusion of apple pomace blends had a coefficient of determination (R^2) of 0.88. The ANOVA showed that the quadratic model was significant (P<0.05), whereas the lack of fit was not significant (P>0.05) for MFR.

The MFR varied between 1.5 to 3.2 kg/h. The effects of screw speed and apple pomace on MFR is shown in Figure 2.4. Increasing the screw speed from 100 to 200 rpm significantly (P<0.05) increased the mass flow rate. Such behavior is expected as drag flow in extruder has been shown to be proportional to the screw speed and hence, higher screw speeds results in higher mass flow rate, due to greater ability to convey the material along the extruder barrel (Harper, 1981). Increasing the apple pomace content from 0 to 20% also increased the mass flow rate. This could be due to decrease in the apparent viscosity. Further increase in screw speed and apple pomace level resulted in decrease in MFR. This could be due to increase in the pomace fiber which limits the availability of water for starch leading to increase in apparent viscosity and hence decrease in MFR.

2.4.3 Effect of processing conditions on specific mechanical energy

SME is a good quantitative parameter that allows direct comparison of different combinations of extrusion conditions such as feed rate, screw speed and torque. The amount of mechanical energy input during extrusion has a direct role in macromolecular transformations and interactions of different components in the feed materials. Macromolecular transformations may be starch conversion or changes in protein structure which consequently determines the rheological properties of the melt (Aguilar-Palazuelos et al., 2012).

A multiple linear regression equation of second-order polynomial model was generated for SME. The regression model shown in Table 2.4 allowed prediction of the effects of independent variables on SME (Y_{SME}).

Apple pomace level (X_1) and temperature (X_2) had highly significant negative linear effect (P<0.05), whereas moisture content (X_4) had highly significant positive

linear effect (P<0.05) on SME followed by a positive quadratic effects (P<0.05) of apple pomace level (X_1^2) and screw speed (X_3^2).

ANOVA for SME of quadratic model is given in Table 2.5. Acceptable coefficient of determination value (R^2=0.91) was obtained for significant model (P<0.05) with significant lack of fit variation. Although the lack of fit was significant, the coefficient of variation was found to be at the level of 15.4%. On the basis of analysis of variance, the selected models adequately represented the data for SME.

The SME in this study varied from 52 to 146 W-h/kg. Figure 2.5(A-C) shows the response surface graph of SME *versus* moisture content and apple pomace level, temperature and apple pomace level, and moisture content and temperature, respectively. SME decreased with increase in apple pomace level up to a certain extent. With further increase in apple pomace level, SME increased. Increase in pomace levels progressively may decrease the starch content. Moreover, the high fiber content has a tendency to bind more water, resulting in less available water for starch (Mir et al., 2015). Viscosity increased with decreasing water content and hence SME increased. A decrease in SME with increase in temperature was also observed. High temperatures are usually associated with a decrease in the melt viscosity inside the extruder, which in turn reduces the energy input of the extruder. According to Ludewig (1989), with increase in screw speed, SME generally increases because the changes in energy input to the screw are typically in greater order of magnitude than the decrease in torque associated with the decrease in apparent viscosity due to shear thinning behavior of the non-Newtonian materials. With increase in feed moisture content viscosity decreases which ultimately leads to reduced SME (Chang et al., 1999; Hsieh et al., 1991).

2.4.4 Effect of processing conditions on torque

The torque indicates the resistance load on the motor. The torque required to turn the extrusion screw is related to its speed, feed filled in the extruder and the viscosity of the feed material in the screw channel (Harper et al., 1989). Multiple regression equation for torque (Y_{Tor}) is given in Table 2.4.

The torque was influenced significantly (P<0.05) by negative linear effects of apple pomace (X_1), temperature (X_2), screw speed (X_3) and moisture content (X_4) suggesting that increase in the levels of these variables resulted in decreased torque. The response was analyzed using ANOVA and the data are presented in Table 2.6. Examination of the model shows a good fit with R^2 equal to 0.92 for the torque. The ANOVA showed that the linear model was significant (P<0.05), whereas lack of fit was not significant (P>0.05) for torque.

The result on torque values obtained from this study varied from 7 to 19.78 N.m. The response surface (Figure 2.6(A)) shows that moisture content had a dominant effect on torque, whereas screw speed seems to have a minor effect. A sharp decrease in torque with increasing moisture content suggests more water is available for starch gelatinization resulting in reduction in apparent viscosity. Increasing moisture content and screw speed decreases the torque (Figure 2.6(B)). Reduction in torque can be attributed to reduced friction in the extruder as a result of increase in feed moisture. This indicated that increasing moisture content or screw speed reduces the difficulty of processing.

Similar results were found by Onwulata et al. (1994) for twin screw extrusion of corn meal and by Chang and El-Dash (2003) for cassava extrusion. Filli et al. (2012) also

reported decrease in torque with increase in screw speed and feed moisture during single screw extrusion of millet-soybean mixture. According to Guha et al. (1997), increase in screw speed decreased the magnitude of torque due to (a) decrease in the filled length, and (b) increase in the shear rate that reduces the apparent viscosity of the extrudate as the mass inside the extruder behaves as a pseudoplastic material (Bhattacharya et al., 1992; Harper, 1981) that shows shear thinning behavior. Torque is sensitive to changes in operating conditions and therefore appeared to be a good control variable.

2.4.5 Effect of processing conditions on die pressure

The regression analysis results indicate that die pressure was highly significant ($P < 0.05$) on linear terms of temperature (X_2), screw speed (X_3) and moisture content (X_4). The regression equation obtained for die pressure (Y_P) is shown in Table 2.4.

The negative coefficient of the first order term of temperature (X_2), screw speed (X_3) and moisture content (X_4) indicated that die pressure increased with decrease of temperature, screw speed and moisture content. ANOVA for the model as fitted (Table 2.6) shows significance ($P<0.05$) with a significant lack of fit. The coefficient of variation was found to be 11.15% for die pressure. Examination of model shows good fit with R^2 equal to 0.92. Despite a significant lack of fit, the model developed for pressure appeared to be adequate.

The measured die pressure in extrusion cooking of apple pomace blend ranged from 9 to 16 MPa. Die pressure decreased with an increase in temperature. The effects of extrusion temperature on pressure are consistent with earlier published data (Altan et al., 2008b; Singh et al., 2007; Singh et al., 1998) The decrease in pressure with increase in temperature may be attributed to the decrease in viscosity of molten blend. Increase in die

temperature with increase in feed moisture affects the viscosity of mass through the extruder that leads to decrease in die pressure value. As shown in Figure 2.7, increasing moisture content and screw speed resulted in decrease in the die pressure.

2.4.6 Effect of processing conditions on dough temperature

Multiple regression equation for dough temperature at the metering zone (Y_{TMZ}) and die (Y_{TD}) are shown in Table 2.4.

ANOVA for the model as fitted (Table 2.7) shows significance ($P<0.05$) whereas lack of fit for dough temperature at metering zone was not significant ($P>0.05$) and lack of fit for dough temperature at die was significant. The response surface regression model on dough temperature yielded excellent fits with coefficient of determination R^2 equal to 0.94 for dough temperature at the metering zone and R^2 equal to 0.92 for dough temperature at the die. The coefficient of variation, which indicates the relative dispersion of the experimental points from the predictions of the model, was found to be 1.74% and 2.94% for dough temperature at the metering zone and at the die, respectively. Based on the analysis of variance and coefficient of determination, the model developed for dough temperature appeared to be adequate. It was observed that dough temperature was significantly ($P<0.05$) dependent on linear term of extrusion temperature.

As expected, as the temperature profile increased, the dough temperatures increased concurrently. Although the barrel temperature was maintained constant according to the experimental runs, the dough temperature measured at the metering zone of the extruder and at the die was much higher because of viscous dissipation. The dough temperature at the die varied from about 119 to 164 ^{o}C and at the metering zone it varied

within 113 to 146 ^{o}C. It appeared, however, that the blend had no effect on dough temperature.

2.5 Conclusions

Response surface design was used to highlight the effects of apple pomace level, barrel and die temperature, screw speed, and moisture content of the blends on the system parameters. An increase in screw speeds increased the SME and the apparent viscosity. Barrel and die head temperature affected the apparent viscosity, SME, torque, dough temperature and die pressure. At the maximum temperature, in combination with screw speed and AP inclusion, there was a significant ($P<0.05$) decrease in apparent viscosity. Apple pomace also affected the apparent viscosity, SME, MFR and torque. The stability and quality of extrusion are mainly detected by the measurements of the motor torque and the die pressure. The torque, barrel pressure, and SME decreased as the moisture content was increased because water acts as a plasticizer that decreases viscosity. The viscosity was the highest for the material with a 17% moisture content operated at lowest screw speed, because greater friction was generated by shearing action of the material at low screw speed. The results showed the influence of AP level and other processing conditions on the system parameters during single screw extrusion. This study will help in formulating and understanding physico-chemical changes during extrusion of apple pomace-soy flour-corn grits blends.

Table 2.1 Ingredient composition of blends and the mean proximate composition of each (values in the parentheses represent standard error).

Feed ingredients	**Mass of ingredients (g kg^{-1})**				
	Blend I	**Blend II**	**Blend III**	**Blend IV**	**Blend V**
Defatted soy flour	500	450	400	350	300
Corn grits	500	500	500	500	500
Apple pomace	0	50	100	150	200
Proximate analysis					
Protein (% db)	28.53 (0.03)	25.56 (0.11)	23.38 (0.18)	20.63 (0.04)	19.14 (0.12)
Fiber (% db)	2.26 (0.12)	3.25 (0.31)	4.34 (0.24)	5.43 (0.10)	6.41 (0.14)
Fat (% db)	1.17 (0.06)	1.23 (0.02)	1.34 (0.11)	1.41 (0.02)	1.56 (0.05)
Ash (% db)	4.43 (0.13)	4.27 (0.03)	3.98 (0.10)	3.73 (0.03)	3.49 (0.02)
NFE (% db)	52.95 (0.17)	51.65 (0.08)	50.36 (0.03)	49.06 (0.13)	47.77 (0.06)

db, dry basis

Table 2.2 Independent numerical variables and their levels.

Numerical variable	**Symbol**	**Coded Variable Levels**				
		-2	**-1**	**0**	**1**	**2**
Apple pomace (%)	X_1	0	5	10	15	20
Temperature (°C)	X_2	100	110	120	130	140
Screw speed (rpm)	X_3	100	125	150	175	200
Moisture content (% wb)	X_4	14	15.5	17	18.5	20

Table 2.3 Experimental design layout.

Run	Coded Variable				Actual Variable			
	x_1	x_2	x_3	x_4	X_1 (%)	X_2 (°C)	X_3 (rpm)	X_4 (% wb)
1	1	-1	1	-1	15	110	175	15.5
2	-1	1	1	1	5	130	175	18.5
3	0	0	0	2	10	120	150	20
4	1	-1	-1	1	15	110	125	18.5
5	0	0	0	-2	10	120	150	14
6	1	-1	1	1	15	110	175	18.5
7	1	-1	-1	-1	15	110	125	15.5
8	-1	-1	-1	1	5	110	125	18.5
9	0	0	-2	0	10	120	100	17
10	0	2	0	0	10	140	150	17
11	-1	1	-1	-1	5	130	125	15.5
12	-1	1	-1	1	5	130	125	18.5
13	2	0	0	0	20	120	150	17
14	-1	-1	-1	-1	5	110	125	15.5
15	1	1	-1	-1	15	130	125	15.5
16	0	0	0	0	10	120	150	17
17	1	1	1	-1	15	130	175	15.5
18	0	0	0	0	10	120	150	17
19	1	1	-1	1	15	130	125	18.5
20	0	0	0	0	10	120	150	17
21	0	0	2	0	10	120	200	17
22	-2	0	0	0	0	120	150	17
23	1	1	1	1	15	130	175	18.5
24	0	-2	0	0	10	100	150	17
25	-1	-1	1	1	5	110	175	18.5
26	-1	1	1	-1	5	130	175	15.5
27	-1	-1	1	-1	5	110	175	15.5

wb, wet basis

Table 2.4 Best-fit response surface models after excluding the insignificant terms for apparent viscosity, mass flow rate, specific mechanical energy, torque, die pressure, and temperature at metering zone and at die.

Response surface model	R^2	**Adj** R^2	**Pred** R^2	**Adeq precision**
$Y_{AV} = 45755.72 - 194.51X_1 - 189.81X_2 - 189.45X_3 - 1120.73X_4 + 0.29X_3^2$	0.96	0.91	0.77	16.77
$Y_{MFR} = 0.87 + 0.21X_1 + 0.09X_3 - 0.005X_1^2 - 0.0002X_3^2$	0.88	0.75	0.34	8.67
$Y_{SME} = 438.17 - 17.81X_1 - 0.79X_2 + 6.66X_4 + 0.26X_1^2 + 0.009X_3^2$	0.91	0.79	0.45	10.28
$Y_{Tor} = 74.24 - 0.21X_1 - 0.19X_2 - 0.03X_3 - 1.81X_4$	0.92	0.90	0.87	26.97
$Y_P = 63.71 - 0.18X_2 - 0.02X_3 - 1.72X_4$	0.92	0.90	0.87	23.91
$Y_{TMZ} = 38.14 + 0.83X_2$	0.94	0.92	0.90	33.67
$Y_{TD} = -10.37 + 1.34X_2$	0.92	0.90	0.87	29.80

Table 2.5 Analysis of variance (ANOVA) for apparent viscosity, SME and MFR.

Source	Apparent Viscosity					MFR				SME			
	df	**SS**	**MS**	***F*-value**	***P*-value**	**SS**	**MS**	***F*-value**	***P*-value**	**SS**	**MS**	***F*-value**	***P*-value**
Model	14	21669526.85	1547823.35	20.33	< 0.0001	3.79	0.27	6.48	0.0013	16278.76	1162.77	8.16	0.0004
X_1	1	1055573.47	1055573.47	13.86	0.0029	1.54	1.54	36.73	< 0.0001	5538.91	5538.91	38.88	< 0.0001
X_2	1	3408719.58	3408719.58	44.77	< 0.0001	0.00	0.00	0.00	0.949	3034.92	3034.92	21.30	0.001
X_3	1	9276401.74	9276401.74	121.83	< 0.0001	1.34	1.34	32.16	0.0001	1.36	1.36	0.01	0.924
X_4	1	6387374.57	6387374.57	83.89	< 0.0001	0.00	0.00	0.00	0.958	5539.85	5539.85	38.89	< 0.0001
X_1X_2	1	24085.32	24085.32	0.32	0.5842	0.03	0.03	0.60	0.4531	57.26	57.26	0.40	0.538
X_1X_3	1	138200.84	138200.84	1.82	0.2028	0.01	0.01	0.30	0.5957	45.32	45.32	0.32	0.583
X_1X_4	1	55176.98	55176.98	0.72	0.4113	0.02	0.02	0.45	0.5136	28.70	28.70	0.20	0.662
X_2X_3	1	162173.49	162173.49	2.13	0.1701	0.01	0.01	0.17	0.6907	0.45	0.45	0.00	0.956
X_2X_4	1	167186.65	167186.65	2.20	0.1642	0.08	0.08	1.90	0.1936	14.46	14.46	0.10	0.756
X_3X_4	1	33030.17	33030.17	0.43	0.5226	0.00	0.00	0.00	0.9467	151.55	151.55	1.06	0.323
X_1^2	1	36.45	36.45	0.00	0.9829	0.37	0.37	8.75	0.012	891.79	891.79	6.26	0.028
X_2^2	1	993.01	993.01	0.01	0.911	0.00	0.00	0.00	0.9601	13.27	13.27	0.09	0.765
X_3^2	1	720754.61	720754.61	9.47	0.0096	0.44	0.44	10.52	0.007	717.20	717.20	5.03	0.045
X_4^2	1	8465.21	8465.21	0.11	0.7446	0.00	0.00	0.06	0.8035	17.98	17.98	0.13	0.729
Residual	12	913714.86	76142.91	-	-	0.50	0.04	-	-	1709.54	142.46	-	-
Lack of Fit	10	904648.62	90464.86	19.96	0.0486	0.49	0.05	7.60	0.1218	1705.51	170.55	84.56	0.012
Pure Error	2	9066.25	4533.12	-	-	0.01	0.01	-	-	4.03	2.02	-	-

df, degrees of freedom; SS, sum of squares; MS, mean squares

Table 2.6 Analysis of variance (ANOVA) for torque and die pressure.

Source	Torque					Die Pressure			
	df	**SS**	**MS**	***F*-value**	***P*-value**	**SS**	**MS**	***F*-value**	***P*-value**
Model	4	310.98	77.75	60.69	< 0.0001	247.05	2995.13	10.44	< 0.0001
X_1	1	26.81	26.81	20.93	0.0001	802.02	802.02	2.80	0.1087
X_2	1	90.19	90.19	70.40	< 0.0001	5991.25	5991.25	20.88	0.0002
X_3	1	17.98	17.98	14.03	0.0011	2294.27	2294.27	8.00	0.0098
X_4	1	176.00	176.00	137.39	< 0.0001	2892.98	2892.98	10.08	0.0044
Residual	22	28.18	1.28	-	-	6311.80	286.90	-	-
Lack of Fit	20	27.93	1.40	10.96	0.0868	5956.56	297.83	1.68	0.4397
Pure Error	2	0.25	0.13	-	-	355.24	177.62	-	-

df, degrees of freedom; SS, sum of squares; MS, mean squares

Table 2.7 Analysis of variance (ANOVA) for dough temperature at metering zone and at die.

Source	Dough Temperature (Metering Zone)					Dough Temperature (Die)			
	df	**SS**	**MS**	***F*-value**	***P*-value**	**SS**	**MS**	***F*-value**	***P*-value**
Model	4	1676.37	419.09	80.64	< 0.0001	4363.52	1090.88	62.03	< 0.0001
X_1	1	12.56	12.56	2.42	0.1342	16.82	16.82	0.96	0.3387
X_2	1	1636.50	1636.50	314.89	< 0.0001	4338.28	4338.28	246.70	< 0.0001
X_3	1	12.33	12.33	2.37	0.1378	0.01	0.01	0.00	0.9835
X_4	1	14.98	14.98	2.88	0.1037	8.41	8.41	0.48	0.4965
Residual	22	114.34	5.20	-	-	386.87	17.59	-	-
Lack of Fit	20	113.85	5.69	23.52	0.0415	381.46	19.07	7.05	0.1313
Pure Error	2	0.48	0.24	-	-	5.41	2.70	-	-

df, degrees of freedom; SS, sum of squares; MS, mean squares

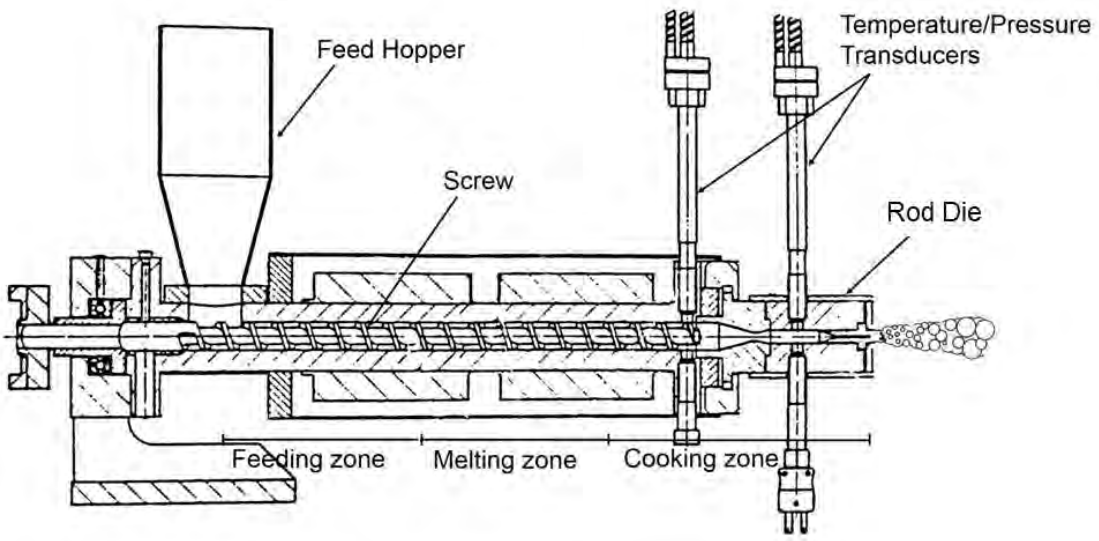

Figure 2.1 Schematic diagram of a single screw extruder

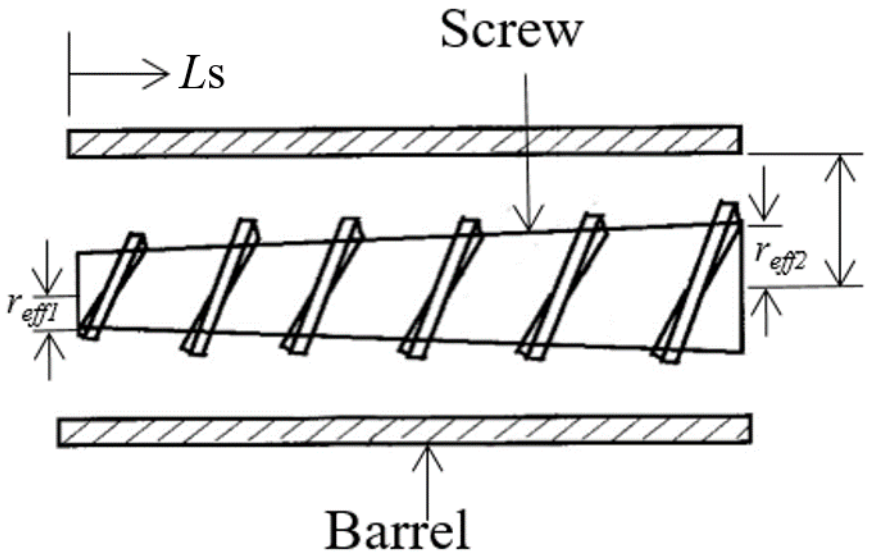

Figure 2.2 Schematic diagram of a section of single screw

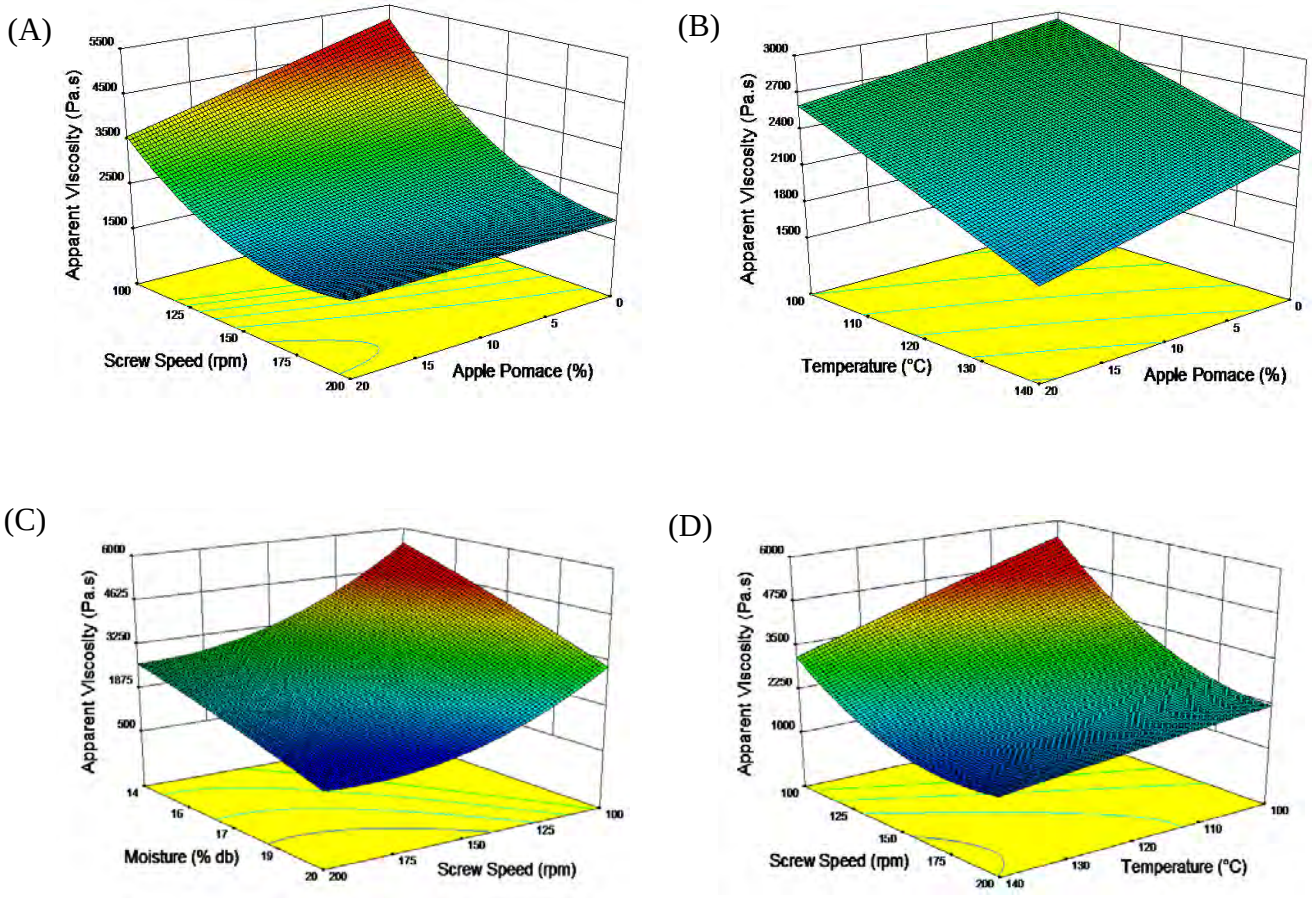

Figure 2.3 Response surface plots for apparent viscosity (A) Screw speed and apple pomace at 120 ^{o}C temperature and 17% moisture content; (B) Temperature and apple pomace at 150 rpm screw speed and 17% moisture content; (C) Moisture and screw speed at 10% apple pomace and 120 ^{o}C temperature; (D) Screw speed and temperature at 10% apple pomace and 17% moisture content

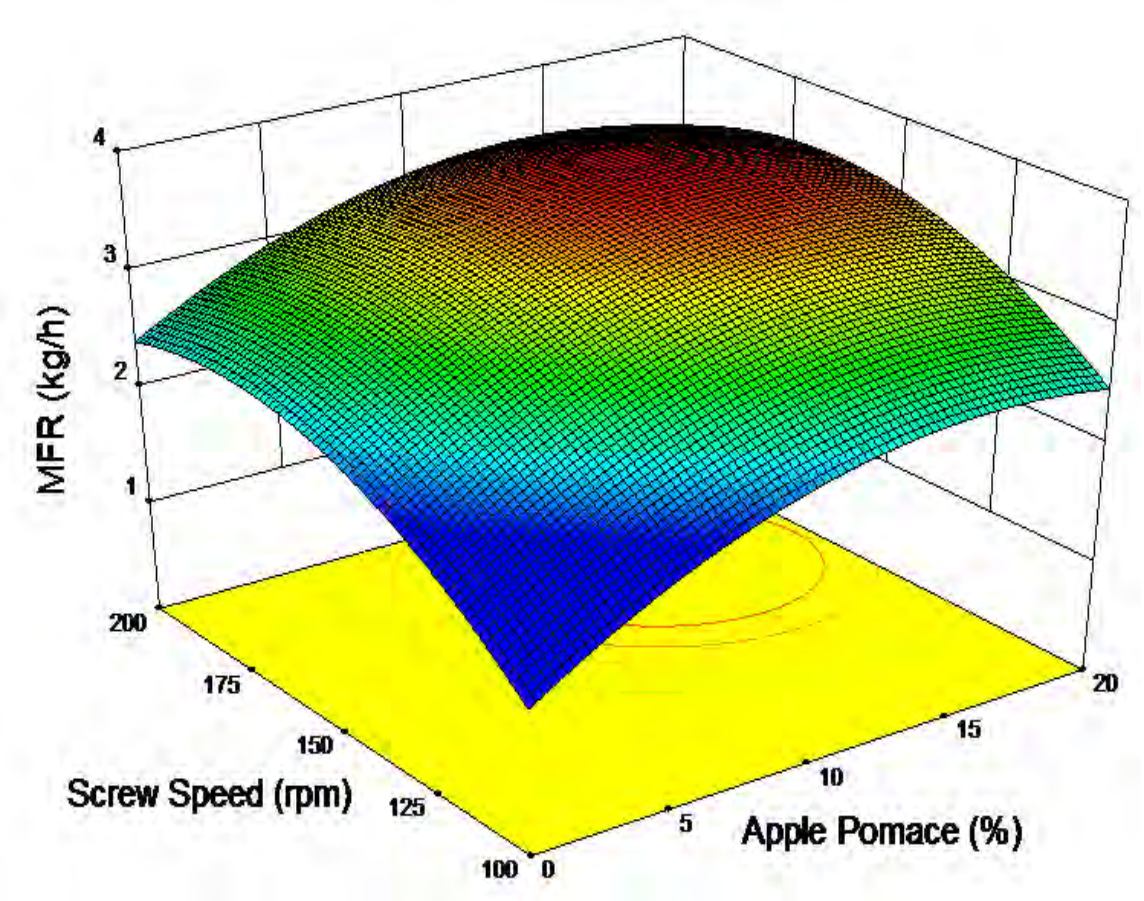

Figure 2.4 Response surface for mass flow rate as a function of screw speed and apple pomace content at 120 ^{o}C temperature and 17% moisture content.

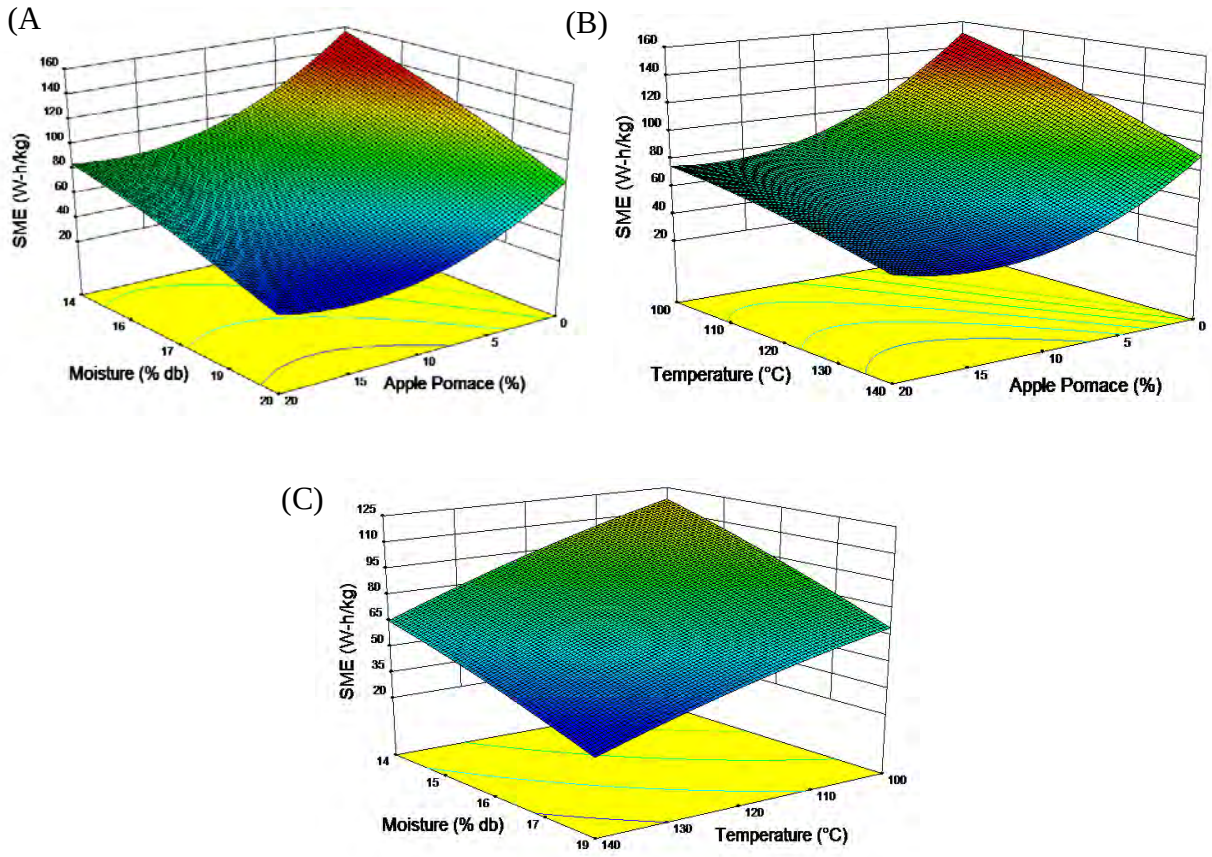

Figure 2.5 Response surface plots for specific mechanical energy (A) Apple pomace and moisture at 120 ^{o}C and 150 rpm screw speed. (B) Temperature and apple pomace at 150 rpm screw speed and 17% moisture content. (C) Moisture and temperature and at 150 rpm screw speed and 10% moisture content

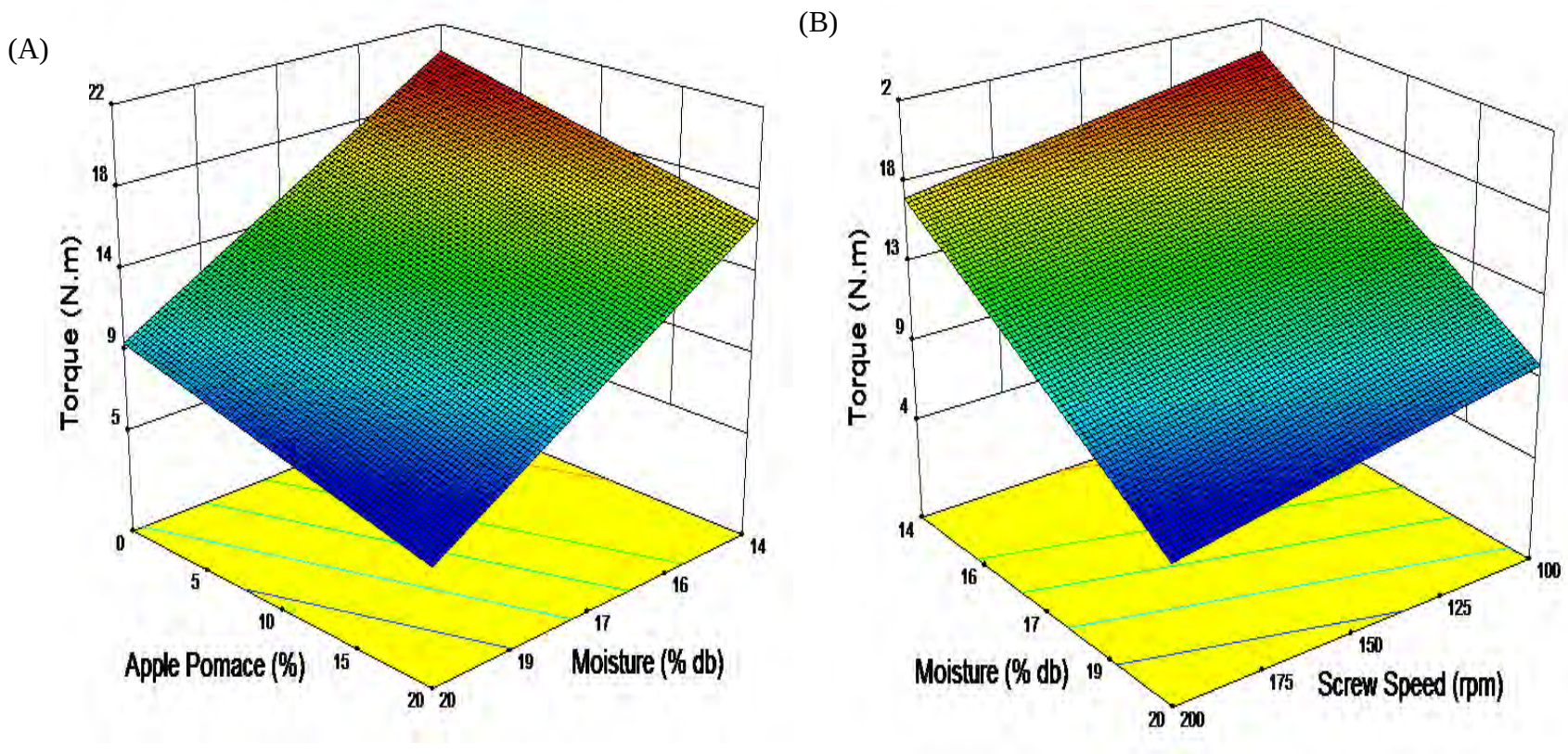

Figure 2.6 Response surface plots for torque (A) Apple pomace and moisture at 120 ^{o}C temperature and 150 rpm screw speed; (B) Moisture and screw speed at 120 ^{o}C temperature and 10% apple pomace level

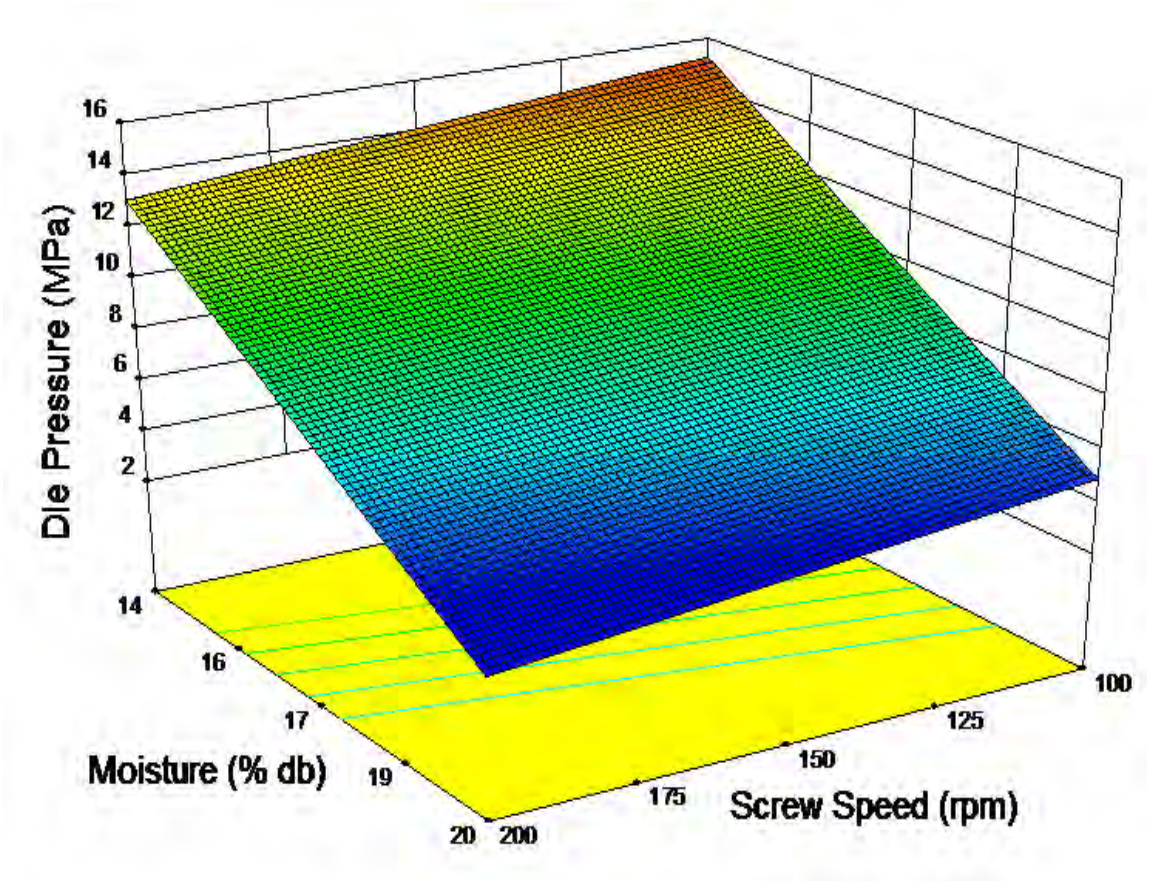

Figure 2.7 Response surface for die pressure as a function of screw speed and moisture at 120 ^{o}C temperature and 10% apple pomace level.

CHAPTER 3 †

Single Screw Extrusion of Apple Pomace Enriched Blends: Extrudate Characteristics and Determination of Optimum Processing Conditions

3.1 Abstract

Response surface methodology was used to investigate the single screw extrusion of apple pomace-defatted soy flour-corn grits blends and the product properties. Five different blends at a level of 0-20 % w/w apple pomace (AP) were extrusion cooked with varied barrel and die temperature (100-140 ^{o}C), screw speed (100-200 rpm) and feed moisture (14-20 % wet basis). Increasing AP content in the blends significantly (P<0.05) increased the bulk density, total phenolic content and the antioxidant activity of the extrudates. The expansion ratio increased with 5% pomace level of inclusion compared with control (0% AP) but decreased significantly (P<0.05) at higher levels of inclusion (10%-20%). Moisture content had quadratic influence on water absorption and solubility indices. Optimal extrusion cooking conditions most likely to produce AP enriched extruded snack food were at 140^{o}C, 20% moisture content and 200 rpm. The results indicated active interaction between apple pomace and starch during expansion process.

3.2 Introduction

Extrusion is a high-temperature short time process in which final product is obtained by a combination of several unit operations such as heating, mixing, shearing and forcing the material through a die in a single step (Singh and Muthukumarappan, 2017b). The thermomechanical action during extrusion brings about gelatinization of starch, denaturation of protein and inactivation of enzymes, microbes and many anti-

† Singha, P., Muthukumarappan, K., (2017). Single screw extrusion of apple pomace enriched blends: Extrudate characteristics and determination of optimum processing conditions Food Science and Technology International (Under review)

nutritional factors; all this occurs in a shear environment, resulting in a plasticized continuous mass (Bhattacharya and Prakash, 1994). The rate and extent of heating, mixing, shearing, and compressing of the materials inside the barrel, and subsequently the die, is strongly related to the properties of the raw materials and process conditions used (Singh and Muthukumarappan, 2017a).

Extrusion cooking has become a well-established industrial technology, with a wide range of food and feed applications. In addition to the usual benefits of heat processing, extrusion offers the possibility of modifying the functional properties of food ingredients and/or of expanding them (Cheftel, 1986). Extrusion is a versatile and very efficient technology in food processing (Singh, 2016).

Cereals such as corn are gluten free, easily accessible and have a high starch content, which can give excellent expansion characteristics. For this reason, corn in different form has been widely used as raw material for extrusion (Singha et al., 2017). Most cereal-based snack products consumed by children are made from corn and hence there is a need to improve the nutritional value of this kind of food (Pastor-Cavada et al., 2011). Combining corn with legumes increases both the amount and quality of the protein (Young, 1991). Legumes such as soybean are rich source of protein that can be used to improve the diet of millions of people. Typically, on a wet basis, stored mature soybeans contains 13 % water and hence it contains about 35% protein, 17% oil, 31% carbohydrate, and 4.4% ash (Liu, 1997). Soybean in different forms (Bookwalter et al., 1971; Chiang, 2007; Iwe et al., 2001a; Lobato et al., 2011; Singh and Muthukumarappan, 2014a; Singh and Muthukumarappan, 2016; Yu et al., 2014) have been extensively used as a major ingredient during food and feed extrusion. Addition of soybean can act as a

good source of protein in formulated food products besides offering other functional, nutritional and health benefits (Friedman and Brandon, 2001). The defatted soybean flour, a by-product of oil processing industry, has been used to increase the protein content of the extruded snacks (Alam et al., 2016).

The by-product that remains after the extraction of juice from apple is known as pomace. It is rich in cell wall residues which are not digested by enzymes in the human body. These are classified as dietary fiber (Hwang et al., 1998). It is also composed of carbohydrates, small amounts of protein, fat and ash (Sudha et al., 2007). AP is also a good source of phytochemicals primarily phenolic acids and flavonoids. Some of the phenolic compounds identified in AP have been correlated with antioxidant capacities using various methods (DPPH, hydroxyl and superoxide anion radical scavenging activity, FRAP) and thereby confirming that the AP is a potential source of antioxidants (Diñeiro et al., 2009). The common applications of this by-product are the direct disposal to soil in a landfill and for extraction of pectins (gelling agent and stabilize). It has also been incorporated in bakery products (bread, muffins, and cookies) (Masoodi et al., 2002; Min et al., 2010; Wang and Thomas, 1989) to achieve more desirability than those baked with bran. Still the very large volumes produced each year exceed existing uses and new applications for AP are required. It may find use as a valuable food additive due to the high levels of fiber and antioxidants it contains.

Although apple pomace has been combined with other flours such as rice and corn (Mir et al., 2015; O'Shea et al., 2013; Reis et al., 2014) in extrusion, there have been no studies on the efficacy of extruding blends of defatted soy flour, apple pomace and corn grits for the development of a healthy snacks. Therefore, the objectives of this study

were, (a) to include apple pomace as a value-added ingredient into an extruded formulation using a single screw extruder, (b) to assess the product quality characteristics of the extruded snack via observing bulk density, expansion ratio, color, water absorption and solubility indices, total phenolic content and antioxidant activity, and (c) to optimize both the process and the formulation to create a snack with maximum phenolic content and expansion ratio.

3.3 Materials and Methods

3.3.1 Raw materials and blend preparation

Apple pomace (AP) powder obtained from Tree Top, Inc. (Selah, WA) was stored at -18°C. The initial moisture content of AP powder was 10.08% (wet basis). The proximate composition of the AP powder was: 4.14% protein, 2.79% fat, 2.00% ash, 24.73% total fiber and 0.20% nitrogen free extract (NFE, dry basis). Corn grits (CG) was obtained from Bob's Red Mill (Milwaukie, OR). The initial moisture content of CG was 11.46% (wet basis). The proximate composition of the CG was: 6.78% protein, 1.69% fat, 2.26% ash, 1.02% total fiber and 81.80% NFE (dry basis). Defatted soy flour (DSF) was obtained from Hodgson Mill, Inc. (Effingham, Il). The initial moisture content of DSF was 7.75% (wet basis). The proximate composition of the DSF powder was: 53.94% protein, 0.77% fat, 6.87% ash, 2.93% total fiber and 26.09% NFE (dry basis). Table 3.1 shows the five different blend compositions (Blend I to V) of AP, DSF and CG. Depending on the experimental design, water was added to the blends. The ingredients were mixed in a laboratory scale mixer (KitchenAid Professional 5 Plus, Troy, Ohio, USA) for 10 minutes and stored overnight at ambient temperature for

moisture stabilization. The moisture content of the prepared blends was determined by using the method 44-19 (AACC, 2000).

3.3.2 Extrusion processing

After mixing and conditioning, the blends were randomly extruded using a 19.18 mm barrel inner diameter, single-screw laboratory extruder (Brabender Intelli-Torque Plasti-Corder®, South Hackensack, NJ) at Food Innovation Center, University of Nebraska, Lincoln, NE which was powered by a 7.5-HP motor with an operating range of screw speeds from 0 to 225 rpm. The extruder had a barrel with length to diameter ratio of 20:1. A uniform 19.05 mm pitch screw having 381 mm screw length, a 19.05 mm constant outside (top of flight) diameter, a 3.81 mm initial screw feed depth, an 11.43 mm initial screw root diameter, and a screw compression ratio (feed channel depth to metering channel depth) of 1.5:1 was used in the experiments.

3.3.3 Experimental design and statistical analysis

Experiments were conducted using the central composite rotatable design which was developed using Design-Expert 8.0.7.1 (Stat-Ease, Minneapolis, MN, USA), consisting four numerical independent variables namely apple pomace (x_1), temperature (x_2), screw speed (x_3) and moisture content (x_4) each at five levels as shown in Table 3.2. The temperature profiles in the barrel and die sections (hereafter will be referred to as temperature) are represented in Table 3.3. Three replicates were taken (optional) at the design center (0, 0, 0) and the total number of observations were 27 [24 (axial points) and 3 (center points)]. The experimental design and the codes for the processing variables have been reported in Table 3.3.

Second-order polynomial regression models were established for the dependent variables to fit experimental data for each response using statistical software Design-Expert 8 (Stat-Ease Inc., Minneapolis, MN). A second-order polynomial regression models were established for the dependent variables to fit experimental data for each response.

$$y_i = b_0 + \sum_{i=1}^{a} b_i x_i + \sum_{i=1}^{a} b_{ii} x_{i^2} + \sum_{i=1}^{a} \sum_{j=1}^{a} b_{ij} x_i x_j \quad (3.1)$$

where y_i is the predicted response; b_0 is the interception coefficient; b_i, b_{ii}, and b_{ij} are coefficients of the linear, quadratic, and interaction terms; and x_i is the independent variables studied. The fitness of the model was evaluated and the interactions between the independent and dependent variables were identified by using an analysis of variance (ANOVA) presented in Table 3.7 to Table 3.9. The goodness of fit of the second-order equation was expressed by the coefficient of determination (R^2) and its statistical significance was determined by the F-test. Three-dimensional response surfaces were used to visualize the interactive effects of the independent variables. A Pearson's correlation matrix on product responses was carried out using SPSS 23.0 (SPSS Inc., Chicago, IL, USA) to determine correlation coefficients between parameters.

3.3.4 Evaluation of Product Properties

3.3.4.1 Expansion ratio

Expansion ratio was calculated as the cross-sectional area of extrudate divided by the cross- sectional area of the die opening. An average diameter of ten samples was measured with a Vernier caliper (Neiko 01407A) to determine the expansion ratio of each set of samples.

3.3.4.2 Bulk density

Bulk density was determined as the ratio of the mass of extrudates that they filled up to a given bulk volume and measured using a standard bushel tester (Seedburo Equipment Company, Chicago, IL) following the method recommended by USDA (USDA, 1999).

3.3.4.3 Color

Color of extrudates was measured using Minolta Spectrophotometer (CM-2500d, Minolta Co. Ltd, Japan) and total color difference (ΔE) were determined following Singha and Muthukumarappan (2016). The *L** value indicates the lightness, 0–100 representing dark to light color. The *a** value gives the degree of the red-green color, with a higher positive *a** value indicating more red color. The *b** value indicates the degree of the yellow-blue color, with a higher positive *b** value indicating more yellow color.

3.3.4.4 Water absorption and solubility indices

Extrudates were ground to fine powders using a coffee grinder (Black & Decker ® Corporation, Towson, MD, USA). The ground extrudates (2.5 g) was suspended in distilled water (30 mL) in a tarred 50 mL centrifuge tube. The suspension was stirred intermittently and centrifuged at 3000×g for 10 min. The supernatant was decanted into a tarred aluminum cup and dried at 135^{o}C for 2 h. The weight of the gel remaining in the centrifuge tube was measured. The water absorption index (WAI, g/g) and water solubility index (WSI, %) were calculated as mentioned by Singh and Muthukumarappan (2016).

3.3.4.5 Total phenolic content and antioxidant activity

For determining the total phenolic content (TPC) and the antioxidant activity (AA), samples were extracted using the method described by Khan et al. (2013). 1 g of ground extrudates was mixed with 10 ml of methanol followed by shaking at low speed for 1 h and then centrifuged at 3000×g for 20 min. The supernatant was decanted and the residue was re-extracted as described above. The two supernatants were combined and stored at -20^{o}C until analysis for TPC and AA. TPC of extrudates was determined using Folin-Ciocalteau method (Singleton et al., 1999) with some modification. 50 µl methanol extract of sample (i.e. the supernatant) was added with 3.5 ml distilled water and 150 µl Folin-Ciocalteau reagent. The solution was vortexed and incubated for 30 min. Thereafter, absorbance of solution was measured at 760 nm against blank. Blank solution consisted only 3.5 ml distilled water and 150 µl Folin-Ciocalteau reagent. Gallic acid was used as positive control (standard) and linear regression curve between absorbance and concentration was drawn for the standard. This standard curve with gallic acid concentration from 0 to 0.5 mg/ml was used for calculating the concentration of sample and data was expressed in mg Gallic acid equivalent (GAE)/100 g dry basis. This analysis was done in triplicate.

AA was measured by the method described by Brand-Williams et al. (1995) 2,2–diphenyl- 1-picrylhydrazyl (DPPH) (Sigma, St. Louis, MO, USA) solution was prepared by adding 7.9 mg of DPPH in 200 ml ethanol. 125 µl methanol extract was mixed with 2 ml ethanol and 0.5 ml of this solution was added with 3 ml DPPH. The solution was vortexed and incubated at room temperature for 30 min. Absorbance of solution and

control (DPPH) was measured at 517 nm against blank (ethanol). Results were expressed as μmol Trolox equivalent (TE)/100 g dry basis. Samples were analyzed in triplicate.

3.4 Results and Discussion

3.4.1 Effect of processing conditions on expansion ratio (ER)

The regression equation for ER is given in Table 3.5. It was observed that AP (X_1) had significant negative linear effect ($P<0.05$) on ER followed by a positive linear effect of screw speed (X_3) ($P<0.05$). Temperature (X_2) and moisture content (X_4) showed no significant effect ($P>0.05$) on ER. ANOVA was performed to study the effects of independent variables on expansion. Regression model fitted to experimental results of ER showed correlation of determination R^2 equal to 0.65. Table 3.7 shows that the model was significant ($P<0.05$) whereas lack-of fit was not significant ($P>0.05$).

The amount of expansion in food depends on the difference between the vapor pressure of water and the atmospheric pressure, as well as the ability of the exiting product to sustain expansion (Khan et al., 2015). The ER of the pomace enriched extrudates varied between 1.27 and 1.98. Expansion of the extrudates increased with increase in screw speed. In our previous study, (Singha and Muthukumarappan, 2017a) we have found out that with increase in the screw speed the apparent viscosity during extrusion of the same formulation decreased. This means with increase in screw speed the shear stress also increased giving rise to a more developed and uniform dough with better expansion properties at the die exit. With the increase in shear stress gelatinization of starch also increases which is an important factor for expansion (Case et al., 1992; Chinnaswamy and Hanna, 1988). The results demonstrate that screw speed is a powerful variable for changing the expansion properties of the product.

There was slight increase in ER at AP level 5% (ER=1.82) compared to control (0% AP, ER=1.75) However, further increase in AP resulted in decrease in ER of extrudates (Figure 3.1). Apple pomace contains insoluble fiber which lowers the affinity between the starch and insoluble fiber. Insoluble fibers also have higher hydrophilic properties, which have been shown to absorb more water, therefore modifying the glass transition temperature of the melt. It also can lower expansion by adhering to the bubble structure and consequently puncturing the cell, reducing cell extensibility (van der Sman and Broeze, 2013). O'Shea et al. (2013) investigated the inclusion of apple pomace in a puffed snack. The authors reported similar results to those reported in the study, a decrease in expansion properties of the extruded material as the AP inclusions increased. As reported by Chang et al. (1998), such behavior can be due to dilution effect of AP on starch content therefore reducing the starches swelling ability and also due to rupture of cell walls of the bubbles by the fibers.

3.4.2 Effect of processing conditions on bulk density (BD)

The regression equation for BD is shown in Table 3.5. Apple pomace (X_1) had a significant positive linear effect (P<0.05), whereas temperature (X_2) and screw speed (X_3) had significant negative linear effects (P<0.05) on BD. BD was significantly affected (P<0.05) by quadratic term of moisture content (X_4^2). The interactions of temperature (X_2) and screw speed (X_3), and temperature (X_2) and moisture content (X_4) were also found to be significant (P<0.05).

ANOVA for BD of quadratic model is given in Table 3.8. Regression analysis indicated that the fitted model had a significant coefficient of determination (R^2) of 0.93

in the experimental data. Table 3.8 shows that the model for BD was significant ($P<0.05$) whereas lack of fit was not significant ($P>0.05$).

From commercial standpoint, bulk density of extruded products is very important quality attribute because most extruded product are filled by weight and not by volume. If BD varies during production, either the pack will be underfilled or overfilled. In addition to controlling the correct packaging, good control in bulk density will also ensure that the product texture is within the required quality limits. The BD of the pomace enriched extrudates varied between 310 to 521 kg m^{-3}. Figure 3.2(A & B) shows the dependence of BD on the AP, temperature, screw speed and moisture content. BD increased with increasing AP level. The effect may be attributed to reduced expansion and fiber distribution within the expanded network of starch in extrudates (Guy, 2001). The negative correlation between BD and ER (Table 3.6) confirms that products with higher bulk density have lower expansion and vice versa.

A negative influence of temperature was found on BD of pomace enriched extrudates. Similar results was observed during extrusion of carrot pomace (Alam et al., 2016), grape pomace (Altan et al., 2008c), pea grits (Singh et al., 2007) based snacks. When the melt exits the die nozzle at high temperature it expands more and have more volume than the extrudates exiting at low temperature (Singh and Muthukumarappan, 2014b) and hence results in decrease in BD. It was also observed that an increase in screw speed resulted in an extrudate with lower density. A low screw speed is associated with high residence time; consequently, the food material inside the extruder receives more input of thermal energy in a low shear environment. High input of thermal energy due to high residence time due to low screw speeds leads to the creation of enhanced

level of superheated steam, hence the product will have good expansion which creates flaky and porous structures due to formation of air cells (Bhattacharya, 1997) and hence the products become lighter.

Moisture content had quadratic effect on the BD. The BD initially decreased with moisture content, which may be due to proper gelatinization and higher expansion, whereas further increase in BD may be because of reduction in elasticity of dough and lower expansion (Ding et al., 2006; Ding et al., 2005). It also reflects its influence on the rheological characteristics of the material. As observed in our previous study, (Singha and Muthukumarappan, 2017a) higher screw speeds lowered the melt viscosity of the mix increasing the elasticity of the dough. This may have resulted in reduction in the density of the extrudate (Ding et al., 2006).

3.4.3 Effect of processing conditions on color parameters and total color change

Multiple linear regression equations for color parameters (L^*, a^* and b^* values) and total color change (ΔE) are shown in Table 3.5. The color parameter L^* of the extrudates was significantly ($P<0.05$) affected by linear terms of apple pomace (X_1) and moisture content (X_4). The effect of temperature (X_2) and screw speed (X_3) was not significant ($P>0.05$).

ANOVA for the color parameters and total color change are given in Table 3.9. The coefficients of determination (R^2) for L^*, a^*, b^* color parameters and ΔE were 0.60, 0.44, 0.58 and 0.61, respectively. Table 3.9 shows that the model for the color parameters and total color difference was significant ($P<0.05$) whereas lack of fit was not significant ($P>0.05$).

The extrudates had color values in the ranges of: L^* 35.50–60.03; a^* 8.23–13.88; and b^* 15.61–34.01. Among the color parameters, the L^* (Figure 3.3) and b^* values showed marked changes due to addition of apple pomace only. Reduction in whiteness, as evident with the decreased L^* values, indicates darker samples. L^* value negatively correlated (Table 3.6) with antioxidant activity and total phenolic content. b^* value decreased with increase in temperature which suggests that yellowness increased. A possible explanation could be degradation of anthocyanin pigment.

Total color change in extruded products ranged between 30.06 and 52.29. Color change is one of the quality indicators for protein-based cooked materials in the food and feed industries (Brown et al., 2015). It is also an indication of the components present in the food materials. Only linear effect of apple pomace was significant ($P<0.05$), as confirmed in the ANOVA table (Table 3.9). There are many reactions that take place during extrusion cooking that affect color. The most common are nonenzymatic browning reactions and pigment degradation. However, for this study the most significant factor for the dark color of the extrudates is AP.

3.4.4 Effect of processing conditions on water absorption and solubility indices

Multiple regression equations were generated relating to water absorption index (WAI) and water solubility index (WSI) to coded levels of the variables (Table 3.5). Fitted models shown in Table 3.5 indicates significant negative linear effect ($P<0.05$) of temperature (X_2) and positive linear effect ($P<0.05$) of moisture content (X_4) on WAI. Moisture content (X_4^2) had significant negative quadratic effect ($P<0.05$) on WAI whereas it had significant positive quadratic effect ($P<0.05$) on WSI. Interaction of apple

pomace and temperature (X_1X_2) had significant positive effect (P<0.05) on WAI and significant negative effect (P<0.05) on WSI.

ANOVA for the models WAI and WSI is summarized in Table 3.8. As indicated in ANOVA table, second order models for WAI and WSI were found to be significant (P<0.05). The lack-of-fit was found to be non-significant (P>0.05) for WAI and WSI. The coefficients of determination (R^2) for water absorption and solubility indices were 0.79 and 0.82, respectively.

The WAI measures the volume occupied by the granule or starch polymer after swelling in excess water. While the WSI determines the amount of free polysaccharide or polysaccharide released from the granule after addition of excess water. The WAI ranged from 2.97 to 4.88 g/g for the extrudates. The effect of temperature and moisture content on the WAI is shown by the response surface graph in Figure 3.4. Increasing temperature significantly (P<0.05) decreased the WAI of extrudates. A decrease in WAI with increasing temperature was probably due to decomposition or degradation of starch (Pelembe et al., 2002). Ding et al. (2006) also stated that the WAI decreases with increasing temperature if dextrinization or starch melting prevails over the gelatinization phenomenon. As the moisture content increased from 14 to 20% (wet basis), WAI also increased. Degradation of starch is mainly responsible for water absorption (Singh and Muthukumarappan, 2016).

The WSI is a parameter that indicates the total degradation undergone by starch granules. It is combination effect of gelatinization and dextrinization followed by solubilization of starch. Also, WSI is often used as an indicator of degradation of molecular components (Yang et al., 2008), which measures the degree of starch

conversion during extrusion and the amount of soluble polysaccharides released from the starch component after extrusion process (Agathian et al., 2014). In the present study, the quadratic effect of moisture content on WSI is well correlated by the response surface plot as shown in Figure 3.5. The WSI varied between 7.80 % and 17.02 %. Moisture content had quadratic effect on WSI. Initially, WSI decreased with increase in moisture content and then with further increase in moisture content, the WSI was found to increase. As stated before, WSI is a measure of the extent of starch degradation. Lower values of WSI means there is minor degradation of starch i.e. less numbers of soluble molecules are present in the extrudates. It has been found that high water content in extrusion processes can diminish protein denaturation and starch degradation (Rodriguez-Miranda et al., 2012). In general, the WAI and WSI values reflect the hydrophobic and hydrophilic nature of the extrudates which can be influenced by degree of starch degradation, protein denaturation, and the hydration dynamics of fiber (Brown et al., 2015).

3.4.5 Effect of processing conditions on total phenolic content (TPC) and antioxidant activity (AA)

The equations for first-order polynomial models employed to predict the TPC and AA of the extrudates are given in Table 3.5. Regression analysis showed that TPC and AA were significantly ($P<0.05$) affected by positive linear terms of pomace level, temperature and moisture content. Phenolic content was not significantly ($P>0.05$) affected by screw speed. Interaction effect between pomace level, temperature and moisture content was not observed. Analysis of variance for the models are summarized in Table 3.7. The lack-of-fit was found to be non-significant ($P>0.05$) for both TPC and

AA with low coefficients of variation (12.78% and 12.98%, respectively) of the predicted models. The coefficients of determination for TPC and AA were found to be 0.84 and 0.82, respectively.

The concentration of TPC ranged from 15.33 to 105.65 g GAE/100 g dry sample. Changes in TPC values with moisture content and pomace level are shown in Figure 3.6. The TPC values increased with increase in pomace level. The fact can be explained by the increased initial phenolic content in the extrudates. This increase seems likely a normal consequence of the high temperature, water-stress and wounding (Fernando Reyes et al., 2007) that could possibly favor the synthesis of enzymes in the metabolic pathway that are responsible for increased production of phenolic compounds (Saltveit, 1998). Highest TPC value was observed at 20% pomace level (maximum pomace level in this study). The phenolic content of the pomace enriched extrudates increased with the rise in moisture content. This variation can be explained by the fact that phenolics can be modified as their solubility and functional group properties are altered (Yang et al., 2008). An increase in phenolic content with feed moisture content was also observed for cereal based extrudates containing carrot powder (Ozer et al., 2006).

Increasing the extrusion temperature could have detrimental effect on the phenolic content. Many studies on extrusion of beans (Korus et al., 2007), oats (Wani and Kumar, 2016) have shown reduction in phenolic content with rise in extrusion temperature. However, extrusion temperature in this study exhibited an opposite effect on TPC. The increase of extrusion temperature slightly increased the phenolic content of pomace-enriched extrudates. Extrusion cooking has been found to increase the total phenolic content in broccoli-corn extrudates (Bisharat et al., 2015), barley-tomato-grape pomace

extrudates at temperature ranging from 140-160^{o}C (Altan et al., 2009b) and whole grains (Zielinski et al., 2001). Apart from apple pomace addition, antioxidant compounds as well as antioxidant activity change in extruded products could also be attributed to the release of phenolic compounds from cell walls (e.g., breaking complex polyphenols into low molecular weight phenolic compounds), to interaction of phenolics with protein, and to formation of Maillard reaction products as a result of high extrusion temperatures (Nayak et al., 2011; Stojceska et al., 2009).

Response surface plot for AA as functions of temperature and AP level is given in Figure 3.7. AA of the extrudates increased with increase in AP level. An increase in AA with increasing pomace level can be attributed to phenolic compound in apple pomace. This result was also supported by significant increase of TPC by increasing level of apple pomace. A positive correlation (R=0.99) was found between phenolic content and antioxidant activity of pomace enriched extrudates. A decrease in the moisture of the blend resulted in lower AA values. This could be due to the shearing effects which would be more destructive on the compounds with antioxidant activities other than phenolic compounds at drier extrusion conditions (Ozer et al., 2006). The presence of a greater amount of moisture in the sample would lead to a gentler processing in the extruder barrel. An increase in extrusion temperature also led to increase in the AA values. The reason for the high antioxidant potential of the extrudates can be partly accounted for the presence of the high molecular weight products of Maillard reaction which are formed at higher temperatures and act as antioxidants. Furthermore, it can also be stated that at low temperature formation of oxidation-promoting compounds is favored which reduces the antioxidant activity (Nicoli et al., 1999).

3.5 Optimization

The optimization tool from the design expert software was utilized to derive the optimal formulations based on response results evaluated. This was implemented by choosing the maximum limits for both the favorable factors and desirable responses, i.e. apple pomace (factor), water solubility index (response), expansion ratio (response), total phenolic content (response), and antioxidant activity (response). Undesirable response (bulk density) was minimized to produce formulations with a more pleasing texture. The remaining factors were left in range, and all outstanding responses were left out of this procedure. By applying the desirability function method, covering our criteria, the solution obtained for the optimum conditions to produce the apple pomace-soy flour-corn grits extrudates had a desirability value of 0.88. The optimum apple pomace level, barrel and die temperature, screw speed and moisture content estimated were 13%, 140°C, 200 rpm and 20%, respectively. By applying these optimal conditions, an edible extrudate with a BD equal to 244.32 kg m^{-3}, ER of 1.8, WAI of 4.6 g/g, WSI of 17%, AA of 343.40 μmol TE/100 g dry basis, TPC of 108.63 mg GAE/100 g dry basis, L^* 41.80, a^* of 10.97, b^* of 18.11 and ΔE of 45.03 could be produced.

3.6 Conclusions

Extrusion cooking of the blends from apple pomace, defatted soy flour and corn grits were investigated in a single-screw extruder. It is evident that blend of fruit byproducts can be utilized in ready-to-eat snacks. Increasing apple pomace content resulted in an increase in the bulk density, antioxidant activity and total phenolic content and a decrease in the expansion ratio of the extrudate. Optimal extrusion cooking conditions most likely to produce apple pomace-defatted soy flour-corn grits products

suitable for a snack food was predicted by the response surface models found. Optimum extrusion conditions of 140°C barrel and die temperature, 200 rpm screw speed, 20% moisture content (wet basis) and apple pomace: defatted soy flour: corn grits (13:37:50) blend having desirability value of 88.0% could be used to develop a good quality acceptable snack. Additionally, inclusion of apple pomace into extruded products helped to identify a use for a byproduct that is currently little valorized.

Table 3.1 Ingredient composition of blends and the mean proximate composition of each (values in the parentheses represent standard error).

Feed ingredients	**Mass of ingredients (g kg^{-1})**				
	Blend 1	**Blend 2**	**Blend 3**	**Blend 4**	**Blend 5**
Apple Pomace	0	50	100	150	200
Defatted Soy Flour	500	450	400	350	300
Corn Grits	500	500	500	500	500
Proximate analysis					
Protein (% db)	28.53 (0.03)	25.56 (0.11)	23.38 (0.18)	20.63 (0.04)	19.14 (0.12)
Fiber (% db)	2.26 (0.12)	3.25 (0.31)	4.34 (0.24)	5.43 (0.10)	6.41 (0.14)
Fat (% db)	1.17 (0.06)	1.23 (0.02)	1.34 (0.11)	1.41 (0.02)	1.56 (0.05)
Ash (% db)	4.43 (0.13)	4.27 (0.03)	3.98 (0.10)	3.73 (0.03)	3.49 (0.02)
NFE (%db)	52.95 (0.17)	51.65 (0.08)	50.36 (0.03)	49.06 (0.13)	47.77 (0.06)

db = dry basis; NFE = Nitrogen Free Extract

Table 3.2 Independent numerical variables and their levels.

Numerical variables	**Symbol**	**Coded variable levels**				
		-2	**-1**	**0**	**1**	**2**
Apple Pomace (%)	X_1	0	5	10	15	20
Temperature (°C)	X_2	100	110	120	130	140
Screw speed (rpm)	X_3	100	125	150	175	200
Moisture content (%wb)	X_4	14.0	15.5	17.0	18.5	20.0

wb = wet basis

Table 3.3 Temperature profiles of the barrel and die section.

Temperature (^{o}C)	**Temperature profile in the extruder (^{o}C)**
100	60/80/100/100
110	60/80/110/110
120	60/80/120/120
130	60/80/130/130
140	60/80/140/140

Table 3.4 Experimental design layout.

Run	Coded variables				Actual variables			
	x_1	x_2	x_3	x_4	X_1 (%)	X_2 (^{o}C)	X_3 (rpm)	X_4 (% wb)
1	1	-1	1	-1	15	110	175	15.5
2	-1	1	1	1	5	130	175	18.5
3	0	0	0	2	10	120	150	20.0
4	1	-1	-1	1	15	110	125	18.5
5	0	0	0	-2	10	120	150	14.0
6	1	-1	1	1	15	110	175	18.5
7	1	-1	-1	-1	15	110	125	15.5
8	-1	-1	-1	1	5	110	125	18.5
9	0	0	-2	0	10	120	100	17.0
10	0	2	0	0	10	140	150	17.0
11	-1	1	-1	-1	5	130	125	15.5
12	-1	1	-1	1	5	130	125	18.5
13	2	0	0	0	20	120	150	17.0
14	-1	-1	-1	-1	5	110	125	15.5
15	1	1	-1	-1	15	130	125	15.5
16	0	0	0	0	10	120	150	17.0
17	1	1	1	-1	15	130	175	15.5
18	0	0	0	0	10	120	150	17.0
19	1	1	-1	1	15	130	125	18.5
20	0	0	0	0	10	120	150	17.0
21	0	0	2	0	10	120	200	17.0
22	-2	0	0	0	0	120	150	17.0
23	1	1	1	1	15	130	175	18.5
24	0	-2	0	0	10	100	150	17.0
25	-1	-1	1	1	5	110	175	18.5
26	-1	1	1	-1	5	130	175	15.5
27	-1	-1	1	-1	5	110	175	15.5

Table 3.5 Best-fit response surface models after excluding the insignificant terms for ER, BD, WAI, WSI, L*, a*, b*, AA and TPC.

Parameters	Response surface model	R^2	P value
ER	$Y_{ER} = 0.62 - 0.03X_1 + 0.004X_3$	0.65	<0.0001
BD (kg/m^3)	$Y_{BD} = 797.31 + 39.08X_1 - 2.18X_2 - 8.62X_3 + 0.09X_2X_3 - 1.73X_2X_4 + 5.07X_4^2$	0.93	<0.0001
WAI (g/g)	$Y_{WAI} = -9.74 - 0.08X_2 + 2.01X_4 + 0.003X_1X_2 - 0.09X_4^2$	0.79	0.0262
WSI (%)	$Y_{WSI} = 284.47 - 0.04X_1X_2 + 0.66X_4^2$	0.82	0.0131
L*	$Y_{L^*} = 74.97 - 0.89X_1 - 1.23X_4$	0.60	0.0004
a*	$Y_{a^*} = 9.04 + 0.03X_3$	0.44	0.0098
b*	$Y_{b^*} = 52.76 - 0.47X_1 - 1.34X_2 + 0.05X_3 - 0.99X_4$	0.58	0.0005
ΔE	$Y_{\Delta E} = 19.49 + 0.85X_1$	0.61	0.0002
AA (μmol TE/100 g)	$Y_{AA} = -449.36 + 9.39X_1 + 2.64X_2 + 14.99X_4$	0.82	<0.0001
TPC (mg GAE/100g)	$Y_{TPC} = -152.94 + 3.11X_1 + 0.85X_2 + 4.88X_4$	0.84	<0.0001

ER = expansion ratio, BD = bulk density, WAI = water absorption index, WSI = water solubility index, L* = lightness, a* = red-green color, b* = yellow-blue color, ΔE = total color difference, AA = antioxidant activity, TPC = total phenolic content, TE = Trolox Equivalent, GAE = Gallic Acid Equivalent

Table 3.6 Correlation coefficients between product properties.

	ER	BD	*L**	*a**	*b**	ΔE	WAI	WSI	AA	TPC
ER	1	-0.521**	0.504**	0.184^{ns}	0.214^{ns}	-0.527**	-0.171^{ns}	-0.245^{ns}	-0.356^{ns}	-0.337^{ns}
BD		1	-0.196^{ns}	-0.360^{ns}	-0.011^{ns}	0.236^{ns}	-0.005^{ns}	-0.019^{ns}	-0.185^{ns}	-0.197^{ns}
L*			1	0.091^{ns}	0.162^{ns}	-0.983**	0.380^{ns}	-0.127^{ns}	-0.667**	-0.672**
a*				1	0.397*	-0.045^{ns}	-0.193^{ns}	0.277^{ns}	-0.090^{ns}	-0.046^{ns}
b*					1	-0.189^{ns}	-0.173^{ns}	-0.005^{ns}	-0.179^{ns}	-0.169^{ns}
ΔE						1	-0.389*	0.218^{ns}	0.626**	0.631**
WAI							1	-0.521**	-0.252^{ns}	-0.282^{ns}
WSI								1	-0.022^{ns}	-0.028^{ns}
AA									1	0.991**
TPC										1

ER = expansion ratio, BD = bulk density, L* = lightness, a* = red-green color, b* = yellow-blue color, ΔE = total color difference, WAI = water absorption index, WSI = water solubility index, AA = antioxidant activity, TPC = total phenolic content

*significant at P<0.05

**significant at P<0.01

^{ns}not significant.

Table 3.7 Analysis of variance for expansion ratio (ER), antioxidant activity (AA) and total phenolic content (TPC).

	ER					AA				TPC			
Source	**df**	**SS**	**MS**	***F*-value**	***P*-value**	**SS**	**MS**	**F-value**	**P-value**	**SS**	**MS**	***F*-value**	***P*-value**
Model	4	0.84	0.21	10.08	< 0.0001	81786.56	20446.64	25.21	< 0.0001	8825.26	2206.32	29.84	< 0.0001
X_1	1	0.59	0.59	28.45	< 0.0001	52899.50	52899.50	65.22	< 0.0001	5787.13	5787.13	78.27	< 0.0001
X_2	1	0.04	0.04	1.96	0.1756	16755.54	16755.54	20.66	0.0002	1741.03	1741.03	23.55	< 0.0001
X_3	1	0.20	0.20	9.42	0.0056	6.02	6.02	0.01	0.9321	10.52	10.52	0.14	0.7096
X_4	1	0.01	0.01	0.48	0.4948	12125.50	12125.50	14.95	0.0008	1286.58	1286.58	17.40	0.0004
Residual	22	0.46	0.02	-	-	17843.05	811.05	-	-	1626.57	73.93	-	-
Lack of Fit	20	0.46	0.02	17.60	0.0551	17571.01	878.55	6.46	0.1424	1601.59	80.08	6.41	0.1434
Pure Error	2	0.00	0.00	-	-	272.04	136.02	-	-	24.98	12.49	-	-

df, degrees of freedom; SS, sum of squares; MS, mean squares

Table 3.8 Analysis of variance for bulk density (BD), water absorption index (WAI) and water solubility index (WSI).

		BD				WAI				WSI			
Source	**df**	**SS**	**MS**	***F*-value**	***P*-value**	**SS**	**MS**	***F*-value**	***P*-value**	**SS**	**MS**	***F*-value**	***P*-value**
Model	14	90557.93	6468.42	12.13	< 0.0001	4.88	0.35	3.17	0.0262	158.24	11.30	3.79	0.0131
X_1	1	3994.53	3994.53	7.49	0.018	0.09	0.09	0.78	0.3935	3.35	3.35	1.12	0.3103
X_2	1	41834.03	41834.03	78.44	< 0.0001	2.57	2.57	23.38	0.0004	10.90	10.90	3.66	0.08
X_3	1	16051.46	16051.46	30.10	0.0001	0.00	0.00	0.01	0.9433	3.04	3.04	1.02	0.3325
X_4	1	2006.80	2006.80	3.76	0.0763	0.53	0.53	4.79	0.0492	0.26	0.26	0.09	0.7718
X_1X_2	1	243.57	243.57	0.46	0.512	0.53	0.53	4.82	0.0486	70.12	70.12	23.52	0.0004
X_1X_3	1	150.90	150.90	0.28	0.6045	0.00	0.00	0.04	0.8379	1.06	1.06	0.35	0.5628
X_1X_4	1	2362.25	2362.25	4.43	0.0571	0.09	0.09	0.84	0.3769	2.28	2.28	0.77	0.3986
X_2X_3	1	8479.07	8479.07	15.90	0.0018	0.02	0.02	0.14	0.7103	0.11	0.11	0.04	0.8496
X_2X_4	1	10830.26	10830.26	20.31	0.0007	0.15	0.15	1.40	0.2593	7.36	7.36	2.47	0.1421
X_3X_4	1	271.78	271.78	0.51	0.489	0.01	0.01	0.11	0.7487	0.27	0.27	0.09	0.7674
X_1^2	1	558.70	558.70	1.05	0.3263	0.07	0.07	0.67	0.4274	0.45	0.45	0.15	0.7042
X_2^2	1	773.87	773.87	1.45	0.2516	0.00	0.00	0.01	0.912	1.26	1.26	0.42	0.5282
X_3^2	1	168.70	168.70	0.32	0.5842	0.02	0.02	0.15	0.7075	0.78	0.78	0.26	0.6172
X_4^2	1	2771.03	2771.03	5.20	0.0417	0.79	0.79	7.17	0.0201	47.26	47.26	15.85	0.0018
Residual	12	6399.80	533.32	-	-	1.32	0.11	-	-	35.77	2.98	-	-
Lack of Fit	10	6176.71	617.67	5.54	0.1626	0.94	0.09	0.50	0.8139	29.76	2.98	0.99	0.6017
Pure Error	2	223.09	111.54	-	-	0.38	0.19	-	-	6.02	3.01	-	-

df, degrees of freedom; SS, sum of squares; MS, mean squares

Table 3.9 Analysis of variance for color parameters (l*, a*, and b*) and total color difference (ΔE).

			L*				***a****		
Source	**df**	**SS**	**MS**	**F-value**	**P-value**	**SS**	**MS**	**F-value**	**P-value**
Model	4	557.85	139.46	8.11	0.0004	18.11	4.53	4.33	0.0098
X_1	1	472.21	472.21	27.46	< 0.0001	0.23	0.23	0.22	0.6422
X_2	1	0.05	0.05	0.00	0.9561	0.00	0.00	0.00	0.9465
X_3	1	4.13	4.13	0.24	0.6292	15.17	15.17	14.50	0.001
X_4	1	81.46	81.46	4.74	0.0405	2.70	2.70	2.58	0.1222
Residual	22	378.37	17.20	-	-	23.01	1.05	-	-
Lack of Fit	20	353.05	17.65	1.39	0.4998	21.89	1.09	1.95	0.393
Pure Error	2	25.32	12.66	-	-	1.12	0.56	-	-

			b*				**Δ*E***		
Source	**df**	**SS**	**MS**	**F-value**	**P-value**	**SS**	**MS**	**F-value**	**P-value**
Model	4	267.10	66.78	7.70	0.0005	499.92	124.98	8.63	0.0002
X_1	1	131.01	131.01	15.10	0.0008	437.06	437.06	30.20	< 0.0001
X_2	1	44.48	44.48	5.13	0.0338	2.31	2.31	0.16	0.6936
X_3	1	38.61	38.61	4.45	0.0465	1.35	1.35	0.09	0.7632
X_4	1	53.00	53.00	6.11	0.0217	59.20	59.20	4.09	0.0555
Residual	22	190.88	8.68	-	-	318.44	14.47	-	-
Lack of Fit	20	155.35	7.77	0.44	0.8725	299.45	14.97	1.58	0.4591
Pure Error	2	35.53	17.77	-	-	18.98	9.49	-	-

df, degrees of freedom; SS, sum of squares; MS, mean squares

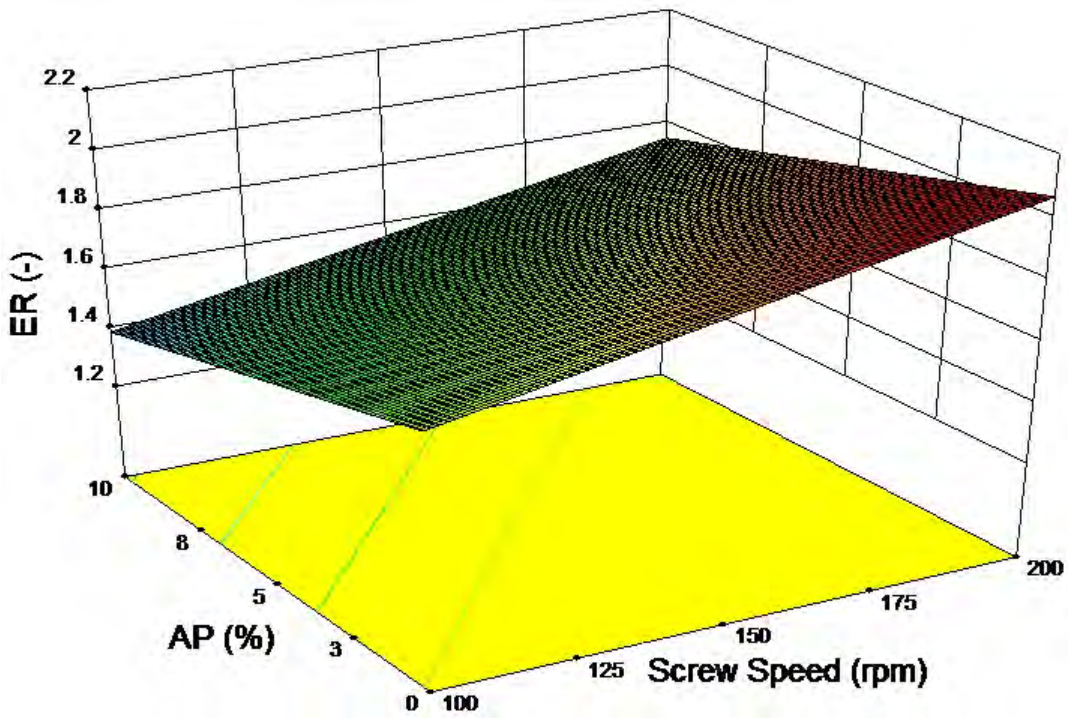

Figure 3.1 Effect of apple pomace level and screw speed on expansion ratio of pomace enriched extrudates

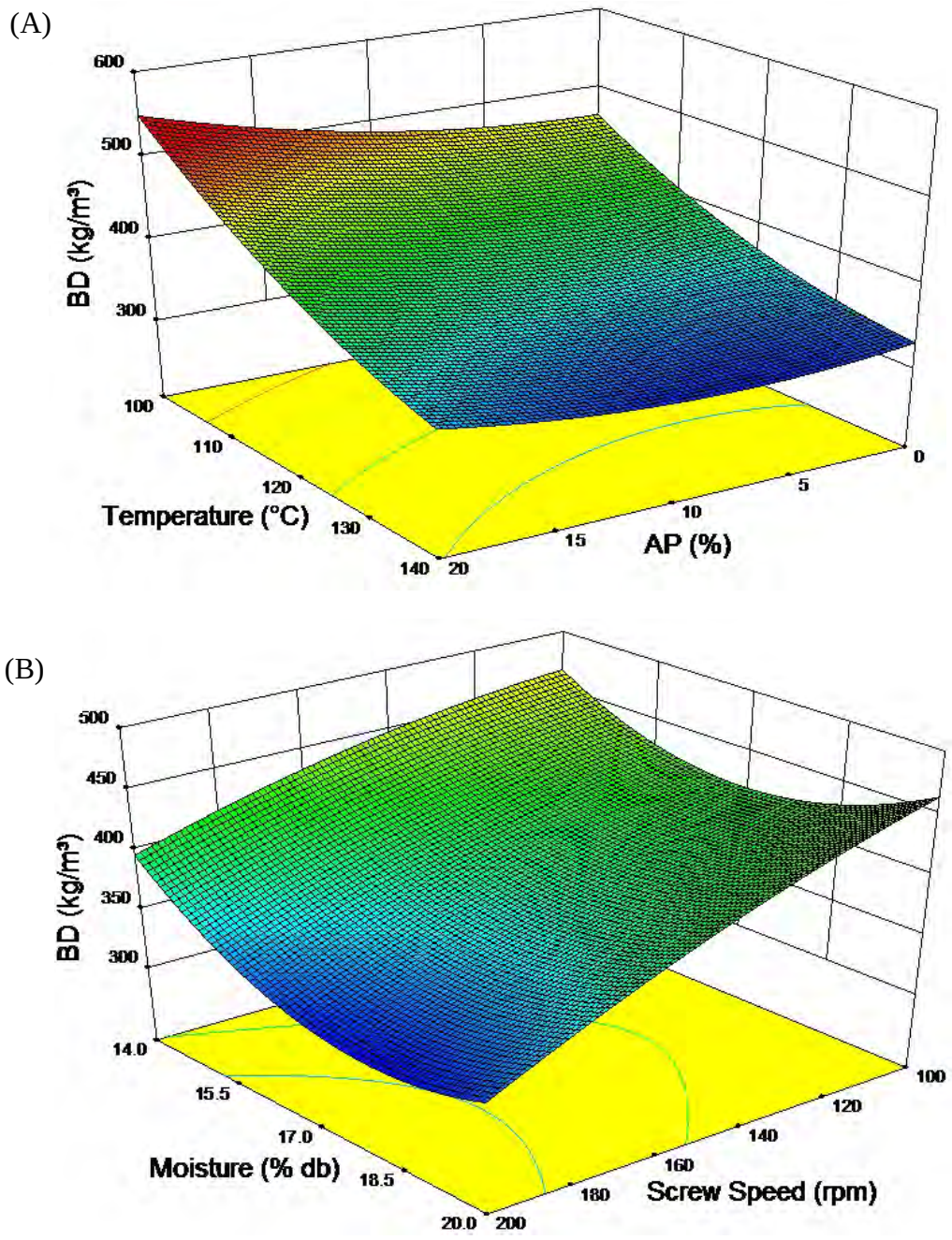

Figure 3.2 (a) Effect of extrusion temperature and apple pomace level, and (b) effect of moisture content and screw speed on bulk density of pomace enriched extrudates

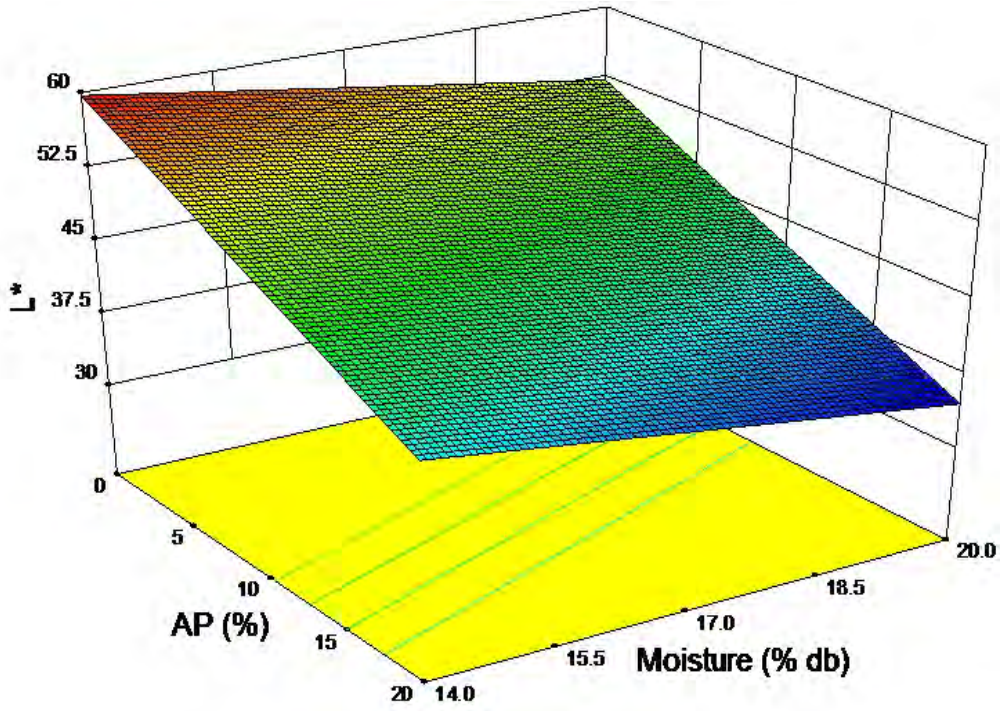

Figure 3.3 Effect of apple pomace level and moisture content on L* of pomace enriched extrudates

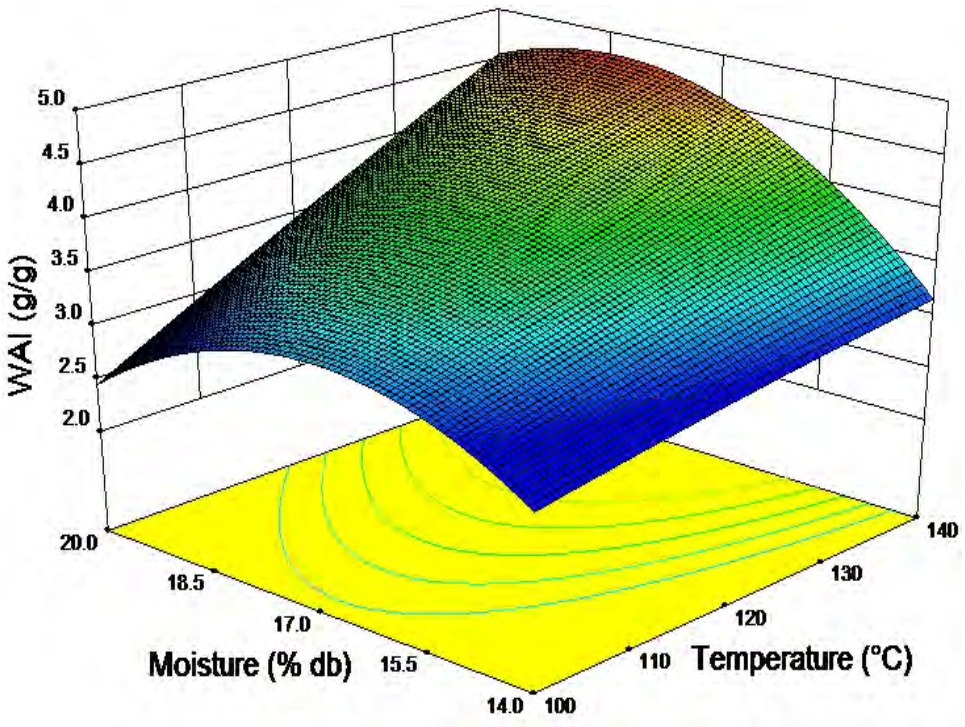

Figure 3.4 Effect of extrusion temperature and moisture content on water absorption activity of pomace enriched extrudates

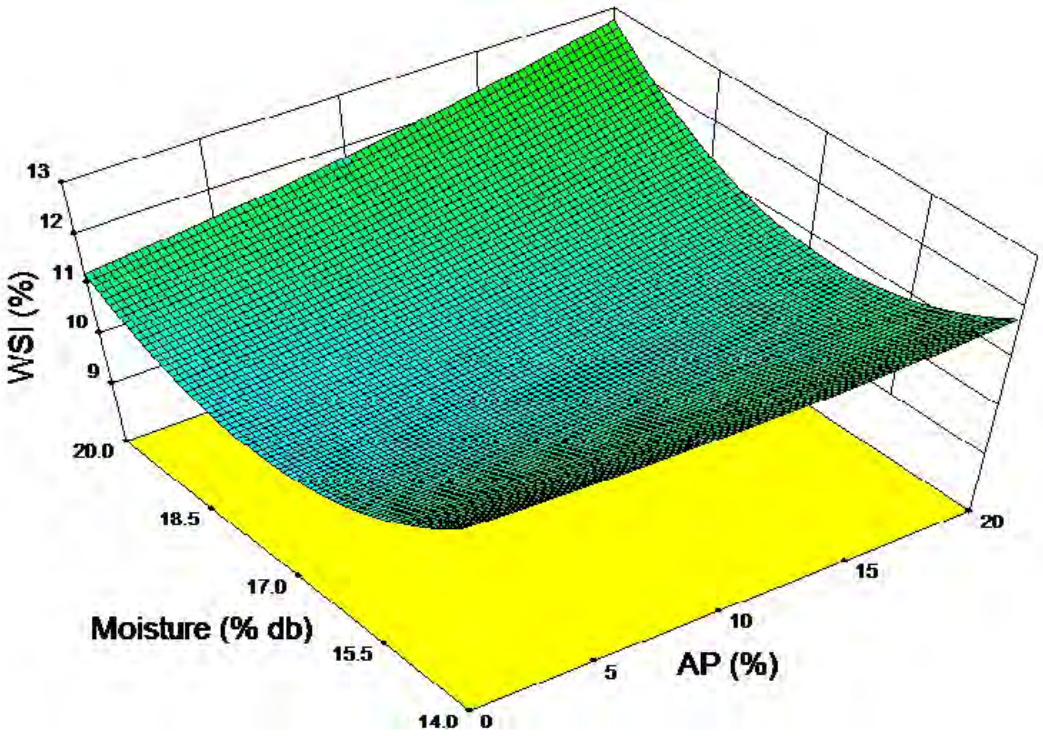

Figure 3.5 Effect of moisture content and apple pomace level on water solubility index of pomace enriched extrudates

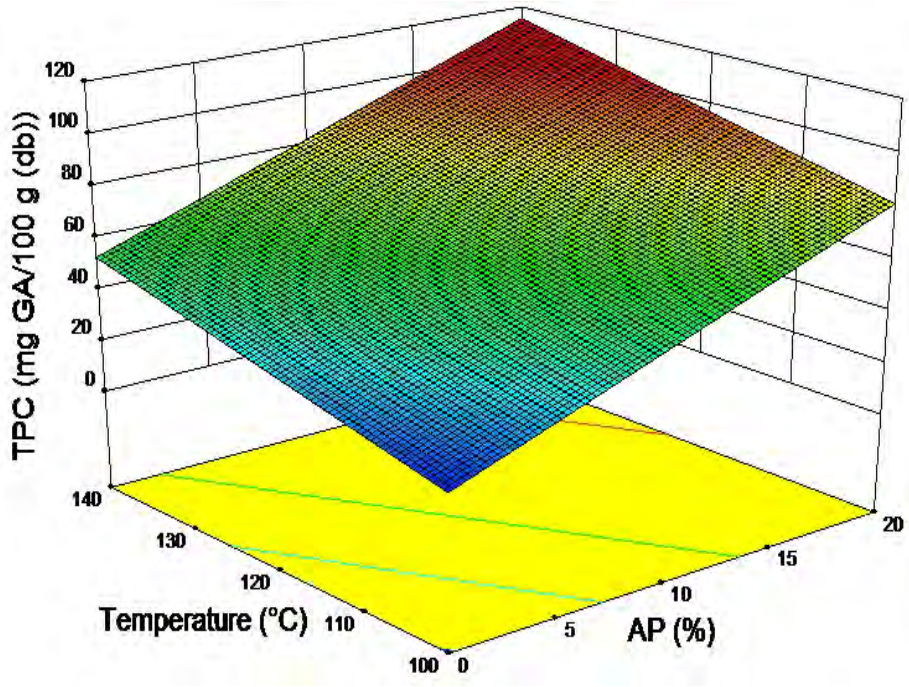

Figure 3.6 Effect of extrusion temperature and apple pomace level on total phenolic content of pomace enriched extrudates

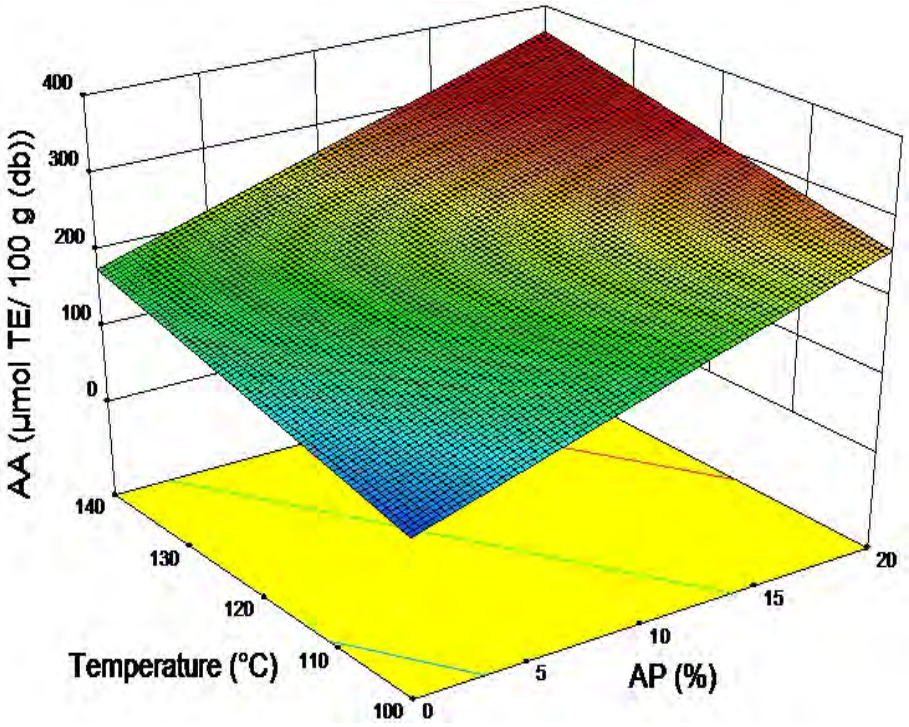

Figure 3.7 Effect of extrusion temperature and apple pomace level on antioxidant activity of pomace enriched extrudates

CHAPTER 4

Textural and Structural Characterization of Extrudates Containing Blends of Apple Pomace, Defatted Soy Flour and Corn Grits

4.1 Abstract

The study was aimed to investigate the textural and structural characteristics of protein and fiber rich extruded snacks. Corn extrudates supplemented with apple pomace (0-20%) and defatted soy flour (30-50%) were prepared though extrusion processing. The effect of apple pomace levels and processing conditions (extrusion temperature, screw speed and moisture content) on the extrudates' textural properties, cellular structure and molecular conformation were studied. Results show that increase in the apple pomace level (0 to 20%) and extrusion temperature (100 to 140^{o}C) decreased the hardness of the extrudates. The hardness ranged between 7.57 and 15.23 N. Lower hardness was associated with higher crispness and brittleness. The internal microstructure of the extrudates was examined through scanning electron microscope. Higher temperature and shear were responsible for the damage of the cellular structure of the extrudates. Presence of air cells indicated crisper extrudates. The changes in the molecular distribution in the extrudates were determined using Fourier Transform Infrared Spectroscopy (FTIR). FTIR showed that there were significant changes in the carbohydrate components and the structure of the proteins on extrusion, with consequent effects on the expansion and density of the extruded product. This study showed the variation in internal structure and molecular bonds and their effect on the texture of the extrudates.

4.2 Introduction

Snacks are mostly smaller than a regular meal and usually eaten between meals. According to a survey, children in the North America eat snacks on an average of six times per day (Statista, 2014). With the increasing hectic lifestyle people are turning more to ready-to-eat snack foods (Stojceska et al., 2009). Along with good taste sensory characteristics such as texture is also important from consumer point of view (Corradini and Peleg, 2006). The success or failure of new extruded snack food product depends on the sensory attributes, where texture is the primary parameter (Anton and Luciano, 2007). In particular, textural attributes of crispness and crunchiness are the most important descriptors making snack foods popular among consumers (Bouvier et al., 1997). Barrett and Peleg (1992) reported that many starch-based extruded foods were inherently cellular and brittle, and these products exhibited a classical brittle failure mechanism as the consequence of their cellularity and lack of structural resiliency. They are described as "crispy/crunchy" because of a complex failure mechanism that involves the repetitive deformation and fracturing of cell structure, specifically individual cells and brittle cell walls. Mechanical strength, cellular structure, and fracturability have been extensively studied for extruded products in the past (Conti-Silva et al., 2012; Dogan and Kokini, 2007; Luyten et al., 2005). Gao and Tan (1996) related the texture of food products to their structural characteristics, such as cell size, cell density and uniformity. They reported that in expanded food products, three-dimensional structures of an intricate network of interconnected air cells and cell walls formed during the puffing process resulted in products that were generally quite cellular, porous and low in density. Food

texture was the result of microstructure, which depends on the influence of physical forces on chemical components (Choi et al., 2004).

The high temperature-short time extrusion process has been used in the food industry to produce directly and/or indirectly expanded extruded foods. Directly expanded extrudates vary in structural properties and mechanical properties depending on formulation and extrusion conditions (Sunderland, 1996). Characterization of cellular structure may provide information that helps control the extrusion process to produce a desired and uniform extrudate product.

Scanning Electron Microscopy (SEM) is often used to analyze the internal geometrical structure of a food product including how finely or coarsely the material is subdivided, the number of cells per unit cross-sectional area and the surface appearance in details (Gao and Tan, 1996). Ryu and Walker (1994) used a scanning electron microscope to observe cell structure of wheat flour extrudates produced with different compositions, and reported that the type of emulsifiers and the addition of sucrose and shortening powder significantly influenced structural parameters. Tan et al. (1997) characterized cellular structure of puffed corn meal extrudates using SEM images, measuring the run length (number of pixels at 0 gray scale, i.e., distance along a void), and from these, calculated average run length and standard deviation, variance, and third moment of run lengths, and reported that most of these parameters were good indications of directly measured cell size and uniformity. There are many advantages of using SEM for this purpose compared to light microscopy and transmission electron microscopy. SEM has a large depth of field allowing many areas of sample to be in focus at the same time. SEM also produces images of high resolution, which means that closely spaced

features can be examined at a high magnification. Combination of higher magnification, larger depth of focus, greater resolution, and ease of sample observation makes SEM one of the most heavily used instruments in research today.

Fourier transform infrared spectroscopy (FTIR) provides unique insights into the protein conformation changes (Barth, 2007; Cordeiro et al., 2006; Kong and Yu, 2007) and has been used to examine changes in protein-polysaccharide interaction in extruded snacks (Guerrero et al., 2014). Therefore, FTIR may be a useful technique to examine the process-induced changes to components in the formulations.

In our previous studies (Singha and Muthukumarappan, 2017a; Singha and Muthukumarappan, 2017b), we have given detailed explanation on the effects of extrusion processing on the systems parameters, physico-chemical and antioxidant properties of blends containing apple pomace, defatted soy flour and corn grits. The objectives of this study are: (a) To examine the textural properties of the extrudates, (b) To characterize the cellular structure of the extrudates, and (c) To analyze the changes in the molecular conformation after extrusion.

4.3 Materials and Methods

4.3.1 Raw materials and blend preparation

Apple pomace powder was obtained from Tree Top, Inc. (Selah, WA) and stored at -18°C until use. The initial moisture content of AP powder was 10.08% (wb). The proximate composition of the AP powder was: 4.14% protein, 2.79% fat, 2.00% ash, 24.73% total fiber and 0.2% NFE (dry basis). Corn grits was obtained from Bob's Red Mill (Milwaukie, OR). The initial moisture content of CG was 11.46% (wb). The proximate composition of the CG was: 6.78% protein, 1.6 % fat, 2.26% ash, 1.02% total

fiber and 81.8 % NFE (dry basis). Defatted soy flour was obtained from Hodgson Mill, Inc. (Effingham, Il). The initial moisture content of DSF was 7.75% (wb). The proximate composition of the DSF powder was: 53.94% protein, 0.77% fat, 6.87% ash, 2.93% total fiber and 26.09% NFE (dry basis). Apple pomace, defatted soy flour and corn grits were mixed in to five different compositions (Blend I to V) following our previous study (Singha and Muthukumarappan, 2017a). In short, AP was varied between 0 to 20% w/w, DSF was varied between 30-50% w/w and CG was kept constant at 50% w/w. Depending on the experimental runs the moisture content of the blends were adjusted. Moisture content of the blends was varied between 14 to 20% (dry basis). The ingredients were mixed in a laboratory scale mixer (KitchenAid Professional 5 Plus, Troy, Ohio, USA) for 10 minutes and stored overnight at ambient temperature for moisture stabilization. The moisture content of the prepared blends was determined by using the method 44-19 (AACC, 2000). The ingredient composition and proximate of the blends are mentioned in Table 4.1.

4.3.2 Extrusion processing

After mixing and conditioning, the blends were randomly extruded using a 19.18 mm (0.755 in.) barrel inner diameter (i.d.), single-screw laboratory extruder (Brabender Intelli-Torque Plasti-Corder®, South Hackensack, NJ) at Food Innovation Center, University of Nebraska, Lincoln, NE which was powered by a 7.5-HP motor with an operating range of screw speeds from 0 to 225 rpm. A screw compression ratio (feed channel depth to metering channel depth) of 1.5:1 was used in the experiment. On exiting the die, samples were collected and allowed to cool at ambient temperature and then

stored in airtight bags at <4 ^{o}C until analyzed. Moisture content of stored extrudates was between 2.5 and 4.5% (wet basis).

4.3.3 Experimental design and statistical analysis

Experiments were conducted using the central composite rotatable design (CCRD) consisting four numerical independent variables namely apple pomace (X_1), temperature (X_2), screw speed (X_3) and moisture content (X_4) each at five levels as shown in Table 4.2. The experimental design and the codes for the processing variables have been reported in Table 4.3. Textural properties such as hardness, crispness and brittleness measured as the peak force, slope and distance, respectively were taken as the responses of the designed experiments. The goodness of the fit and the significance of linear, quadratic and interaction effects of each factor on the responses were examined by performing analysis of variance (ANOVA) (Table 4.5). Statistical analysis was conducted using Design-Expert 8.0.7.1 (Statease, Minneapolis, MN, USA). Pearson's correlation coefficient (r) was also applied to establish specific correlations using SPSS (16.0) statistical software.

4.3.4 Texture analysis

The textural proprieties of the extrudates were evaluated by a downward force on extrudates of length 50 mm sitting in a guillotine deformed by a Warner–Bratzler blade using a texture analyzer (TA.HD*Plus* Texture Analyzer, Hamilton, MA) equipped with 750 kg load cell. The pretest speed was 1 mm/s, test speed was 1.50 mm per second and post-test speed was 2 mm/s under 50 N of force. All results were expressed in N, and each experiment was repeated at least ten times. The following parameters were measured: peak force (hardness), slope (crispness) and distance (brittleness).

4.3.5 Scanning electron microscopy

Structural observation was carried using a Hitachi-S3400 N (Tokyo, Japan) scanning electron microscope (SEM) operated at 10kV. Extrudate samples cut radially and approximately 2 mm thickness were mounted on stubs and 10 nm gold was coated using a CrC-150 sputtering system set to a pressure of 5-10 millitorr. Magnification of 35×, 500×, 1000× and 1500× were used to study the internal structure of the extrudates. At least two samples for each treatment showing similar images were used for the results.

4.3.6 Infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) spectra were gathered as an average of 70 scans using a Nicolet 380 ATR-FTIR spectrophotometer (Thermo Nicolet 380) in the range 4000-700 cm^{-1} with a 1 cm^{-1} resolution. The peak position was analyzed using OMNIC software.

4.4 Results and Discussion

4.4.1 Effect of processing conditions on textural characteristics of extrudates

To determine textural property of the extrudates, the maximum peak force (measure of hardness), slope (measure of crispness) and distance (measure of brittleness) were measured. The regression equations for peak force (PF), slope (S) and distance (Di) as textural attributes after removing the insignificant terms are shown in Table 4.4.

ANOVA results for quadratic models of the textural attributes are given in Table 4.5. Acceptable coefficient of determination (R^2 = 0.9962, R^2 = 0.8682, R^2 = 0.9842) were obtained for significant models of peak force, slope and distance, respectively. Whereas lack-of-fit were not significant for all the textural attributes ($P>0.05$). The peak force, slope and distance of extrudates were dependent on temperature and apple pomace

level. Peak force of extrudates was significantly affected by linear and quadratic effects of temperature and apple pomace level ($P<0.05$). The linear effect of apple pomace level and linear and quadratic effects of temperature were found to be significant for slope and distance ($P<0.05$). Screw speed had significant linear effect on the slope ($P<0.05$). Moisture content had no significant ($P>0.05$) effect on the textural attributes of the extrudates. The correlation between the textural properties are represented in Table 4.6.

Hardness is described as the maximum force required to fracture or break the product on the texture analyzer. As described by Dehghan-Shoar et al. (2010), hardness correlates well with the initial bite or texture of hardness when a person begins to bite into a snack. The influence of operating conditions on the changes in peak force of extrudates is shown in Figure 4.1(a). Maximum peak force as measured by hardness of the extrudates varied between 7.57 and 15.23 N. A decrease in product hardness with increasing temperature was observed. Altan et al. (2008c) and Ryu and Walker (1995) also observed that breaking strength decreased at higher temperature of during extrusion of barley-grape pomace blend and wheat flour, respectively. Yuliani et al. (2006) reported that an increase in temperature would decrease the melt viscosity, causing more bubble growth and decreased bubble wall thickness, although some bubbles may collapse and fracture. In our previous study (Singha and Muthukumarappan, 2017a), viscosity was found to decrease with increase in the extrusion temperature. This resulted in a lower bulk density and hence lower hardness of extrudates. Apple pomace is known to contain a high quantity of pectin, which may have resulted in the decrease in the hardness of the end product by acting as a lubricant and thus producing a crispier rather than a harder snack (van der Sman and Broeze, 2013).

Crispness is typically a textural attribute for snack foods and baked products. The slope before the first major fracturability peak was taken as the crispness of the extrudates (Jackson et al., 1996). The lower the slope, the crisper the product is considered. The slope appeared to decrease with increasing temperature, indicating an increase in crispness. (Figure 4.1(b)). As temperature was increased, the hardness decreased which means that crispness of extrudates increased. This is also evident from the negative correlation between the peak force and the slope ($R=-0.846$, $P<0.01$). It is noted that when progressive increase in temperatures resulted in pores in the structure due to formation of air cells and the surface appeared flaky and porous and hence decreased hardness (Bhattacharya and Choudhury, 1994) as seen in this study. Therefore, a crispy texture was obtained with increasing temperature due to decrease in hardness. Duizer and Winger (2006) stated that less force is required in breaking a product that is very crisp. Therefore, it is expected to have a crispy texture while increasing temperature whereby less force is required to break a product that is very crisp.

The distance to break the extrudate is an indication of brittleness, with the shortest distance being the most brittle product. When the product cracks at a shorter distance it is said to be more brittle than the one which cracks at longer distance. The measured value of distance for the extrudates was in the range of 0.47-0.98 mm. The effect of apple pomace and temperature on the distance is shown in Figure 4.1(c). Distance was found to have significant positive correlation with hardness ($R=0.986$, $P<0.01$). This indicates that low hardness value with increased temperature would produce lower distance to break and thus higher brittleness of extrudates.

4.4.2 Structural observations

Several factors affect the internal structure of extrudates. Since in our study we have varied the extrusion temperature, pomace level, screw speed and moisture content of the blends, it will be difficult to generalize the changes in internal structure due to one specific factor. The SEM images clearly reveal the detailed microstructure of the extrudates (Figure 4.2 to Figure 4.6). Roughly circular cell and variation in cell area are visible. The extrudates have variation in cell area and distribution mostly affected by AP level, temperature and screw speed.

Figure 4.2(a) and (b) reveal the surface and internal structure of the extrudates containing only DSF and CG (50:50 ratio) extruded at 120^{o}C, 150 rpm and 15% moisture content. The extrudates has been previously characterized by high expansion ratio (ER = 1.48) and moderately low bulk density (BD = 397.88 kg/m^{3}) (Singha and Muthukumarappan, 2017b). Presence of large air cells were detected. Minor damage to the structure was observed as the extrusion temperature was moderate.

Figure 4.3(a-f) illustrates the surface and internal microstructure of extrudates containing 5% AP. Fibrous structure resulting from the presence of pomace is visible. The extrudates depicted in Figure 4.3(a-d) were extruded at 110^{o}C. While the ones in Figure 4.3(a) and (b) were extruded at a screw speed of 125 rpm and 18.5% moisture content, and the products shown in Figure 4.3(c) and (d) were extruded at a screw speed of 175 rpm and 15.5% moisture content. The products extruded at 175 rpm (Figure 4.3(c) and (d)) had more number of air cells and had the highest expansion ratio. Compared to these, the extrudates shown in Figure 4.3(c) and (f) were more damaged as the extrusion

temperature was 130^{o}C. It also suggests that the extrudate expanded and then shrunk on the surface.

Addition of AP increased the fiber content in the extrudates (Figure 4.4 to Figure 4.6). The presence of deep fissures and open space can also be observed from cell wall components of the extrudates along with the organized structure. The flat surface can also be observed, which represent flakey structures of extrudates (Figure 4.4(c)). Micrographs in Figure 4.3 to Figure 4.6 reflects presence of small open space between the cells. Elongated outgrowth can be visualized in structural orientation. The micrograph reveals the accumulation of well-organized cellular structure that has led to the formation of organized structure. The presence of fiber and protein as well as decreased starch content is more likely to be responsible for the compact structure (Figure 4.6(b)).

At extrusion temperature of 130^{o}C, micrograph reveals the presence of open spaces between the fibers, and also certain small fibrous structures are visible (Figure 4.5(c) and (d)). High temperature and screw speed were responsible for extruded products with large number of flattened and sheared granules. A compact flakes mass of fibers can also be visualized in Figure 4.6. Lue et al. (1990) extruded corn meal mixed with dietary fiber, and examined the microstructure of the resulting products. They reported that the degree of expansion was associated with the size of air cells inside of extrudates and the external structure of products, which was dependent on the source and amount of dietary fiber.

4.4.3 Infrared spectroscopy

FTIR spectroscopy is a useful tool in elucidating the functional groups of organic macromolecules, as well as understanding the bonding interactions of these functional

groups. The technique works on the fact that bonds and groups of bonds vibrate at characteristic frequencies. A molecule that is exposed to infrared rays absorbs infrared energy at frequencies that are characteristic to that molecule. Thus, FTIR spectroscopy is utilized to provide key information on the nature of molecular forces that dominate the transformation of the carbohydrate and protein during the extrusion (Zhang et al., 2009).

All spectra showed a broad peak in the OH stretching region of 3300–3200 cm^{-1}. Figure 4.7 shows the FTIR spectra of AP, DSF and CG. The FTIR spectra of un-extruded blend containing 50% DSF and 50% CG have been compared with the spectra of the extruded blend in Figure 4.8. The intensity of the peaks diminished after extrusion as can be seen in the amide regions between 1500 and 1650 cm^{-1} and the -OH region. Figure 4.9 shows the FTIR spectra of other extrudates. For the extrudates, the -OH stretching region had a triangular shape as a result of a large contribution from -NH stretching vibrations (symmetric 3180, asymmetric 3330 cm^{-1}) superimposing on the -OH band. The peaks, arising from carbohydrates between 2890 and 2970 cm^{-1}, are associated with asymmetric and symmetric stretching modes of $-CH_3$, respectively. The lack of an absorption band in the range of 1740 to 1745 cm^{-1} for all extruded samples indicated the absence of carbonyl groups (C=O). This indicates that the C=O region disappeared after extrusion. The C=O region was detected in the raw ingredients (Figure 4.7). The next band, which is centered near 1628 cm^{-1}, is associated to the amide I functional group from proteins. The characteristic vibration frequencies of the amide I band arise from the overlapping secondary structural components of the polypeptide chains in the proteins from the soy/corn mix. The components of amide I arise from the C=C stretching and N–H bending modes and also from the C=O stretching mode. These bands are more

specifically assigned to the β-sheet structure of the protein. The peaks at 1072 cm^{-1} are related to the C–O stretching vibrations of fiber.

4.5 Conclusions

A central composite design using RSM successfully described the effect of independent variables (apple pomace level, extrusion temperature, screw speed and moisture content) on the textural properties of the extrudates. The pomace level and extrusion temperature significantly influenced the hardness, crispness and brittleness of the final extruded products. Microstructure of the extrudates analyzed by scanning electron microscopy indicated that the cell shape was roughly circular and exhibited variations in cell size from one sample to another. The presence of air cells is directly related to products' physical and textural properties. A compact cellular structure produced denser product. The FTIR spectra of the raw ingredients, unextruded blends and extrudates were determined. Spectra of the various extrudates could be distinguished by different peaks and slight peak shifts for the amide and carbohydrate bands. Distinct protein rich and protein free domains were observed. The differing formulations and extrusion processing conditions produced extruded products with different cell structure features, which in turn would be expected to lead to different textural perceptions. The results outlined in this study provides unique information on the effect of extrusion process on the textural and structural properties of the extrudates. It also provides valuable information on the molecular backbone of the raw materials and the extrudates

Table 4.1 Ingredient composition of blends and the mean proximate composition of each (values in the parentheses represent standard error).

Feed ingredients	**Mass of ingredients (g kg^{-1})**				
	Blend I	**Blend II**	**Blend III**	**Blend IV**	**Blend V**
Defatted soy flour	500	450	400	350	300
Corn grits	500	500	500	500	500
Apple pomace	0	50	100	150	200
Proximate analysis					
Protein (% db)	28.53 (0.03)	25.56 (0.11)	23.38 (0.18)	20.63 (0.04)	19.14 (0.12)
Fiber (% db)	2.26 (0.12)	3.25 (0.31)	4.34 (0.24)	5.43 (0.10)	6.41 (0.14)
Fat (% db)	1.17 (0.06)	1.23 (0.02)	1.34 (0.11)	1.41 (0.02)	1.56 (0.05)
Ash (% db)	4.43 (0.13)	4.27 (0.03)	3.98 (0.10)	3.73 (0.03)	3.49 (0.02)
NFE (% db)	52.95 (0.17)	51.65 (0.08)	50.36 (0.03)	49.06 (0.13)	47.77 (0.06)

db, dry basis; NFE, nitrogen free extract

Table 4.2 Independent numerical variables and their levels.

Numerical variable	**Symbol**	**Coded variable levels**				
		-2	**-1**	**0**	**1**	**2**
Apple pomace (%)	X_1	0	5	10	15	20
Temperature (°C)	X_2	100	110	120	130	140
Screw speed (rpm)	X_3	100	125	150	175	200
Moisture content (% wb)	X_4	14	15.5	17	18.5	20

wb, wet basis

Table 4.3 Experimental design layout.

Run	Coded variables				Actual variables			
	x_1	x_2	x_3	x_4	X_1 (%)	X_2 (^{o}C)	X_3 (rpm)	X_4 (% wb)
1	1	-1	1	-1	15	110	175	15.5
2	-1	1	1	1	5	130	175	18.5
3	0	0	0	2	10	120	150	20.0
4	1	-1	-1	1	15	110	125	18.5
5	0	0	0	-2	10	120	150	14.0
6	1	-1	1	1	15	110	175	18.5
7	1	-1	-1	-1	15	110	125	15.5
8	-1	-1	-1	1	5	110	125	18.5
9	0	0	-2	0	10	120	100	17.0
10	0	2	0	0	10	140	150	17.0
11	-1	1	-1	-1	5	130	125	15.5
12	-1	1	-1	1	5	130	125	18.5
13	2	0	0	0	20	120	150	17.0
14	-1	-1	-1	-1	5	110	125	15.5
15	1	1	-1	-1	15	130	125	15.5
16	0	0	0	0	10	120	150	17.0
17	1	1	1	-1	15	130	175	15.5
18	0	0	0	0	10	120	150	17.0
19	1	1	-1	1	15	130	125	18.5
20	0	0	0	0	10	120	150	17.0
21	0	0	2	0	10	120	200	17.0
22	-2	0	0	0	0	120	150	17.0
23	1	1	1	1	15	130	175	18.5
24	0	-2	0	0	10	100	150	17.0
25	-1	-1	1	1	5	110	175	18.5
26	-1	1	1	-1	5	130	175	15.5
27	-1	-1	1	-1	5	110	175	15.5

Table 4.4 Best-fit response surface models after excluding the insignificant terms for PF, S and D_i.

Parameters	**Response surface model**	R^2	***P* value**
Coded			
PF (N)	$Y_{PF} = 10.19 - 0.65x_1 - 1.86x_2 + 0.17x_1x_2 - 0.21x_1^2 + 0.35x_2^2$	0.9962	<0.0001
S (N/mm)	$Y_S = 16.61 + 0.12x_1 + 0.30x_2 + 0.12x_3 - 0.17x_2x_3 - 0.13x_2^2$	0.8682	0.0250
Di (mm)	$Y_{Di} = 0.61 - 0.05x_1 - 0.11x_2 + 0.01x_1x_2 + 0.03x_2^2$	0.9842	<0.0001
Actual			
PF (N)	$Y_{PF} = 94.06 - 0.40X_1 - 1.09X_2 + 0.003X_1X_2 - 0.008X_1^2 + 0.004X_2^2$	0.9962	<0.0001
S (N/mm)	$Y_S = -39.99 + 0.29X_1 + 0.50X_2 + 0.15X_3 - 0.0007X_2X_3 - 0.001X_2^2$	0.8682	0.0250
Di (mm)	$Y_{Di} = 8.66 - 0.03X_1 - 0.10X_2 + 0.0003X_1X_2 + 0.0003X_2^2$	0.9842	<0.0001

PF = peak force, S = slope, Di = distance

Table 4.5 Analysis of variance for peak force, slope and distance.

Response	Source	df	Sum of squares	Mean squares	F-value	P-value
Peak force	Regression	14	98.98	7.07	222.45	< 0.0001
	Lack-of-fit	10	0.38	0.04	18.56	0.0522
	Pure error	2	0.00	0.03		
	Residual	12	0.38	0.00		
	Total	26	99.36			
Slope	Regression	14	4.44	0.32	5.64	0.0024
	Lack-of-fit	10	0.65	0.07	6.14	0.1481
	Pure error	2	0.02	0.06		
	Residual	12	0.67	0.01		
	Total	26	5.11			
Distance	Regression	14	0.41	0.03	53.40	< 0.0001
	Lack-of-fit	10	0.01	0.00	19.33	0.0502
	Pure error	2	0.00	0.00		
	Residual	12	0.01	0.00		
	Total	26	0.41			

Table 4.6 Correlation coefficients between product properties.

	Peak force	**Slope**	**Distance**
Peak force	1	-.789**	.986**
Slope		1	-.846**
Distance			1

**Significant at P<0.01

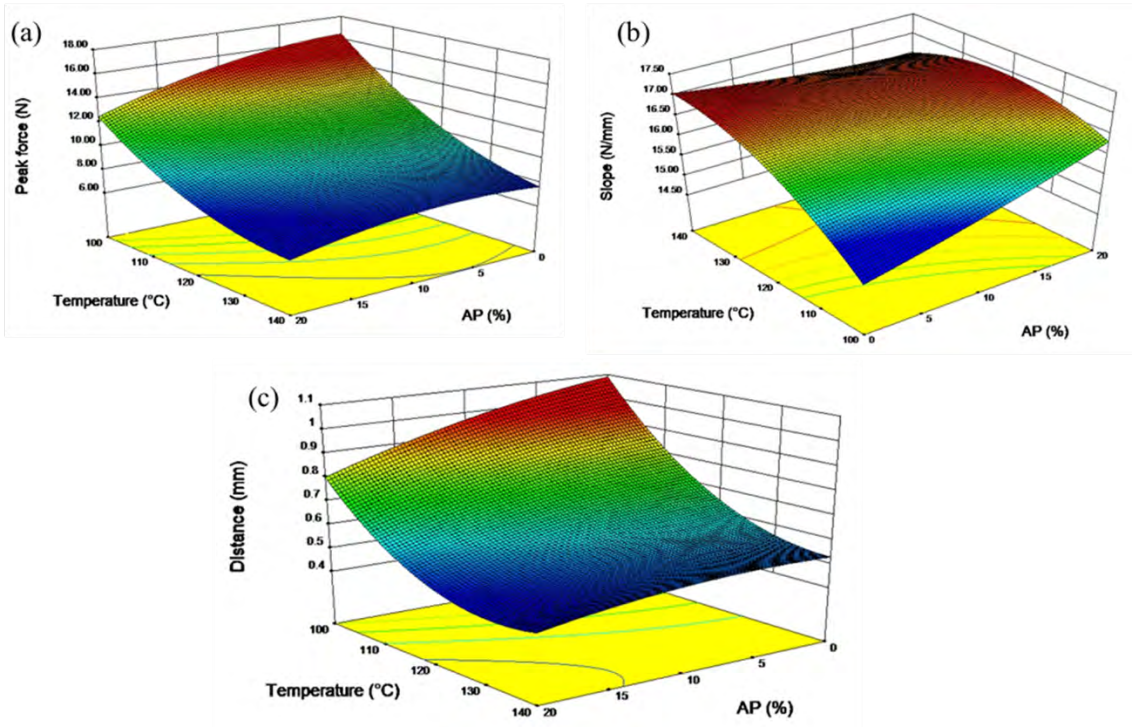

Figure 4.1 Response surface plots for the effect of temperature and AP level on the (a) peak force, (b) slope, and (c) distance

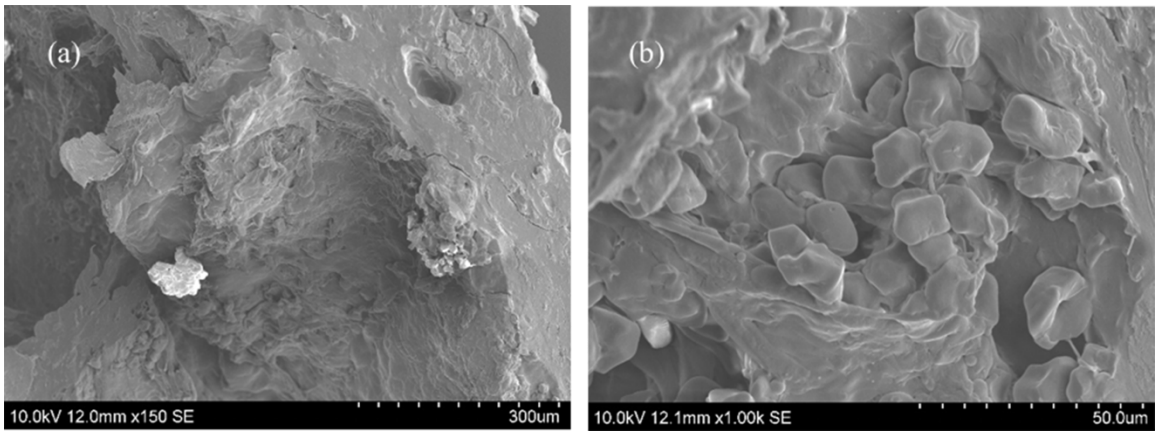

Figure 4.2 SEM micrographs of the extrudates containing 0% AP extruded at a temperature of 120^{o}C, screw speed 150 rpm and moisture content of 17%. (a) 35× magnification and (b) 1000× magnification

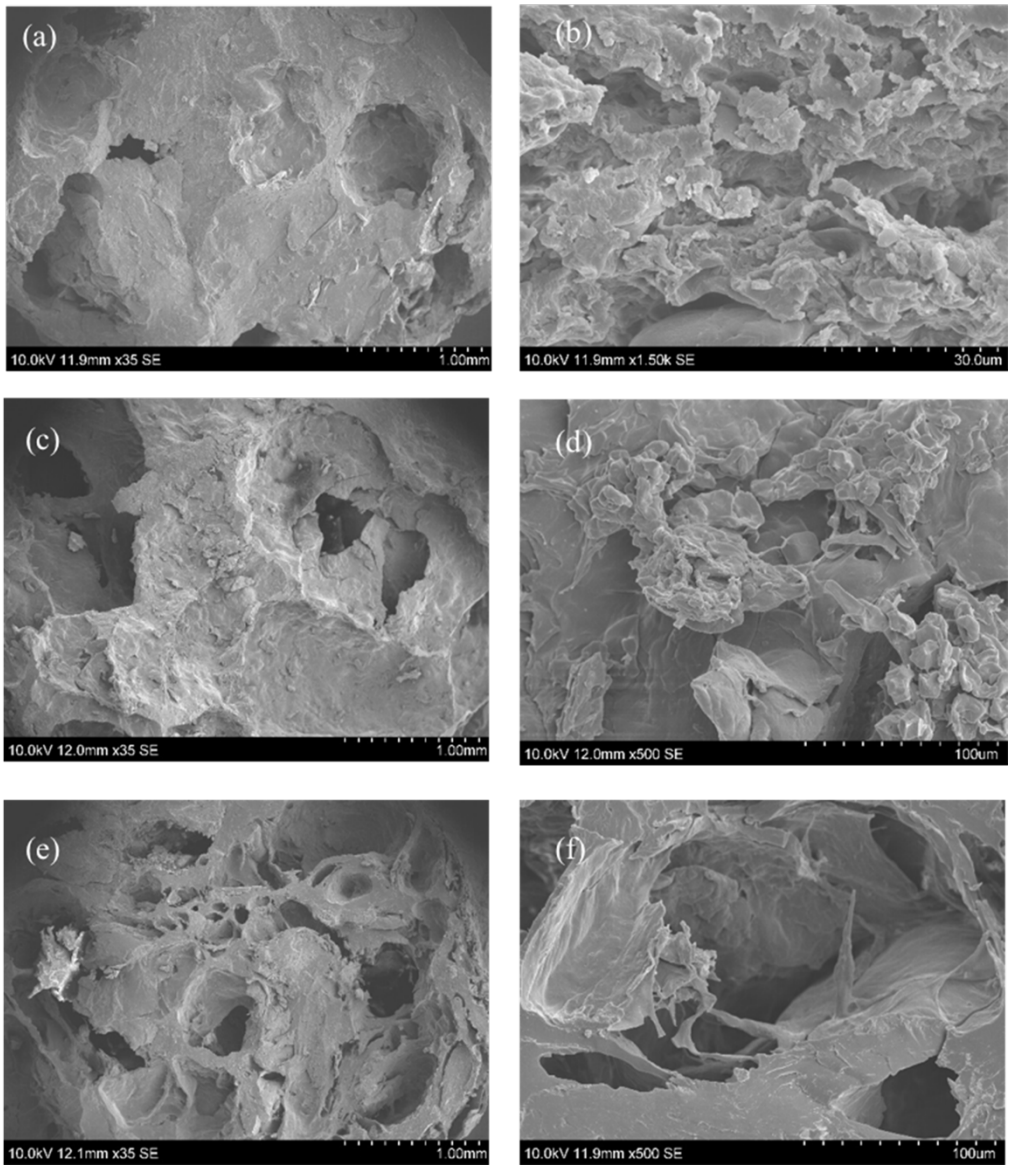

Figure 4.3 SEM micrographs of the extrudates containing 5% AP extruded at a temperature of 110^{o}C, screw speed 125 rpm and moisture content of 18.5% (a) 35× magnification and (b) 1000× magnification; a temperature of 110^{o}C, screw speed 175 rpm and moisture content of 15.5% (c) 35× magnification and (d) 1000× magnification and a temperature of 130^{o}C, screw speed 175 rpm and moisture content of 15.5% (e) 35× magnification and (f) 1000× magnification

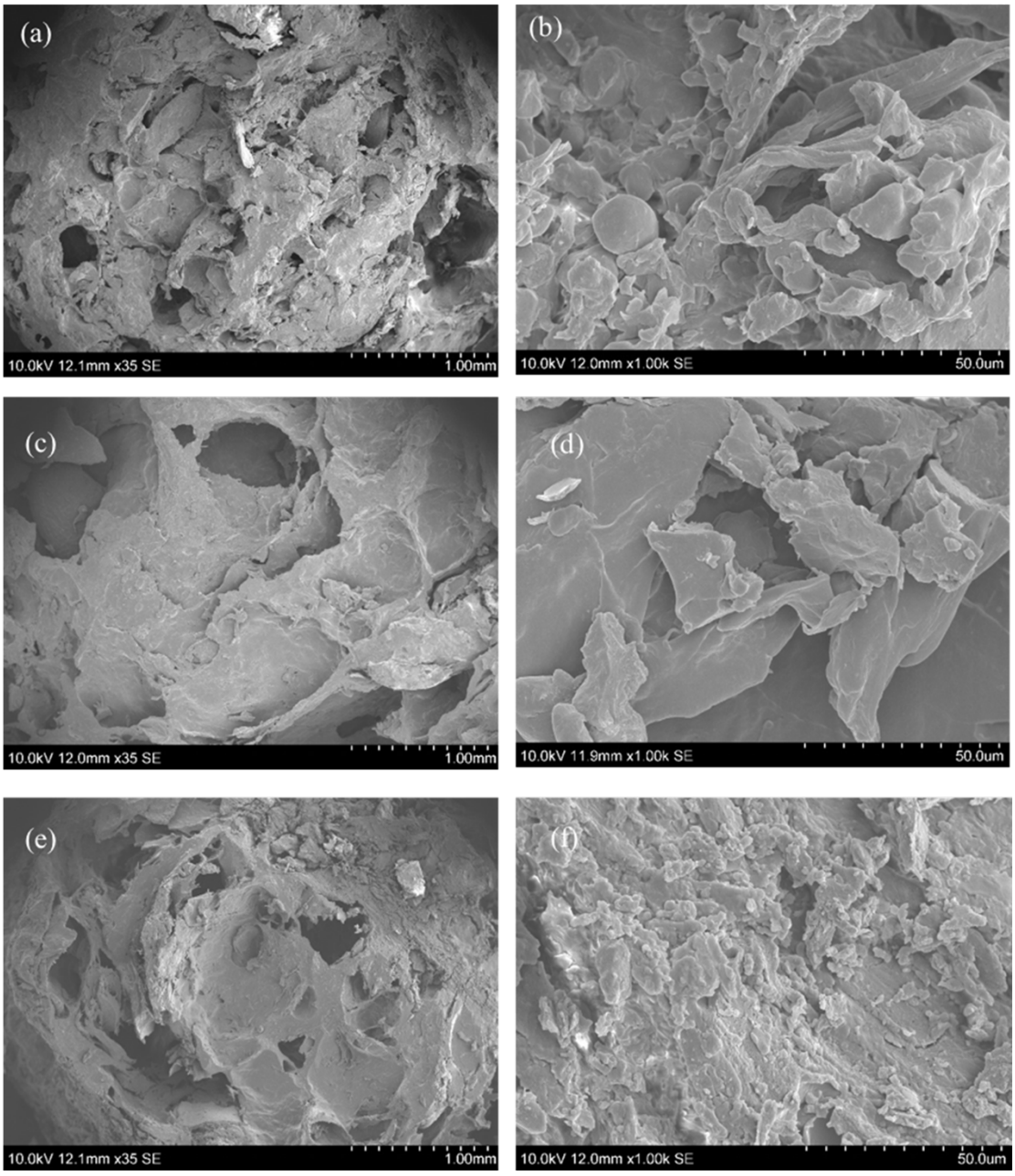

Figure 4.4 SEM micrographs of the extrudates containing 10% AP extruded at a temperature of 120^{o}C, screw speed 150 rpm and moisture content of 17% (a) 35× magnification and (b) 1000× magnification; a temperature of 120^{o}C, screw speed 200 rpm and moisture content of 17% (c) 35× magnification and (d) 1000× magnification; a temperature of 140^{o}C, screw speed 150 rpm and moisture content of 17% (e) 35× magnification and (f) 1000× magnification

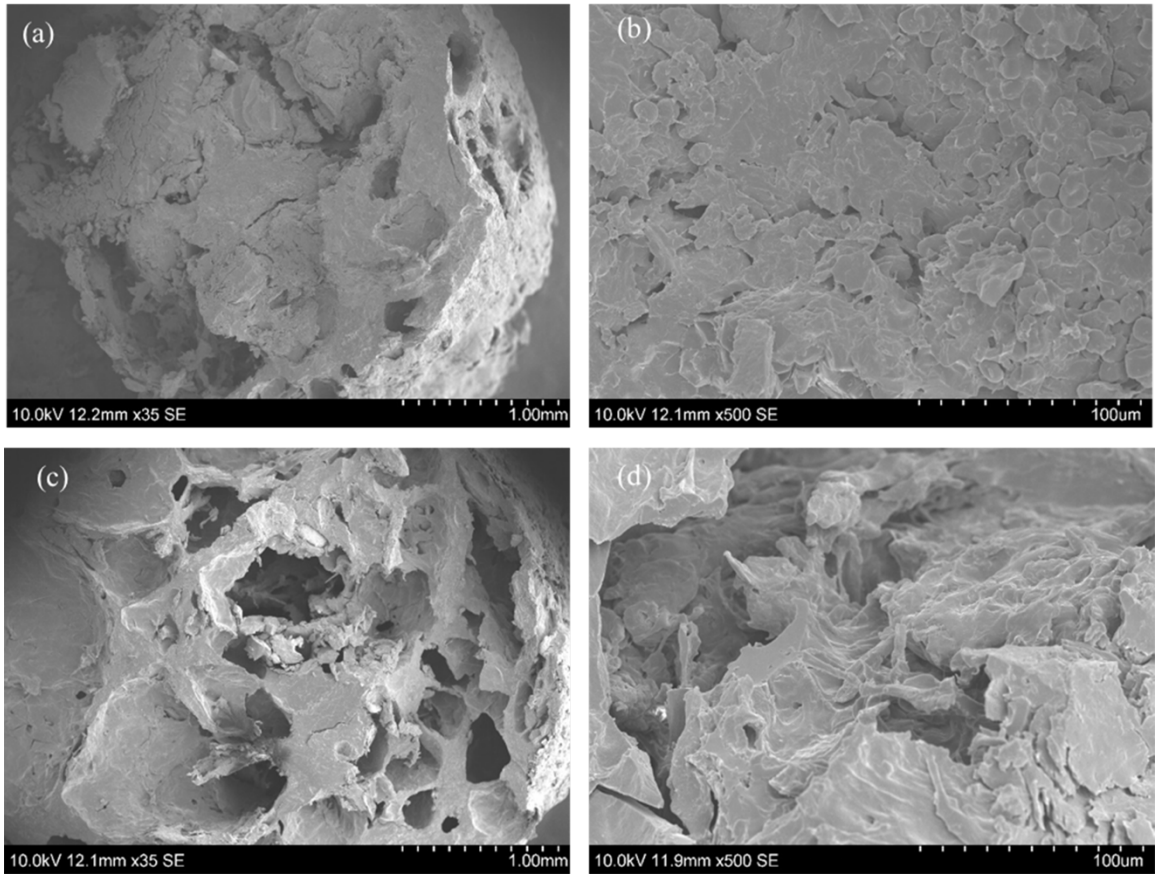

Figure 4.5 SEM micrographs of the extrudates containing 15% AP extruded at a temperature of 110^{o}C, screw speed 175 rpm and moisture content of 15.5% (a) 35× magnification and (b) 1000× magnification; a temperature of 130^{o}C, screw speed 125 rpm and moisture content of 18.5% (c) 35× magnification and (d) 1000× magnification

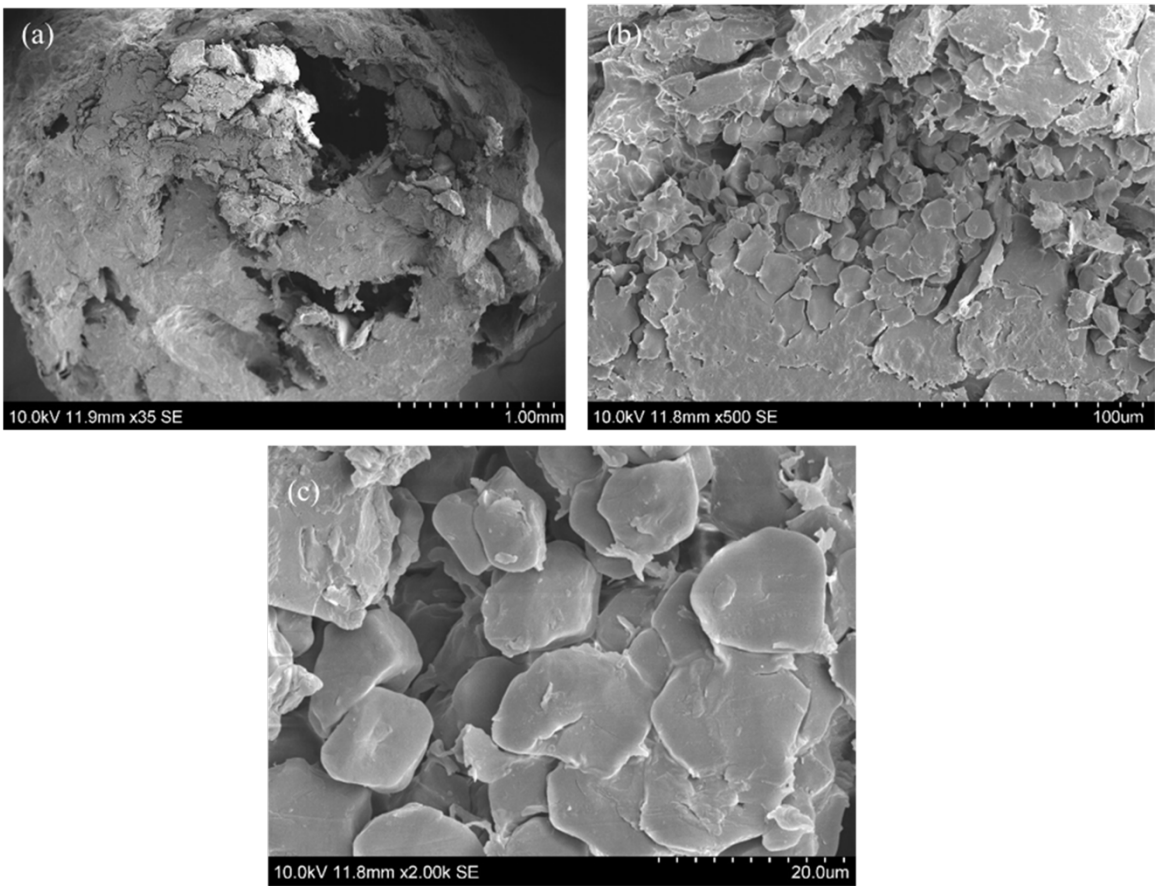

Figure 4.6 SEM micrographs of the extrudates containing 20% AP extruded at a temperature of 120^{o}C, screw speed 150 rpm and moisture content of 17%

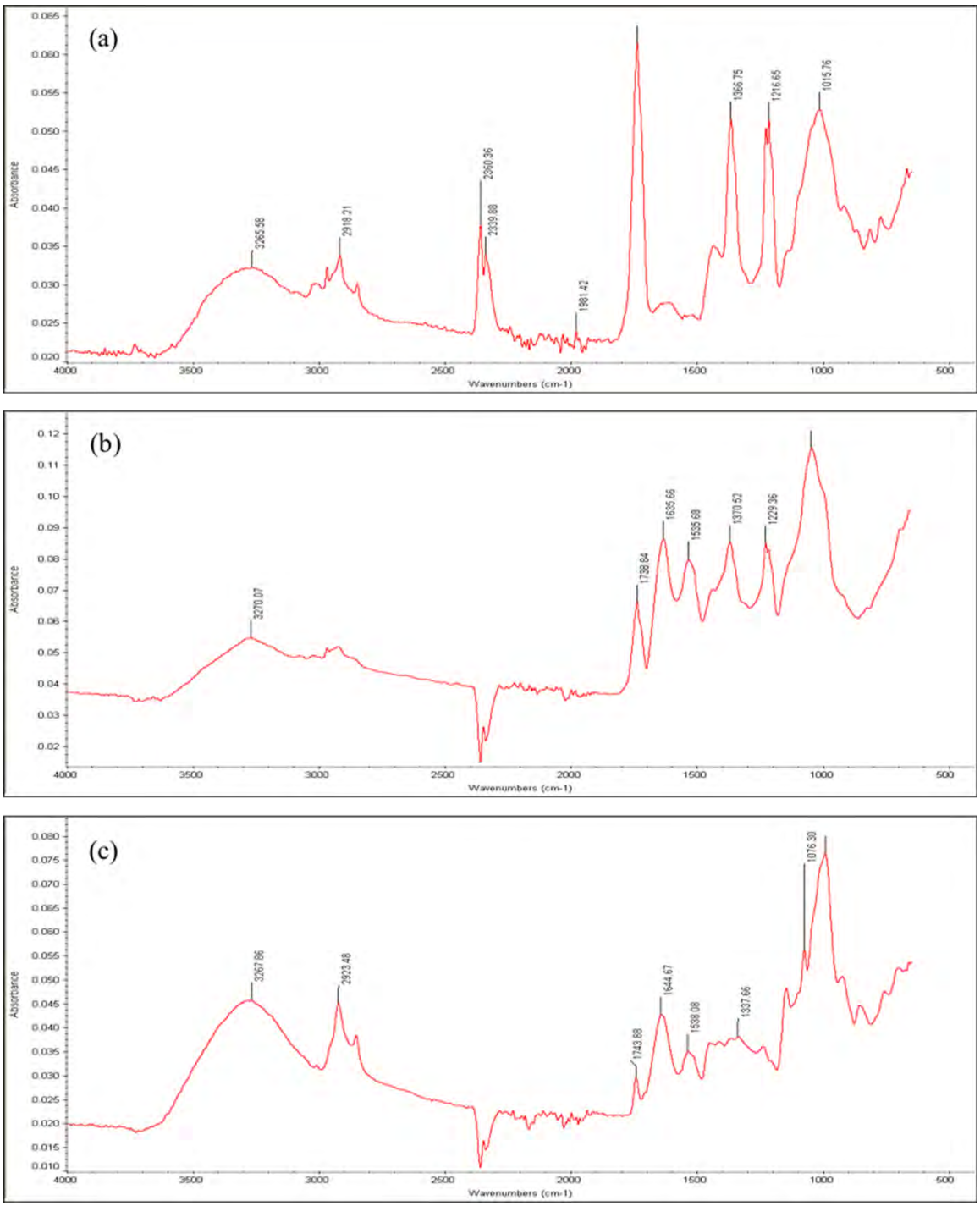

Figure 4.7 FTIR spectra of raw ingredients (a) apple pomace, (b) defatted soy flour, (c) corn grits

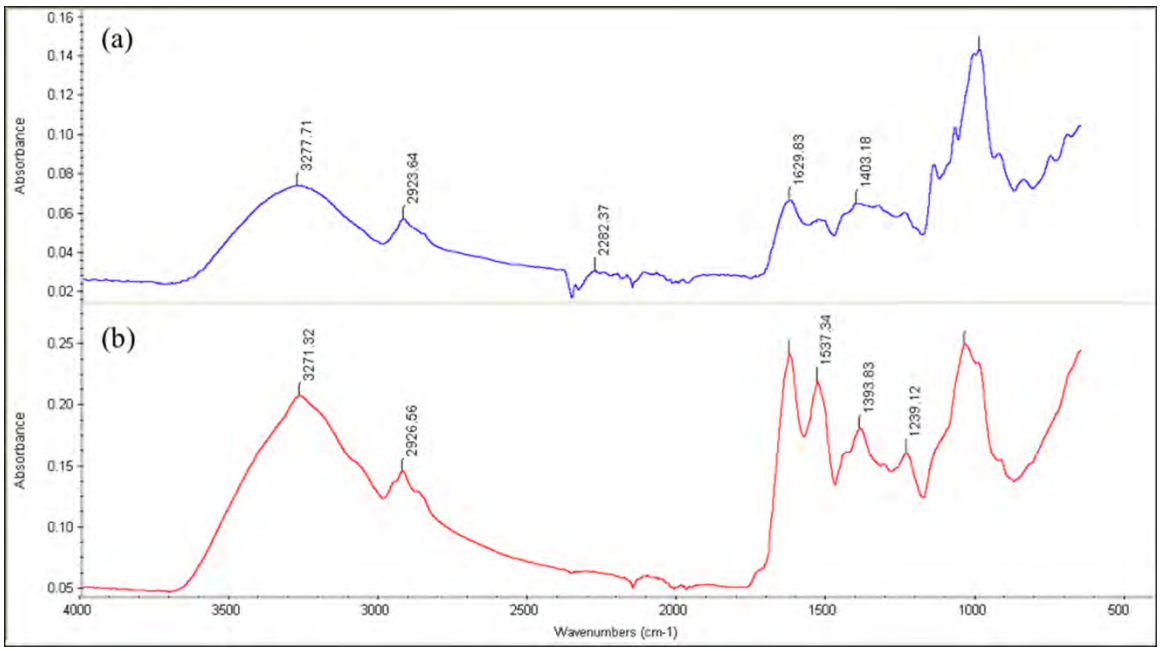

Figure 4.8 FTIR spectra of (a) extrudates containing 0% AP extruded at temperature 120^{o}C, screw speed 150 rpm and 17% moisture content and (b) the unextruded blend containing 50:50 w/w DSF and CG (bottom)

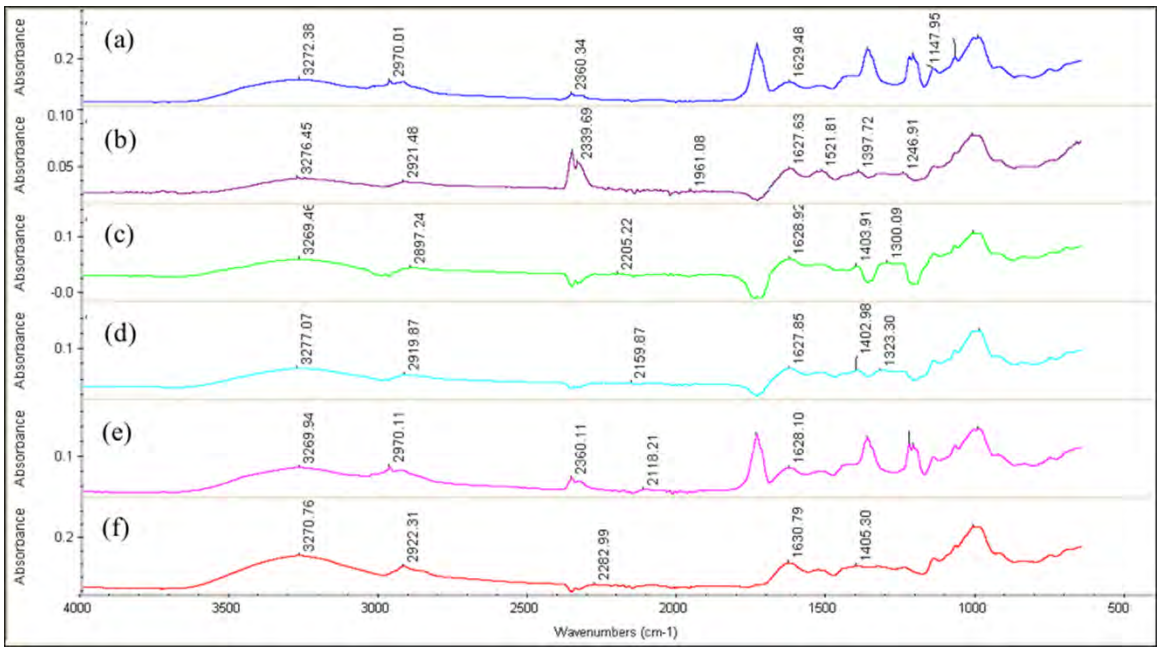

Figure 4.9 FTIR spectra of the extrudates containing (a) 10% AP and extruded at temperature 120^{o}C, screw speed 150 rpm and 17% moisture content, (b) 15% AP and extruded at temperature 110^{o}C, screw speed 175 rpm and 15.5% moisture content, (c) 5% AP and extruded at temperature 110^{o}C, screw speed 125 rpm and 18.5% moisture content, (d) 10% AP and extruded at temperature 140^{o}C, screw speed 150 rpm and 17% moisture content. (e) 5% AP and extruded at temperature 130^{o}C, screw speed 125 rpm and 15.5% moisture content, (f) 20% AP and extruded at temperature 120^{o}C, screw speed 150 rpm and 17% moisture content

CHAPTER 5 †

Influence of Processing Conditions on Apparent Viscosity and System Parameters during Extrusion of Distiller's Dried Grains Based Snacks

5.1 Abstract

A combination of different levels of distillers dried grains processed for food application (FDDG), garbanzo flour and corn grits were chosen as a source of high-protein and high-fiber extruded snacks. A four-factor central composite rotatable design was adopted to study the effect of FDDG level, moisture content of blends, extrusion temperature, and screw speed on the apparent viscosity, mass flow rate or MFR, torque, and specific mechanical energy or SME during the extrusion process. With increase in the extrusion temperature from 100 to 140^{o}C, apparent viscosity, specific mechanical energy and torque value decreased. Increase in FDDG level resulted in increase in apparent viscosity, SME and torque. FDDG had no significant effect (P>0.05) on mass flow rate. SME also increased with increase in the screw speed which could be due to the higher shear rates at higher screw speeds. Screw speed and moisture content had significant negative effect (P<0.05) on the torque. The apparent viscosity of dough inside the extruder and the system parameters were affected by the processing conditions. This study will be useful for control of extrusion process of blends containing these ingredients for the development of high-protein high-fiber extruded snacks.

5.2 Introduction

Extrusion cooking is a popular food processing technique that have been extensively used to produce protein-rich and fiber-rich products. Extrusion process is a

† Singha, P., Muthukumarappan, K., Krishnan, P., (2017). Influence of Processing Conditions on Apparent Viscosity and System Parameters during Extrusion of Distiller's Dried Grains Based Snacks. Food Science and Nutrition,00:1-10, doi: 10.1002/fsn3.534

combination of cooking, mixing and forming, resulting in good quality direct expanded product. It is widely used in industries owing to its characteristic high throughput and automatic control (Singh and Muthukumarappan, 2014b). During extrusion, the interactive effects of temperature, shearing forces, and moisture content of the blend transform the feed ingredients at macroscopic and microscopic levels leading to structural changes of protein and starch (Brown et al., 2015). Starch is gelatinized, protein is denatured and enzymes, microbes and many anti-nutritional factors are inactivated.

The extent of mixing, shearing and compressing of the materials, and the rate of heating inside the extruder and die, depends on the raw materials and process conditions used (Singh and Muthukumarappan, 2016). The extrusion process also depends on the pressure developed inside the die and the degree to which the screw is filled (Singh and Muthukumarappan, 2014a). Hence, it is important to understand the rheological changes encountered by an ingredient melt inside the barrel. Each material that flows inside the extruder has its own properties and behaves differently. The behavior can be quantified by determining mass flow and energy responses. Apparent viscosity is one of the most important rheological properties which has a direct impact on the quality of a final product. A continuous monitoring system are often utilized to measure the apparent viscosity during the extrusion process (Chen et al., 1978; Lam and Flores, 2003). It is a good indicator of the dough's behavior and the changes during processing. Protein denaturation and polysaccharide gel formation can affect viscosity during extrusion (Bhattacharya and Hanna, 1986). Separate attachments have been employed to measure the dough rheology of wheat, corn, and soybeans using straight tube viscometers (Harper et al., 1971), cylindrical dies of different lengths (Harmann and Harper, 1974), capillary

die rheometers (Singh and Muthukumarappan, 2017a, b) and viscoamylographs (Remsen and Clark, 1978) attached to food extruders. Not only the feed material but the quality of extrudates depends on the type of extruder used, choice of screw configuration, moisture content of the feed, temperature profile in the barrel and die, and screw speed (Singha and Muthukumarappan, 2017a).

Dry-milling process involved in corn ethanol production, produces distillers dried grains (DDG) and distillers dried solubles (DDS). Distillers dried grains with solubles (DDGS) is produced after mixing and drying these two co-products (Singh, 2016). DDGS contain high levels of protein since most of the starch is removed (Rosentrater and Krishnan, 2006). It is usually used as cattle feed. However, few studies have been reported on its application in human food (Rasco and McBurney, 1989; Rosentrater and Krishnan, 2006; Wu et al., 1987). The growing interest in the health benefit of protein and fiber justifies exploring the use of DDGS as a protein and fiber supplement in food products. DDGS supplementation will improve the nutritive value of food products by enriching their protein and fiber content, and expand the use of the co-product from alcohol fermentation (Tsen et al., 1982). DDG and DDGS have been extensively used as a protein source for the development of pet food and aquafeed. Few studies have also been reported on the addition of DDG in snack foods. Reddy and Stoker (1993) added DDGS in wheat flour for the preparation of noodles and baked foods.

Cereal or grains are the primary source of most extruded snack foods because of their high expansion properties. However, they tend to be low in protein and essential nutrients. Usually, cereals lack lysine as the essential amino acid but have sufficient sulfur containing amino acids. Legumes on the other hand, are rich in lysine and deficient

in sulfur containing amino acids. When combined together, the proteins of cereals and legumes complement one another to produce a protein of a better quality. Corn in varied forms has been widely used as raw material for extrusion. It is ideal for extrusion since it has a high starch content, which facilitates the expansion process. It is gluten-free and contains protein, fiber, vitamins and unsaturated fatty acids. Compared to wheat, oat, and rice, corn has higher phytochemical content such as phenolic compounds which have anticarcinogenic effects (I.O et al., 2014). Pulse crops (garbanzo, lentils, dry beans, lupin, and various types of beans) are excellent source of protein, complex carbohydrates, fibers, essential vitamins and minerals. Additionally, they are low in fat and sodium content, no cholesterol, and high in phenolics and bioactive compounds (Roy et al., 2010). Inclusion of pulses and legumes increases the protein content and improves potential nutritional content owing to increase in protein digestibility. This was reported by Tiwari et al. (2011) while studying the addition of pigeon pea to wheat flour based biscuits and, de la Hera et al. (2012) while studying the effects of addition of legume flour to traditional cereal based flours. Madhumitha and Prabhasankar (2011) reported that there was an improvement in the nutritional value of pasta by adding black gram flour and mentioned that the processing of food material increases the value and shelf life of the product. Extrusion treatment of lentil flours has also been linked with increase in some bioactive components (Morales et al., 2015).

Garbanzo flour contain moderately high protein (17–22 %), low fat (6.48 %), high available carbohydrate (50 %) and crude fiber contents of 3.82 % (Alajaji and El-Adawy, 2006). Garbanzo has significant amounts of calcium, potassium, phosphorus, zinc, magnesium and iron. Garbanzo is known to reduce cholesterol and blood glucose levels

(Singh and Singh, 1992). They are increasingly used in healthy diets to promote general well-being and to reduce the risks of cardiovascular diseases and diabetes. Development of garbanzo-based snacks could provide enhanced uses for chickpea. Processing of garbanzo into extruded snacks is limited (Bhattacharya and Prakash, 1994; Meng et al., 2010; Shirani and Ganesharanee, 2009). Little information is available on the effect of extrusion on sytem parameters using garbanzo as one of the ingredients (Meng et al., 2010).

Blending of DDGS and garbanzo flour will serve as a good raw material for gluten-free healthy alternative snacks for a health-conscious population. For our study, DDGS was processed for food application and was named FDDG. We blended garbanzo flour, FDDG and corn grits at different levels. Our objective was to study the effect of the feed moisture content, screw speed, and barrel and die temperature, on the apparent viscosity and the system parameters (specific mechanical energy, torque and mass flow rate) during extrusion cooking.

5.3 Materials and Methods

5.3.1 Raw materials and blend preparation

Distiller's dried grains with solubles (DDGS) was obtained from Glacial Lakes Energy LLC, Watertown, SD. It was then washed, freeze dried, steam/pressure sterilized, oven toasted and ground to make a wholesome food-grade ingredient. The processing of DDGS for food application was done following method described by Rosentrater and Krishnan (2006). The DDGS processed specifically for food application studies is referred to as FDDG henceforth. The initial moisture content of FDDG was 0.70%. The proximate composition of FDDG was: 35.12 % protein, 0.53 % fat, 1.24 % ash, 35.00 %

fiber and 13.03% nitrogen free extract (dry basis). The FDDG was stored at -20°C until further use. Garbanzo flour (GF) was purchased from Hyvee, Brookings, SD. The initial moisture content of GF was 10.08%. The proximate composition of the GF was: 22.42 % protein, 5.94 % fat, 2.70 % ash, 6.56 % fiber and 54.63 % nitrogen free extract (dry basis). Corn grits (CG) was obtained from Bob's Red Mill (Milwaukie, OR). The initial moisture content of CG was 11.46 %. The proximate composition of the CG was: 6.00 % protein, 1.50 % fat, 2.00 % ash, 0.90 % fiber and 78.14 % nitrogen free extract (dry basis). The different ingredients i.e. FDDG, GF and CG were mixed into five different compositions (Blend I to V) as shown in Table 5.1. Water was added to the blends to make 14 to 20% (wet basis) final moisture depending on the experimental runs (Table 5.2). The ingredients were mixed in a laboratory scale mixer (KitchenAid Professional 5 Plus, Troy, Ohio, USA) for 10 minutes. For moisture stabilization, the blends were stored overnight at ambient temperature. The moisture content of the prepared blends was determined by using the method 44-19 (AACC, 2000). The proximate composition of blends is shown in Table 5.1.

5.3.2 Extrusion processing

Post conditioning, the blends were randomly extruded using a single-screw laboratory extruder (Brabender Intelli-Torque Plasti-Corder®, South Hackensack, NJ) having a barrel inner diameter of 19.18 mm. A screw compression ratio of 1.5:1 was used in the experiments. A pictorial representation of the single screw extruder is shown in Figure 5.1 (Singh and Muthukumarappan, 2014b). Pressure at the die, and net torque exerted on the extruder drive (N-m) were measured. Stock thermocouples (model 05-00-317, C. W. Brabender) were inserted into the barrel and die to measure the dough

temperature. Extrudate samples were collected every 30s to determine the mass flow rate (g/s) by the method described by Rosentrater et al. (2005). SME (W-h/kg) consumption was calculated (Lam and Flores, 2003) as:

$$SME = \frac{\Omega.\omega}{MFR\left[\frac{3600}{1000}\right]} \tag{5.1}$$

where Ω is the net torque exerted on the extruder drive (N-m), ω is the angular velocity of the screw (rad/s) and *MFR* is the mass flow rate of dough (mass throughput, g/s).

The apparent viscosity of the dough in the extruder was calculated by approximating extruder behavior as that of a coaxial viscometer but corrected for the tapered screw geometry (Figure 5.2) of the extruder barrel (Konkoly, 1997; Lam, 1996; Rogers, 1970). As discussed by Lam and Flores (2003), the shear stress (τ_s) at the screw surface (N/m^2) and the shear rate ($\dot{\gamma}_s$, 1/s) were calculated from the following equations:

$$\tau_s = \Omega / (2.\pi.(r_{coor})^2.L_s) = C_{ss}\Omega \tag{5.2}$$

$$\dot{\gamma}_s = (2.\omega.r_b^2) / (r_b^2 - (r_{coor}^2)) = C_{sr}\omega \tag{5.3}$$

where r_{corr} is the radius correction due to the screw's frustum geometry

$$r_{coor} = \left(\sqrt{(r_{eff\,1}^2 + r_{eff\,1}r_{eff\,2} + r_{eff\,2}^2 / 3}\right) \tag{5.4}$$

r_{eff} is the effective radius (m), Ω is the net torque exerted on the screw (N m), *L*s is the screw length in the axial direction (m), ω is the angular velocity of the screw (rad/s), C_{ss} is an empirical correction factor for shear stress (which is 10321.5 for this study), $\dot{\gamma}_s$ is the shear rate at the screw surface (1/s), r_b is the inner barrel radius (m), and C_{sr} is the

empirical correction factor for shear rate (which is 3.48 for this study). The calibration value for this extruder have been calculated from the calculation reported elsewhere (Lam and Flores, 2003). The apparent viscosity was calculated by taking the ratio of Eq. (5.2) to Eq. (5.3).

$$\eta_{app} = \frac{\tau_s}{\dot{\gamma}_s} = \left(\frac{C_{ss}}{C_{sr}}\right)\left(\frac{\Omega}{\omega}\right) \tag{5.5}$$

where η_{app} is the apparent viscosity of the dough in the extruder (Pa.s),

5.3.3 Experimental design and statistical analysis

Experiments were conducted using the central composite rotatable design which was developed using Design-Expert 8.0.7.1 (Statease, Minneapolis, MN, USA). Four numerical independent variables namely FDDG (X_1), temperature (X_2), screw speed (X_3) and moisture content (X_4) each at five levels were taken as shown in Table 5.2. Three replicates were taken (optional) at the design center (0, 0, 0) and the total number of observations were 27 [24 (axial points) and 3 (center points)]. The experimental design and the codes for the processing variables are shown in Table 5.3.

Mass flow rate (Y_{MFR}), specific mechanical energy (Y_{SME}), apparent viscosity of dough (Y_{AV}), and torque (Y_{Tor}) were taken as the responses of the designed experiments. A second-order polynomial regression models were established for the dependent variables to fit experimental data for each response.

$$y_i = b_0 + \sum_{i=1}^{a} b_i x_i + \sum_{i=1}^{a} b_{ii} x_{i^2} + \sum_{i=1}^{a}\sum_{j=1}^{a} b_{ij} x_i x_j \tag{5.6}$$

where y_i is the predicted response; b_0 is the interception coefficient; b_i, b_{ii}, and b_{ij} are coefficients of the linear, quadratic, and interaction terms; and x_i is the independent

variables studied. The fitness of the model was evaluated and the interactions between the independent and dependent variables were identified by using an analysis of variance (ANOVA) presented in Table 5.5 and Table 5.6. The goodness of fit of the second-order equation was expressed by the coefficient of determination (R^2) and its statistical significance was determined by the F-test. Three-dimensional response surfaces were used to visualize the interactive effects of the independent variables.

5.4 Results and Discussion

5.4.1 Effect of processing conditions on apparent viscosity

The fitted model shown in Table 5.4 had a significant coefficient of determination (R^2) of 0.82. The second order model (Table 5.5) for apparent viscosity was significant (P<0.05), whereas lack-of-fit was not significant (P>0.05). The selected model adequately represented the data for apparent viscosity. FDDG (X_1) had significant (P<0.05) positive linear effect, whereas temperature (X_2) and screw speed (X_3) had significant negative linear effects (P<0.05) on apparent viscosity of the dough inside the extruder. Temperature also showed significant negative quadratic effect (P<0.05) suggesting that viscosity decreased with excessive increase of temperature.

The apparent viscosity varied between 1654 and 4397 Pas. Although in this study, moisture content did not have any significant effect (P>0.05) on apparent viscosity, the highest viscosity was observed at the lowest moisture content of the blend which was 14%. Response surface plots of apparent viscosity at different FDDG level, temperature and screw speed are shown in Figure 5.3a and Figure 5.3b. The apparent viscosity of the dough increased with higher levels of FDDG. Increasing the percentage of FDDG in the blend also increased the protein content. The rise in FDDG content changed the overall

chemical composition and the dough functionality which may have resulted in the higher apparent viscosity. Bhattacharya and Hanna (1986) also reported an increase in viscosity when the percentage of soy protein content in the blend was increased during extrusion of corn-soy mix.

Temperature had a significant quadratic effect (P<0.05) on the apparent viscosity (Figure 5.3(B)). It was observed that the apparent viscosity of the dough decreased when temperature was increased and at very high temperature there was a sharp decrease in apparent viscosity. The apparent viscosity decreased with increase in the screw speed from 100 rpm to 200 rpm (Figure 5.3), indicating shear thinning behavior of the dough. (Singh and Muthukumarappan, 2017b) This happens due to increased shear rates and molecular degradation (Singh and Muthukumarappan, 2017b). Similar observations were also reported by Chinnaswamy and Hanna (1990),

5.4.2 Effect of processing conditions on mass flow rate (MFR)

The drag flow developed by screw rotation inside extruder and the pressure developed due to constriction at the die influences the mass flow rate (Ludewig, 1989). Multiple regression equation for MFR (Y_{MFR}) in terms of coded variables is shown in Table 5.4. ANOVA for the model of MFR as fitted (Table 5.5) shows that the model was significant (P<0.05) whereas lack of fit was not significant (P>0.05). The response surface regression model on MFR yielded a good fit with a coefficient of determination (R^2 = 0.81) for the extrudates. Regression analyses showed that MFR was significantly (P<0.05) affected by linear effect of moisture content (X_1) and quadratic effect of screw speed (X_3). Interaction effects of temperature and screw speed (X_2X_3), and screw speed and moisture content (X_3X_4) were also observed.

The MFR varied between 1.67 to 2.28 kg/h. The effect of screw speed and moisture content on MFR are shown in Figure 5.4. Increasing the screw speed from 100 to 200 rpm significantly (P<0.05) increased the MFR. Such behavior is expected as there is a proportional relationship between drag flow in extruder and the screw speed. For this reason, higher screw speeds means higher mass flow rate and there is greater ability for the material to move along the extruder barrel (Harper, 1981). Increasing the moisture content from 14 to 20% also significantly (P<0.05) increased the MFR. High moisture content aides in gelatinization of the dough and thus apparent viscosity decreases which has been observed in this study. This explains the increase in MFR with increase in moisture content of the dough. No significant effect (P>0.05) of FDDG on the MFR was observed.

5.4.3 Effect of processing conditions on specific mechanical energy (SME)

A multiple linear regression equation of SME (Y_{SME}) in terms of coded levels is shown in Table 5.4. Linear terms of FDDG (X_1), temperature (X_2), screw speed (X_3) and moisture content (X_4) had significant effects (P<0.05) on SME. Temperature had a significant negative quadratic effect (P<0.05) on SME. SME increased with increase in temperature, while excessive increase of temperature resulted in decrease of SME. The fitted quadratic model had a coefficient of determination (R^2) of 0.89. The model (Table 5.6) for SME was significant (P<0.05), whereas lack of fit was not significant (P>0.05).

The amount of mechanical energy input during extrusion has a direct role in macromolecular transformations and interactions of different components in the feed materials. The SME in this study varied from 61 to 164 W-h/kg. Figure 5.5a and Figure 5.5b shows the response surface graph of SME *versus* moisture content and screw speed,

and FDDG level and temperature, respectively. SME increased with increase in FDDG level. This could be due to reduced starch content in the dough containing higher FDDG. Furthermore, we have observed previously that viscosity increased with increase in FDDG. This indicates that higher energy and pressure is required during the extrusion of the blends containing higher percent of FDDG. The high fiber content has a tendency to bind more water, resulting in reduced availability of water for starch (Mir et al., 2015). SME increased initially with increase in temperature but further increase in temperature resulted in decrease in SME. High temperatures are normally associated with a decrease in the melt viscosity inside the extruder, which in turn reduces the energy input of the extruder. According to Ludewig (1989), with increase in screw speed, the SME generally increases. This is because the magnitude of change in energy input to the screw is typically greater than the decrease in torque associated with the decrease in apparent viscosity due to shear thinning behavior of the non-Newtonian materials. The high SME at high screw speed and low barrel temperature was also observed by Meng et al. (2010) during extrusion of chickpea and whey protein based blends. Increase in feed moisture during extrusion is associated with decrease in viscosity which ultimately leads to reduced SME (Chang et al., 1999; Hsieh et al., 1991). Such findings are in agreement with Bhattacharya and Hanna (1987) and Filli et al. (2012).

5.4.4 Effect of processing conditions on torque

The multiple regression equation for torque (Y_{Tor}) in terms of coded variable is given in Table 5.4. The torque was influenced significantly (P<0.05) by negative linear effects of FDDG (X_1), temperature (X_2), screw speed (X_3) and moisture content (X_4) suggesting that increase in the levels of these variables resulted in decrease in torque.

Temperature also had significant negative quadratic effect ($P<0.05$) on torque indicating excessive increase in temperature reduced the torque of the extruder. Interaction effects of FDDG and screw speed (X_1X_3) and temperature and moisture content (X_2X_4) were also observed. The responses were analyzed using ANOVA and the data are presented in Table 5.6. Examination of the model shows a good fit with R^2 equal to 0.95 for the torque. The linear model was significant ($P<0.05$), whereas lack of fit was not significant ($P>0.05$) for torque.

Torque increases with increase in FDDG level (Figure 5.6(A)) which also means that it decreases with increase in garbanzo flour. This is in agreement with findings of Bhattacharya and Prakash (1994). The torque during extrusion ranged between 9 and 19 N.m and high torque was associated with low screw speed. The effect of screw speed on torque also depends on the level of temperature. At high extrusion temperature, the response surface plot (Figure 5.6(B)) shows that torque value is almost constant with change in screw speed but at low extrusion temperature torque increased with decrease in screw speed. Filli et al. (2012) also reported a decrease in torque with increase in screw speed and feed moisture during single screw extrusion of millet-soybean mixture. Since the blends showed shear thinning behavior inside the extruder, the net torque required by the screw to convey the dough through the extruder decreased significantly. According to Guha et al. (1997), the decrease in the magnitude of torque with increase in screw speed can be explained by the reduced degree of fill in the extruder. Decrease in torque with increasing moisture content (Figure 5.6(C)) suggests more water is available for starch gelatinization resulting in reduction in apparent viscosity. Melt viscosity is low at high moisture contents and hence less torque will be required to work the material in the screw

channels. Reduction in torque can be attributed to reduced friction in the extruder because of increase in feed moisture. This indicated that increasing moisture content or screw speed reduces the difficulty of processing. Similar results were found by Onwulata et al. (1994) during twin screw extrusion of corn meal and by Chang and El-Dash (2003) during extrusion of cassava.

5.5 Conclusions

The apparent viscosity, MFR, torque, and SME were shown to be significantly influenced by the extruder operating conditions. FDDG level and extrusion temperature significantly affected the apparent viscosity, torque and SME. Apparent viscosity increased with the increase in FDDG content. Higher feed moisture and higher extrusion temperature reduced the viscosity. With increase in the screw speed and feed moisture content the MFR also increased. Increasing the moisture of the blends and the extrusion temperature resulted in a decrease in torque. High SME was observed at high screw speed and low extrusion temperature.

Table 5.1 Ingredient composition of blends.

Feed ingredients	**Percentage of ingredients (% db)**				
	Blend I	**Blend II**	**Blend III**	**Blend IV**	**Blend V**
FDDG	0	5	10	15	20
Garbanzo flour	40	35	30	25	20
Corn grits	60	60	60	60	60
Proximate analysis					
Protein (% db)	13.79	15.06	16.34	17.62	18.90
Fiber (% db)	3.45	4.86	6.27	7.67	9.08
Fat (% db)	3.59	3.30	3.00	2.71	2.41
Ash (% db)	2.53	2.44	2.36	2.27	2.19
NFE (% db)	76.64	74.34	72.03	69.73	67.42

FDDG = Distiller's grains processed for food application, db = dry basis

Table 5.2 Independent numerical variables and their levels.

Numerical variable	**Symbol**	**Coded variable levels**				
		-2	**-1**	**0**	**1**	**2**
FDDG (%)	x_1	0	5	10	15	20
Temperature (°C)	x_2	100	110	120	130	140
Screw speed (rpm)	x_3	100	125	150	175	200
Moisture content (% wb)	x_4	14	15.5	17	18.5	20

wb = wet basis

Table 5.3 Experimental design layout.

Run	Coded variable				Actual Variable			
	x_1	x_2	x_3	x_4	X_1 (%)	X_2 (°C)	X_3 (rpm)	X_4 (% wb)
1	-1	-1	1	1	5	110	175	18.5
2	1	1	1	1	15	130	175	18.5
3	0	0	2	0	10	120	200	17
4	1	-1	1	1	15	110	175	18.5
5	-1	-1	-1	1	5	110	125	18.5
6	0	2	0	0	10	140	150	17
7	-1	-1	-1	-1	5	110	125	15.5
8	1	-1	-1	-1	15	110	125	15.5
9	2	0	0	0	20	120	150	17
10	0	0	0	-2	10	120	150	14
11	1	1	-1	1	15	130	125	18.5
12	0	0	-2	0	10	120	100	17
13	-1	1	1	-1	5	130	175	15.5
14	0	0	0	2	10	120	150	20
15	0	0	0	0	10	120	150	17
16	1	1	-1	-1	15	130	125	15.5
17	0	0	0	0	10	120	150	17
18	0	-2	0	0	10	100	150	17
19	-1	1	-1	1	5	130	125	18.5
20	1	-1	-1	1	15	110	125	18.5
21	-1	-1	1	-1	5	110	175	15.5
22	-2	0	0	0	0	120	150	17
23	1	-1	1	-1	15	110	175	15.5
24	-1	1	1	1	5	130	175	18.5
25	-1	1	-1	-1	5	130	125	15.5
26	1	1	1	-1	15	130	175	15.5
27	0	0	0	0	10	120	150	17

wb = wet basis

Table 5.4 Best-fit response surface models in terms coded variables after excluding the insignificant terms for apparent viscosity (AV), mass flow rate (MFR), specific mechanical energy (SME) and torque (Tor).

Response surface model	R^2	Adj R^2
$Y_{AV} = 3020.38 + 371.45x_1 - 239.75x_2 - 430.65x_3 - 339.85x_2^2$	0.82	0.61
$Y_{MFR} = 1.93 + 0.04x_4 + 0.06x_2x_3 - 0.05x_3x_4 + 0.04x_3^2$	0.81	0.58
$Y_{SME} = 134.54 + 7.24x_1 - 8.24x_2 + 11.13x_3 - 11.09x_4 - 13.03x_2^2$	0.89	0.76
$Y_{Tor} = 15.6 + 0.77x_1 - 1.47x_2 - 1.25x_3 - 1.33x_4 - 0.76x_1x_3 - 1.17x_2x_4 - 1.47x_2^2$	0.95	0.89

Table 5.5 Analysis of variance for apparent viscosity and mass flow rate (MFR)

	Apparent Viscosity					MFR			
Source	**df**	**SS**	**MS**	***F*-value**	***P*-value**	**SS**	**MS**	***F*-value**	***P*-value**
Model	14	15343599.13	1095971.37	3.9220	0.0114	0.2220	0.0159	3.5378	0.0172
X_1**-FDDG**	1	3311400.79	3311400.79	11.8501	0.0049	0.0006	0.0006	0.1274	0.7274
X_2**-Temperature**	1	1379553.43	1379553.43	4.9368	0.0463	0.0005	0.0005	0.1006	0.7566
X_3**-Screw Speed**	1	4450944.93	4450944.93	15.9280	0.0018	0.0172	0.0172	3.8378	0.0738
X_4**-Moisture**	1	1091398.09	1091398.09	3.9056	0.0716	0.0312	0.0312	6.9675	0.0216
X_1X_2	1	367469.23	367469.23	1.3150	0.2738	0.0205	0.0205	4.5783	0.0536
X_1X_3	1	172289.06	172289.06	0.6165	0.4476	0.0003	0.0003	0.0610	0.809
X_1X_4	1	47959.95	47959.95	0.1716	0.6860	0.0018	0.0018	0.3959	0.541
X_2X_3	1	203505.23	203505.23	0.7283	0.4102	0.0580	0.0580	12.9321	0.0037
X_2X_4	1	4701.64	4701.64	0.0168	0.8989	0.0082	0.0082	1.8219	0.2020
X_3X_4	1	135458.33	135458.33	0.4847	0.4995	0.0387	0.0387	8.6264	0.0124
X_1^2	1	413947.77	413947.77	1.4813	0.2470	0.0081	0.0081	1.8135	0.2030
X_2^2	1	2463928.70	2463928.70	8.8173	0.0117	0.0138	0.0138	3.0719	0.1051
X_3^2	1	18756.19	18756.19	0.0671	0.8000	0.0390	0.0390	8.6929	0.0122
X_4^2	1	817.44	817.44	0.0029	0.9578	0.0170	0.0170	3.8039	0.0749
Residual	12	3353294.49	279441.21	-	-	0.0538	0.0045	-	-
Lack of Fit	10	2537336.80	253733.68	0.6219	0.7520	0.0310	0.0031	0.2719	0.9365
Pure Error	2	815957.69	407978.84	-	-	0.0228	0.0114	-	-

Table 5.6 Analysis of variance for specific mechanical energy (SME) and torque.

	SME					Torque			
Source	**df**	**SS**	**MS**	***F*-value**	***P*-value**	**SS**	**MS**	***F*-value**	***P*-value**
Model	14	15402.4562	1100.1754	6.7475	0.0010	254.0629	18.1473	16.1637	< 0.0001
X_1**-FDDG**	1	1258.3786	1258.3786	7.7178	0.0167	14.0558	14.0558	12.5194	0.0041
X_2**-Temperature**	1	1629.9350	1629.9350	9.9966	0.0082	51.8246	51.8246	46.1598	< 0.0001
X_3**-Screw Speed**	1	2972.2487	2972.2487	18.2291	0.0011	37.4933	37.4933	33.3951	< 0.0001
X_4**-Moisture**	1	5468.2265	5468.2265	33.5372	< 0.0001	42.4260	42.4260	37.7886	< 0.0001
X_1X_2	1	4.2068	4.2068	0.0258	0.8751	3.0900	3.0900	2.7523	0.1230
X_1X_3	1	35.6478	35.6478	0.2186	0.6485	9.1869	9.1869	8.1828	0.0143
X_1X_4	1	10.9873	10.9873	0.0674	0.7996	0.6546	0.6546	0.5831	0.4599
X_2X_3	1	228.0132	228.0132	1.3984	0.2599	2.5868	2.5868	2.3041	0.1549
X_2X_4	1	45.8293	45.8293	0.2811	0.6057	22.0873	22.0873	19.6730	0.0008
X_3X_4	1	16.2969	16.2969	0.1000	0.7573	0.1693	0.1693	0.1508	0.7046
X_1^2	1	147.8367	147.8367	0.9067	0.3598	1.6217	1.6217	1.4444	0.2526
X_2^2	1	3621.7322	3621.7322	22.2125	0.0005	46.2688	46.2688	41.2113	< 0.0001
X_3^2	1	538.2015	538.2015	3.3008	0.0943	0.1660	0.1660	0.1478	0.7073
X_4^2	1	268.1656	268.1656	1.6447	0.2239	1.4779	1.4779	1.3164	0.2736
Residual	12	1956.5932	163.0494	-	-	13.4726	1.1227	-	-
Lack of Fit	10	1834.2774	183.4277	2.9992	0.2759	9.6126	0.9613	0.4981	0.8151
Pure Error	2	122.3158	61.1579	-	-	3.8600	1.9300	-	-

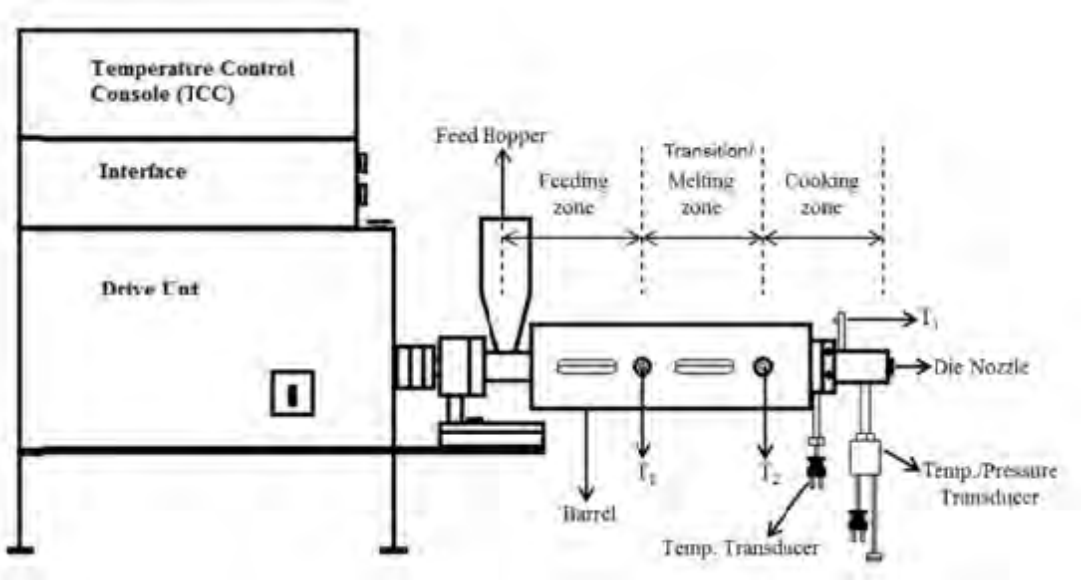

Figure 5.1 Schematic diagram of a single screw extruder (Source:Singh and Muthukumarappan (2014b))

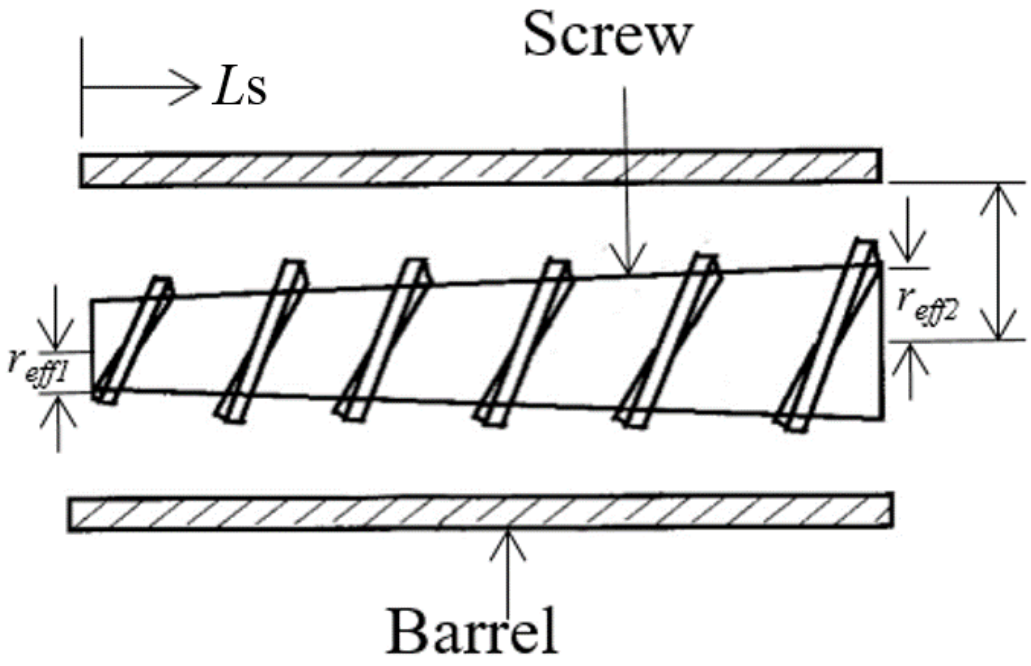

Figure 5.2 Schematic diagram of a section of single screw (Source: Singha and Muthukumarappan (2016))

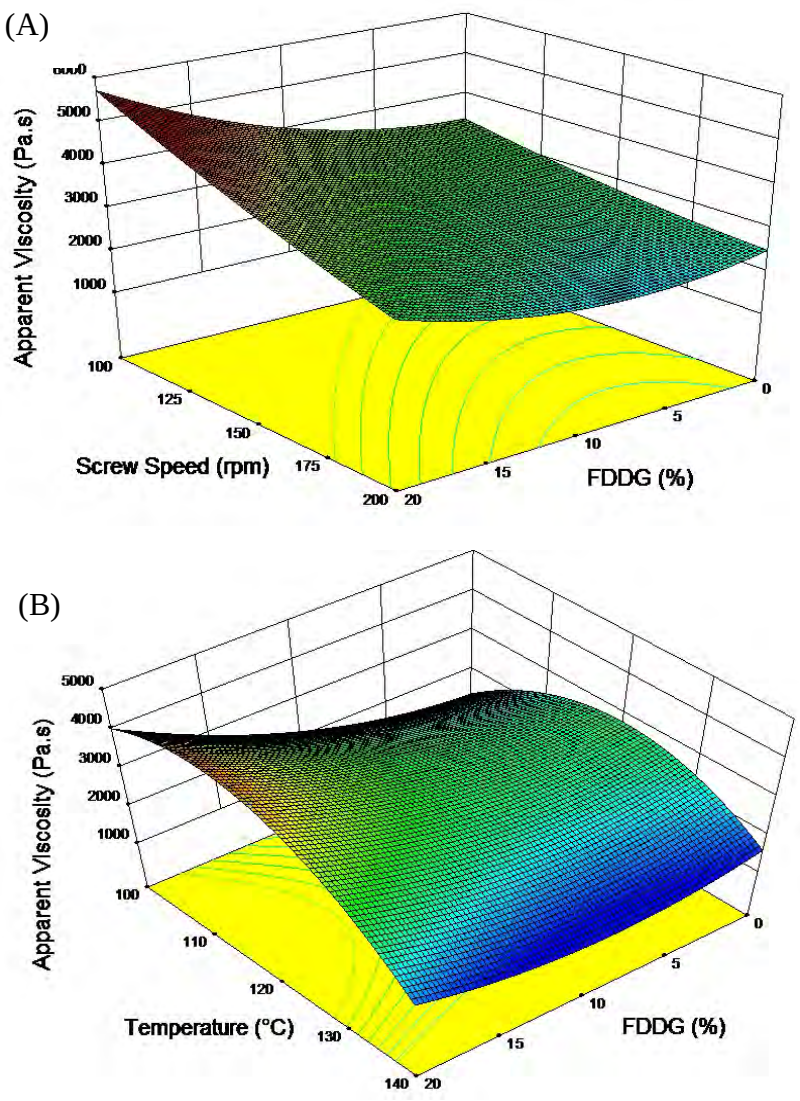

Figure 5.3 Response surface plots for apparent viscosity as a function of (A) Screw speed and FDDG at 120^{o}C temperature and 17% moisture content; (B) Temperature and FFDG at 150 rpm screw speed and 17% moisture content

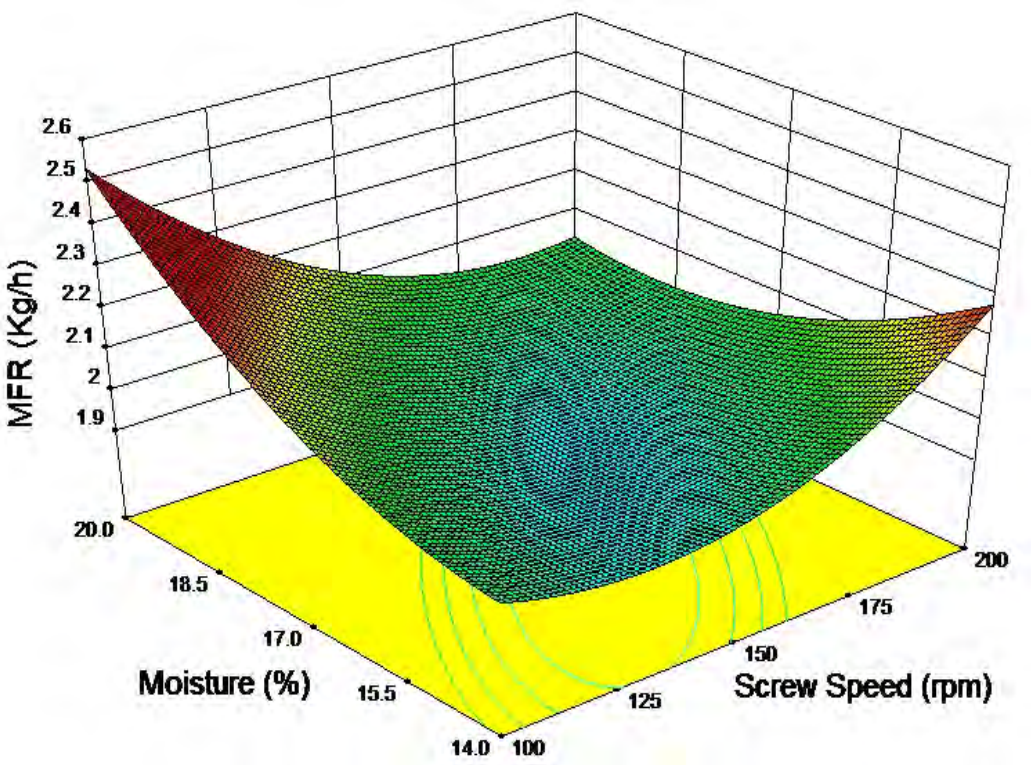

Figure 5.4 Response surface for mass flow rate as a function of screw speed and moisture content at 120^{o}C temperature and 10% FDDG

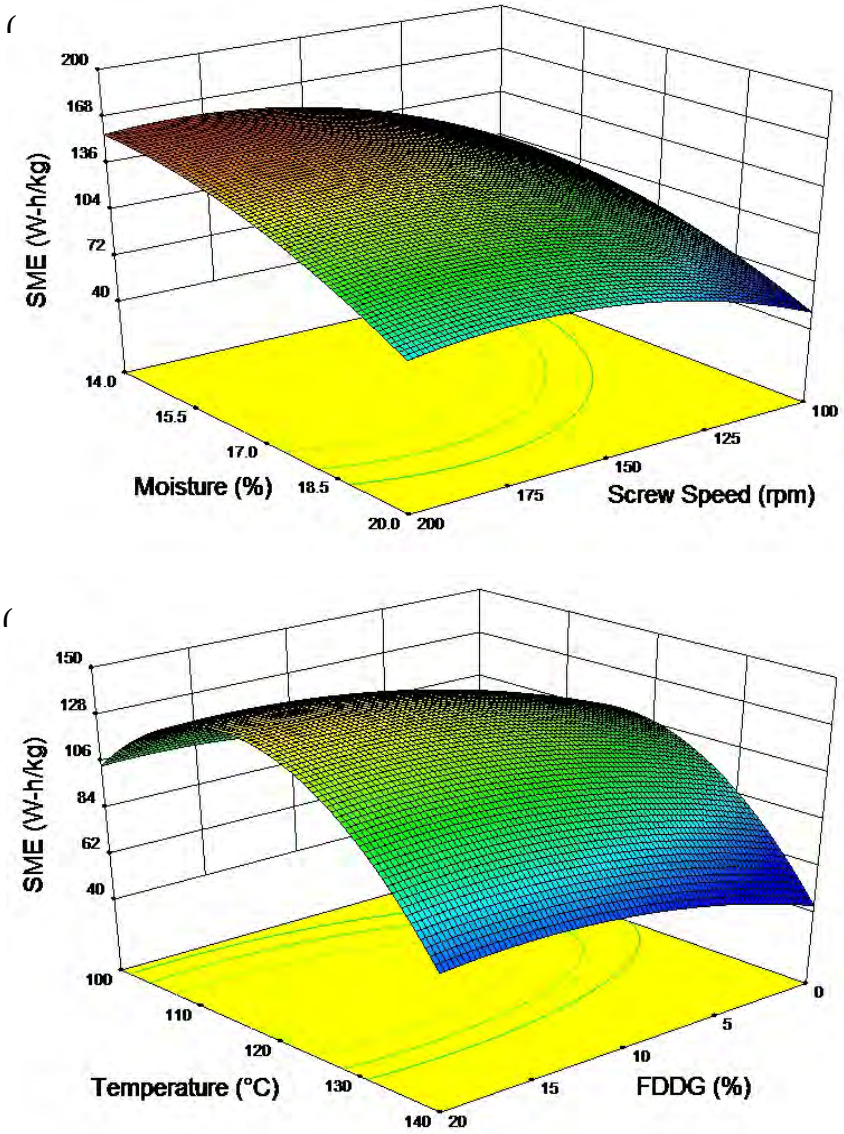

Figure 5.5 Response surface plots for specific mechanical energy as a function of (A) Moisture content and screw speed at 120^{o}C and 10% FDDG level; (B) Temperature and FDDG at 150 rpm screw speed and 17% moisture content

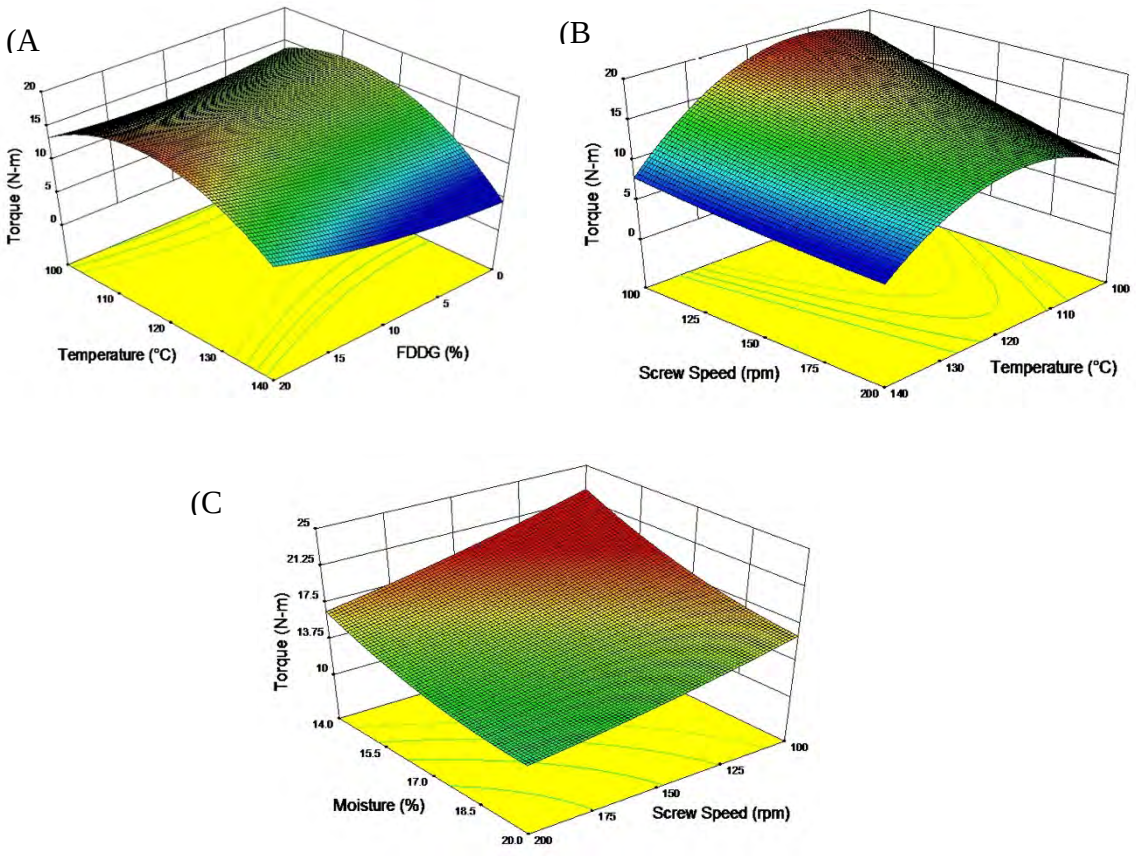

Figure 5.6 Response surface plots for torque as a function of (A) FDDG and temperature at 17% moisture content and 150 rpm screw speed; (B) Screw speed and temperature at 17% moisture content and 10% FDDG level; (C) Moisture content and screw speed at 10% FDDG level and 120^{o}C temperature

CHAPTER 6 [†]

Physicochemical and Nutritional Properties of Extrudates from Food Grade Distiller's Dried Grains, Garbanzo Flour and Corn Grits

6.1 Abstract

Distiller's dried grains and garbanzo flour were blended with corn grits for the development of expanded snacks using a single screw extruder. Distiller's dried grains was processed for food application and termed as FDDG. Effects of FDDG level (0-20%) and extrusion process parameters such as barrel and die temperature (100–140°C), screw speed (100–200 rpm) and feed moisture content (14-20% wb) on the physical properties (expansion ratio, bulk density, color parameters), functional properties (water absorption and solubility indices) and nutritional properties (total dietary fiber, soluble and insoluble dietary fiber and protein content) of the extrudates were investigated and optimized using response surface methodology. FDDG incorporation had a significant effect on the total dietary fiber, color parameters and the functional properties of the extrudate snacks. Desirable expanded extrudates with high level of total dietary fiber and protein were obtained with blends containing 20% FDDG at 140^{o}C extrusion temperature, 167 rpm screw speed and 19% feed moisture content. Results indicate garbanzo flour and FDDG can be successfully blended with corn grits to produce nutritious gluten-free extruded snacks which is high in protein and dietary fiber.

[†] Singha P and Muthukumarappan K. (2017) Physicochemical and nutritional properties of extrudates from food grade distiller's dried grains, garbanzo flour and corn grits. Journal of Food Science (Under Review).

6.2 Introduction

Modern life is characterized by limited free time and long working hours, making it difficult for most people to have proper meals resulting in an increase in consumer preference for ready-to-eat products. This lifestyle is now demanding for single-portion, portable, and healthier snack products (Euromonitor International, 2015). Growing numbers of young population are attracted to snack products which are particularly tasty and easy to be consumed. According to a report published by Mintel (2015), in USA, 94% of consumers snack at least once a day, while more than 50% are likely to snack 2 to 3 times a day. As snacking is replacing traditional meals, 33% of consumers are snacking on healthier foods more than ever before. Therefore, food industries have increased the production of ready-to-eat products using several processes. One such well-established food processing technique in industries is extrusion. It is a high temperature-short time process which is characterized by continuous cooking, mixing and forming (Singh and Muthukumarappan, 2017b) and produces direct expanded materials with high quality. Extrusion is flexible in the production of new products, such as cereal baby foods, breakfast cereals, snack foods, bakery products, pastas, etc. The extrusion process offers the ability to choose the ingredients and the ways of processing them. Manufacturers use this process to produce healthier snacks with varied ingredients. Moreover, the extrusion process eliminates some of the naturally occurring toxins (Cazzaniga et al., 2001; Elias-Orozco et al., 2002) and reduces the micro-organisms present in the final product, thereby making them safer for consumption. According to IMARC (2017), the global extruded snack food market reached a value of around US$ 50 Billion in 2016, growing at a compound annual growth rate of around 3% during 2009-2016.

During the extrusion process, the screw exerts shearing action on starch and protein- based materials and along with high-temperature transforms the material to a viscoelastic mass. At the exit of the die, the pressure drops suddenly and the emerging material expands (Singha and Muthukumarappan, 2017a). The physical properties of the product (density, expansion, texture, etc.) relies on the material composition (such as presence of protein, starch, fiber and moisture), and the processing conditions during extrusion.

The most widely consumed extruded snacks are made primarily with cereals/grains due to their good expansion characteristics. However, they tend to be low in protein and many other nutrients. Legumes are an excellent source of protein, fiber and many nutrients. To cater to the millennial's demand of vegan protein, food industries are developing protein isolates from unconventional legume sources. Garbanzo or chickpea have a high (40–50%) starch content (Huang et al., 2007; Wang and Daun, 2004), which may favor an extrusion process to produce directly expanded snack foods. It has good nutritional value with almost 20-28% protein and protein efficiency ratio of 2.64. Few studies report processing of garbanzo into extruded snacks (Batistuti et al., 1991; Geetha et al., 2012; Meng et al., 2010; Milán-Carrillo et al., 2000; Shirani and Ganesharanee, 2009). Researchers (Bhattacharya and Prakash, 1994; Shirani and Ganesharanee, 2009) reported that incorporation of garbanzo into rice flour decreased product expansion. Previous studies on extrusion of blends containing garbanzo dealt with relatively simple raw material compositions. In those studies, effects of extrusion processing on the important aspect of the nutritional properties (protein digestibility, functionality, and antioxidant) and physical attributes (sensory characteristics) of

extrudates of pulse-based flours are not investigated. Furthermore, garbanzo is high in amino acid lysine but low in sulfur containing amino acids.

Distiller's dried grains (DDG) is a coproduct of ethanol production. There has been enormous use of DDG for aqua feed and cattle food (Singh and Muthukumarappan, 2014a; Singh and Muthukumarappan, 2014b). However, in the past decade, ethanol production has dramatically increased causing a surplus of distiller's grains and saturating the market. The use of DDG, which is high in both protein and fiber, to fortify extruded snacks is one option to reduce the excess of DDG while enhancing its economic value. However, very few references (Kim et al., 1989; Shukla et al., 2005) are available in literature relating to the application of DDG in extruded snacks. Perhaps in combination with legumes, useful and alternative route for incorporation of DDG will be established.

We hypothesize that high-protein high-fiber corn-based snacks can be produced employing garbanzo flour and distiller's dried grain processed for food application (FDDG). We also hypothesize that garbanzo flour and FDDG may enhance the nutritional and physical characteristics of the corn-based processed food. The physiological function of the major compounds in the processed foods are strongly affected by the processing conditions. Therefore, it is essential to develop a process capable of advancing the utilization potential of pulses or pulse-derived products with enhanced nutritional and physical characteristics and sensory attributes of the processed food**.**

The objectives of the present study were (i) development of corn extrudates enriched with garbanzo flour and FDDG, and (ii) investigate the physical, functional and

nutritional properties of the extruded snack, (iii) optimize the extrusion process using response surface methodology.

6.3 Materials and methods

6.3.1 Raw materials and blend preparation

Distiller's dried grains with solubles (DDGS) was collected from Glacial Lakes Energy LLC, Watertown, SD. It was then washed, freeze dried, steam/pressure sterilized, oven toasted and ground to make a wholesome food-grade product specially for this study. Solubles were removed during the processing of DDGS for food application and was done following method described by Rosentrater and Krishnan (2006). We will be referring the DDGS processed specifically for food application as FDDG, henceforth. The FDDG was stored at -20 °C until further use. Garbanzo flour (GF) was purchased from a local store in Brookings, SD. Corn grits (CG) was obtained from Bob's Red Mill (Milwaukie, OR). The corn grits were ground in a mill (Perten Lab Mill 3610) and passed through 40 mesh screens. The analysis of chemical compositions like crude protein, crude fat, crude fiber, ash and moisture content of FDDG, GF and CG were carried out by standard methods (AOAC International, 2012). The percentage of carbohydrates were found by deduction method. The blends of feed ingredients were obtained per the experimental design. The w/w ratios of FDDG, GF and CG in the blends were 0:40:60, 5:35:60, 10:30:60, 15:25:60 and 20:20:60. All the flours were mixed in a laboratory scale mixer (KitchenAid Professional 5 Plus, Troy, Ohio, USA). The moisture content of the blends was determined by standard method (AACC, 2000). Prior to processing, the blends were rehydrated to each required moisture content level (Table 6.1) by calculated

amount of water sprayed on to the feed. The blend was then kept in sealed polyethylene bags for 24 h for uniform distribution of moisture.

6.3.2 Extrusion processing

The blends were randomly extruded using a 19.18 mm (0.755 in.) barrel inner diameter (i.d.), single-screw laboratory extruder (Brabender Intelli-Torque Plasti-Corder®, South Hackensack, NJ) which was powered by a 7.5-HP motor with an operating range of screw speeds from 0 to 225 rpm. The extruder had a barrel with length to diameter ratio of 20:1. A screw compression ratio (feed channel depth to metering channel depth) of 1.5:1 was used in the experiments. Extrudates were collected and air dried before further analysis.

6.3.3 Evaluation of product properties

6.3.3.1 Expansion ratio

Expansion ratio was calculated as the cross-sectional area of extrudate divided by the cross- sectional area of the die opening. An average diameter of ten samples was measured with a digital caliper to determine the expansion ratio of each set of samples.

6.3.3.2 Bulk density

Bulk density was determined as the ratio of the mass of extrudates that they filled up to a given bulk volume and measured using a standard bushel tester (Seedburo Equipment Company, Chicago, IL) following the method recommended by USDA (USDA, 1999).

6.3.3.3 Color

Color of extrudates was measured using Minolta Spectrophotometer (CM-2500d, Minolta Co. Ltd, Japan) and total color difference (ΔE) were determined following

Singha and Muthukumarappan (2016). The *L** value indicates the lightness or brightness, 0–100 representing darkness to lightness. The *a** value gives the degree of the red-green color, with a higher positive *a** value indicating more red color. The *b** value indicates the degree of the yellow-blue color, with a higher positive *b** value indicating more yellowness.

6.3.3.4 Water absorption and solubility indices

Extrudates were ground to fine powders using a coffee grinder (Black & Decker ® Corporation, Towson, MD, USA). The ground extrudates (2.5 g) was suspended in distilled water (30 mL) in a tarred 50 mL centrifuge tube. The suspension was stirred intermittently and centrifuged at 3000×*g* for 10 min. The supernatant was decanted into a tarred aluminum cup and dried at 135 ^{o}C for 2 h. The weight of the gel remaining in the centrifuge tube was measured. The water absorption index (WAI, g/g) and water solubility index (WSI, %) were calculated as mentioned by (Singh and Muthukumarappan, 2016).

6.3.3.5 Total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF)

Total dietary fiber was measured in the laboratory by the AOAC approved method 991.43 (AOAC, 1992). Grounded extrudate samples were suspended in duplicate in MES/TRIS buffer (0.05 M, pH 8.2 at 24°C) and incubated sequentially with, (i) heat-stable α-amylase (95-100°C, 30 min) to favor gelatinization, hydrolysis and de-polymerization of starch, (ii) protease (60°C, 30 min) to solubilize and depolymerize proteins, and (iii) amyloglucosidase (60°C, 30 min, pH 4.5) to hydrolyze starch fragments to glucose. The enzyme digest was then treated with four volumes of 95%

ethanol (1 h) to precipitate soluble fiber. The alcohol-treated digest was filtered through borosilicate sintered glass crucibles (40-90 μm) that had been previously matted with celite, dried, and weighed. The total dietary fiber residue present in the crucible was washed with alcohol and acetone, dried overnight (105°C), and weighed. One duplicate from each sample was used for ash determination (650°C in muffle furnace) and the other for protein determination. Total dietary fiber percent (TDF, g/100g DW) was calculated as:

$$TDF = \frac{\frac{R_1 + R_2}{2} - p - A - B}{\frac{m_1 + m_2}{2}} \times \frac{100}{DW} \times 100 \quad (6.1)$$

where, R_1 and R_2 are the residue weights (g) from m_1 and m_2, respectively, m_1 and m_2 are the weights (g) of duplicate samples, A is the ash weight (g) from R_1, p is the protein weight (g) from $_{R2}$, B is the blank and DW is percentage dry weight (g) of sample.

$$B = \frac{BR_1 + BR_2}{2} - BP - BA \quad (6.2)$$

where, BR_1 and BR_2 are the weights (g) of blank residues, BP is the weight (g) of protein from BR_1 and BA is the weight (g) of ash from BR_2.

To determine the soluble dietary fiber (SDF) and insoluble dietary fiber (IDF), duplicate samples were incubated with enzymes as described earlier. IDF was filtered and then residue was washed with warm distilled water. Combined solution of filtrate and water washings were precipitated with 4 volumes of 95% ethanol for SDF determination. Both SDF and IDF residues were corrected for protein, ash and blank, for the final calculation of SDF and IDF values using Equation (6.1) and Equation (6.2).

6.3.4 Experimental design and statistical analysis

Response surface methodology (RSM) was adopted in the design of experimental combinations. The central composite rotatable design consisted four numerical independent variables (Table 6.2). The variables had values of: X_1 (percentage of FDDG) = 0, 5, 10, 15 and 20; X_2 (barrel and die temperature) = 100, 110, 120, 130 and 140°C; X_3 (screw speed) = 100, 125, 150, 175 and 200 rpm; and X_4 (moisture content) = 14%, 15.5%, 17%, 18.5% and 20%. Three replicates were taken (optional) at the design center (0, 0, 0) and the total number of observations were 27 [24 (not center points) and 3 (center points)]. The experimental design and the codes for the processing variables have been reported in Table 6.3. The responses studied were expansion ratio, bulk density, color parameters and overall color changes, water absorption index, water solubility index and total dietary fiber. The goodness of the fit and the significance of linear, quadratic and interaction effects of each factor on the responses were examined by performing analysis of variance (ANOVA). Statistical analysis was conducted using Design-Expert 8.0.7.1 (Statease, Minneapolis, MN, USA). Pearson's correlation coefficient (r) was also applied to establish specific correlations using SPSS (16.0) statistical software.

6.4 Results and Discussion

The chemical composition of the garbanzo flour, FDDG and corn grits are given in Table 6.1. The reported values are means of triplicate samples with standard deviations. Figure 6.1 shows the images of some of the extruded snacks.

6.4.1 Expansion ratio

The expansion ratio (ER) of the extruded snacks ranged between 1.45 and 3.56 with the adequate precision of 7.617 and R^2 of 0.8314. The highest value of ER was obtained with a FDDG:GF ratio of 5:35 at 15.5 % moisture content, 130 °C temperature and 175 rpm screw speed, whereas the lowest value was with 10:30 at 17 % moisture content, 120 °C temperature and 150 rpm screw speed. The equations for quadratic models employed to predict the ER of the extrudates are given in Table 6.4 and Table 6.5. Table 6.6 shows the ANOVA of the response function ER in terms of the coded variables. The results indicated that the feed moisture content had linear effect and FDDG had quadratic effect on the expansion ratio, whereas the screw speed had both the linear and quadratic terms.

As illustrated in Figure 6.2(a), the expansion ratio was high at low moisture content and decreased as the moisture content in the blends increased. According to Oke et al. (2012), drag force increases when moisture content decreases and therefore exerts more pressure at the die resulting in greater expansion of extrudate at the exit. The increase in expansion at higher screw speed can be attributed to the shearing effect of the screw which causes protein molecules to be stretched farther apart, weakening bonds and resulting in a puffer product (Filli et al., 2012). In addition to that, shearing effect causes starch to gelatinize which favored expansion (Chinnaswamy and Hanna, 1988).

Initially, with increase in the level of the FDDG in the blends the expansion ratio of the extrudates decreased (Figure 6.2(b)). This could be due to the overall increase in the protein to starch ratio in the blends which also affect the starch gelatinization. On further increase in the FDDG level (beyond 10%), there was an increase in the ER. This

increase was more pronounced at higher screw speed (more than 175 rpm). The extrudates' expansion is also governed by the dough viscosity and elasticity. In our previous study (Singha and Muthukumarappan, 2017a), we have reported that apparent viscosity increased with increase in the FDDG level in the blends during extrusion. This increased viscosity led to lowering rate of contraction on cooling and more mechanical energy into the melt. These favors the expansion process since they do not allow the cellular matrix to collapse under high vapor pressure (Ilo et al., 1996; Moraru and Kokini, 2003). Thus, lower density products with increased bubble growth are produced.

6.4.2 Bulk density

Bulk density (BD) is the other important property of the extrudates commonly influenced by expansion phenomena occurring upon exiting the extrudates from die section (Brown et al., 2015). The bulk density of the extruded snacks ranged from 98.3 to 445.5 kg m^{-3} with the adequate precision of 13.968 and R^2 of 0.7513. The highest value of BD was obtained with FDDG:GF ratio of 5:35 at 18.5 % moisture content, 110°C temperature and 125 rpm screw speed, whereas the lowest value was with 15:25 at 15.5% moisture content, 130°C temperature and 175 rpm screw speed. Table 6.7 shows the ANOVA of the response function BD in terms of the coded variables. The first order terms of temperature, screw speed and moisture content were significant ($P<0.05$). The resulting polynomial after removing the non-significant terms are shown in Table 6.4 and Table 6.5.

The product’s BD increased with increasing feed moisture, whereas decreased with increasing screw speed and barrel temperature. This has also been reported by Meng et al. (2010). Decrease in the BD with increase in extrusion temperature was also

observed during extrusion of blends of rice and chickpea flours (Bhattacharya and Prakash, 1994). An increase in the barrel temperature will increase the degree of superheating of water in the extruder encouraging bubble formation. This will result in decrease in melt viscosity as reported in our previous study (Singha and Muthukumarappan, 2017a) leading to reduced density. Response surface plot in Figure 6.2(c) shows the effect of screw speed and moisture content on the BD. Increase in screw speed resulted in an extrudate with lower density. Higher screw speeds may be expected to lower melt viscosity of the mix (Fletcher et al., 1985) increasing the elasticity of the dough, resulting in a reduction in the density of the extrudate. Increase in screw speed increased product expansion (and hence bulk density decreased) during extrusion of rice meal (Lee and McCarthy, 1996). Increase in feed moisture content resulted in increase in BD of the corn-based extrudates. Ding et al. (2005) studied the effect of extrusion conditions on physicochemical properties of rice based snacks and feed moisture was found to be main factor affecting the extrudate expansion.

6.4.3 Color parameters and total color change

Color is an important characteristic of extruded foods. Color changes during the extrusion process can provide important information about the degree of thermal treatment (Chen et al., 1991). Multiple linear regression equations for color parameters (L^*, a^* and b^* values) and total color change (ΔE) are shown in Table 6.4 and Table 6.5.

The ANOVA for the L^* are shown in Table 6.8. FDDG had significant linear and quadratic ($P<0.05$) effect, and temperature had significant quadratic effect ($P<0.05$) on the L^* values. Interaction effects between FDDG and temperature, and temperature and screw speed were also found to be significant ($P<0.05$), as confirmed in the ANOVA

(Table 6.8). The lightness (L^*) values varied from 64.69 to 91.25%. The L^* value decreased with increase in FDDG content in the blends. It is known that reducing sugars and proteins (amino acids) in foods can react under high processing temperatures to promote non-enzymatic browning (Maillard reaction), which results in darkening of the final product. Corn grits are high in sugars (Wang and Ryu, 2013) and both garbanzo flour and FDDG are high in protein (amino acids). Therefore, the observed decrease in brightness may be attributed to the Maillard reaction. Reduction in whiteness, as evidenced in decrease in L^*values, indicates darker samples.

The ANOVA for a^* (redness) are shown in Table 6.9. Redness 'a^*' of the extruded samples ranged from 4.05 to 6.57. Screw speed and moisture content significantly ($P<0.05$) affected redness (Table 6.9). Redness increased as the moisture content increased, which confirms that whiteness decreased. Redness in food relates to loss of whiteness (Iwe et al., 2000).

Yellowness values (b^*) of extruded samples ranged from 32.79 to 41.88. The ANOVA for b^* (redness) are shown in Table 6.9. Screw speed had a significant effect on yellowness. Yellowness increased as the screw speed increased. Shearing effect increases with increase in the screw speed and this may favor formation of colored compounds.

Color difference (ΔE) was used to represent the color change between the blends and the extrudates. The ANOVA for ΔE are shown in Table 6.8. Screw speed had a significant linear effect ($P<0.05$) and temperature had a significant quadratic effect ($P<0.05$) on the color difference. The response surface plot for color changes with temperature and FDDG is represented in Figure 6.2(d). Since, snack products containing garbanzo flour, FDDG and corn grits are not common in the market place, there might

not be a standard value for color development of an acceptable snack made from these ingredients. The color data is an important value for future product development of snacks containing garbanzo flour, FDDG and corn grits.

6.4.4 Water absorption index

The water absorption index (WAI) is related to the degree of starch gelatinization (Ding et al., 2005). It measures the volume occupied by the starch after swelling in excess water, which maintains the integrity of starch in aqueous dispersion (Mason and Hoseney, 1986). Multiple linear regression equations for WAI are shown in Table 6.4 and Table 6.5. Table 6.6 shows the ANOVA of the response function WAI in terms of the coded variables. The coefficient estimates of WAI model showed that the first order term of screw speed and the second-order term of FDDG were significant ($P<0.05$). An interaction effect of FDDG and screw speed was also found to be significant ($P<0.05$).

In this study, the WAI was in the range of 5.02 and 6.97 g/g dry solid with the adequate precision 7.944 of and R^2 of 0.8614. The effect of GF: FDDG and screw speed on WAI is shown in Figure 6.3(a). It was interpreted that there is decrease in WAI at high screw speed. At low screw speed with increase in FDDG level the WAI increased. This could be due to increased availability of fiber in the FDDG which has higher water absorption capacity. A decrease in WAI with increase in screw speed has been reported in the literature during extrusion soy white flakes-based blends (Singh and Muthukumarappan, 2016). High shear rate (high screw speed) reduces the availability of undamaged polymer chains. As a result, the availability of hydrophilic groups which can bind water molecules are low which is responsible for lower values of WAI.

6.4.5 Water solubility index

The water solubility index (WSI) measures the amount of soluble component released from starch after extrusion (Ding et al., 2006) and is often used as an indicator of degradation of molecular components. Multiple linear regression equations for WAI are shown in Table 6.4 and Table 6.5. Table 6.6 shows the ANOVA of the response function WSI in terms of the coded variables. The coefficient estimates of WSI model showed that the first order term of screw speed and the second-order term of FDDG were significant ($P<0.05$).

The WSI ranged from 7.85 to 14.75 with the adequate precision of 6.381 and R^2 of 0.8192. Maximum WSI was recorded at moisture content of 18.5%, temperature 130°C and screw speed 175 rpm. The WSI value significantly ($P<0.05$) increased with increase in the extrusion speed and at very low level of FDDG (Figure 6.3(b)). The increase of screw speed induced a sharp increase of specific mechanical energy. The high mechanical shear degraded macromolecules, so the molecular weight of starch granules decreased. Consequently, the WSI increased because starch granules were then more soluble in water (Smith, 1992). Mezreb et al. (2003) found that the WSI increased significantly when screw speed was increased from 200 to 300 rpm for wheat extrudates and from 300 to 500 rpm for corn extrudates.

6.4.6 Total dietary fiber

Multiple linear regression equations for the total dietary fiber (TDF) are shown in Table 6.4 and Table 6.5. ANOVA in Table 6.7 confirms that the model is significant ($P<0.001$) with insignificant lack of fit ($P>0.05$). Good coefficient of determination

(R^2=0.9605) was obtained with adequate precision of 43.874. The coefficient estimates of the TDF model showed that only the first order term of FDDG was significant (P<0.05).

The TDF ranged between 4.53 and 11.24 (g/100 g DW) % in the extruded snacks. The response surface plot in Figure 6.3(c) depicts the variation in TDF with changes in FDDG content and temperature. The TDF content of garbanzo flour and FDDG was 19.75 g/100 g and 44.73 g/100g, respectively. Hence, contribution of TDF from FDDG was more than GF. Extrudates with higher level of FDDG had higher percentage of TDF content. The FDDG level also had significant effect (P<0.050 on the SDF and IDF (results not shown). An increase in SDF in the extruded products was observed compared to those in the raw blends. The SDF in the raw blends ranged between 0.97 to 1.37 (g/100 g DW) and in the extruded products ranged between 2.06 and 5.54. (g/100 g DW). On the other hand, there was a slight decrease in the IDF of the extruded products. The IDF in the raw blends ranged between 3.3 to 11.6 (g/100 g DW) and in the extruded products ranged between 1.78 and 5.7 (g/100 g DW).

6.4.7 Relationship between the properties

The degree of puffing of the extrudates as it exits the die nozzle can be estimated from the ER and BD values. While expansion ratio considers expansion only in the direction perpendicular to the extrudate flow, bulk density considers expansion in all directions (Falcone and Phillips, 1988). Gujska and Khan (1991) suggested that the degree of expansion affects the density, fragility and overall texture of extruded products (Anton et al., 2009). In our study, negative correlation (r= -0.794, P<0.01) was found between ER and BD. Negative correlation between ER and BD have been reported by many researchers during extrusion cooking (Chevanan et al., 2007). The WAI and WSI

were found to have a strong negative correlation (r= -0.936, P<0.01). A negative correlation was found between TDF and *L** (r= -0.622, P<0.01). Our results showed that with increase in the FDDG level in the extruded products the lightness (*L**) value decreased, whereas the TDF of the extrudates increased. Total color change had a negative correlation (r= -0.697, P<0.01) with yellowness whereas a positive correlation (r= 0.544, P<0.01) was found with redness.

6.4.8 Optimization and model verification

The independent variables were optimized numerically using Design Expert v8. FFDG level was kept maximum and the remaining independent variables were kept in range during optimization. The goals were assigned to each response parameters. The WAI and WSI were kept in range; the TDF and ER were at maximum. The optimization resulted in 55 solutions and the top 10 solutions are shown in Table 6.10. Validation of the predicted responses was carried out by extruding the blends at three different optimum conditions as shown in Table 6.10. To check the variability of the predicted responses, two-tailed, one sample t -test was carried out. Good agreement was found between the predicted and experimental values. Results of the t -test demonstrated no significant difference between the values of recorded responses and the predicted responses. The experimental values for solution #2 were very close to the predicted values. The optimum condition was: 20% FDDG, 139.67^{o}C barrel temperature, 166.50 rpm screw speed and 18.98% moisture content. The extruded snacks produced at the optimum condition had a TDF content of 11.04 g/100 g DW and a crude protein content of 15.91%.

6.5 Conclusions

A corn-based extruded snack containing varying percent of FDDG and garbanzo flour has been produced. Product properties studied were expansion ratio, bulk density, water solubility and absorption indices, color parameters and total color change, and total dietary fiber. FDDG level in the blends had significant effect on the total dietary fiber, expansion ratio, lightness value, and water solubility and absorption indices. Extrusion screw speed and moisture content of the blends had significant effect on the expansion ratio. Strong negative correlation was found between expansion ratio and bulk density, and between water absorption and water solubility indices. Optimization of the processing conditions to achieve high expansion ratio and total dietary fiber content in the extruded snacks was done. The optimized combinations of independent variables found were FDDG (20%), extrusion temperature (~140^{o}C), screw speed (~166 rpm) and feed moisture content (~19%). Based on this study, it was clearly shown that some physicochemical and nutritional properties of the extruded snacks could be significantly influenced by raw materials and extrusion processing conditions. Nutritious high-protein high-fiber extruded snacks were developed. This study provides a new research on the physico-chemical properties of corn-based extruded snacks containing FDDG and garbanzo flour.

Table 6.1 Chemical composition of the raw materials before the extrusion process.

Component(g/100g)	**FDDG**	**GF**	**CG**
Moisture	0.70±0.03	7.75±0.07	11.46±.01
Protein	35.12±0.11	22.52±0.09	6.0±0.04
Fat	0.53±0.05	5.94±0.11	1.5±0.08
Ash	1.24±0.06	2.7±0.14	2.0±0.16
Nitrogen free extract	54.18±0.09	54.63±0.05	78.14±0.07
Total dietary fiber	44.73±0.76	22.3±1.20	0.83±0.49
Soluble	1.4 ±1.06	3.4±1.47	0.02±1.73
Insoluble	44.33±0.96	18.9±1.08	0.81±1.18

FDDG – Distiller's dried grains processed for food application; GF - garbanzo flour; CG - corn grits

Values in the column are mean ± SD (n=3)

Table 6.2 Independent numerical and categorical variables and their levels.

Numerical variables	**Symbol**	**Coded variable levels**				
		-2	**-1**	**0**	**1**	**2**
FDDG (%)	X_1	0	5	10	15	20
Temperature (°C)	X_2	100	110	120	130	140
Screw speed (rpm)	X_3	100	125	150	175	200
Moisture content (% wb)	X_4	14.0	15.5	17.0	18.5	20.0

FDDG – Distiller's dried grains processed for food application; wb – wet basis

Table 6.3 Experimental design layout.

Run	Coded variables				Actual variables			
	x_1	x_2	x_3	x_4	X_1 (%)	X_2 (%)	X_3 (°C)	X_4 (rpm)
1	-1	1	-1	1	5	18.5	110	175
2	1	1	1	1	15	18.5	130	175
3	0	0	0	2	10	17.0	120	200
4	1	1	-1	1	15	18.5	110	175
5	-1	1	-1	-1	5	18.5	110	125
6	0	0	2	0	10	17.0	140	150
7	-1	-1	-1	-1	5	15.5	110	125
8	1	-1	-1	-1	15	15.5	110	125
9	2	0	0	0	20	17.0	120	150
10	0	-2	0	0	10	14.0	120	150
11	1	1	1	-1	15	18.5	130	125
12	0	0	0	-2	10	17.0	120	100
13	-1	-1	1	1	5	15.5	130	175
14	0	2	0	0	10	20.0	120	150
15	0	0	0	0	10	17.0	120	150
16	1	-1	1	-1	15	15.5	130	125
17	0	0	0	0	10	17.0	120	150
18	0	0	-2	0	10	17.0	100	150
19	-1	1	1	-1	5	18.5	130	125
20	1	1	-1	-1	15	18.5	110	125
21	-1	-1	-1	1	5	15.5	110	175
22	-2	0	0	0	0	17.0	120	150
23	1	-1	-1	1	15	15.5	110	175
24	-1	1	1	1	5	18.5	130	175
25	-1	-1	1	-1	5	15.5	130	125
26	1	-1	1	1	15	15.5	130	175
27	0	0	0	0	10	17.0	120	150

Table 6.4 Best-fit response surface models in terms of coded variables after excluding the insignificant terms for ER, BD, L*, a*, b*, ΔE, WAI, WSI and TDF.

Parameters	**Response surface model**	R^2	***P* value**
ER	$Y_{ER} = 1.81 + 0.21x_3 - 0.34x_4 + 0.27x_1^2 + 0.17x_3^2$	0.8314	0.0084
BD (kg/m^3)	$Y_{BD} = 212.21 - 41.88x_2 - 29.42x_3 + 58.07x_4$	0.7513	<0.0001
L*	$Y_{L^*} = 76.34 - 4.28x_1 + 2.99x_1x_2 + 1.82x_2x_3 + 2.13x_1^2 - 1.75x_2^2$	0.8979	0.0006
a*	$Y_{a^*} = 6.77 - 0.7x_3 + 0.67x_4$	0.4463	0.0089
b*	$Y_{b^*} = 31.47 + 2.67x_3$	0.5336	0.0016
Δ*E*	$\Delta E = 3.80 - 1.61x_3 + 2.09x_1x_3 + 1.94x_2^2$	0.7727	0.0354
WAI (g/g)	$Y_{WAI} = 5.64 - 0.46x_3 - 0.20x_1x_3 + 0.17x_1^2$	0.8614	0.0031
WSI (%)	$Y_{WSI} = 11.88 + 1.50x_3 - 0.64x_1^2$	0.8192	0.0119
TDF (%)	$Y_{TDF} = 8.19 + 1.46x_1$	0.9605	<0.0001

ER = expansion ratio, BD = bulk density, L* = lightness, a* = red-green color, b* = yellow-blue color, ΔE = total color difference, WAI = water absorption index, WSI = water solubility index, L* = lightness, TDF = total dietary fiber

Table 6.5 Best-fit response surface models in terms of actual variables after excluding the insignificant terms for ER, BD, L*, a*, b*, ΔE, WAI, WSI and TDF.

Parameters	**Response surface model**	R^2	***P* value**
ER	$Y_{ER} = 53.60 - 0.13X_3 - 2.84X_4 + 0.01X_1^2 + 0.0003X_3^2$	0.8314	0.0084
BD (kg/m^3)	$Y_{BD} = 257.41 - 4.19X_2 - 1.18X_3 + 38.71X_4$	0.7513	<0.0001
L*	$Y_{L^*} = -77.69 - 4.88X_1 + 0.06X_1X_2 + 0.007X_2X_3 + 0.085X_1^2 - 0.017X_2^2$	0.8979	0.0006
a*	$Y_{a^*} = 7.08 - 0.03X_3 + 0.45X_4$	0.4463	0.0089
b*	$Y_{b^*} = 20.56 + 0.11X_3$	0.5336	0.0016
Δ*E*	$\Delta E = 236.32 - 0.10X_3 + 0.02X_1X_3 + 0.02X_2^2$	0.7727	0.0354
WAI (g/g)	$Y_{WAI} = 14.18 - 0.14X_3 - 0.0016X_1X_3 + 0.0067X_1^2$	0.8614	0.0031
WSI (%)	$Y_{WSI} = 60.23 + 0.285X_3 - 0.025X_1^2$	0.8192	0.0119
TDF (%)	$Y_{TDF} = 4.84 + 0.29X_1$	0.9605	<0.0001

ER = expansion ratio, BD = bulk density, L* = lightness, a* = red-green color, b* = yellow-blue color, ΔE = total color difference, WAI = water absorption index, WSI = water solubility index, L* = lightness, TDF = total dietary fiber

Table 6.6 Analysis of variance for expansion ratio, water absorption index and water solubility index.

		Expansion ratio				Water absorption index				Water solubility index			
Source	**df**	**SS**	**MS**	***F*-value**	***P*-value**	**SS**	**MS**	***F*-value**	***P*-value**	**SS**	**MS**	***F*-value**	***P*-value**
Model	14	6.82	0.49	4.23	0.0084	7.96	0.57	5.33	0.0031	86.81	6.20	3.88	0.0119
X_1**- FDDG**	1	0.20	0.20	1.69	0.2174	0.18	0.18	1.67	0.2205	1.24	1.24	0.77	0.3960
X_2 **- Temperature**	1	0.53	0.53	4.56	0.0541	0.11	0.11	1.01	0.3339	0.50	0.50	0.31	0.5877
X_3 **- Screw speed**	1	1.10	1.10	9.52	0.0095	5.18	5.18	48.52	< 0.0001	54.16	54.16	33.93	< 0.0001
X_4 **- Moisture**	1	2.71	2.71	23.49	0.0004	0.02	0.02	0.14	0.7117	0.26	0.26	0.16	0.6936
X_1X_2	1	0.11	0.11	0.91	0.3578	0.04	0.04	0.35	0.5672	1.22	1.22	0.76	0.3998
X_1X_3	1	0.03	0.03	0.24	0.6359	0.64	0.64	5.96	0.0311	6.80	6.80	4.26	0.0614
X_1X_4	1	0.33	0.33	2.87	0.1161	0.09	0.09	0.80	0.3888	0.86	0.86	0.54	0.4772
X_2X_3	1	0.07	0.07	0.63	0.4442	0.37	0.37	3.45	0.0878	1.39	1.39	0.87	0.3699
X_2X_4	1	0.01	0.01	0.07	0.7994	0.05	0.05	0.42	0.5270	0.03	0.03	0.02	0.8934
X_3X_4	1	0.06	0.06	0.49	0.4966	0.01	0.01	0.09	0.7713	0.01	0.01	0.00	0.9549
X_1^2	1	1.51	1.51	13.14	0.0035	0.59	0.59	5.55	0.0363	8.61	8.61	5.40	0.0386
X_2^2	1	0.20	0.20	1.72	0.2144	0.02	0.02	0.21	0.6585	0.14	0.14	0.09	0.7691
X_3^2	1	0.62	0.62	5.36	0.0391	0.23	0.23	2.12	0.1712	1.52	1.52	0.95	0.3489
X_4^2	1	0.38	0.38	3.28	0.0953	0.12	0.12	1.10	0.3148	3.86	3.86	2.42	0.1460
Residual	12	1.38	0.12	-	-	1.28	0.11	-	-	19.15	1.60	-	-
Lack of Fit	10	1.02	0.10	0.56	0.7849	0.83	0.08	0.37	0.8830	11.63	1.16	0.31	0.9175
Pure Error	2	0.37	0.18	-	-	0.45	0.22	-	-	7.53	3.76	-	-

df, degrees of freedom; SS, sum of squares; MS, mean squares

Table 6.7 Analysis of variance for bulk density and total dietary fiber.

Source		Bulk density				Total dietary fiber			
	df	**SS**	**MS**	***F*-value**	***P*-value**	**SS**	**MS**	***F*-value**	***P*-value**
Model	4	147323.04	36830.76	16.62	< 0.0001	51.05	12.76	133.77	< 0.0001
X_1**- FDDG**	1	3534.26	3534.26	1.59	0.2199	51.01	51.01	534.71	< 0.0001
X_2 **- Temperature**	1	42085.85	42085.85	18.99	0.0003	0.02	0.02	0.19	0.6665
X_3 **- Screw speed**	1	20776.51	20776.51	9.37	0.0057	0.01	0.01	0.10	0.7606
X_4 **- Moisture**	1	80926.41	80926.41	36.51	< 0.0001	0.01	0.01	0.10	0.7556
Residual	22	48765.95	2216.63	-	-	2.10	0.10	-	-
Lack of Fit	20	42020.59	2101.03	0.62	0.7743	2.09	0.10	17.89	0.0542
Pure Error	2	6745.36	3372.68	-	-	0.01	0.01	-	-

df, degrees of freedom; SS, sum of squares; MS, mean squares

Table 6.8 Analysis of variance for lightness (L*) and color change (ΔE).

Source	df	*L**				*ΔE*			
		SS	**MS**	***F*-value**	***P*-value**	**SS**	**MS**	***F*-value**	***P*-value**
Model	14	1025.77	73.27	7.54	0.0006	339.45	24.25	2.91	0.0354
X_1**- FDDG**	1	440.50	440.50	45.32	< 0.0001	16.78	16.78	2.02	0.1810
X_2 **- Temperature**	1	10.80	10.80	1.11	0.3126	0.28	0.28	0.03	0.8586
X_3 **- Screw speed**	1	2.61	2.61	0.27	0.6135	62.51	62.51	7.51	0.0179
X_4 **- Moisture**	1	43.42	43.42	4.47	0.0562	30.82	30.82	3.70	0.0783
$X_1 X_2$	1	143.76	143.76	14.79	0.0023	24.34	24.34	2.93	0.1129
$X_1 X_3$	1	20.88	20.88	2.15	0.1684	70.07	70.07	8.42	0.0133
$X_1 X_4$	1	38.38	38.38	3.95	0.0702	9.67	9.67	1.16	0.3022
$X_2 X_3$	1	52.71	52.71	5.42	0.0382	0.38	0.38	0.05	0.8346
$X_2 X_4$	1	22.52	22.52	2.32	0.1539	0.00	0.00	0.00	0.9883
X_3X_4	1	0.30	0.30	0.03	0.8641	29.05	29.05	3.49	0.0863
X_1^2	1	97.11	97.11	9.99	0.0082	21.77	21.77	2.62	0.1317
X_2^2	1	65.26	65.26	6.71	0.0236	80.27	80.27	9.65	0.0091
X_3^2	1	0.00	0.00	0.00	0.9882	28.45	28.45	3.42	0.0892
X_4^2	1	7.58	7.58	0.78	0.3946	1.74	1.74	0.21	0.6559
Residual	12	116.63	9.72	-	-	99.86	8.32	-	-
Lack of Fit	10	113.97	11.40	8.56	0.1091	98.25	9.83	12.21	0.0780
Pure Error	2	2.66	1.33	-	-	1.61	0.80	-	-

df, degrees of freedom; SS, sum of squares; MS, mean squares

Table 6.9 Analysis of variance for redness (a*) and yellowness (b*) values.

Source		*a**				*b**			
	df	**SS**	**MS**	***F*-value**	***P*-value**	**SS**	**MS**	***F*-value**	***P*-value**
Model	4	26.39	6.60	4.43	0.0089	185.91	46.48	6.29	0.0016
X_1**- FDDG**	1	2.20	2.20	1.48	0.2373	8.39	8.39	1.14	0.2980
X_2 **- Temperature**	1	1.68	1.68	1.13	0.2993	0.00	0.00	0.00	0.9840
X_3 **- Screw speed**	1	11.61	11.61	7.80	0.0106	170.51	170.51	23.09	< 0.0001
X_4 **- Moisture**	1	10.90	10.90	7.32	0.0129	7.01	7.01	0.95	0.3405
Residual	22	32.74	1.49	-	-	162.47	7.39	-	-
Lack of Fit	20	32.36	1.62	8.50	0.1104	150.21	7.51	1.23	0.5436
Pure Error	2	0.38	0.19	-	-	12.26	6.13	-	-

df, degrees of freedom; SS, sum of squares; MS, mean squares

Table 6.10 Solutions for optimum conditions and validation.

Solution #	FDDG (%)	Temperature (^{o}C)	Screw Speed (rpm)	Moisture (% wb)	ER (-)	WAI (-)	WSI (%)	TDF (g/100 DW)
1	20.00	139.92	152.16	19.91	3.57	5.57398	13.2378	11.20
2	20.00	139.67	166.50	18.98	3.56	5.50571	13.2327	11.18
3	20.00	134.55	174.44	20.00	3.88	5.20295	14.6448	11.17
4	20.00	140.00	183.35	19.31	4.14	5.2299	14.7463	11.17
5	20.00	138.60	171.78	18.66	3.56	5.48542	13.2106	11.17
6	20.00	139.93	181.48	18.51	3.85	5.36924	13.8214	11.16
7	20.00	137.57	192.05	19.00	4.22	5.19593	14.7452	11.15
8	20.00	130.46	184.11	19.76	3.92	5.09722	14.7443	11.15
9	20.00	137.35	119.60	14.11	3.56	6.9102	7.85184	11.14
10	20.00	137.98	121.15	14.00	3.60	6.80709	8.19708	11.14
Validation								
2	20.00	139.67	166.50	18.98	4.36	5.28	14.74	11.04
3	20.00	134.55	174.44	20.00	4.01	5.27	14.75	10.57
8	19.98	130.46	184.11	19.76	3.54	6.33	9.65	11.03

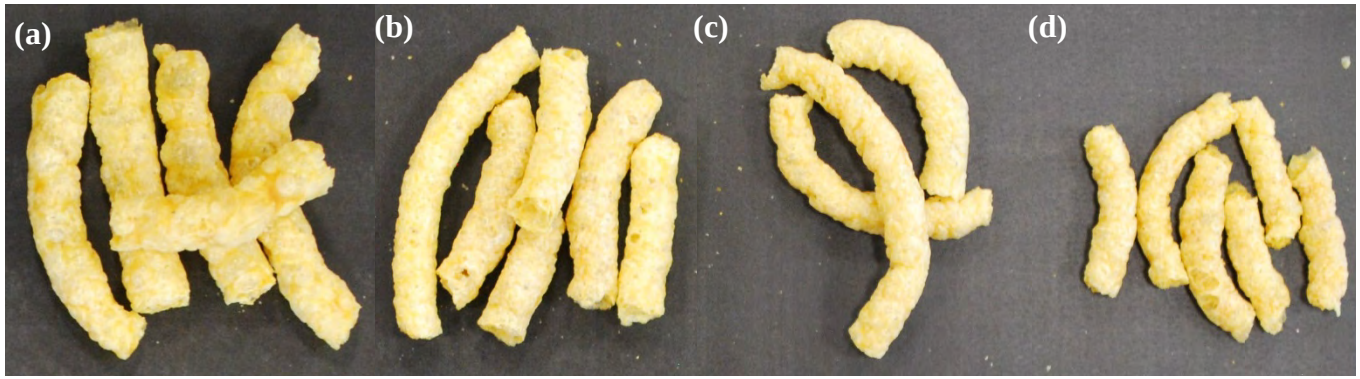

Figure 6.1 Extruded corn-based snacks containing (a) 5% FDDG, (b) 10% FDDG, (c) 15% FDDG, and (d) 20% FDDG

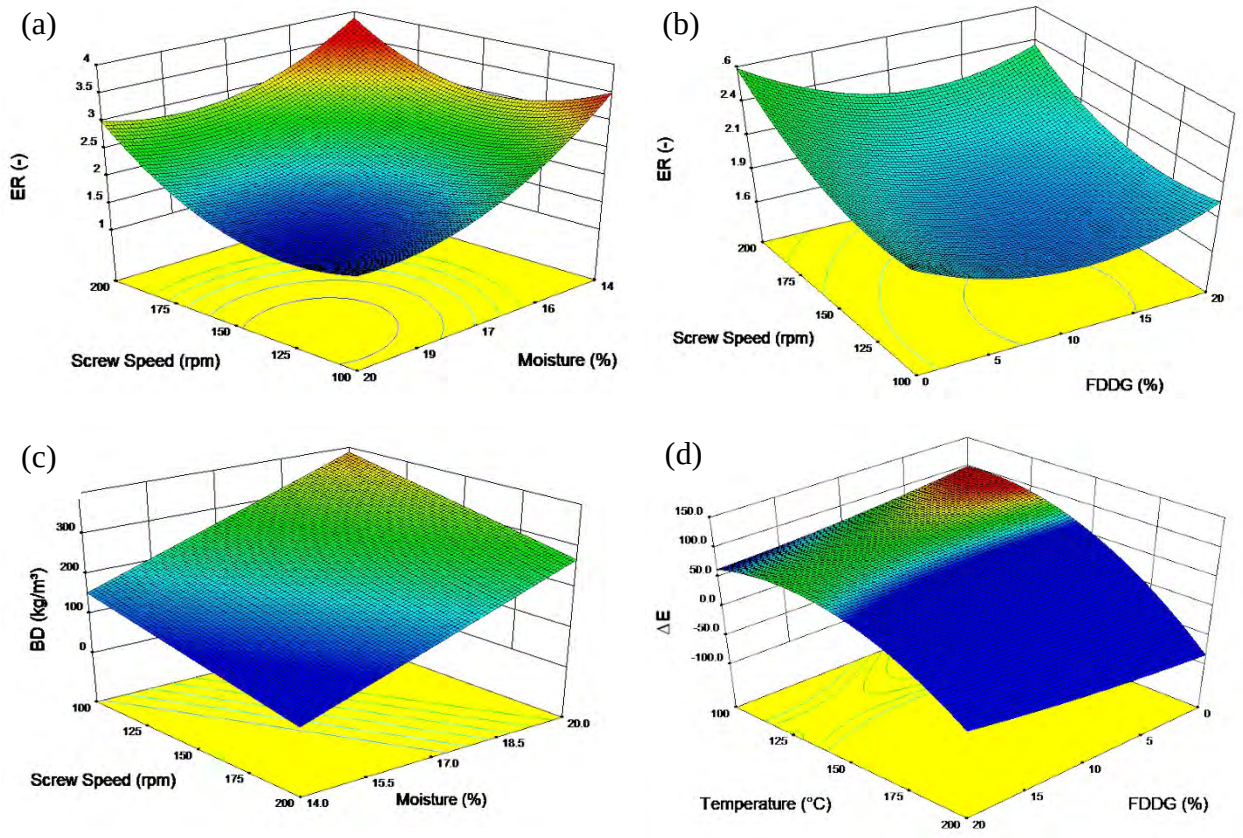

Figure 6.2 Response surface graphs illustrating the effects of (a) screw speed and moisture on the ER, (b) screw speed and FDDG on the ER, (c) temperature and moisture content on the BD, and (d) temperature and FDDG on the color change of the extruded snacks

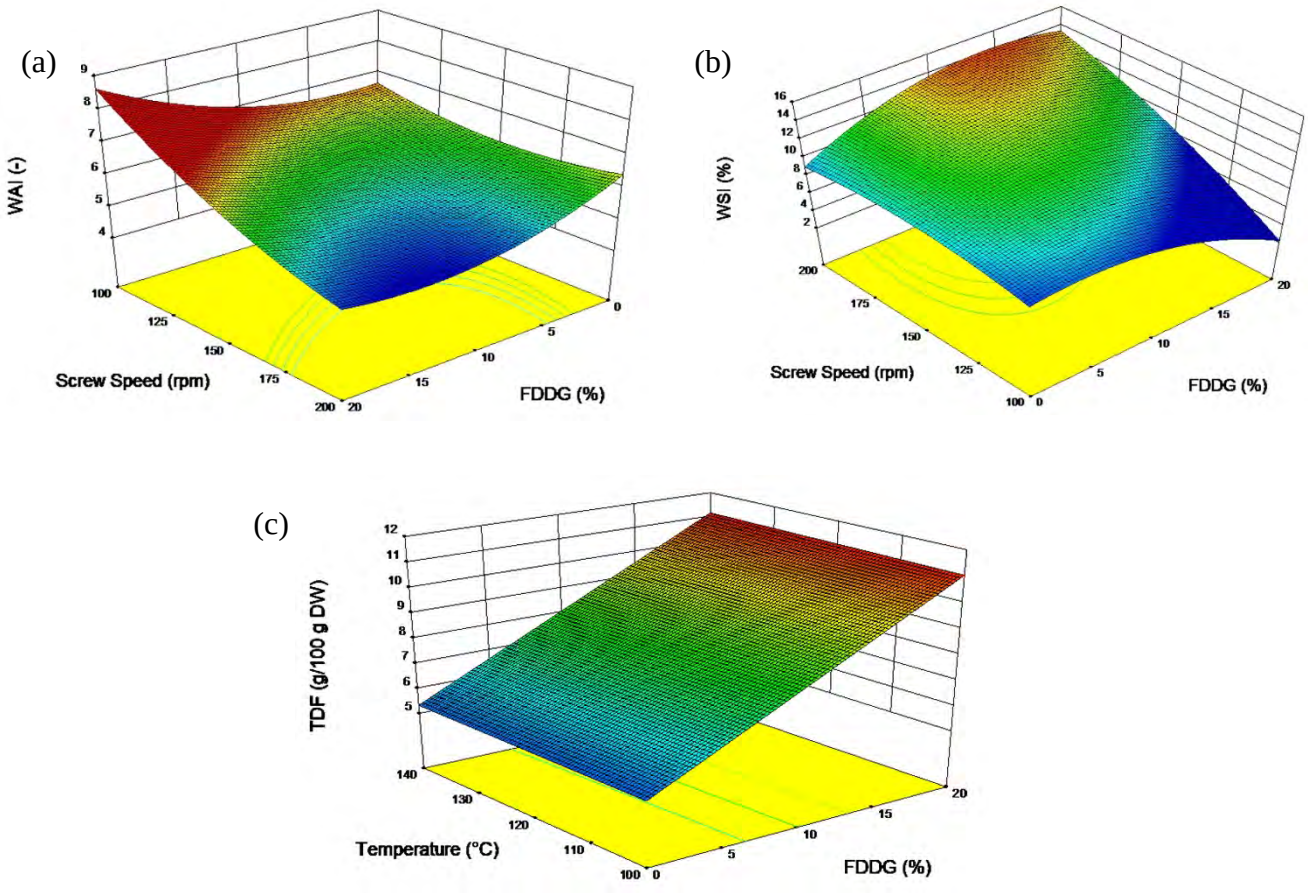

Figure 6.3 Response surface graphs illustrating the effects of (a) screw speed and FDDG on the water absorption index, (b) screw speed and FDDG on the water solubility index, and (c) FDDG on the TDF of the extruded snacks

CHAPTER 7

Twin Screw Extrusion of Soy Flour and Corn Grits Blends Enriched with Apple Pomace: Extrudate Characteristics and Determination of Optimum Processing Conditions

7.1 Abstract

The apple pomace-defatted soy flour-corn grits blends were extruded in a conical counter rotating twin-screw extruder. Response surface methodology using a central composite design was used to evaluate the effects of independent variables, namely apple pomace level (0-20%), die and barrel temperature (100-140^{o}C), screw speed (100–200 rpm) and feed moisture content (14-20% wet basis) on the product responses (expansion ratio, bulk density, water absorption index, water solubility index, textural properties, color parameters, total phenolic content and antioxidant activity). The product responses were most affected by changes in moisture content, pomace level and to a lesser extent by screw speed. Incorporation of apple pomace significantly enhanced the antioxidant properties of the extruded snacks. The optimum conditions to develop acceptable snacks were found to be a pomace level of 15%, extrusion temperature of 110^{o}C, screw speed of 175 rpm screw speed and feed moisture content of 15.5%. Structural observation of the extruded snacks was also conducted. Apple pomace level and processing conditions significantly affected the internal structure of the extrudates. Porous structure exhibited crispness and compact structure indicated dense product. FTIR analysis was carried out to understand the changes in the polysaccharide and amide bonds in the extrudate snacks. The results indicate that acceptable extrudates can be produced from blends of apple pomace, defatted soy flour and corn grits.

7.2 Introduction

Extrusion is a high-temperature short time process in which final product is obtained by a combination of several unit operations such as heating, mixing, shearing and forcing the material through a die in a single step (Singh and Muthukumarappan, 2017b). The thermomechanical action during extrusion brings about gelatinization of starch, denaturation of protein and inactivation of enzymes, microbes and many anti-nutritional factors; all this occurs in a shear environment, resulting in a plasticized continuous mass (Bhattacharya and Prakash, 1994). The rate and extent of heating, mixing, shearing, and compressing of the materials inside the barrel, and subsequently the die, is strongly related to the properties of the raw materials and process conditions used (Singh and Muthukumarappan, 2017a).

Cereals such as corn are gluten free, easily accessible and have a high starch content, which can give excellent expansion characteristics. For this reason, corn in different form has been widely used as raw material for extrusion (Singha and Muthukumarappan, 2017a). Most cereal-based snack products consumed by children are made from corn (Pastor-Cavada et al., 2011). Combining corn with legumes increases both the amount and quality of the protein (Young, 1991). Legumes such as soybean are rich source of protein that can be used to improve the diet of millions of people. Typically, on a wet basis, stored mature soybeans contains 13% water and hence it contains about 35% protein, 17% oil, 31% carbohydrate, and 4.4% ash (Liu, 1997). Soybean in different forms (Bookwalter et al., 1971; Chiang, 2007; Iwe et al., 2001a; Lobato et al., 2011; Singh and Muthukumarappan, 2014a; Singh and Muthukumarappan, 2016; Yu et al., 2014) have been extensively used as a major ingredient during food and

feed extrusion. Addition of soybean can act as a good source of protein in formulated food products besides offering other functional, nutritional and health benefits (Friedman and Brandon, 2001). The defatted soybean flour, a by-product of oil processing industry, has been used to increase the protein content of the extruded snacks (Alam et al., 2016).

Apple pomace (AP) is a press residue obtained after the extraction of juice from apple. It is rich in cell wall residues which are not digested by enzymes in the human body. These are classified as dietary fiber (Hwang et al., 1998). It is also composed of carbohydrates, small amounts of protein, fat and ash (Sudha et al., 2007). AP is also a good source of phytochemicals primarily phenolic acids and flavonoids. The common applications of this by-product are the direct disposal to soil in a landfill and for extraction of pectins (gelling agent and stabilize). It has also been incorporated in bakery products (bread, muffins, and cookies) (Masoodi et al., 2002; Min et al., 2010; Wang and Thomas, 1989) to achieve more desirability than those baked with bran. Still the very large volumes produced each year exceed existing uses and new applications for AP are required. It may find use as a valuable food ingredient due to the high levels of fiber and antioxidants it contains.

Apple pomace has been combined with other flours such as rice and corn (Mir et al., 2015; O'Shea et al., 2013; Reis et al., 2014) in extrusion. In our previous study, using single screw extruder we extruded blends of defatted soy flour, apple pomace and corn grits for the development of a healthy snacks. Since most snack food companies use twin screw extruder for manufacturing snacks, we extruded the blends using conical counter rotating twin screw extruder.

The objectives of this study were, (a) to assess the product quality characteristics of the extruded snack such as bulk density, expansion ratio, color, water absorption and solubility indices, texture and internal structure, and (c) to optimize both the process and the formulation to create extrudates with maximum expansion ratio, crispness and antioxidant properties.

7.3 Materials and Methods

7.3.1 Raw materials and blend preparation

Apple pomace (AP) powder was obtained from Tree Top, Inc. (Selah, WA) and was stored at -18°C until use. The initial moisture content of AP powder was 10.08% (wet basis). The proximate composition of the AP powder was: 4.14% protein, 2.79% fat, 2.00% ash, 24.73% total fiber and 0.20% nitrogen free extract (NFE, dry basis). Corn grits (CG) was obtained from Bob's Red Mill (Milwaukie, OR). The initial moisture content of CG was 11.46% (wet basis). The proximate composition of the CG was: 6.78% protein, 1.69% fat, 2.26% ash, 1.02% total fiber and 81.80% NFE (dry basis). Defatted soy flour (DSF) was obtained from Hodgson Mill, Inc. (Effingham, Il). The initial moisture content of DSF was 7.75% (wet basis). The proximate composition of the DSF powder was: 53.94% protein, 0.77% fat, 6.87% ash, 2.93% total fiber and 26.09% NFE (dry basis). The different ingredients i.e. AP, DSF and CG were mixed into five different compositions (Blend I to V) as shown in Table 7.1. Water was added to the blends to make 14 to 20% (wet basis) final moisture depending on the experimental runs (Table 7.2 and Table 7.3). The ingredients were mixed in a laboratory scale mixer (KitchenAid Professional 5 Plus, Troy, Ohio, USA) for 10 minutes. For moisture stabilization, the blends were stored

overnight at ambient temperature. The moisture content of the prepared blends was determined by using the method 44-19 (AACC, 2000).

7.3.2 Extrusion processing

A C W Brabender conical, counter-rotating twin screw extruder was used. The general screw geometry was: length 330.7 mm, diameter 32 mm and flight depth 3.75 mm. The 15-40-002 mixing-counter rotating screw pair configuration was used. The extruder had a barrel with length to diameter ratio of 13:1. The extruder had four temperature control zones on the barrel. The pressure at the die during extrusion was measured with a pressure transducer (Brabender, NJ, USA). A thermocouple was used for the product temperature measurement at the die. All extrusion variables shown in the control panel (barrel-, screw-, die- and product-temperature, die pressure, screw speed and percent torque), were displayed and stored simultaneously every 20 s on Dell-PC computer. The product temperature during extrusion was adjusted by varying the temperatures in barrel sections, screw and die.

7.3.3 Experimental design

A central composite rotatable design of four variables at five levels each with six central point combinations was developed using Design-Expert 8.0.7.1 (Stat-Ease, Minneapolis, MN, USA). Independent variables included apple pomace level (X_1), temperature (X_2) and screw speed (X_3) and feed moisture content (X_4). Coded levels for independent variables are shown in Table 7.2. Outline of experimental design with the coded and uncoded levels is presented in Table 7.3. Response variables were system parameters such as die pressure and torque and extrudate characteristics such as expansion ratio, bulk density, WSI, WAI, hardness and color parameters.

7.3.4 Effect of extrusion processing conditions

7.3.4.1 System parameter analysis

Die pressure was measured using a pressure transducer (Brabender, USA). Readings were recorded every 20 s for at least 2 min and average values were expressed as MPa. Torque was recorded every 20 s for at least 2 min and average values were expressed as Nm.

7.3.4.2 Expansion ratio

Expansion ratio was calculated as the cross-sectional area of extrudate divided by the cross- sectional area of the die opening. An average diameter of ten samples was measured with a digital caliper to determine the expansion ratio of each set of samples.

7.3.4.3 Bulk density

Bulk density was determined as the ratio of the mass of extrudates that filled up to a given bulk volume. It was measured using a standard bushel tester (Seedburo Equipment Company, Chicago, IL) following the method recommended by USDA (USDA, 1999).

7.3.4.4 Water absorption and solubility indices

Extrudates were ground to fine powders using a coffee grinder (Black & Decker® Corporation, Towson, MD, USA). The ground extrudates (2.5 g) were suspended in distilled water (30 mL) in a tarred 50 mL centrifuge tube. The suspension was stirred intermittently and centrifuged at 3000×*g* for 10 min. The supernatant was decanted into a tarred aluminum cup and dried at 135^{o}C for 2 h. The weight of the gel remaining in the centrifuge tube was measured. The water absorption index (WAI, g/g) and water

solubility index (WSI, %) were calculated as mentioned by Singh and Muthukumarappan (2016).

7.3.4.5 Texture

The peak force as an indication of hardness was measured with a TA-XT2 Texture Analyzer (Stable Micro Systems Texture Analyzer TAXT2, Texture Technologies Corp., England) employed with a Warner Bratzler probe 12 x 7cm (HDP/BS) with maximum load of 5kg. The probe was set to move at a pre-test speed of 1.5 m/s, speed test of 2.0 m/s and speed post-test of 10 m/s. The curve was recorded and analyzed by Texture Exponent 32 software program (version 3.0). The slope (N/mm) and distance (mm) at which the extrudates breaks were measured from force–distance curve and evaluated as crispness and brittleness, respectively. Mean of ten measurements were recorded.

7.3.4.6 Color

Color of extrudates was measured using Minolta Spectrophotometer (CM-2500d, Minolta Co. Ltd, Japan) and total color difference (ΔE) was determined following Singha and Muthukumarappan (2016). The L^* value indicates the lightness, 0–100 representing dark to light color. The a^* value gives the degree of the red-green color, with a higher positive a^* value indicating more red color. The b^* value indicates the degree of the yellow-blue color, with a higher positive b^* value indicating more yellow color.

7.3.4.7 Total phenolic content

For determining the total phenolic content (TPC), samples were extracted using the method described by Khan et al. (2013). 1 g of ground extrudates was mixed with 10 ml of methanol followed by shaking at low speed for 1 h and then centrifuged at 3000×g

for 20 min. The supernatant was decanted and the residue was re-extracted as described above. The two supernatants were combined and stored at -20^{o}C until analysis for TPC. TPC of extrudates was determined using Folin-Ciocalteau method (Singleton et al., 1999) with some modification. 50 µl methanol extract of sample (i.e. the supernatant) was added with 3.5 ml distilled water and 150 µl Folin-Ciocalteau reagent. The solution was vortexed and incubated for 30 min. Thereafter, absorbance of solution was measured at 760 nm against blank. Blank solution consisted only 3.5 ml distilled water and 150 µl Folin-Ciocalteau reagent. Gallic acid was used as positive control (standard) and linear regression curve between absorbance and concentration was drawn for the standard. This standard curve with gallic acid concentration from 0 to 0.5 mg/ml was used for calculating the concentration of sample and data was expressed in mg Gallic acid equivalent (GAE)/100 g dry basis. This analysis was done in triplicate and the mean value was reported.

7.3.4.8 Antioxidant activity

Antioxidant activity (AA) was measured by the method described by Brand-Williams et al. (1995). 2,2–diphenyl- 1-picrylhydrazyl (DPPH) (Sigma, St. Louis, MO, USA) solution was prepared by adding 7.9 mg of DPPH in 200 ml ethanol. 1 g of ground extrudates was mixed with 10 ml of methanol followed by shaking at low speed for 1 h and then centrifuged at 3000×g for 20 min. The supernatant was decanted and the residue was re-extracted as described above. The two supernatants were combined and stored at -20^{o}C until analysis. 125 µl methanol extract was mixed with 2 ml ethanol and 0.5 ml of this solution was added with 3 ml DPPH. The solution was vortexed and incubated at room temperature for 30 min. Absorbance of solution and control (DPPH) was measured

at 517 nm against blank (ethanol). Results were expressed as μmol Trolox equivalent (TE)/100 g dry basis. Samples were analyzed in triplicate and the mean value was reported.

7.3.4.9 Scanning electron microscopy

Structural observation was carried using a Hitachi-S3400 N (Tokyo, Japan) scanning electron microscope (SEM) operated at 10kV. Extrudate samples cut radially and approximately 2 mm thickness were mounted on stubs and 10 nm gold was coated using a CrC-150 sputtering system set to a pressure of 5-10 millitorr. At least two samples for each treatment showing similar images were used for the results.

7.3.4.10 Infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) spectra were gathered as an average of 70 scans using a Nicolet 380 ATR-FTIR spectrophotometer (Thermo Nicolet 380) in the range 4000-700 cm^{-1} with a 1 cm^{-1} resolution. The peak position was analyzed using OMNIC software.

7.3.5 Statistical analysis

Analysis of variance (ANOVA) test was carried out using commercial statistical package, Design Expert 8.0 (State-Ease Inc., Minneapolis, USA) to determine the significance at different levels. Same software was used for the generation of response surface plots. A Pearson's correlation coefficient matrix on system parameters and product responses was carried out using SPSS 23.0 (SPSS Inc., Chicago, IL, USA) in order to determine correlation coefficient between parameters

7.4 Results and Discussion

7.4.1 Effect of extrusion processing conditions on the systems parameters

Multiple linear regression equations for die pressure are shown in Table 7.4 and Table 7.5. The experimental data fit the response equation with a high degree of significance (Table 7.6). However, the lack of fit was not significant ($P>0.05$). The results from the ANOVA indicated that the temperature and moisture content had significant linear effect ($P<0.05$) and AP level, screw speed and moisture content had significant quadratic effect ($P<0.05$) on the die pressure.

The die pressure ranged between 4 and 17 MPa. The die pressure decreased with an increase in moisture content and die temperature (Figure 7.1(a)). This phenomenon is attributed to decrease in viscosity of melt due to fragmentation of swollen and gelatinized starch granules (Singh and Smith, 1997). Increase in die temperature with increase in feed moisture affects the viscosity of mass through the extruder that leads to decrease in die pressure value. Feed moisture had a lubricating effect on molten starch inside the extruder that may affect the heat generation simultaneously with decrease in die pressure. These observations are in also agreement with the work reported by Govindasamy et al. (1997) and Singh et al. (2007). Statistical analysis revealed that die pressure was positively correlated ($r=0.656$, $P<0.01$) with torque and negatively correlated ($r=-0.634$, $P<0.01$) with bulk density (Table 7.9). These results indicate that high pressure naturally offers low density product. Increase in extruder pressure directly increases expansion while decreasing density of product. This is also evident from the positive correlation of die pressure and expansion ratio ($r=0.655$, $P<0.01$)

Multiple linear regression equations for torque are shown in Table 7.4 and Table 7.5. Table 7.6 shows the ANOVA of the response function torque. Only the temperature had significant linear effect ($P<0.05$) on the torque. The torque ranged between 11.3 and 65.4 N.m. Torque decreased with an increase in die temperature. These observations are in agreement with the work reported by Bhattacharya (1997).

7.4.2 Effect of extrusion processing conditions on the expansion ratio

Multiple linear regression equations for expansion ratio (ER) are shown in Table 7.4 and Table 7.5. ANOVA for quadratic model as fitted to experimental results of ER showed significance ($P<0.05$). However, model showed lack-of-fit was not significant ($P>0.05$) (Table 7.6). AP level had significant linear effect ($P<0.05$) and feed moisture content had significant quadratic effect ($P<0.05$) on the expansion ratio.

The ER of the extrudates ranged between 1.22 and 2.12. The highest value of ER was obtained with a AP:DSF ratio of 10:40 at 14 % moisture content, 120°C temperature and 150 rpm screw speed, whereas the lowest value was with 15:35 at 18.5 % moisture content, 110°C temperature and 175 rpm screw speed. Response surface plot in Figure 7.1(b) shows the variation of ER with AP and moisture content. Initially with increase in moisture content there was a slight decrease in ER. Upon further increase in moisture content, the ER was found to increase slightly. Expansion ratio decreased with increase in AP level. Apple pomace contains insoluble fiber which lowers the affinity between the starch and insoluble fiber. Insoluble fibers also have higher hydrophilic properties, which have been shown to absorb more water, therefore modifying the glass transition temperature of the melt. It also can lower expansion by adhering to the bubble structure and consequently puncturing the cell, reducing cell extensibility (van der Sman and

Broeze, 2013). O'Shea et al. (2013) investigated the inclusion of apple pomace in a puffed snack. The authors reported similar results to those found in the study, a decrease in expansion properties of the extruded material as the AP inclusions increased. As reported by Chang et al. (1998), such behavior can be due to dilution effect of AP on starch content therefore reducing the starches swelling ability and also due to rupture of cell walls of the bubbles by the fibers.

7.4.3 Effect of extrusion processing conditions on the bulk density

Bulk density (BD) is an important property of the extrudates commonly influenced by expansion phenomena occurring upon exiting the extrudates from die section (Brown et al., 2015). Multiple linear regression equations for BD are shown in Table 7.4 and Table 7.5. ANOVA for quadratic model as fitted to experimental results of BD showed significance ($P<0.05$). However, model showed lack-of-fit was not significant ($P>0.05$) (Table 7.6). The first order term of AP and second order term of moisture content were found to be significant ($P<0.05$). An interaction effect of AP and temperature was also found to be significant ($P<0.05$).

The bulk density of the extrudates ranged from 324.46 to 553.96 kg m^{-3}. The highest value of BD was obtained with AP:DSF ratio of 10:40 at 17 % moisture content, 120°C temperature and 100 rpm screw speed, whereas the lowest value was with 5:45 at 18.5% moisture content, 110°C temperature and 175 rpm screw speed. Effect of moisture content and AP level on the BD can be found in the response surface plot (Figure 7.1(c)). The BD increased with increase in the AP level. The effect may be attributed to reduced expansion and fiber distribution within the expanded network of starch in extrudates (Guy, 2001). The negative correlation between BD and ER (Table 7.9) confirms that

products with higher bulk density have lower expansion and vice versa. Moisture content had quadratic effect on the BD. The BD decreased with moisture content, which may be due to proper gelatinization and higher expansion (Ding et al., 2006; Ding et al., 2005). It also reflects its influence on the rheological characteristics of the material. As observed in our previous study, (Singha and Muthukumarappan, 2017a) higher moisture content lowered the melt viscosity of the mix increasing the elasticity of the dough. This may have resulted in reduction in the density of the extrudate (Ding et al., 2006).

7.4.4 Effect of extrusion processing conditions on the water absorption index

The water absorption index (WAI) is an index that relates to the degree of starch gelatinization (Ding et al., 2005). Multiple linear regression equations for WAI are shown in Table 7.4 and Table 7.5. ANOVA for quadratic model as fitted to experimental results of WAI showed significance ($P<0.05$). However, model showed lack-of-fit was not significant ($P>0.05$) (Table 7.6). The first order terms of temperature and moisture content and the second-order term of screw speed were found to be significant ($P<0.05$). An interaction effect of the moisture content with AP level and temperature were also found to be significant ($P<0.05$).

In this study, the WAI was in the range of 3.1 and 3.5 g/g dry solid. Increase in feed moisture was observed to result in significant decrease in WSI of extrudate. These observations are consistent with previous studies (Harper, 1981). WAI measures the volume occupied by the granule or starch polymer after swelling in excess water. Extrusion process parameters had significant effect on WAI, as increase in feed moisture and temperature significantly decreased the WAI of extrudates (Figure 7.1(d)). Increase in die temperature was observed to cause a slight decrease in extrudate WAI. A decrease

in WAI with increasing temperature was probably due to decomposition or degradation of starch (Altan et al., 2008a). Ding et al. (2006) also stated that the WAI decreases with increasing temperature if dextrinization or starch melting prevails over the gelatinization phenomenon. Simultaneously, it can be said that WAI increases when the moisture content and temperature are kept low. The increase in WAI of extruded products is related to the hydrophilic nature of the raw ingredient, which could be affected by changes in molecular conformation exposing amino acid chains previously enclosed, thus providing water interactions (Paredes-LÓPez et al., 1991).

7.4.5 Effect of extrusion processing conditions on the water solubility index

The water solubility index (WSI) measures the amount of soluble component released from starch after extrusion (Ding et al., 2006) and is often used as an indicator of degradation of molecular components. Multiple linear regression equations for WSI are shown in Table 7.4 and Table 7.5. The experimental data fit the response equation with a high degree of significance (Table 7.6). However, the lack of fit was not significant ($P>0.05$). It was observed that the first order term of AP level and moisture content and the second-order term of moisture content were significant ($P<0.05$). An interaction effect of the moisture content with temperature and screw speed were also found to be significant ($P<0.05$).

The WSI ranged from 10.6 to 14.5%. Statistical analysis revealed that WSI was negatively correlated with WAI ($r=-0.438$, $P<0.05$) and hardness ($r=-0.473$, $P<0.05$) (Table 7.9) (Figure 7.1(e)). Increased feed moisture was observed to result in a significant decrease in the WSI of extrudate. It has been found that high water content in extrusion processes can diminish protein denaturation and starch degradation (Rodriguez-Miranda

et al., 2012). Slight increase in WSI of the extrudates was also observed when the AP level increased. Such increase in WSI means there is increase in degradation of starch i.e. more numbers of soluble molecules are present in the extrudates.

7.4.6 Effect of extrusion processing conditions on the textural properties

The maximum peak force (hardness), distance (brittleness) and slope (crispness) were measured to determine textural property of extrudates. Multiple linear regression equations for peak force, distance and slope are shown in Table 7.4 and Table 7.5. ANOVA for quadratic model as fitted to experimental results of hardness showed significance ($P<0.05$). The lack of fit was not significant ($P>0.05$) for all textural properties (Table 7.7). It was found that AP level and temperature had significant linear effects ($P<0.05$) and screw speed and temperature had significant quadratic effects ($P<0.05$) on the hardness. Increasing feed moisture content significantly increased the hardness of the extrudate initially. Previous studies have also reported that hardness of extrudate increased as the feed moisture content increased (Badrie and Mellowes, 1991; Liu et al., 2000). Further increase in moisture content led to decrease in the hardness. Hardness of extrudates decreased with increase in temperature (Figure not shown). Ding et al. (2005) reported that increasing temperature would decrease melt viscosity, but it also increases the vapor pressure of water. This favors the bubble growth which is the driving force for expansion that produces low density products and thus decreasing hardness of extrudate.

The distance to break the extrudate is an indication of brittleness, with the shortest distance being the most brittle product. Product cracks when brittleness increases. Multiple linear regression equations for distance are shown in Table 7.4 and Table 7.5.

ANOVA for linear model as fitted to experimental results of distance showed significance ($P<0.05$) (Table 7.7). The measured value of distance for the extrudates was in the range of 0.52–1.25 mm. Increasing apple pomace level decreased distance and hence increased brittleness of samples. Altan et al. (2008c) also reported similar finding during twin screw extrusion of grape pomace.

Crispness is typically a textural attribute for snack foods and baked products. The lower the slope, the crisper the product is considered. Multiple linear regression equations for slope are shown in Table 7.4 and Table 7.5. ANOVA for linear model as fitted to experimental results of slope showed significance ($P<0.05$) (Table 7.7). The values of slope measured as crispness varied between 5.66 and 16.48 N/mm for the extrudates. Increasing apple pomace content increased the slope of force–time curve. This means that highest pomace content causes less crispy extrudate. This might be the result of the effect of fiber in the apple pomace. Fiber reduces the cell size, probably by causing premature rupture of gas cells, which reduces the overall expansion and results in less porous structure (Lue et al., 1991; Yanniotis et al., 2007).

7.4.7 Effect of extrusion processing conditions on the color parameters

Color is an important characteristic of extruded foods (Figure 7.2). Color changes during the extrusion process can provide important information about the degree of thermal treatment (Chen et al., 1991). Multiple linear regression equations for lightness (L^*) are shown in Table 7.4 and Table 7.5. ANOVA for quadratic model as fitted to experimental results of L^* showed significance ($P<0.05$). The lack of fit was not significant ($P>0.05$) (Table 7.7). Moisture content had both linear and quadratic significant ($P<0.05$) effect, and AP had significant linear effect ($P<0.05$) on the lightness

values. The *L** values varied from 33.96 to 63.79%. The *L** value decreased with increase in AP content and moisture content (Figure 7.3(a)). It is known that reducing sugars and proteins (amino acids) in foods can react under high processing temperatures to promote non-enzymatic browning (Maillard reaction), which results in darkening of the final product. The observed decrease in brightness may be attributed to the Maillard reaction. Reduction in whiteness, as evidenced in decrease in *L**values, indicates darker samples.

Multiple linear regression equations for lightness (*L**) are shown in Table 7.4 and Table 7.5. ANOVA for quadratic model as fitted to experimental results of for *a** (redness) showed significance (P<0.05) (Table 7.7). The lack of fit was not significant (P>0.05). Moisture content had significant linear effect (P<0.05) whereas AP had both significant linear and quadratic effects (P<0.05) on the *a** values of the extrudates. Redness '*a**' of the extruded samples ranged from 4.06 to 9.15. Apple pomace has a redness value of 15.06. Hence it is expected that with increase in the AP level the redness of the extrudates will also increase. The redness of the extrudates also increased as the moisture content increased, which confirms that whiteness decreased. Redness in food relates to loss of whiteness (Iwe et al., 2000).

Multiple linear regression equations for yellowness (*b**) are shown in Table 7.4 and Table 7.5. ANOVA for quadratic model as fitted to experimental results of for *b** showed significance (P<0.05) (Table 7.7). The lack of fit was not significant (P>0.05). Only AP had a significant effect (P<0.05) on yellowness. Yellowness values (*b**) of extruded samples ranged from 17.35 to 28.10. The yellowness value of apple pomace is

low 9.15. Hence, yellowness of the extruded snacks decreased with increase in the AP level.

Color difference (ΔE) was used to represent the color change between the blends and the extrudates. Multiple linear regression equations for ΔE are shown in Table 7.4 and Table 7.5. The ΔE ranged from 27.42 to 53.08. ANOVA for quadratic model as fitted to experimental results of ΔE showed significance ($P<0.05$). The lack of fit was not significant ($P>0.05$) (Table 7.7). Moisture content had a significant linear and quadratic effect ($P<0.05$) on the color difference. The response surface plot for color changes with moisture content and temperature is represented in Figure 7.3(b). The color data is an important value for future product development of snacks containing AP, DSF and corn grits.

7.4.8 Effect of processing conditions on the total phenolic content

Multiple linear regression equations for total phenolic content (TPC) are shown in Table 7.4 and Table 7.5. ANOVA for the linear model as fitted to experimental results of TPC showed significance ($P<0.05$) (Table 7.8). The lack of fit was not significant ($P>0.05$). Regression analysis showed that TPC was significantly ($P<0.05$) affected by apple pomace level, temperature and moisture content.

The concentration of total phenolic content (TPC) of the extrudates ranged from 35.16 to 109.94 g GAE/100 g dry sample. The TPC values increased with increase in pomace level. The phenolic content of AP was 403.67 μg GAE/100 g dry solid. Hence, any variation in the pomace level in the blends affected the TPC content in the extruded snacks. The phenolic content of the extrudates increased with the rise in moisture content. This variation can be explained by the fact that phenolics can be modified as their

solubility and functional group properties are altered (Yang et al., 2008). An increase in phenolic content with feed moisture content was also observed for cereal based extrudates containing carrot powder (Ozer et al., 2006).

Previous studies of extrusion of beans (Korus et al., 2007), oats (Wani and Kumar, 2016) have shown reduction in phenolic content with rise in extrusion temperature. We found that with increase in the extrusion temperature the TPC of the extrudates also increased i.e., temperature was not detrimental to the TPC. Increase of TPC with temperature was also reported by Bisharat et al. (2015) and Zielinski et al. (2001). The high extrusion temperature favored release of phenolic components.

7.4.9 Effect of processing conditions on the antioxidant activity

DPPH assay was employed for antioxidant quantitation. Multiple linear regression equations for antioxidant activity (AA) are shown in Table 7.4 and Table 7.5. ANOVA for the linear model as fitted to experimental results of AA showed significance ($P<0.05$) (Table 7.8). The lack of fit was not significant ($P>0.05$). Regression analysis showed that AA was significantly ($P<0.05$) affected by apple pomace level, temperature and moisture content.

The AA of the extrudates ranged from 55.6 to 317.0 μmol TE/100 g dry sample. AA of the extrudates increased with increase in AP level. An increase in AA with increasing pomace level can be attributed to phenolic compound in apple pomace. High values of linear coefficient indicate that phenolics are major contributor to the antioxidant activity (Figure 7.4). A decrease in the moisture of the blend resulted in lower AA values. This could be due to the shearing effects which would be more destructive on the compounds with antioxidant activities other than phenolic compounds at drier extrusion

conditions (Ozer et al., 2006). The presence of a greater amount of moisture in the sample would lead to a gentler processing in the extruder barrel. An increase in extrusion temperature also led to increase in the AA values. The reason for the high antioxidant potential of the extrudates can be partly accounted for the presence of the high molecular weight products of Maillard reaction which are formed at higher temperatures and act as antioxidants. Furthermore, it can also be stated that at low temperature formation of oxidation-promoting compounds is favored which reduces the antioxidant activity (Nicoli et al., 1999).

7.4.10 Extrudates analysis by SEM and FTIR

The SEM images in Figure 7.5 clearly reveal the detailed internal structure of the extruded products. The SEM images show that there are differences in the internal structure of the extrudates at different processing conditions. In Figure 7.5(a), the hollow round pores are air cells within the extruded products. This structure depicts that the product has high crispness value. The extruded product contains 5 % AP and crispness value is 16.15 N/mm which is close to highest crispness obtained in the experiment. Figure 7.5(b) shows that the structure of extrudates is compact and less porous structure. The extrudate snack contains 20% AP and expansion ratio was 1.44. Increasing the level of AP level resulted in a less expanded, more compact texture in the final snack products. The internal structure of extruded product in Figure 7.5(c) shows presence of many small air cells. The structure is also not compact and implies that the product has good expansion ratio and crispness. The extruded product had highest expansion ratio of 2.12 and a crispness value of 11.49 N/mm. Figure 7.5(d) shows the internal structure of

extrudates with 0% AP. Presence of air cells with thin cell wall resulted in an expansion ratio of 1.77 and crispness of 16.48 N/mm of the extruded snack.

Fourier transform infrared spectroscopy (FTIR) provides unique insights into the protein conformation changes (Mangavel et al., 2001; Subirade et al., 1998) and has been used to examine changes in protein-polysaccharide interaction in extruded snacks (Guerrero et al., 2014). Therefore, FTIR is a useful technique to examine the process-induced changes to components in the formulations. FTIR revealed the changes in the chemicals bonds present in the extruded snacks due to different processing conditions. FTIR spectra of the extruded snack containing 50% DSF and 50% CG (Figure 7.6(top)) is compared with the unextruded blend in Figure 7.6(bottom). The spectral changes for polysaccharides and pectin region (1200–900 cm^{-1}) and amide II region (1741 and 1540 cm^{-1}) were more noticeable in the extruded snacks containing higher level of AP (Figure 7.7). The peak associated with C-O stretching of pectin (1230 cm^{-1}) is seen in the extruded snack. Spectral changes observed after extrusion are indicative of process induced changes. The spectral fluctuations seen in the amide I and II regions are directly related to the protein backbone conformation and is most commonly used for the quantitative analysis of the secondary structure in protein (Kong and Yu, 2007; Krimm and Bandekar, 1986). During the high temperature, high shear extrusion process, process-induced changes and degradation of food components occur. Extrusion causes changes of protein structure (Verbeek and van den Berg, 2010).

7.4.11 Optimization and model verification

The independent variables were optimized numerically using Design Expert v8. AP level was kept maximum and the remaining independent variables were kept in range

during optimization. The goals were assigned to each response parameters. The WAI and WSI were kept in range; the ER, *L**, crispness and brittleness was kept at maximum; and hardness, BD, *a** and *ΔE* were kept minimum. The optimization resulted in 42 solutions and the top 10 solutions are shown in Table 7.10. Validation of the predicted responses was carried out by extruding the blends at three different optimum conditions as shown in Table 7.10. To check the variability of the predicted responses, two-tailed, one sample t -test was carried out. Good agreement was found between the predicted and experimental values. Results of the t -test demonstrated no significant difference between the values of recorded responses and the predicted responses. The experimental values for solution number 1 were very close to the predicted values. The optimum condition was: 15% AP, 130^{o}C barrel temperature, 175 rpm screw speed and 15.5% moisture content.

7.5 Conclusions

The product responses were affected by pomace level and moisture content. The results showed that varying levels of apple pomace could be incorporated into an extruded corn-based snack depending on the desired texture of the final product. Optimal twin screw extrusion cooking conditions most likely to produce apple pomace-defatted soy flour-corn grits products suitable for a snack food was predicted by the response surface models found. After validation, blends of 15% apple pomace extruded at 110^{o}C, 175 rpm and 15.5 % moisture content extruded was selected as the optimum condition. The findings of this study demonstrate the feasibility of developing value-added products from apple pomace, defatted soy flour and corn grits by extrusion processing.

Table 7.1 Ingredient composition of blends and the mean proximate composition of each (values in the parentheses represent standard error).

Feed ingredients	**Mass of ingredients (g kg^{-1})**				
	Blend I	**Blend II**	**Blend III**	**Blend IV**	**Blend V**
Defatted soy flour	500	450	400	350	300
Corn grits	500	500	500	500	500
Apple pomace	0	50	100	150	200
Proximate analysis					
Protein (% db)	28.53 (0.03)	25.56 (0.11)	23.38 (0.18)	20.63 (0.04)	19.14 (0.12)
Fiber (% db)	2.26 (0.12)	3.25 (0.31)	4.34 (0.24)	5.43 (0.10)	6.41 (0.14)
Fat (% db)	1.17 (0.06)	1.23 (0.02)	1.34 (0.11)	1.41 (0.02)	1.56 (0.05)
Ash (% db)	4.43 (0.13)	4.27 (0.03)	3.98 (0.10)	3.73 (0.03)	3.49 (0.02)
NFE (% db)	52.95 (0.17)	51.65 (0.08)	50.36 (0.03)	49.06 (0.13)	47.77 (0.06)

db, dry basis; NFE, nitrogen free extract

Table 7.2 Independent numerical variables and their levels.

Numerical variable	**Symbol**	**Coded variable levels**				
		-2	**-1**	**0**	**1**	**2**
Apple pomace (%)	X_1	0	5	10	15	20
Temperature (°C)	X_2	100	110	120	130	140
Screw speed (rpm)	X_3	100	125	150	175	200
Moisture content (% wb)	X_4	14	15.5	17	18.5	20

wb, wet basis

Table 7.3 Experimental design layout.

Run	Coded variables				Actual variables			
	x_1	x_2	x_3	x_4	X_1 (%)	X_2 (%)	X_3 (°C)	X_4 (rpm)
1	-1	1	-1	1	5	130	125	18.5
2	0	2	0	0	10	140	150	17.0
3	-1	-1	1	1	5	110	175	18.5
4	2	0	0	0	20	120	150	17.0
5	1	-1	-1	1	15	110	125	18.5
6	1	1	1	1	15	130	175	18.5
7	1	1	1	-1	15	130	175	15.5
8	-2	0	0	0	0	120	150	17.0
9	0	0	0	-2	10	120	150	14.0
10	1	1	-1	1	15	130	125	18.5
11	-1	1	-1	-1	5	130	125	15.5
12	-1	-1	1	-1	5	110	175	15.5
13	0	0	2	0	10	120	200	17.0
14	-1	1	1	-1	5	130	175	15.5
15	-1	1	1	1	5	130	175	18.5
16	1	-1	-1	-1	15	110	125	15.5
17	0	0	-2	0	10	120	100	17.0
18	1	1	-1	-1	15	130	125	15.5
19	0	0	0	0	10	120	150	17.0
20	-1	-1	-1	-1	5	110	125	15.5
21	0	-2	0	0	10	100	150	17.0
22	-1	-1	-1	1	5	110	125	18.5
23	0	0	0	0	10	120	150	17.0
24	0	0	0	0	10	120	150	17.0
25	0	0	0	2	10	120	150	20.0
26	1	-1	1	-1	15	110	175	15.5
27	1	-1	1	1	15	110	175	18.5

Table 7.4 Best-fit response surface models in terms of coded variables after excluding the insignificant terms for DP, To, ER, BD, WAI, WSI, PF, Di, S, L*, a*, b*, ΔE, TPC and AA.

Parameters	**Response surface model**	R^2	***P* value**
DP (MPa)	$Y_{DP} = 5.28 - 1.42x_2 - 1.04x_4 + 1.09x_1^2 + 0.91x_3^2 + 1.95x_4^2$	0.8458	0.0053
T_o (N.m)	$Y_{To} = 36.98 - 12.76x_2$	0.6369	0.0001
ER (-)	$Y_{ER} = 1.34 - 0.13x_1 + 0.12x_1x_2 + 0.13x_4^2$	0.8720	0.0020
BD (kg/m^3)	$\mathrm{BD} = 487.64 + 27.89x_1 - 34.03x_1x_2 - 27.96x_4^2$	0.8165	0.0128
WAI (g/g)	$Y_{WAI} = 3.26 + 0.04x_2 + 0.03x_4 - 0.04x_1x_4 + 0.06x_2x_4 - 0.03x_3^2$	0.8328	0.0080
WSI (%)	$Y_{WSI} = 11.21 + 0.0.24x_1 - 0.49x_4 - 0.29x_2x_4 - 0.29x_3x_4 + 0.45x_4^2$	0.8311	0.0085
PF (N)	$Y_{PF} = 9.39 - 0.54x_1 - 0.59x_2 + 0.51x_2x_4 - 0.68x_2^2 - 0.62x_3^2$	0.8081	0.0159
D_i (mm)	$Y_{Di} = 0.81 + 0.12x_1 + 0.11x_4$	0.6663	<0.0001
S (N/mm)	$Y_S = 10.25 - 2.10x_1 - 0.89x_2 - 0.96x_4$	0.6540	<0.0001
L*	$Y_{L^*} = 52.25 - 2.52x_1 - 3.91x_4 - 2.69x_4^2$	0.8082	0.0159
a*	$Y_{a^*} = 7.94 + 0.64x_1 + 0.39x_4 - 0.66x_1^2$	0.8332	0.0080
b*	$Y_{b^*} = 22.31 - 1.33x_1$	0.3672	0.0328
Δ*E*	$\Delta E = 35.38 + 3.86x_4 + 2.51x_4^2$	0.7970	0.0208
TPC (g GAE/100 g dry sample)	$Y_{TPC} = 70.65 + 15.40x_1 + 8.74x_2 + 7.32x_4$	0.8389	<0.0001
AA (µmol TE/100 g dry sample)	$Y_{AA} = 224.35 + 46.95x_1 + 26.24x_2 + 22.48x_4$	0.8209	<0.0001

DP= die pressure, To = torque, ER = expansion ratio, BD = bulk density, WAI = water absorption index, WSI = water solubility index, PF = peak force, Di = distance, S = slope, L* = lightness, a* = red-green color, b* = yellow-blue color, ΔE = total color difference, TPC = total phenolic content, AA = antioxidant activity

Table 7.5 Best-fit response surface models in terms of actual variables after excluding the insignificant terms for DP, To, ER, BD, WAI, WSI, PF, Di, S, L*, a*, b*, ΔE, TPC and AA.

Parameters	**Response surface model**	R^2	***P* value**
DP (MPa)	$Y_{DP} = 478.25 - 1.59X_2 - 35.58X_4 + 0.04X_1^2 + 0.001X_3^2 + 0.87X_4^2$	0.8458	0.0053
T_o (N.m)	$Y_{To} = 212.87 - 1.28X_2$	0.6369	0.0001
ER (-)	$Y_{ER} = 18.21 - 0.23X_1 + 0.002X_1X_2 + 0.06X_4^2$	0.8720	0.0020
BD (kg/m^3)	$Y_{BD} = -2441.8 + 44.78X_1 - 0.68X_1X_2 - 12.43X_4^2$	0.8165	0.0128
WAI (g/g)	$Y_{WAI} = 10.41 - 0.07X_2 - 0.37X_4 + 0.005X_1X_4 + 0.004X_2X_4 - 0.00005X_3^2$	0.8328	0.0080
WSI (%)	$Y_{WSI} = 40.57 - 0.03X_1 - 3.58X_4 - 0.02X_2X_4 - 0.008X_3X_4 + 0.19X_4^2$	0.8311	0.0085
PF (N)	$Y_{PF} = -30.31 - 0.54X_1 + 0.81X_2 + 0.03X_2X_4 - 0.007X_2^2 - 0.0009X_3^2$	0.8081	0.0159
D_i (mm)	$Y_{Di} = -0.49 + 0.02X_1 + 0.07X_4$	0.6663	<0.0001
S (N/mm)	$Y_S = 35.20 - 0.42X_1 - 0.09X_2 - 0.64X_4$	0.6540	<0.0001
L*	$Y_{L^*} = -387.44 + 1.97X_1 + 47.44X_4 - 1.19X_4^2$	0.8082	0.0159
a*	$Y_{a^*} = -16.65 + 0.76X_1 - 1.63X_4 - 0.03X_1^2$	0.8332	0.0080
b*	$Y_{b^*} = 30.49 - 0.27X_1$	0.3672	0.0328
Δ*E*	$Y_{\Delta E} = 428.69 - 44.47X_4 + 1.12X_4^2$	0.7970	0.0208
TPC (g GAE/100 g dry sample)	$Y_{TPC} = -152.52 + 3.08X_1 + 0.87X_2 + 4.88X_4$	0.8389	<0.0001
AA (µmol TE/100 g dry sample)	$Y_{AA} = -444.36 + 9.39X_1 + 2.64X_2 + 14.99X_4$	0.8209	<0.0001

DP= die pressure, To = torque, ER = expansion ratio, BD = bulk density, WAI = water absorption index, WSI = water solubility index, PF = peak force, Di = distance, S = slope, L* = lightness, a* = red-green color, b* = yellow-blue color, ΔE = total color difference, TPC = total phenolic content, AA = antioxidant activity

Table 7.6 Analysis of variance for die pressure, torque, expansion ratio (ER), bulk density (BD), water absorption index (WAI) and water solubility index (WSI).

Response	**Source**	**df**	**Sum of squares**	**Mean squares**	***F*-value**	***P*-value**
Die pressure	Regression	14	1.43	0.10	5.84	0.0020
	Lack-of-fit	10	0.18	0.02	1.06	0.5786
	Pure error	2	0.03	0.02		
	Residual	12	0.21	0.02		
	Total	26	1.64			
Torque	Regression	14	82224.00	5873.14	3.81	0.0128
	Lack-of-fit	10	11230.97	1123.10	0.31	0.9170
	Pure error	2	7245.71	3622.85		
	Residual	12	18476.68	1539.72		
	Total	26	100700.68			
ER	Regression	14	0.18	0.01	4.27	0.0080
	Lack-of-fit	10	0.02	0.00	0.35	0.8961
	Pure error	2	0.01	0.01		
	Residual	12	0.04	0.00		
	Total	26	0.21			
BD	Regression	14	82224.00	5873.14	3.81	0.0128
	Lack-of-fit	10	11230.97	1123.10	0.31	0.9170
	Pure error	2	7245.71	3622.85		
	Residual	12	18476.68	1539.72		
	Total	26	100700.68			
WAI	Regression	14	0.18	0.01	4.27	0.0080
	Lack-of-fit	10	0.02	0.00	0.35	0.8961
	Pure error	2	0.01	0.01		
	Residual	12	0.04	0.00		
	Total	26	0.21			
WSI	Regression	14	15.75	1.13	4.22	0.0085
	Lack-of-fit	10	2.55	0.25	0.78	0.6792
	Pure error	2	0.65	0.33		
	Residual	12	3.20	0.27		
	Total	26	18.95			

Table 7.7 Analysis of variance for hardness, brittleness, crispness, lightness (L*), redness (a*), yellowness (b*) and total color change (ΔE).

Response	Source	df	Sum of squares	Mean squares	*F*-value	*P*-value
Peak force	Regression	14	39.97	2.86	3.61	0.0159
	Lack-of-fit	10	8.49	0.85	1.69	0.4284
	Pure error	2	1.00	0.50		
	Residual	12	9.49	0.79		
	Total	26	49.47			
Distance	Regression	4	0.64	0.16	10.98	< 0.0001
	Lack-of-fit	20	0.31	0.02	1.89	0.4027
	Pure error	2	0.02	0.01		
	Residual	22	0.32	0.01		
	Total	26	0.97			
Slope	Regression	4	147.44	36.86	10.39	< 0.0001
	Lack-of-fit	20	69.19	3.46	0.78	0.6993
	Pure error	2	8.83	4.42		
	Residual	22	78.02	3.55		
	Total	26	225.46			
*L**	Regression	14	920.96	65.78	3.61	0.0159
	Lack-of-fit	10	201.51	20.15	2.36	0.3337
	Pure error	2	17.04	8.52		
	Residual	12	218.56	18.21		
	Total	26	1139.51			
*a**	Regression	14	31.99	2.29	4.28	0.0080
	Lack-of-fit	10	5.88	0.59	2.23	0.3494
	Pure error	2	0.53	0.26		
	Residual	12	6.40	0.53		
	Total	26	38.40			
*b**	Regression	4	52.40	13.10	3.19	0.0328
	Lack-of-fit	20	88.18	4.41	4.14	0.2125
	Pure error	2	2.13	1.07		
	Residual	22	90.31	4.11		
	Total	26	142.71			
ΔE	Regression	14	786.65	56.19	3.37	0.0208
	Lack-of-fit	10	184.67	18.47	2.35	0.3348
	Pure error	2	15.69	7.84		
	Residual	12	200.35	16.70		
	Total	26	987.00			

Table 7.8 Analysis of variance for total phenolic content (TPC) and antioxidant activity (AA).

Response	Source	df	Sum of squares	Mean squares	F-value	P-value
TPC	Regression	4	5497.65	1374.41	28.65	< 0.0001
	Lack-of-fit	20	1030.23	51.51	4.09	0.2147
	Pure error	2	25.21	12.60		
	Residual	22	1055.43	47.97		
	Total	26	6553.08			
AA	Regression	4	81786.56	20446.64	25.21	< 0.0001
	Lack-of-fit	20	17571.01	878.55	6.46	0.1424
	Pure error	2	272.04	136.02		
	Residual	22	17843.05	811.05		
	Total	26	99629.60			

Table 7.9 Correlation coefficients between product properties.

	DP	To	ER	BD	WAI	WSI	PF	Di	S	*L**	*a**	*b**	*ΔE*	*TPC*	*AA*
DP	1	0.656**	0.655**	-0.634**	-0.268^{ns}	0.347^{ns}	0.193^{ns}	-0.152^{ns}	0.303^{ns}	0.125^{ns}	-0.270^{ns}	-0.242^{ns}	-0.107^{ns}	-0.265^{ns}	-0.476*
To		1	0.367^{ns}	-0.393*	-0.307^{ns}	0.033^{ns}	0.255^{ns}	0.065^{ns}	0.157^{ns}	0.121^{ns}	-0.061^{ns}	-0.223^{ns}	-0.130^{ns}	-0.236^{ns}	-0.383*
ER			1	-0.908**	-0.123^{ns}	0.271^{ns}	0.317^{ns}	-0.331^{ns}	0.481*	-0.066^{ns}	-0.030^{ns}	-0.008^{ns}	0.167^{ns}	-0.404*	-0.543**
BD				1	0.131^{ns}	-0.155^{ns}	-0.443*	0.195^{ns}	-0.472*	0.170^{ns}	-0.074^{ns}	0.076^{ns}	-0.260^{ns}	0.321^{ns}	0.421*
WAI					1	-0.438*	0.172^{ns}	0.400*	-0.165^{ns}	-0.333^{ns}	-0.060^{ns}	-0.139^{ns}	0.353^{ns}	0.234^{ns}	0.207^{ns}
WSI						1	-0.473*	-.245^{ns}	-0.102^{ns}	0.063^{ns}	0.094^{ns}	-0.213^{ns}	-0.126^{ns}	0.269^{ns}	0.120^{ns}
PF							1	-.059^{ns}	0.696**	0.011^{ns}	-0.028^{ns}	0.287^{ns}	0.086^{ns}	-0.431*	-0.437*
Di								1	-0.727**	-0.697**	0.341^{ns}	-0.698**	0.627**	0.589**	0.564**
S									1	0.525**	-0.347^{ns}	0.639**	-0.410*	-0.725**	-0.728**
L*										1	-0.676**	0.641**	-0.980**	-0.552**	-0.587**
a*											1	-0.247^{ns}	0.638**	0.570**	0.594**
b*												1	-0.530**	-0.593**	-0.481*
ΔE													1	0.413*	0.469*
TPC														1	0.897**
AA															1

DP= die pressure, To = torque, ER = expansion ratio, BD = bulk density, WAI = water absorption index, WSI = water solubility index, PF = peak force, Di = distance, S = slope, L* = lightness, a* = red-green color, b* = yellow-blue color, ΔE = total color difference, TPC = total phenolic content, AA = antioxidant activity

**Significant at $p<0.01$

*Significant at $p<0.05$

ns – not Significant

Table 7.10 Solutions for optimum conditions and validation.

Solution number	AP (%)	Temperature (^{o}C)	Screw Speed (rpm)	Moisture (%)	ER (-)	BD (kg m^{-3})	WAI (g g^{-1})	WSI (%)	Hardness (N)	Brittleness (mm)	Crispness (N mm^{-1})	*ΔE* (-)	*TPC* (µg GAE/ 100 DW)	*AA* (µmol TE /100 g DW)
1	15.00	130.00	175.00	15.50	1.51	446.72	3.15	13.88	6.50	0.79	8.37	36.36	92.16	275.74
2	15.00	130.00	174.36	15.51	1.51	447.27	3.16	13.85	6.54	0.79	8.36	36.36	92.15	275.89
3	14.85	129.96	171.48	15.50	1.51	447.46	3.16	13.75	6.68	0.79	8.41	36.31	91.51	274.15
4	15.00	130.00	168.26	15.50	1.52	446.88	3.16	13.68	6.82	0.79	8.33	36.29	91.74	275.57
5	14.87	130.00	174.99	15.55	1.50	449.98	3.16	13.80	6.53	0.79	8.39	36.36	91.92	275.32
6	15.00	130.00	166.10	15.50	1.52	447.11	3.17	13.62	6.90	0.80	8.32	36.26	91.63	275.57
7	15.00	128.52	173.26	15.50	1.51	448.60	3.16	13.71	6.85	0.79	8.49	36.11	91.32	271.80
8	14.53	128.38	175.00	15.50	1.51	450.51	3.16	13.70	6.80	0.77	8.71	36.09	90.05	267.06
9	14.98	130.00	158.46	15.50	1.51	449.09	3.17	13.43	7.12	0.80	8.28	36.16	91.12	275.26
10	15.00	130.00	159.04	15.51	1.51	449.35	3.17	13.43	7.11	0.80	8.27	36.17	91.22	275.60
Validation														
1	15.00	130.00	175.00	15.50	1.54	447.32	3.16	13.84	6.68	0.77	8.68	35.89	95.67	294.12
5	14.87	130.00	174.99	15.55	1.53	452.87	3.15	13.64	6.67	0.78	8.55	34.18	94.44	287.54
9	14.98	130.00	158.46	15.50	1.52	454.63	3.18	13.22	7.02	0.79	8.89	37.65	94.08	284.37

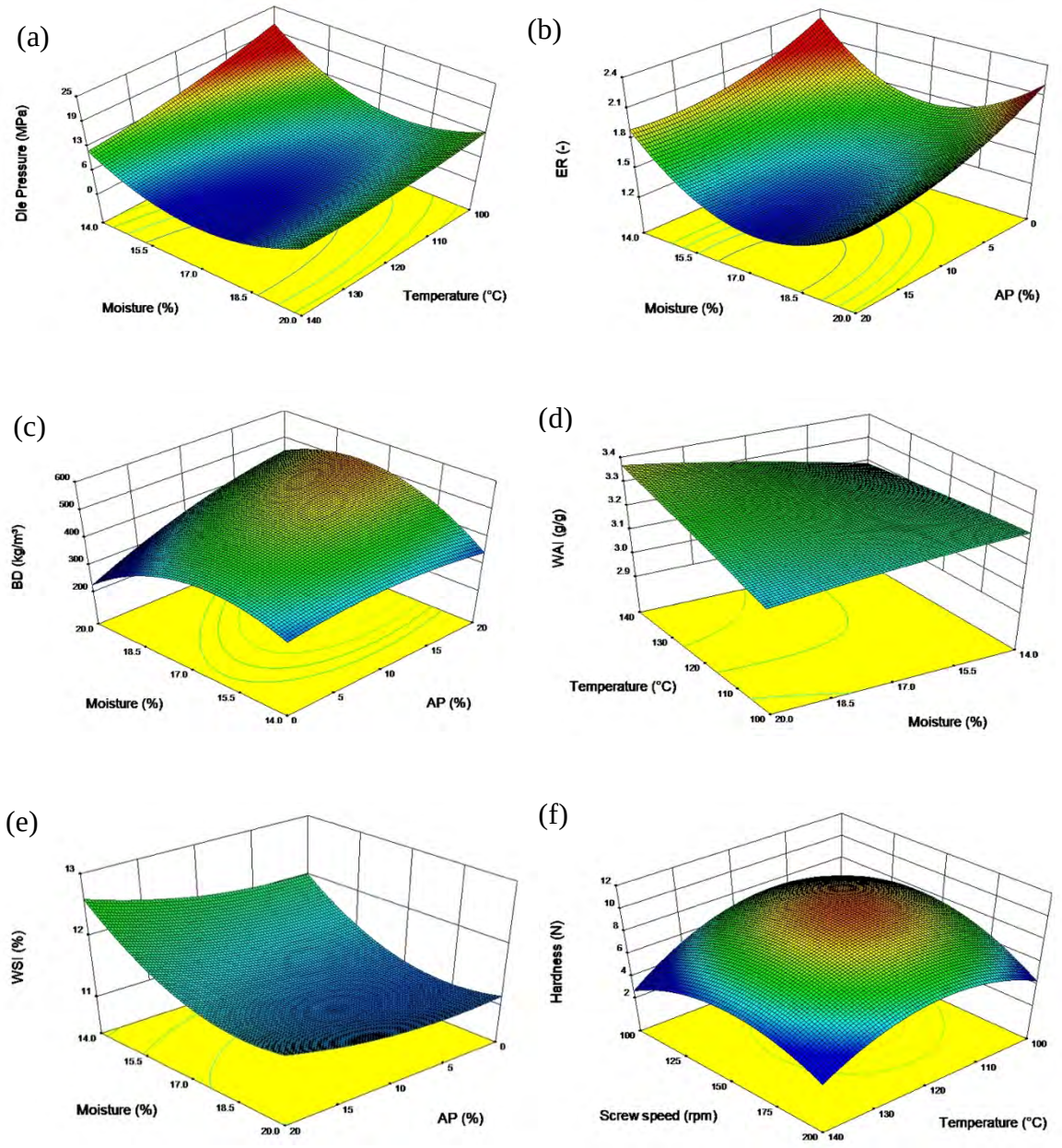

Figure 7.1 Response surface graphs illustrating the effects of (a) temperature and moisture on the die pressure, (b) moisture and AP on the ER, (c) moisture and AP on the BD, and (d) temperature and moisture on the WAI, (e) moisture and AP on the WSI, (f) screw speed and temperature on the hardness of the extruded snacks

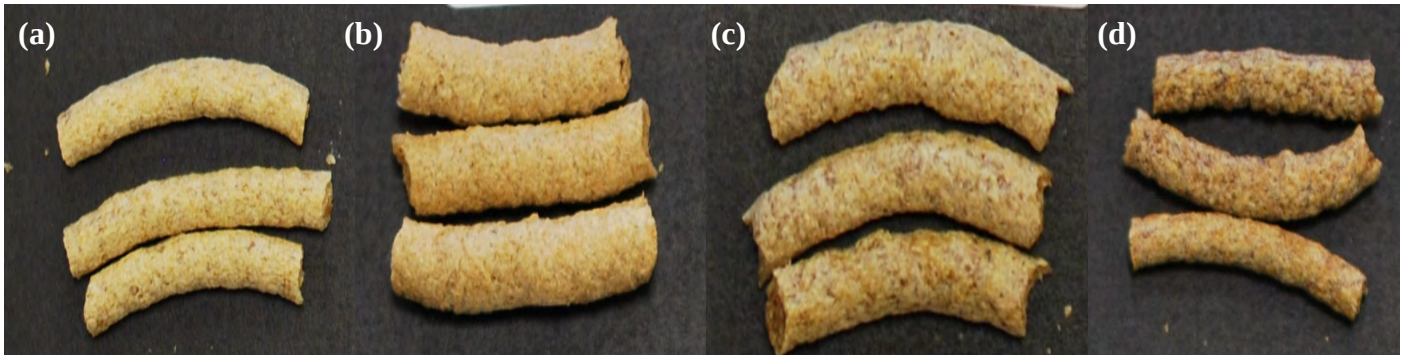

Figure 7.2 Corn-based snacks extruded under different processing conditions containing (a) 5% apple pomace, (b) 10% apple pomace, (c) 15% apple pomace, (d) 20% apple pomace

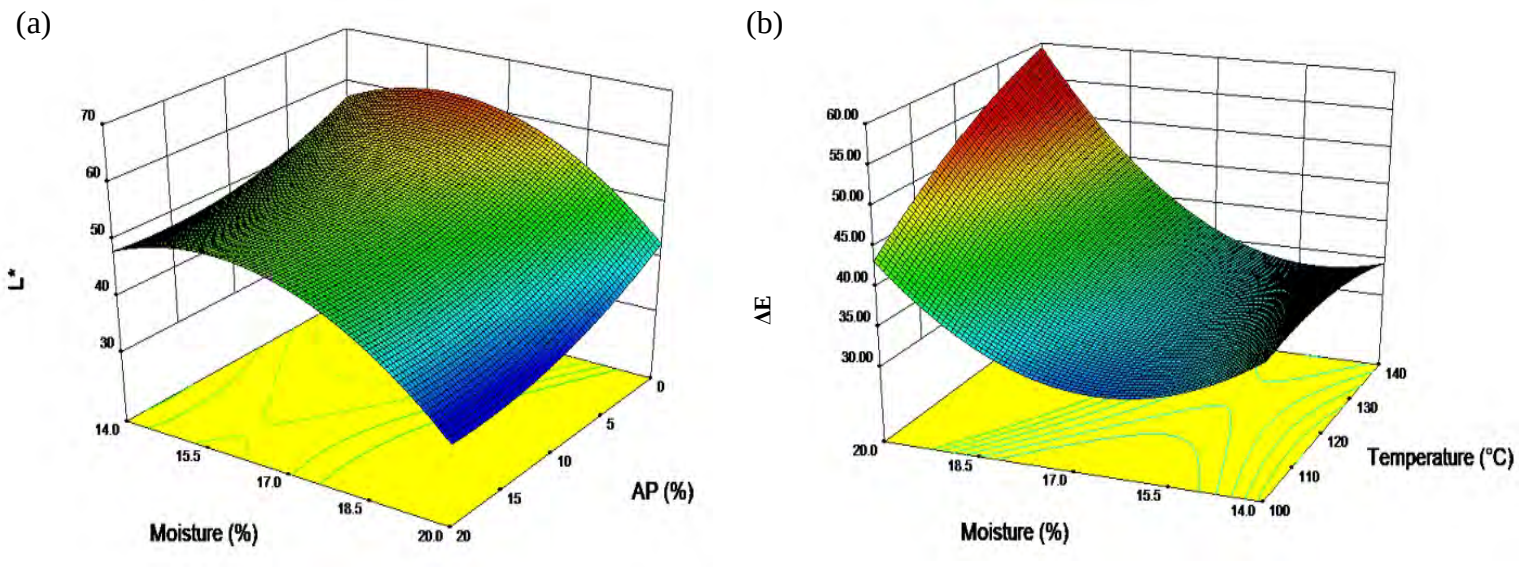

Figure 7.3 Response surface graphs illustrating the effects of (a) moisture and AP on the lightness, (b) temperature and moisture content on the total color change of the extruded snacks

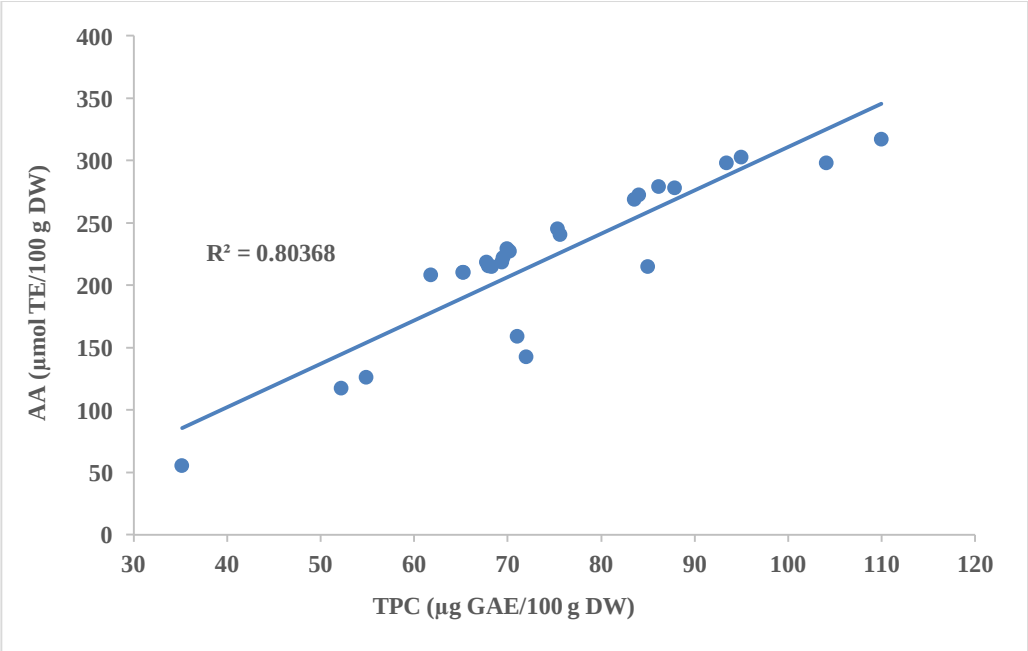

Figure 7.4 Correlation between total phenolic content and antioxidant activity measured by DPPH

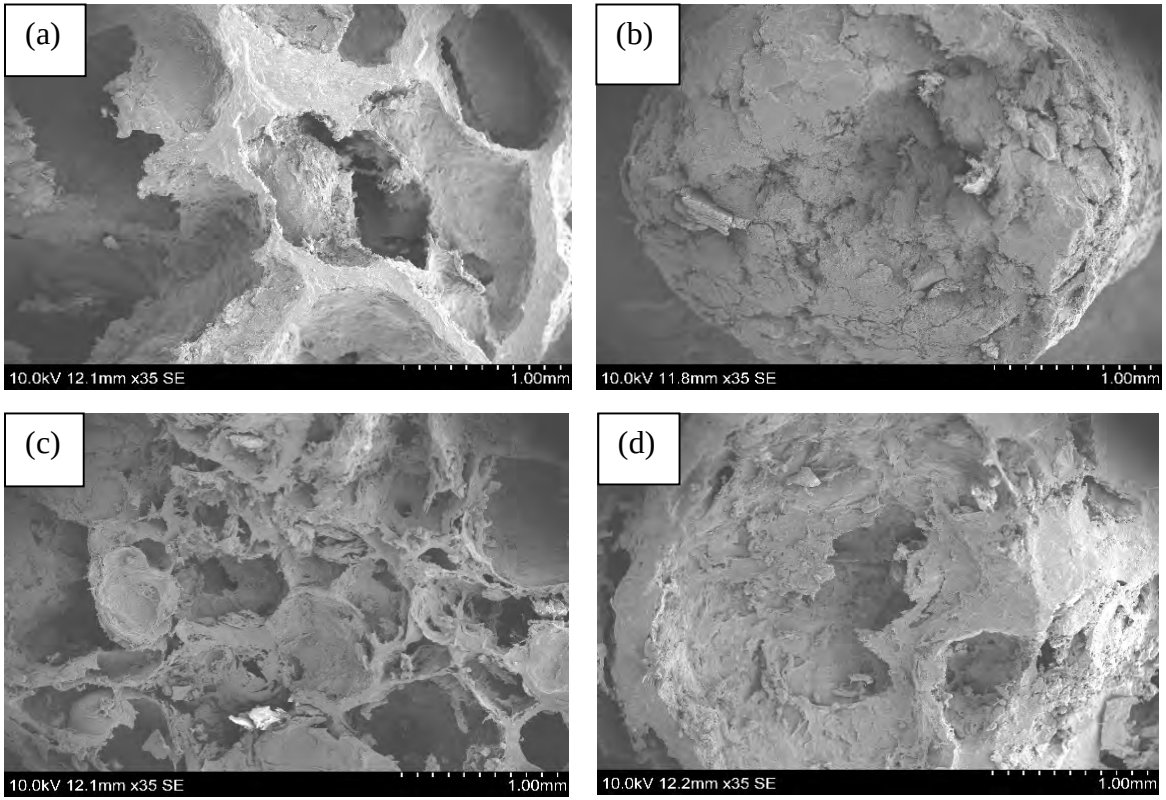

Figure 7.5 SEM micrographs of extruded snacks containing (a) 5% AP and 18.5 % moisture content extruded at 110 ^{o}C and 175 rpm screw speed, (b) 20 % AP and 17 % moisture content extruded at 120 ^{o}C and 150 rpm screw speed, (c) 10 % AP and 14 % moisture content extruded at 120 ^{o}C and 150 rpm screw speed, (d) 0 % AP and 17 % moisture content extruded at 120 ^{o}C and 150 rpm screw speed

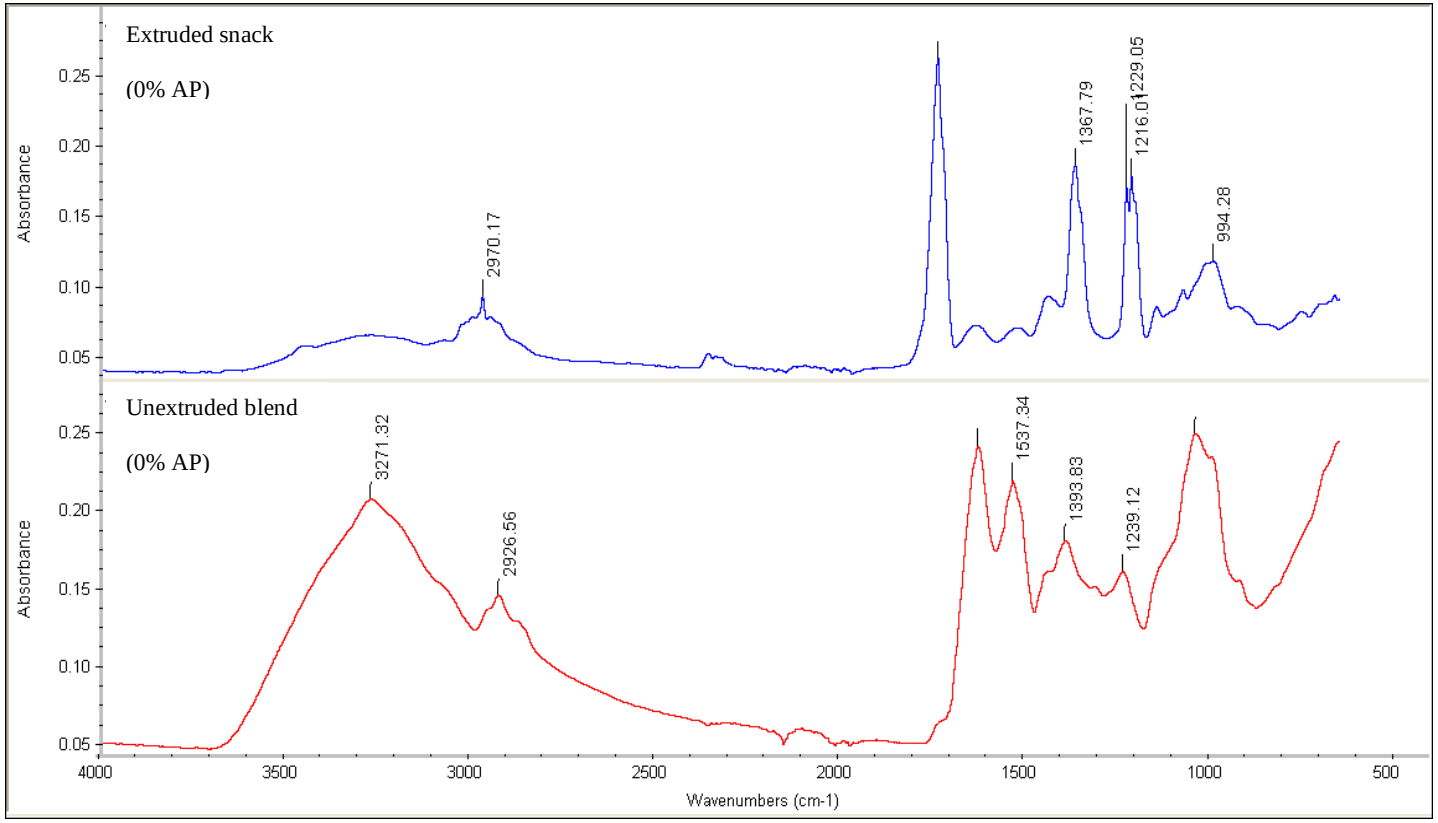

Figure 7.6 FTIR spectra of extruded snack enriched with 15% AP and extruded at 130^{o}C temperature and 175 rpm screw speed

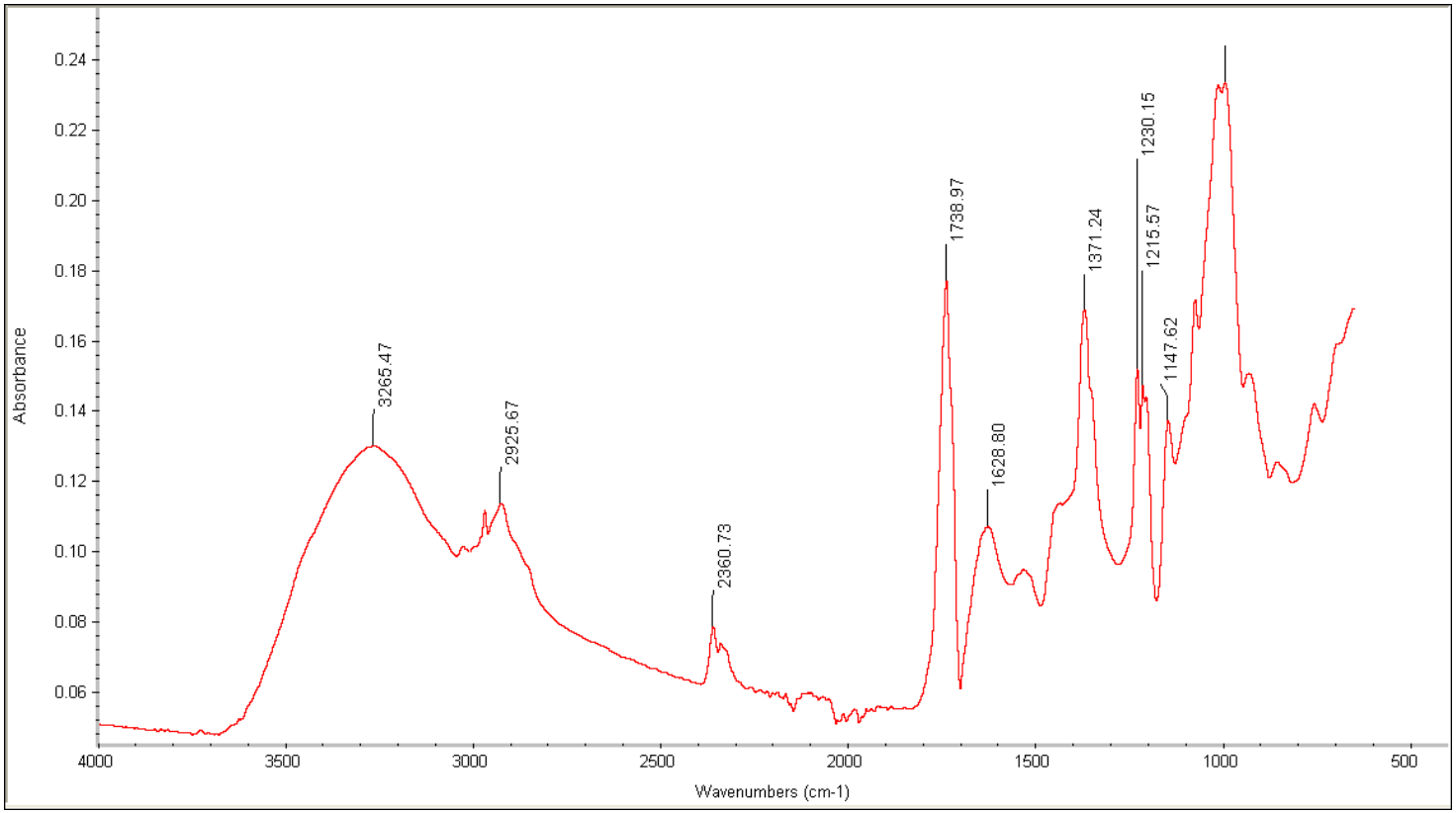

Figure 7.7 FTIR spectra of extruded snack enriched with 15% AP and extruded at 130^{o}C temperature and 175 rpm screw speed

CHAPTER 8

Influence of Extrusion Process and Grape Pomace Addition on the Properties of Soy and Corn-Based Extrudates

8.1 Abstract

Different ratio of grape pomace and defatted soy flour were blended with corn grits and extruded using conical twin screw extruder. Response surface methodology was used to investigate the effect of grape pomace level and extrusion processing conditions on the product properties. Five different blends at a level of 0-15% w/w grape pomace (GP) were extrusion cooked with varied barrel and die temperature (100-160^{o}C), screw speed (100-250 rpm) and feed moisture (15-25 % wet basis). Physical and functional properties of the extrudates were analyzed. The expansion ratio, water solubility index, hardness, lightness, yellowness significantly decreased with increase in the GP level in the blends. Whereas increasing the GP content in the blends significantly (P<0.05) increased the bulk density, water absorption index and total color change of the extrudates. Screw speed had significant effect on all the properties except crispness of the extrudates. Temperature had significant effect on the textural properties. The results indicated active interaction between grape pomace and starch during expansion process.

8.2 Introduction

The United States ranks 4th in the worldwide production of wines preceded by Italy, France and Spain (OIV, 2017). NASS reports that US has 1,049,600 grape bearing acres producing an average of 7.4 tons per acre, valued at $5.76 billion at the farm gate, of which wine grapes account for $3.5 billion. California accounts for over 90 percent of domestic grapes for making U.S. wines. NASS forecast California's wine grape

production at 8.0 billion pounds (or 4.0 million tons) in 2017 followed by Washington for 520 million pounds (or 260,000 tons) (USDA, 2017). Therefore, tremendous amounts of wine grape pomace (GP) are available annually in the Pacific Northwest region of the United States. GP consists mainly of peels (skins), seeds and stems and accounts for about 20–25% of the weight of the grape crushed for wine production (Yu and Ahmedna, 2013). Common uses of GP includes extraction of grape seed oils, and the production of citric acid, methanol, ethanol, and xanthan via fermentation (Deng et al., 2011).

GP is a fiber and polyphenol-rich by-product of wine making. It also contains significant amount of lipid, proteins, and minerals. GP contains up to 75% of dietary fiber and over 60% of GP dry matter is non-digestible (Bravo and Saura-Calixto, 1998). The non-digestible GP dietary fiber includes pectin, cellulose, Klason lignin and polyphenols (Deng et al., 2011; González-Centeno et al., 2010; Llobera and Cañellas, 2007). GP is rich in extractable phenolic antioxidants (10–11% of dry weight) (Makris et al., 2007). Anthocyanins, catechins, procyanidins, flavonol glycosides, phenolic acids and stilbenes are the principal phenolic constituents found in GP (Rodríguez Montealegre et al., 2006). Polyphenol composition of GP depends on the grape variety. The red varieties are usually rich in anthocyaninins (Yu and Ahmedna, 2013). Numerous studies have demonstrated the antioxidant and health promoting effects of phenolic compounds present in grapes and wine, particularly in relation to cardiovascular diseases (Scalbert et al., 2005).

Extruded snacks contribute as an important part for many consumers in daily nutrient and calories intake. With the growing awareness of the beneficial effects of healthy diet on the quality of life as well as on cost-effectiveness of health care, the snack food industry is facing the challenge of developing new products with special health

enhancing characteristics. GP has the potential to serve as an important source of insoluble fiber and phenolic antioxidant for functional food development. Altan et al. (2008c), extruded blends of GP and barley and studied the product's properties. While optimization of extrusion process suggested an addition of 4.47-6.57% GP could produce acceptable extrudates, sensory analysis revealed extrudates containing 2% GP had highest consumer preference. Biologically important monomers and dimers in GP were reported to have increased considerably after extrusion across a temperature and screw speed range of 160-190^{o}C and 100-200 rpm, respectively (Khanal et al., 2009). Extrusion of GP alone will not produce acceptable extrudates. It is possible to produce extrudates combining GP with cereal or legume flours. Defatted soy flour (DSF) and corn grits (CG) may provide a good matrix for the extrusion process along with nutritional value to the snack products (Ali et al., 1996; Hardacre et al., 2006; Kellor, 1974). The objectives of this study were (i) to document the effects of raw material (feed) composition and process conditions on physical and functional properties of extrudates from grape pomace, defatted soy flour, and corn grits using a twin-screw extruder; and (2) to determine, using response surface methodology (RSM), the extrusion conditions (feed composition and process conditions) most likely to produce extrudates with textural properties suitable for an expanded snack food.

8.3 Materials and Methods

8.3.1 Raw materials and blend preparation

Grape pomace (CP) was provided by G3 Enterprise (Modesto, CA). It was dried in oven at 40^{o}C for 24 hr. The dried pomace was grinded and stored at -18°C until use. The initial moisture content of dried GP powder was 4.36% (wet basis). The proximate

composition of the GP was: 12.35% protein, 0.22% fat, 6.36% ash, 31.22% total fiber and 45.49% NFE (dry basis). Corn grits (CG) was obtained from Bob's Red Mill (Milwaukie, OR). The initial moisture content of CG was 11.46% (wet basis). The proximate composition of the CG was: 6.78% protein, 1.69% fat, 2.26% ash, 1.02% total fiber and 81.80% NFE (dry basis). Defatted soy flour (DSF) was obtained from Hodgson Mill, Inc. (Effingham, Il). The initial moisture content of DSF was 7.75% (wet basis). The proximate composition of the DSF powder was: 53.94% protein, 0.77% fat, 6.87% ash, 2.93% total fiber and 26.09% NFE (dry basis). The different ingredients i.e. GP, DSF and CG were mixed into five different compositions (Blend I to V) as shown in Table 8.1. Water was added to the blends to adjust the final moisture content (15 to 25% wb) depending on the experimental runs (Table 8.2 and Table 8.3). The ingredients were mixed in a laboratory scale mixer (KitchenAid Professional 5 Plus, Troy, Ohio, USA) for 10 minutes. For moisture stabilization, the blends were stored overnight at ambient temperature. The moisture content of the prepared blends was determined by using the method 44-19 (AACC, 2000). The analysis of chemical compositions like crude protein, crude fat, crude fiber, ash and moisture content of GP, DSF and CG were carried out by standard methods (AOAC International, 2012).

8.3.2 Extrusion processing

A conical counter-rotating twin screw extruder was used. The general screw geometry was: length 330.7 mm, diameter 32 mm and flight depth 3.75 mm. The 15-40-002 mixing-counter rotating screw pair configuration was used. The extruder had a barrel with length to diameter ratio of 13:1. The extruder had four temperature control zones on the barrel. The pressure at the die during extrusion was measured with a pressure

transducer (Brabender NJ, USA). A thermocouple was used for the product temperature measurement at the die. All extrusion variables shown in the control panel (barrel-, screw-, die- and product-temperature, die pressure, screw speed and percent torque), were displayed and stored simultaneously every 20 s on Dell-PC computer. The product temperature during extrusion was adjusted by varying the temperatures in barrel sections, screw and die.

8.3.3 Experimental design

A central composite rotatable design of four variables at five levels each with six central point combinations was developed using Design-Expert 8.0.7.1 (Stat-Ease, Minneapolis, MN, USA). Independent variables included grape pomace level (X_1), temperature (X_2), screw speed (X_3) and moisture content (X_4). Coded levels for independent variables are shown in Table 8.2. Outline of experimental design with the coded and uncoded levels is presented in Table 8.3. Response variables were expansion ratio, bulk density, water absorption and solubility indices, textural properties, color parameters, total dietary fiber and anthocyanin content.

8.3.4 Determination of physico-chemical properties of the extrudates

8.3.4.1 Expansion ratio

Expansion ratio was calculated as the cross-sectional area of extrudate divided by the cross- sectional area of the die opening. An average diameter of ten samples was measured with a digital caliper to determine the expansion ratio of each set of samples.

8.3.4.2 Bulk density

Bulk density was determined as the ratio of the mass of extrudates that they filled up to a given bulk volume and measured using a standard bushel tester (Seedburo

Equipment Company, Chicago, IL) following the method recommended by USDA (USDA, 1999).

8.3.4.3 Water absorption and solubility indices

Extrudates were ground to fine powders using a coffee grinder (Black & Decker® Corporation, Towson, MD, USA). The ground extrudates (2.5 g) was suspended in distilled water (30 mL) in a tarred 50 mL centrifuge tube. The suspension was stirred intermittently and centrifuged at 3000×*g* for 10 min. The supernatant was decanted into a tarred aluminum cup and dried at 135^{o}C for 2 h. The weight of the gel remaining in the centrifuge tube was measured. The water absorption index (WAI, g/g) and water solubility index (WSI, %) were calculated as mentioned by Singh and Muthukumarappan (2016).

8.3.4.4 Texture

The peak force as an indication of hardness was measured with a TA-XT2 Texture Analyzer (Stable Micro Systems Texture Analyzer TAXT2, Texture Technologies Corp., England) employed with a Warner Bratzler probe 12 x 7cm (HDP/BS) with maximum load of 5kg. The probe was set to move at a pre-test speed of 1.5 m/s, speed test of 2.0 m/s and speed post-test of 10 m/s. The curve was recorded and analyzed by Texture Exponent 32 software program (version 3.0). The slope (N/mm) and distance (mm) at which the extrudates breaks were measured from force–distance curve and evaluated as crispness and brittleness, respectively. Ten measurements were performed on each sample.

8.3.4.5 Color

Color of extrudates was measured using Minolta Spectrophotometer (CM-2500d, Minolta Co. Ltd, Japan). The *L** value indicates the lightness, 0–100 representing dark to light color. The *a** value gives the degree of the red-green color, with a higher positive *a** value indicating more red color. The *b** value indicates the degree of the yellow-blue color, with a higher positive *b** value indicating more yellow color.

8.3.4.6 Determination of Total Phenolic Content and Antioxidant Acitivity

For determining total phenolic content (TPC) and (AA), samples were extracted using the method described by Khan et al. (2013). 1 g of ground extrudates was mixed with 10 ml of methanol followed by shaking at low speed for 1 h and then centrifuged at 3000×g for 20 min. The supernatant was decanted and the residue was re-extracted as described above. The two supernatants were combined and stored at -20 ^{o}C until analysis for TPC and AA. TPC of extrudates was determined using Folin-Ciocalteau method (Singleton et al., 1999) with some modification. 50 µl methanol extract of sample (i.e. the supernatant) was added with 3.5 ml distilled water and 150 µl Folin-Ciocalteau reagent. The solution was vortexed and incubated for 30 min. Thereafter, absorbance of solution was measured at 760 nm against blank. Blank solution consisted only 3.5 ml distilled water and 150 µl Folin-Ciocalteau reagent. Gallic acid was used as positive control (standard) and linear regression curve between absorbance and concentration was drawn for the standard. This standard curve with gallic acid concentration from 0 to 0.5 mg/ml was used for calculating the concentration of sample and data was expressed in mg Gallic acid equivalent (GAE)/100 g dry basis. This analysis was done in triplicate.

AA was measured by the method described by Brand-Williams et al. (1995) 2,2–diphenyl- 1-picrylhydrazyl (DPPH) (Sigma, St. Louis, MO, USA) solution was prepared by adding 7.9 mg of DPPH in 200 ml ethanol. 125 µl methanol extract was mixed with 2 ml ethanol and 0.5 ml of this solution was added with 3 ml DPPH. The solution was vortexed and incubated at room temperature for 30 min. Absorbance of solution and control (DPPH) was measured at 517 nm against blank (ethanol). Results were expressed as µmol Trolox equivalent (TE)/100 g dry basis. Samples were analyzed in triplicate.

8.3.5 Statistical analysis

Analysis of variance (ANOVA) test was carried out using commercial statistical package, Design Expert 8.0 (State-Ease Inc., Minneapolis, USA) to determine the significance at different levels. Same software was used for the generation of response surface plots. A Pearson's correlation coefficient matrix on system parameters and product responses was carried out using SPSS 23.0 (SPSS Inc., Chicago, IL, USA) to determine correlation coefficient between the parameters.

8.4 Results and Discussion

8.4.1 Expansion ratio

Multiple linear regression equations for expansion ratio (ER) are shown in Table 8.4 and Table 8.5. It was observed that GP (X_1) had significant negative linear effect ($P<0.05$) on ER followed by a positive linear effect of screw speed (X_3) ($P<0.05$). ANOVA for linear model as fitted to experimental results of ER showed significance ($P<0.05$). However, model showed lack-of-fit was not significant ($P>0.05$) (Table 8.6). Regression model fitted to experimental results of ER showed correlation of determination R^2 equal to 0.7327.

The ER of the extrudates ranged between 1.42 and 3.34. The highest value of ER of 3.34 was obtained for the extrudates containing 50% w/w DSF and 50% w/w CG. However, an ER of 3.15 was obtained for snack containing 3.75 % GP extruded at 115^{o}C temperature, 213 rpm screw speed and 22.5% moisture content. Expansion of the extrudates decreased with increase in GP level and increased with increase in screw speed. Negative correlation was found between GP level and ER (Table 8.8). Grape pomace contains insoluble fiber which lowers the affinity between the starch and insoluble fiber. Insoluble fibers also have higher hydrophilic properties, which have been shown to absorb more water, therefore modifying the glass transition temperature of the melt. It also can lower expansion by adhering to the bubble structure and consequently puncturing the cell, reducing cell extensibility (van der Sman and Broeze, 2013). The ER increased with increase in screw speed. During extrusion, with increase in screw speed viscosity decreases with favors expansion (Singha and Muthukumarappan, 2017a).

8.4.2 Bulk density

Bulk density (BD) is an important property of the extrudates commonly influenced by expansion phenomena occurring upon exiting the extrudates from die section (Brown et al., 2015). Multiple linear regression equations for BD are shown in Table 8.4 and Table 8.5. ANOVA for linear model as fitted to experimental results of BD showed significance ($P<0.05$). However, model showed lack-of-fit was not significant ($P>0.05$) (Table 8.6). The first order terms of GP (X_1) and screw speed (X_3) were found to be significant ($P<0.05$). Regression model fitted to experimental results of ER showed correlation of determination R^2 equal to 0.5923.

The bulk density of the extrudates ranged from 84.37 to 378.65 kg m^{-3}. The highest value of BD was obtained for extrudates containing 11.25% GP extruded at, 115 °C temperature, 138 rpm screw speed and 17.5 % moisture content. The BD increased with increase in the GP level. The effect may be attributed to reduced expansion and fiber distribution within the expanded network of starch in extrudates (Guy, 2001). The negative correlation between BD and ER (Table 8.8) confirms that products with higher bulk density have lower expansion and vice versa. Screw speed influences the rheological characteristics of the material. As observed in our previous study, (Singha and Muthukumarappan, 2017a) increasing the screw speed lowered the melt viscosity of the mix increasing the elasticity of the dough. This may have resulted in reduction in the density of the extrudate (Ding et al., 2006).

8.4.3 Water absorption index

The water absorption index (WAI) hat relates to the degree of starch gelatinization (Ding et al., 2005). Multiple linear regression equations for WAI are shown in Table 8.4 and Table 8.5. ANOVA for quadratic model as fitted to experimental results of WAI showed significance ($P<0.05$). However, model showed lack-of-fit was not significant ($P>0.05$) (Table 8.6). The first order terms of temperature and screw speed and the second-order term of GP were found to be significant ($P<0.05$). Regression model fitted to experimental results of ER showed correlation of determination R^2 equal to 0.8309.

In this study, the WAI was in the range of 3.3 and 4.14 g/g dry solid. WAI measures the volume occupied by the granule or starch polymer after swelling in excess water. Response surface graph for change in WAI with GP level and screw speed is

shown in Figure 8.1. Moisture content didn't have any significant effect on the WAI of the extrudates. Extrusion process parameters had significant effect on WAI, as increase in temperature significantly decreased the WAI of extrudates. A decrease in WAI with increasing temperature was probably due to decomposition or degradation of starch (Altan et al., 2008a). Ding et al. (2006) also stated that the WAI decreases with increasing temperature if dextrinization or starch melting prevails over the gelatinization phenomenon. Simultaneously, it can be said that WAI increases when the moisture content and temperature are kept low. The increase in WAI of extruded products is related to the hydrophilic nature of the raw ingredient, which could be affected by changes in molecular conformation exposing amino acid chains previously enclosed, thus providing water interactions (Paredes-LÓPez et al., 1991). As increased screw speed results in an increased SME, that would ultimately result in more breakdown of the starch. This suggests that high screw speed renders more soluble products (Altan et al., 2009a). GP contains fiber, carbohydrates, and proteins components that can provide more hydrophilic forces to compete for water than the starch (Altan et al., 2008c), which could be another explanation to the higher WAI found in extrudates with GP. WAI is an indicator of the hydrophilic groups and their gel-forming capability within the starch matrix. High WAI values indicate that the extrudates can hold water with lesser solubility. This is a valuable property in processing breakfast cereal products, which helps to increase bowl life.

8.4.4 Water solubility index

The water solubility index (WSI) measures the amount of soluble component released from starch after extrusion (Ding et al., 2006) and is often used as an indicator of

degradation of molecular components. Multiple linear regression equations for WSI are shown in Table 8.4 and Table 8.5. The experimental data fit the response equation with a high degree of significance (Table 8.6). However, the lack of fit was not significant ($P>0.05$). It was observed that the first order terms of GP level and screw speed were significant ($P<0.05$) on the WSI.

The WSI ranged from 6.78 to 16.75%. Statistical analysis revealed that WSI was negatively correlated with WAI (Table 8.8). WSI was also negatively correlated with GP level and BD. Decrease in WSI of the extrudates was observed when the GP level increased. Addition of GP provides insoluble fiber and other hydrophilic groups that can interact with starch to reduce the overall gelatinization, and hold water instead of being solubilized. In fact, cherry pomace in swelled starch granules trapes water and lowers WSI (Wang et al., 2016). However, soluble sugars within the GP could also lead to an increase in WSI since they are more dextrinized components. The overall WSI values are likely a balance between the WSI from the starch components and from the GP components. The lower WSI of GP extrudates was consistent with their higher WAI, which was further proved by the negative correlation (-0.55) between WSI and WAI (Table 8.8). With increase in screw speed the WSI was found to decrease. The WSI depends on quantity of solubles which increases due to the degradation of starch. Wen et al. (1990) indicated that screw speed had a direct effect on polysaccharide size distribution. A higher screw speed resulted in more fragmentation than a lower screw speed. A degradation of amylose and amylopectin molecules of maniac starch through chain splitting has been reported by Colonna et al. (1983).

8.4.5 Textural properties

The maximum peak force was measured to determine the hardness of the extrudates. Multiple linear regression equations for peak force are shown in Table 8.4 and Table 8.5. ANOVA for quadratic model as fitted to experimental results of peak force showed significance ($P<0.05$). The lack of fit was not significant ($P>0.05$) (Table 8.7). GP, temperature and screw speed had significant linear effects ($P<0.05$) on the peak force.

Peak force (hardness) of extrudates decreased with increase in temperature (Figure not shown). Ding et al. (2005) reported that increasing temperature would decrease melt viscosity, but it also increases the vapor pressure of water. This favors the bubble growth which is the driving force for expansion that produces low density products and thus decreasing hardness of extrudate. Similar results were also reported by Altan et al. (2008c). Increase in GP level in the blends resulted in decrease in the peak force of the extrudates. This could be due to increase in non-digestable fiber content with increase in GP. A negative correlation was observed between peak force and GP level (Table 8.8).

The distance to break the extrudates is an indication of brittleness, with the shortest distance being the most brittle product. Product cracks when brittleness increases. Multiple linear regression equations for distance are shown in Table 8.4 and Table 8.5. ANOVA for linear model as fitted to experimental results of distance showed significance ($P<0.05$) (Table 8.7). The lack of fit was not significant ($P>0.05$) (Table 8.7). GP, temperature and screw speed had significant linear effects ($P<0.05$) on the distance. The measured value of distance for the extrudates was in the range of 0.61–1.33

mm. Increasing GP level in the blends decreased the distance and hence increased brittleness of samples. Altan et al. (2008c) also reported similar finding during twin screw extrusion of grape pomace. Peak force was positively correlated (Table 8.8) with distance suggesting less brittle products have are low in hardness.

Crispness is typically a textural attribute for snack foods and baked products. The lower the slope, the crisper the product is considered. Multiple linear regression equations for the slope are shown in Table 8.4 and Table 8.5. ANOVA for linear model as fitted to experimental results of slope showed significance ($P<0.05$). Only temperature had significant linear effect ($P<0.05$) on the slope. The values of slope measured as crispness varied between 5.78 and 12.78 N/mm for the extrudates. Crispness increased with increase in the extrusion temperature. Similar results was reported by Garg and Singh (2010) during extrusion of soy/rice blends

8.4.6 Color parameters

Color is an important characteristic of extruded foods. Color changes during the extrusion process can provide important information about the degree of thermal treatment (Chen et al., 1991). Images of the extrudates are represented in Figure 8.2. Multiple linear regression equations for the color parameters and total color change are shown in Table 8.4 and Table 8.5. ANOVA for quadratic model as fitted to experimental results of lightness (L^*) showed significance ($P<0.05$). The lack of fit was not significant ($P>0.05$) for all color parameters (Table 8.7). Grape pomace level had significant linear effect ($P<0.05$) on the L^* values. The L^* values varied from 25.78 to 89.72. The L^* values decreased with increase in GP level. It is known that reducing sugars and proteins (amino acids) in foods can react under high processing temperatures to promote

nonenzymatic browning (Maillard reaction), which results in darkening of the final product. The observed decrease in brightness may be attributed to the Maillard reaction. Reduction in whiteness, as evidenced in decrease in *L**values, indicates darker samples.

Redness '*a**' of the extruded samples ranged from 4.25 to 13.65. The ANOVA for *a** (redness) is shown in Table 8.7. Linear effect of GP was found to be significant ($P<0.05$) on the *a** values of the extrudates. The redness value of the extrudates increased as the GP level increased. The increase in a value of the extrudates may also be associated with Maillard reaction and destruction of heat sensitive pigments.

Yellowness values (*b**) of extruded samples ranged from 6.41 to 32.05. The ANOVA for *b** (yellowness) is shown in Table 8.7. GP had a significant linear effect ($P<0.05$) and temperature had significant quadratic effect on yellowness. Yellowness of the extrudates decreased with increase in the GP level (Figure 8.3(a)). The yellowness decreased initially as the extrusion temperature increased. Further increase in temperature resulted in increase in the yellowness. The *L** and *b** values were negatively correlated with GP level, whereas *a** values were positively correlated with GP level (Table 8.8).

Color difference (ΔE) was used to represent the color change between the blends and the extrudates. The ΔE ranged from 4.01 to 51.00. ANOVA for quadratic model as fitted to experimental results of ΔE showed significance ($P<0.05$). The lack of fit was not significant ($P>0.05$). GP had significant linear and quadratic effects ($P<0.05$) on the color difference of the extrudates (Figure 8.3(b)).

8.4.7 Total phenolic content of the extrudates

The equations for quadratic models employed to predict the TPC and AA of the extrudates are given in Table 8.4 and Table 8.5. Regression analysis showed that TPC

and AA were significantly ($P<0.05$) affected by linear term of screw speed and linear and quadratic terms of GP level. Phenolic content was not significantly ($P>0.05$) affected by temperature and moisture content. Any interaction effect between the independent variables was also not observed.

Total polyphenolic content (TPC) of the dried GP was 523.1 ± 4.11 mg GAE/100g DW. TPC of the blends ranges from 51.01 ± 3.48 to 194.56 ± 5.17 mg GAE/100g DW. The concentration of TPC of the extrudates ranged from 48.06 to 187.06 g GAE/100 g dry sample. The loss of polyphenolics depended on the extrusion processing conditions. The TPC of the extrudates depended on the GP level in the blends. As the GP level in the blends increased the TPC also increased in the extrudates. Slight decrease in TPC was also observed as the screw speed increased. An increase in screw speed generates greater mechanical energy to the system and shearing effect increases. Although increased screw speed reduces residence time, the shearing effect becomes more dominant on the destruction of phenolics in blends during extrusion. Changes in TPC with GP and screw speed is shown in Figure 8.4(a)

AA of the extrudates increased with increase in GP level. An increase in AA with increasing GP level can be attributed to phenolic compound in grape pomace. This result was also supported by significant increase of TPC by increasing level of grape pomace. A positive correlation (Table 8.8) was found between phenolic content and antioxidant activity of pomace enriched extrudates. Response surface plot for AA as functions of screw speed and GP level is given in Figure 8.4(b). The AA values were affected negatively with increase in the screw speed which could probably be due to destruction of antioxidant compounds. Similar reduction in AA was also observed by Ozer et al. (2006)

during extrusion of corn flour. Results indicated that most of the polyphenolics and antioxidant compounds retained in extrusion processing and thus could contribute to the nutritional quality of extrudates.

8.5 General comparison of twin screw extrusion soy-corn blends containing apple pomace and grape pomace

Apple and grape pomace both were successfully extruded along with blends of defatted soy flour and corn grits. Antioxidant properties of the extrudates enhanced after addition of the pomaces. However, TPC and AA improved to a greater degree upon addition of GP than that obtained after addition of AP to the blends. At 15% pomace addition, TPC and AA values were 94.97 mg GAE/100 dry solid and 302.88 μg TE/100 g dry solid for AP enriched extrudates, respectively. While at 15% pomace level, TPC and AA values for GP extrudates were 187.06 mg GAE/100 dry solid and 357.06 μg TE/100 g dry solid, respectively. The color of the extrudates significantly varied and largely depended on the AP and GP level. GP extrudates were darker and more reddish in color compared to AP extrudates. At 15% pomace addition, L^*, a^* and b^* values for AP extrudates were 41.92, 9.15 and 20.77, respectively. Whereas the L^*, a^* and b^* values for GP extrudates were 25.78, 13.65 and 6.41, respectively. AP and GP had significant effect on the ER, BD, WSI and hardness of the extrudates. At 15% AP addition, ER was 1.53 and at 15% GP addition ER was 1.42. However, a maximum ER of 3.15 was achieved at GP level of 3.75 extruded at 115^{o}C, 213 rpm and 22.5% moisture content. Interestingly, maximum ER of 2.11 was achieved at 10% AP level in the blends extruded at 120^{o}C, 150 rpm and 14% moisture content.

8.6 Conclusions

Extrusion cooking of the blends from grape pomace, defatted soy flour and corn grits were investigated in a conical twin screw extruder. It is evident that blend of fruit byproducts can be incorporated in cereal and legume flour during extrusion. The product responses were mostly affected by GP level and extrusion temperature. At 3.75% GP level, extrudate expansion ratio of 3.15 has been achieved. The color of the extrudates were darker due to grape pomace addition. Increasing grape pomace content resulted in an increase in the bulk density, and a decrease in the expansion ratio and lightness of the extrudate. The results showed that varying levels of grape pomace could be incorporated into an extruded snack depending on the desired texture of the final product. The data obtained is helpful to compare nutritional as well as physical properties of extrudates containing AP. The findings of this study demonstrate the feasibility of developing value-added products from grape pomace, defatted soy flour and corn grits by extrusion processing.

Table 8.1 Ingredient composition of blends.

Feed ingredients	**Mass of ingredients (g kg^{-1})**				
	Blend 1	**Blend 2**	**Blend 3**	**Blend 4**	**Blend 5**
Grape Pomace	0	37.5	75.0	112.5	150
Defatted soy Flour	500	463	425	388	350
Corn Grits	500	500	500	500	500

Table 8.2 Independent numerical variables and their levels.

Numerical variables	**Symbol**	**Coded variable levels**				
		-2	**-1**	**0**	**1**	**2**
Apple Pomace (%)	X_1	0	3.75	7.50	11.25	15.0
Moisture content (%wb)	X_2	15.0	17.5	20.0	22.5	25.0
Screw speed (rpm)	X_3	100	138	175	213	250
Temperature (°C)	X_4	100	115	130	145	160

wb, wet basis

Table 8.3 Experimental design layout.

Run	Coded variables				Actual variables			
	x_1	x_2	x_3	x_4	X_1 (%)	X_2 (°C)	X_3 (rpm)	X_4 (%)
1	-1	-1	1	1	3.75	115.0	213	23
2	-1	1	1	-1	3.75	145.0	213	18
3	1	-1	1	1	11.25	115.0	213	23
4	-1	-1	-1	-1	3.75	115.0	138	18
5	-1	1	1	1	3.75	145.0	213	23
6	0	0	0	-2	7.5	130.0	175	15
7	1	-1	1	-1	11.25	115.0	213	18
8	1	1	-1	1	11.25	145.0	138	23
9	-1	-1	-1	1	3.75	115.0	138	23
10	2	0	0	0	15	130.0	175	20
11	0	0	0	0	7.5	130.0	175	20
12	0	0	2	0	7.5	130.0	250	20
13	1	-1	-1	1	11.25	115.0	138	23
14	1	1	-1	-1	11.25	145.0	138	18
15	-2	0	0	0	0	130.0	175	20
16	0	0	0	0	7.5	130.0	175	20
17	1	1	1	-1	11.25	145.0	213	18
18	-1	-1	1	-1	3.75	115.0	213	18
19	0	0	0	2	7.5	130.0	175	25
20	1	1	1	1	11.25	145.0	213	23
21	1	-1	-1	-1	11.25	115.0	138	18
22	0	2	0	0	7.5	160.0	175	20
23	-1	1	-1	1	3.75	145.0	138	23
24	-1	1	-1	-1	3.75	145.0	138	18
25	0	-2	0	0	7.5	100.0	175	20
26	0	0	-2	0	7.5	130.0	100	20
27	0	0	0	0	7.5	130.0	175	20

Table 8.4 Best-fit response surface models in terms of coded variables after excluding the insignificant terms for ER, BD, WAI, WSI, PF, Di, S, L*, a*, b*, ΔE, TPC and AA.

Parameters	**Response surface model**	R^2	***P* value**
ER (-)	$Y_{ER} = 2.23 - 0.40x_1 + 0.11x_3$	0.7327	<0.0001
BD (kg/m^3)	$\mathrm{BD} = 199.26 + 22.54x_1 - 50.93x_3$	0.5923	0.0004
WAI (g/g)	$Y_{WAI} = 3.49 + 0.11x_2 - 0.07x_3 - 0.07x_1x_3 + 0.08x_1x_4 - 0.07x_1^2$	0.8309	0.0085
WSI (%)	$Y_{WSI} = 11.22 - 1.32x_1 - 0.63x_3$	0.6622	<0.0001
PF (N)	$Y_{PF} = 7.10 - 0.0.60x_1 - 0.68x_2 + 0.641x_3$	0.6117	0.0002
D$_i$ (mm)	$Y_{Di} = 1.01 - 0.08x_1 - 0.09x_2 - 0.06x_3$	0.6463	<0.0001
S (N/mm)	$Y_S = 9.11 + 1.09x_2$	0.4629	0.0065
L*	$Y_{L^*} = 48.61 - 10.32x_1$	0.6529	<0.0001
a*	$Y_{a^*} = 9.01 + 2.99x_1$	0.8692	<0.0001
b*	$Y_{b^*} = 15.27 - 2.65x_1 + 3.06x_2^2$	0.7670	0.0397
Δ*E*	$\Delta E = 39.67 + 7.31x_1 + 3.45x_1x_4 + 3.46x_1^2$	0.8690	0.0023
TPC (g GAE/100 g dry sample)	$Y_{TPC} = 125.86 + 30.47x_1 - 3.69x_3 - 2.63x_1^2$	0.0720	<0.0001
AA (µmol TE/100 g dry sample)	$Y_{AA} = 295.86 + 35.67x_1 + 13.69x_3 + 6.34x_1^2$	0.0720	<0.0001

ER = expansion ratio, BD = bulk density, WAI = water absorption index, WSI = water solubility index, PF = peak force, Di = distance, S = slope, L* = lightness, a* = red-green color, b* = yellow-blue color, ΔE = total color difference, TPC = total phenolic content, AA = antioxidant activity

Table 8.5 Best-fit response surface models in terms of actual variables after excluding the insignificant terms for ER, BD, WAI, WSI, PF, Di, S, L*, a*, b*, ΔE, TPC and AA.

Parameters	**Response surface model**	R^2	***P* value**
ER (-)	$Y_{ER} = 2.61 - 0.11X_1 - 0.003X_3$	0.7327	<0.0001
BD (kg/m^3)	$Y_{BD} = 521.38 + 6.01X_1 - 1.36X_3$	0.5923	0.0004
WAI (g/g)	$Y_{WAI} = 9.71 - 0.19X_2 - 0.009X_3 + 0.0005X_1X_3 + 0.0008X_1X_4 + 0.005X_1^2$	0.8309	0.0085
WSI (%)	$Y_{WSI} = 10.40 - 0.35X_1 - 0.05X_3$	0.6622	<0.0001
PF (N)	$Y_{PF} = 18.17 - 0.16X_1 - 0.05X_2 - 0.02X_3$	0.6117	0.0002
D$_i$ (mm)	$Y_{Di} = 2.57 - 0.02X_1 - 0.006X_2 - 0.002X_3$	0.6463	<0.0001
S (N/mm)	$Y_S = -8.05 + 0.07X_2$	0.4629	0.0065
L*	$Y_{L^*} = 60.38 - 2.75X_1$	0.6529	<0.0001
a*	$Y_{a^*} = 0.65 + 0.79X_1$	0.8692	<0.0001
b*	$Y_{b^*} = 151.86 - 0.66X_1 + 0.01X_2^2$	0.7670	0.0397
Δ*E*	$Y_{\Delta E} = 124.33 - 0.51X_1 + 0.37X_1X_4 - 0.25X_1^2$	0.8690	0.0023
TPC (g GAE/100 g dry sample)	$Y_{TPC} = 67.00 + 10.57X_1 - 0.11X_3 - 0.19X_1^2$	0.0720	<0.0001
AA (µmol TE/100 g dry sample)	$Y_{AA} = 237.00 + 15.02X_1 - 0.15X_3 - 0.25X_1^2$	0.0720	<0.0001

ER = expansion ratio, BD = bulk density, WAI = water absorption index, WSI = water solubility index, PF = peak force, Di = distance, S = slope, L* = lightness, a* = red-green color, b* = yellow-blue color, ΔE = total color difference, TPC = total phenolic content, AA = antioxidant activity

Table 8.6 Analysis of variance for expansion ratio (ER), bulk density (BD), water absorption index (WAI), water solubility index (WSI) and peak force.

Response	Source	df	Sum of squares	Mean squares	F-value	P-value
ER	Regression	4	4.36	1.09	15.08	< 0.0001
	Lack-of-fit	20	1.50	0.07	1.55	0.4639
	Pure error	2	0.10	0.05		
	Residual	22	1.59	0.07		
	Total	26	5.96			
BD	Regression	4	85777.39	21444.35	7.99	0.0004
	Lack-of-fit	20	57154.13	2857.71	3.04	0.2768
	Pure error	2	1882.17	941.09		
	Residual	22	59036.31	2683.47		
	Total	26	144813.69			
WAI	Regression	14	0.80	0.06	4.21	0.0085
	Lack-of-fit	10	0.10	0.01	0.28	0.9303
	Pure error	2	0.07	0.03		
	Residual	12	0.16	0.01		
	Total	26	0.97			
WSI	Regression	4	123.42	30.86	10.78	< 0.0001
	Lack-of-fit	20	49.83	2.49	0.38	0.9036
	Pure error	2	13.13	6.57		
	Residual	22	62.96	2.86		
	Total	26	186.38			
Peak force	Regression	4	29.73	7.43	8.6644175	0.0002
	Lack-of-fit	20	16.22	0.81	0.6109281	0.7804
	Pure error	2	2.65	1.33		
	Residual	22	18.87	0.86		
	Total	26	48.60			

Table 8.7 Analysis of variance for distance, slope, lightness (L*), redness (a*), yellowness (b*) and total color change (ΔE)

Response	Source	df	Sum of squares	Mean squares	F-value	P-value
Distance	Regression	4	0.49	0.12	10.05	< 0.0001
	Lack-of-fit	20	0.24	0.01	0.80	0.6902
	Pure error	2	0.03	0.01		
	Residual	22	0.27	0.01		
	Total	26	0.75			
Slope	Regression	4	46.77	11.69	4.74	0.0065
	Lack-of-fit	20	49.37	2.47	1.01	0.6119
	Pure error	2	4.90	2.45		
	Residual	22	54.27	0.01		
	Total	26	101.04			
*L**	Regression	4	2699.30	674.82	10.35	< 0.0001
	Lack-of-fit	20	1398.41	69.92	3.85	0.2262
	Pure error	2	36.32	18.16		
	Residual	22	1434.74	65.22		
	Total	26	4134.04			
*a**	Regression	4	218.17	54.54	36.54	< 0.0001
	Lack-of-fit	20	32.41	1.62	7.60	0.1226
	Pure error	2	0.43	0.21		
	Residual	22	32.84	1.49		
	Total	26	251.01			
*b**	Regression	14	565.14	40.37	2.82	0.0397
	Lack-of-fit	10	141.74	14.17	0.95	0.6166
	Pure error	2	29.96	14.98		
	Residual	12	171.69	14.31		
	Total	26	736.83			
ΔE	Regression	14	2222.46	158.75	5.69	0.0023
	Lack-of-fit	10	296.95	29.69	1.56	0.4531
	Pure error	2	38.09	19.04		
	Residual	12	335.04	27.92		
	Total	26	2557.50			

Table 8.8 Correlation coefficients between GP and product properties.

	GP	ER	BD	WAI	WSI	PF	Di	S	*L**	*a**	*b**	*ΔE*	TPC	AA
GP	1	-0.812**	0.290^{ns}	0.258^{ns}	-0.473*	-0.422*	-0.429*	0.233^{ns}	-0.786**	0.926**	-0.479*	0.708**	0.982**	0.982**
ER		1	-0.583**	-0.195^{ns}	0.557**	0.235^{ns}	0.239^{ns}	-0.048^{ns}	0.764**	-0.738**	0.463*	-0.722**	-0.835**	-0.835**
BD			1	0.117^{ns}	-0.423*	0.214^{ns}	0.239^{ns}	-0.134^{ns}	-0.263^{ns}	0.167^{ns}	-0.253^{ns}	0.249^{ns}	0.343^{ns}	0.343^{ns}
WAI				1	-0.549**	-0.210^{ns}	-0.371^{ns}	0.364^{ns}	-0.082^{ns}	0.253^{ns}	-0.098^{ns}	0.044^{ns}	0.266^{ns}	0.266^{ns}
WSI					1	0.037^{ns}	0.070^{ns}	-0.064^{ns}	0.484*	-0.344^{ns}	0.114^{ns}	-0.453*	-0.578**	-0.578**
PF						1	0.811**	-0.652**	0.283^{ns}	-0.415*	0.048^{ns}	-0.246^{ns}	-0.374^{ns}	-0.374^{ns}
Di							1	-0.823**	0.289^{ns}	-0.451*	-0.013^{ns}	-0.293^{ns}	-0.380^{ns}	-0.380^{ns}
S								1	-0.154^{ns}	0.192^{ns}	0.022^{ns}	0.148^{ns}	0.209^{ns}	0.209^{ns}
L*									1	-0.702**	0.412*	-0.979**	-0.825**	-0.825**
a*										1	-0.375^{ns}	0.654**	0.886**	0.886**
b*											1	-0.285	-0.430*	-0.430*
ΔE												1	0.749**	0.749**
TPC													1	1.000**
AA														1

GP = grape pomace, ER = expansion ratio, BD = bulk density, WAI = water absorption index, WSI = water solubility index, PF = peak force, Di = distance, S = slope, L* = lightness, a* = red-green color, b* = yellow-blue color, ΔE = total color difference, TPC = total phenolic content, AA = antioxidant activity

**Significant at p<0.01

*Significant at p<0.05

ns – not Significant

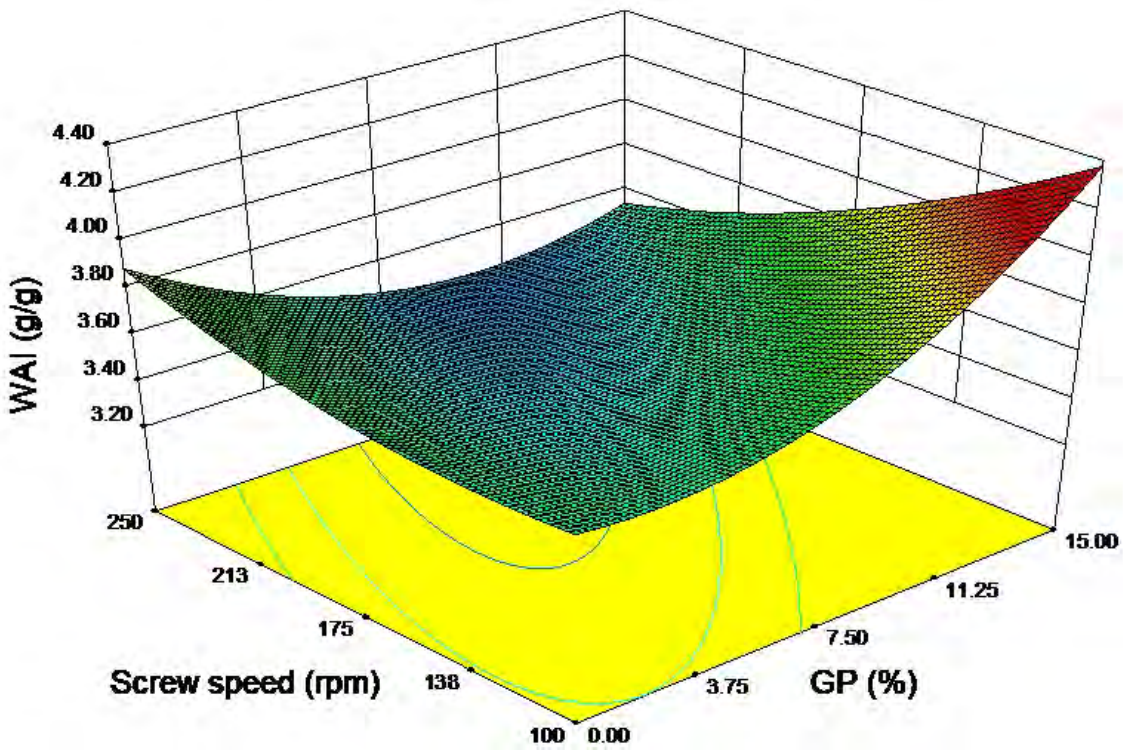

Figure 8.1 Response surface graphs illustrating the effects of screw speed and grape pomace on the water absorption index of the extrudate

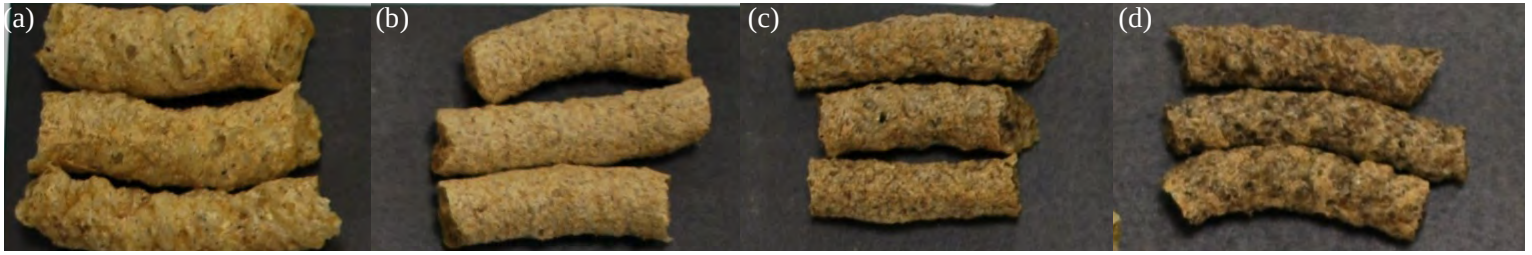

Figure 8.2 Corn-based extrudates extruded under different processing conditions containing (a) 3.75% grape pomace, (b) 7.5% grape pomace, (c) 11.25% grape pomace, (d) 15% grape pomace

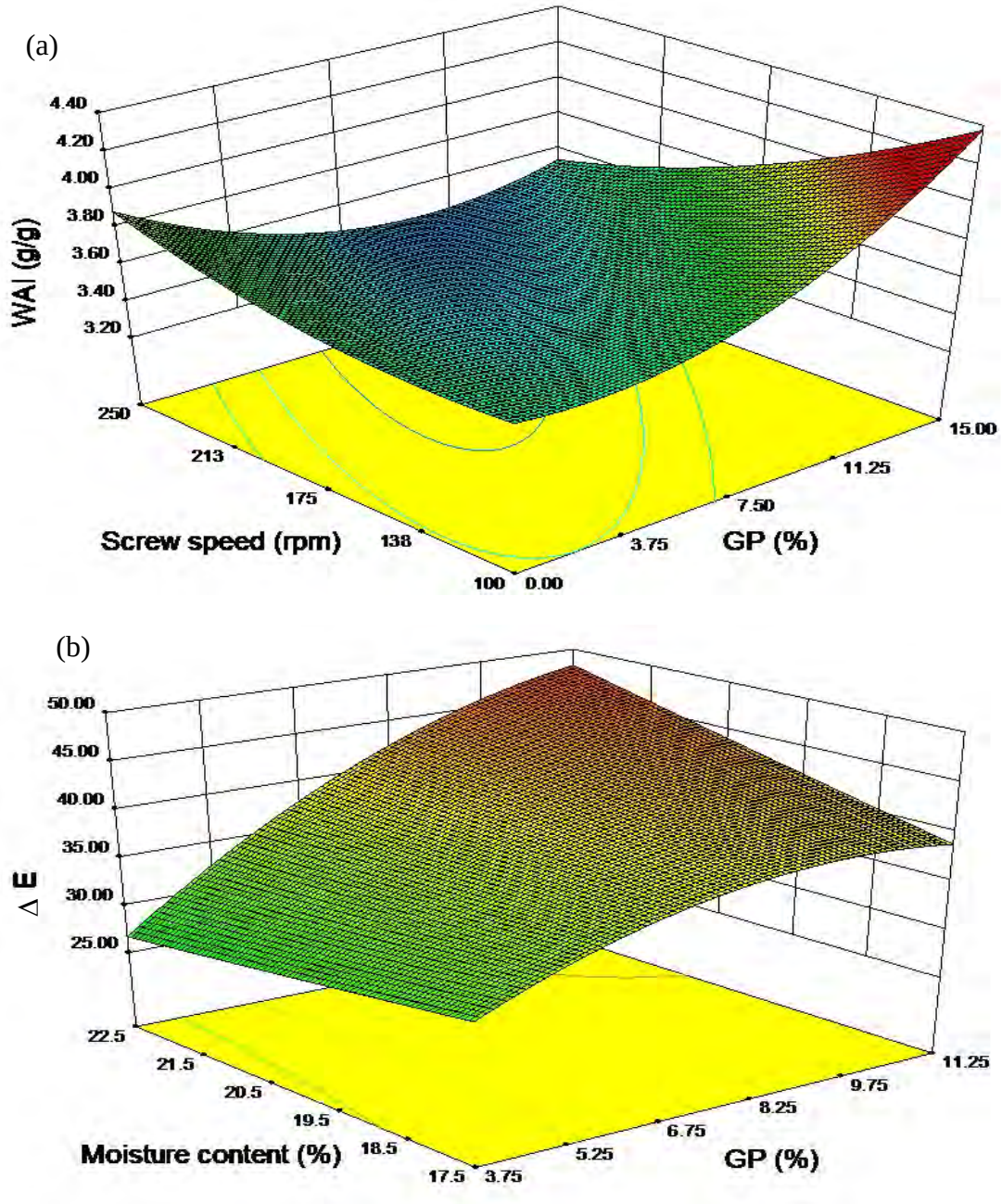

Figure 8.3 Response surface graphs illustrating (a) the effects of screw speed and grape pomace level on the yellowness value of the extrudates, (b) the effects of moisture content and grape pomace level on total color change

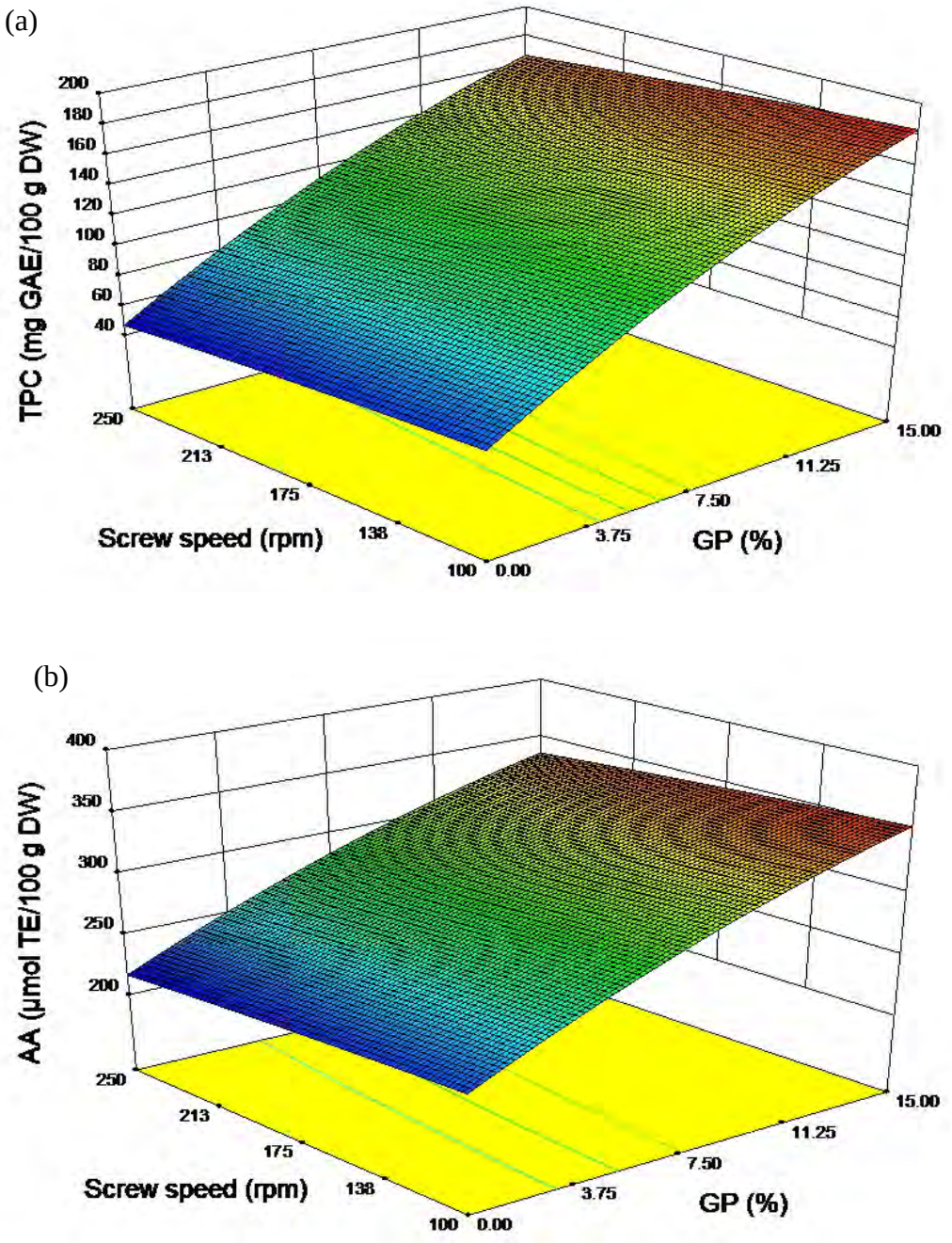

Figure 8.4 Response surface graphs illustrating the effects of screw speed and grape pomace level on the total phenolic content and antioxidant activity of the grapes

CHAPTER 9

Conclusions

The primary objective of this study was to investigate the feasibility of developing extruded expanded snacks by incorporating food industry by-products and co-products in to legume and cereal flours. The secondary objective was to understand the effect of extrusion processing conditions on the systems parameters and on the physical and functional properties of the extruded snacks.

Utilization of food waste is not only economical for the producer but also prevents environmental complications that can arise due to disposal. This dissertation studied the physico-chemical characteristics of extruded snacks containing two fruit by-products, namely apple and grape pomace. Rheological studies during extrusion of apple pomace (AP), defatted soy flour (DSF) and corn grits (CG) blends showed that increase in AP results in decrease in apparent viscosity, whereas increase in DSF results in increase in the apparent viscosity of the melt. Comparison of single-screw extrusion (SSE) and twin-screw extrusion (TSE) of the blends showed that Torque required during SSE (range 7 to 19.78 N.m) of the blends was much lower compared to that during TSE process (11.3 and 65.4 N.m). Hence, mechanical energy required was lower during SSE compared to that of TSE. Also, die pressure was comparatively high during TSE than during SSE. While maximum ER of extrudates obtained during SSE of the blends was 1.98, during TSE maximum ER of extrudates was 2.12. The antioxidant properties of the extrudates produced using TSE was comparatively higher than that produced using SSE. Extrudates containing 20% AP, showed 4% increase in TPC during TSE than during

SSE. Useful information on the structural changes in the extrudates during SSE and TSE have been reported.

In the grape pomace (GP) study, TSE of blends of GP, DSF and CG were explored. GP addition produced darker extruded products owing to the red-purple color of the pomace. However, an ER of 3.15 was obtained for snack containing 3.75 % GP extruded at 115^{o}C temperature, 213 rpm screw speed and 22.5% moisture content. Increasing grape pomace content resulted in an increase in the bulk density, and a decrease in the expansion ratio and lightness of the extrudate. TPC and AA improved to a greater degree upon addition of GP in the DSF-CG blends than that obtained after addition of AP to the blends. At 15% pomace addition, TPC and AA values were 94.97 mg GAE/100 dry solid and 302.88 μg TE/100 g dry solid for AP enriched extrudates, respectively. While at 15% pomace level, TPC and AA values for GP extrudates were 187.06 mg GAE/100 dry solid and 357.06 μg TE/100 g dry solid, respectively. The color of the extrudates significantly varied and largely depended on the AP and GP level. The findings of this study demonstrate the feasibility of developing value-added products from GP, DSF and CG by extrusion processing.

This dissertation also investigated a possible use of ethanol industry co-product, DDGS, in human food. Ethanol industry co-product, corn-DDGS was processed for food application (FDDG) and varying proportion of FDDG and garbanzo flour (GF) were mixed with CG for the extrusion studies. FDDG was a source of both protein and dietary fiber. The effect of FDDG and GF ratios and the extrusion processing conditions on the system parameters, physico-chemical and nutritional properties were investigated. During SSE of the FDDG, GF and CG blends, torque required was between 9 to 19 N.m which is

almost similar to that obtained during SSE of AP, DSF and CG blends (7 to 19.78 N.m). An interesting observation was that FDDG incorporation favored the expansion of the extrudates which is an important property for development of puffed snack. FDDG incorporation produced fiber rich extruded products. TDF content increased with increase in the FDDG level in the blends. Optimized extruded product had an ER of 4.36, TDF content of 11.04 g/100 g DW and a crude protein content of 15.9%.

This dissertation highlights that it is possible to include fruit by-products and ethanol industry co-products into food products. Incorporation of the by-products and co-products can improve texture and functional properties of the products. It also highlights the importance of understanding the behavior of the raw materials during extrusion process and the structure-texture relationship. Physical and functional properties of the extrudates depends on the raw materials, extrusion type, and processing conditions. The by-products and co-products have the potential to be applied to a selection of cereal and sweet products i.e. cakes, pretzels, pastries and confectionary.

CHAPTER 10

Recommendation for Future Work

This research has demonstrated several important findings. Meanwhile, it also showed some ideas of interest for future research and development, which are summarized as follows:

1. One avenue for future study could be replacing corn grits with other starch rich cereals without changing the source of protein and fiber. Sensory analysis of the extruded products will be an important study.
2. Studying the effect of other extrusion parameters such as different screw configuration, die nozzles, feed rate and varying the particle size of raw ingredients.
3. Further studies on the internal structure and changes in molecular conformation can be done by varying any one processing conditions such as extrusion temperature, screw speed, feed moisture content, etc.
4. One avenue for further study would be to develop viscosity model for the conical twin screw extruder using different die nozzle. This will help in analyzing the changes in apparent viscosity in the die during extrusion.
5. Future research in using different post processing methods such as frying might be useful in developing acceptable snacks. Addition of flavors could improve consumer preference.
6. It is relevant to investigate possible scale-up of the extrusion studies which might find potential application in snack food industries.

REFERENCES

AACC, (2000). Method 44-19, Moisture-Air Oven Method, Drying at 135°C, *AACC Approved Methods*, 10th ed. American Association of Cereal Chemists, St. Paul, MN.

Abbott, J., O'Palka, J., McGuire, C.F., (1991). Dried Distillers'Grains with Solubles: Particle Size Effects on Volume and Acceptability of Baked Products. Journal of food science 56(5), 1323-1326.

Adom, K.K., Liu, R.H., (2002). Antioxidant Activity of Grains. Journal of agricultural and food chemistry 50(21), 6182-6187.

Agathian, G., Semwal, A.D., Sharma, G.K., (2014). Optimization of barrel temperature and kidney bean flour percentage based on various physical properties of extruded snacks. Journal of Food Science and Technology.

Aguilar-Palazuelos, E., Zazueta-Morales, J.d.J., Harumi, E.N., Martínez-Bustos, F., (2012). Optimization of extrusion process for production of nutritious pellets. Food Science and Technology (Campinas) 32(1), 34-42.

Alajaji, S.A., El-Adawy, T.A., (2006). Nutritional composition of chickpea (Cicer arietinum L.) as affected by microwave cooking and other traditional cooking methods. Journal of Food Composition and Analysis 19(8), 806-812.

Alam, M.S., Pathania, S., Sharma, A., (2016). Optimization of the extrusion process for development of high fibre soybean-rice ready-to-eat snacks using carrot pomace and cauliflower trimmings. LWT - Food Science and Technology 74, 135-144.

Ali, Y., Hanna, M.A., Chinnaswamy, R., (1996). Expansion Characteristics of Extruded Corn Grits. LWT - Food Science and Technology 29(8), 702-707.

Altan, A., Mccarthy, K.L., Maskan, M., (2008a). Evaluation of snack foods from barley – tomato pomace blends by extrusion processing. Journal of Food Engineering 84, 231-242.

Altan, A., McCarthy, K.L., Maskan, M., (2008b). Extrusion cooking of barley flour and process parameter optimization by using response surface methodology. Journal of the Science of Food and Agriculture 88(9), 1648-1659.

Altan, A., McCarthy, K.L., Maskan, M., (2008c). Twin-screw extrusion of barley–grape pomace blends: Extrudate characteristics and determination of optimum processing conditions. Journal of Food Engineering 89, 24-32.

Altan, A., McCarthy, K.L., Maskan, M., (2009a). Effect of Extrusion Cooking on Functional Properties and in vitro Starch Digestibility of Barley-Based Extrudates from Fruit and Vegetable By-Products. Journal of food science 74(2), E77-E86.

Altan, A., McCarthy, K.L., Maskan, M., (2009b). Effect of extrusion process on antioxidant activity, total phenolics and β-glucan content of extrudates developed from barley-fruit and vegetable by-products. International Journal of Food Science & Technology 44(6), 1263-1271.

Alvarez-Martinez, L., Kondury, K.P., Harper, J.M., (1988). A General Model for Expansion of Extruded Products. Journal of food science 53(2), 609-615.

Anton, A.A., Gary Fulcher, R., Arntfield, S.D., (2009). Physical and nutritional impact of fortification of corn starch-based extruded snacks with common bean (Phaseolus

vulgaris L.) flour: Effects of bean addition and extrusion cooking. Food Chemistry 113(4), 989-996.

Anton, a.a., Luciano, F.B., (2007). Instrumental Texture Evaluation of Extruded Snack Foods: a Review Evaluación Instrumental De Textura En Alimentos Extruidos: Una Revisión. Ciencia y Tecnologia Alimentaria 5, 245-251.

AOAC International, (2012). *Official Methods of Analysis of AOAC International* (19th ed). AOAC International, Gaithersburg, MD.

Badrie, N., Mellowes, W.A., (1991). Texture and Microstructure of Cassava (Manihot esculenta Crantz) Flour Extrudate. Journal of food science 56(5), 1319-1322.

Baranowski, J.D., Davidson, P.M., Nagel, C.W., Branen, A.L., (1980). Inhibition of saccharomyces cerevisiae by naturally occurring hydroxycinnamates. Journal of food science 45(3), 592-594.

Barrett, A.H., Peleg, M., (1992). Extrudate Cell Structure-Texture Relationships. Journal of food science 57(5), 1253-1257.

Barron, M., McDonough, C., Rooney, L.W., (2002). *The effects of a rice based emulsifier on extruded corn meal*. AACC meeting, Montréal, Québec, Canada.

Barth, A., (2007). Infrared spectroscopy of proteins. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1767(9), 1073-1101.

Batistuti, J.P., Barros, R.M.C., Arêas, J.A.G., (1991). Optimization of Extrusion Cooking Process for Chickpea (Cicer arietinum, L.) Defatted Flour by Response Surface Methodology. Journal of food science 56(6), 1695-1698.

Bhattacharya, M., Hanna, M.A., (1986). Viscosity modelling of dough in extrusion. International Journal of Food Science & Technology 21(2), 167-174.

Bhattacharya, M., Hanna, M.A., (1987). Kinetics of Starch Gelatinization During Extrusion Cooking. Journal of Food Science 52(3), 764-766.

Bhattacharya, S., (1997). Twin-screw extrusion of rice-green gram blend: Extrusion and extrudate characteristics. Journal of Food Engineering 32(1), 83-99.

Bhattacharya, S., Choudhury, G.S., (1994). Twin-screw extrusion of rice flour: Effect of extruder length-to-diameter ratio and barrel temperature on extrusion parameters and product characteristics. Journal of Food Processing and Preservation 18(5), 389-406.

Bhattacharya, S., Das, H., Das, A.N., (1992). Rheological Behaviour During Extrusion of Blends of Minced Fish and Wheat Flour. Journal of Food Engineering 15, 123-134.

Bhattacharya, S., Prakash, M., (1994). Extrusion of blends of rice and chick pea flours: A response surface analysis. Journal of Food Engineering 21(3), 315-330.

Bisharat, G.I., Lazou, A.E., Panagiotou, N.M., Krokida, M.K., Maroulis, Z.B., (2015). Antioxidant potential and quality characteristics of vegetable-enriched corn-based extruded snacks. Journal of Food Science and Technology 52(7), 3986-4000.

Bookwalter, G.N., Mustakas, G.C., Kwolek, W.F., McGhee, J.E., Albrecht, W.J., (1971). Full-Fat Soy Flour Extrusion Cooked: Properties and Food Uses. Journal of food science 36(1), 5-9.

Bouvier, J.M., Bonneville, R., Goullieux, A., (1997). Instrumental methods for the measurement of extrudate crispness. Agro-Food-Industry Hi-Tech 8, 16-19.

Brand-Williams, W., Cuvelier, M.E., Berset, C., (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology 28(1), 25-30.

Bravo, L., (2007). Dietary fibre composition, antioxidant capacity and physicochemical properties of a fibre-rich product from cocoa (*Theobroma cacao* L.). Food Chemistry 104(3), 948-954.

Bravo, L., Saura-Calixto, F., (1998). Characterization of Dietary Fiber and the In Vitro Indigestible Fraction of Grape Pomace. American Journal of Enology and Viticulture 49(2), 135.

Brown, M.L., Fallahi, P., Muthukumarappan, K., Singha, P., Sindelar, S., (2015). A Comparative Study of the Effects of Non-starch Polysaccharide Gums on Physical Properties of Single-screw Extruded Aquafeed. Journal of Food Processing & Technology 06, 457.

Case, S.E., Hamann, D.D., Schwartz, S.J., (1992). Effect of starch gelatinization on physical properties of extruded wheat-and corn-based products. Cereal Chemistry 69, 401-404.

Cazzaniga, D., Basilico, J.C., Gonzalez, R.J., Torres, R.L., de Greef, D.M., (2001). Mycotoxins inactivation by extrusion cooking of corn flour. Letters in Applied Microbiology 33(2), 144-147.

Chang, Y.K., El-Dash, A.A., (2003). Effects of acid concentration and extrusion variables on some physical characteristics and energy requirements of cassava starch. Brazilian Journal of Chemical Engineering 20(2), 129-137.

Chang, Y.K., Martinez-Bustos, F., Park, T.S., Kokini, J.L., (1999). The influence of specific mechanical energy on cornmeal viscosity measured by an on-line system during twin-screw extrusion. Brazilian Journal of Chemical Engineering 16(3), 285-295.

Chang, Y.K., Silva, M.R., Gutkoski, L.C., Sebio, L., Da Silva, M.A.A.P., (1998). Development of extruded snacks using jatobá (*Hymenaea stigonocarpa* Mart) flour and cassava starch blends. Journal of the Science of Food and Agriculture 78(1), 59-66.

Chavan, J.K., Kadam, S.S., Salunkhe, D.K., Beuchat, L.R., (1987). Biochemistry and technology of chickpea(Cicer arietinumL.) seeds. C R C Critical Reviews in Food Science and Nutrition 25(2), 107-158.

Cheftel, J.C., (1986). Nutritional Effects of Extrusion-Cooking. Food Chemistry 20, 263-283.

Chen, A.H., Jao, Y.C., Larkin, J.W., Goldstein, W.E., (1978). Rheological Model of Soy Dough in Extrusion. Journal of Food Process Engineering 2, 337-342.

Chen, H., Rubenthaler, G.L., Leung, H.K., Baranowski, J.D., (1988). Chemical, Physical, and Baking Properties of Apple Fiber Compared with Wheat and Oat Bran. Cereal Chemistry 65(3), 244-247.

Chen, J., Serafin, F.L., Pandya, R.N., Daun, H., (1991). Effects of Extrusion Conditions on Sensory Properties of Corn Meal Extrudates. Journal of food science 56(1), 84-89.

Chevanan, N., Rosentrater, K.A., Muthukumarappan, K., (2007). Twin-Screw Extrusion Processing of Feed Blends Containing Distillers Dried Grains with Solubles (DDGS). Cereal Chemistry 84(5), 428-436.

Chiang, A., (2007). Protein-Protein Interaction of Soy Protein Isolate from Extrusion Processing, *Department of Food Science*, University of Missouri-Columbia, p. 105.

Chinnaswamy, R., Hanna, M.A., (1988). Relationship between amylose content and extrusion-expansion properties of corn starches. Cereal Chemistry 65, 138-143.

Chinnaswamy, R., Hanna, M.A., (1990). Relationship between viscosity and expansion properties of variously extrusion-cooked corn grain components. Food Hydrocolloids 3(6), 423-434.

Choi, I.D., Phillips, R.D., Jeong, H.S., (2004). Cellular Structure of Peanut-Based Extruded Snack Products Using Scanning Electron Microscopy. Journal of Texture Studies 35(4), 353-370.

Colonna, P., Melcion, J.P., Vergnes, B., Mercier, C., (1983). Flow, mixing and residence time distribution of maize starch within a twin-screw extruder with a longitudinally-split barrel. Journal of Cereal Science 1(2), 115-125.

Conti-Silva, A.C., Bastos, D.H.M., Arêas, J.A.G., (2012). The effects of extrusion conditions and the addition of volatile compounds and flavour enhancers to corn grits on the retention of the volatile compounds and texture of the extrudates. International Journal of Food Science & Technology 47(9), 1896-1902.

Cordeiro, Y., Kraineva, J., Suarez, M.C., Tempesta, A.G., Kelly, J.W., Silva, J.L., Winter, R., Foguel, D., (2006). Fourier Transform Infrared Spectroscopy Provides a Fingerprint for the Tetramer and for the Aggregates of Transthyretin. Biophysical Journal 91(3), 957-967.

Corradini, M.G., Peleg, M., (2006). Direction Reversals in the Mechanical Signature of Cellular Snacks: A Measure of Brittleness? Journal of Texture Studies 37(5), 538-552.

de la Hera, E., Ruiz-París, E., Oliete, B., Gómez, M., (2012). Studies of the quality of cakes made with wheat-lentil composite flours. LWT - Food Science and Technology 49(1), 48-54.

Dehghan-Shoar, Z., Hardacre, A.K., Brennan, C.S., (2010). The physico-chemical characteristics of extruded snacks enriched with tomato lycopene. Food Chemistry 123(4), 1117-1122.

Deng, Q., Penner, M.H., Zhao, Y., (2011). Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins. Food Research International 44(9), 2712-2720.

Diñeiro, G.Y., Valles, B.S., Picinelli, L.A., (2009). Phenolic and antioxidant composition of by-products from the cider industry: Apple pomace. Food Chemistry 117(4), 731-738.

Ding, Q.-B., Ainsworth, P., Plunkett, A., Tucker, G., Marson, H., (2006). The effect of extrusion conditions on the functional and physical properties of wheat-based expanded snacks. Journal of Food Engineering 73(2), 142-148.

Ding, Q.-B., Ainsworth, P., Tucker, G., Marson, H., (2005). The effect of extrusion conditions on the physicochemical properties and sensory characteristics of rice-based expanded snacks. Journal of Food Engineering 66(3), 283-289.

Dogan, H., Kokini, J.L., (2007). Psychophysical Markers for Crispness and Influence of Phase Behavior and Structure. Journal of Texture Studies 38(3), 324-354.

Dotzel, M.M., (1999). Food labeling: health claims; soy protein and coronary heart disease. Food and Drug Administration, HHS. Final rule, in: Food and Drug Administration, H. (Ed.), Federal Register, pp. 57700-57733.

Duizer, L.M., Winger, R.J., (2006). Instrumental Measures of Bite Forces Associated with Crisp Products. Journal of Texture Studies 37(1), 1-15.

Eden, J.A., (2001). Managing the menopause: phyto-oestrogens or hormone replacement therapy. Ann Med 33, 4-6.

Elias-Orozco, R., Castellanos-Nava, A., Gaytán-Martínez, M., Figueroa-Cárdenas, J.D., Loarca-Piña, G., (2002). Comparison of nixtamalization and extrusion processes for a reduction in aflatoxin content. Food Additives and Contaminants 19(9), 878-885.

Endres, J.G., (2001). *Soy Protein Products: Characteristics, Nutritional Aspects, and Utilization*. AOCS Press, Champaign.

Erickson, D.R., (1995). *Practical Handbook of Soybean Processing and Utilization*. AOCS Press, United Soybean Board, Champaign, St. Louis.

Esposito, E., Rotilio, D., Dimatteo, V., Digiulio, C., Cacchio, M., Algeri, S., (2002). A review of specific dietary antioxidants and the effects on biochemical mechanisms related to neurodegenerative processes. Neurobiology of Aging 23(5), 719-735.

Euromonitor International, (2015). Sweet and savoury snacks in the US.

Falcone, R.G., Phillips, R.D., (1988). Effects of Feed Composition, Feed Moisture, and Barrel Temperature on the Physical and Rheological Properties of Snack-like Products Prepared from Cowpea and Sorghum Flours by Extrusion. Journal of food science 53(5), 1464-1469.

Fernando Reyes, L., Emilio Villarreal, J., Cisneros-Zevallos, L., (2007). The increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue. Food Chemistry 101(3), 1254-1262.

Filli, K.B., Nkama, I., Jideani, V.A., Ibok, I.U., (2012). System Parameters and Product Properties Responses During Extrusion of Fura from Millet-Soybean Mixtures. Nigerian Food Journal 30(1), 82-100.

Firkins, J.L., Berger, L.L., Fahey, G.C., (1985). Evaluation of Wet and Dry Distillers Grains and Wet and Dry Corn Gluten Feeds for Ruminants. Journal of Animal Science 60(3), 847.

Fletcher, S.I., Richmond, P., Smith, A.C., (1985). An experimental study of twin-screw extrusion-cooking of maize grits. Journal of Food Engineering 4(4), 291-312.

Friedman, M., Brandon, D.L., (2001). Nutritional and Health Benefits of Soy Proteins†. Journal of agricultural and food chemistry 49(3), 1069-1086.

Gao, X., Tan, J., (1996). Analysis of expanded-food texture by image processing part i: Geometric properties. Journal of Food Process Engineering 19(4), 425-444.

Garg, S.K., Singh, D.S., (2010). Optimization of extrusion conditions for defatted soy-rice blend extrudates. Journal of Food Science and Technology 47(6), 606-612.

Geetha, R., Mishra, H.N., Srivastav, P.P., (2012). Twin screw extrusion of kodo millet-chickpea blend: process parameter optimization, physico-chemical and functional properties. Journal of Food Science and Technology 51(11), 3144-3153.

Ghiselli, A., Nardini, M., Baldi, A., Scaccini, C., (1998). Antioxidant Activity of Different Phenolic Fractions Separated from an Italian Red Wine. Journal of agricultural and food chemistry 46(2), 361-367.

González-Centeno, M.R., Rosselló, C., Simal, S., Garau, M.C., López, F., Femenia, A., (2010). Physico-chemical properties of cell wall materials obtained from ten

grape varieties and their byproducts: grape pomaces and stems. LWT - Food Science and Technology 43(10), 1580-1586.

Gorinstein, S., Zachwieja, Z., Folta, M., Barton, H., Piotrowicz, J., Zemser, M., Weisz, M., Trakhtenberg, S., Màrtín-Belloso, O., (2001). Comparative Contents of Dietary Fiber, Total Phenolics, and Minerals in Persimmons and Apples. Journal of agricultural and food chemistry 49(2), 952-957.

Govindasamy, S., Campanella, O.H., Oates, C.G., (1997). Enzymatic hydrolysis of sago starch in a twin-screw extruder. Journal of Food Engineering 32(4), 403-426.

Guerrero, P., Kerry, J.P., de la Caba, K., (2014). FTIR characterization of protein–polysaccharide interactions in extruded blends. Carbohydrate Polymers 111, 598-605.

Guha, M., Ali, S.Z., Bhattacharya, S., (1997). Twin-screw extrusion of rice flour without a die: Effect of barrel temperature and screw speed on extrusion and extrudate characteristics. Journal of Food Engineering 32(3), 251-267.

Gujska, E., Khan, K., (1991). Feed Moisture Effects on Functional Properties, Trypsin Inhibitor and Hemagglutinating Activities of Extruded Bean High Starch Fractions. Journal of food science 56(2), 443-447.

Guy, R., (2001). Raw materials for extrusion cooking., in: Guy, R. (Ed.), *Extrusion Cooking: Technologies and Applications*. CRC Press, Cornwall, England, pp. 5-27.

Halpern, M.J., Dahlgren, A.L., Laakso, I., Seppänen-Laakso, T., Dahlgren, J., McAnulty, P.A., (1998). Red-wine Polyphenols and Inhibition of Platelet Aggregation:

Possible Mechanisms, and Potential Use in Health Promotion and Disease Prevention. Journal of International Medical Research 26(4), 171-180.

Ham, G.A., Stock, R.A., Klopfenstein, T.J., Larson, E.M., Shain, D.H., Huffman, R.P., (1994). Wet corn distillers byproducts compared with dried corn distillers grains with solubles as a source of protein and energy for ruminants. Journal of Animal Science 72(12), 3246.

Hardacre, A.K., Clark, S.M., Riviere, S., Monro, J.A., Hawkins, A.J., (2006). Some Textural, Sensory and Nutritional Properties of Expanded Snack Food Wafers Made from Corn, Lentil and Other Ingredients. Journal of Texture Studies 37(1), 94-111.

Harmann, D.V., Harper, J.M., (1974). Modeling a Forming Foods Extruder. Journal of Food Science 39(6), 1099-1104.

Harper, J.M., (1978). Food extrusion. CRC Critical Review in Food Science and Nutrition 11(2), 155-215.

Harper, J.M., (1981). Basics of extrusion and the effects of extrusion on food quality., in: Harper, J.M. (Ed.), *Extrusion of Foods.* CRC Press, Boca Raton, Florida, p. 212.

Harper, J.M., Mercier, C., Linko, P., (1989). Extrusion cooking. St. Paul, Minnesota, USA: American Association of Cereal Chemists Inc.

Harper, J.M., Rhodes, T.P., Wanniger, L.A., (1971). Viscosity Model for Cooked Cereal Doughs, *AICHE Symposium Series.* American Institute of Chemical Engineers, New York, NY, pp. 40-43.

Hauck, B.W., (1988). Is the Single-screw extruder about to become a dinosaur? Petfood Industry 30(2), 16.

Hsieh, F., Huff, H.E., Lue, S., Stringer, L., (1991). Twin screw extrusion of sugar-beet fiber and corn meal. Lebensmitted-Wissenschaft Unter- suchung-Technology. 24, 495-500.

Huang, J., Schols, H., Vansoest, J., Jin, Z., Sulmann, E., Voragen, A., (2007). Physicochemical properties and amylopectin chain profiles of cowpea, chickpea and yellow pea starches. Food Chemistry 101(4), 1338-1345.

Huber, G., (2001). Snack foods from cooking extruders, in: Lusas, E.W., Rooney, L.W. (Eds.), *Snack Food Processing*. CRC Press, Boca Raton, FL, pp. 315-367.

Hwang, J.-K., Choi, J.-S., Kim, C.-J., Kim, C.-T., (1998). Solubilization of apple pomace by extrusion. Journal of Food Processing Preservation 22, 477-491.

I.O, A., Silungwe, H., Takalani, T., A. Anyasi, T., Udeh, H., Omolol, A., (2014). Antioxidant-Rich Natural Grain Products and Human Health.

Ilo, S., Tomschik, U., Berghofer, E., Mundigler, N., (1996). The Effect of Extrusion Operating Conditions on the Apparent Viscosity and the Properties of Extrudates in Twin-Screw Extrusion Cooking of Maize Grits. LWT - Food Science and Technology 29, 593-598.

IMARC, (2017). Extruded Snack Food Market – Global Industry Trends, Share, Size, Growth, Opportunity and Forecast 2017-2022. International Market Analysis Research and Consult.

Iwe, M.O., van Zuilichem, D.J., Ngoddy, P.O., (2000). Color of single-screw extruded blends of soy-sweet potato flour – A response surface analysis. Plant Foods for Human Nutrition 55(2), 159-168.

Iwe, M.O., Van Zuilichem, D.J., Ngoddy, P.O., (2001a). Extrusion cooking of blends of soy flour and sweet potato flour on specific mechanical energy (SME), extrudate, temperature and torque. Journal of Food Processing and Preservation 25(4), 251-266.

Iwe, M.O., Van Zuilichem, D.J., Ngoddy, P.O., Ariahu, C.C., (2001b). Residence time distribution in a single-screw extruder processing soy-sweet potato mixtures. Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology 34(7), 478-483.

Jackson, J.C., Bourne, M.C., Barnard, J., (1996). Optimization of Blanching for Crispness of Banana Chips Using Response Surface Methodology. Journal of food science 61(1), 165-166.

Janssen, L.P.B.M., (1978). *Twin screw extrusion*. Elsevier Scientific, Amsterdam.

Janssen, L.P.B.M., (1989). *Engineering Aspects of Food Extrusion*. American Association of Cereal Chemists, Inc., Minnesota, USA.

Jung, J., Cavender, G., Zhao, Y., (2014). Impingement drying for preparing dried apple pomace flour and its fortification in bakery and meat products. Journal of Food Science and Technology 52(9), 5568-5578.

Kaisangsri, N., Kowalski, R.J., Wijesekara, I., Kerdchoechuen, O., Laohakunjit, N., Ganjyal, G.M., (2016). Carrot pomace enhances the expansion and nutritional quality of corn starch extrudates. LWT - Food Science and Technology 68, 391-399.

Karkle, E.L., Keller, L., Dogan, H., Alavi, S., (2012). Matrix transformation in fiber-added extruded products: Impact of different hydration regimens on texture, microstructure and digestibility. Journal of Food Engineering 108(1), 171-182.

Katsube, N., Iwashita, K., Tsushida, T., Yamaki, K., Kobori, M., (2003). Induction of Apoptosis in Cancer Cells by Bilberry (Vaccinium myrtillus) and the Anthocyanins. Journal of agricultural and food chemistry 51(1), 68-75.

Kellor, R.L., (1974). Defatted soy flour and grits. Journal of the American Oil Chemists' Society 51(1), 77A-80A.

Khan, I., Yousif, A., Johnson, S.K., Gamlath, S., (2013). Effect of sorghum flour addition on resistant starch content, phenolic profile and antioxidant capacity of durum wheat pasta. Food Research International 54(1), 578-586.

Khan, M.A., Mahesh, C., Semwal, A.D., Sharma, G.K., (2015). Effect of Virgin Coconut Meal (VCM) on the development of rice-based extruded snacks. International Journal of Advanced Research 3(10), 717-725.

Khanal, R.C., Howard, L.R., Prior, R.L., (2009). Procyanidin content of grape seed and pomace, and total anthocyanin content of grape pomace as affected by extrusion processing. Journal of food science 74, H174-182.

Kim, C.H., Maga, J.A., Martin, J.T., (1989). PROPERTIES of EXTRUDED DRIED DISTILLER GRAINS (DDG) and FLOUR BLENDS. Journal of Food Processing and Preservation 13(3), 219-231.

Klopfenstein, T., (1996). Distillers grains as an energy source and effect of drying on protein availability. Animal Feed Science and Technology 60(3-4), 201-207.

Kong, J., Yu, S., (2007). Fourier Transform Infrared Spectroscopic Analysis of Protein Secondary Structures. Acta Biochimica et Biophysica Sinica 39(8), 549-559.

Kong, J.-M., Chia, L.-S., Goh, N.-K., Chia, T.-F., Brouillard, R., (2003). Analysis and biological activities of anthocyanins. Phytochemistry 64(5), 923-933.

Konkoly, A.M., (1997). Rheological characterization of commercially available cream cheese and physical properties of corn and peanut composite flour extrudates. M.S. thesis., *Department of Agricultural Engineering*. Iowa State University, Ames, Iowa, USA.

Korus, J., Gumul, D., Czechowska, K., (2007). Effect of extrusion on the phenolic composition and antioxidant activity of dry beans of Phaseolus vulgari L. Food Technol. Biotechnol 45(2), 139-146.

Krimm, S., Bandekar, J., (1986). Vibrational Spectroscopy and Conformation of Peptides, Polypeptides, and Proteins. 38, 181-364.

Lam, C.D., Flores, R.A., (2003). Effect of Particle Size and Moisture Content on Viscosity of Fish Feed. Cereal Chemistry 80, 20-24.

Lam, C.M.D., (1996). Effects of processing parameters and material on fish feed extrusion with momentum transfer consideration. M.S. thesis., *Department of Agricultural Engineering*. Iowa State University, Ames, Iowa, USA.

Lee, S.Y., McCarthy, K.L., (1996). Effect of screw configuration and speed on rtd and expansion of rice extrudate. Journal of Food Process Engineering 19(2), 153-170.

Liu, K., (1997). Chemistry and Nutritional Value of Soybean Components. 25-113.

Liu, K., (2004). *Soybeans as Functional Foods and Ingredients*. AOCS Press, Columbia.

Liu, Y., Hsieh, F., Heymann, H., Huff, H.E., (2000). Effect of Process Conditions on the Physical and Sensory Properties of Extruded Oat-Corn Puff. Journal of food science 65(7), 1253-1259.

Llobera, A., Cañellas, J., (2007). Dietary fibre content and antioxidant activity of Manto Negro red grape (Vitis vinifera): pomace and stem. Food Chemistry 101(2), 659-666.

Lobato, L.P., Anibal, D., Lazaretti, M.M., Grossmann, M.V.E., (2011). Extruded puffed functional ingredient with oat bran and soy flour. LWT - Food Science and Technology 44(4), 933-939.

Ludewig, H.G., (1989). *Extrusion Cooking*. American Association of Cereal Chemists. Inc., St. Paul, Minnesota, USA.

Lue, S., Hsieh, F., Huff, H.E., (1991). Extrusion cooking of corn meal and sugar beet fiber: effects on expansion properties, starch gelatinization, and dietary fiber content. Cereal Chemistry 68, 227-234.

Lue, S., Hsieh, F., Peng, I.C., Huff, H.E., (1990). Expansion of corn extrudates containing dietary fiber: a microstructure study. LWT - Food Science and Technology 23, 165-173.

Lusas, E.W., Rhee, K.C., (1995). Soybean Protein Processing and Utilization, in: Erickson, D.R. (Ed.), *Practical Handbook of Soybean Processing and Utilization*. AOCS Press, St. Louis.

Luyten, H., J. Plijter, J., Van Vliet, T.O.N., (2005). Crispy/Crunchy Crusts of Cellular Solid Foods: A Literature Review with Discussion. Journal of Texture Studies 35(5), 445-492.

Madhumitha, S., Prabhasankar, P., (2011). Influence of Additives on Functional and Nutritional Quality Characteristics of Black Gram Flour Incorporated Pasta. Journal of Texture Studies 42(6), 441-450.

Maga, J., Kim, C., (1989). Co-extrusion of rice flour with dried fruits and fruit juice concentrates. LWT - Food Science and Technology 22(182-187).

Maga, J.A., Van Everen, K.E., (1989). Chemical and sensory properties of wholewheat pasta products supplemented with wheat-derived dried distillers grain (ddg). Journal of Food Processing and Preservation 13(1), 71-78.

Makris, D.P., Boskou, G., Andrikopoulos, N.K., (2007). Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. Journal of Food Composition and Analysis 20(2), 125-132.

Mangavel, C., Barbot, J., Popineau, Y., Guéguen, J., (2001). Evolution of Wheat Gliadins Conformation during Film Formation: A Fourier Transform Infrared Study. Journal of agricultural and food chemistry 49(2), 867-872.

Mason, W.R., Hoseney, R.C., (1986). Factors Affecting the Viscosity of Extrusion-Cooked Wheat Starch. Cereal Chemistry 63, 436-441.

Masoodi, F.A., Sharma, B., Chauhan, G.S., (2002). Use of apple pomace as a source of dietary fiber in cakes. Plant Foods for Human Nutrition 57(2), 121-128.

Meng, X., Threinen, D., Hansen, M., Driedger, D., (2010). Effects of extrusion conditions on system parameters and physical properties of a chickpea flour-based snack. Food Research International 43(2), 650-658.

Meuser, F., Pfaller, W., van Lengerich, B., (1987). Technological aspects regarding specific change to the characteristic properties of extrudates by HTST-extrusion

cooking, in: O'Connor, C. (Ed.), *Extrusion Technology for the Food Industry*. Elsevier Applied Science, London.

Mezreb, K., Goullieux, A., Ralainirina, R., Queneudec, M., (2003). Application of image analysis to measure screw speed influence on physical properties of corn and wheat extrudates. Journal of Food Engineering 57(2), 145-152.

Milán-Carrillo, J., Reyes-Moreno, C., Armienta-Rodelo, E., Carábez-Trejo, A., Mora-Escobedo, R., (2000). Physicochemical and Nutritional Characteristics of Extruded Flours from Fresh and Hardened Chickpeas (Cicer arietinum L). LWT - Food Science and Technology 33(2), 117-123.

Min, B., Bae, I.Y., Lee, H.G., Yoo, S.-H., Lee, S., (2010). Utilization of pectin-enriched materials from apple pomace as a fat replacer in a model food system. Bioresource technology 101(14), 5414-5418.

Mintel, (2015). A snacking nation: 94% of americans snack daily.

Mir, S.A., Bosco, S.J.D., Shah, M.A., Santhalakshmy, S., Mir, M.M., (2015). Effect of apple pomace on quality characteristics of brown rice based cracker. Journal of the Saudi Society of Agricultural Sciences.

Morales, P., Berrios, J.D.J., Varela, A., Burbano, C., Cuadrado, C., Muzquiz, M., Pedrosa, M.M., (2015). Novel fiber-rich lentil flours as snack-type functional foods: an extrusion cooking effect on bioactive compounds. Food Funct. 6(9), 3135-3143.

Moraru, C.I., Kokini, J.L., (2003). Nucleation and Expansion During Extrusion and Microwave Heating of Cereal Foods. Comprehensive Reviews in Food Science and Food Safety 2, 147-165.

Mussatto, S.I., Dragone, G., Roberto, I.C., (2006). Brewers' spent grain: generation, characteristics and potential applications. Journal of Cereal Science 43(1), 1-14.

Nayak, B., Berrios, J.D.J., Powers, J.R., Tang, J., (2011). Effect of Extrusion on the Antioxidant Capacity and Color Attributes of Expanded Extrudates Prepared from Purple Potato and Yellow Pea Flour Mixes. Journal of food science 76(6), C874-C883.

Nicoli, M.C., Anese, M., Parpinel, M., (1999). Influence of processing on the antioxidant properties of fruit and vegetables. Trends in Food Science & Technology 10(3), 94-100.

O'Shea, N., Arendt, E., Gallagher, E., (2013). Enhancing an Extruded Puffed Snack by Optimising Die Head Temperature, Screw Speed and Apple Pomace Inclusion. Food and Bioprocess Technology 7(6), 1767-1782.

O'Shea, N., Ktenioudaki, A., Smyth, T.P., McLoughlin, P., Doran, L., Auty, M.A.E., Arendt, E., Gallagher, E., (2014). Physicochemical assessment of two fruit by-products as functional ingredients: (apple and orange pomace). Journal of Food Engineering.

OIV, (2017). Global economic vitiviniculture data, Paris.

Oke, M.O., Awonorin, S.O., Sanni, L.O., Asiedu, R., Aiyedun, P.O., (2012). Effect of Extrusion Variables on Extrudates Properties of Water Yam Flour - a Response Surface Analysis. Journal of Food Processing and Preservation 37, 456-473.

Onwulata, C.I., Mulvaney, S.J., Hsieh, F., (1994). System analysis as the basis for control of density of extruded cornmeal. Food Control 5(1), 39-48.

Ozer, E.A., Herken, E.N., Guzel, S., Ainsworth, P., Ibanoglu, S., (2006). Effect of extrusion process on the antioxidant activity and total phenolics in a nutritious snack food. International Journal of Food Science and Technology 41(3), 289-293.

Paredes-LÓPez, O., Ordorica-Falomir, C., Olivares-VÁZquez, M.R., (1991). Chickpea Protein Isolates: Physicochemical, Functional and Nutritional Characterization. Journal of food science 56(3), 726-729.

Pastor-Cavada, E., Drago, S.R., González, R.J., Juan, R., Pastor, J.E., Alaiz, M., Vioque, J., (2011). Effects of the addition of wild legumes (*Lathyrus annuus* and *Lathyrus clymenum*) on the physical and nutritional properties of extruded products based on whole corn and brown rice. Food Chemistry 128(4), 961-967.

Pelembe, L.A.M., Erasmus, C., Taylor, J.R.N., (2002). Development of a Protein-rich Composite Sorghum–Cowpea Instant Porridge by Extrusion Cooking Process. LWT - Food Science and Technology 35(2), 120-127.

Poltronieri, F., Arêas, J.A.G., Colli, C., (2000). Extrusion and iron bioavailability in chickpea (Cicer arietinum L.). Food Chemistry 70(2), 175-180.

Rasco, B.A., McBurney, W.J., (1989). Human food product produced from dried distillers' spent cereal grains and solubles.

Reddy, J.A., Stoker, R., (1993). Bakery product from distiller's grain.

Reis, S.F., Rai, D.K., Abu-Ghannam, N., (2014). Apple pomace as a potential ingredient for the development of new functional foods. International Journal of Food Science & Technology 49(7), 1743-1750.

Remsen, C.H., Clark, J.P., (1978). A Viscosity Model for a Cooking Dough. Journal of Food Process Engineering 2, 39-64.

Riaz, M.N., (2000a). *Extruders in Food Applications.* CRC Press.

Riaz, M.N., (2000b). Introduction to extruders and their principles, in: Riaz, M.N. (Ed.), *Extruders in Food Applications*. Technomic Publishing Company, Inc, Lancaster, Pennysylvania, USA, pp. 1-24.

Riaz, M.N., (2007). Extruders and Expanders in Pet Food, Aquatic and Livestock Feeds. Agrimedia GmbH, Clenze, Germany.

Rice-Evans, C., Miller, N., Paganga, G., (1997). Antioxidant properties of phenolic compounds. Trends in Plant Science 2(4), 152-159.

Rodríguez Montealegre, R., Romero Peces, R., Chacón Vozmediano, J.L., Martínez Gascueña, J., García Romero, E., (2006). Phenolic compounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm climate. Journal of Food Composition and Analysis 19(6-7), 687-693.

Rodriguez-Miranda, J., Delgado-Licon, E., Hernández-Santos, B., Reyes-Jaquez, D., Aguilar-Palazuelos, E., Medrano-Roldan, H., Navarro-Cortez, R.O., Castro-Rosas, J., Gomez-Aldapa, C.A., (2012). The effect of pregelatinized potato starch on the functional properties of an extruded aquafeed. Journal of Animal Production Advances 2(7), 335-344.

Rogers, M.G., (1970). Rheological Interpretation of Brabender Plasti-Corder (Extruder Head) Data. Industrial & Engineering Chemistry Process Design and Development 9(1), 49-52.

Rosentrater, K.A., Krishnan, P.G., (2006). Incorporating Distillers Grains in Food Products. Cereal Foods World 51(2), 52-60.

Rosentrater, K.A., Richard, T.L., Bern, C.J., Flores, R.A., (2005). Small-Scale Extrusion of Corn Masa By-Products. Cereal Chemistry 82, 436-446.

Roy, F., Boye, J.I., Simpson, B.K., (2010). Bioactive proteins and peptides in pulse crops: Pea, chickpea and lentil. Food Research International 43(2), 432-442.

Ryu, G.H., Walker, C.E., (1994). Cell Structure of Wheat Flour Extrudates Produced with Various Emulsifiers. LWT - Food Science and Technology 27(5), 432-441.

Ryu, G.H., Walker, C.E., (1995). The Effects of Extrusion Conditions on the Physical Properties of Wheat Flour Extrudates. Starch - Stärke 47(1), 33-36.

Saltveit, M.E., (1998). Heat-Shock and Fresh Cut Lettuce, *Perishables Handling Quarterly Issue No. 95*, pp. 5-6.

Scalbert, A., Manach, C., Morand, C., Rémésy, C., Jiménez, L., (2005). Dietary polyphenols and the prevention of diseases. Critical reviews in food science and nutrition 45, 287-306.

Shalini, R., Gupta, D.K., (2010). Utilization of pomace from apple processing industries: a review. Journal of Food Science and Technology 47(4), 365-371.

Shirani, G., Ganesharanee, R., (2009). Extruded products with Fenugreek (Trigonella foenum-graecium) chickpea and rice: Physical properties, sensory acceptability and glycaemic index. Journal of Food Engineering 90(1), 44-52.

Shukla, C.Y., Muthukumarappan, K., Julson, J.L., (2005). Effect of Single-Screw Extruder Die Temperature, Amount of Distillers' Dried Grains With Solubles

(DDGS), and Initial Moisture Content on Extrudates. Cereal Chemistry Journal 82(1), 34-37.

Simic, M.G., Jovanovic, S.V., (1994). Inactivation of Oxygen Radicals by Dietary Phenolic Compounds in Anticarcinogenesis. 547, 20-32.

Singh, B., Sekhon, K.S., Singh, N., (2007). Effects of moisture, temperature and level of pea grits on extrusion behaviour and product characteristics of rice. Food Chemistry 100(1), 198-202.

Singh, N., Smith, A.C., (1997). A comparison of wheat starch, whole wheat meal and oat flour in the extrusion cooking process. Journal of Food Engineering 34(1), 15-32.

Singh, N., Smith, A.C., Frame, N.D., (1998). Effect of process variables and monoglycerides on extrusion of maize grits using two sizes of extruder. Journal of Food Engineering 35(1), 91-109.

Singh, S.K., (2016). Understanding the Effect of Extrusion Processing Parameters on Physical, Nutritional and Rheological Properties of Soy White Flakes Based Aquafeed in a Single Screw Extruder, p. 252.

Singh, S.K., Muthukumarappan, K., (2014a). Effect of Different Extrusion Processing Parameters on Physical Properties of Soy White Flakes and High Protein Distillers Dried Grains-Based Extruded Aquafeeds. Journal of Food Research 3(6), 107-123.

Singh, S.K., Muthukumarappan, K., (2014b). Single Screw Extrusion Processing of Soy White Flakes Based Catla Feed. Journal of Food Research 4(1), 1-9.

Singh, S.K., Muthukumarappan, K., (2016). Effect of feed moisture, extrusion temperature and screw speed on properties of soy white flakes based aquafeed: a response surface analysis. J Sci Food Agric 96(6), 2220-2229.

Singh, S.K., Muthukumarappan, K., (2017a). Rheological Characterization and CFD Simulation of Soy White Flakes Based Dough in a Single Screw Extruder. Journal of Food Process Engineering 40(2).

Singh, S.K., Muthukumarappan, K., (2017b). A Viscosity Model for Soy White Flakes-Based Aquafeed Dough in a Single Screw Extruder. Journal of Food Process Engineering 40(2).

Singh, U., Singh, B., (1992). Tropical grain legumes as important human foods. Economic Botany 46(3), 310-321.

Singh, U., Subrahmanyam, N., Kumar, J., (1991). Cooking quality and nutritional attributes of some nely developed cultivars of chickpea (Cicer arietinum). Journal of the Science of Food and Agriculture 55(1), 37-46.

Singha, P., Muthukumarappan, K., (2016). Quality changes and freezing time prediction during freezing and thawing of ginger. Food Sci Nutr 4(4), 521-533.

Singha, P., Muthukumarappan, K., (2017a). Effects of processing conditions on the system parameters during single screw extrusion of blend containing apple pomace. Journal of Food Process Engineering 40(4), 1-11.

Singha, P., Muthukumarappan, K., (2017b). Single screw extrusion of apple pomace enriched blends: Extrudate characteristics and determination of optimum processing conditions. Food Science and Technology International (*Under review*).

Singha, P., Muthukumarappan, K., Krishnan, P., (2017). Influence of processing conditions on apparent viscosity and system parameters during extrusion of distiller's dried grains-based snacks. Food Science & Nutrition.

Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. 299, 152-178.

Smith, A.C., (1992). Studies on the physical structure of starch-based materials in the extrusion cooking process., in: Kokini, J.L., Ho, C.-T., Karwe, M.V. (Eds.), *Food Extrusion Science and Technology*. Marcel Dekker, Inc., New York, pp. 570-618.

STAT, F., Database in www.fao.org.

Statista, (2014). North America: consumers snack consumption 2014 (feed-based).

Stojceska, V., Ainsworth, P., Plunkett, A., İbanoğlu, Ş., (2009). The effect of extrusion cooking using different water feed rates on the quality of ready-to-eat snacks made from food by-products. Food Chemistry 114(1), 226-232.

Subirade, M., Kelly, I., Guéguen, J., Pézolet, M., (1998). Molecular basis of film formation from a soybean protein: comparison between the conformation of glycinin in aqueous solution and in films. International Journal of Biological Macromolecules 23(4), 241-249.

Sudha, M.L., Baskaran, V., Leelavathi, K., (2007). Apple pomace as a source of dietary fiber and polyphenols and its effect on the rheological characteristics and cake making. Food Chemistry 104(2), 686-692.

Sunderland, R., (1996). Production of third-generation snacks. Cereal Foods World 41(1), 12-14.

Tan, J., Zhang, H., Gao, X., (1997). Sem Image Processing for Food Structure Analysis. Journal of Texture Studies 28(6), 657-672.

Tiwari, B.K., Brennan, C.S., Jaganmohan, R., Surabi, A., Alagusundaram, K., (2011). Utilisation of pigeon pea (Cajanus cajan L) byproducts in biscuit manufacture. LWT - Food Science and Technology 44(6), 1533-1537.

Torres, J.L., Varela, B., García, M.T., Carilla, J., Matito, C., Centelles, J.J., Cascante, M., Sort, X., Bobet, R., (2002). Valorization of Grape (Vitis vinifera) Byproducts. Antioxidant and Biological Properties of Polyphenolic Fractions Differing in Procyanidin Composition and Flavonol Content. Journal of agricultural and food chemistry 50(26), 7548-7555.

Tsen, C.C., Eyestone, W., Weber, J.L., (1982). Evaluation of the quality of cookies supplemented with distillers' dried grain flours. Journal of food science 47, 684-685.

USDA, (1999). Practical procedures for grain handlers: Inspecting Grain. United States Department of Agriculture-Grain Inspection, Packers and Stockyards Administration.

USDA, (2017). Fruit and Tree Nuts Outlook. Economic Research Service.

van der Sman, R.G.M., Broeze, J., (2013). Structuring of indirectly expanded snacks based on potato ingredients: A review. Journal of Food Engineering 114(4), 413-425.

van Zuilichem, D.J., Stolp, W., Janssen, L.P.B.M., (1983). Engineering aspects of single- and twin-screw extrusion-cooking of biopolymers. Journal of Food Engineering 2(3), 157-175.

Verbeek, C.J.R., van den Berg, L.E., (2010). Extrusion Processing and Properties of Protein-Based Thermoplastics. Macromolecular Materials and Engineering 295(1), 10-21.

Vlachopoulos, J., Strutt, D., (2013). Polymer processing. Materials Science and Technology 19(9), 1161-1169.

Waller, J., Klopfenstein, T., Poos, M., (1980). Distillers Feeds as Protein Sources for Growing Ruminants. Journal of Animal Science 51(5), 1154.

Wang, H., Cao, G., Prior, R.L., (1997). Oxygen Radical Absorbing Capacity of Anthocyanins. Journal of agricultural and food chemistry 45(2), 304-309.

Wang, H.J., Thomas, R.L., (1989). Direct Use of Apple Pomace in Bakery Products. Journal of food science 54(3), 618-620.

Wang, N., Daun, J.K., (2004). The chemical composition and nutritive value of Canadian pulses, *Canadian Grain Commission report*.

Wang, S., Kowalski, R.J., Kang, Y., Kiszonas, A.M., Zhu, M.-J., Ganjyal, G.M., (2016). Impacts of the Particle Sizes and Levels of Inclusions of Cherry Pomace on the Physical and Structural Properties of Direct Expanded Corn Starch. Food and Bioprocess Technology 10(2), 394-406.

Wang, Y.-Y., Ryu, G.-H., (2013). Physicochemical and antioxidant properties of extruded corn grits with corn fiber by CO2 injection extrusion process. Journal of Cereal Science 58(1), 110-116.

Wani, S.A., Kumar, P., (2016). Effect of Extrusion on the Nutritional, Antioxidant and Microstructural Characteristics of Nutritionally Enriched Snacks. Journal of Food Processing and Preservation 40(2), 166-173.

Weigel, J., Loy, D., Kilmer, L., (1997). Feed Co-products of the Dry CornMilling Process, *Renewable Fuels Association and National Corn Growers Association*, Washington DC and St Louis, MO.

Wen, L.F., Rodis, P., Wasserman, B.P., (1990). Starch fragmentation and protein insolubilization during twin screw extrusion of corn meal. Cereal Chemistry 67(3), 268-275.

Wijngaard, H.H., Rößle, C., Brunton, N., (2009). A survey of Irish fruit and vegetable waste and by-products as a source of polyphenolic antioxidants. Food Chemistry 116(1), 202-207.

Wiseman, H., (1997). Dietary phytoestrogens: disease prevention versus potential hazards. Nutrition & Food Science 97(1), 32-38.

Wu, Y.V., (1994). Determination of Neutral Sugars in Corn Distillers' Dried Grains, Corn Distillers' Dried Solubles, and Corn Distillers' Dried Grains with Solubles. Journal of agricultural and food chemistry 42(3), 723-726.

Wu, Y.V., Youngs, V.L., Warner, K., Bookwalter, G.N., (1987). Evaluation of spaghetti supplemented with corn distillers' dried grains. Cereal Chem 64, 437.

Yagci, S., Gogus, F., (2010). Effect of Incorporation of Various Food By-products on Some Nutritional Properties of Rice-based Extruded Foods. Food Science and Technology International 15(6), 571-581.

Yang, S.-h., Peng, J., Lui, W.-B., Lin, J., (2008). Effects of adlay species and rice flour ratio on the physicochemical properties and texture characteristic of adlay-based extrudates. Journal of Food Engineering 84(3), 489-494.

Yanniotis, S., Petraki, A., Soumpasi, E., (2007). Effect of pectin and wheat fibers on quality attributes of extruded cornstarch. Journal of Food Engineering 80(2), 594-599.

Young, V.R., (1991). Soy protein in relation to human protein and amino acid nutrition. J Am Diet Assoc 91(7), 828-835.

Yu, J., Ahmedna, M., (2013). Functional components of grape pomace: their composition, biological properties and potential applications. International Journal of Food Science & Technology 48(2), 221-237.

Yu, L., Meng, Y., Ramaswamy, H.S., Boye, J., (2014). Residence Time Distribution of Soy Protein Isolate and Corn Flour Feed Mix in a Twin-Screw Extruder. Journal of Food Processing and Preservation 38, 573-584.

Yuliani, S., Torley, P.J., D'Arcy, B., Nicholson, T., Bhandari, B., (2006). Effect of extrusion parameters on flavour retention, functional and physical properties of mixtures of starch and d-limonene encapsulated in milk protein. International Journal of Food Science and Technology 41(s2), 83-94.

Zhang, M., Liang, Y., Pei, Y., Gao, W., Zhang, Z., (2009). Effect of Process on Physicochemical Properties of Oat Bran Soluble Dietary Fiber. Journal of food science 74(8), C628-C636.

Zhang, Y.Z., (1998). *The Extrusion and Application on Food*. LightIndustry Press, Beijing, China.

Zielinski, H., Kozlowska, H., Lewczuk, B., (2001). Bioactive compounds in the cereal grains before and after hydrothermal processing. Innovative Food Science & Emerging Technologies 2(3), 159-169.