2016

Understanding the Impact of Non-thermal Processing and CO2 Assisted Extrusion on Antioxidant, Textural and Functional Properties of Corn, Sorghum and Apple Pomace Based Extrudates

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UNDERSTANDING THE IMPACT OF NON-THERMAL PROCESSING AND CO_2 ASSISTED EXTRUSION ON ANTIOXIDANT, TEXTURAL AND FUNCTIONAL PROPERTIES OF CORN, SORGHUM AND APPLE POMACE BASED EXTRUDATES

BY

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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

2016

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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.

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I dedicate this dissertation to

- My respected parents and parents-in-law
- My beloved wife Indra and my son Gauresh (Hanu)

ACKNOWLEDGEMENTS

I, hereby, acknowledge my profound gratitude to my advisor and chairman of advisory committee Dr. K. Muthukumarappan (Muthu), Distinguished Professor for his esteemed guidance, constant encouragement, unfailing support, critical and constructive evaluation of this manuscript and generous facilities provided by him proved a boon for me throughout in making this work a success. He always motivated me to be a proactive, initiative, multidisciplinary and multidirectional like him. I would be more than happy if I have adopted more than half of his skills.

Words of acknowledgement cannot fathom the efforts put by the members of my advisory committee, Dr. Zhengrong Gu, Dr. Padmanaban Krishnan (Padu) and Dr. Michael Adelaine. I feel deeply indebted, as they have been a constant source of inspiration with professional competence and generous attitude. I am highly thankful to Dr. Padu for making available the facilities of the Seed Technology laboratory in order to determine the total dietary fiber for the present investigation.

I am extremely gratified to Dr Haridas for helping in running the hydrodynamic cavitator in Dairy Science Department for the present study. I also extend my gratitude to Ahsan who constantly worked on UHPLC equipment to get it fixed and also helped me in running the samples. I express my sincere gratitude to Simon Deon, Animal Science for her kind support in running my samples in protein analyzer. I really appreciate the gracious aid by Reza in running my samples for electron microscopy. I am also grateful to Scott, Derek and John for their technical support in fabricating the tools during my present investigation.

I would specially thank to Dr. Jeyam Subbiah, Professor for providing me the facility to work on pulsed electric field equipment in University of Nebraska-Lincon and of course the help rendered by Krish, Dustin and Kiat to run the equipment is duly acknowledged.

I am very much obliged to the faculty members of the Department, specially Dr. Van Kelley, Head of Department and Dr. James Julson for their encouragement during the entire course of study. I also express my heartfelt thanks to Susan, Candy and Jasmeen for their service rendered during my study and research.

Genuine appreciation goes to Sushil for providing timely support in learning Solid Works and ANSYS software. I also acknowledge the memorable help and support of Bipin during my research. I do thank every other person who directly or indirectly aided the cause of preparing my dissertation.

No words of gratitude will be able to express my feeling towards my wife, Indra for looking after my son and taking care of household chores. The patience shown by her is most humbly acknowledged. The good wishes, blessing and words of encouragement rendered by my parents, parents-in-law, my brother, my uncles and aunts inspired me a lot to accomplish the present work successfully.

Last but not the least, I wish to thank the ‘Almighty God’ Who has been helping through thins and thicks of my life.

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ABBREVIATIONS

%	percentage
°C	degree Celsius
β	flow number
µ	micro
ρ	density
Å	angstrom
atm	atmospheric pressure
AA	antioxidant activity
AAD	absolute average deviation
ANN	artificial neural network
ANOVA	analysis of variance
AP	adequate precision
AP	apple pomace
APR	apple pomace ratio
BBD	Box-Behnken design
cm	centimeter
cP	centipoise
C	carbon
CF	corn flour
CH	cavitator holes
CO_2	carbon dioxide
df	degree of freedom

DAD	diode-array detector
DC	direct current
DM	drying method
DNA	deoxyribonucleic acid
DOE	design of experiment
DPPH	2,2-diphenyl-1-picrylhydrazyl
DSC	differential scanning calorimetry
DW	dry weight
EFI	electric field intensity
ER	expansion ratio
F	farad
FM	feed moisture
FR	flow rate
FT	fermentation time
FWR	flour to water ratio
g	gravitational acceleration
g	gram
GHz	giga hertz
GAE	gallic acid equivalent
GOPOD	glucose oxidase/peroxidase
h	hour
H	hydrogen
Hz	hertz

HC	hydrodynamic cavitation
HCl	hydrogen chloride
i.e.	that is
IDF	insoluble dietary fiber
IU	international unit
IVSD	in-vitro starch digestibility
kg	kilo gram
kHz	kilo hertz
kJ	kilo jule
kV	kilo voltage
K	cavitation number
K	kelvin
KCl	potassium chloride
KOH	potassium hydroxide
l	liter
LOF	lack of fit
m	meter
mg	milligram
min	minute
ml	milliliter
mm	millimeter
M	molarity
MAE	microwave assisted extraction

MC	moisture content
MES	2(N-morpholino) ethanesulfonic acid
MHz	mega hertz
MPa	mega pascal
MS	mean square
MSE	mean square error
MW	microwave
nm	nanometer
N	newton
OH	hydroxyl
P	pressure
PEF	pulsed electric field
PFE	pressurized fluid extraction
PTFE	Polytetrafluoroethylene
PWR	pomace to water ratio
rpm	revolution per minute
R_e	Reynold number
RMSE	root mean square error
ROS	reactive oxygen species
RS	resistant starch
RSM	response surface methodology
s	second
SA	sinapic acid

SDF	soluble dietary fiber
SEM	scanning electron microscopy
SF	sorghum flour
SFE	supercritical fluid extraction
SS	sum of squares
SS	screw speed
SSE	single screw extruder/extrusion
t	treatment time
T	temperature
TDF	total dietary fiber
TE	trolox equivalent
TPC	total phenolic content
TRIS	tris(hydroxymethyl)aminomethane
TSE	twin screw extruder/extrusion
U	enzyme unit
UA	ultrasonication amplitude
UAE	ultrasound assisted extraction
UC	ultrasound cavitation
UHPLC	ultra high performance liquid chromatography
UI	ultrasonication intensity
UT	ultrasonication time
UV	ultraviolet
v/v	volume by volume

V	voltage
w/v	weight by volume
wb	wet basis
W_e	Weber number
W	wattage
WAI	water absorption index
WSI	water solubility index

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ABSTRACT

UNDERSTANDING THE IMPACT OF NON-THERMAL PROCESSING AND CO_2 ASSISTED EXTRUSION ON ANTIOXIDANT, TEXTURAL AND FUNCTIONAL PROPERTIES OF CORN, SORGHUM AND APPLE POMACE BASED EXTRUDATES

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Currently, there is a worldwide demand for antioxidant rich foods in diet, especially ready to eat foods, i.e. cereals and snacks. In recent years, the research has got momentum to valorize the underutilized crops, i.e. millet, sorghum and industrial food by-products, i.e. fruit pomace to incorporate into main stream of human diet due to their enrichment in nutritional as well as bioactive compounds like phenolic and flavonoid antioxidants. However, most of the phenolic compounds in plant are present in the bound form with the carbohydrates, lignin, pectin and proteins which reduces their ability to function as good antioxidants. Therefore, in the first part of study, selected non-thermal technologies were compared to liberate the bound phenolic acids in the sorghum flour (SF) and apple pomace (AP) in order to enhance their total content in raw materials prior to secondary processing. Starting with the natural fermentation followed by the batch ultrasonication, SF and AP showed a significant ($p<0.05$) improvement in the total phenolic content (TPC) and antioxidant activity (AA). The increased TPC and AA were observed as 21.8%, 33.9% and 40.3%, 93% for SF and AP, respectively. Drying as the final unit operation of this process, the microwave drying of ultrasonicated AP showed higher TPC and AA when compared to the oven drying.

After getting improved TPC and AA using the batch ultrasonication process, SF and AP were treated with the continuous ultrasonicator in order to investigate the release of phenolics at the pilot scale. The investigation revealed that the continuous ultrasonication (UC) further improved 15.8% TPC, 6.3% AA in SF and 3.8% TPC, 1.4% AA in AP with compared to the batch process. Although the ultrasonication time and intensity observed in the continuous process were less, similar fermentation time (12 h SF, 24 h AP) and sample concentration (10%w/v SF, 5%w/v AP) were found at the optimum conditions for both the ultrasonication process.

Pulsed electric field (PEF) as a second non-thermal technology was employed to investigate its effect on the TPC, AA of the SF and AP. The optimized conditions of fermentation time of SF and AP from UC were taken into consideration for further research. The mild intensity PEF improved the TPC and AA in both the materials, significantly at higher sample concentration (45%w/v SF, 12.5%w/v AP), however the relative increase was less when compared to the continuous ultrasonication process at its optimum conditions. PEF intensity, treatment time and sample concentration were observed as the important parameters to impact the phenolic release in SF and AP. The TPC, AA for SF and AP were observed 11.5%, 5.9% and 5.7%, 5%, respectively less when compared to the continuous ultrasonication.

As an alternative to ultrasonication, hydrodynamic cavitation (HC), the third non-thermal technology was compared with the previous two for TPC and AA of SF and AP. As investigation revealed, HC increased the TPC, AA in SF and AP by 39.5%, 38.6% and 42%, 97%, respectively when compared to the controls. Both the materials had almost equivalent TPC and higher AA than that found for the continuous ultrasonication.

Though the optimum sample concentration of SF for HC was same as UC, AP sample concentration (8.75%w/v) for HC was found higher than that reported for UC (5%w/v). Therefore, among the three, HC was selected as the best non-thermal process in order to release the phenolic compounds in SF and AP.

After selecting the best non-thermal process that increased the phenolics in SF and AP, the second part of study was conducted to retain the maximum phenolics along with the improved textural and functional properties in SF and AP extrudates during extrusion process. Corn flour (CF) and hydrodynamic cavitated sorghum flour, apple pomace blend was extruded for three levels of apple pomace ratio APR (10%, 20%, 30%), feed moisture FM (25%, 30%, 35%wb), extrusion temperature T (80, 110, 140ºC) and screw speed SS (100, 150, 200 rpm). At the optimum conditions of 30% APR, 25%wb FM, 132ºC T and 108 rpm SS, TPC and AA observed in the extrudates were 62.5% and 67.3%, respectively higher than that of the control extrudates (without HC). The expansion ratio, brittleness, crispness, water solubility index (WSI), IVSD, TDF and soluble dietary fiber (SDF) increased whereas hardness and water absorption index (WAI) significantly ($p<0.05$) decreased in the extrudates when compared to that of the control extrudates.

In another modified extrusion process, carbon dioxide was injected with the same blend of CF, SF and AP. At the optimum conditions of 30% APR, 25%wb FM, 97ºC T and 100 rpm SS, TPC and AA of the extrudates produced with CO_2, exhibited 12% and 7% more TPC and AA, respectively with compared to that of the control extrudates (without CO_2). It was observed that the extrudates produced in latter extrusion process possessed improved TPC, AA, hardness, brittleness, crispness, WAI and WSI, however

due to CO_2 injection, ER, IVSD and TDF slightly decreased with compared to that of the extrudates developed in the former extrusion process.

The whole study concluded that the natural fermentation followed by the hydrodynamic cavitation and CO_2 assisted extrusion produced the corn flour, sorghum flour and apple pomace based extrudates with improved TPC, AA, textural, nutritional and functional properties.

CHAPTER 1

Introduction and Background

1.1 Introduction

Health and nutrition is the most demanding and challenging field in this era and has potentiality to be continued in the future as well. Biomedical research has shown that oxidative stress, i.e. constant attack by oxygen free radicals and reactive oxygen species (ROS), leads to initiation and growth of many major diseases like aging, such as wrinkled skin, gray hair, balding, and bodily stiffness. Oxidative damage results about 85% of chronic and degenerative diseases (Bellé et al., 2004). Diseases caused by oxygen free radicals and ROS are cancer, atherosclerosis, heart disease, cerebrovascular, stroke, Diabetes mellitus rheumatoid arthritis, osteoporosis, ulcers, sunburn, cataracts, and aging (Gülçın et al., 2003, Halliwell, 1992). The human body has an antioxidant defense system that deactivates these highly reactive free radicals. Antioxidants enzymes, made in the body, and antioxidant nutrients, found in foods, turn free radicals into harmless or waste products that are eliminated through the liver. Therefore, these antioxidant nutrients are functional components of food that have additional health benefits (Amić et al., 2003, Chu et al., 2002, Oboh, 2005a, Oboh, 2005b, Sun et al., 2002).

Presently, there is a word wide demand for antioxidant rich foods and especially ready to eat foods like cereals and snacks. In recent years, the research has got momentum to valorize the underutilized crops, i.e. millet, sorghum and industrial food by-products, i.e. fruit pomace to incorporate into main stream of human diet due to their enrichment in nutritional as well as bioactive compounds like phenolic and flavonoid antioxidants. As in previous researches, different cereals have been identified and

accepted as functional foods and nutraceuticals because of good sources of dietary fiber, proteins, energy, minerals, vitamins, and antioxidants required for human health (Charalampopoulos et al., 2002). Cereals contain linoleic acid, fiber, vitamin E, selenium, folate, phytoestrogens, and several phenolic acids with antioxidant properties (Ötles and Cagindi, 2006, Truswell, 2002). Sorghum (*Sorghum bicolor* L.) is a gluten-free cereal that has the highest content of phenolic compounds among cereals (Cardoso et al., 2015). Whole grain sorghum flour (SF) contains phenolic compounds mainly in the forms of phenolic acids and flavonoids (Hahn et al., 1984). These compounds have potentiality to impact positively on human health because of their antioxidant and antiradical properties (Awika and Rooney, 2004).

Apple pomace (AP), a solid residue obtained after the extraction of juice from apple, is a byproduct from juice and cider processing industries. As a common application, AP is directly disposed to soil in a landfill, and for pectin recovery usage (gelling agent, stabilizer and source of dietary fiber). These applications are not sufficient to utilize the 1.3 million metric tons of AP produced in the United Sates every year (Jung et al., 2014); therefore, studies have got momentum to valorize the AP for other purposes also. AP as a rich source of antioxidant compounds could be used for increasing the stability of foods by preventing lipid peroxidation and also for protecting oxidative damage in living systems by scavenging oxygen radicals. AP contains numerous phytochemicals in the form of simple sugars, pectin, and natural antioxidants (Bhushan et al., 2008) and is also composed of dietary fibers, carbohydrates, small amount of proteins, fat, and ash (Sudha et al., 2007). The amount of total phenolic compounds varies greatly in between flesh and peel of apple. Peels contains higher quantity of

phenolic compounds. The procyanidins, catechin, epicatechin, chlorogenic acid, phloridzin and quercetin conjugates are commonly found in apple peels (Diñeiro et al., 2009).

SF and AP has potential source of polyphenolic compounds and most of these compounds are present in bound forms with carbohydrates, lignin, pectin and proteins (Acosta-Estrada et al., 2014, Ajila et al., 2011). This bound nature of polyphenolics as glycosides reduces their ability to function as good antioxidants. Therefore, release of these bound phenolics can improve their health functionality. There are several processes or pretreatments that enhance the liberation of bound phenolics. Germination, malting, fermentation and thermo-mechanical processes such as microwave drying, extrusion cooking and alkaline hydrolysis are most popular (Acosta-Estrada et al., 2014).

Fermentation brings about numerous biochemicals, nutritional and organoleptic changes in the raw materials including the breakdown of certain constituents (Murekatete et al., 2012, Oboh and Amusan, 2009). Fermentation is one of the pretreatments to release the bound phenolics in plant materials. In past few years, fermentation has been used for improving the antioxidant properties in different food materials (Ajila et al., 2011, Fernandez-Orozco et al., 2009, Gassara et al., 2012, Hegde et al., 2006, Kayodé et al., 2013, Oboh et al., 2009, Oboh and Amusan, 2009).

In recent years, application of non-thermal technologies in food processing area in order to extract the bioactive compounds and to enhance the nutritional and functional properties in foods has been widely used. Ultrasound assisted extraction of phenolic compounds in different solvents from different foods has been extensively investigated (Ajila et al., 2011, Cheok et al., 2012, Cheok et al., 2013, Garcia-Castello et al., 2015,

Ghafoor et al., 2009, Jabbar et al., 2015, Khan et al., 2010, Ma et al., 2009, Ma et al., 2008, Muñiz-Márquez et al., 2013, Rodrigues et al., 2008, Szydłowska-Czerniak et al., 2015, Vasantha Rupasinghe et al., 2011, Virot et al., 2010, Wang et al., 2008, Wang et al., 2013). However the extraction of phenolics in water as solvent are limited (Golmohamadi et al., 2013, González-Centeno et al., 2015, Pan et al., 2011). Ultrasonication separates starch from protein matrix and breaks down these molecules (Zhao et al., 2008) resulting in releasing of bound phenolics with protein and other components.

Pulsed electric field (PEF) treatment has been studied broadly for extracting bioactive compounds from raw material (Boussetta et al., 2014, Fincan, 2015, Gachovska et al., 2010, Leong et al., 2016, Lin et al., 2012, López et al., 2008, López-Giral et al., 2015, Luengo et al., 2013, Odriozola-Serrano et al., 2009b, Teh et al., 2015, Turk et al., 2012, Vallverdú-Queralt et al., 2012, Wang et al., 2012, Wiktor et al., 2015, Xue and Farid, 2015, Zhao et al., 2011), extension of food storage shelf life by food sterilization and enzyme inactivation (Boulaaba et al., 2014, Puértolas et al., 2010, Salvia-Trujillo et al., 2011, Wu et al., 2014, Zhao et al., 2009), maintaining physical–chemical property and nutritional values of foods (Barba et al., 2015, Cortés et al., 2006, Elez-Martínez and Martín-Belloso, 2007, Gachovska et al., 2010, Morales-de la Peña et al., 2010, Odriozola-Serrano et al., 2009a, Oms-Oliu et al., 2009, Sánchez-Moreno et al., 2005, Sánchez-Vega et al., 2015). Nevertheless, research related to application of PEF in order to release bound phenolics in sorghum flour (SF) and apple pomace is missing.

As another cavitation process, hydrodynamic cavitation has been used for sterilization of food (Milly et al., 2007), microbial cell disruption (Balasundaram and

Harrison, 2006a, Balasundaram and Harrison, 2006b, Balasundaram and Pandit, 2001, Save et al., 1997), water disinfection (Arrojo et al., 2008, Jyoti and Pandit, 2001, Jyoti and Pandit, 2003, Mezule et al., 2009), wastewater treatment (Pradhan and Gogate, 2010, Sivakumar and Pandit, 2002, Wang and Zhang, 2009), and enzymatic hydrolysis of oil (Sainte Beuve and Morison, 2010). Application of HC in the area of food processing in order to increase in nutritional or functional values is lacking.

Retaining and enhancing the nutritional quality of food during food processing is always a potentially important area for research. Degradation of nutritional quality, owing to high temperature, is a challenging problem in most traditional cooking methods. Extruded snacks, where starch is the major component, will have more nutritional value if incorporated with fiber enriched flours containing antioxidants. But, on the other hand, high temperature extrusion process also results in loss of phenolics (Cardoso et al., 2015, Gujral et al., 2012, Leyva-Corral et al., 2016, Ti et al., 2015, Wani and Kumar, 2015, Korkerd et al., 2016). There can be two ways to get the nutritional or antioxidants rich snacks or cereals. First, antioxidants can be enhanced in raw material by liberating the phenolics using some pretreatments prior to extrusion and second, antioxidants can be retained in extrudates snacks during extrusion by using modified extrusion process.

Previously, many researchers have demonstrated the potential use of CO_2 injection to improve the structure formation (Bilgi Boyaci et al., 2012, Ferdinand et al., 1992, Ferdinand et al., 1990), expansion (Jeong and Toledo, 2004, Ondo et al., 2013, Singkhornart et al., 2014), appearance (Wang and Ryu, 2013a) and physicochemical properties (Singkhornart et al., 2013) of extrudates. However, little attention has been

devoted to the impact of CO_2 injection on antioxidant properties of extruded products (Wang and Ryu, 2013b).

1.2 Objectives

The primary objective of this study was to develop a non-thermal process to enhance the TPC and AA in SF and AP by liberating bound phenolics and to improve TPC, AA, textural and functional properties in the final extrudates using different process technologies. The hypothesis of the study was that non-thermal pretreatment would enhance the TPC and AA in SF and AP prior to extrusion and thereby loss of phenolics during extrusion process might be counterbalanced by already improved TPC and AA. In addition to this, modified extrusion process might retain maximum TPC and AA in the final extrudates. The specific objectives of this study were to:

1. Understand the influence of sequential treatment of natural fermentation, batch ultrasonication and extrusion on phenolic of sorghum extrudate (Chapter 2)
2. Understand the continuous ultrasonication to improve total phenolic content and antioxidant activity in sorghum flour and its comparison with batch ultrasonication (Chapter 3)
3. Study the modeling of natural fermentation followed by continuous ultrasonication to improve total phenolic content and antioxidant activity in sorghum flour using response surface methodology and artificial neural network (Chapter 4)
4. Study the effect of drying methods and batch ultrasonication in improving the antioxidant activity and total phenolic content of apple pomace powder (Chapter 5)
5. Understand the effect of sequential treatments of natural fermentation and ultrasonication followed by microwave drying on bioactive content of apple pomace

and the thermal, textural and functional water absorption characteristics of their extrudates (Chapter 6)

6. Understand the influence of pulsed electric field to release bound phenolics in fermented sorghum flour and apple pomace (Chapter 7)
7. Study the application of hydrodynamic cavitation to improve antioxidant activity in fermented sorghum flour and apple pomace (Chapter 8)
8. Understand the effect of extrusion process on antioxidant, textural and functional properties of hydrodynamic cavitated corn flour, sorghum flour and apple pomace based extrudates (Chapter 9)
9. Study the effect of CO_2 assisted extrusion process on antioxidant, textural and functional properties of hydrodynamic cavitated corn flour, sorghum flour and apple pomace based extrudates (Chapter 10)

The above mentioned objectives were investigated in nine different phases and presented in chapters 2 through 10.

1.3 Literature review

1.3.1 Need of phenolic compounds in human diet

A number of highly reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), nitric oxide radical ($NO^{\bullet}$), and alkyl peroxyl ($ROO^{\bullet}$) are regularly produced in the human body. These radicals can damage lipids, proteins and DNA and participate in pathogenesis and ageing (Santos-Buelga and Scalbert, 2000). Phenolic compounds, together with other natural compounds (vitamins C and E, and carotenoids), scavenge free radicals, inhibit oxidative enzymes and thus contribute to the defense. The antioxidant activity of phenolic compounds is

mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals (Bors et al., 2001). Epidemiological studies have shown that some phenolic compounds consumption is associated with a reduced risk for developing chronic diseases, such as coronary heart disease, cancer, diabetes, and Alzheimers's disease, linked to their free radical scavenging activities (Khokhar and Magnusdottir, 2002, Willett, 2002, Yang et al., 2004). Another interesting property of phenolic compounds, notably hydroxyanthraquinones and hydroxynapthoquinones, is their cathartic effect (Clifford, 2000). Cathartic compounds are believed to give a better feeling and help to deal with difficult emotions and eliminate them.

The most common phenolic compounds, i.e. phenolic acids and flavonoids are generally found as soluble conjugated (glycosides) and insoluble forms (Nardini et al., 2002). In nature, phenolic acids occur mostly in the bound or insoluble forms while flavonoids present as glycosides with a single or multiple sugar moieties linked through an OH group (O-glycosides) or through carbon-carbon bonds (C-glycosides). The insoluble bound phenolics have demonstrated a significantly higher antioxidant capacity compared to free and soluble conjugated phenolics (Chandrasekara and Shahidi, 2010). Fruits and vegetables have most of their phytochemicals in the free or soluble conjugate forms and only an average of 24% of the total phenolics are comprised of bound phenolics in these food matrices (Adom and Liu, 2002). On the contrary, whole cereal grains contains most of the phenolic compounds in the insoluble bound forms which are covalently bound to cell wall structural components such as cellulose, hemicellulose (e.g. arabinoxylans), lignin, pectin and rod-shaped structural proteins (Wong, 2006) as graphically described in Figure 1.1. The phenolic acids are classified as hydroxybenzoic

and hydroxycinnamic acid derivatives (Figure 1.2). These phenolic acids form ether linkages with lignin through their hydroxyl groups in the aromatic ring and ester linkages with structural carbohydrates and proteins through their carboxylic group (Bhanja et al., 2009). The consumption of free or bound phenolic compounds depends on the desired health impact. Dietary intake of bound forms will have a chemopreventive activity against colon cancer. Moreover, dietary intake of free and soluble conjugated forms are more rapidly absorbed in the stomach and small intestine and facilitate with other health benefits such as inhibition activities against oxidation of low density lipoprotein cholesterol and liposomes (Chandrasekara and Shahidi, 2011). Therefore the release of bound phenolics prior to intake will be necessary.

1.3.2 Phenolic acids in sorghum and apple pomace

The phenolic content and profile in sorghum are more diverse and higher than those observed in wheat, barley, rice, maize, rye, and oats (Ragaee et al., 2006). Almost all classes of phenolics are found in sorghum (Awika and Rooney, 2004, Dykes et al., 2005) where phenolic acids and flavonoids are major phenolic compounds. The phenolic acids identified in sorghum are major amounts of the protocatechuic and ferulic acids and small amounts of the p-coumaric, syringic, vanillic, gallic, caffeic, cinnamic, and p-hydroxybenzoic (Svensson et al., 2010). Phenolic acids in sorghum are mostly bound to arabinoxylans chains or lignin (Dykes and Rooney, 2006). These bound phenolic acids are not hydrolyzed by human digestive enzymes that decrease their bioavailability, but are fermented by the microbiota of the colon (Hole et al., 2012).

Phenolic compounds found in apple pomace depending on its varieties were chlorogenic acid, protocatechuic acid, *p*-coumaric acid, ferulic acid, caffeic acid, benzoic

acid, salicylic acid, t-cinnamic acid, vanillic acid, *o*-coumaric acid, gentisic acid, quinic acid, gallic acid, ellagic acid and synapic acid (Bhushan et al., 2008, Çam and Aaby, 2010, Cetkovic et al., 2008, Schieber et al., 2003, Suárez et al., 2010). Polyphenols mostly presented in pomace are responsible for the antioxidant activity and can easily be extracted for food fortification or nutraceutical product development. Apple pomace can therefore become an inexpensive and readily available source of dietary antioxidants.

Knowledge about techniques for improving the bioavailability of phenolic acids in sorghum and apple pomace is incipient. The processing of grains can play a key role in improving this bioavailability. The study results exhibited that fermentation, different extraction and cooking processes can significantly increase the free phenolic acids (Azmir et al., 2013, Hole et al., 2012, N'Dri et al., 2013, Soria and Villamiel, 2010), thereby improving their bioavailability in plant materials. The effects of other types of processing on the profile of phenolic acids in sorghum and apple pomace need to be studied.

1.3.3 Effect of fermentation on phenolic compounds and other nutritional and functional properties

Fermentation, probably the best among several processing technologies including cooking, germination and milling, have been put into practice to improve the nutritional properties of cereals (Pugalenthi and Vadivel, 2005). Fermentation offers food preservation, improved food safety, enhanced flavor and acceptability, increased variety in the diet, improved nutritional value, reduction in anti-nutritional compounds, and improved functional properties (Singh et al., 2015). Recently, this unit operation has been

applied to the production and extraction of bioactive compounds in the foods (Abd Razak et al., 2015, Wang et al., 2015).

As instances of microbial fermentations, Dlamini et al. (2007) investigated that four types of sorghum fermented with lactic acid culture for 24 h showed reduction in total phenols and antioxidant activity (AA) by 33% and 52%. These changes probably involved associations between the tannins, phenols, proteins and other compounds in the grain. In addition, Towo et al. (2006) also reported the reduction in phenolic compounds by fermentation of sorghum gruels with starter culture for 16 h. The decrease in phenolic compounds during fermentation could also be due to the acidic environment that may result in abstraction of hydride ions and rearrangement of the phenolic structures. During fermentation, enzymes such as amylases, xylanases, and proteases derived from the grain and microbes contribute to the modification of grain composition (Katina et al., 2007). Ajila et al. (2011) used *Phanerocheate chrysosporium* in solid state fermentation of apple pomace in order to improve the TPC and AA. They observed 11% and 91% increase in TPC and AA, respectively. These results can be explained by the fact that levels of bioactive compounds can be modified during fermentation by the metabolic activity of microbes. Also fermentation-induced structural break down of cereal cell walls may occur, leading to the liberation and/or synthesis of various bioactive compounds.

Kayodé et al. (2013) studied the effect of germination (0-72 h) and natural fermentation (0-72 h) on phenolic acids of type III tannin sorghum. They reported that 14 h fermentation increased TPC and AA in sorghum flour. The decarboxylation of phenolics that may occur during the spontaneous fermentation could enhance the antioxidant properties of processed sorghum flour. (Fernandez-Orozco et al., 2009)

compared naturally fermented and induced fermented (with *Lactobacillus plantarum*) chickpea flour for TPC after 48 h fermentation period. They found increase in TPC for both the fermented samples however the TPC of naturally fermented flour was observed to be 40.5% higher with compared to that of induced fermented. Table 1.1 shows the details of phenolic compounds extracted from different plant materials using fermentation process.

Fermentation also brings improvement in physical as well as functional properties. Pranoto et al. (2013) compared natural fermentation with the fermentation inoculated by *Lactobacillus plantarum* at 37 °C for 36 h. Both the fermentations increased IVSD by 71.48% and 65.63%, respectively. Fermentation causes proteolysis of the protein matrix surrounding the starch granules in order to release the starch granules that are easily hydrolyzed by amylase and thereby increasing the digestibility of the starch. On the other hand, fermentation can break large molecular weight starch granules into smaller molecular weight granules which are more susceptible to enzymatic hydrolysis due to higher specific surface area that is exposed to the enzymes, leading to increase in starch digestibility. Granito et al. (2001) observed the effect of natural fermentation of flour and whole bean seeds on the nutrients and anti-nutritional factors. Within the studied fermentation processes (42°C and 48 h), whole grain natural fermentation is very promising, due to improved properties as well as its lower cost. Alka et al. (2012) also studied the effect of natural fermentation on physicochemical parameters of sorghum. Bulk density and water absorption capacity decreased whereas starch digestibility of sorghum increased with fermentation period (0-36 h).

1.3.4 Extraction of phenolic compounds from plant materials

1.3.4.1 Supercritical fluid extraction

Supercritical fluid extraction (SFE) is an extraction technique that separates desired components from a solid or liquid using a supercritical fluid as the extracting solvent. Carbon dioxide is the most commonly used solvent with SFE as it has low critical temperature (31.1 °C) and pressure (74 bars) that makes it an extremely attractive solvent for use with SFE (Temelli and Güçlü-Üstündağ, 2005). In the supercritical region, CO_2 diffuses like a gas and easily penetrates the solid matrix and is able to dissolve solutes like a liquid. The solubility of a compound in a supercritical fluid tends to increase with increasing fluid density. As pressure increases above the critical pressure, the density of the fluid increases; hence, solubility increases with pressure. The ability to control solubility through pressure is one of the main features that distinguishes supercritical fluids from traditional liquid solvent.

Several researchers have used SFE to extract phenolics from a number of products including spearmint (Bimakr et al., 2011), blueberry (Paes et al., 2014), grape marc (Da Porto et al., 2014), peach palm pulp (Espinosa-Pardo et al., 2014), blackberry (Pasquel Reátegui et al., 2014) and cherry seeds (Santos et al., 2015).

High critical pressure, its associated hazards and the expensive equipment are the major disadvantages of SFE. Supercritical CO_2 behaves as a nonpolar, lipophilic solvent, whose extraction performance is limited by polarity.

1.3.4.2 Pressurized fluid extraction

This method is also known as accelerated fluid extraction, enhanced solvent extraction, and high pressure solvent extraction (Nieto et al., 2010). In pressurized fluid

extraction (PFE), high pressure is applied to the liquid solvents at temperatures greater than their normal boiling point to increase their extractability. The combined use of high pressures (3.3-20.3 MPa) and temperatures (40-200 °C) promotes higher analyte solubility and mass transfer rate and, also decreases the viscosity and surface tension of solvents, thus improving extraction rate (Ibañez et al., 2012). However, phenolic compounds are easily oxidized at high temperatures so it is very important to make sure that they should not degrade under the proposed PFE conditions. In recent years, PFE has been successfully applied to the extraction of phenolic compounds from different plant materials such as soybean (Rostagno et al., 2004), pasta (Moret et al., 2014), pine nuts (Ruiz-Aceituno et al., 2014), blackberry (Machado et al., 2015) and rice (Setyaningsih et al., 2016). Individual phenolic compounds such as gallocatechin, catechin, epicatechin gallate, caffeic acid, chlorogenic acid, and myricetin and total phenolic contents were recovered from various parts of *Anatolia propolis* using PFE at optimum condition (40 °C, 10.3 MPa for 15 min) (Erdogan et al., 2011).

Despite the advantages of PFE, it has not been widely used as an extraction technique due to several key limitations including low analyte selectivity, large solvent volume usage during the rinsing step and high equipment costs. At elevated temperature and pressure, extraction occurs much faster but selectivity is significantly reduced due to solubility of other undesirable components and therefore an additional clean-up step is often required after extraction.

1.3.4.3 Microwave assisted extraction

Microwave, a form on non-ionizing electromagnetic energy, can be absorbed, reflected or transmitted to a material in the form of waves at a frequency ranging from

approximately 300 MHz to 300 GHz. Domestic microwave units typically operate at a frequency of 2450 MHz while the Federal Communications Commission has approved a frequency of 915 MHz for industrial applications. Microwave power that is transmitted through the bulk of a material can be absorbed and converted to heat. There are two proposed mechanisms of microwave heating: dipole rotation and ionic polarization. The mechanism of dipole rotation is based on the fact that many molecules exist as electric dipoles. When placed in an electromagnetic field, dipoles attempt to orient themselves according to the polarity of the field and thus constant rotation of the molecules causes frictional heat. Ionic polarization occurs when movement of ionic compounds is accelerated in response to an alternating electric field. The kinetic energy generated by the movement of the ions is converted to thermal energy. Above mentioned mechanisms clearly indicate the use of dielectric materials and solvents having a permanent dipole moment efficiently in microwaves.

The extraction mechanism of microwave assisted extraction (MAE) is supposed to involve three sequential steps described by (Alupului et al., 2012). First, separation of solutes from active sites of sample matrix under increased temperature and pressure; second, diffusion of solvent across sample matrix; third, release of solutes from sample matrix to solvent. Microwave extraction using water as solvent was as efficient as conventional processes to extract phenolics from different plant materials.

Mirzajani et al. (2010) reported increase in recovery of withaferin from *Withania somnifera* Dunal. with increase in temperature below 90°C. Longer extraction time recovered more withaferin however long duration at higher temperature decreased the yield. Liazid et al. (2010) also investigated that TPC and AA of pine seeds increased with

increase in temperature up to 75°C. They also concluded that ultrasonic assisted extraction yielded more phenolics with compared to MAE. Additionally, phenolic yield of coffee bean decreased after 5 min extraction time and above 50°C (Upadhyay et al., 2012). However, Inglett et al. (2010) and Sutivisedsak et al. (2010) observed higher phenolics from buckwheat and bean, respectively at higher extraction temperature (150°C). According to Shao et al. (2011), flavonoids yield increased in *Perilla Frutescens* leaves with increase in microwave power, solvent to material ratio and irradiation time up to a certain level with no significant change further. Hayat et al. (2010) and Upadhyay et al. (2012) also observed increase in phenolics of mandarin pomace and coffee bean, respectively with increase in power. Latter authors also reported that water as solvent gave higher yield with compared to ethanol and methanol as solvents. Zheng et al. (2011) concluded that total phenolic yield in pomegranate peel had a positive linear relationship with the extraction time. The details of phenolic compounds extracted from different plant materials using MAE are shown in Table 1.2.

1.3.4.4 Ultrasound assisted extraction

Ultrasound is high frequency sound (>20 kHz) that is greater than the upper limit of human detection. Two levels of ultrasound, i.e. low-intensity (< 1 W/cm^2) and high-intensity ultrasound (10-1000 W/cm^2) are used in the food processing industry. Low-intensity and high frequency (100 kHz-1 MHz) ultrasound is most commonly applied as an analytical technique to provide information on the physicochemical properties of food such as firmness, ripeness, sugar content, acidity, etc (Demirdöven and Baysal, 2008). On the contrary, the power levels used in low frequency (16-100 kHz) applications are so large that they are used to alter the food properties either physically or chemically

(McClements, 1995). Ultrasound-assisted extraction (UAE) involves passing ultrasonic energy in the form of compressive and shear waves through a liquid solvent containing the solid particles. This constant production of compressive and shearing forces gives rise to a phenomenon known as cavitation. Bubbles grow during the rarefying phase of the sound wave and collapse during the compression phase. The implosion of cavitation bubbles leads to energy accumulations in hot spots, generating extreme temperatures (5000 K), pressures (1000 atm) with heating and cooling rate above 10^{10} K/s (Bhaskaracharya et al., 2009). Cavitation is defined as the whole process of bubble nucleation, growth and collapse. These rapid localized increases in pressure and temperature are responsible for the disruption of cellular membranes, thereby facilitating the leaching of desired components out of the cell wall. The extraction mechanism by ultrasound involves two main types of physical phenomena, (a) the diffusion across the cell wall and (b) rinsing the contents of cell after breaking the walls (Mason, 1996). Moisture content of sample, particle size and solvent are very important factors for obtaining efficient and effective extraction. Furthermore, temperature, pressure, frequency and time of sonication are the governing factors for the action of ultrasound.

Ultrasonication can be applied in analytical chemistry in two ways: directly to the sample or indirectly through the walls of the sample container. Direct application is achieved through ultrasonic probes by immersing it into sample and performing ultrasonication directly over the solution without any barrier to be crossed by the ultrasonication wave other than the solution itself. Indirect application is performed, generally, using an ultrasonication bath.

The advantages of UAE include reduction in extraction time, energy and use of solvent. Ultrasound energy for extraction facilitates more effective mixing, faster energy transfer, reduced thermal gradients and extraction temperature, selective extraction, reduced equipment size, faster response to process extraction control, quick start-up, increased production and also eliminates process steps (Chemat et al., 2008).

Many researchers applied ultrasound in order to extract the bioactive compounds from different plant materials. Virot et al. (2010) observed the sonication durable and temperature as most influent variables to extract the antioxidants from apple pomace as TPC increased linearly as sonication time and temperature increased. The same effect was noticed with ultrasonic power but with a less predominant influence. González-Centeno et al. (2015) also found a gradual and significant increase of the phenolic content and antioxidant capacity of the grape pomace extracts as the temperature increased from 20°C to 50°C. Pan et al. (2011) reported that total phenolic yields of pomegranate peel significantly improved with increased intensity level and treatment time during continuous ultrasonication process, however the TPC and AA of raspberry puree decreased after 10 min of extraction time without having any effect of ultrasonic frequency (Golmohamadi et al., 2013). Cheok et al. (2013) reported that mangosteen hull required longer sonication time and higher amplitude regardless of the extraction solvent used, whereas, Garcia-Castello et al. (2015) observed that time had a positive effect but it was not significant at the higher time range for the extraction of flavonoids from grapefruit pomace. They also reported insignificant effect of temperature on flavonoids. Temperature has two opposite effects, it enhances mass transfer during extraction but also promotes higher degradation rates. Jabbar et al. (2015) asserted the remarkable

effects of extraction time, temperature and solvent concentration on the extraction yields of TPC and AA of carrot pomace. Regarding individual phenolic compounds, Ma et al. (2009) investigated that p-hydroxybenzoic acid among all detected phenolic acids in citrus peel suffering from ultrasonic treatment was the most unstable. Table 1.3 shows the details of phenolic compounds extracted from different plant materials using UAE.

In addition to above, Park et al. (2006), , Zhao et al. (2008), Shewale and Pandit (2009) and Claver et al. (2010) used ultrasonication for extraction of starch, protein, glucose and polysaccharides, respectively from sorghum. Patero and Augusto (2015) applied ultrasonication to enahance the hydration of sorghum.

1.3.4.5 Pulsed-electric field extraction

Pulsed electric field (PEF) is a technology that has been extensively investigated in recent years for its applications in food processing. The principle of PEF is to destroy cell membrane structure and to increase mass transfer for enhancing extraction and decreasing extraction time. PEF has been applied to improve release of intracellular compounds from plant tissue with the help of increasing cell membrane permeability (electroporation) (Toepfl et al., 2006). During suspension of a living cell in electric field, an electric potential passes through the membrane of the cell and separates molecules according to their charge in the cell membrane. It has a treatment chamber consisting of two electrodes where plant materials are placed. Depending on the design of treatment chamber PEF process can operate in either continuous or batch mode. The key variables involved in a PEF process are electric field strength, pulse duration or pulse width, pulse number, treatment time, wave form of the pulse, treatment temperature and properties of the materials to be treated.

Usually, a simple circuit with exponential decay pulses is used for PEF treatment of plant materials. Higher intensity fields (15–40 kV/cm, 5–100 pulses, 40 to 700 μs, 1.1 to 100 Hz) (Zulueta et al., 2010) are more effective towards microbial inactivation, while low and medium intensity fields (0.6–2.6 V/cm, 5–100 pulses, short treatment time within 10^{-4}–10^{-2} s, 1 Hz) have been successfully used for enhancing mass transfer in solid foods (Corrales et al., 2008).

Vallverdú-Queralt et al. (2012) repored that PEF intensity of 2 kV/cm may cause lethal damage to cells due to irreversible loss of cell membrane permeability properties resulted in increase in polyphenol content and antioxidant capacity during PEF treatment of tomato. Luengo et al. (2013) investigated that the extraction of polyphenols from orange peel was improved significantly by increasing the pressing time and electric field strength from 1 to 7 kV/cm. Xue and Farid (2015) also reported that polyphenol yield in white button mushroom increased linearly with increase in intensity On the other hand, Wiktor et al. (2015) observed that higher intensity (5 kV/cm) and pulse number (100) caused the degradation of phenolic compunds in apple tissue. Moreover the increase of the PEF specific energy intake above 40 kJ/kg caused a significant decrease in phenolic content due to insufficient energy to deactivate the polyphenol oxidase. Teh et al. (2015) also reported that the use of higher voltage (30 V) and PEF time decreased the amount of phenolics in the canola seed cake extract. The details of phenolic compounds extracted from different plant materials using PEF is shown in Table 1.4.

1.3.4.6 Hydrodynamic cavitation

Mechanically induced cavitation is referred to as hydrodynamic cavitation (HC). It is induced mechanically when fluids are pressurized and depressurized while flowing

around or through an obstacle in the flow field. The cavitator had specially designed rotors with surface indentations that influenced the flow trajectory of the samples inside the cavitator. The flow trajectory and the variable pressure zones created due to the flow pattern lead to the formation, expansion and collapsing of the microscopic bubbles giving off shockwaves into the liquid medium. The high shear and heat generated by shockwaves and internal liquid friction, respectively creates temperature uniformly throughout the entire liquid without having hot and cold space. Physical stresses resulting from acoustic or hydrodynamic cavitation are understood to be the mechanisms responsible for cellular inactivation. Biological entities in the immediate area of a cavitation event endure stresses that induce severe damage to cell walls (Badve et al., 2015).

Some of the advantages which can be offered with the use of HC reactors are reduced energy consumption, achieving microbial lethality at reduced temperatures and maintaining fresh-like product quality during processing (Gogate, 2010). The main factors which determine the formation of the hydrodynamic cavitation and its effectiveness can be divided into three parts. The first part consists of parameters which determine the structural characteristics of the reactor, i.e. the size and shape of the cavitation inducer and the flow chamber. The second part includes parameters characterizing the properties of the liquid medium, i.e. viscosity, density, surface tension and the dissolved gas contents. The third part includes technological process parameters such as processing time, temperature and pressure. The technological efficiency of the cavitation process depends on the cumulative effect of the above mentioned parameters (Ozonek and Lenik, 2011).

The appearance of cavitation in the liquid can be written in the following form (Cai et al., 2009):

$$f\left(\frac{l_1}{l},\ldots,\frac{l_n}{l},K,R_e,W_e\right)=0 \tag{1.1}$$

where: l, l_1, l_2,…l_n are linear values defining the size, shape, location of the body, and in addition its surface condition microbubble dimensions and solid particles constituting the cavitation nucleus; K, R_e, W_e are Cavitation, Reynolds and Weber numbers characteristic for cavitation.

Increasing the Reynolds number (R_e) and Weber number (W_e) is usually accompanied by an increase in Cavitation number (K).

As a dimensionless parameter characterizing the cavitation conditions in hydraulic systems, the cavitation number K has generally been used to relate the flow conditions with the cavitation intensity (Save et al., 1997). It is defined in the following form:

$$K=\frac{P-P_v}{\frac{1}{2}\rho v_0^2} \tag{1.2}$$

where P is fully recovered downstream pressure, P_v is the vapor pressure of the liquid, ρ is the density of liquid and v_0 is the velocity of the liquid at the constriction.

Though, cavitation can be achieved even at higher cavitation numbers, for maximum benefit from the reactor, the flow conditions and the geometry should be adjusted in such a way that the cavitation number lies in the range of 0.1 to 1. For smaller cavitation numbers (K), the number of bubbles produced per unit time increases as well as the intensity of the cavitation process (Ozonek and Lenik, 2011).

The intensity of the generated cavitation significantly depends on the geometry of the component causing the cavitation. The geometry can be described by the geometric numbers characteristic of the hydrodynamic flow conditions (Ozonek and Lenik, 2011). These are parameterised as follows

$$\alpha = \frac{total\ sum\ of\ \ all\ the\ hole\ cicumferences}{sum\ of\ \ hole\ area\ on\ the\ rotor\ \ plate}\left[mm^{-1}\right] \tag{1.3}$$

$$\beta = \frac{sum\ of\ \ the\ hole\ area\ on\ the\ rotor\ \ plate}{cross\ \ \text{sec}tion\ area\ of\ \ the\ pipe} \tag{1.4}$$

The geometric parameter α has an influence on the shape of the stream developing behind the inducer. The value β is often referred to as the flow number, its magnitude significantly affects the cavitation number and thus determines the intensity of the resulting cavitation. Cavitation number decreases with an increase in α and decrease in β.

Numerous researchers have investigated the applications of hydrodynamic cavitation in food sector. Milly et al. (2007) applied hydrodynamic cavitation reactor for sterilization of tomato juice, apple juice and skim milk. Hydrodynamic cavitation has also been reported to induce adequate destructive forces to inactivate vegetative cells of bacteria, yeast, and heat-resistant bacterial spores. Balasundaram and Harrison (2006a) investigated the application of hydrodynamic cavitation for the partial disruption of *Escherichia coli* cells. Sainte Beuve and Morison (2010) found hydrodynamic cavitation to be very effective in producing both oil-in-water and water-in-oil emulsions.

For a scientific problem, the research outcome using laboratory-scale equipment may not always be useful in large-scale processing. Such problems can be overwhelmed by appropriate alternatives that are suitable for large scale processing. Experimental and theoretical studies have shown that hydrodynamic cavitation may be suitable for large-

scale processing in food industry as an alternative to acoustic cavitation for processing large volumes of liquid media. The concept of the hydrodynamic cavitation fully meets the established requirements of the frequency and intensity of ultrasound in food processing (Ashokkumar et al., 2011).

1.3.4.7 Conventional and liquid CO_2 assisted extrusion processing

The extrusion process has wide applications in the production of snacks, breakfast cereals, textured vegetable proteins and animal feeds. Extrusion is a continuous process in which feed materials is subjected to mixing, shearing, heating, cooking and shaping. Extrusion cooking is an attractive process in food industry with the advantages of versatility, high productivity, quality, low cost, energy efficiency, absence of effluents and possibility of product design (Tumuluru et al., 2013).

Extrusion cooking may influence dietary fiber, protein, vitamins, and other nutrients both positively and negatively in the food (Singh et al., 2007). Similarly, it can change phenolic content in food products with two opposite effects. First, decomposition of heat-labile phenolic compounds and polymerization of some phenolic compounds, incline to decrease the extractable phenolic content. Secondly, due to disruption of cell wall matrices and breaking of high molecular weight complexed polyphenols during extrusion, extractability of phenolic compounds is improved (Wang et al., 2014b). The net effect of extrusion on total phenolic content depends on which effect is predominant. Furthermore, extrusion cooking induces alteration in physicochemical and functional properties which also depend on raw material and extrusion process variables such as feed moisture, screw speed and configuration, die geometry, temperature and time (Sarawong et al., 2014).

Several studies have shown the positive impact of extrusion on total phenolic content (TPC) and antioxidant activity (AA) of starchy-fiber food extrudates. Sarawong et al. (2014) investigated the effect of feed moisture FM (20% and 50%) and screw speed SS (200 and 400 rpm) on physicochemical properties, resistant starch (RS) and antioxidant capacities of banana flour. They reported that low FM and higher SS produced the extrudates low in RS, water absorption index (WAI) and having high total dietary fiber (TDF), water solubility index (WSI), TPC and AA. Lower starch degradation due to less shear rate at lower SS conditions caused higher RS. Higher FM during extrusion cooking resulted in a lower degree of starch gelatinization thus resulting in an increase in WAI and a decrease in WSI. The decreased torque development associated with shorter resistance time at increased SS and lower FM affected more dominantly the phenolic content than the effect of the increased shearing. Furthermore, Bisharat et al. (2015) reported an increase in TPC and AA of corn flour, broccoli flour and olive paste based extrudates with increase in temperature (140-180°C) and decrease in feed moisture (14-19%wb). It was attributed to the release of phenolic compounds from cell walls and to the interaction of phenolics with protein resulting formation of Maillard's reaction products as a result of high extrusion temperatures. The higher moisture content presumably promoted phenolic polymerization, which affected extractability of phenols and reduced antioxidant activity. Sharma et al. (2012) observed decrease in TPC while increase in AA with elevation in temperature (140-180°C) and feed moisture (15-20%wb). A change in the extrusion temperature and moisture could have led to the formation of different amounts of Maillard browning products which was responsible for the increase in AA despite of TPC reduction. Korkerd et al. (2016) also

reported similar impact of feed moisture content and barrel temperature on TPC and AA of protein-starch-fiber based extrudates. High temperature increased the chance of oxidation of phenolics resulted in decrease the yield of TPC. However, AA increased due to production of Maillard's reaction products.

On the other hand, studies demonstrated that extrusion decreased the TPC and AA of extrudates. Extrusion cooking significantly reduced ABTS antioxidant activity by 83% to 87%, for the sorghum products when compared to that of the raw grains (Dlamini et al., 2007). Gujral et al. (2012) also reported 50% reduction in TPC and AA of brown rice upon extrusion at 100°C. However WAI, WSI and expansion ratio (ER) increased with increase in temperature. Similarly, Yang et al. (2014) observed a decrease in TPC and AA of brown rice and maize when extruded in the range of 110-140°C because of decomposition of more TPC at higher temperatures. Wani and Kumar (2015) also reported the decrease in TPC and AA after extrusion of fenugreek seed, fenugreek leave powder, oats, dried green pea, rice and corn flour blend material. Moreover, Leyva-Corral et al. (2016) extruded the mixtures of oat flour (60%), apple pomace (14%) and potato starch (26%) in a single screw extruder. Compared with the unprocessed mixture, a retention between 79.9 and 97.1% of total phenolic content was observed whereas individual phenolic compounds were retained between 57 and 71% for chlorogenic acid, 55-64% for caffeic acid, 38-51% for p-coumaric acid, 25-28% for ferulic acid, 56-70% for rutin, and 46-76% for phloridzin. Most of the individual phenolic compounds showed higher stability at 140 °C with intermediate feed moisture content (25-26%wb).

In recent years, a new extrusion process involving the injection of carbon dioxide into the extruder barrel as the blowing agent instead of steam has been introduced. Many

researchers studied the different applications of CO_2 assisted extrusion in food sector. Masatcioglu et al. (2013) produced extrudates from corn flour supplemented with tomato, green tea, and ginseng powder by conventional extrusion and CO_2 injection methods at 80 °C. Higher tomato powder supplementation levels resulted in higher total phenolic compound levels and antioxidant activities. The antioxidant activity of the green tea-supplemented extrudates produced by the CO_2 injection process was significantly higher than the ones produced by conventional extrusion at the same extrusion temperature. Wang and Ryu (2013b) studied the physicochemical and antioxidant properties of extruded corn grits with corn fiber by CO_2 injection extrusion process. Extrusion with CO_2 injection restrained the decrease of the TPC at higher melt temperature (120 °C) that may be attributed to the cooling effect of the CO_2. Extrudates with higher WAI and lower WSI were obtained with CO2 injection Extrusion condition did not significantly influence the dietary fiber content, which might result from the lower melt temperature employed in this study. Table 1.5 exhibited the details of phenolic compounds extracted from different plant materials using extrusion process.

In addition to above, CO_2 assisted extrusion has widely been used to improve the textural and functional properties of extrudates. Jeong and Toledo (2004) tested the feasibility of twin-screw extrusion for pre-gelatinized rice flour at low temperature (60°C) using CO_2 for expansion and other properties of extrudates. Expansion ratio, porosity and WSI of extrudates increased while WAI decreased with increasing CO_2 injection pressure. Higher CO_2 injection pressure resulted in more starch fragmentation during the extrusion process than at lower CO_2 injection pressure. (Wang and Ryu, 2013a) studied physical properties of extruded corn grits with corn fiber by CO_2 injection

extrusion at a temperature range of 90-120°C. They reported that the lower melt temperature (90 and 105°C) with the CO_2 injection produced smoother extrudats with lower fracturability and the more uniform cross sectional microstructure. Ondo et al. (2013) also reported that the extrusion process (95-120°C) with CO_2 gas injection produced cornmeal-based extrudates with unique porous structure and texture. The finding showed that the use of CO_2 can help to produce extrudates with uniform cellular texture. WAI and WSI values of extrudates with CO_2 gas injection were significantly higher compared to those without CO_2 injection. Myat (2013) and Singkhornart et al. (2013) also observed that CO_2 injection significantly affected WAI, WSI, specific length, expansion ratio, bulk density, mechanical properties and color of all extruded products from germinated wheat-barley and corn starch, respectively. Singkhornart et al. (2014) studied the influence of germination and extrusion with CO_2 injection on physicochemical properties of wheat extrudates and found increased specific length, lightness and the WSI. They reported that CO_2 injection of extrudate does not favor the Maillard's reaction which can avoid excessive loss of available amino acid.

1.3.4.8 Effect on pomace inclusion on nutritional and functional properties of extrudates

Selani et al. (2014) included pineapple pomace in the range of 0-21% and observed that hardness, yellowness, water absorption, and bulk density of extrudates remained unaffected when pomace level of 10.5% incorporated with corn flour. O'Shea et al. (2014) reported the optimal extrusion conditions and apple pomace (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. (2009) 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. (2008b) 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. On the other hand, Kumar et al. (2010) observed decreased expansion ratio, hardness, WSI and increased WAI of extruded product with increase in carrot pomace (10-30%) level into the rice flour. The similar impact of carrot pomace (5-15%) inclusion in corn starch on WAI, WSI and ER were observed by Kaisangsri et al. (2016). Large numbers of hydroxyl groups in carrot pomace structure interact with the hydrogen bonds of water and thus may help bind more water to the fiber, thus increasing the WAI with increased pomace level.

Table 1.1 Phenolics extracted by fermentation from different plant materials

Material	Medium used	Process conditions	Phenolic yield	References
Pearl millet (*Pennisetum typhoidium*)	Natural	30°C, 36 h	38.5% reduction in polyphenols	Elyas et al. (2002)
Sorghum (*Sorghum bicolor* (L.) Moench) gruel	Local starter culture	30°C, 16 h	Reduced 57% total phenols	Towo et al. (2006)
Sorghum (*Sorghum bicolor* (L.) Moench)	Lactic acid culture	25°C, 24 h	Reduced 33% total phenols and 52% AA	Dlamini et al. (2007)
Chickpea (*C. arietinum cv. Blanco lechoso*)	Natural and with *Lactobacillus plantarum*	37°C, 48 h	TPC increased by 311% in natural and 192% with inoculum	Fernandez-Orozco et al. (2009)
Bambara groundnut (*V. subterranea* L. verdc), African yam bean (*S. stenocarpa* Harms), Pigeon pea (*C. cajan* L. Millsp) and Kidney bean (*P. vulgaris* L.)	Natural	37°C, 4 days	Increased 92%, 68%, 25% and 42%, respectively total free phenol Increased 40%, 6%, 2% and 7%, respectively AA	Oboh et al. (2009)
Buckwheat (*Fag-opyrum esculentum*) Wheat (*Triticum aestivum*) Barley (*Hordeum vulgare*)	*Saccharomyces cerevisiae* and *Lactobacillus rhamnosus*	30°C and 37°C, respectively, 24 h	Increased 5% TPC with *S. cerevisiae* and 17% with *L. rhamnosus* Increased 13.5% TPC with *S. cerevisiae* and 27% with *L. rhamnosus* Increased 12.8% TPC with *S. cerevisiae* and 22.6% with *L. rhamnosus*	Đorđević et al. (2010)
Apple pomace	*Phanerocheate chrysosporium*	37°C, 14 days	Increased 11% TPC and 91% AA	Ajila et al. (2011)
Rice bran	*Rhizopus oryzae*	30°C, 24 h	Increased 358% TPC	Oliveira et al. (2012)
Sorghum (*Sorghum bicolor* (L.) Moench)	Natural	Germinated flour, 45% (w/w) in water, 27°C, 14 h	Increased 24% TPC	Kayodé et al. (2013)

AA: antioxidant activity, TPC: total phenolic content

Table 1.2 Phenolics extracted by supercritical fluid extraction from different plant materials

Material	Process conditions	Phenolic yield	References
Mandarin pomace (*Citrus reticulata* Blanco cv Kinnow)	water:material 125:1, 250 W, 5 min	Increased 26% phenolic content	Hayat et al. (2010)
Buckwheat (*Fagopyrum esculentum* Möench)	water:material 50:1, 150°C	13.2 mg GAE/g TPC with 100% water 6.47 mg GAE/g TPC with 100% ethanol	Inglett et al. (2010)
Pine (*Pinus pinaster*) seeds	water:material 25:1, 75°C, 20 min	MAE: 3.1 mg/100g UAE: 5.0 mg/100g	Liazid et al. (2010)
Withania somnifera Dunal.	methanol:water 25:75, 17 ml methanol, 68°C, 150 s	Recovered 88.2% withaferin	Mirzajani et al. (2010)
Bean (*Phaseolus vulgaris* L.)	water:material 49:1, 100°C	3 times those by conventional extraction	Sutivisedsak et al. (2010)
Perilla Frutescens leaves	water: material 17:1, 600 W, 23 min, 8.4 pH	MAE: 6.07 mg/g flavonoids Soxhlet: 2.69 mg/g flavonoids	Shao et al. (2011)
Pomegranate (*Punica granatum*) peel	water:material 20:1, 600 W, 60 s	210.4 mg GAE/g	Zheng et al. (2011)
Green coffee bean	800 W, 50°C, 5 min	Increased 9% TPC	Upadhyay et al. (2012)

GAE: gallic acid equivalent, MAE: microwave assisted extraction, TPC: total phenolic content, UAE: ultrasonication assisted extraction, water: material (v/w)

Table 1.3 Phenolics extracted from different plant materials using ultrasound assisted extraction

Material	Process conditions	Phenolic yield	References
Coconut (*Cocos nucifera*) shell powder	25 kHz, 0.5 W/cm^2, ethanol:peel 50:1, 30°C, 15 min, pH 6.5	Increased phenolic content 3 times of control	Rodrigues et al. (2008)
Orange (*Citrus sinensis* L.) peel	25 kHz, 150 W, ethanol:peel 4:1, 40°C, 30 min	Increased 10.9% TPC and 40% AA	Khan et al. (2010)
Apple pomace	0.142 W/g, ethanol:water 10:1, 40°C, 45 min	Increased 20% TPC	Virot et al. (2010)
Pomegranate peel	20 kHz, 59 W/cm^2, water:peel 50:1	Increased 15% TPC and 24 % AA	Pan et al. (2011)
Mangosteen (*Garcinia mangostana* Linn.)	20 kHz, 80% amplitude, methanol:sample 20:1, 25 min	Increased 9% TPC	Cheok et al. (2013)
Raspberry ((*Rubus idaeus*) puree	20 kHz, 150 ml, 10 min	Increased 17% AA	Golmohamadi et al. (2013)
Grapefruit (*Citrus paradisi* L.) waste	40 kHz, solvent:sample 8:1, 25°C, 55 min	50% TPC and 66% AA higher than conventional	Garcia-Castello et al. (2015)
Grape (*Vitis vinifera* L.) pomace	55 kHz, 23 W/cm^2, water:material 20:1, 200 ml, 20°C	230 mg GAE/100g Conventional: 77.5 mg GAE/100g	González-Centeno et al. (2015)
Carrot (*Daucus carota* L.) pomace	20 kHz, 48 W/cm^2, ethanol:sample 48:1, 34°C, 17 min	316.9 mg/g	Jabbar et al. (2015)
Mustard (*Sinapis alba*) seed	40 kHz, solvent:sample 5:1, power:time 4:1	28.5 mg SA/g, Conventional: 23.5 mg SA/g	Szydłowska-Czerniak et al. (2015)

AA: antioxidant activity, GAE: gallic acid equivalent, SA: sinapic acid, TPC: total phenolic content, solvent:sample (v/w)

Table 1.4 Phenolics extracted from different plant materials using pulsed electric field

Material	Process conditions	Phenolic yield	References
Grapes (*Vitis vinifera* var. Tempranillo)	5 kV/cm EFI, 1 Hz, 1.8 kJ/kg, 25 g, 30°C	Increased 17% total phenols and 15.5% anthocyanins	López et al. (2008)
Strawberry (*Fragaria ananassa* Duch, cultivar Camarosa) juice	35 kV/cm, 232 Hz, 1 μs, 250 bipolar pulses, 60 ml/min	Increased 102% anthocyanins and 100% AA	Odriozola-Serrano et al. (2009b)
Cabbage	2.5 kV/cm, 1μF, 1 Hz, 15 μs, 50 pulses, 42 g mash of cabbage	Increased 2 times anthocyanins	Gachovska et al. (2010)
Apple juice Apple pomace	650 V/cm, 32 kJ/kg, 200 Hz, 100 μs,232 pulses, 4400 kg/h	Increased 8.8% polyphenols in juice No effect in pomace	Turk et al. (2012)
Tomato (*Licopersicon esculentum* Mill. cv. Daniella)	1.2 kV/cm,4 μs, 0.1 Hz, 30 pulses	Increased 44% polyphenol	Vallverdú-Queralt et al. (2012)
Orange (*Citrus sinensis* from late cultivar Valencia) peel	7 kV/cm, 3.77 kJ/kg, 3 μs, 1 Hz, 40 g chopped peel	Increased 159% TPC and 192% AA	Luengo et al. (2013)
Flaxseed (*L. usitatissimum*, cultivar Baladin) hulls	20 kV/cm, 300kJ/kg, 0.33 Hz, 10 ms, ethanol/water:sample 25:1	Increased 80% polyphenols	Boussetta et al. (2014)
Spearmint (*Mentha x spicata* L.)	3 kV/cm, 99 pulses, 4 kJ/kg, mannitol:sample 4:1	Increased 25% TPC	Fincan (2015)
Graciano grape	7.4 kV/cm, 400 Hz, 20 μs	Increased 48.5% anthocyanins and 11% AA	López-Giral et al. (2015)
Canola seed (*Brassica napus*) cake	1.9 kV/cm, 30 Hz, 30 V, 20 μs, 900 pulses, 10 kJ, ethanol:cake 10:1	2.62 g GAE/100 g TPC Increased 38.6 % AA	Teh et al. (2015)
Apple (*Malus domestica* var. "Ligol") tissues	1.85 kV/cm, 0.25 μF, 10 μs, 10 pulses, 27.4 ml	Increased 33% AA	Wiktor et al. (2015)
White button mushroom (*Agaricus bisporus*)	38.4 kV/cm, 800 Hz, 2 μs, 136 bipolar pulses, 85°C, water:sample 9:1,	Increased 51% polyphenols	Xue and Farid (2015)
Grape (*Vitis vinifera* L.) juice	1.5 kV/cm, 20 μs, 50 Hz, 243 pulses, 14.5 kJ/kg, 200 g grape mash	Increased 22% TPC	Leong et al. (2016)

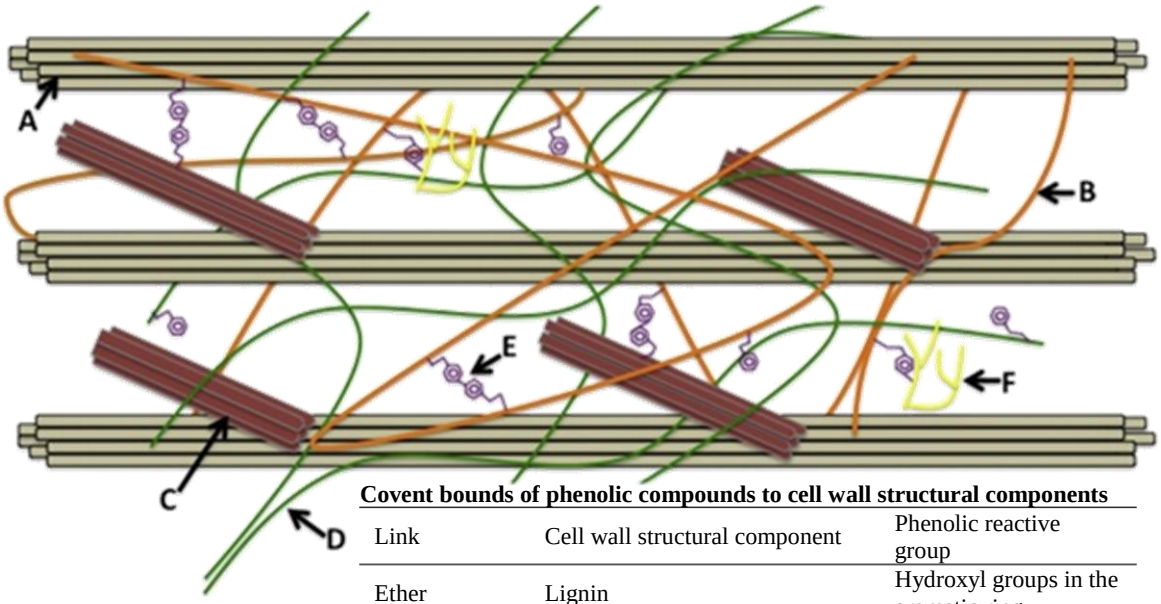
AA: antioxidant activity, EFI: electric field intensity, GAE: gallic acid equivalent, TPC: total phenolic content, solvent:sample (v/w)

Table 1.5 Impact of extrusion process on phenolics of different plant materials

Material	Process conditions	Phenolic yield	References
Corn flour, corn starch, oat flour, chickpea flour, carrot powder, hazelnut	TSE, 11-15%wb, 22-26 kg/h feed rate, 110°C, 220-340 rpm	No effect on TPC Decreased AA	Ozer et al. (2006)
Buckwheat (*F. esculentum*)	TSE, 20%wb, 13.5 kg/h, 120-200°C, 500 rpm,	Decreased 3 times TPC, 10% AA	Zieliński et al. (2006)
Bean (*Phaseolus vulgaris* L.)	SSE, 14 & 20%wb, 120 & 180°C, 90 rpm	Increased 14% TPC	Korus et al. (2007)
Barley flour, tomato pomace (0-12.7%), grape pomace (0-12.7%)	TSE, 2 kg/h, 133-167°C, 133-217 rpm	Decreased TPC, AA Increased starch digestibility	Altan et al. (2009)
Corn starch, bean flours (*Phaseolus vulgaris* L.) (15-45%)	TSE, 1.8 kg/h, 22%wb, 160°C, 150 rpm	Decreased TPC, AA	Anton et al. (2009)
Corn flour, bean flour (60%)	SSE, 14.5-18%wb, 150-190°C, 90 rpm	Decreased TPC, AA	Delgado-Licon et al. (2009)
Wheat flour, corn starch, brewer's spent grain, red cabbage	TSE, 15%wb, 25 kg/h, 200 rpm, 120 °C	Increased TPC, AA, ER, BD, WAI, TDF Decreased WSI	Stojceska et al. (2009)
Corn starch, craneberry pomace (30-50%)	TSE, 30%wb, 150-190, 150 & 200 rpm	Increased 30-34% AA	White et al. (2010)
Brown rice (*Oryza sativa* L.)	SSE, 33 kg/h, 5%wb, 100°C, 100 rpm	Decreased TPC, AA Increased WSI, WAI, ER	Gujral et al. (2012)
Barley (*Hordeum vulgare* L.)	TSE, 20 kg/h, 15% & 20%wb, 150 & 180°C, 400 rpm	Decreased TPC Increased AA	Sharma et al. (2012)
Corn flour, tomato powder, ginseng powder, green tea	TSE, 20%wb, 80-130°C, 250 rpm, 1MPa CO_2	Increased TPC, AA compared to conventional extrusion	Masatcioglu et al. (2013)
Chestnut	TSE, 7 kg/h, 200 rpm, 120 & 140°C, 25 & 28%wb,	Increased 21.2% TPC	Obiang-Obounou and Ryu (2013)

Corn grits, corn fiber (70-100%)	TSE, 30%wb, 6 kg/h, 90-120°C, 150 rpm, 12 l/h CO_2 injection	Reduced TPC, WSI Stable AA, TDF Increased WAI	Wang and Ryu (2013b)
Maize starch, bilberry	TSE, 180-720 rpm, 18-28%wb, 100-160°C	No effect on TPC and AA	Hirth et al. (2014)
Rice flour, wheat semolina, apple pomace	SSE, 60 rpm, 190°C	Increased TPC, AA Decreased TDF	Reis et al. (2014)
Banana (*Musa spp.*)) flour	TSE, 20%wb, 2 kg/h, 130°C, 400 rpm	Increased TDF, WSI, TPC, AA Decreased RS, WAI	Sarawong et al. (2014)
Sweet potato (*Ipomoea batatas* L.) flour	TSE, 10%wb, 10.5 kg/h, 110°C, 250 rpm	Increased 13% TPC, 16% AA, WAI, WSI	Soison et al. (2014)
Brown rice and maize	TSE, 3.6 kg/h, 25%wb, 110-140°C, 100 rpm	Decreased TPC, AA	Yang et al. (2014)
Corn flour, broccoli flour, olive paste	TSE, 14-19%wb, 140-180°C, 150-250 rpm	Increased TPC, AA	Bisharat et al. (2015)
Sorghum (*Sorghum bicolor* L.)	TSE, 12%wb	Decreased flavanones and flavones	Cardoso et al. (2015)
Lentil flour (*Lens culinaris* L.)	TSE, 20 kg/h, 17%wb, 160°C, 500 rpm	Increased polyphenols	Morales et al. (2015)
Black rice (*Oryza sativa* var. Heiyounian)	TSE, 25 kg/h, 12-17%wb, 120°C, 200 rpm	Increased 12.6% TPC and 19.7% AA	Ti et al. (2015)
Fenugreek seed, fenugreek leave powder, oats, dried green pea, rice and corn flour	TSE, 12%wb, 110°C, 200 rpm	Decreased TPC, AA	Wani and Kumar (2015)
Defatted soybean meal, germinated brown rice meal, mango peel fiber and corn grit	SSE, 13-15%wb, 160-180°C, 180 rpm	Decreased 29% TDF, 51.5% TPC Increased 330% AA	Korkerd et al. (2016)
Oat flour, potato starch, apple pomace (14%)	SSE, 23-29%wb, 115-165°C, 180 rpm	Decreased 3-20% TPC No effect on AA	Leyva-Corral et al. (2016)

AA: antioxidant activity, BD: bulk density, ER: expansion ratio, RS: resistant starch, SSE: single screw extruder, TDF: total dietary fiber, TPC: total phenolic content, TSE: twin screw extruder, WAI: water absorption index, WSI: water solubility index

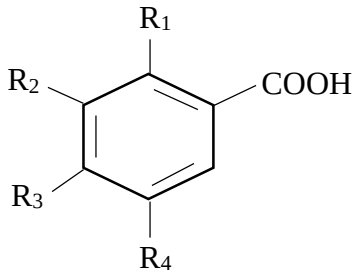


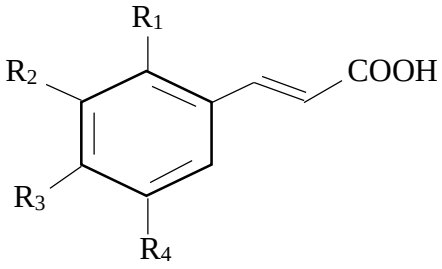
Covent bounds of phenolic compounds to cell wall structural components

Link	Cell wall structural component	Phenolic reactive group
Ether	Lignin	Hydroxyl groups in the aromatic ring
Ester	Structural carbohydrates Proteins	Carboxylic group

Source: (Acosta-Estrada et al., 2014)

Figure 1.1 Representation of primary cell wall structure of plant material and cross-linking between structural components and phenolic compounds, (A) Cellulose, (B) Hemicellulose, (C) Structural proteins, (D) Pectin, (E) Phenolic acids, (F) Lignin





	Hydroxybenzoic acids					**Hydroxycinnamic acids**			
	R_1	**R_2**	**R_3**	**R_4**		**R_1**	**R_2**	**R_3**	**R_4**
Gallic acid	H	OH	H	OH	Caffeic acid	H	OH	OH	H
Gentisic acid	OH	H	H	OH	Ferulic acid	H	OCH_3	OH	H
Salicylic acid	OH	H	H	H	*o*-coumaric acid	OH	H	H	H
p-hydroxybenzoic acid	H	H	H	OH	*p*-coumaric acid	H	H	H	H
Syringic acid	H	OCH_3	OH	OCH_3	Sinapic acid	H	OCH_3	OCH_3	OCH_3
Protocatechuic acid	H	OH	OH	H	Vanillic acid	H	OCH_3	OCH_3	H

Figure 1.2 Structure of major phenolic acids present in foods

CHAPTER 2

Effect of natural fermentation and ultrasonication on phenolic compounds and textural properties of sorghum extrudates[1]

2.1 Abstract

Extrusion cooking results in loss of the nutritive values in several food products. Therefore, an approach to enhance the total phenolic content (TPC) and antioxidant activity (AA) in the sorghum flour using non-thermal processing was investigated prior to extrusion. Sorghum flour was naturally fermented for 0 h, 12 h, and 24 h followed by the ultrasonication at 30, 55, and 80 W/cm^2 ultrasonication intensity (UI) for 3, 4, and 5 min ultrasonication time (UT). For ultrasonication, fermented flour to water ratio (FWR) was kept as 10, 15, and 20% (w/v). Maximum TPC and AA of the sorghum flour were observed after 12 h fermentation time (FT), and were decreased significantly with further increase in the fermentation time. Increasing FWR and UI during ultrasonication resulted in decreasing TPC and AA significantly. Ultrasonication time did not have any significant effect on the TPC and AA of the sorghum flour. AA was positively correlated with the TPC of the sorghum flour for different FT, FWR and UI. At the optimum pretreatment condition, TPC and AA were found as 77.8 mg GAE/ 100g DW, and 178.8 µmol TE/100g DW, respectively. The optimally pretreated sorghum extrudates had improved textural and functional properties. Pretreatment reduced the crystalline fraction amount and stability of the starch crystallites in the sorghum flour.

[1] Lohani, U. C. and Muthukumarappan, K. 2016. Effect of natural fermentation and ultrasonication on phenolic compounds and textural properties of sorghum extrudates. Journal of Cereal Science. *Under review*.

2.2 Introduction

Presently, it's a worldwide demand for the antioxidant rich foods in diet, especially ready to eat foods, i.e. cereals and snacks. In recent years, various cereals have been identified and accepted as the functional foods and nutraceuticals because of the good sources of dietary fiber, proteins, energy, minerals, vitamins, and antioxidants required for the human health. Sorghum is one of these cereals that contains phenolic compounds mainly in the forms of phenolic acids and flavonoids (Hahn et al., 1984). These compounds have potentiality to impact positively on the human health because of their antioxidant and antiradical properties (Awika and Rooney, 2004). Sorghum utilization can be improved by incorporating it into mainstream human diet in different innovative ways. Extrusion is the one of the relatively inexpensive, energy-efficient and an easily operated ways to produce a wide range of ready to eat products in the form of cereals and snacks.

From our preliminary experiments, it was observed that even the mild extrusion resulted in more than 45% loss of total phenolic content (TPC) and antioxidant activity (AA) in final extrudates. Dlamini et al. (2007) also reported diminution in the total phenolics and antioxidant activity in the sorghum during extrusion. There can be two ways to get the nutritional or antioxidants rich snacks. First, antioxidants can be enhanced in the raw material by liberating the bound phenolics using some pretreatments prior to extrusion and second, antioxidants can be retained in extrudates snacks during extrusion by using modified extrusion process. In this paper, an approach has been made to investigate the pretreatment method to enhance the TPC and AA in sorghum flour.

In past few years, fermentation has been used for improving the antioxidant properties in legumes (Oboh et al., 2009), and rice bran (Hegde et al., 2006), but fermentation reduces the TPC and AA in sorghum (Dlamini et al., 2007, Svensson et al., 2010) but improves the functional properties, i.e. starch-protein digestibility, water absorption index and water solubility index (Pranoto et al., 2013). During microbial fermentation, polyphenol oxidase activity degrades the phenolics in cereals, and also acidic environment during fermentation causes the abstraction of hydride ions and rearrangement of phenolic structures (Towo et al., 2006). These results were supported by our preliminary trials where sorghum flour inoculated with pure culture of *Lactobacillus plantarum* showed less phenolic content with compared to that of naturally fermented. Therefore, natural fermentation was opted in this study as a pretreatment of sorghum flour prior to ultrasonication. In natural fermentation, phenolic could be bound to proteins, starches and other components in the aqueous fermentation environment and thus it reduces the extractability of phenolic compounds (Taylor and Duodu, 2015). Hypothetically, natural fermentation of sorghum flour followed by ultrasonication will increase the total phenolics and antioxidant activity because ultrasonication separates starch from protein matrix and breaks down these molecules (Zhao et al., 2008) resulting in release of bound phenolics with protein and other components. Our comprehensive literature review revealed that there has been very few detailed report on the use of fermentation and ultrsonication in combination to liberate phenolic compounds in plant materials (Ajila et al., 2011) and particularly none in case of sorghum flour. Therefore, keeping in view the above facts, the objective of the study was to observe the influence of sequential treatments of fermentation and ultrasonication on TPC and AA of sorghum

flour. Also the sorghum extrudates from pretreated (fermented and ultrasonicated) flour were compared with those from control (un-pretreated) sorghum flour in terms of TPC, AA, thermal, textural (hardness, brittleness, crispness) and functional (water absorption index, water solubility index) properties.

2.3 Materials and Methods

Sorghum flour (SF) provided by ADM Milling Co. (Overland Park, KS) was stored at -20°C. The moisture content, fat, protein, ash and carbohydrates presented in flour were 9.33%, 3.19%, 9.51%, 1.12% and 76.85%, respectively. For natural fermentation, sorghum was prepared by adding 45 g flour in 100 ml distilled water. The fermentation was carried out in a controlled environment with temperature 30±1°C for 0 h, 12 h, and 24 h. The pH of fermented slurry corresponding to fermentation times were 6.0, 5.5 and 4.1, respectively. Fermented slurry was further diluted to keep the flour to water ratio (FWR) as 10%, 15%, and 20% (w/v). Diluted slurry was then subjected to ultrasonication at 30, 55, and 80 W/cm^2 intensity for 3, 4, and 5 min. Ultrasonicated samples were oven dried at 40°C and stored at -20°C for TPC and AA analysis.

2.3.1 Extraction of samples

The extraction of sample for determining TPC, AA and phenolic characterization was done using the method described by Khan et al. (2013). For determining TPC and AA, 1 g of SF 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°C until analysis for TPC and AA.

Free phenolic acid extraction was performed by adding 10 ml of 80% (v/v) aqueous methanol into 2 g of SF. Mixture was shaken in a shaking water bath for 1 h at 25°C. After centrifugation at 3000×*g* for 20 min, the supernatant was decanted and the extraction was repeated as described above. The two supernatants were combined, evaporated to near dryness and reconstituted with methanol to a final volume of 10 ml.

2.3.1.1 Total phenolic content (TPC)

TPC of SF was determined using Folin–Ciocalteu method (Singleton et al., 1999) with some modification. 50 µl methanol extract of sample was added with 3.5 ml distilled water and 150 µl Folin-Ciocalteu reagent. The solution was vortexed and incubated for 30 min. Thereafter, absorbance of solution was measured at 760 nm against blank. Blank solution contained all the components that were present in the sample except the methanol extract. Gallic acid was used as positive control (standard) and linear regression curve between absorbance and concentration was drawn for the standard. This standard curve was used for calculating the concentration of sample and data was expressed in mg Gallic acid equivalent (GAE)/100 g dry weight (DW). This analysis was done in six replications.

2.3.1.2 Antioxidant activity (AA)

Extinction of DPPH is a free radical scavenging activity which was measured using spectrophotometric method described by Brand-Williams et al. (1995). 2,2-diphenyl-1-picrylhydrazyl (DPPH) 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 for 30 min. Thereafter, 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 weight (DW). Samples were analyzed in six replications.

2.3.1.3 Free phenolic acid characterization

Analysis of sample extracts was carried out using Thermo Scientific, Dionex Ultimate 3000 UHPLC system (Bannockburn, IL, United States) equipped with diode-array detector (DAD) and C_{18} column (150 mm × 4.6 mm) packed with 5 μm particles. The samples were injected with a mobile-phase flow rate of 800 μl/min. Gradient elution was carried out with a solvent system of water/acetic acid (99.8:0.2 v/v) as mobile phase A and acetonitrile/acetic acid (99.8:0.2 v/v) as mobile phase B. The total run time was 12 min, and the gradient elution was as follows: 0.0−3.0 min, B 10-25%; 3.0−4.5 min, B 25−45%; 4.5−6.5 min, B 45−65%; 6.5−8.0 min, B 65−85%; 8.0−9.0 min, B 85−100%.; 9.0−12.0 min, B 100−10%. All the solvents were filtered through 0.22 μm PTFE filters prior to inject. The column was maintained at 30°C while the autosampler was thermostated at 4°C. The system was controlled by Thermo Scientific Dionex Chromeleon 7 software. Benzoic acid and cinnamic acid derivatives were detected at 280 nm and 320 nm, respectively. The concentrations of phenolic acids were calculated from peak areas in comparison to calibration curves of the respective standards and were expressed as μg/g DW.

2.3.2 Moisture content

Moisture content of SF powder was determined by air oven standard methods recommended by AOAC (1980). Initially 5 g of sample in triplicate was dried in hot air oven at 130-133°C for 2 h. After drying, dried sample was again weighed. Following formula is used for calculating the moisture content (MC).

$$\text{MC (\%wb)} = \frac{W_i - W_f}{W_i} \times 100 \quad (2.1)$$

W_i= initial weight of sample (5 g),

W_f= weight of sample after drying, g

2.3.3 Scanning electron microscopy

The microstructure of control, and optimally cavitated sorghum flour was examined using a scanning electron microscope (SEM) (Hitachi-S3400 N, Tokyo, Japan). Small amounts of samples were mounted on SEM specimen stubs by using double-sided adhesive tape. Each powder sample was coated with 10 Å thick layer of gold in a sputter coater before being scanned and photographed at 1500× magnification.

2.3.4 Extrusion cooking

Control sorghum flour and cavitated sorghum flour at optimum condition with 20% moisture content were extruded in a single screw extruder (Brabender Plasti-corder, model PL 2000, South Hackensack, NJ) at 90°C die temperature and 90 rpm screw speed. Compression ratio, die dimension, and length to diameter ratio of barrel were kept constant as 3:1, 3 mm, and 20:1, respectively.

2.3.5 Texture analysis

Extrudates from control SF and cavitated SF at optimum conditions were subjected to hardness, brittleness and crispness using TA-XTPlus Texture Analyzer (Texture Technologies Corp., Hamilton, MA) as per method described by Altan et al. (2008b). The peak force as an indication of hardness was measured with a TA-XTPlus Texture Analyzer (Texture Technologies Corp., Hamilton, MA) using 3-point bend test. The test speed was 2 mm/s and the distance between two supports was kept as 22 mm. The curve between force vs distance was plotted and analyzed by Texture Exponent 32

software program (version 3.0). The slope (N/mm) and distance (mm) at which a product breaks were measured from force–distance curve and evaluated as crispness and brittleness, respectively (Texture Technologies, a). Both the sample extrudates were replicated 10 times. The radial expansion ratio of the extrudates was measured as the ratio of the diameter of the extrudates to the diameter of the die orifice.

2.3.6 Water absorption index (WAI) and water solubility index (WSI)

To determine the WAI and WSI of extrudates, the methodology proposed by Anderson (1982) was followed. Ground extrudates (2.5 g) was suspended in distilled water (30 ml) in a tarred 60 ml centrifuge tube. The suspension was stirred intermittently and centrifuged at 3000×*g* for 10 min. The supernatant was decanted into a tarred aluminums cup and dried at 135°C for 2 h. The weight of the gel remaining in the centrifuge tube was measured. The WAI and WSI were calculated by

$$\text{WAI}=\frac{W_g}{W_{ds}}\times 100 \tag{2.2}$$

where, WAI is water absorption index, W_g is the weight of gel (g), and W_{ds} is the weight of dry sample (g).

$$\text{WSI}=\frac{W_{ss}}{W_{ds}}\times 100 \tag{2.3}$$

where, WSI is the water solubility index (%), W_{ss} is the weight of dry solids of supernatant (g), and W_{ds} is the weight of dry sample (g).

2.3.7 Gelatinization properties

Differential scanning thermal analysis of control SF, optimally cavitated SF and extrudates developed from both was performed using the method described by Mahasukhonthachat et al. (2010). Analysis was performed using a differential scanning

calorimeter (DSC) (TA Instruments Model Q2000, New Castle, DE, USA) previously calibrated with indium. The extrudates were ground and sieved through a 295-µm screen. Powder samples of 10 mg were weighed directly into DSC hermetic aluminum pans, and 20µl of distilled water was added using a micro-syringe. The sample pans were sealed and allowed to equilibrate for overnight at 4°C. The heating rate was 10°C/min, from 20 to 130°C with nitrogen flushing (20 cm^3/min). A sealed empty pan was used as reference for all measurements. Gelatinization peak temperature and the enthalpy of the endotherm for gelatinization were measured and calculated using TA Universal Analysis, version 5.5.17 (TA Instruments, New Castle, DE, USA).

2.3.8 Statistical analysis

Full factorial design was used for experimental plan and results were compared by analysis of variance (ANOVA) using SPSS (16.0) statistical software. Optimization of independent variables was obtained using statistical software package (Design Expert 9.0.3.1, Stat-Ease Inc., USA). All data were reported as mean ± standard deviation of replicates. Tukey's tests were used to compare the significant differences of the mean values with the family error rate held at 0.05.

2.4 Results and Discussion

2.4.1 Effect of Fermentation time and ultrasonication on TPC and AA

It is explicated from ANOVA that fermentation time (FT) had significant ($p<0.05$) effect on TPC and AA. Interaction effect of FT with FWR and UI on TPC and AA was found significant ($p<0.05$) (Table 2.1). With increased fermentation time from 0 h to 12 h, TPC and AA of ultrasonicated sorghum flour significantly ($p<0.05$) increased by 31.4% and 28.8%, respectively (Figure 2.1). Up to 12 h of fermentation, phenols

bounded with protein and starch were released by ultrasonication. Decarboxylation of hydroxycinnamic acids also caused increase in TPC and AA. It has been reported that the carboxyl group exerted a negative effect on the antioxidant capacity of phenolic acids (Kayodé et al., 2013). It is obvious from Table 2.2 that concentrations of cinnamic acid derived phenolic acids after 12h fermentation followed by ultrasonication (pretreated SF) were higher. Further increase in fermentation time up to 24 h resulted in significant ($p<0.05$) decrease in TPC and AA (Figure 2.1). This reduction might be due to hydrolysis of the glycosidic bonds of bound phenolics and degradation of bound phenolics during fermentation, therefore TPC and AA were not enhanced by further ultrasonication. TPC and AA of sorghum flour were observed as 67.7 mg GAE/100g DW and 155.7 µmol TE/100g DW, respectively on average after 12 h fermentation followed by ultrasonication (Figure 2.1).

2.4.2 Effect of flour to water ratio on TPC and AA

It is indicated by ANOVA that FWR and its interaction with UI had significant ($p<0.05$) effect on TPC and AA of sorghum flour (Table 2.1). TPC and AA decreased significantly ($p<0.05$) by 7.67% and 8.92%, respectively with increase in FWR from 10% (w/v) to 15% (w/v) (Figure 2.1). Further increase in FWR did not exhibit significant ($p>0.05$) decrease in TPC but AA of sorghum flour decreased significantly ($p<0.05$). Similar results were found by Gribova et al. (2008) for AA of bearberry leaves. With increase in FWR, increased viscosity of solution caused non-uniform transmittance of ultrasound energy to the whole sample solution at a given ultrasonication intensity. This resulted in less release of phenolics and therefore also less AA was determined. TPC and

AA of sorghum flour were determined as 61.87 mg GAE/100g DW, and 147.91 µmol TE/100g DW, respectively on average at 10% (w/v) flour to water ratio (Figure 2.1).

2.4.3 Effect of ultrasonication intensity on TPC and AA

It is depicted from ANOVA that UI significantly ($p<0.05$) affected TPC and AA, while it's interaction with UT did not show any significant ($p>0.05$) influence on TPC and AA of sorghum flour (Table 2.1). TPC and AA decreased significantly ($p<0.05$) by 8.33% and 9.58%, respectively with increase in ultrasonication intensity from 30 W/cm^2 to 80 W/cm^2 (Figure 2.2). However, for TPC this drop was significant ($p<0.05$) after 55 W/cm^2 UI, while SF did not show significant ($p>0.05$) decrease in AA when UI elevated from 55 W/cm^2 to 80 W/cm^2. Yu et al. (2012) observed a decrease in antioxidant activity of peanut hydrolysate with increase in ultrasonication power. At higher intensity, due to agitation of sample instead of cavitation, bubble cloud density became too large which resulted in rise to shielding effects, coalescence and general bubble-bubble interactions that decreased the overall cavitation efficiency of the process. However, Ma et al. (2008) reported increase in TPC with increase in ultrasonication power for mandarin peel because it contains flavanoids having a higher thermally stability, and are significantly higher than phenolic acids. Sorghum flour had TPC and AA as 60.3 mg GAE/100g DW, and 144.2 µmol TE/100g DW, respectively on average at 30 W/cm^2 ultrasonication intensity (Figure 2.2).

2.4.4 Effect of ultrasonication time on TPC and AA

It is shown by ANOVA that UT didn't have any significant ($p>0.05$) effect on TPC and AA, however, TPC of sorghum flour was significantly ($p<0.05$) affected by three way interactions of UT with FT, FWR, and UI (Table 2.1). Figure 2.2 shows that

TPC and AA varied from 57.6-58.5 mg GAE/100g DW and 135.6-137.5 µmol TE/100g DW, respectively when ultrasonication time increased from 3 min to 5 min. Similar results were also observed by Lohani and Muthukumarappan (2015) for apple pomace powder.

Optimum conditions of process parameters for maximum TPC and AA of sorghum flour was observed as 12 h fermentation time, 10% (w/v) flour to water ratio, 30 W/cm^2 ultrasonication intensity, and 3 min ultrasonication time. TPC and AA of control sorghum were 63.9 mg GAE/100g DW and 133.5 µmol TE/100g DW, respectively and were increased by 21.8% and 33.9%, respectively when pretreated at optimum conditions (Figure 2.3). In support of these results, the individual phenolic acids were quantified in control and pretreated sorghum flour in Table 2.2. All benzoic and cinnamic acid derivatives were found to be higher in pretreated SF with compared to control SF. It was also observed that salicylic acid followed by ferulic and *p*-hydroxybenzoic acid were the major phenolic acids presented in both the SF.

2.4.5 Relationship between TPC and AA

Sorghum flour with higher levels of TPC had a greater AA and these results are confirmed with the findings of Kayodé et al. (2013). The values of AA indicated a positive correlation with the values of TPC of sorghum flour at different fermentation time, flour to water ratio, and ultrasonication intensity. A linear correlation in each case was observed between AA and TPC. The correlation coefficient (r) were 0.979, 0.992, and 0.865 at different FT, FWR, and UI, respectively, which indicated that TPC was the major factor accounting for the antioxidant activity of the sorghum flour.

2.4.6 Effect of fermentation and ultrasonication on TPC and AA of sorghum extrudates

Extrudates from control sorghum flour (without fermentation and ultrasonication) and pretreated sorghum flour at optimum conditions (12 h FT, 10% (w/v) FWR, 30 W/cm^2 UI, and 3 min UT) were analyzed for TPC and AA (Table 2.3). It was observed that TPC and AA of control sorghum extrudates were reduced by 33.3% and 25.5%, respectively with compare to control sorghum flour. These losses were because of degradation of phenolic acids due to high temperature and pressure inside the extruder (Table 2.2). These results are in agreement with the findings of Licata et al. (2014) for sorghum flour. Pretreated sorghum extrudates exhibited more retention of TPC and AA with 6.9% and 8.7%, respectively higher than that of control sorghum. These results are supported by Table 2.2 showing that concentration of benzoic acid and cinnamic acid derived phenolic acids were found higher in pretreated sorghum extrudates as compare to that of control sorghum flour, however *p*-hydroxybenzoic acid and *p*-coumaric acid values of both the samples were not significantly ($p>0.05$) different.

2.4.7 Microstructure analysis of pretreated sorghum flour

The changes in physical structure of control and pretreated samples of sorghum flour at optimum conditions were imaged by scanning electron microscope. Figure 2.4a shows the structure of control sorghum flour, which is a compact and less porous structure. The SEM images revealed that the pretreatment resulted in modification of the cellular structure. Fermentation and ultrasonication damaged the plant cell structure and caused more porosity (Figure 2.4b). Similar results were found by Karki et al. (2010) for soy flakes. Pretreated sample starch granules were more disrupted than control sample

granules which caused increase in surface area and helped in releasing more phenolics which were bound with carbohydrates (Figure 2.4).

2.4.8 Textural analysis of control and pretreated sorghum extrudates

Extrudates from control sorghum and pretreated sorghum flour (Figure 2.5) were analyzed for the maximum peak force (hardness), minimum slope (crispness) and minimum distance (brittleness). For crispness, the lower the slope, the crisper the product is considered and if a product cracks at a smaller deformation, it is more brittle (Altan et al., 2008b). Table 2.3 showed that the pretreatment of sorghum flour prior to extrusion improved the brittleness and crispness of extrudates by 21% and 60%, respectively, while hardness of the pretreated extrudates decreased by 56.5% with compare to that of control sorghum extrudates. Expansion ratio that is attributed to puffing of extrudates was observed to be increased by 5% when sorghum flour was pretreated prior to extrusion.

2.4.9 Effect of pretreatment on WAI and WSI of sorghum extrudates

Water absorption index (WAI) indicates the part of the starch that was not affected by the extrusion cooking and maintained its internal structure (Mason and Hoseney, 1986). WAI is also indirectly related to water-holding capacity, which thus affects product storage stability (Singh and Muthukumarappan, 2014). Pretreated sorghum extrudates exhibited no significant ($p>0.05$) difference in WAI from control extrudates which indicated that pretreatment did not affect the starch integrity of extrudates (Table 2.3).

Water solubility index (WSI) is directly related to the extent of starch gelatinization that occurs inside the extruder (Harper, 1981). More gelatinization of starch, more the

extrudate is digestible. Data in Table 2.3 revealed that pretreated sorghum extrudates had approximately 1.5 times greater WSI than that of control extrudates.

2.4.10 Effect of pretreatment on gelatinization properties of sorghum flour and extrudates

Above data for WSI of pretreated sorghum extrudates is verified by differential scanning calorimeter (DSC) results in Table 2.4. Peak gelatinization temperature and enthalpy was not detected for the pretreated sorghum extrudates which indicated complete starch gelatinization during extrusion cooking. Starch gelatinization temperature and enthalpy of control sorghum flour were in the range of reported values of 66–77°C (Beta et al., 2001) and 4.4–7.8 J/g (Aboubacar and Hamaker, 1999), respectively. Pretreated sorghum flour exhibited lower gelatinization peak temperature and enthalpy than that of control sorghum flour (Table 2.4). Fermentation and ultrasonication pretreatment destroyed the compact crystal structure of the starch and resulted in a much less ordered residue structure with low thermal stability (Figure 2.4b). Extrusion of control sorghum flour caused further broke down of starch molecules and thus decrease in the peak gelatinization temperature. A small enthalpy value indicates that a few ordered structures possible remain after extrusion (Table 2.4).

2.5 Conclusions

Fermentation and ultrasonication pretreatment of sorghum flour played a significant role in enhancing the TPC, and AA. Fermentation time, flour to water ratio, and ultrasonication intensity had significant ($p<0.05$) effect on TPC and AA of sorghum flour but with increase in ultrasonication time, there was no significant ($p>0.05$) effect on TPC and AA. The optimum pretreatment condition for sorghum flour was found as 12 h

fermentation time, 10% (w/v) flour to water ratio, 30 W/cm^2 ultrasonication intensity, and 3 min ultrasonication time. The optimum values of TPC and AA were 21.8% and 33.9% higher than that of control sorghum flour, respectively. AA of sorghum flour showed the linear correlation with TPC. From the microstructural analysis, fermentation and ultrasonication pretreatment caused more cell collapse and cell disruption resulting in release of phenolics from the bound structure.

Pretreated sorghum extrudates at optimum conditions had more TPC and AA than that of control flour. Pretreatment of sorghum flour exhibited the more brittleness, crispness and less hardness of extrudates. WSI and DSC data revealed that starch in pretreated sorghum extrudates was completely gelatinized resulting increase in digestibility. Thus pretreated sorghum flour can be used as raw ingredients to formulate cereal extrudates rich in texture, functional and antioxidant activity.

Table 2.1 Variance analysis for total phenolic content (TPC) and antioxidant activity (AA) of sorghum flour

Sources	**df**		***SS***	**MS**	**F-value**	**p-value**
FT	2	TPC	11720	5860	334.4	0.000*
		AA	50575	25287	1452	0.000*
FWR	2	TPC	1917	958.8	54.7	0.000*
		AA	18285	9142	524.8	0.000*
UI	2	TPC	1056	528.4	30.2	0.000*
		AA	8025.6	4012.8	230.3	0.000*
UT	2	TPC	33.1	16.5	0.9	0.391
		AA	88.3	44.2	2.51	0.110
FT*FWR	4	TPC	8430	2107	120.3	0.000*
		AA	1621	405.4	23.3	0.000*
FT*UI	4	TPC	775.7	193.9	11.1	0.000*
		AA	302.5	75.6	4.34	0.002*
FT*UT	4	TPC	138.4	34.6	1.9	0.101
		AA	95.8	23.9	1.37	0.245
FWR*UI	4	TPC	217.3	54.3	3.1	0.017*
		AA	145.3	36.3	2.08	0.085
FWR*UT	4	TPC	420.5	105.1	5.9	0.000*
		AA	38.6	9.65	0.55	0.696
UI*UT	4	TPC	64.7	16.2	0.9	0.452
		AA	159.9	39.9	2.29	0.061
FT*FWR*UI	8	TPC	392.9	49.1	2.8	0.006*
		AA	355.3	44.4	2.55	0.012*
FT*FWR*UT	8	TPC	281.6	35.2	2.0	0.048*
		AA	67.9	8.49	0.48	0.864
FT*UI*UT	8	TPC	1059.6	132.4	7.5	0.000*
		AA	37.3	4.66	0.27	0.976
FWR*UI*UT	8	TPC	1375.7	171.9	9.8	0.000*
		AA	84.3	10.5	0.61	0.773
FT*FWR*UI*UT	16	TPC	795.3	49.7	2.8	0.000*
		AA	142.7	8.92	0.51	0.938

*significant at 5% level of significance, df: degree of freedom, FT: fermentation time, FWR: flour to water ratio, MS: mean square, *SS*: sum of squares, UI: ultrasonication intensity, UT: ultrasonication time

Table 2.2 Phenolic profile of control and pretreated sorghum flours (SF) and their extrudates (μg/g DW)

Compounds	Control SF	Pretreated SF	Control sorghum extrudate	Pretreated sorghum extrudate
Benzoic acids				
Protocatechuic acid	6.18±0.11^{a}	7.88±0.19^{c}	4.93±0.12^{d}	7.18±0.16^{e}
p-Hydroxybenzoic acid	13.3±0.32^{a}	15.9±0.21^{b}	9.57±0.18^{c}	13.4±0.31^{a}
Cinnamic acids				
Caffeic acid	10.2±0.19^{a}	14.9±0.29^{b}	8.33±0.23^{c}	13.3±0.27^{d}
p-coumaric acid	4.87±0.21^{a}	6.38±0.32^{b}	2.13±0.11^{c}	5.36±0.24^{a}
Ferulic acid	13.4±0.28^{a}	18.1±0.26^{b}	8.69±0.23^{c}	15.5±0.13^{d}
Salicylic acid	22.8±0.23^{a}	26.9±0.34^{b}	14.5±0.25^{c}	24.4±0.27^{d}

Means±SD in the same row with different letters are significantly different ($p<0.05$)

Table 2.3 Nutritional, textural and functional analysis of control and pretreated sorghum extrudates (12 h fermentation time, 10%w/v flour to water ratio, 30 W/cm^2 ultrasonication intensity and 3 min ultrasonication time)

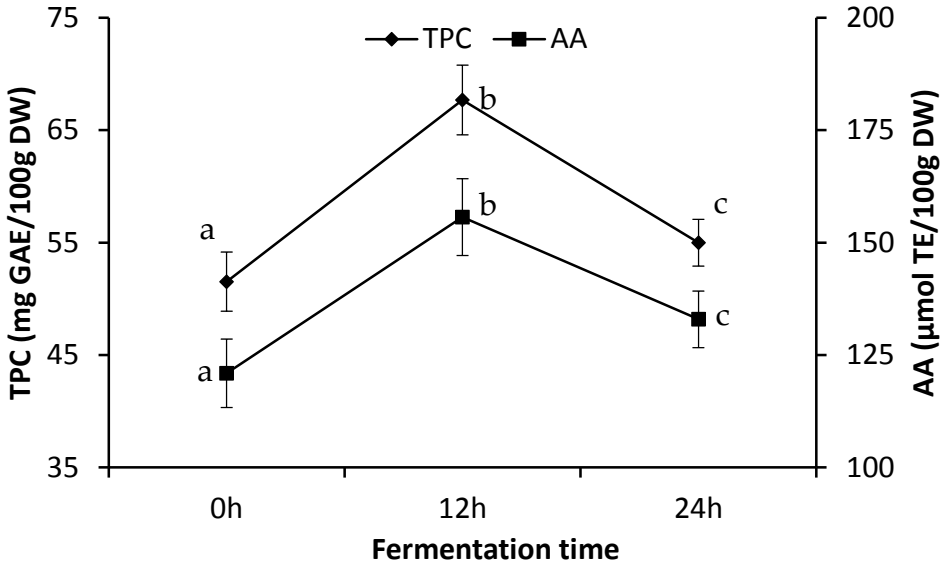
Extrudates	TPC* (mg GAE/ 100g DW)	AA* (µmol TE/100g DW)	Brittleness (mm)*	Hardness (N)*	Crispness (N/mm)*	Expansion ratio*	WAI	WSI* (%)
Control	42.6±0.48	99.5±4.18	4.45±0.16	4.87±0.59	1.88±0.05	1.34±0.03	3.22±0.12	5.88±0.17
Pre-treated	68.3±0.83	145.1±3.92	3.52±0.50	2.11±0.55	0.76±0.07	1.41±0.04	3.05±0.10	15.1±0.59

*mean±SD values in column are significantly different at 5% level of significance, AA: antioxidant activity, TPC: total phenolic content, WAI: water absorption index, WSI: water solubility index

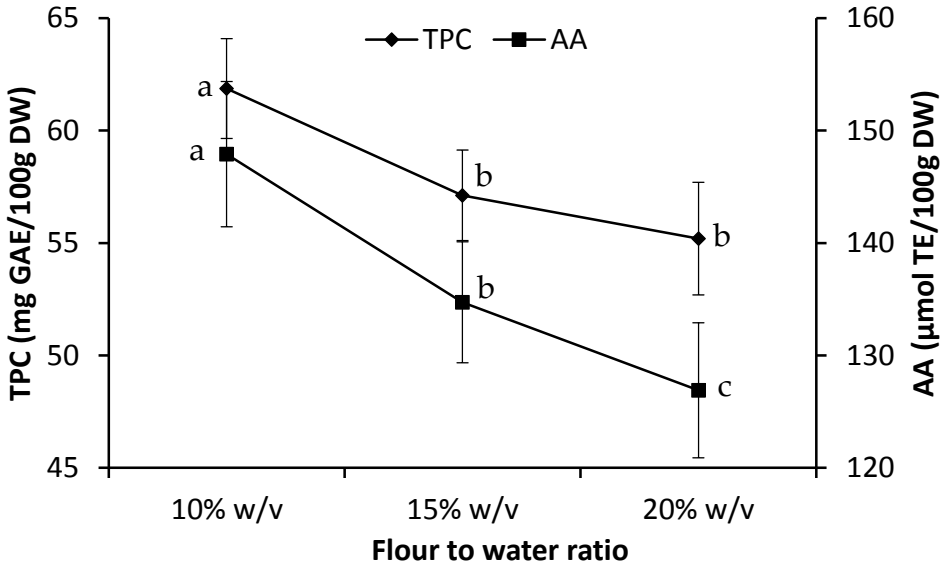
Table 2.4 Thermal properties of control and pretreated sorghum flour and extrudates

Samples	Starch gelatinization	
	Peak temperature (°C)	**Enthalpy (J/g)**
Control flour	69.75±1.19	5.38±0.98
Pre-treated flour	62.50±1.03	2.28±0.63
Control extrudates	56.73±1.27	0.54±0.06
Pre-treated extrudates	ND	ND

ND: Not detected

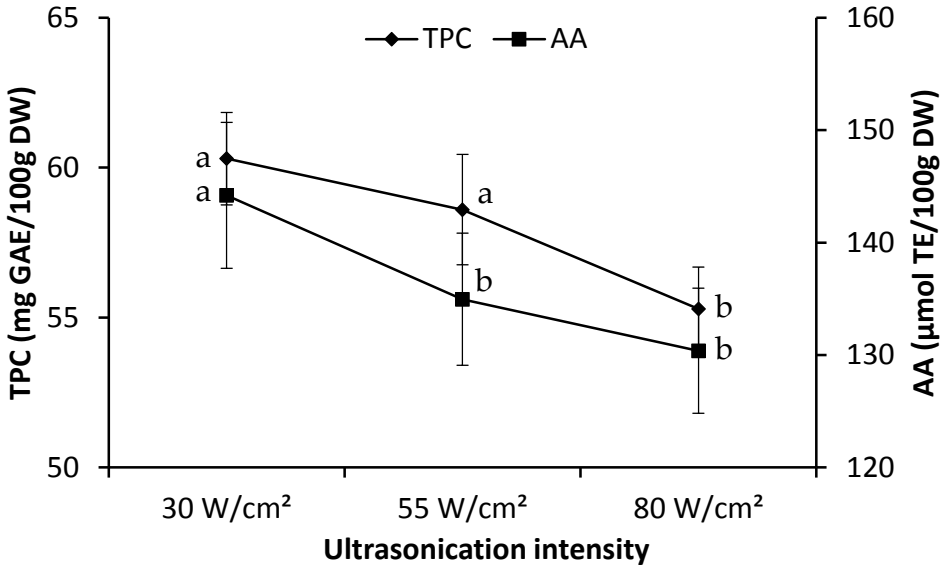


(a)

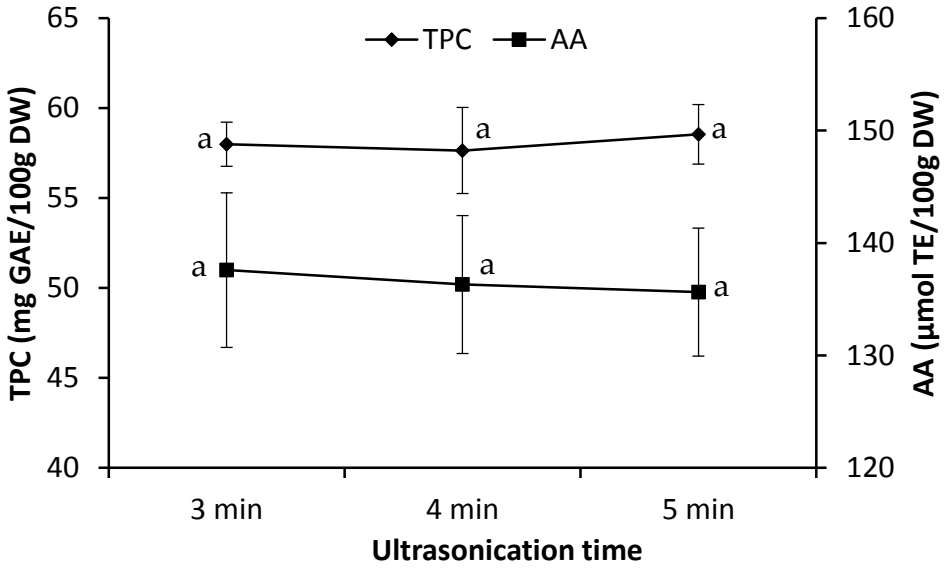


(b)

Figure 2.1 Main effect of fermentation time (a) and flour to water ratio (b) on total phenolic content (TPC) and antioxidant activity (AA) of sorghum flour. Values with the different letters at different points in the same line are significantly ($p < 0.05$) different



(a)



(b)

Figure 2.2 Main effect of ultrasonication intensity (a) and ultrasonication time (b) on total phenolic content (TPC) and antioxidant activity (AA) of sorghum flour. Values with the different letters at different points in the same line are significantly ($p < 0.05$) different

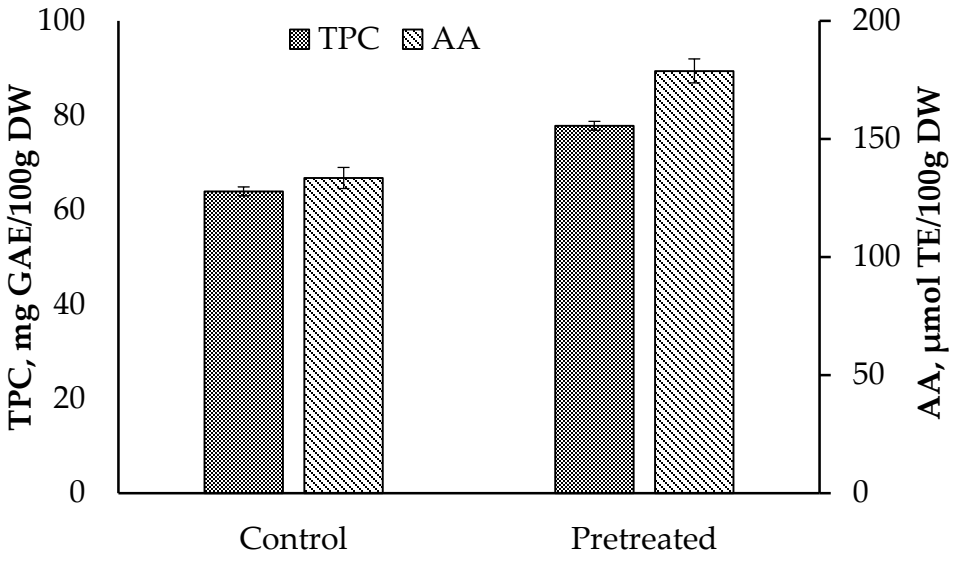
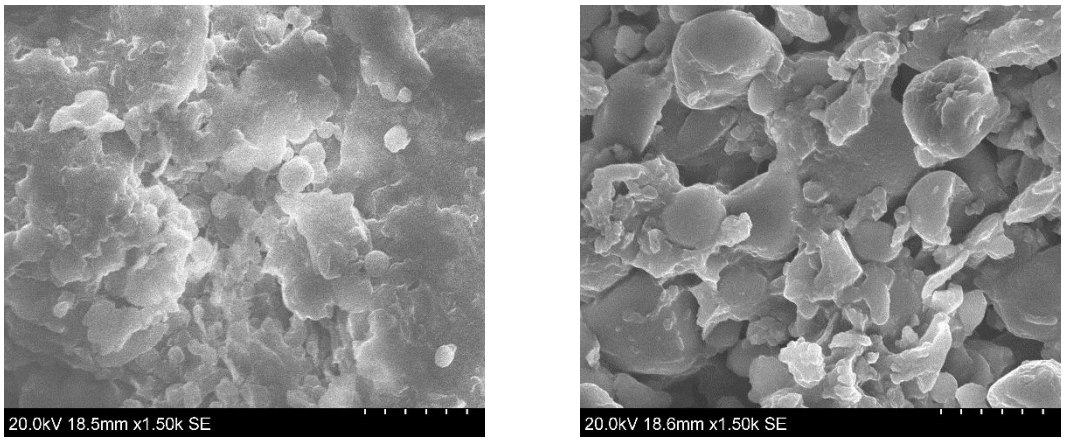


Figure 2.3 Comparison of total phenolic content (TPC) and antioxidant activity (AA) of control sorghum flour with that of pretreated at optimum conditions (12 h fermentation time, 10% (w/v) flour to water ratio, 37 W/cm^2 ultrasonication intensity, and 3 min ultrasonication time)



(a) (b)

Figure 2.4 Scanning electron micrographs (×1500 magnification) of control (a), and pretreated sorghum flour (b) (12 h fermentation time, 10% (w/v) flour to water ratio, 30 W/cm^2 ultrasonication intensity, and 3 min ultrasonication time)

(a) (b)

Figure 2.5 Images of extrudates from (a) control and (b) pretreated sorghum flour

CHAPTER 3

Study of continuous ultrasonication to improve total phenolic content and antioxidant activity in sorghum flour and its comparison with batch ultrasonication[2]

3.1 Abstract

Ultrasonic technology was applied to release the phenolics bound with starch and protein matrix in order to enhance total phenolic content (TPC) and antioxidant activity (AA) of sorghum flour. Both the continuous and batch ultrasonication were implied with independent variables such as flour to water ratio (FWR), ultrasonication intensity (UI), and ultrasonication time (UT) with an additional variable as flow rate (FR) in continuous ultrasonication. All the process variables showed a significant effect on the corresponding ultrasonication process. The Box–Behnken Design provided satisfactory mathematical models which accurately explain the behavior of both the systems; allowing to predict TPC and AA of the sorghum flour. The optimal conditions for continuous ultrasonication were a FWR of 10% w/v, an UI of 20 W/cm^2, a FR of 15 ml/s, and 130 s UT which predicted a maximum values of 70.88 mg GAE/100g DW for TPC and 143.98 µmol TE/100g DW for AA. Regarding batch ultrasonication, the maximum predicted values were 65.61 mg GAE/100g DW and 141.04 µmol TE/100g DW for TPC and AA, respectively at the optimum conditions of 10% w/v FWR, 30 W/cm^2 UI, and 200 s UT. When comparing with the batch ultrasonication, continuous process saved 35% time and 33% of energy consumption to obtain comparatively higher TPC and AA of sorghum

[2] Lohani, U. C. and Muthukumarappan, K. 2016. Study of continuous ultrasonication to improve total phenolic content and antioxidant activity in sorghum flour and its comparison with batch ultrasonication. Ultrasonication Sonochemistry. *Under second review.*

flour. Ultrasonication improved the free phenolic acid content by releasing the bound phenolics in the sorghum flour.

3.2 Introduction

In recent years, the research areas to develop the ready to eat food or snacks with higher antioxidant activity have been demanded and increased. Antioxidant rich foods have potential role to fight against cancer, atherosclerosis, heart disease, cerebrovascular, stroke, Diabetes mellitus rheumatoid arthritis, osteoporosis, ulcers, sunburn, cataracts, and aging (Gülçın et al., 2003). Sorghum is one of the crops that contains phenolic compounds mainly in the forms of phenolic acids and flavonoids (Hahn et al., 1984). These compounds have potentiality to impact positively on human health because of their antioxidant and antiradical properties (Awika and Rooney, 2004). Sorghum utilization can be improved by incorporating it into mainstream human diet in different innovative ways such as extrusion and baking. Most of the phenolic compounds in plant are present in bound forms with carbohydrates, lignin, pectin and proteins (Acosta-Estrada et al., 2014, Ajila et al., 2011). This bound nature of phenolics as glycosides reduces their ability to function as good antioxidants. Therefore, by liberating these bound phenolics using some pretreatments, antioxidants rich sorghum flour can be introduced to human diet.

In past few years, ultrasound assisted extraction of phenolic compounds from pomegranate peel (Pan et al., 2011), mustard (Szydłowska-Czerniak et al., 2015), carrot pomace (Jabbar et al., 2015), and grape pomace (González-Centeno et al., 2015), has been extensively investigated but use of ultrasonication to enhance the phenolics itself in food is limited (Lohani and Muthukumarappan, 2015) and is none in case of sorghum.

The mechanism for ultrasonic is the cavitation of bubbles upon the propagation of the acoustic waves. Collapse of bubbles can produce physical, chemical, and mechanical effects, which results in the disruption of biological cell walls to facilitate the release of extractable compounds and thus increases the total phenolics and antioxidant activity. Ultrasonication separates starch from protein matrix and breaks down these molecules (Zhao et al., 2008) resulting in releasing of bound phenolics with protein and other components.

Even though batch ultrasonication proved to be effective in extracting the phenolics in food materials, it poses considerable difficulty for scale up. In addition to this, the influence of larger sample size and flow rate as additional parameter on phenolic release in continuous ultrasonication would be of interest. Hypothetically, higher sample concentration, less intensity and less time would be required to get the equivalent TPC and AA in sorghum flour. Therefore, based on these rationales, the objectives of the study were to understand the continuous ultrasonication behavior to enhance the TPC and AA in sorghum flour and to compare it with batch ultrasonication process.

3.3 Materials and Methods

Sorghum flour (SF) provided by ADM Milling Co. (Overland Park, KS) was stored at -20°C. For continuous process, ultrasonic device (UIP1000hd, Hielscher Inc., NJ, USA) with 20 kHz frequency, 1000 W power and a sonotrode of 22 mm tip diameter was used. The effective volume of flow cell after intruding sonotrode was 165 ml. For continuous ultrasonication, 100 ml distilled water was added to 10 g, 20 g and 30 g flour to keep the flour to water ratio (FWR) as 10%, 20%, and 30% (w/v). Sorghum slurry was then subjected to ultrasonication at 20, 40, and 60 W/cm^2 ultrasonication intensity (UI)

for 90, 120, and 150 s. Flow rate (FR) of slurry during ultrasonication was varied from 4 ml/s to 30 ml/s. Intensity was determined using calibration curve between amplitude and intensity given in the manual.

Ultrasonic processor (VC 505, Sonics and Materials Inc., CT, USA) with 20 kHz frequency, 500 W power and a horn of 13 mm tip diameter was used for batch ultrasonication. Sorghum flour was batch ultrasonicated for 10%, 15%, and 20% (w/v) FWR at 30, 55, and 80 W/cm^2 intensity for 120, 180, and 240 s ultrasonication time (UT). Intensity was calculated dividing the ultrasonic power (recorded directly) by the emitting surface of the probe. Ultrasonicated samples were oven dried at 40°C till their constant weight and were stored at -20°C for TPC and AA analysis. All the experimental variables were selected for both the ultrasonication processes on the basis of preliminary trials. Sample volumes of 200 ml and 2000 ml were taken for batch and continuous ultrasonication, respectively. The sorghum flour without ultrasonication was taken as control for comparison.

3.3.1 Extraction of samples

The extraction of sample for determining TPC, AA and phenolic characterization was done using the method described by Khan et al. (2013). For determining TPC and AA, 1 g of SF 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°C until analysis for TPC and AA.

Free phenolic acid extraction was performed by adding 10 ml of 80% (v/v) aqueous methanol into 2 g of SF. Mixture was shaken in a shaking water bath for 1 h at

25°C. After centrifugation at 3000×*g* for 20 min, the supernatant was decanted and the extraction was repeated as described above. The two supernatants were combined, evaporated to near dryness and reconstituted with methanol to a final volume of 10 ml.

3.3.1.1 Total phenolic content (TPC)

TPC of SF was determined using Folin–Ciocalteu method (Singleton et al., 1999) with some modification. 50 µl methanol extract of sample was added with 3.5 ml distilled water and 150 µl Folin-Ciocalteu reagent. The solution was vortexed and incubated for 30 min. Thereafter, absorbance of solution was measured at 760 nm against blank. Blank solution contained all the components that were present in the sample except the methanol extract. Gallic acid was used as positive control (standard) and linear regression curve between absorbance and concentration was drawn for the standard. This standard curve was used for calculating the concentration of sample and data was expressed in mg Gallic acid equivalent (GAE)/100 g dry weight (DW). This analysis was done in six replications.

3.3.1.2 Antioxidant activity (AA)

Extinction of DPPH is a free radical scavenging activity which was measured using spectrophotometric method described by Brand-Williams et al. (1995). 2,2-diphenyl-1-picrylhydrazyl (DPPH) 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 for 30 min. Thereafter, 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 weight (DW). Samples were analyzed in six replications.

3.3.1.3 Free phenolic acid characterization

Analysis of sample extracts was carried out using Thermo Scientific, Dionex Ultimate 3000 UHPLC system (Bannockburn, IL, United States) equipped with diode-array detector (DAD) and C_{18} column (150 mm × 4.6 mm) packed with 5 μm particles. The samples were injected with a mobile-phase flow rate of 800 μl/min. Gradient elution was carried out with a solvent system of water/acetic acid (99.8:0.2 v/v) as mobile phase A and acetonitrile/acetic acid (99.8:0.2 v/v) as mobile phase B. The total run time was 12 min, and the gradient elution was as follows: 0.0−3.0 min, B 10-25%; 3.0−4.5 min, B 25−45%; 4.5−6.5 min, B 45−65%; 6.5−8.0 min, B 65−85%; 8.0−9.0 min, B 85−100%.; 9.0−12.0 min, B 100−10%. All the solvents were filtered through 0.22 μm PTFE filters prior to inject. The column was maintained at 30°C while the autosampler was thermostated at 4°C. The system was controlled by Thermo Scientific Dionex Chromeleon 7 software. Benzoic acid and cinnamic acid derivatives were detected at 280 nm and 320 nm, respectively. The concentrations of phenolic acids were calculated from peak areas in comparison to calibration curves of the respective standards and were expressed as μg/g DW.

3.3.2 Moisture content

Moisture content of SF powder was determined by air oven standard methods recommended by AOAC (1980). Initially 5 g of sample in triplicate was dried in hot air oven at 130-133°C for 2 h. After drying, dried sample was again weighed. Following formula is used for calculating the moisture content (MC).

$$\text{MC (\%wb)} = \frac{W_i - W_f}{W_i} \times 100 \qquad (3.1)$$

W_i= initial weight of sample (5 g),

W_f= weight of sample after drying, g

3.3.3 Experimental design

A Box-Behnken design was applied for both the ultrasonication methods to determine the effects and the optimum levels of the above parameters. The effects were studied at three experimental levels –1, 0, and +1. A total of 30 and 17 experiments were required for continuous and batch ultrasonication, respectively as described in Table 3.1 and Table 3.2, available as supplementary data. The experimental data were analyzed by the response surface regression procedure and the parameters obtained from the response surface methodology (RSM) analysis were substituted into the following second-order polynomial model equation.

$$Y_i = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_{ii}^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \qquad (3.2)$$

where Y_i is the predicted response; β_0 is the interception coefficient; β_i, β_{ii}, and β_{ij} are coefficients of the linear, quadratic, and interaction terms; X_i and X_j are the variables; and k is the number of independent parameters (k= 4 and 3 for continuous and batch ultrasonication, respectively).

3.3.4 Statistical analysis

Design expert 9 statistical software package (Stat-Ease Inc., USA) was used to analyze the experimental data. Multiple regression analysis and analysis of variance (ANOVA) were used to evaluate the experimental data. The modeling was started with a quadratic model including linear, squared, interaction terms. Significant terms in the model for each response were found by ANOVA. The adequacy and quality of the models were examined by evaluating the lack of fit (LOF), the coefficient of determination R^2, adjusted R^2, predicted R^2, coefficient of variance and the Fisher test

value (F-value) obtained from the ANOVA. Derringer's desired function methodology was used to generate optimal conditions for continuous (FWR, UI, FR and UT) and batch ultrasonication (FWR, UI and UT) on the TPC and AA of sorghum flour. The TPC and AA were determined under the optimal conditions. To determine the validity of the models, the experimental and predicted values were compared. Correspondence between the predicted (X_{pred}) and the experimental (X_{exp}) values was evaluated by calculating the adequate precision (*AP*) and the absolute average deviation (*AAD*).

$$AP = \frac{X_{pred}^{\max} - X_{pred}^{\min}}{\sqrt{\frac{P - \sigma^2}{n}}} > 4 \tag{3.3}$$

$$AAD = \frac{\sum\left(\frac{\left|X_{\exp} - X_{pred}\right|}{X_{\exp}}\right)}{n} \times 100 \tag{3.4}$$

where n is the number of experimental runs, *P* is the number of significant terms of the mathematical model including the model constant, and σ^2 is the mean square residual value from the ANOVA analysis.

3.4 Results and Discussion

3.4.1 Model fitting

A response surface methodology approach was conducted to determine the effect of continuous and batch ultrasonication on TPC and AA of sorghum flour. In accordance with the experimental design, the observed and predicted response values for TPC and AA from continuous and batch ultrasonication are indicated in Table 3.3 and Table 3.4, respectively.

For continuous ultrasonication, the observed values of TPC and AA were found in range of 52.85-69.92 mg GAE/100 g DW and 91.40-143.31 µmol TE/100 g DW, respectively. The observed values ranged from 36.08 to 66.01 mg GAE/100 g DW for TPC and 89.84 to 140.44 µmol TE/100 g DW for AA in case of batch ultrasonication. According to these experiment ranges, among the 30 process conditions for continuous ultrasonication, the highest TPC and AA were obtained at 10% (w/v) FWR, 20 W/cm^2 UI, 17 ml/s FR, and 90 s UT (Run 1), whereas, for batch ultrasonication, 10% (w/v) FWR, 30 W/cm^2 UI, and 240 s UT (Run 4) were obtained for maximum TPC and AA of sorghum flour.

The regression coefficients of mathematical model analyzed by RSM describing the TPC and AA of sorghum flour as a function of FWR (X_1), UI (X_2), FR (X_3), and UT (X_4) for continuous ultrasonication are depicted in Table 3.5. Similarly, for batch ultrasonication, regression coefficients for TPC and AA as a function of FWR (X_1), UI (X_2), and UT (X_3) are indicated in Table 3.5. The results of the analysis of variance are also summarized to show the significance of the regression coefficients, the goodness of fit, and the adequacy and quality of the models.

It is obvious from Table 3.5 that p-values of lack of fit were not significant ($p>0.05$) for TPC and AA values for both continuous and batch ultrasonication which indicated their suitability to predict the variations within the system. Higher coefficient of determination ($R^2 >0.97$) and lower coefficient of variance ($\leq$2.87%) exhibited goodness of fit for all four mathematical models. The values of adjusted determination coefficient ($R^2_{adj} > 0.96$) were high and confirmed that the model was highly significant. Moreover, the predicted coefficient of determination (R^2_{pred}) value was found to be close to the R^2.

Furthermore, the experimental data showed a good fit with Eq. (1), since all four regression models were statistically significant ($p < 0.05$).

FWR, UI, FR, and UT showed significant ($p<0.05$) linear and quadratic effect on TPC and AA of sorghum flour for continuous ultrasonication (Table 3.5). Also for batch ultrasonication, significant ($p<0.01$) linear effects of FWR, and UT, and linear and quadratic effect of UI on TPC and AA were observed, whereas FWR, and UT had significant ($p<0.05$) quadratic effect only on AA of sorghum flour. Pinelo et al. (2005) also reported the significant linear effect of solvent to solid ratio on TPC and antiradical activity of grape pomace. González-Centeno et al. (2014) found the significant linear effect of ultrasound power on total phenolics and antioxidant capacity of grape pomace. González-Centeno et al. (2014) reported significant linear effect of ultrasonication time on phenolics from grape pomace, whereas, Ghafoor et al. (2009), and Yang et al. (2010) explicated that not only the linear but also the quadratic effect of ultrasonication time were significant on grape seed, and *Citrus aurantium* flower, respectively. In contrast, Tabaraki and Nateghi (2011) did not observe any significant ($p>0.05$) effect of ultrasonication time on TPC and AA of rice bran. For continuous ultrasonication, UI and FR also exhibited the significant ($p<0.01$) interactive effect on TPC.

The values of the regression coefficients presented in Table 3.5 were used in the final predictive model equations after discarding the non-significant terms. Thus, these equations were assumed to best describe the relationships between the experimental variables and the response factors.

3.4.2 Model accuracy

Models were further validated by comparing predicted values with experimental values. In addition to correlation coefficient and coefficient of variance, the accuracy of model was also evaluated by adequate precision (*AP*), absolute average deviation (*AAD*), residuals and influence plots for the experimental data.

AP compares the range of the predicted values at the design points with the average prediction error, indicating adequate model discrimination when values are greater than 4.0. For continuous ultrasonication, *AP* values for TPC and AA were obtained as 31.71 and 131.02, respectively, while for batch ultrasonication, these values were found as 72.32 and 106.85. Therefore, because of a signal to noise ratio greater than 4, the proposed models were able to be used within the experimental conditions investigated.

Furthermore, *AAD* between the predicted and observed data was evaluated for the accuracy of the model, where values ought to be as small as possible. *AAD* values for TPC and AA were calculated as 0.81%, and 0.79%, respectively for continuous ultrasonication, while these values for batch ultrasonication were determined as 1.44%, and 0.56%, respectively. These results also showed that the four proposed models were adequate to explain the outcome of the both ultrasonication process.

Predicted versus actual plots of TPC and AA for both the ultrasonication process are shown in Figure 3.1. The predicted values obtained were quite close to the experimental values, and the points of all predicted and experimental response values fall very close to the 45° line, indicating an adequate agreement between real data and the data obtained from the models. By constructing internally studentized residuals plot,

experimental data were analyzed for satisfactory and good fit of the developed models and it is obvious from the plots that all the data points lay within the limits (Figure 3.2).

3.4.3 Interpretation of response surface model and contour plots

Three-dimensional response surface plots and two-dimensional contour plots were obtained on the basis of the model equations mentioned above to explicate the correlation between independent and dependent variables studied in this study. Both types of plots presented the effects of two independent variables on the response factor, keeping others at level-coded zero.

3.4.3.1 Effect of ultrasonication variables on TPC of sorghum flour

The effect of FWR, UI, and UT on TPC of sorghum flour for continuous and batch ultrasonication is shown in Figure 3.3. With regard to the combined effect by FWR and UI for continuous ultrasonication (Figure 3.3a), maximum TPC was obtained in SF up to 25% (w/v) FWR at low UI (20 W/cm^2), whereas TPC started decreasing after 15% (w/v) FWR during batch ultrasonication at its low UI (30 W/cm^2) (Figure 3.3d). This result was attributed to the early stage agitation instead of cavitation with the effect of UI in batch process when bubble cloud density became too large resulted in rise to shielding effects, coalescence and general bubble-bubble interactions that decreased the overall cavitation efficiency of the process. TPC deceased gradually by 12% with increase in FWR from 10% to 30% (w/v) at higher UI (60 W/cm^2) for continuous process (Figure 3.3a). Similar result was obtained for bath process at higher UI (80 W/cm^2) with increment in FWR from 10% to 20% (w/v) (Figure 3.3d). Although, the maximum TPC was obtained at low ultrasonication intensity with lower concentration of sample for both

the processes, continuous ultrasonication released 8% more TPC in sorghum flour at 33% less UI and for 67% more FWR. These results were in contrast with the findings of Carrera et al. (2012), González-Centeno et al. (2014) and Pan et al. (2011) who observed increase in TPC of grape, grape pomace and pomegranate peel, respectively with increase in ultrasound power. Carrera et al. (2012) and Tabaraki and Nateghi (2011) also reported the reduction or no significant change in TPC of grape and rice bran, respectively with increase in sample concentration in solvent. With increase in FWR, the viscosity of solution increased and because of that the ultrasound energy was not transmitted uniformly to the whole solution at a given ultrasonication intensity. The lower the flour to water ratio, the greater the driving force within the solid resulted in increase of diffusion rate. The main effect of the FWR was to modify the solubility and equilibrium constants and thus increased the TPC to a maximum at the lowest FWR.

The trend observed for TPC of sorghum flour upon simultaneous variation of FWR and UT is exhibited in Figure 3.3b and Figure 3.3e for continuous and batch ultrasonication, respectively. At low FWR (10 w/v), continuous process produced SF with maximum TPC in 110 s, whereas maximum TPC was obtain in 210 s during batch ultrasonication. Stagnant sample in batch process caused the accumulation of acoustic energy near the probe which transmitted slowly in the whole sample resulted in more input of energy. On the other hand, continuous flow of sample allowed the transmittance of acoustic energy efficiently and uniformly in the whole sample. With increase in FWR from 10 to 30% (w/v), TPC of sorghum flour during continuous process depleted by 9% regardless the UT (Figure 3.3b), whereas for batch process, TPC decreased by 19% with increase in FWR from 10 to 20% (w/v) regardless the UT (Figure 3.3e). Even though,

low FWR and higher duration indicated the maximum TPC in sorghum flour for both the processes, continuous ultrasonication provided 20% more TPC at 91% less time with compared to batch ultrasonication. These findings are in agreement with Carrera et al. (2012) and Jabbar et al. (2015) who also reported insignificant change in TPC of grapes and carrot pomace, respectively at longer extraction time.

Finally, the plot of TPC as affected by UI and UT for continuous and batch ultrasonication are shown in Figure 3.3c and 1f, respectively. For continuous process, TPC increased by 3% when UT increased from 90 s to 100 s followed by insignificant ($p>0.05$) change in TPC at low UI (20 W/cm^2) (Figure 3.3c). However, for batch process, UT didn't have any significant ($p>0.05$) effect on TPC at low UI (30 W/cm^2). Having said that, TPC gradually increased by 6% with increase in UT from 120 s to 240 s at higher UI (80 W/cm^2) (Figure 3.3f). Low UI in batch ultrasonication could not provide enough acoustic energy in standstill sample to get transmitted into the whole sample. Both the processes exhibited a declined trend in TPC with increase in UI regardless the UT though the effect of UI on TPC was observed more severe for batch ultrasonication (Figure 3.3c and Figure 3.3f). Though both the processes exhibited the higher TPC in sorghum flour at low UI, continuous ultrasonication released 8% more TPC at 33% less UI consuming 43% less time with compared to the batch process.

The response surface of the effect of FR with FWR, UI and UT for continuous ultrasonication is shown in Figure 3.4. Maximum TPC was obtained at low FWR (10% w/v) (Figure 3.4a) and low UI (20 W/cm^2) (Figure 3.4b) with moderate values of FR ranged from 15-20 ml/s. Figure 3.4c exhibits the combined effect of FR and UT on TPC. As observed, a gradual increase of UT up to 130 s resulted in increased TPC by 10% at a

FR level of 15-17 ml/s, followed by an insignificant change. Lowest flow rate might cause the overheating of sample due to elongation in sample probe contact time for each cycle. On the other hand, sample-probe contact time was too short for each cycle to transmit the acoustic energy to the sample resulted in less cavitation.

3.4.3.2 Effect of ultrasonication variables on AA of sorghum flour

As explicated in response surface plots for AA (Figure 3.5), the ultrasonication variables for continuous and batch process, i.e. FWR, UI and UT affected the response factors in a way similar to that observed for the TPC. These results supported the claims that AA of the plant extracts is associated substantially with their TPC.

AA decreased significantly ($p<0.05$) with increase in FWR and UI for both continuous (Figure 3.5a) and batch (Figure 3.5d) ultrasonication. Both the response surface plots also show that this effect of one variable was regardless of other. Even though maximum AA was obtained at low FWR and UI for both the ultrasonication, continuous process exhibited comparatively 7% more AA in sorghum flour at 33% less UI. Similar results were found by Gribova et al. (Gribova et al., 2008) for AA of bearberry leaves. Tabaraki and Nateghi (2011) also reported no significant change in scavenging activity of DPPH of rice bran at higher liquid to solid ratio.

For continuous ultrasonication, AA of sorghum flour gradually increased by 10% with increase in UT from 90 s to 150 s at higher FWR (30% w/v). This relation was less effective at low FWR (10% w/v) though there was no significant ($p>0.05$) increase in AA at higher level of UT (150 s) (Figure 3.5b). Similar results were observed for batch ultrasonication where maximum AA was found at low FWR when UT increased up to 130 s, followed by no change (Figure 3.5e). These results are in accordance with the

finding of Jabbar et al. (2015) and Pan et al. (2011) who also reported no significant change in antioxidant capacity of carrot pomace and pomegranate peel, respectively at longer extraction time.

Figure 3.5c and Figure 3.5f shows the effect of UI and UT on AA of sorghum flour for continuous and batch ultrasonication, respectively. For both the processes, maximum AA was observed at low UI and higher UT, nevertheless, no significant change in AA was observed after 130 s of ultrasonication time during continuous process (Figure 3.5c). For the batch process, lower UI (30 W/cm^2) favored increase in AA by 8% with increase in UT up to 180 s, followed by no change at all (Figure 3.5f).

It is obvious from Figure 3.5 that AA of sorghum flour ranged from 140-143 µmol TE/100 g DW was observed at 33% and 28% less UI and UT, respectively during continuous ultrasonication as compare to those of batch process. This result was attributed to the amount of TPC presented in the sample.

Three dimensional plots for AA of sorghum flour showing the effect of FR along with FWR, UI and UT in continuous ultrasonication are depicted in Figure 3.4. AA was found maximum for the moderate FR values ranged from 15-20 ml/s at low FWR (10% w/v) and UI (20 W/cm^2) when UT increased up to 130 s, followed by stability in AA data.

3.4.3.3 Optimization of the ultrasonication processes and validation

Optimal process conditions were investigated for continuous and batch ultrasonication and to determine the maximum TPC and AA of sorghum flour using Derringer's desired function methodology. This algorithm varies on a scale of 0–1, where 0 represents a completely undesirable response, and 1 depicts the most desirable one.

Specifically, the global desirability values of 0.98 and 0.97 were observed when optimum conditions were obtained for continuous and batch ultrasonication by maximizing the response factors. Table 3.6 indicates the optimum conditions for both the ultrasonication process along with predicted and experimental TPC and AA values. The predicted results matched well with the experimental results which validated the RSM model, indicating Box-Behnken design could be effectively used to optimize the process parameters for both ultrasonication processes on TPC and AA of sorghum flour.

It is obvious from Table 3.6 that approximately 6% and 2% more TPC and AA, respectively were obtained using continuous ultrasonication as compare to batch process. With compare to control SF, continuous ultrasonicated SF had 11% and 7.9% more TPC and AA, respectively. Furthermore, corresponding to these results, continuous ultrasonication interestingly reduced 33% UI and 35% UT, providing less time and low energy consumption with compare to batch ultrasonication. Pan et al. (2011) reported that maximum phenolic content and antioxidant capacity from pomegranate peel were found at 59.2 W/cm^2 ultrasound intensity during continuous process.

Increase in phenolic content during ultrasonication was due to release of bound phenolics in sorghum flour. Table 3.7 depicts that ultrasonicated sorghum flours had significantly ($p<0.05$) more benzoic acid and cinnamic acid derived phenolic acids than that of control SF. However, *p*-coumaric acid and salicylic acid were not significantly ($p>0.05$) different in control SF and batch ultrasonicated SF. It was also observed that total starch, crude protein and crude fiber of control SF, continuous and batch ultrasonicated SF at optimum conditions varied from 72.36-73.02 g/100g DW, 11.13-

11.89 g/100g DW and 1.23-1.27 g/100g DW. It indicates that ultrasonication didn't have any significant effect on starch, protein and fiber.

3.5 Conclusions

In the present study, continuous and batch ultrasonication were used to release the bound phenolics resulted in enhanced TPC and AA in sorghum flour. Although the maximum TPC and AA was obtained at low ultrasonication intensity with lower concentration of sample for both the processes, continuous ultrasonication released 8% and 7% more TPC and AA, respectively in sorghum flour at 33% less UI and for 67% more FWR. Furthermore both the processes exhibited the higher TPC and AA in sorghum flour at low UI, continuous ultrasonication released 8% more TPC and equivalent AA at 33% less UI consuming 43% and 27% less time, respectively with compared to the batch process. As far as flow rate was concerned for continuous process, moderate flow rate provided the maximum TPC and AA in sorghum flour. RSM was used to optimize the ultrasonication variables, i.e. flour to water ratio, ultrasonication intensity, and ultrasonication time for batch process and additionally flow rate as a variable in continuous ultrasonication. Developed models for both the ultrasonication processes were adequate and precise with the experimental data. At optimum condition, continuous ultrasonicated SF had 11% and 7.9% more TPC and AA, respectively with compare to control SF. Phenolic characterization revealed that salicylic acid followed by ferulic, hydroxybenzoic and caffeic acids mainly contributed to the TPC of SF. Although, continuous ultrasonication is in the pipeline of those technologies which are prone to be commercialized, it saves time and reduces energy consumption with compare to batch process to enhance the phenolic in sorghum flour. Feasibility of the process at industrial

scale can be enhanced by including some pretreatment methods, i.e. fermentation, malting prior to ultrasonication to improve the extraction of phenolics.

Table 3.1 Box-Behnken experimental design for continuous ultrasonication

Run	FWR	UI	FR	UT	Run	FWR	UI	FR	UT
1	-1	-1	0	0	16	0	0	+1	+1
2	+1	0	0	-1	17	0	0	-1	+1
3	+1	0	+1	0	18	0	0	0	0
4	0	+1	0	-1	19	0	+1	0	+1
5	-1	0	0	-1	20	0	-1	-1	0
6	0	0	+1	-1	21	+1	0	0	+1
7	+1	0	-1	0	22	-1	+1	0	0
8	0	0	0	0	23	0	-1	0	-1
9	-1	0	0	+1	24	0	-1	+1	0
10	-1	0	+1	0	25	+1	+1	0	0
11	0	0	0	0	26	0	+1	-1	0
12	-1	0	-1	0	27	+1	-1	0	0
13	0	0	0	0	28	0	-1	0	+1
14	0	0	-1	-1	29	0	0	0	0
15	0	+1	+1	0	30	0	0	0	0

Variables	**Levels**		
	-1	**0**	**+1**
Flour to water ratio (FWR, %w/v)	10	20	30
Ultrasonication intensity (UI, W/cm^2)	20	40	60
Flow rate (FR, ml/s)	4	17	30
Ultrasonication time (UT, s)	90	120	150

Table 3.2 Box-Behnken experimental design for batch ultrasonication

Run	FWR	UI	UT	Run	FWR	UI	UT
1	-1	0	-1	10	+1	-1	0
2	0	0	0	11	+1	0	-1
3	0	-1	+1	12	+1	0	+1
4	-1	-1	0	13	0	+1	-1
5	0	+1	+1	14	0	0	0
6	0	0	0	15	+1	+1	0
7	0	0	0	16	-1	+1	0
8	-1	0	+1	17	0	0	0
9	0	-1	-1				

Variables	Levels		
	-1	0	+1
Flour to water ratio (FWR, %w/v)	10	15	20
Ultrasonication intensity (UI, W/cm^2)	30	55	80
Ultrasonication time (UT, s)	120	180	240

Table 3.3 Observed and predicted values of total phenolic content (TPC) and antioxidant activity (AA) of sorghum flour for continuous ultrasonication

Exp run	TPC (mg GAE/100g DW)		AA (µmol TE/100g DW)		Exp run	TPC (mg GAE/100 g DW)		AA (µmol TE/100 g DW)	
	Observed	Predicted	Observed	Predicted		Observed	Predicted	Observed	Predicted
1	69.92±1.79	69.76	143.31±4.12	143.08	16	59.38±1.67	59.87	111.31±4.21	110.66
2	54.74±0.81	55.56	93.01±4.18	94.14	17	60.63±1.22	61.07	115.01±4.77	114.59
3	55.05±0.79	55.06	95.47±4.57	95.81	18	64.49±0.87	65.36	117.71±4.09	113.47
4	52.85±1.78	53.69	92.55±4.01	92.92	19	59.04±0.96	58.91	104.78±4.32	104.41
5	61.18±0.82	61.52	118.52±4.68	118.46	20	65.58±1.23	66.45	127.41±3.92	128.67
6	55.48±1.21	55.25	99.04±4.34	98.09	21	57.13±1.47	58.44	104.33±4.00	105.90
7	56.85±1.44	56.70	101.59±4.88	101.28	22	60.95±1.88	61.06	114.64±4.39	114.85
8	64.21±1.21	65.36	113.30±4.11	113.47	23	63.31±0.91	63.38	121.11±4.44	121.34
9	66.21±0.99	67.02	129.67±4.27	130.05	24	59.41±0.83	61.23	122.10±4.29	122.94
10	62.43±0.97	62.35	120.52±4.09	120.70	25	53.47±1.17	53.83	91.40±4.17	90.24
11	67.14±1.01	65.36	112.74±3.98	113.47	26	54.38±1.54	54.20	98.48±4.22	99.15
12	63.87±1.19	63.95	125.35±3.94	124.87	27	62.36±1.38	62.45	120.82±4.68	119.23
13	65.30±0.88	65.36	111.14±4.57	113.47	28	67.45±1.19	66.55	133.71±4.71	133.21
14	57.57±1.57	57.30	104.52±4.31	103.80	29	65.60±1.50	65.36	110.90±3.96	113.47
15	55.40±1.11	56.16	94.99±4.13	95.24	30	65.68±1.63	65.36	115.05±4.08	113.47

Response experimental results are reported as mean ± standard deviation (n= 6)

Table 3.4 Observed and predicted values of total phenolic content (TPC) and antioxidant activity (AA) of sorghum flour for batch ultrasonication

Exp run	TPC (mg GAE/100g DW)		AA (µmol TE/100g DW)	
	Observed	**Predicted**	**Observed**	**Predicted**
1	50.14±1.02	49.39	115.87±4.11	115.89
2	49.63±1.47	47.22	123.16±4.54	120.38
3	61.83±1.09	61.67	130.39±4.28	130.14
4	66.01±1.74	66.54	140.44±3.99	140.24
5	41.64±1.08	41.42	101.37±3.97	101.16
6	45.43±1.33	47.22	122.22±4.08	120.38
7	46.22±1.51	47.22	117.86±4.18	120.38
8	55.75±1.04	55.35	126.82±4.31	126.85
9	55.92±1.21	56.12	119.47±4.22	119.48
10	57.32±1.87	56.72	119.41±4.07	119.47
11	39.04±1.44	39.42	95.02±3.98	94.78
12	43.46±1.11	44.20	105.64±4.29	105.42
13	36.08±1.03	36.23	90.18±4.19	90.23
14	46.82±1.19	47.22	120.89±4.27	120.38
15	36.46±1.38	35.91	89.84±4.31	89.86
16	46.63±1.24	47.22	111.87±4.09	111.63
17	48.02±1.64	47.22	118.22±4.15	120.38
Control	**63.91±0.97**		**133.48±3.19**	

Response experimental results are reported as mean ± standard deviation (n= 6)

Table 3.5 Regression coefficients and statistical parameters describing the effect of the independent variables on total phenolic content (TPC) and antioxidant activity (AA) of sorghum flour for continuous and batch ultrasonication

Model term	Regression coefficient estimated				
	Continuous ultrasonication			Batch ultrasonication	
	TPC	AA		TPC	AA
β_0	10.4189***	112.4380***	β_0	85.1256***	65.9398***
β_1	0.6126***	-1.6573***	β_1	-2.2813***	0.7879***
β_2	-0.0787***	-1.1287***	β_2	-0.8877***	-0.0708***
β_3	0.4140***	0.4782***	β_3	0.2061***	0.8260***
β_4	0.8529***	0.8739***	β_{11}	0.0522*	-0.0918**
β_{11}	-0.0179***	0.0127*	β_{22}	0.0049***	-0.0045**
β_{22}	-0.0045***	0.0053***	β_{33}	-0.0004*	-0.0020***
β_{33}	-0.0240***	-0.0241***	β_{12}	-0.0030	-0.0020
β_{44}	-0.0033***	-0.0029***	β_{13}	-0.0001	-0.0003
β_{12}	0.0001	-0.0009	β_{23}	-0.00006	0.00004
β_{13}	-0.0007	-0.0025			
β_{14}	-0.0022	0.0001			
β_{23}	0.0069***	0.0017			
β_{24}	0.0008	-0.0001			
β_{34}	0.0005	0.0011			
Adequacy of mathematical model					
p (Lack of fit)	0.6783	0.9834		0.7934	0.9983
R^2	0.9805	0.9906		0.9882	0.9929
R^2_{adj}	0.9623	0.9818		0.9730	0.9837
R^2_{perd}	0.9216	0.9745		0.9462	0.9881
CV (%)	1.51	1.58		2.87	1.56
p (F value)	0.000	0.000		0.000	0.000

Significant at *p<0.1, **p<0.05, ***p<.0.01, adj: adjusted, pred: predicted, CV: coefficient of Variance

Table 3.6 Estimated optimum conditions, predicted and experimental values of responses for continuous and batch ultrasonication

Ultrasonication	Optimum conditions				Response variables			
	FWR (%w/v)	UI (W/cm^2)	FR (ml/s)	UT (s)	TPC, mg GAE/100 g DW		AA, µmol TE/100 g DW	
					Predicted	Actual	Predicted	Actual
Continuous	10	20	15	130	71.03	70.88±1.79	144.67	143.98±3.58
Batch	10	30	-	200	66.37	65.61±1.45	141.74	141.04±3.23
Control						63.91±0.97		133.48±3.19

Response experimental results are reported as mean ± standard deviation (n= 6), AA: antioxidant activity, FR: flow rate, FWR: flour to water ratio, TPC: total phenolic content, UI: ultrasonication intensity, UT: ultrasonication time

Table 3.7 Phenolic profile of continuous and batch ultrasonicated sorghum flours (SF) (μg/g DW)

Compounds	Control SF	Continuous ultrasonicated SF	Batch ultrasonicated SF
Benzoic acids			
Protocatechuic acid	6.18±0.11[a]	7.11±0.12[b]	6.74±0.14[c]
p-Hydroxybenzoic acid	13.3±0.22[a]	14.8±0.29[b]	13.9±0.21[c]
Cinnamic acids			
Caffeic acid	10.2±0.19[a]	13.5±0.17[b]	12.6±0.23[c]
p-coumaric acid	4.87±0.13[a]	5.53±0.11[b]	4.94±0.11[a]
Ferulic acid	13.4±0.28[a]	16.7±0.21[b]	14.9±0.19[c]
Salicylic acid	22.8±0.20[a]	24.5±0.18[b]	22.5±0.15[a]

Means±SD in the same row with different letters are significantly different ($p<0.05$)

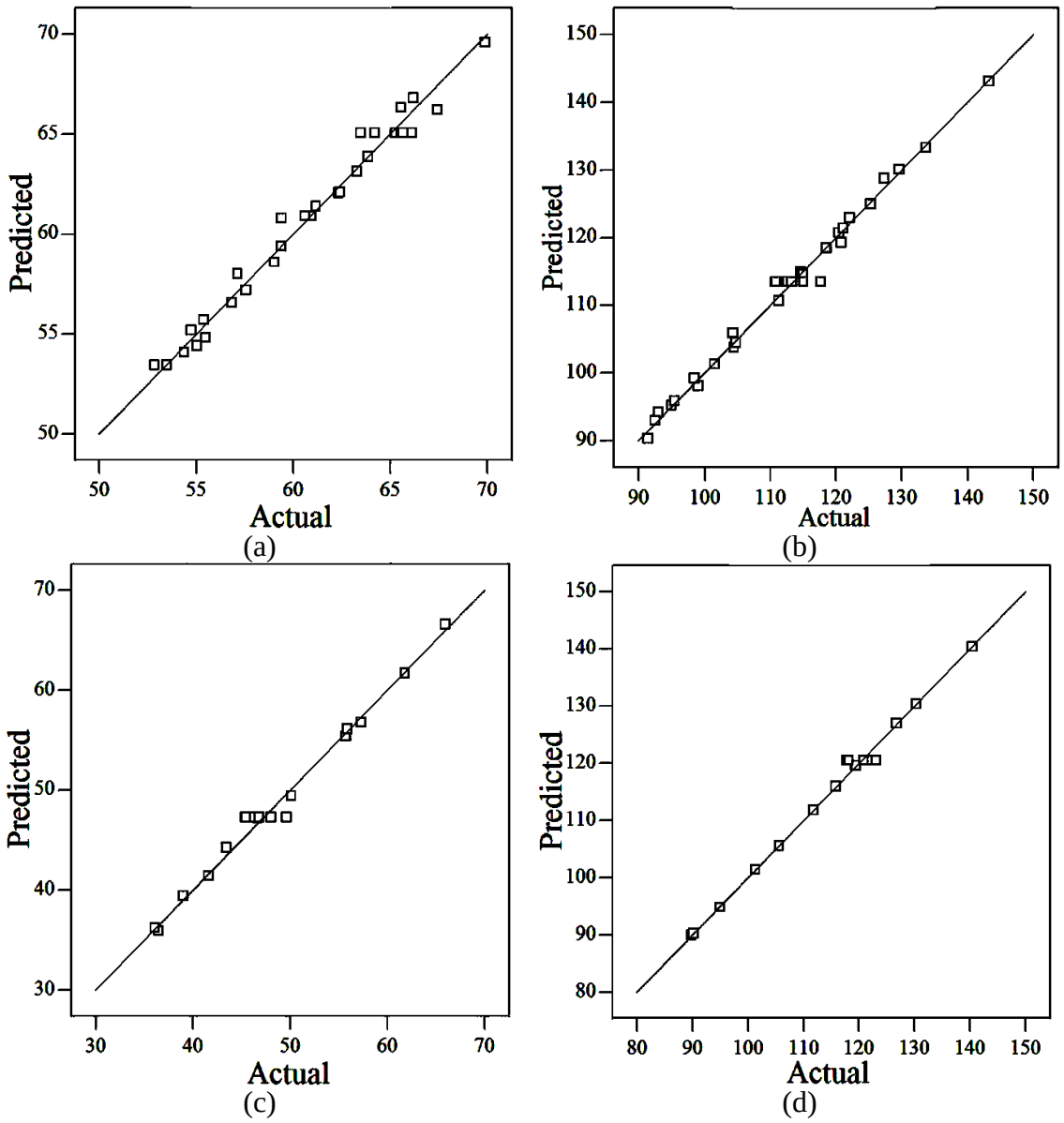


Figure 3.1 Predicted vs actual data plot of a) total phenolic content (TPC), b) antioxidant activity (AA) for continuous ultrasonication and c) TPC, d) AA for batch ultrasonication

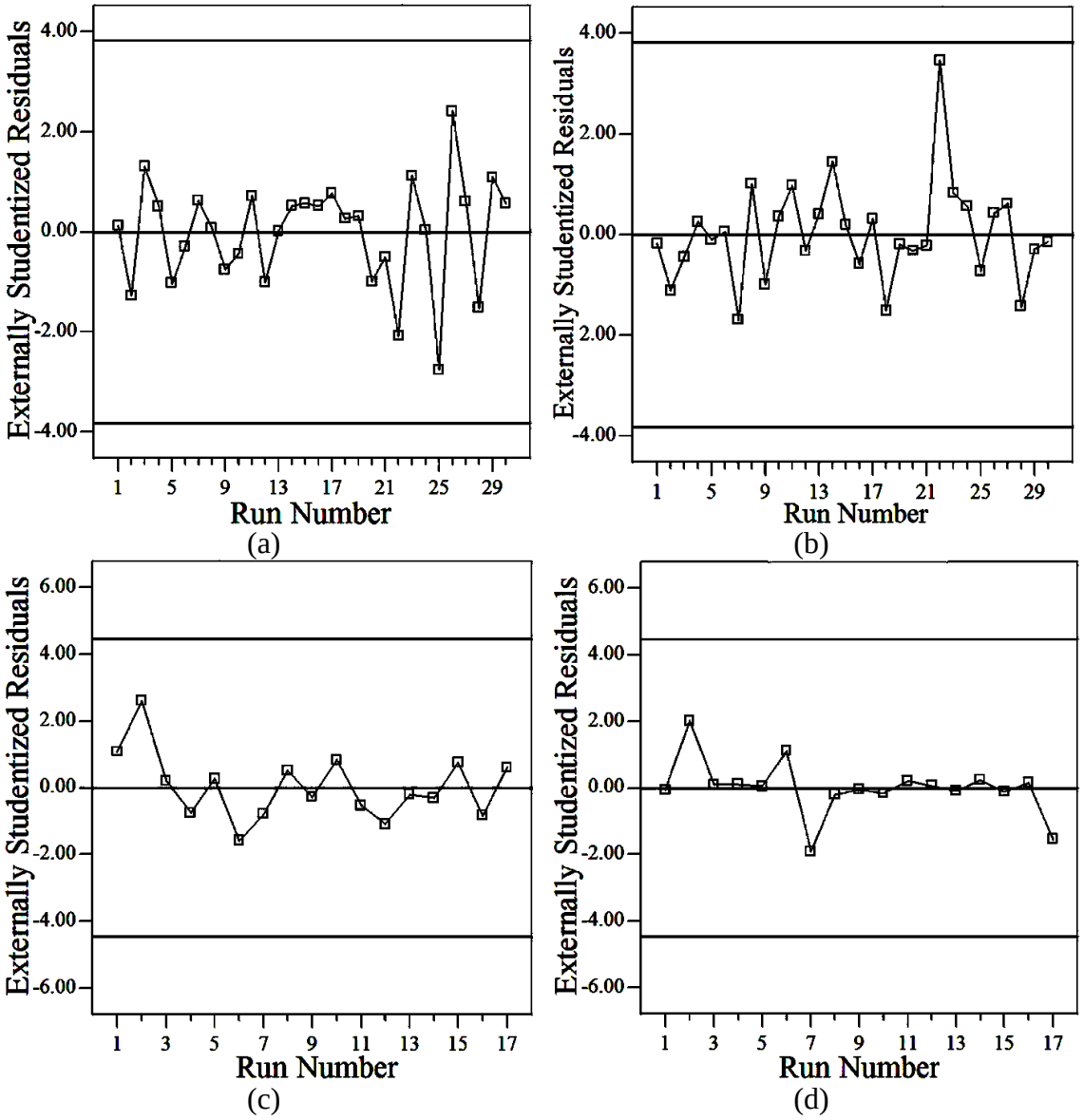


Figure 3.2 Diagnostic plots of a) total phenolic content (TPC), b) antioxidant activity (AA) for continuous ultrasonication and c) TPC, d) AA for batch ultrasonication

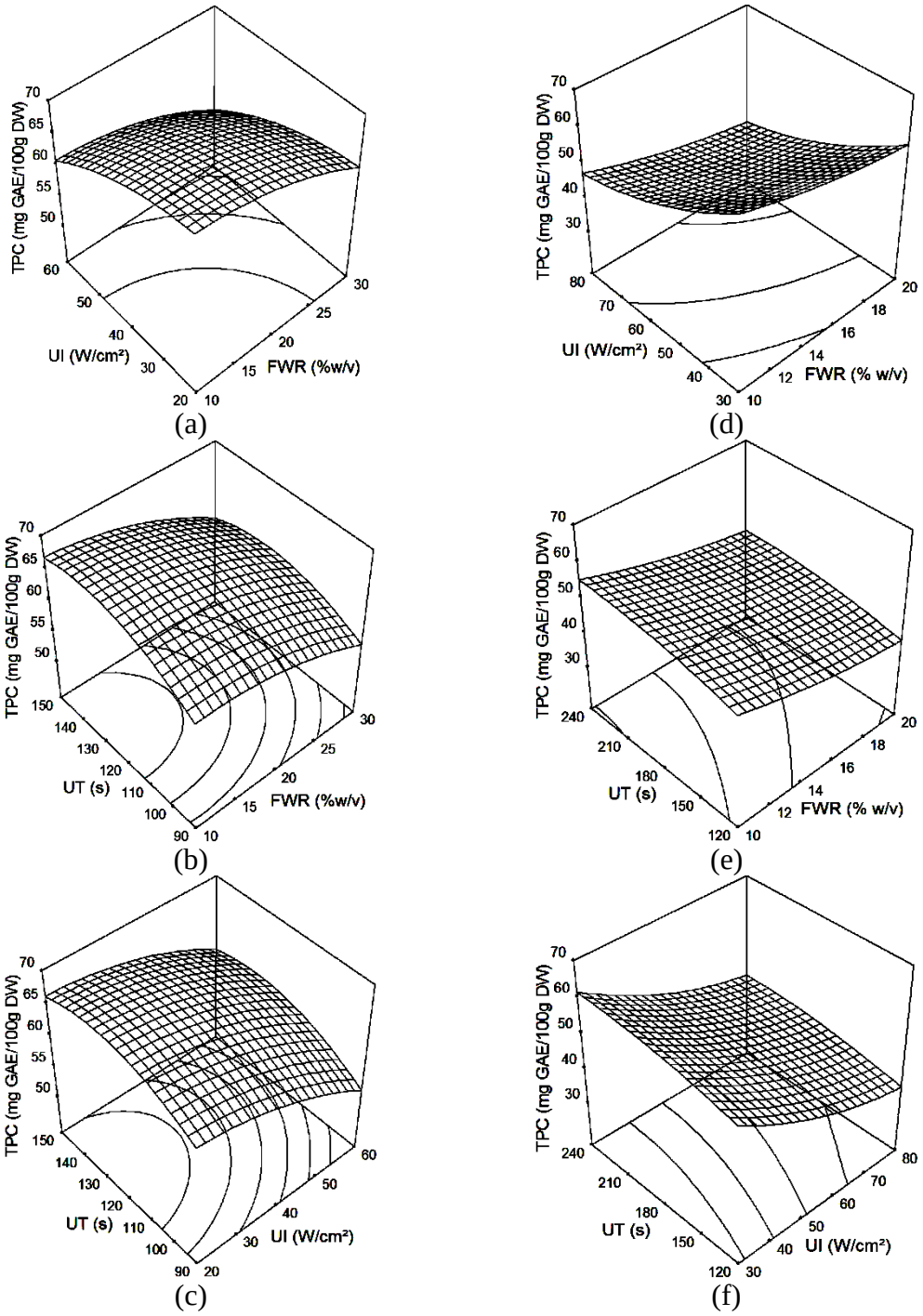


Figure 3.3 Response surface plots of total phenolic content (TPC) of sorghum flour as affected by flour to water ratio (FWR), ultrasonication intensity (UI), and ultrasonication time (UT) for (a, b, c) continuous ultrasonication and (d, e, f) batch ultrasonication at 0 level of corresponding third variable

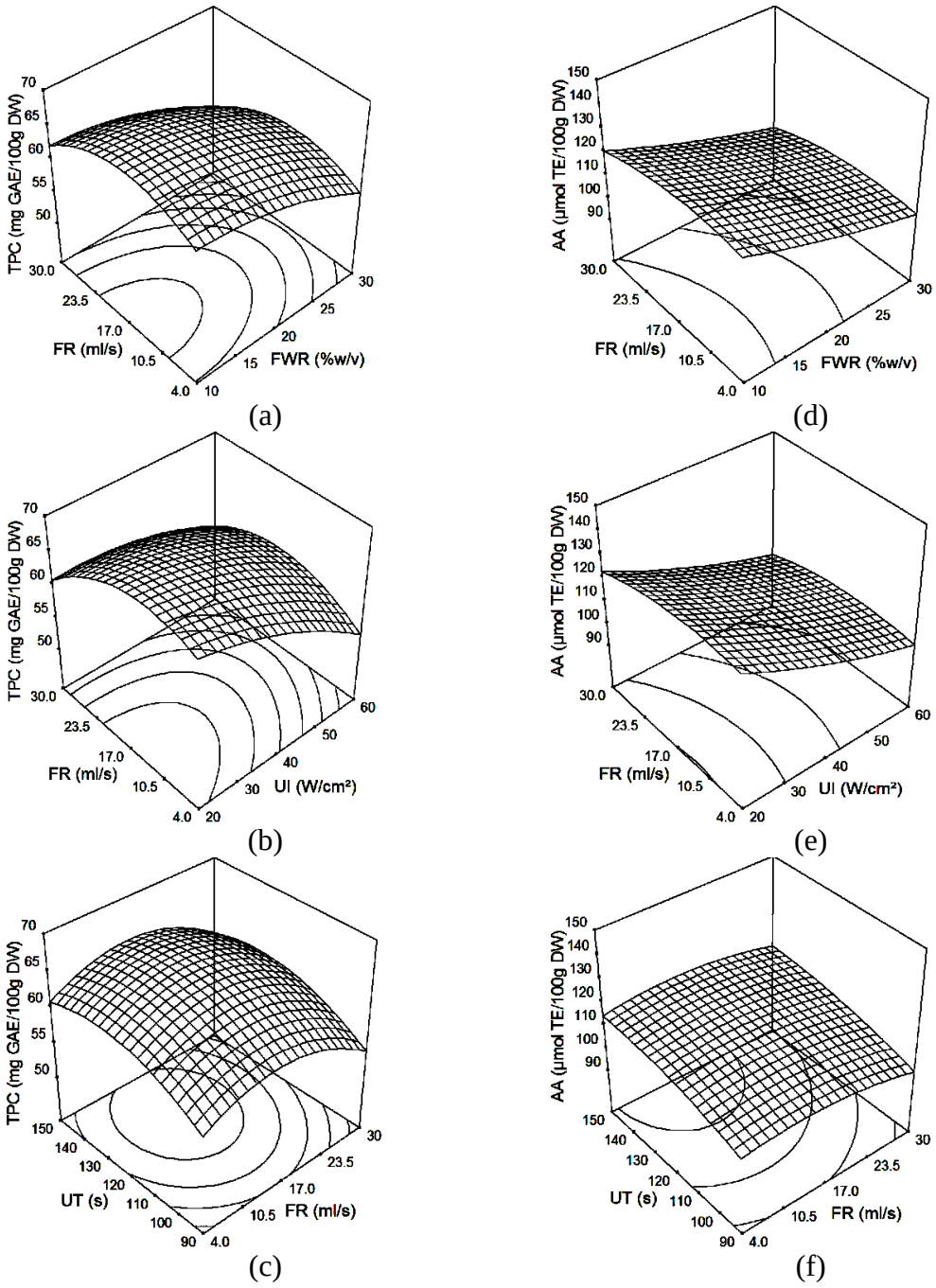


Figure 3.4 Resp**o**nse surface plots of total phenolic content (TPC) (a, b, c) and antioxidant activity (AA) (d, e, f) of sorghum flour as affected by combination of flour to water ratio (FWR), ultrasonication intensity (UI), and ultrasonication time (UT) with flow rate (FR) for continuous ultrasonication at 0 level of corresponding third variable

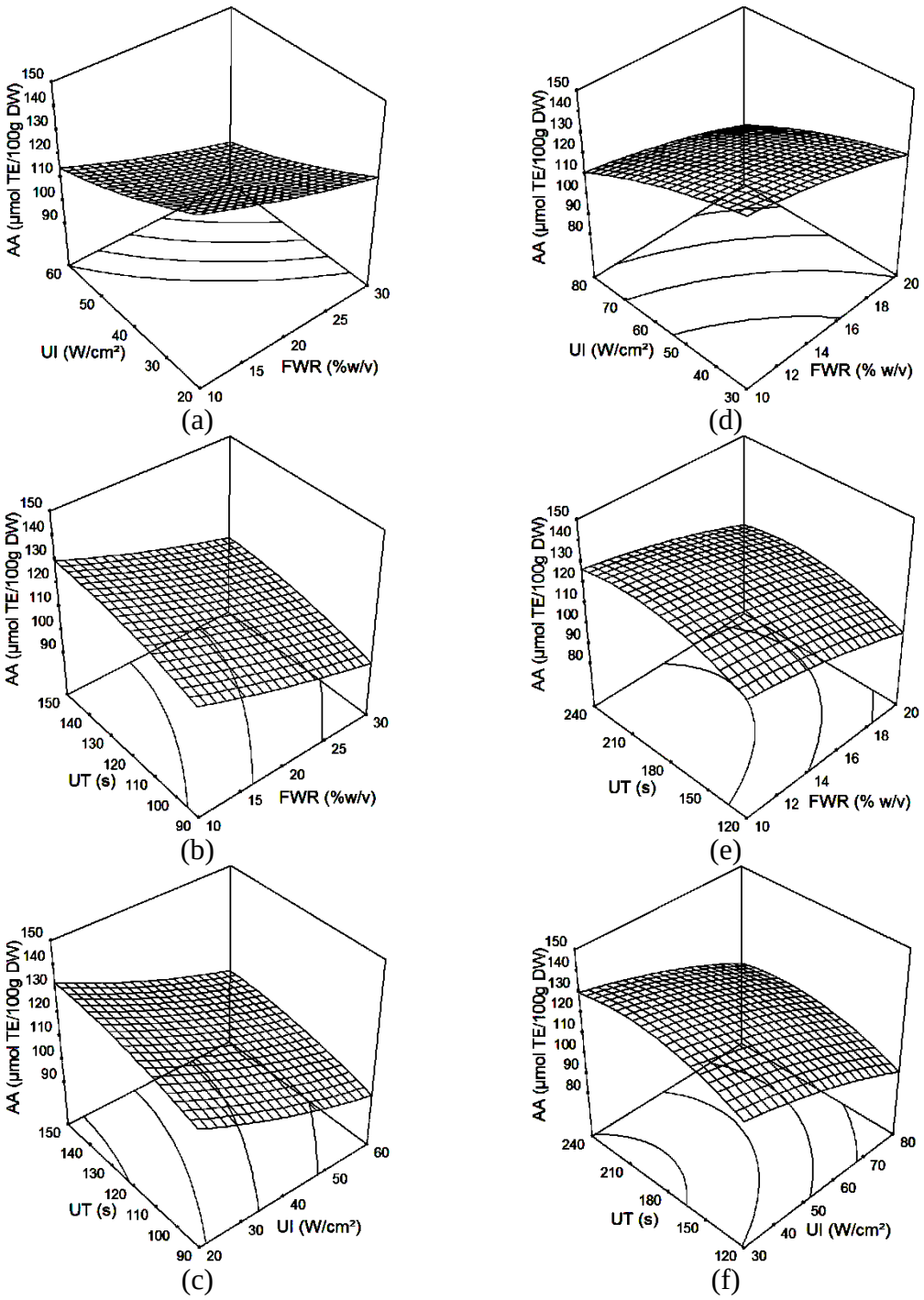


Figure 3.5 Response surface plots of antioxidant activity (AA) of sorghum flour as affected by flour to water ratio (FWR), ultrasonication intensity (UI), and ultrasonication time (UT) for (a, b, c) continuous ultrasonication and (d, e, f) batch ultrasonication at 0 level of corresponding third variable

CHAPTER 4

Modelling of continuous ultrasonication to improve total phenolic content and antioxidant activity in sorghum flour: A comparison between response surface methodology and artificial neural network[3]

4.1 Abstract

Fermentation followed by continuous ultrasonication was applied to release the bound phenolics and in turn to enhance the total phenolic content (TPC) and antioxidant activity (AA) of sorghum flour (SF). TPC and AA increased with decrease in fermentation time (FT) and flour to water ratio (FWR). Furthermore, by decreasing flow rate (FR) and ultrasonication intensity (UI), TPC and AA were increased in SF. The present study is to evaluate and compare the prediction efficiencies of response surface methodology (RSM) and artificial neural network (ANN) based models on TPC and AA. The influence of process variables was investigated by Box–Behnken design and multi-layer perceptron neural network with the topology of 5-8-1. The optimum conditions for maximum TPC and AA were obtained as 12 h FT, 10% (w/v) FWR, 20 W/cm^2 UI, 4 ml/s FR and 120 s UT. The values observed for TPC and AA at optimum conditions were 90.1 mg GAE/100g DW and 190.1 µmol TE/100g DW, respectively, while these values for control SF were observed as 63.9 mg GAE/100g DW and 133.5 µmol TE/100g DW. Based on the validation data set, RSM and ANN were statistically compared by root mean squared error, coefficient of determination (R^2) and absolute average deviation. For RSM and ANN models, R^2 were found as 0.950 and 0.996, respectively for TPC, while

[3] Lohani, U. C. and Muthukumarappan, K. 2016. Modelling of continuous ultrasonication to improve total phenolic content and antioxidant activity in sorghum flour: A comparison between response surface methodology and artificial neural network. International Journal of Food Engineering. *Under Review*.

for AA, the values were 0.989 and 0.998, respectively. Both models showed good predictions in this study, nevertheless the ANN model was more precise compared to the RSM model. All process variables showed the significant effect on responses.

4.2 Introduction

In recent years, different cereals have been identified and accepted as functional foods and nutraceuticals because of good sources of dietary fibers, proteins, minerals, vitamins, and antioxidants required for human health (Charalampopoulos et al., 2002). Sorghum is one of the crops that contains phenolic compounds mainly in the forms of phenolic acids and flavonoids (Hahn et al., 1984). These compounds have potentiality to impact positively on human health because of their antioxidant and antiradical properties (Awika and Rooney, 2004). Sorghum utilization can be improved by incorporating it into mainstream human diet in different innovative ways such as extrusion and baking. Most of the phenolic compounds in plant are present in bound form with carbohydrates, lignin, pectin and proteins (Acosta-Estrada et al., 2014, Ajila et al., 2011). This bound nature of phenolics as glycosides reduces their ability to function as good antioxidants. Therefore, by liberating these bound phenolics using some pretreatments, antioxidants rich sorghum flour can be introduced to human diet.

In past decade, fermentation has been used for improving the antioxidant properties in legumes (Oboh et al., 2009) and rice bran (Hegde et al., 2006). During microbial fermentation, polyphenol oxidase activity degrades the phenolics in cereals, and also acidic environment during fermentation causes the abstraction of hydride ions and rearrangement of phenolic structures (Towo et al., 2006). These results were supported by our preliminary trials where sorghum flour inoculated with pure culture of

Lactobacillus plantarum showed less phenolic content with compared to that of naturally fermented. Therefore, natural fermentation was opted in this study as a pretreatment of sorghum flour prior to ultrasonication.

In past few years, ultrasound assisted extractions of phenolic compounds from pomegranate peel (Pan et al., 2011), mangosteen (Cheok et al., 2012, Cheok et al., 2013), mustard (Szydłowska-Czerniak et al., 2015), carrot pomace (Jabbar et al., 2015), and grape pomace (González-Centeno et al., 2015), have been extensively investigated but use of ultrasonication to enhance the phenolics itself in food is limited (Lohani and Muthukumarappan, 2015) and is none in case of sorghum. The mechanism for ultrasonic is the cavitation of bubbles upon the propagation of the acoustic waves. Collapse of bubbles can produce physical, chemical, and mechanical effects, which results in the disruption of biological cell walls to facilitate the release of extractable compounds and thus increases the total phenolics and antioxidant activity. Ultrasonication separates starch from protein matrix and breaks down these molecules (Zhao et al., 2008) resulting in releasing of phenolics bound with protein and other components.

RSM is an empirical modeling system applied for developing, improving, and optimizing complex processes. For decades, it has been using in various research areas including ultrasonication with satisfactory outcomes (González-Centeno et al., 2014, Pinelo et al., 2005, Prakash Maran et al., 2013, Tabaraki and Nateghi, 2011, Yang et al., 2010). It has the advantage of reducing the number of experimental runs, which is sufficient to provide statistically acceptable results. On the other hand, in the past few years, ANN also has been used as a most popular artificial learning tool with a wide applications range included ultrasonication (Maran and Priya, 2015, Milić et al., 2013,

Tao et al., 2014, Toboc and Lavric, 2012). Betiku et al. (2014), Maran and Priya (2015), and Milić et al. (2013) showed that ANN gave better results than RSM both in terms of data fitting and predictive ability.

Continuous ultrasonication is in the pipeline of those technologies which are prone to be commercialized. For scaling up, the laboratory process should be optimized and validated for the developed model. Therefore, the present work on continuous ultrasonication is focused on modeling the total phenolic content (TPC) and antioxidant activity (AA) of sorghum flour using two modeling tools (Artificial neural network, ANN and Response surface methodology, RSM).

Therefore, keeping in view the above facts, the objectives of the study were

1) To observe the effect of continuous ultrasonication variables on TPC and AA of sorghum flour
2) To optimize the fermentation time, flour to water ratio (FWR), ultrasonication intensity (UI), flow rate (FR) and ultrasonication time (UT) for TPC and AA of sorghum flour
3) To develop the artificial neural network model for prediction capabilities on TPC and AA of sorghum flour
4) To compare the results predicted by ANN with those of RSM technique

4.3 Materials and Methods

Sorghum flour provided by ADM Milling Co. (Overland Park, KS) was stored at -20°C before experimental utilization. For natural fermentation, sorghum sample was prepared by adding 45 g flour in 100 ml distilled water. The sample was fermented (in the presence of lactic acid bacteria) in a controlled environment with temperature 30±1°C for 12 h, 24 h, and 36 h fermentation time (FT). Fermented slurry was further diluted to

keep the flour to water ratio (FWR) as 10 %, 15 %, and 20 % (w/v). Diluted slurry (2000 ml) was then subjected to continuous ultrasonication (UIP1000hd, Hielscher Inc., NJ, USA) at 20, 25, and 30 W/cm^2 ultrasonication intensity (UI) for 120, 180, and 240 s ultrasonication time (UT). Flow rate (FR) of slurry during ultrasonication was varied from 4 ml/s to 17 ml/s. Ultrasonicated samples were oven dried at 40°C till their constant weight and were stored at -20°C for TPC and AA analysis.

4.3.1 Extraction of samples

The extraction of sample for determining TPC, AA and phenolic characterization was done using the method described by Khan et al. (2013). For determining TPC and AA, 1 g of SF 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°C until analysis for TPC and AA.

Free phenolic acid extraction was performed by adding 10 ml of 80% (v/v) aqueous methanol into 2 g of SF. Mixture was shaken in a shaking water bath for 1 h at 25°C. After centrifugation at 3000×*g* for 20 min, the supernatant was decanted and the extraction was repeated as described above. The two supernatants were combined, evaporated to near dryness and reconstituted with methanol to a final volume of 10 ml.

4.3.1.1 Total phenolic content (TPC)

TPC of SF was determined using Folin–Ciocalteu method (Singleton et al., 1999) with some modification. 50 µl methanol extract of sample was added with 3.5 ml distilled water and 150 µl Folin-Ciocalteu reagent. The solution was vortexed and incubated for 30 min. Thereafter, absorbance of solution was measured at 760 nm against blank. Blank

solution contained all the components that were present in the sample except the methanol extract. Gallic acid was used as positive control (standard) and linear regression curve between absorbance and concentration was drawn for the standard. This standard curve was used for calculating the concentration of sample and data was expressed in mg Gallic acid equivalent (GAE)/100 g dry weight (DW). This analysis was done in six replications.

4.3.1.2 Antioxidant activity (AA)

Extinction of DPPH is a free radical scavenging activity which was measured using spectrophotometric method described by Brand-Williams et al. (1995). 2,2-diphenyl-1-picrylhydrazyl (DPPH) 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 for 30 min. Thereafter, 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 weight (DW). Samples were analyzed in six replications.

4.3.1.3 Free phenolic acid characterization

Analysis of sample extracts was carried out using Thermo Scientific, Dionex Ultimate 3000 UHPLC system (Bannockburn, IL, United States) equipped with diode-array detector (DAD) and C_{18} column (150 mm × 4.6 mm) packed with 5 µm particles. The samples were injected with a mobile-phase flow rate of 800 µl/min. Gradient elution was carried out with a solvent system of water/acetic acid (99.8:0.2 v/v) as mobile phase A and acetonitrile/acetic acid (99.8:0.2 v/v) as mobile phase B. The total run time was 12 min, and the gradient elution was as follows: 0.0−3.0 min, B 10-25%; 3.0−4.5 min, B

25−45%; 4.5−6.5 min, B 45−65%; 6.5−8.0 min, B 65−85%; 8.0−9.0 min, B 85−100%.; 9.0−12.0 min, B 100−10%. All the solvents were filtered through 0.22 μm PTFE filters prior to inject. The column was maintained at 30°C while the autosampler was thermostated at 4°C. The system was controlled by Thermo Scientific Dionex Chromeleon 7 software. Benzoic acid and cinnamic acid derivatives were detected at 280 nm and 320 nm, respectively. The concentrations of phenolic acids were calculated from peak areas in comparison to calibration curves of the respective standards and were expressed as μg/g DW.

4.3.2 Moisture content

Moisture content of SF powder was determined by air oven standard methods recommended by AOAC (1980). Initially 5 g of sample in triplicate was dried in hot air oven at 130-133°C for 2 h. After drying, dried sample was again weighed. Following formula is used for calculating the moisture content (MC).

$$\text{MC (\%wb)} = \frac{W_i - W_f}{W_i} \times 100 \qquad (4.1)$$

W_i= initial weight of sample (5 g),

W_f= weight of sample after drying, g

4.3.3 Experimental design

4.3.3.1 Response surface methodology

Response surface methodology (RSM) is an empirical statistical modeling technique used for multiple regression analysis by quantitative data obtained from experiments. In central composite rotatable design (CCRD), the corner points of two parameters, i.e. ultrasonication intensity and flow rate, were found to be very extreme and unapproachable because the experimental lower level of both the parameters were the

least values that could be achieved by the ultrasonicator. Therefore, Box-Behnken design (BBD) was applied to determine the effects of the experimental parameters at three levels –1, 0, and +1. A total of 46 experimental runs were required for continuous ultrasonication as described in Table 4.1. The experimental data were analyzed by the response surface regression procedure and the parameters obtained from the RSM analysis were substituted into the following second-order polynomial model equation.

$$Y_i = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_{ii}^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \quad (4.2)$$

where Y_i is the predicted response; β_0 is the interception coefficient; β_i, β_{ii}, and β_{ij} are coefficients of the linear, quadratic, and interaction terms; X_i and X_j are the variables; and k is the number of independent parameters (k= 5 in this study).

The statistical software package (Design Expert 9.0.3.1, Stat-Ease Inc., USA) was used to generate design of experiment (DOE), perform the statistical analysis and develop a model.

4.3.3.2 Artificial neural network

Artificial neural network (ANN) can be used as an alternative to the polynomial regression based modeling tool, which provides the modeling of complex nonlinear relationships. A feed forward back-propagation multilayer ANN was developed in Matlab, R2010a (The MathWorks, Inc., MA, USA). The topology of ANN structure consisted of an input layer with 5 neurons which represented the aforementioned five experimental factors, namely FT, FWR, UI, FR, and UT; an output layer with 1 neuron which represented either TPC or AA. Furthermore, the optimal number of neuron in the hidden layer was determined by varying its number from 5–12. The residual between

expected and actual data was computed with mean squared error (MSE) according to the following equation:

$$MSE = \frac{1}{n}\sum_{i=1}^{n}(Y_{i,\exp} - Y_{i,pred})^2 \quad (4.3)$$

where, $Y_{i,exp}$ and $Y_{i,pred}$ are experimental and predicted data, respectively, and n is the number of experimental runs.

In this study, a tan-sigmoid transfer function (tansig) at hidden layer and a linear transfer function (purelin) at output layer were applied (Tao et al., 2014). The Levenberg–Marquardt back-propagation algorithm was used for network training (Maran and Priya, 2015).

4.3.4 Statistical analysis

The statistical significance of RSM and ANN models were evaluated by comparing predicted response with experimental responses using various descriptive statistical analysis such as coefficient of determination (R^2), and root mean square error (RMSE). Correspondence between the predicted ($Y_{i,pred}$) and the experimental ($Y_{i,exp}$) values was also evaluated by calculating the absolute average deviation (*AAD*).

$$AAD = \frac{\sum_{i=1}^{n}\left(\frac{\left|Y_{i,\exp} - Y_{i,pred}\right|}{Y_{i,\exp}}\right)}{n} \times 100 \quad (4.4)$$

where n is the number of experimental runs. Derringer's desired function methodology was used to generate optimal conditions for ultrasonication process variables on TPC and AA of sorghum flour.

4.4 Results and Discussion

4.4.1 Modelling and optimization using RSM

Results obtained by performing the continuous experiments according to the BBD matrix are presented in Table 4.2 and Table 4.3. The ANOVA results suggest that the models for TPC and AA were significant (Table 4.4 and Table 4.5), as it is evident from the F-values with a low probability value ($p<0.0001$). Both the models were further checked for the goodness of fit by the correlation coefficients ($R^2>0.94$) that indicated statistically significance of both regression models. The predicted correlation coefficients ($R^2_{pred}>0.89$) also showed good agreement with the adjusted correlation coefficient ($R^2_{adj}>0.82$) for both the models. Low coefficient of variance ($CV<3.60$) suggested not only a high degree of precision but also a good deal of reliability in conducted experiments. For TPC and AA models, the adequate precision (AP) values that indicates signal to noise ratio were found to be greater than 4, which depicted best fitness of the quadratic models. Insignificant lack of fit values ($p>0.05$) implied validity of the both the quadratic models. Low RMSE (<2.5) and *AAD* (<2.05) values expressed that RSM model can be used adequately to describe the relationship of input variables with TPC and AA (Table 4.4 and Table 4.5). A regression analysis of the model equations for TPC and AA shows that the main effects of the independent process variables were highly significant ($p<0.01$). For TPC, the quadratic terms of UI, FR, and UT had significant ($p<0.01$) effect, whereas only quadratic effect of UT was significant ($p<0.01$) for AA of sorghum flour. There was no significant ($p>0.05$) interaction effects of independent variables observed for both TPC and AA (Table 4.4 and Table 4.5).

The effect of FT, FWR, UI, and UT on TPC of sorghum flour for continuous ultrasonication is shown in Figure 4.1. With regard to the combined effect by FT and FWR (Figure 4.1a), TPC decreased with increase in FT from 12 to 36 h at the lowest FWR. This relation became more effective as FWR increased up to 20% (w/v). Significant decrease in TPC were also observed with increase in FWR from 10 to 20% (w/v) at low FT and this effect was more noticeable at higher FT. Apparently, the maximum TPC was obtained at low fermentation time with minimum concentration of sample. These results were in agreement with the findings of Dlamini et al. (2007), Svensson et al. (2010) and Towo et al. (2006) who also reported negative effect on TPC due to fermentation. Up to 12 h of fermentation, phenolics bounded with protein and starch were released by ultrasonication. Further increase in fermentation time up to 36 h resulted in decrease in TPC. This reduction was due to hydrolysis of the glycosidic bonds of bound phenolics and their degradation during fermentation, therefore TPC were not improved by further ultrasonication. The decrease in phenolic compounds during fermentation could also be due to the acidic environment that might result in abstraction of hydride ions and rearrangement of the phenolic structures. Carrera et al. (2012), Cheok et al. (2013) and Tabaraki and Nateghi (2011) also reported the reduction or no significant change in TPC of grape, *Garcinia mangostana* Linn. hull and rice bran, respectively with increase in sample concentration in solvent. With increase in FWR, the viscosity of solution increased and because of that the ultrasound energy was not transmitted uniformly in to the whole solution. The lower the flour to water ratio, the greater the driving force within the solid resulted in increase of diffusion rate. The main

effect of the FWR was to modify the solubility and equilibrium constants and thus increased the TPC to a maximum at the lowest FWR.

The trend observed for TPC of sorghum flour upon simultaneous variation of UI and FR is exhibited in Figure 4.1b. There was a small decrease in TPC with increase in UI from 20 to 30 W/cm^2 at the lowest FR (4 ml/s). This linear relation became more effective as FR increased up to 17 ml/s. Quadratic effect of UI and FR on TPC was also substantial. Significant decrease in TPC were also observed with increase in FR from 4 to 17 ml/s at low UI (20 W/cm^2) and this effect was more noticeable at higher UI. Overall, low FR and intensity indicated the maximum TPC in sorghum flour. These results were in contrast with the findings of Carrera et al. (2012), González-Centeno et al. (2014) and Pan et al. (2011) who observed increase in TPC of grape, grape pomace and pomegranate peel, respectively with increase in ultrasound power. At higher intensity, agitation of solution instead of cavitation caused degradation of phenolic compounds that resulted in reduction of TPC. Under these conditions, bubble cloud density becomes too large, giving rise to shielding effects, coalescence and in general bubble-bubble interactions which decrease the overall efficiency of the process. At low flow rate, the sample remained in contact with ultrasonication probe for longer time for each cycles regardless the total ultrasonication time, hence it resulted an increase in mass flow rate as well as TPC of sorghum flour.

Finally, the plot of TPC as affected by UI and UT for continuous ultrasonication is shown in Figure 4.1c. At low UI, TPC increased consistently with increase in UT from 120 to 180 s and with further increase in UT up to 240 s, it showed insignificant ($p>0.05$) change in TPC. More ultrasonication time at higher UI resulted in noticeable decrease in

TPC of sorghum flour. Evidently, low UI and moderate duration indicated the maximum TPC in sorghum flour. These findings are in agreement with Carrera et al. (2012) and Jabbar et al. (2015) who also reported insignificant change in TPC of grapes and carrot pomace, respectively at longer extraction time. Ultrasonication led to an increase in the effective diffusivity of the mass transfer process and this effect was maximum at short times.

As explicated in response surface plots for AA of sorghum flour (Figure 4.1), the ultrasonication variables, i.e. FT, FWR, UI, FR and UT affected the response factors in a way similar to that observed for the TPC. These results supported the claims that AA of the plant extracts is associated substantially with their TPC. AA decreased significantly ($p<0.05$) with increase in FT and FWR for continuous ultrasonication (Figure 4.1d). Response surface plot also shows that this linear effect of one variable was regardless of other. Maximum AA resulted at low FT and FWR is supported by the results found by Gribova et al. (2008) for AA of bearberry leaves in terms of water to solid ratio. Tabaraki and Nateghi (2011) also reported no significant change in scavenging activity of DPPH of rice bran at higher liquid to solid ratio.

At low UI, AA of sorghum flour decreased with increase in FR from 4 ml/s to 17 ml/s. This linear effect appeared to be more effective at higher UI (Figure 4.1e). Similarly, UI had the negative impact on AA at a given FR. Apparently, maximum AA of sorghum flour was observed at low UI and FR.

Maximum AA was found at low UI when UT increased up to 180 s, followed by no significant change (Figure 4.1f). This result are in accordance with the finding of Jabbar

et al. (2015) and Pan et al. (2011) who also reported no significant change in antioxidant capacity of carrot pomace and pomegranate peel, respectively at longer extraction time.

The optimum conditions for maximum TPC and AA were obtained as 12 h FT, 10% (w/v) FWR, 20 W/cm^2 UI, 4 ml/s FR and 120 s UT. The values observed for TPC and AA at optimum conditions (Run 1) were 90.1 mg GAE/100g DW and 190.1 µmol TE/100g DW, respectively (Table 4.6). With compared to the control sorghum flour (without fermentation and ultrasonication), these TPC and AA values were 41% and 42.4% more, respectively. Increase in phenolic content during ultrasonication was due to release of bound phenolics in sorghum flour. Table 4.7 depicts that ultrasonicated sorghum flours had significantly ($p<0.05$) more benzoic acid and cinnamic acid derived phenolic acids than that of control SF. Salicylic acid followed by ferulic acid were the major phenolic acids found in ultrasonicated SF.

4.4.2 Prediction of ANN model on TPC and AA of SF

As stated above, feed-forward back-propagation network was selected to predict the TPC and AA yields during fermentation and ultrasonication of sorghum flour. The whole dataset generated by BBD in Table 4.1 was randomly divided into training, validation and test subsets during development of ANN models. The numbers of samples used for training, validation and test subsets were 28, 9 and 9, respectively.

In this study, the optimization of the topology of the ANN models was limited to the optimal number of the neurons in the hidden layer. A series of topologies was examined in order to determine the optimum number of neurons in the hidden layer and these values were varied from 5 to 12 (Figure 4.2). The optimal topologies for predicting the TPC and AA were obtained according to the minimized performance function based

on MSE. Table 4.8 presents the optimal topologies of ANN models for the predictions of TPC, AA and the corresponding weights and biases values. For predicting both TPC and AA, the numbers of neurons in the hidden layers of ANN models were 8. As a result, the TPC and AA predicted by ANN can be written as:

$$Y_n(x_n) = purelin(LW^{(2,1)} \log sig(IW^{(1,1)} x_n + b^{(1)}) + b^{(2)}) \quad (4.5)$$

where Y_n denotes either TPC or AA predicted by ANN models, x_n are the input variables, $IW^{(1,1)}$ is the input weight matrix, $LW^{(2,1)}$ is the layer weight matrix, $b^{(1)}$ and $b^{(2)}$ are the biases, whose destinations are the hidden layer and output layer, respectively. The predicted values of TPC and AA from developed models are listed in Table 4.2 and Table 4.3, respectively.

The scatter diagrams comparing experimental data with the computed neural network data in training, testing and validation networks are shown in Figure 4.3. Scattering of almost all the data around the 45° line and higher values of R^2 (>0.99) indicated excellent compatibility between the experimental results and ANN predicted data.

Three statistical parameters, including R^2, RMSE, and *AAD* were employed to evaluate the performance of the developed ANN models for TPC and AA. The results calculated using the experimental data derived from BBD. The developed ANN models could fit the experimental data with satisfactory accuracy, since both ANN models of TPC and AA had high values of R^2 (0.9792 and 0.9881, respectively), low values of RMSE (1.19 and 1.78, respectively) and AAD (0.85 and 0.49, respectively).

4.4.3 Comparison of RSM and ANN models

In order to validate and test the extrapolative capability of both the ANN and RSM models, experiments were conducted for 10 new trials, consisting of combinations of experimental factors, which does not belong to the training data sets. The experimental and predicted values of the response for both the ANN and RSM models are given in Table 4.6. The performance of ANN and RSM models were statistically measured by root mean square error (RMSE), coefficient of determination (R^2) and absolute average deviation (*AAD*).

These results indicated that the RSM prediction had a greater deviation than of ANN for both TPC and AA. Both RSM and ANN models provided good quality predictions in this study, nevertheless the ANN showed a superiority over RSM for both data fitting and estimation capabilities. RSM has the advantage of giving a regression equation for prediction and showing the effect of experimental factors and their interactions on responses in comparison with ANN. However, the main limitation of RSM that it assumes only quadratic non-linear correlation, but ANN can inherently capture almost any form of non-linearity, it can easily overcome the limitation of RSM and this methodology does not require a standard experimental design to build the model. In this study, the performance of the neural network for predicting the TPC and AA of fermented and ultrasonicated sorghum flour was found to be very impressive.

4.5 Conclusions

In this study, fermentation followed by continuous ultrasonication was used to increase TPC and AA of sorghum flour. The effects of fermentation time, flour to water ratio, ultrasonication intensity, flow rate and ultrasonication time were investigated using

RSM and ANN methods. Maximum TPC and AA were obtained at low fermentation time with minimum concentration of sample. Low FR and intensity indicated the maximum TPC and AA in sorghum flour. At optimum conditions, TPC and AA of ultrasonicated sorghum flour were observed 41% and 42.4%, respectively more than that of control sorghum flour. Phenolic characterization revealed that salicylic acid followed by ferulic, hydroxybenzoic and caffeic acids mainly contributed to the TPC of SF. Ultrasonication released the bound phenolics from the starch-protein matrix of fermented sorghum flour and thus increased the free phenolic content. The performance and modeling capabilities of RSM and ANN models were statistically determined (RMSE, R^2 and *AAD*) and compared with actual values. The ANN models were found to have higher predictive capability than RSM model. Based on the findings, ANN can be used for prediction of TPC and AA of sorghum flour under different experimental conditions of fermentation and ultrasonication.

Table 4.1 Box-Behnken experimental design for continuous ultrasonication

Run	FT	FWR	UI	FR	UT	Run	FT	FWR	UI	FR	UT
1	0	-1	1	0	0	24	1	0	0	1	0
2	0	0	1	-1	0	25	-1	0	0	0	-1
3	0	1	0	1	0	26	0	0	0	0	0
4	-1	0	-1	0	0	27	1	0	0	-1	0
5	0	0	0	0	0	28	0	1	0	0	1
6	0	0	0	1	1	29	1	0	0	0	1
7	0	0	-1	0	1	30	0	1	1	0	0
8	0	0	0	-1	1	31	0	0	1	0	1
9	1	0	-1	0	0	32	0	0	-1	1	0
10	0	0	0	0	0	33	0	1	-1	0	0
11	0	-1	0	0	-1	34	-1	0	0	-1	0
12	0	0	1	1	0	35	0	0	0	0	0
13	1	-1	0	0	0	36	0	0	1	0	-1
14	-1	0	0	1	0	37	0	0	-1	-1	0
15	0	1	0	0	-1	38	1	0	1	0	0
16	0	-1	0	1	0	39	0	0	0	0	0
17	1	1	0	0	0	40	0	0	0	1	-1
18	0	-1	-1	0	0	41	-1	-1	0	0	0
19	0	0	0	-1	-1	42	0	0	0	0	0
20	-1	0	1	0	0	43	-1	0	0	0	1
21	0	0	-1	0	-1	44	1	0	0	0	-1
22	0	-1	0	0	1	45	-1	1	0	0	0
23	0	1	0	-1	0	46	0	-1	0	-1	0

Variables	Levels		
	-1	0	+1
Fermentation time (FT, h)	12	24	36
Flour to water ratio (FWR, %w/v)	10	15	20
Ultrasonication intensity (UI, W/cm^2)	20	25	30
Flow rate (FR, ml/s)	4	10.5	17
Ultrasonication time (UT, s)	120	180	240

Table 4.2 Observed and predicted values of total phenolic content TPC of sorghum flour by response surface methodology (RSM) and artificial neural network (ANN) for continuous ultrasonication

Exp run	TPC (mg GAE/100g DW)			Exp run	TPC (mg GAE/100g DW)		
	Observed	Predicted			Observed	Predicted	
		RSM	ANN			RSM	ANN
1	75.10±0.45	69.89	74.45	24	62.10±1.08	62.88	63.13
2	64.07±0.78	66.24	61.59	25	78.26±0.77	77.54	78.21
3	65.38±0.92	65.40	66.01	26	78.31±0.86	77.24	76.99
4	89.65±0.47	88.91	90.26	27	69.02±0.76	69.25	67.37
5	81.30±0.65	77.24	76.99	28	68.46±0.84	69.60	68.91
6	70.53±0.81	70.15	70.51	29	68.93±0.51	67.07	69.42
7	81.39±0.89	82.00	81.34	30	66.21±0.69	60.02	65.90
8	78.02±0.69	76.54	77.96	31	62.04±0.83	64.41	62.10
9	76.36±1.08	75.18	76.36	32	79.24±0.66	77.53	79.24
10	72.99±0.87	77.24	76.99	33	75.27±0.49	76.44	75.24
11	76.82±0.58	76.43	76.87	34	84.82±1.01	83.82	85.52
12	58.72±0.76	60.24	58.76	35	79.35±0.99	77.24	76.99
13	72.04±1.02	73.93	72.78	36	57.86±0.58	60.24	58.05
14	78.10±1.00	77.65	78.06	37	85.13±0.91	84.08	85.06
15	64.14±0.72	65.30	63.83	38	55.50±0.73	56.67	55.62
16	76.19±0.91	76.08	76.23	39	74.22±0.81	77.24	76.99
17	60.45±0.64	61.63	60.43	40	65.92±0.89	66.07	65.94
18	86.47±0.57	88.61	87.09	41	86.29±0.96	87.32	86.25
19	73.16±0.93	72.22	73.09	42	77.41±0.74	77.24	76.99
20	70.68±0.82	72.28	70.41	43	82.31±0.67	82.10	82.24
21	77.17±0.74	77.79	77.23	44	65.60±0.53	63.23	65.60
22	80.92±0.62	80.52	80.91	45	77.25±0.79	77.58	77.11
23	70.30±0.93	71.33	70.24	46	81.80±0.93	82.70	80.31
				Control	**63.91±0.97**		

Response experimental results are reported as mean ± standard deviation (n= 6), control: sorghum flour without fermentation and ultrasonication

Table 4.3 Observed and predicted values of antioxidant activity (AA) of sorghum flour by response surface methodology (RSM) and artificial neural network (ANN) for continuous ultrasonication

Exp run	AA (µmol TE/100g DW)			Exp run	AA (µmol TE/100g DW)		
	Observed	Predicted			Observed	Predicted	
		RSM	ANN			RSM	ANN
1	141.98±4.15	145.20	141.91	24	131.30±3.87	134.18	131.97
2	146.50±4.28	141.62	146.42	25	158.03±4.03	157.93	158.02
3	141.52±3.98	140.66	141.50	26	148.95±4.38	155.27	154.81
4	188.04±4.55	188.17	188.04	27	142.76±4.17	145.50	142.78
5	156.99±3.97	155.27	154.81	28	141.46±4.29	142.21	141.46
6	146.96±4.12	148.44	146.94	29	137.89±4.43	136.59	138.66
7	170.06±4.29	170.47	170.85	30	121.51±3.96	124.32	122.31
8	158.01±3.89	158.95	157.87	31	132.24±3.77	133.49	132.20
9	157.63±3.99	156.01	158.05	32	168.05±4.28	168.31	168.05
10	153.36±4.09	155.27	154.81	33	162.98±4.12	163.20	162.99
11	154.27±4.34	152.35	154.21	34	174.93±4.08	175.43	174.91
12	135.66±4.28	130.60	135.61	35	150.10±3.91	155.27	154.81
13	148.65±4.17	148.16	148.95	36	122.78±3.89	123.87	122.78
14	164.74±4.11	165.38	164.73	37	178.20±4.07	178.64	178.21
15	133.55±4.29	133.90	132.37	38	119.97±4.37	120.23	119.92
16	158.56±4.31	158.98	157.57	39	162.32±4.51	155.27	154.81
17	129.89±4.54	128.82	129.92	40	138.14±4.26	139.05	137.83
18	180.42±3.87	181.05	180.40	41	179.33±4.14	178.74	179.72
19	149.53±3.63	149.89	149.52	42	159.37±3.73	155.27	154.81
20	147.21±4.47	149.21	147.16	43	168.78±4.08	168.03	168.79
21	161.38±4.11	161.63	161.38	44	128.91±3.95	128.24	129.22
22	164.00±4.23	162.48	164.41	45	160.53±4.25	159.36	160.52
23	150.61±3.98	150.29	150.58	46	169.75±4.19	170.70	169.25
				Control	**133.48±3.19**		

Response experimental results are reported as mean ± standard deviation (n= 6)

Table 4.4 Regression coefficients and ANOVA describing the effect of the independent variables on total phenolic content of sorghum flour for continuous ultrasonication

Model term	df	Coefficient estimated	*SS*	MS	F-value	p-value
β_0	20	34.62	2965.38	148.27	21.15	0.0001**
β_1	1	0.23	860.83	860.83	122.80	0.0001**
β_2	1	-0.52	485.85	485.85	69.31	0.0001**
β_3	1	3.41	1233.69	1233.69	175.98	0.0001**
β_4	1	0.64	157.16	157.16	22.42	0.0001**
β_5	1	0.39	70.87	70.87	10.11	0.0039**
β_{12}	1	-0.01	1.63	1.63	0.23	0.6335
β_{13}	1	-0.008	0.88	0.88	0.13	0.7256
β_{14}	1	-0.0006	0.0095	0.0095	0.0014	0.9709
β_{15}	1	-0.0002	0.13	0.13	0.018	0.8935
β_{23}	1	0.02	1.32	1.32	0.19	0.6675
β_{24}	1	0.005	0.12	0.12	0.018	0.8953
β_{25}	1	0.0001	0.011	0.011	0.0015	0.9693
β_{34}	1	0.004	0.075	0.075	0.011	0.9186
β_{35}	1	-0.00	0.0002	0.0002	0.0000	0.9951
β_{45}	1	-0.0001	0.015	0.015	0.0002	0.9632
β_{11}	1	-0.009	14.84	14.84	2.12	0.1581
β_{22}	1	-0.03	5.95	5.95	0.85	0.3656
β_{33}	1	-0.11	62.63	62.63	8.93	0.0062**
β_{44}	1	-0.06	56.36	56.36	8.04	0.0089**
β_{55}	1	-0.0009	104.26	104.26	14.87	0.0007**
Residual	25		175.25	7.01		
Lack of fit	20		125.96	6.30	0.64	0.7848
Pure error	5		49.30	9.86		
R^2		0.9442				
R^2_{adj}		0.8996				
R^2_{pred}		0.8170				
CV, %		3.60				
AP		18.02				
RMSE		1.95				
AAD		2.03				

Significant at *p<0.05, **p<0.01, adj: adjusted, pred: predicted, AAD: absolute average deviation, *AP*: adequate precision, CV: coefficient of Variance, df: degree of freedom, MS: mean square, RMSE: root mean square error, *SS*: sum of squares

Table 4.5 Regression coefficients and ANOVA describing the effect of the independent variables on AA of sorghum flour for continuous ultrasonication

Model term	df	Coefficient estimated	*SS*	MS	F-value	p-value
β_0	20	199.78	12087.06	604.35	59.87	0.0001**
β_1	1	-1.19	3739.44	860.83	370.45	0.0001**
β_2	1	-0.20	1499.91	485.85	148.59	0.0001**
β_3	1	-1.47	5583.68	1233.69	553.15	0.0001**
β_4	1	-1.17	455.36	157.16	45.11	0.0001**
β_5	1	0.78	331.56	70.87	32.85	0.0001**
β_{12}	1	0.0001	0.0003	0.0003	0.00	0.9957
β_{13}	1	0.01	2.53	0.88	0.25	0.6209
β_{14}	1	-0.004	0.40	0.0095	0.040	0.8436
β_{15}	1	-0.0006	0.78	0.13	0.077	0.7831
β_{23}	1	-0.03	2.30	1.32	0.23	0.6373
β_{24}	1	0.02	1.10	0.12	0.11	0.7444
β_{25}	1	-0.002	0.83	0.011	0.083	0.7762
β_{34}	1	-0.005	0.12	0.075	0.012	0.9147
β_{35}	1	0.0006	0.15	0.0002	0.015	0.9028
β_{45}	1	0.0002	0.028	0.015	0.0003	0.9585
β_{11}	1	-0.005	5.12	14.84	0.51	0.4830
β_{22}	1	-0.03	4.63	5.95	0.46	0.5044
β_{33}	1	-0.04	10.49	62.63	1.04	0.3178
β_{44}	1	0.01	3.36	56.36	0.33	0.5690
β_{55}	1	-0.002	405.23	104.26	40.14	0.0001**
Residual	25		252.36	10.09		
Lack of fit	20		112.53	5.63	0.20	0.9960
Pure error	5		139.83	27.97		
R^2		0.9795				
R^2_{adj}		0.9632				
R^2_{pred}		0.9472				
CV, %		2.09				
AP		31.65				
RMSE		2.34				
AAD		1.09				

Significant at *p<0.05, **p<0.01, adj: adjusted, pred: predicted, AAD: absolute average deviation, *AP*: adequate precision, CV: coefficient of Variance, df: degree of freedom, MS: mean square, RMSE: root mean square error, *SS*: sum of squares

Table 4.6 Optimization, validation and comparison of experimental data set

Run	FT (h)	FWR (% w/v)	UI (W/cm^2)	FR (ml/s)	UT (s)	TPC (mg GAE/100g DW)			AA (µmol TE/100g DW)		
						Actual	Predicted		Actual	Predicted	
							RSM	ANN		RSM	ANN
1	12	10	20	4	120	90.13±0.73	88.64	89.78	190.12±4.21	190.33	189.72
2	36	20	30	17	240	46.44±0.98	43.30	46.30	104.67±3.88	101.58	105.56
3	12	10	25	17	240	82.19±1.11	80.23	81.38	171.27±4.05	172.60	170.94
4	36	20	25	4	120	60.67±0.67	56.61	61.45	126.09±4.54	124.13	125.56
5	12	20	25	4	240	78.43±0.91	76.88	77.96	160.88±4.33	162.19	160.14
6	36	10	25	17	120	65.33±0.55	62.77	66.63	136.11±3.97	131.09	137.23
7	12	15	30	10.5	120	65.57±1.13	66.56	64.77	136.59±4.22	137.16	135.38
8	36	15	20	10.5	240	74.73±1.07	73.65	74.18	149.87±4.31	153.19	148.84
9	12	20	20	17	120	74.89±0.84	71.31	75.45	164.74±4.09	163.51	166.25
10	36	10	30	4	240	64.11±0.91	60.57	65.17	133.39±3.94	134.85	134.41
						RMSE	2.62	0.75		2.39	0.95
						R^2	0.9504	0.9959		0.9898	0.9984
						AAD	3.66	0.99		1.44	0.62

Response experimental results are reported as mean ± standard deviation, AA: antioxidant activity, ANN: artificial neural network, FR: flow rate, FT: fermentation time, FWR: flour to water ratio, RSM: response surface methodology, TPC: total phenolic content, UI: ultrasonication intensity, UT: ultrasonication time

Table 4.7 Phenolic profile of control and ultrasonicated sorghum flours (SF) (μg/g DW)

Compounds	Control SF	Ultrasonicated SF
Benzoic acids		
Protocatechuic acid	6.18±0.11[a]	8.37±0.13[b]
p-Hydroxybenzoic acid	13.3±0.22[a]	18.2±0.24[b]
Cinnamic acids		
Caffeic acid	10.2±0.19[a]	16.8±0.15[b]
p-coumaric acid	4.87±0.13[a]	7.63±0.12[b]
Ferulic acid	13.4±0.28[a]	21.2±0.19[b]
Salicylic acid	22.8±0.20[a]	30.7±0.22[b]

Means±SD in the same row with different letters are significantly different ($p<0.05$)

Table 4.8 Optimal topologies of artificial neural network models for predicting total phenolic content (TPC) and antioxidant activity (AA) and values of weights and biases resulted from network training

	TPC	**AA**
Topology	5^a-8^b-1^c	5^a-8^b-1^c
Input weight matrix (Destination : HL, source: inputs)	$IW^{(1,1)} = \begin{bmatrix} 0.1680 & -2.7201 & -1.6597 & -0.1955 & 0.1269 \\ 4.5031 & -0.3673 & 0.9827 & 4.4896 & -1.2162 \\ -0.4261 & 0.3559 & -0.4439 & 0.0301 & 0.0099 \\ 1.4949 & 2.6424 & -1.0145 & -2.9278 & -1.6874 \\ 1.9915 & -2.2538 & 2.7740 & -3.6589 & 0.0901 \\ 0.9302 & 1.1652 & 0.1393 & -3.4278 & 2.2238 \\ -2.4395 & 1.1581 & 0.5123 & 4.8651 & -0.3309 \\ -2.0447 & -6.2823 & -1.0502 & 0.6047 & -0.1544 \end{bmatrix}$	$IW^{(1,1)} = \begin{bmatrix} 0.5389 & -0.0683 & 1.0409 & -2.4705 & 1.0346 \\ 1.2662 & 1.2023 & 2.6780 & 2.5274 & -1.4126 \\ -1.8753 & 1.3770 & 0.6350 & 2.4254 & -1.5672 \\ 0.7586 & 1.2593 & -2.3712 & -2.1031 & 0.7691 \\ 0.2037 & 0.0013 & 0.1367 & -0.1473 & -0.0376 \\ -0.3595 & 0.2632 & -0.1051 & 0.2934 & -2.9006 \\ 1.3285 & 1.1249 & 1.0483 & 2.4367 & -1.6414 \\ 2.5732 & -1.9021 & -1.4033 & -0.6024 & 1.6629 \end{bmatrix}$
Bias vector (Destination : HL)	$b^{(1)} = \begin{bmatrix} 0.6792 & -2.2079 & -0.2418 & -3.3649 & 1.7372 & 1.8624 & -2.658 & -3.2764 \end{bmatrix}^T$	$b^{(1)} = \begin{bmatrix} 2.6742 & -1.9485 & 1.3349 & -2.6884 & 0.2562 & 0.9717 & 2.0783 & 2.7520 \end{bmatrix}^T$
Layer weight matrix (Destination : OL, source: HL)	$LW^{(2,1)} = \begin{bmatrix} 0.3666 & -0.0636 & 1.1998 & -0.1186 & 0.2582 & 0.1008 & 0.1178 & 0.2058 \end{bmatrix}$	$LW^{(2,1)} = \begin{bmatrix} 0.1043 & -0.2623 & -0.1643 & -0.0208 & -1.9804 & 0.2842 & -0.2504 & 0.1305 \end{bmatrix}$
Bias (Destination : OL)	0.1291	0.1876

a, b, c denote the numbers of neurons in the input, hidden and output layers, respectively; HL: hidden layer, OL: output layer

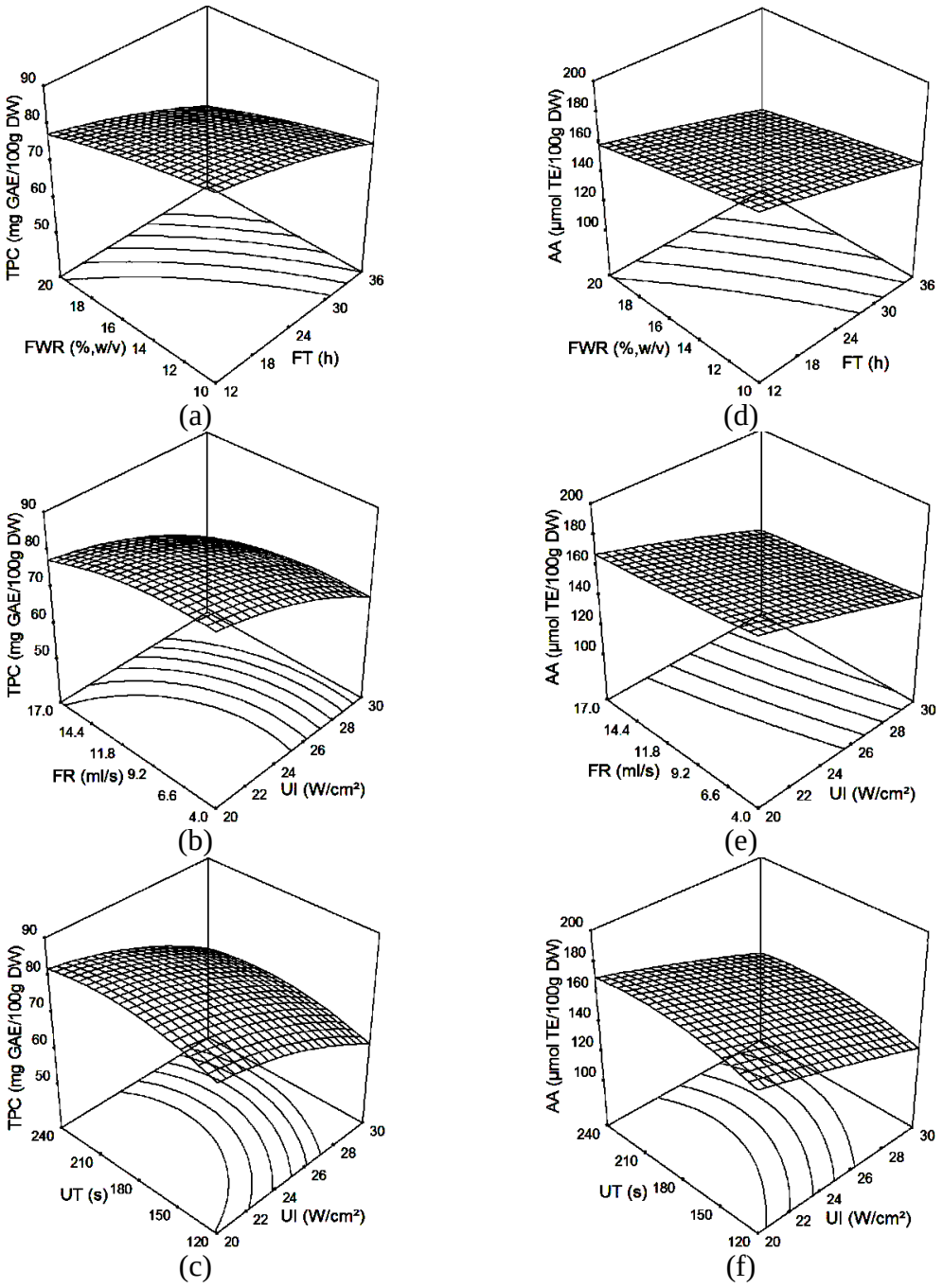


Figure 4.1 Response surface plots of total phenolic content (TPC) (a, b, c) and antioxidant activity (AA) (d, e, f) of sorghum flour as affected by fermentation time (FT), flour to water ratio (FWR), ultrasonication intensity (UI), flow rate (FR) and ultrasonication time (UT) for continuous ultrasonication at 0 level of corresponding third variable

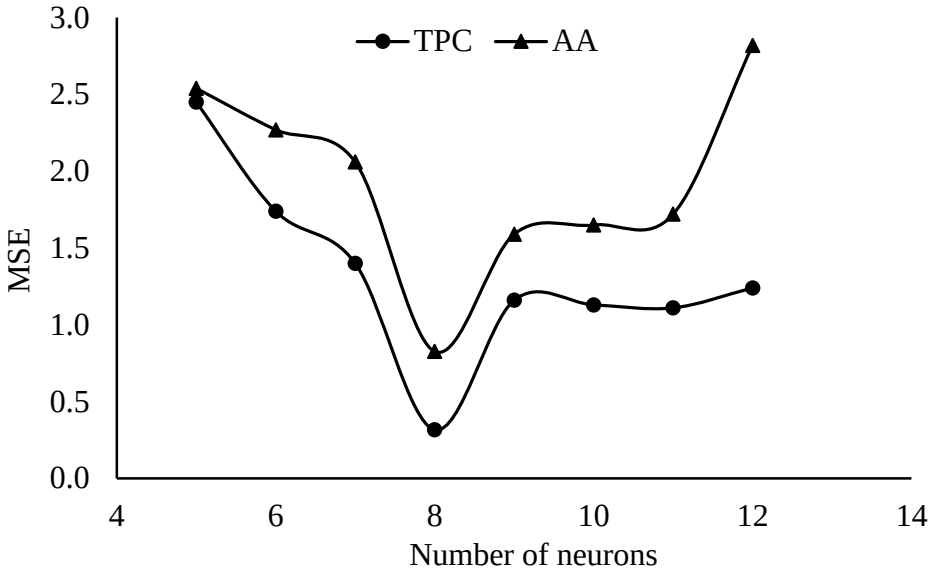


Figure 4.2 Determination of optimum number of neurons in hidden layer for artificial neural network in predicting total phenolic content (TPC) and antioxidant activity (AA) of sorghum flour

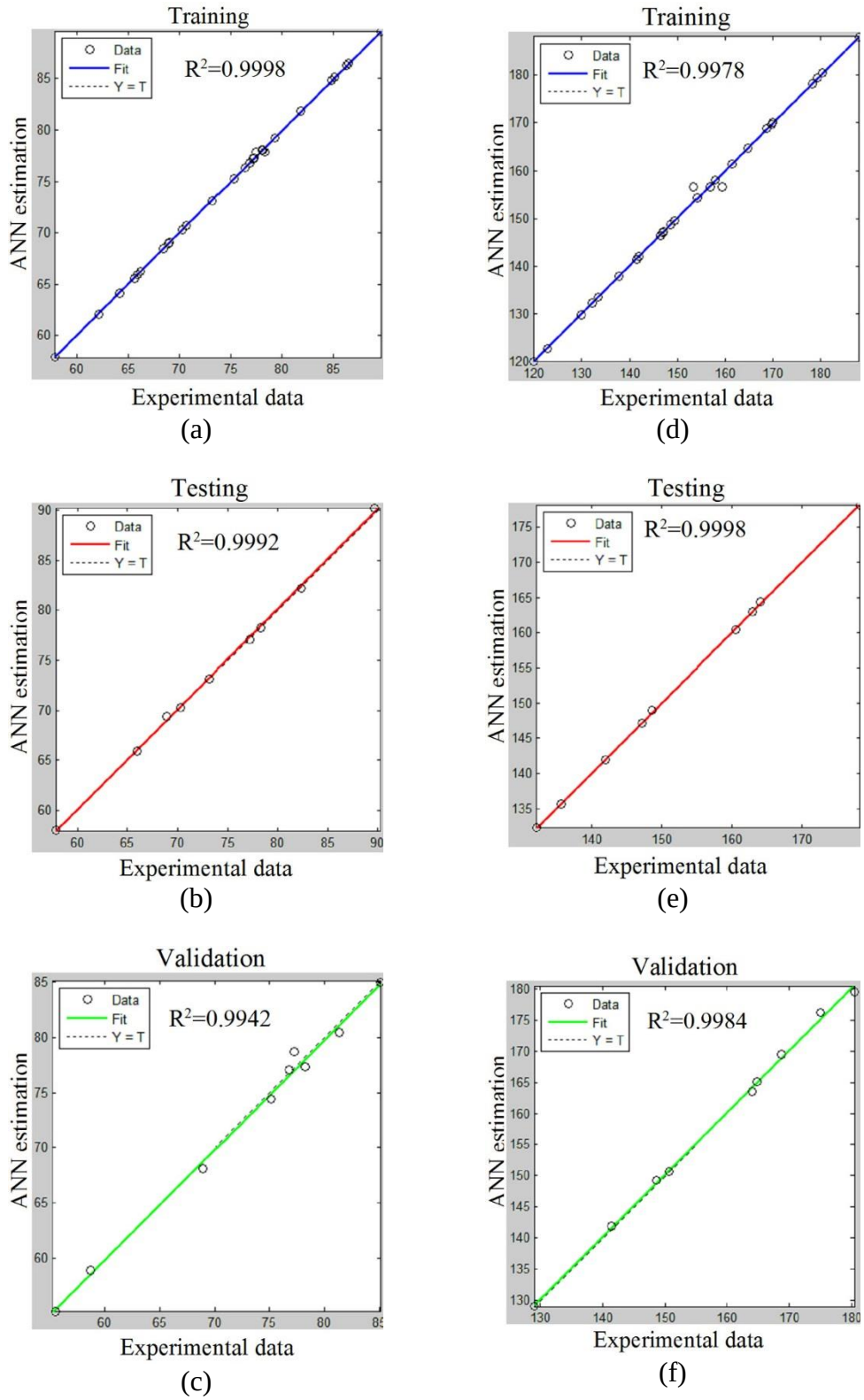


Figure 4.3 Neural network training, testing, and validation set for total phenolic content (a, b, c) and antioxidant activity (d, e, f) of sorghum flour

CHAPTER 5

Effect of drying methods and ultrasonication in improving the antioxidant activity and total phenolic content of apple pomace powder[4]

5.1 Abstract

Although extrusion is a promising process to develop ready to eat cereals and snacks, thermal treatment to raw material during extrusion results in degradation of phenolic compounds. Therefore, an approach was made to enhance the total phenolic content (TPC) and antioxidant activity (AA) of apple pomace (AP) prior to extrusion process. In this study, AP powder was naturally fermented (F) for 12 h and then was subjected to ultrasonication (U) at various conditions [25, 37, and 50 µm ultrasonication amplitude (UA) for 1, 2, and 3 min of ultrasonication time (UT)]. AP was then dried in oven (O) and microwave (MW), separately and thus four drying methods, i.e. O_F-O_U, O_F-MW_U, MW_F-O_U, MW_F-MW_U were used in combinations. Full factorial design was used for experimental plan and results were analyzed using statistical software. It was observed that drying method significantly affected the TPC and AA of AP powder followed by UA. UT did not have any significant effect on TPC, and AA. Maximum TPC, and AA observed for the AP powder dried in MW after fermentation and ultrasonication (MW_F-MW_U) at 50 µm UA for 3 min UT were 372.98 mg GAE/100g DW, and 729.67 µmol TE/100 g DW, respectively. MW_F-MW_U drying exhibited a more prominent disrupted and porous structure of AP powder compared with that of O_F-O_U drying.

[4] Lohani, U. C. and Muthukumarappan, K. 2015. Effect of drying methods and ultrasonication in improving the antioxidant activity and total phenolic content of apple pomace powder. Journal of Food Research, 4 (2): 68-77.

5.2 Introduction

Apple pomace (AP) is a solid residue (25%-30% of total fruit) obtained after the extraction of juice from apple. AP is mainly composed of dietary fibers, carbohydrates, small amount of proteins, fat, and ash (Sudha et al., 2007). It also contains numerous phytochemicals in the form of simple sugars, pectin, and natural antioxidants (Bhushan et al., 2008). The amount of total phenolic compounds (TPC) varies greatly in between flesh and peel of apple. Peels contains higher quantity of phenolic compounds. The procyanidins, catechin, epicatechin, chlorogenic acid, phloridzin and quercetin conjugates are commonly found in Apple peels. In the Apple flesh, catechin, procy anidins, epicatechin and phloridzin are found in much lower concentrations. Some of the phenolic compounds in AP have been correlated with antioxidant activities (AA) using various methods (DPPH, hydroxyl and superoxide anion radical scavenging activity, FRAP) and thereby confirming the AP as a valuable source of antioxidants (Diñeiro et al., 2009).

As a common application, AP is used for direct disposal to soil in a landfill, and for pectin recovery usage (gelling agent, stabilizer and source of dietary fiber). These Applications are not sufficient to utilize the several tones of AP produced every year; therefore, studies have got momentum to valorize the AP for other purposes also. AP as a rich source of antioxidant compounds could be used for increasing the stability of foods by preventing lipid peroxidation and also for protecting oxidative damage in living systems by scavenging oxygen radicals. Extruded snacks, where starch is the major component, will have more nutritional value if incorporated with fiber enriched flours containing antioxidants. But, on the other hand, high temperature extrusion process also

results in loss of nutritive values. From preliminary experiments, it was observed that extrusion at optimum temperature reduced 25-30% TPC and AA of AP. A lot of work has been done in the area of food safety in terms of improving shelf life, prohibiting deterioration, but work related to enhancing the nutritional values in foods during processing is lacking. Therefore, prior to extrusion, an effort is required to enhance the nutritive value, specially, TPC, and AA in AP. In past few years, ultrasonication and fermentation have been used for accelerating the extraction of phenolic antioxidants from AP (Ajila et al., 2011, Ajila et al., 2012, Opalić et al., 2009, Vasantha Rupasinghe et al., 2011). Fermentation also brings about numerous biochemicals, nutritional and organoleptic changes in the raw materials including the breakdown of certain constituents (Murekatete et al., 2012, Oboh and Amusan, 2009). Several methods such as heat treatment and far-infrared radiation have been studied to liberate and activate natural antioxidants (Kim et al., 2008, Turkmen et al., 2005, Xu et al., 2006). However, our comprehensive literature review revealed there has been very few detailed report on the use of microwaves to liberate phenolic compounds in plant materials and particularly none in case of AP. Therefore, keeping in view the above facts, the study was focused on enhancing the TPC and AA in AP powder using fermentation, ultrasonication and microwave (MW) drying technology.

5.3 Materials and Methods

Apple pomace (AP) powder provided by Tree Top, Inc. (Selah, WA) was stored at -20 °C. The initial moisture content of AP powder was 8.75% (wb). Protein, fat, ash, crude fiber and carbohydrate of AP powder were 4.14%, 2.79%, 2.11%, 22.06%, and 60.21%, respectively. For natural fermentation, AP slurry was prepared by adding 12.5 g

AP powder in 100 ml distilled water. The fermentation was carried out in a controlled conditions with temperature 30±1 °C for 12 h. Fermented slurry was dried and further subjected to ultrasonication at 25 μm, 37 μm, and 50 μm amplitude for 1, 2, and 3 min. Sample to water ratio was kept constant as 5% (w/v) as a maximum concentration for ultrasonication from preliminary trials. Ultrasonicated sample was again dried and was stored at -20 °C for TPC and AA analysis. Two methods, i.e. hot air oven (45 °C) and microwave (MW) drying (90 W) were used for drying of sample till its constant weight. Four drying methods, i.e. O_F-O_U, O_F-MW_U, MW_F-O_U, MW_F-MW_U were used in combinations. Hot air oven drying was denoted by 'O', and subscripts 'F', and 'U' indicated 'after fermentation', and 'after ultrasonication', respectively. For example, MW_F-O_U indicates microwave drying after fermentation and hot air drying after ultrasonication. (Table 5.1).

5.3.1 Extraction of samples

The extraction of sample for determining TPC, AA and phenolic characterization was done using the method described by Khan et al. (2013). For determining TPC and AA, 1 g of AP 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°C until analysis for TPC and AA.

5.3.1.1 Total phenolic content (TPC)

TPC of AP was determined using Folin–Ciocalteu method (Singleton et al., 1999) with some modification. 50 μl methanol extract of sample was added with 3.5 ml distilled water and 150 μl Folin-Ciocalteu reagent. The solution was vortexed and incubated for

30 min. Thereafter, absorbance of solution was measured at 760 nm against blank. Blank solution contained all the components that were present in the sample except the methanol extract. Gallic acid was used as positive control (standard) and linear regression curve between absorbance and concentration was drawn for the standard. This standard curve was used for calculating the concentration of sample and data was expressed in mg Gallic acid equivalent (GAE)/100 g dry weight (DW). This analysis was done in six replications.

5.3.1.2 Antioxidant activity (AA)

Extinction of DPPH is a free radical scavenging activity which was measured using spectrophotometric method described by Brand-Williams et al. (1995). 2,2-diphenyl-1-picrylhydrazyl (DPPH) 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 for 30 min. Thereafter, 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 weight (DW). Samples were analyzed in six replications.

5.3.2 Moisture content

Moisture content of AP was determined by air oven standard methods recommended by AOAC (1980). Initially 5 g of sample in triplicate was dried in hot air oven at 130-133°C for 2 h. After drying, dried sample was again weighed. Following formula is used for calculating the moisture content (MC).

$$\text{MC (\%wb)} = \frac{W_i - W_f}{W_i} \times 100 \qquad (5.1)$$

W_i= initial weight of sample (5 g),

W_f= weight of sample after drying, g

5.3.3 Microstructure Evaluation

The microstructure of control, oven dried and MW dried Apple pomace powder was examined using a scanning electron microscope (SEM) (Hitachi-S3400 N, Tokyo, Japan). Small amounts of samples were mounted on SEM specimen stubs by using double-sided adhesive tape. Each powder sample was coated with 10 Å thick layer of gold in a sputter coater before being scanned and photographed at 1000× magnification.

5.3.4 Statistical analysis

Full factorial design was used for experimental plan and results were compared by analysis of variance (ANOVA) using SPSS (16.0) statistical software. All data were reported as mean ± standard deviation of replicates. Tukey's tests were used to compare the significant differences of the mean values with the family error rate held at 0.05.

5.4 Results and Discussion

5.4.1 Effect of drying method on TPC and AA

ANOVA showed that drying methods (DM) had significant ($P<0.05$) effect on TPC and AA followed by interaction effect of DM and UA (Table 5.2). Table 5.3 shows that TPC, and AA increased significantly by 43.7%, and 18.5% on average when AP powder was MW dried after fermentation and ultrasonication as compare to oven drying during both the processes. Some other studies have also reported that the phenolic content was increased after microwave treatment of the plant materials (Boateng et al., 2008, Hayat et al., 2010, Omwamba and Hu, 2010). However, Sharma and Gujral (2011) found decrease in TPC of barley after microwave cooking. Microwave treatment of AP powder cleaved and liberated phenolic compounds, hence resulting in the increase of free

phenolic compounds and enhancement of antioxidant activity. O_F-MW_U, and MW_F-O_U drying methods did not show any significant difference in TPC, while there was no significant difference in AA between O_F-O_U and O_F-MW_U, and MW_F-O_U and MW_F-MW_U drying methods. TPC of AP powder was found least during O_F-O_U drying that may be because of thermal degradation of TPC. When AP powder was MW dried after fermentation, and ultrasonication at 40% UA for 3 min UT, TPC and AA were increased by 60%, and 26.1%, respectively, as compare to oven dried during both the processes (Figure 5.1). Maximum TPC, and AA observed for the AP powder for MW_F-MW_U at 40% UA for 3 min UT were 33%, and 32.2% more than that of control (untreated AP powder), respectively (Figure 5.1). Apart from enhancing the TPC and AA of AP powder, MWF-MWU drying saved 65% of drying time as compared to OF-OU drying (data not shown).

5.4.2 Effect of UA and UT on TPC and AA

It was explicated by ANOVA that there was significant ($P<0.05$) effect of UA, and interaction of UA and drying method on TPC and AA of AP powder (Table 5.2). TPC and AA of AP powder increased significantly with increase in UA from 25 µm to 50 µm (Table 5.3). The reason may be that an increase in UA resulted in disruption of cell compartments facilitating the interaction of phenolic molecules with solvents at reasonably low temperatures. Alighourchi, Barzegar, Sahari, and Abbasi (2013) also observed an increase in TPC of pomegranate juice with increase in ultrasound power. For all drying methods and 3 min of UT, TPC increased nonlinearly with increase in UA from 25 µm to 50 µm, however, only during O_F-MW_U and MW_F-MW_U drying, TPC increased significantly ($p<0.05$) with increase in UA from 25 µm to 37 µm and 37 µm to

50 µm, respectively (Figure 5.2). Except O_F-O_U drying, all drying methods showed the significant ($p<0.05$) difference in TPC values between 25 µm and 50 µm UA (Figure 5.2). With increase in UA, AA also increased for all drying methods except O_F-MW_U method. For this drying method, AA first decrease with increase in UA from 25 µm to 37 µm and thereafter increased with UA up to 50 µm (Figure 5.2). O_F-O_U and O_F-MW_U drying method did not exhibit any significant ($p>0.05$) change in AA of AP powder when UA increased from 25 µm to 50 µm for 3 min UT (Figure 5.2). A significant ($p<0.05$) difference in AA of AP powder was observed between 25 µm and 50 µm UA during MW_F-O_U drying, whereas, MW_F-MW_U drying showed a continuous significant ($p<0.05$) increase in AA with increase in UA in experimental range (Figure 5.2). When MW dried fermented and ultrasonicated AP powder was subjected to an increase in UA from 25 µm to 50 µm for 3 min UT, TPC, and AA were increased by 10.7%, and 80.4%, respectively. Maximum TPC and AA were found as 372.98 mg GAE/100 g DW and 729.67 µmol TE/100 g DW, respectively at 50 µm UA and MW_F-MW_U drying method (Table 5.3).

Table 5.2 and Figure 5.3 shows that there was no significant ($p>0.05$) change in TPC, and AA with increase in UT from 1 to 3 min, although maximum TPC and AA were found for 3 min of UT at 50 µm UA and MW_F-MW_U drying (Table 5.3). The reason of this may be due to short time exposure of AP powder to the ultrasonication.

5.4.3 Effect of Processing on Microstructures of AP Powder

The effect of the drying conditions on the microstructure of treated (fermented and ultrasonicated at 40% amplitude for 3 min) AP powder was carried out by means of SEM. The microphotographs of fresh, O_F-O_U and MW_F-MW_U AP powder are shown in Figure 5.4. Figure 5.4a shows the structure of fresh AP powder, which is a compact and

less porous structure. The SEM images revealed that hot air drying (O_F-O_U) resulted in modification of the cellular structure (Figure 5.4b). MW drying after fermentation and ultrasonication (MW_F-MW_U) damaged the plant cell structure and caused more porosity (Figure 5.4c). Similar results were found by Giri and Prasad (2007) and Han et al. (2010) for mushroom and apple slices, respectively. MW dried sample granules were more disrupted than hot air dried granules which caused increase in surface area and helped in releasing more phenolics from bound structure.

5.4.4 Relationship between TPC and AA

Correlation experiments to predict the antioxidant properties have been performed by many authors. AP powder with higher levels of TPC had a greater antioxidant capacity (Sato et al., 2010, Savatović et al., 2005). The values of AA indicated a positive correlation with the values of TPC of AP powder at different drying method (DM), and ultrasonication amplitude (UA). A linear correlation in each case was observed between AA and TPC. The correlation coefficient (r) were 0.9474, and 0.9960 at different DM and UA, respectively, which indicated that TPC was the major factor accounting for the antioxidant activity of the apple pomace.

5.5 Conclusions

Drying method of AP after fermentation and ultrasonication played a significant role in enhancing the TPC, and AA in AP powder followed by UA. UT did not have any significant effect on TPC, and AA. Higher UA with MW drying during fermentation and ultrasonication process gave the higher values of TPC, and AA. From the microstructural analysis, MW drying caused more cell collapse and cell disruption resulting in release of phenolics from the bound structure. MW drying is also favorable for quick and efficient

drying as compare to oven drying. AA of AP powder showed the linear correlation with TPC. More investigation is required to observe the effect of further increase in UA and UT on TPC and AA of AP powder.

Table 5.1 Independent variable values of the process and their corresponding levels

S. No	Independent variable	Level	Values
1	Drying method	4	O_F-O_U, O_F-MW_U, MW_F-O_U, MW_F-MW_U
2	Ultrasonication amplitude, µm	3	25, 37, 50
3	Ultrasonication time, min	3	1, 2, 3

O: Oven drying, MW: Microwave drying, F: Fermentation, U: Ultrasonication

Table 5.2 Variance analysis for all dependent variables

Source	Dependent variables	Sum of square	df	Mean square	F value	p value
Drying method (DM)	TPC	47302.72	3	157.6757	110.67	0.000*
	AA	678833.34	3	226277.78	58.83	0.000*
Ultrasonication amplitude (UA)	TPC	8732.72	2	4366.36	30.65	0.000*
	AA	187686.93	2	93843.47	24.4	0.000*
Ultrasonication time (UT)	TPC	64.37	2	32.18	0.23	0.798
	AA	1460.91	2	730.45	0.19	0.827
DM*UA	TPC	3958.22	6	659.70	4.63	0.000*
	AA	159417.69	6	26569.62	6.91	0.000*
DM*UT	TPC	238.89	6	39.82	0.28	0.945
	AA	33112.20	6	5518.70	1.43	0.213
UA*UT	TPC	382.54	4	95.64	0.67	0.614
	AA	23506.46	4	5876.61	1.53	0.203
DM*UA*UT	TPC	584.38	12	48.69	0.34	0.978
	AA	53277.46	12	4439.79	1.15	0.332

*significant at 5% level of significance, AA: antioxidant activity, df: degree of freedom, TPC: total phenolic content

Table 5.3 Main effect of independent variables on responses

Independent variables	**Values**	**TPC (mg GAE/ 100g DW)**	**AA (µmol TE/100g DW)**
Drying method	O_F-O_U	291.62±10.29[a]	417.95±25.26[a]
	O_F-MW_U	326.96±12.54[b]	478.45±49.19[b]
	MW_F-O_U	318.92±10.43[b]	578.25±54.69[c]
	MW_F-MW_U	350.20±11.53[c]	618.23±49.11[c]
UA, µm	25	310.86±11.45[a]	469.54±22.87[a]
	37	332.88±9.97[b]	571.18±26.85[b]
	50	322.03±10.36[c]	528.94±33.94[c]
UT, min	1	323.01±10.06[a]	527.37±19.66[a]
	2	321.31±9.45[a]	523.85±13.58[a]
	3	321.45±10.66[a]	518.43±13.69[a]

Values within columns for individual variables with different superscript letters are significantly ($p<0.05$) different, AA: antioxidant activity, O: oven drying, MW: microwave drying, F: fermentation, TPC: total phenolic content, U: ultrasonication, UA: ultrasonication amplitude, UT: ultrasonication time

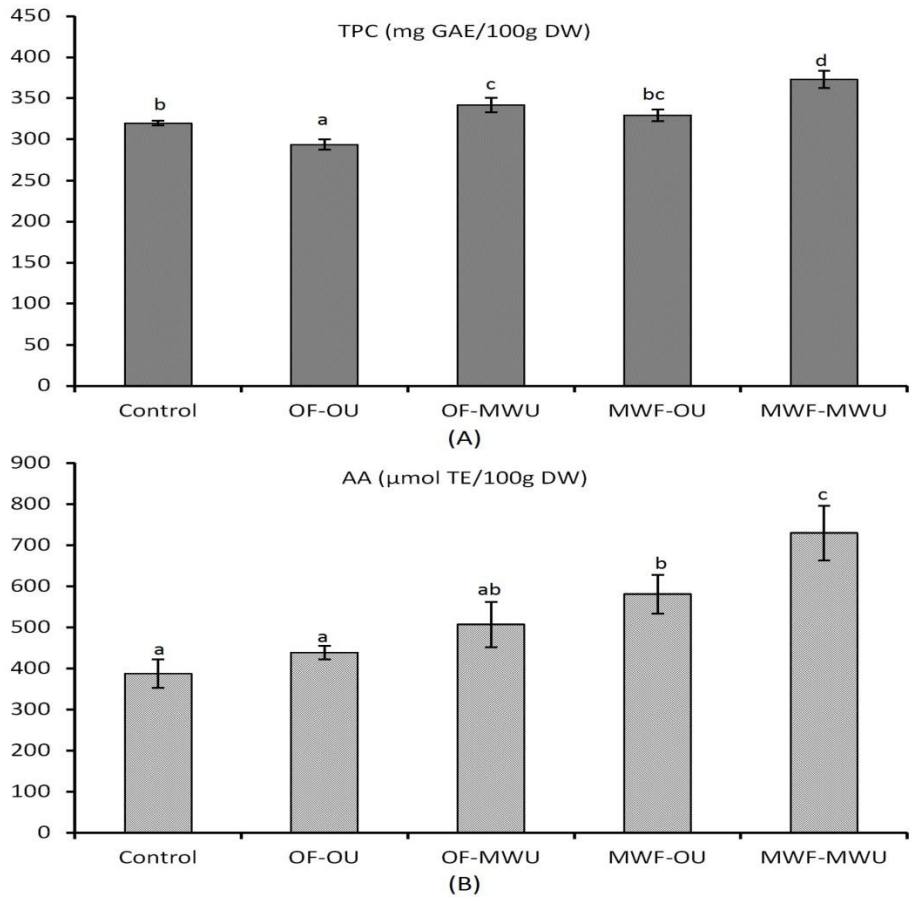


Figure 5.1 Effect of drying methods on total phenolic content (TPC) (A) and antioxidant activity (AA) (B) of apple pomace powder at 50 µm ultrasonication amplitude for 3 min ultrasonication time (O: oven drying, MW: microwave drying, F: fermentation, U: ultrasonication). Bars with different letters are significantly ($p < 0.05$) different

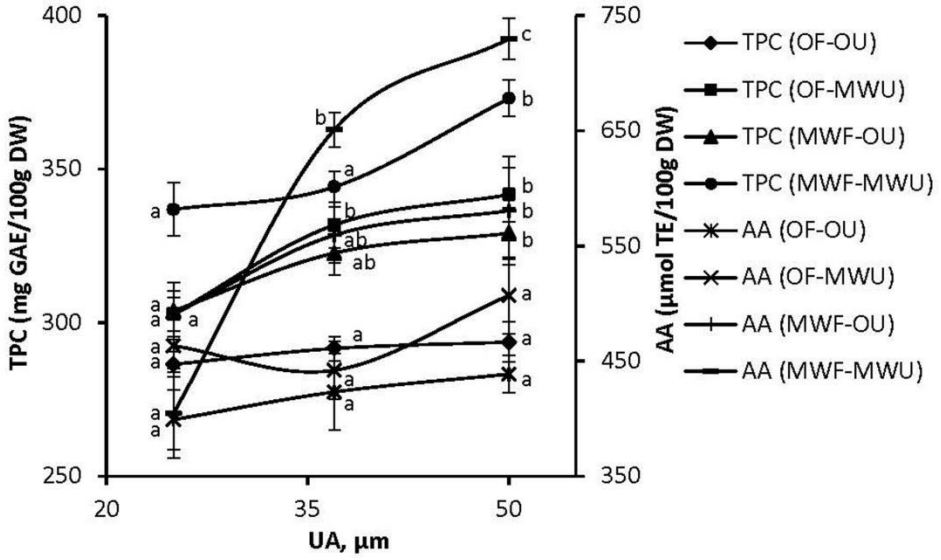


Figure 5.2 Effect of ultrasonication amplitude (UA) on total phenolic content (TPC) and antioxidant activity (AA) of apple pomace powder for 3 min ultrasonication time for all drying methods. Mean values in the same line with different letters are significantly ($p<0.05$) different

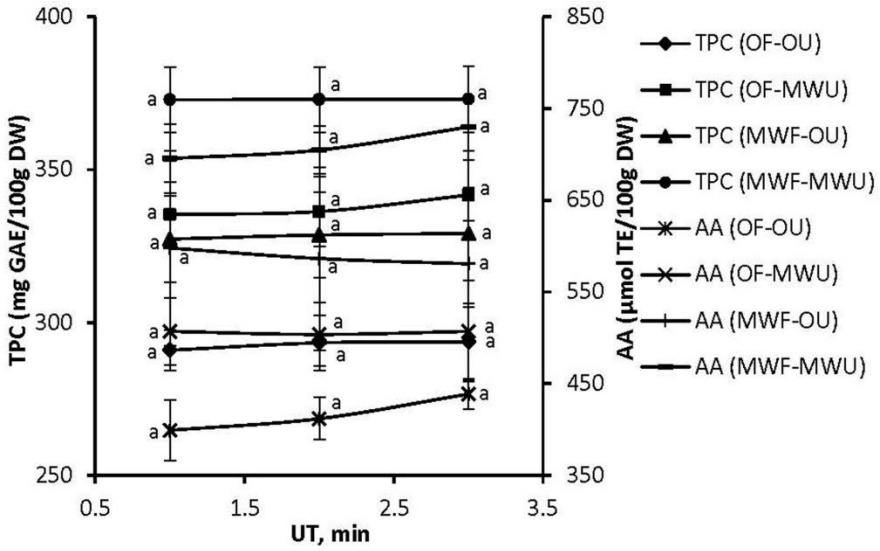


Figure 5.3 Effect of ultrasonication time (UT) on total phenolic content (TPC) and antioxidant activity (AA) of apple pomace at 50 µm ultrasonication amplitude for all drying methods. Mean values in the same line with different letters are significantly ($p<0.05$) different

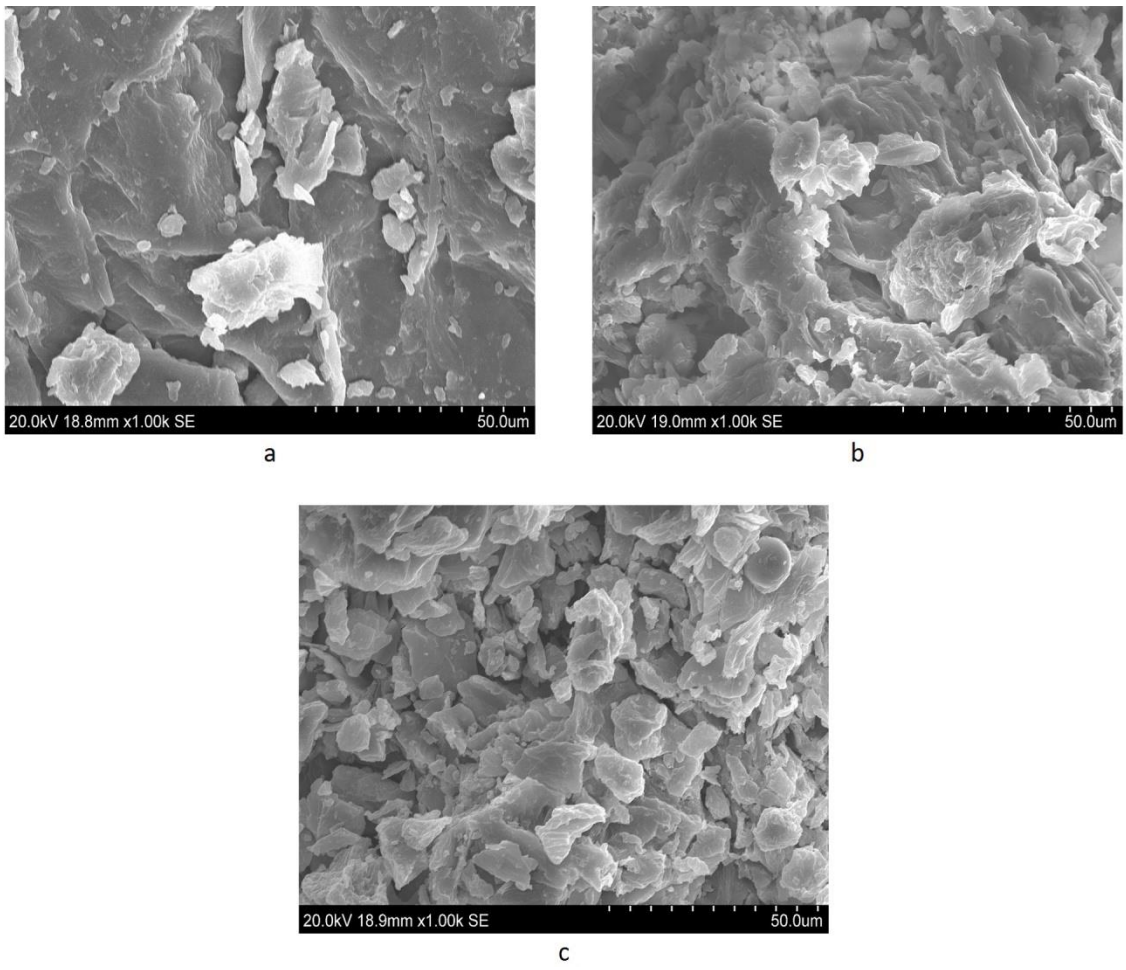


Figure 5.4 Scanning electron micrographs (×1000 magnification) of fresh (a), oven dried O_F-O_U (b), and microwave dried MW_F-MW_U (c) AP powder (12 h fermentation F, ultrasonication U at 50 μm amplitude for 3 min)

CHAPTER 6

Effect of sequential treatments of natural fermentation and ultrasonication followed by microwave drying on bioactive content of apple pomace and the thermal, textural and functional water absorption characteristics of their extrudates[5]

6.1 Abstract

In this study, an approach to enhance the total phenolic content (TPC) and antioxidant activity (AA) in apple pomace using fermentation, ultrasonication and microwave drying was investigated prior to extrusion. Apple pomace (AP) was naturally fermented for 24 h, 48 h, and 72 h, followed by ultrasonication at 22, 37, and 52 W/cm^2 ultrasonication intensity (UI) for 3 min ultrasonication time (UT). For ultrasonication, fermented powder to water ratio (PWR) was kept as 5, 7.5, and 10% (w/v). Fermented and ultrasonicated AP was dried at 90, 270, and 450 W microwave power (MWP) levels. With increase in fermentation time (FT), PWR and MWP, TPC and AA of AP were significantly decreased. Maximum TPC and AA of AP were observed at 37 W/cm^2 UI and were decreased thereafter significantly. AA was positively correlated with TPC of AP for different FT, PWR, UI, and MWP. At optimum pretreatment condition, TPC and AA of AP were found as 424.98 mg GAE/ 100g dry weight (DW), and 846.38 µmol TE/ 100g DW, respectively. Control and pretreated AP were extruded and it was observed that TPC and AA of both the AP extrudates were increased after extrusion but extrudates form pretreated AP had 12%, and 21% more TPC and AA, respectively as compare to that of extrudates from control AP. Extrudates from pretreated AP also had improved

[5] Lohani, U. C. and Muthukumarappan, K. 2016. Effect of sequential treatments of fermentation and ultrasonication followed by microwave drying on bioactive content of Apple Pomace and the thermal, textural and functional water absorption characteristics of their extrudates. International Journal of Food Science & Technology. *Accepted*.

textural (hardness, brittleness, crispness) and functional (water absorption index, water solubility index) properties. Pretreatment reduced crystalline fraction amount and enhanced solubility of AP.

6.2 Introduction

Apple pomace (AP), byproduct from juice and cider processing industries is mostly used for direct disposal to soil in a landfill, and for pectin recovery usage (gelling agent, stabilizer and source of dietary fiber). Despite of that, tons of AP remains unutilized and causes serious environmental threats. Therefore, studies have got momentum to valorize the AP for other purposes also. AP as a rich source of antioxidant compounds could be used for increasing the stability of foods by preventing lipid peroxidation and also for protecting oxidative damage in living systems by scavenging oxygen radicals.

Apple pomace has potential source of polyphenolic compounds and most of these compounds are present in bound forms with carbohydrates, lignin, pectin and proteins (Acosta-Estrada et al., 2014). This bound nature of polyphenolics as glycosides reduces their ability to function as good antioxidants. Therefore, release of these bound phenolics can improve their

health functionality. There are several processes that enhance the liberation of bound phenolics. Germination, malting, fermentation and thermos-mechanical processes such as extrusion cooking and alkaline hydrolysis are most popular (Acosta-Estrada et al., 2014).

Addition of high fiber pomace to extruded products increases antioxidant properties and addresses dietary shortfalls (Reis et al., 2014). Although extrusion increases the antioxidant activity, there were loss of phenolic compounds in AP or AP

inclusive extrudates (Alavi et al., 2014). During extrusion, total phenolic content was decreased due to heat induced polymerization and degradation. Despite of less phenolic content, increase in antioxidant activity of extrudates was because of development of Maillard's reaction products (Reis et al., 2014). Potential detrimental effects of the Maillard's reaction products formed at higher temperatures (Masatcioglu et al., 2013) should also be taken into consideration.

Loss of phenolics can be compensated by enhancing phenolic compounds in raw materials using pretreatments prior to extrusion and can be prohibited during the extrusion by using modified extrusion methods. In this paper, an approach has been made to investigate the pretreatment method to enhance the total phenolic content (TPC) and antioxidant activity (AA) in apple pomace.

Fermentation is one of the pretreatments to release the bound phenolics in plant materials. Ajila et al. (2012) and Gassara et al. (2012) reported that solid-state fermentation improved the polyphenolic content in AP, but the literature related to effect of natural fermentation on AP phenolics are lacking. In natural fermentation, some phenolics can be bound to fiber, proteins, starches and other components in the aqueous fermentation environment and thus may reduce the extractability of phenolic compounds (Taylor and Duodu, 2015). Hypothetically, natural fermentation of apple pomace followed by ultrasonication will increase the total phenolics and antioxidant activity because ultrasonication breaks down composite matrix resulting in releasing of phenolics bound with protein and other components (Zhao et al., 2008).

Our comprehensive literature review revealed that in past few years, ultrasound assisted extraction of phenolic compounds from apple pomace has been extensively

investigated (Ajila et al., 2011, Candrawinata et al., 2014, Grigoras et al., 2013, Pingret et al., 2012) but application of fermentation followed by ultrasonication to enhance the phenolics in apple pomace is limited. Lohani and Muthukumarappan (2015) found significant increase in total phenolic content and antioxidant activity of apple pomace after fermentation followed by ultrasonication and microwave drying but they left the scope of using more level of fermentation time, ultrasonication parameters (intensity, time) and microwave power for further study.

Therefore, keeping in view the above facts, the objective of the study was to observe the influence of sequential treatments of natural fermentation and ultrasonication followed by microwave drying on TPC and AA of AP. Fermentation, ultrasonication and microwave parameters were decided from the research done by Lohani and Muthukumarappan (2015). Also the extrudates from pretreated (fermented and ultrasonicated) AP were compared with those from control (un-pretreated) AP in terms of TPC, AA, thermal, textural (hardness, brittleness, crispness) and functional (water absorption index, water solubility index) properties. Continuous ultrasonication was also compared with batch ultrasonication in terms of TPC and AA.

6.3 Materials and Methods

Apple pomace (AP) provided by Tree Top, Inc. (Selah, WA) was stored at -20°C. For natural fermentation, AP slurry was prepared by adding 12.5 g AP in 100 ml distilled water. The fermentation was carried out in a controlled environment with temperature 30±1°C for 24 h, 48 h and 72 h. Fermented slurry was further diluted to keep the pomace to water ratio (PWR) as 5%, 7.5%, and 10% (w/v). Diluted slurry was then subjected to ultrasonication (VC 505, Sonics and Materials Inc., CT, USA) at 22, 37 and 52 W/cm^2

ultrasonication intensity (UI) for 3 min of ultrasonication time. Ultrasonicated sample was further microwave (MW) dried at 90 W, 270 W and 450 W MW power (MWP) and stored at -20°C for TPC and AA analysis.

For continuous process, ultrasonic device (UIP1000hd, Hielscher Inc., NJ, USA) with 20 kHz frequency, 1000 W power and a sonotrode of 22 mm tip diameter was used. The effective volume of flow cell after intruding sonotrode was 165 ml. AP was fermented for 24 h (optimized condition from batch ultrasonication) and then slurry was further diluted to keep the flour to water ratio (FWR) as 5%, 7.5%, and 10% (w/v). Diluted slurry (2000 ml) was then subjected to ultrasonication at 20, 25, and 30 W/cm^2 ultrasonication intensity (UI) for 120, 150, and 180 s ultrasonication time (UT). Flow rate (FR) of slurry during ultrasonication was kept constant as 4 ml/s from previous study (Chapter 4). Ultrasonicated sample was further microwave (MW) dried at 90 W MW power (optimized condition from batch ultrasonication) and stored at -20°C for TPC and AA analysis.

6.3.1 Extraction of samples

The extraction of sample for determining TPC, AA and phenolic characterization was done using the method described by Khan et al. (2013). For determining TPC and AA, 1 g of AP 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°C until analysis for TPC and AA.

Free phenolic acid extraction was performed by adding 10 ml of 80% (v/v) aqueous methanol into 2 g of AP. Mixture was shaken in a shaking water bath for 1 h at

25°C. After centrifugation at 3000×*g* for 20 min, the supernatant was decanted and the extraction was repeated as described above. The two supernatants were combined, evaporated to near dryness and reconstituted with methanol to a final volume of 10 ml.

6.3.1.1 Total phenolic content (TPC)

TPC of AP was determined using Folin–Ciocalteu method (Singleton et al., 1999) with some modification. 50 µl methanol extract of sample was added with 3.5 ml distilled water and 150 µl Folin-Ciocalteu reagent. The solution was vortexed and incubated for 30 min. Thereafter, absorbance of solution was measured at 760 nm against blank. Blank solution contained all the components that were present in the sample except the methanol extract. Gallic acid was used as positive control (standard) and linear regression curve between absorbance and concentration was drawn for the standard. This standard curve was used for calculating the concentration of sample and data was expressed in mg Gallic acid equivalent (GAE)/100 g dry weight (DW). This analysis was done in six replications.

6.3.1.2 Antioxidant activity (AA)

Extinction of DPPH is a free radical scavenging activity which was measured using spectrophotometric method described by Brand-Williams et al. (1995). 2,2-diphenyl-1-picrylhydrazyl (DPPH) 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 for 30 min. Thereafter, 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 weight (DW). Samples were analyzed in six replications.

6.3.1.3 Free phenolic acid characterization

Analysis of sample extracts was carried out using Thermo Scientific, Dionex Ultimate 3000 UHPLC system (Bannockburn, IL, United States) equipped with diode-array detector (DAD) and C_{18} column (150 mm × 4.6 mm) packed with 5 μm particles. The samples were injected with a mobile-phase flow rate of 800 μl/min. Gradient elution was carried out with a solvent system of water/acetic acid (99.8:0.2 v/v) as mobile phase A and acetonitrile/acetic acid (99.8:0.2 v/v) as mobile phase B. The total run time was 12 min, and the gradient elution was as follows: 0.0−3.0 min, B 10-25%; 3.0−4.5 min, B 25−45%; 4.5−6.5 min, B 45−65%; 6.5−8.0 min, B 65−85%; 8.0−9.0 min, B 85−100%.; 9.0−12.0 min, B 100−10%. All the solvents were filtered through 0.22 μm PTFE filters prior to inject. The column was maintained at 30°C while the autosampler was thermostated at 4°C. The system was controlled by Thermo Scientific Dionex Chromeleon 7 software. Benzoic acid and cinnamic acid derivatives were detected at 280 nm and 320 nm, respectively. The concentrations of phenolic acids were calculated from peak areas in comparison to calibration curves of the respective standards and were expressed as μg/g DW.

6.3.2 Moisture content, total dietary fiber (TDF), soluble dietary fiber (SDF), insoluble dietary fiber (IDF)

Moisture content of AP was determined by air oven standard methods recommended by AOAC (1980). Initially 5 g of sample in triplicate was dried in hot air oven at 130-133°C for 2 h. After drying, dried sample was again weighed. Following formula is used for calculating the moisture content (MC).

$$\text{MC (\%wb)} = \frac{W_i - W_f}{W_i} \times 100 \qquad (6.1)$$

W_i= initial weight of sample (5 g),

W_f= weight of sample after drying, g

Total dietary fiber in all extrudate samples was measured in the laboratory by the AOAC approved method 991.43 (AOAC, 1992). Duplicate samples of milled samples were suspended in MES/TRIS buffer (0.05 M, pH 8.2 at 24°C) and incubated sequentially with heat-stable α-amylase (95-100°C, 30 min) to give gelatinization, hydrolysis and de-polymerization of starch, protease (60°C, 30 min) to solubilize and depolymerize proteins, and amyloglucosidase (60°C, 30 min, pH 4.5) to hydrolyse starch fragments to glucose. The enzyme digestate was then treated with four volumes of 95% ethanol (1 h) to precipitate soluble fiber. The alcohol-treated digestate was filtered through borosilicate sintered glass crucibles (40-90 μm) that had previously been matted with celite, dried, and weighed. The total dietary fiber residue present in the crucible was washed with alcohol and acetone, dried overnight (103°C), and weighed. One duplicate from each sample was used for ash determination (525°C muffle furnace) and the other for protein determination. Total dietary fiber percent (TDF, g/100g DW) was calculated as

$$\text{TDF}=\frac{\frac{R_1+R_2}{2}-p-A-B}{\frac{m_1+m_2}{2}}\times\frac{100}{DW}\times 100 \tag{6.2}$$

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 R_2, B is the blank and DW is percentage dry weight (g) of sample.

$$B=\frac{BR_1+BR_2}{2}-BP-BA \tag{6.3}$$

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 Insoluble dietary fiber (IDF) and soluble dietary fiber (SDF), 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 Eq (6.2) and (6.3).

6.3.3 Scanning electron microscopy

The microstructure of control, and optimally pretreated apple pomace was examined using a scanning electron microscope (SEM) (Hitachi-S3400 N, Tokyo, Japan). Small amounts of samples were mounted on SEM specimen stubs by using double-sided adhesive tape. Each powder sample was coated with 10 Å thick layer of gold in a sputter coater before being scanned and photographed at 1000× magnification.

6.3.4 Extrusion cooking

Control AP and pretreated AP at optimum condition with 20% moisture content were extruded in a single screw extruder (Brabender Plasti-corder, model PL 2000, South Hackensack, NJ) at 120°C die temperature and 90 rpm screw speed. Compression ratio, die dimension, and length to diameter ratio of barrel were kept constant as 3:1, 3 mm, and 20:1, respectively.

6.3.5 Texture analysis

Extrudates from control AP and pretreated AP at optimum conditions were subjected to hardness, brittleness and crispness using TA-XTPlus Texture Analyzer

(Texture Technologies Corp., Hamilton, MA) as per method described by Altan et al. (2008b). The peak force as an indication of hardness was measured with a TA-XTPlus Texture Analyzer (Texture Technologies Corp., Hamilton, MA) using 3-point bend test. The test speed was 2 mm/s and the distance between two supports was kept as 22 mm. The curve between force vs distance was plotted and analyzed by Texture Exponent 32 software program (version 3.0). The slope (N/mm) and distance (mm) at which a product breaks were measured from force–distance curve and evaluated as crispness and brittleness, respectively (Texture Technologies, a). Both the sample extrudates were replicated 10 times. The radial expansion ratio of the extrudates was measured as the ratio of the diameter of the extrudates to the diameter of the die orifice.

6.3.6 Water absorption index (WAI) and water solubility index (WSI)

To determine the WAI and WSI of extrudates, the methodology proposed by Anderson (1982) was followed. Ground extrudates (2.5 g) was suspended in distilled water (30 ml) in a tarred 60 ml centrifuge tube. The suspension was stirred intermittently and centrifuged at 3000×*g* for 10 min. The supernatant was decanted into a tarred aluminums cup and dried at 135°C for 2 h. The weight of the gel remaining in the centrifuge tube was measured. The WAI and WSI were calculated by

$$\text{WAI}=\frac{W_g}{W_{ds}}\times 100 \tag{6.4}$$

where, WAI is water absorption index, W_g is the weight of gel (g), and W_{ds} is the weight of dry sample (g).

$$\text{WSI}=\frac{W_{ss}}{W_{ds}}\times 100 \tag{6.5}$$

where, WSI is the water solubility index (%), W_{ss} is the weight of dry solids of supernatant (g), and W_{ds} is the weight of dry sample (g).

6.3.7 Thermal properties

Differential scanning thermal analysis of control AP, optimally pretreated AP and extrudates developed from both was performed using the method described by Mahasukhonthachat et al. (2010). Analysis was performed using a differential scanning calorimeter (DSC) (TA Instruments Model Q2000, New Castle, DE, USA) previously calibrated with indium. The extrudates were ground and sieved through a 295-µm screen. Powder samples of 10 mg, DM were weighed directly into DSC hermetic aluminum pans, and 20µl of distilled water was added using a micro-syringe. The sample pans were sealed and allowed to equilibrate for overnight at room temperature. Samples were heated from 0 to 300°C with heating rate of 5°C/min. Gelatinization peak temperature and the enthalpy of the endotherm for gelatinization and the peak melting temperature of the amylose–lipid complex and the enthalpy of melting of the amylose–lipid complex were measured and calculated using TA Universal Analysis, version 5.5.17 (TA Instruments, New Castle, DE, USA).

6.3.8 Statistical analysis

Full factorial design was used for experimental plan and results were compared by analysis of variance (ANOVA) using SPSS (16.0) statistical software. Optimization of independent variables was obtained using statistical software package (Design Expert 9.0.3.1, Stat-Ease Inc., USA). All data were reported as mean ± standard deviation of replicates. Tukey's tests were used to compare the significant differences of the mean values with the family error rate held at 0.05.

6.4 Results and Discussion

6.4.1 Effect of Fermentation time and ultrasonication on TPC and AA

It is explicated from ANOVA that fermentation time (FT) had significant ($p<0.05$) effect on TPC and AA (Table 6.1). Interaction effect of FT with PWR, UI and MWP on TPC and AA was also found significant ($p<0.05$). When fermentation time increased from 24 h to 72 h, TPC and AA of ultrasonicated AP significantly ($p<0.05$) decreased by 18.2% and 34%, respectively (Figure 6.1). When AP was subjected to 24 h of fermentation, phenols bounded with protein and starch were released by ultrasonication. Decarboxylation of hydroxycinnamic acids also caused increase in TPC and AA. Kayodé et al. (2013) reported that the carboxyl group exerted a negative effect on the antioxidant capacity of phenolic acids. It is obvious from Table 6.2 that concentrations of cinnamic acid derived phenolic acids, i.e. chlorogenic and salicylic acids after 24h fermentation followed by ultrasonication (pretreated AP) were significantly ($p<0.05$) higher. Further increase in fermentation time up to 72 h induced hydrolysis of the glycosidic bonds of bound phenolics, and degradation of bound phenolics during fermentation and therefore, TPC and AA were not improved by further ultrasonication. These results are in accord with the findings of Ajila et al. (2012) for AP. TPC and AA of AP were observed as 404.4 mg GAE/100g DW, and 803.1 µmol TE/100g DW, respectively on average after 24 h fermentation followed by ultrasonication. TPC and AA of AP after 24 h fermentation were 14% and 41.5% higher ($0<0.05$) than that of control AP (data not shown). These results were in agreement with the findings of Ajila et al. (2012) and Gassara et al. (2012).

6.4.2 Effect of flour to water ratio on TPC and AA

It is indicated by ANOVA that PWR and its interaction with UI and MWP had significant ($p<0.05$) effect on TPC and AA of AP (Table 6.1). With increase in PWR from 5% (w/v) to 7.5% (w/v), TPC and AA of AP significantly ($p<0.05$) decreased by 5.5% and 10%, respectively, however with further increase in PWR, there was no significant ($p>0.05$) drop in TPC and AA of AP (Figure 6.1). Similar results were found by Gribova et al. (2008) for AA of bearberry leaves. With increase in PWR, sample got more concentrated and viscous and thus hindered ultrasound wave to travel uniformly in the whole sample at a given ultrasonication intensity. This resulted in low cavitation and less release of phenolics in the solution. TPC and AA of AP were determined as 383.7 mg GAE/100g DW, and 737.4 µmol TE/100g DW, respectively on average at 5% (w/v) pomace to water ratio.

Like batch ultrasonication, continuous process exhibited the similar effect of PWR on TPC and AA of the AP (Figure 6.2). However, average values of TPC and AA at 5% (w/v) PWR were 3.8% and 1.5% higher with compared to that of batch ultrasonication.

6.4.3 Effect of ultrasonication intensity on TPC and AA

It is depicted from ANOVA that UI along with its interaction with MWP significantly ($p<0.05$) effected TPC and AA of AP (Table 6.1). TPC and AA increased significantly ($p<0.05$) by 4.2% and 5.0%, respectively with increase in UI from 22 W/cm^2 to 37 W/cm^2. Further increase in UI did not show any significant ($p>0.05$) change in TPC an AA (Figure 6.3). Yu et al. (2012) also observed a decrease in antioxidant activity of peanut hydrolysate with increase in ultrasonication power. At higher intensity,

due to agitation of sample instead of cavitation, bubble cloud density became too large which resulted in rise to shielding effects, coalescence and general bubble-bubble interactions that decreased the overall cavitation efficiency of the process. AP had TPC and AA as 368.9 mg GAE/100g DW, and 688.6 µmol TE/100g DW, respectively on average at 37 W/cm^2 ultrasonication intensity.

Continuous ultrasonication exhibited the different behavior of ultrasonication intensity on TPC and AA of the AP. Increasing the UI from 20 to 30 W/cm^2, TPC and AA gradually decreased ($p<0.05$) by 10.9% and 7.2%, respectively (Figure 6.2). The average TPC and AA values of the AP at 20 W/cm^2 were observed as 383.7 mg GAE/100g DW and 701.4 µmol TE/100g DW, respectively.

6.4.4 Effect of microwave power on TPC and AA

It is shown by ANOVA that MWP and its interaction with other experimental parameters had significant ($p<0.05$) effect on TPC and AA of AP (Table 6.1). Figure 6.3 shows that TPC and AA decreased significantly ($p<0.05$) by 15.6% and 30%, respectively with increase in MWP from 90 W to 450 W. At higher power, due to increase in heat, phenolics were degraded and resulted in less TPC and AA of AP. Kammoun Bejar et al. (2011) also reported that TPC of orange peel decreased at higher microwave power level.

6.4.5 Effect of ultrasonication time on TPC and AA

Figure 6.2 represented that the effect of ultrasonication time on TPC and AA of the AP was found insignificant ($p>0.05$) during continuous ultrasonication. The AP had the average 383.7 mg GAE/100g DW TPC and 701.4 µmol TE/100g DW AA at 120 s.

For batch ultrasonication, optimum conditions for TPC and AA of the AP was observed as 24 h fermentation time, 5% (w/v) pomace to water ratio, 37 W/cm^2 ultrasonication intensity, and 90 W microwave power. TPC and AA of control AP were 302.9 mg GAE/100g DW TPC and 438.6 µmol TE/100g DW, respectively and were increased by 40.3% and 93%, respectively when pretreated at optimum conditions (Figure 6.4). Increase in TPC and AA of pretreated AP was dominantly influenced by protocatechuic acid followed by chlorogenic acid and salicylic acid (Table 6.2). The optimum conditions for continuous ultrasonication were obtained as 5% (w/v) PWR, 20 W/cm^2 UI and 120 s UI with the maximum TPC and AA as 441.4 mg GAE/100g DW TPC and 858.6 µmol TE/100g DW, respectively (Figure 6.4).

6.4.6 Relationship between TPC and AA

The values of AA indicated a positive correlation with the values of TPC of apple pomace at different fermentation time, pomace to water ratio and microwave power for batch ultrasonication. A linear correlation in each case was observed between AA and TPC. The correlation coefficient (r) were 0.975, 0.999, and 0.887 at different FT, FWR, and MWP, respectively which indicated that TPC was the major factor accounting for the antioxidant activity of the apple pomace. Correlation experiments to predict the antioxidant properties have been performed by many authors. Candrawinata et al. (2015), and Lohani and Muthukumarappan (2015) also reported high correlation between TPC and AA of apple pomace.

6.4.7 Effect of fermentation and ultrasonication on TPC and AA of AP extrudates

Extrudates from control AP and pretreated AP at optimum conditions (24 h FT, 5% (w/v) PWR, 37 W/cm^2 UI, and 3 min UT) were analyzed for TPC and AA (Table

6.3). It was observed that TPC of extrudates from control AP reduced by 25.5%, whereas AA increased by 15.3% with compare to control AP. Similar results were found by Alavi et al. (2014) for apple pomace. Decrease in TPC of AP was because of degradation of phenolic compounds due to thermo-mechanical treatment during extrusion. On the other hand, AA increased despite the abstraction in TPC due to development of Maillard's reaction products during extrusion. TPC of extrudates from pretreated AP also reduced by 18.6% after extrusion, but it was 14.6% more than that of control AP. Extrudates from pretreated AP exhibited 94.5% more AA as compare to that extrudates from control AP. These results were confirmed by the characterization of phenolic acids. Table 6.2 explicated that concentration of benzoic acid and cinnamic acid derived phenolic acids were found higher in pretreated AP extrudates as compare to that of control AP extrudates, however caffeic acid and ferulic acid values of both the samples were not significantly ($p>0.05$) different.

6.4.8 Microstructure analysis of control and pretreated apple pomace

Figure 6.5 shows the changes in physical structure of control and pretreated samples of apple pomace at optimum conditions were imaged by scanning electron microscope. The structure of control AP, which is a compact and less porous structure (Figure 6.5a). The SEM images revealed that the pretreatment resulted in modification of the cellular structure. Fermentation and ultrasonication damaged the plant cell structure and caused more porosity (Figure 6.5b). Pretreated sample fiber granules were more disrupted than control sample granules which caused increase in surface area and helped in releasing more phenolics which were bound with carbohydrates matrix (Figure 6.5).

6.4.9 Textural analysis of pretreated AP extrudates

Table 6.3 showed that the pretreatment of AP prior to extrusion significantly ($p<0.05$) increased the brittleness and crispness of extrudates by 11.5% and 10.9%, respectively, while hardness of the extrudates from pretreated AP significantly ($p<0.05$) decreased by 29.5% with compare to that of extrudates from control AP. Expansion ratio that is attributed to puffing of extrudates was not significantly ($p>0.05$) increased when AP was pretreated prior to extrusion. However, extrudates obtained from pretreated AP exhibited the smoother surface (Figure 6.6). Pretreatment broke the protein and carbohydrates molecule that made more starch available for gelatinization during extrusion. Pretreatment followed by extrusion also significantly ($p<0.05$) enhanced the soluble fiber in AP (Table 6.4) which increased the elastic properties and hence enhanced the affinity between starch and fiber. During extrusion, elastic matrix was formed upon passing through the die and because of that the gas-retaining capability of starch increased resulting in the formation of stable and fine air cells in the expanded products. Puffer the extrudates, more the air cells they contained and because of that, extrudates had less hardness and thus had more crispness and brittleness. This is in agreement with the results of Altan et al. (2008b) who also found the similar relationship between hardness, crispness and brittleness.

6.4.10 Effect of pretreatment on WAI and WSI of AP extrudates

Extrudates from pretreated AP exhibited 8% lower ($p<0.05$) WAI compared to that of extrudates from control AP (Table 6.3). Water absorption index measures the amount of water absorbed by starch. Pretreatment of AP significantly ($p<0.05$) increased

the soluble fiber (Table 6.4) that decreased the starch molecular degradation because of starch and fiber interaction.

Water solubility index measures the amount of soluble components released during extrusion. Data in Table 6.3 revealed that extrudates from pretreated AP had 10% higher ($p<0.05$) WSI than that of extrudates from control AP. Pretreatment of AP followed by extrusion led to solubilizing the rigid cell wall structure of AP and also increased ($p<0.05$) the solubility of fiber (Table 6.4) and thus enhanced the WSI of extrudates..

6.4.11 Effect of pretreatment on thermal properties of AP and extrudates

Differential scanning calorimetry of control and pretreated AP and their extrudates depicted that pretreatment lowered ($p<0.05$) the melting temperature and enthalpy of AP (Table 6.4). Control AP had more ($p<0.05$) insoluble fiber (Table 6.4) which has more hydrophilic properties and has been attributed to absorb more water and thus needed more energy to absolutely remove the water. Extrudates from pretreated AP exhibited the lower ($p<0.05$) melting temperature and enthalpy than that of extrudates from control AP (Table 6.4). For extrudates, these properties indirectly related to WAI.

6.5 Conclusions

Natural fermentation and ultrasonication pretreatment of AP played a significant role in enhancing the TPC, and AA. Fermentation time, pomace to water ratio, ultrasonication intensity and microwave drying had significant ($p<0.05$) effect on TPC and AA of AP. The optimum pretreatment condition for AP was found as 24 h fermentation time, 5% (w/v) pomace to water ratio, 37 W/cm^2 ultrasonication intensity, and 90 W microwave power for batch ultrasonication. TPC and AA of pretreated AP

were 40.3% and 93% higher than that of control AP, respectively. AA of the AP showed the linear correlation with the TPC for all experimental variables. The TPC and AA of the AP obtained at the optimum conditions of continuous ultrasonication, i.e. 5% (w/v) pomace to water ratio, 20 W/cm^2 ultrasonication intensity and 120 s ultrasonication intensity were 3.9% and 1.4% higher than that of batch process despite of using 46% and 33% less ultrasonication intensity and time, respectively in continuous process.

Extrudates from pretreated AP had more TPC and AA than that of control AP extrudates. Despite the loss of TPC during extrusion, AA of the extrudates from control and pretreated AP increased due to development of Maillard's reaction products. Pretreatment exhibited more brittleness, crispness and less hardness of AP extrudates. WAI and WSI data revealed that extrudates from pretreated AP had more digestibility. DSC data showed that the pretreatment reduced the melting temperature and enthalpy of AP. Overall, this study revealed that the fermented AP followed by the ultrasonication can be used as raw ingredients to formulate the cereal extrudates rich in texture, functional and antioxidant properties.

Table 6.1 Variance analysis for total phenolic content (TPC) and antioxidant activity (AA) of apple pomace

Sources	**df**		***SS***	**MS**	**F-value**	**p-value**
FT	2	TPC	90046.9	45023.5	2902.9	0.000*
		AA	885528.4	442764.2	18443.9	0.000*
PWR	2	TPC	8455.3	4227.6	272.6	0.000*
		AA	141804.2	70902.1	2953.5	0.000*
UI	2	TPC	1509.7	754.9	48.7	0.000*
		AA	28419.9	14209.9	591.9	0.000*
MWP	2	TPC	48915.4	24457.7	1576.9	0.000*
		AA	696640.7	348320.4	14509.7	0.000*
FT*PWR	4	TPC	17288.4	4322.1	278.7	0.000*
		AA	17417.1	4354.3	181.4	0.000*
FT*UI	4	TPC	2504.3	626.1	40.4	0.000*
		AA	27179.8	6794.9	283.1	0.000*
FT*MWP	4	TPC	3478.1	869.5	56.1	0.000*
		AA	21114.4	5278.6	219.9	0.000*
PWR*UI	4	TPC	5471.4	1367.8	88.2	0.000*
		AA	31306.5	7826.6	326.0	0.000*
PWR*MWP	4	TPC	2718.8	679.7	43.8	0.000*
		AA	126123.5	31530.9	1313.5	0.000*
UI*MWP	4	TPC	188.1	47.0	3.03	0.018*
		AA	33787.1	8446.8	351.9	0.000*
FT*PWR*UI	8	TPC	7961.2	995.1	64.2	0.000*
		AA	131732.4	16466.5	685.9	0.000*
FT*PWR*MWP	8	TPC	5414.6	676.8	43.6	0.000*
		AA	99183.7	12397.9	516.4	0.000*
FT*UI*MWP	8	TPC	3387.5	423.4	27.3	0.000*
		AA	107153.8	13394.2	557.9	0.000*
PWR*UI*MWP	8	TPC	4294.3	536.8	34.6	0.000*
		AA	56859.3	7107.4	296.1	0.000*
FT*PWR*UI*MWP	16	TPC	978.2	61.1	3.94	0.000*
		AA	22227.1	1389.2	57.9	0.000*

*significant at 5% level of significance, AA: antioxidant activity, df: degree of freedom, FT: fermentation time, MS: mean square, MWP: microwave power, PWR: pomace to water ratio, *SS*: sum of squares, TPC: total phenolic content, UI: ultrasonication intensity

Table 6.2 Phenolic profile of control and pretreated apple pomace (AP) and their extrudates (mg/100g DW)

Compounds	Control AP	Pretreated AP	Control apple pomace extrudate	Pretreated apple pomace extrudate
Benzoic acids				
Protocatechuic acid	39.2±0.77[a]	70.7±1.41[b]	26.1±0.22[c]	53.1±0.49[d]
Cinnamic acids				
Chlorogenic acid	7.25±0.24[a]	10.3±0.27[b]	4.37±0.11[c]	7.97±0.19[d]
Caffeic acid	13.1±0.32[a]	14.4±0.39[b]	12.3±0.31[c]	13.6±0.31[a]
p-coumaric acid	2.44±0.13[a]	3.81±0.12[b]	1.73±0.09[c]	2.91±0.24[d]
Ferulic acid	6.43±0.29[a]	7.98±0.18[b]	5.16±0.24[c]	6.58±0.13[a]
Salicylic acid	13.2±0.27[a]	18.1±0.21[b]	10.6±0.28[c]	14.7±0.23[d]

Means±SD in the same row with different letters are significantly different ($p<0.05$)

Table 6.3 Nutritional, textural and functional analysis of extrudates form control and pretreated apple pomace

Extrudates	TPC* (mg GAE/100g DW)	AA* (µmol TE/100g DW)	Brittleness (mm)*	Hardness (N)*	Crispness (N/mm)*	Expansion ratio	WAI*	WSI* (%)
Control	224.99±4.52	490.14±4.34	5.04±0.35	11.5±1.10	3.97±0.11	0.98±0.02	4.89±0.13	34.63±0.57
Pre-treated	346.07±5.26	953.58±6.52	4.52±0.21	8.14±0.61	3.58±0.09	1.02±0.03	4.49±0.04	38.10±0.38

*mean±SD values in column are significantly different at 5% level of significance, AA: antioxidant activity, TPC: total phenolic content, WAI: water absorption index, WSI: water solubility index

Table 6.4 Thermal properties and dietary fiber of control and pretreated apple pomace (AP) and their extrudates

Samples	Flow/melting		Dietary fiber (g/100g DW)	
	Peak temperature (°C)	Enthalpy (J/g)	SDF	IDF
Control AP	162.2±2.81[a]	127.9±1.19[a]	10.6±0.09[a]	27.7±0.06[a]
Pretreated AP	149.4±2.03[b]	120.4±1.06[b]	11.9±0.05[b]	26.3±0.06[b]
Control extrudates	137.7±1.93[c]	124.8±0.91[c]	11.6±0.07[c]	26.6±0.05[c]
Pretreated extrudates	112.1±3.01[d]	117.2±0.69[d]	13.2±0.06[d]	24.9±0.05[d]

mean±SD values in column are significantly different at 5% level of significance, IDF: insoluble dietary fiber, SDF: soluble dietary fiber

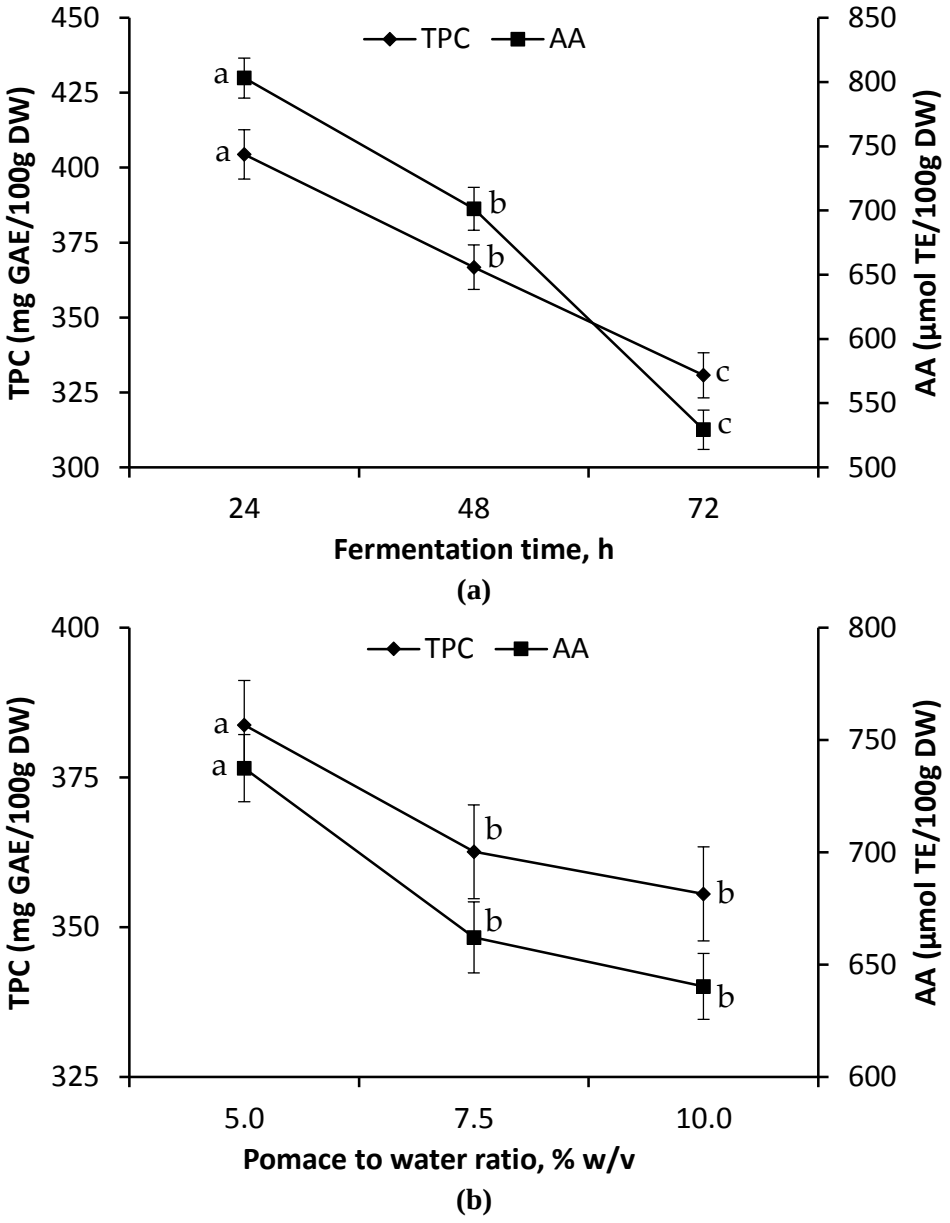


Figure 6.1 Main effect of fermentation time (a) and pomace to water ratio (b) on total phenolic content (TPC) and antioxidant activity (AA) of apple pomace in batch ultrasonication. Values with the different letters at different points in the same line are significantly ($p < 0.05$) different

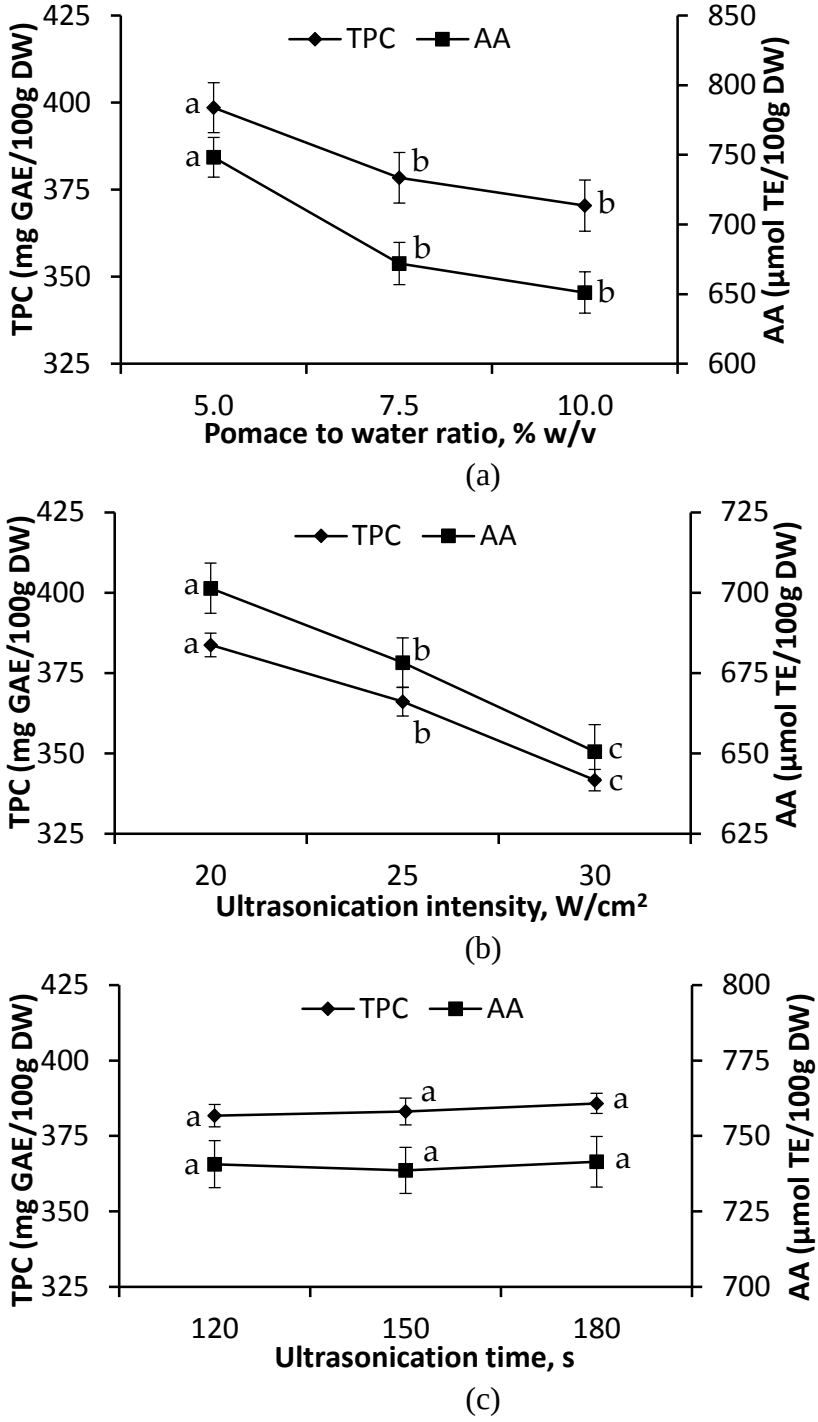


Figure 6.2 Main effect of pomace to water ratio (a), ultrasonication intensity (b) and ultrasonication time (c) on total phenolic content (TPC) and antioxidant activity (AA) of apple pomace in continuous ultrasonication. Values with the different letters at different points in the same line are significantly ($p < 0.05$) different

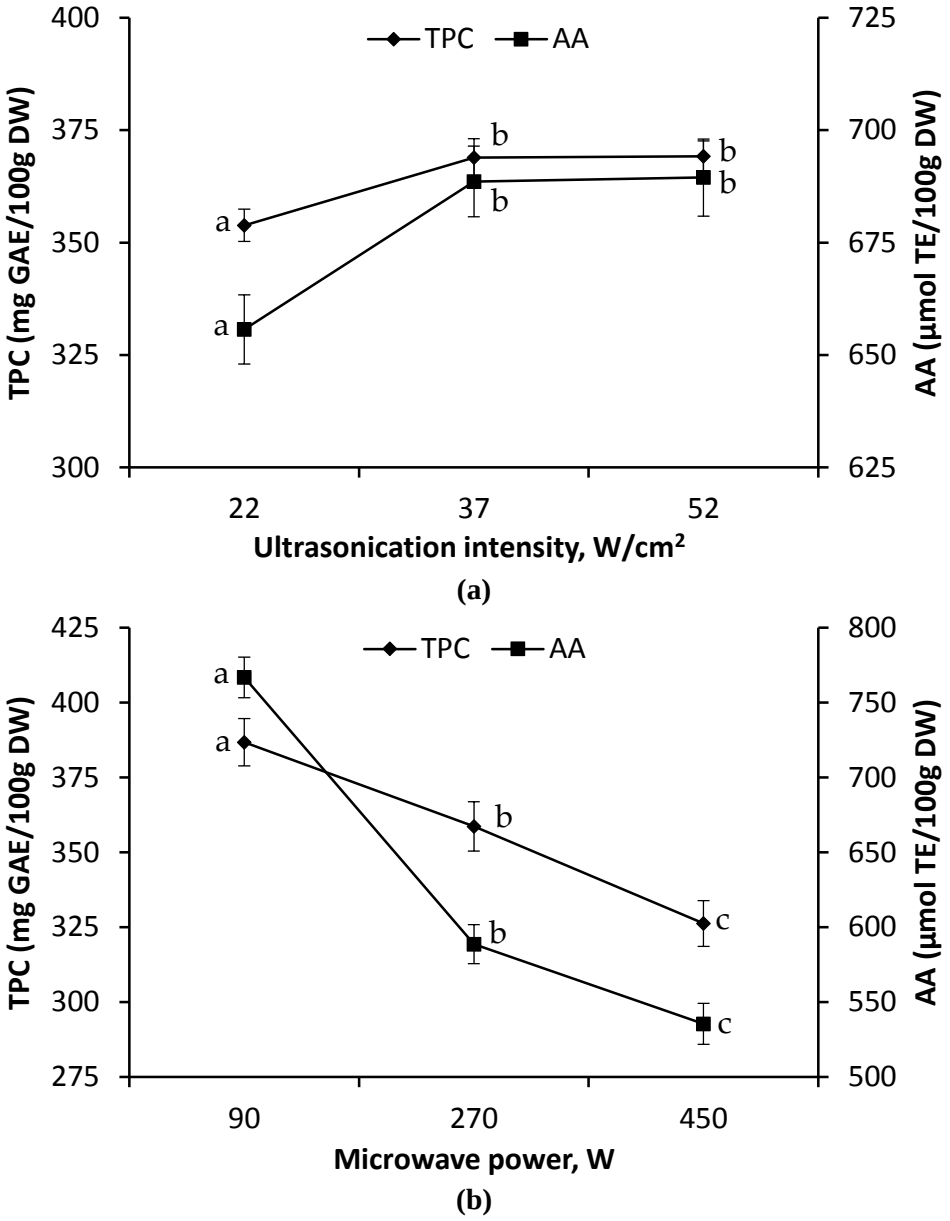


Figure 6.3 Main effect of ultrasonication intensity (a) and microwave power (b) on total phenolic content (TPC) and antioxidant activity (AA) of apple pomace in batch ultrasonication. Values with the different letters at different points in the same line are significantly ($p < 0.05$) different

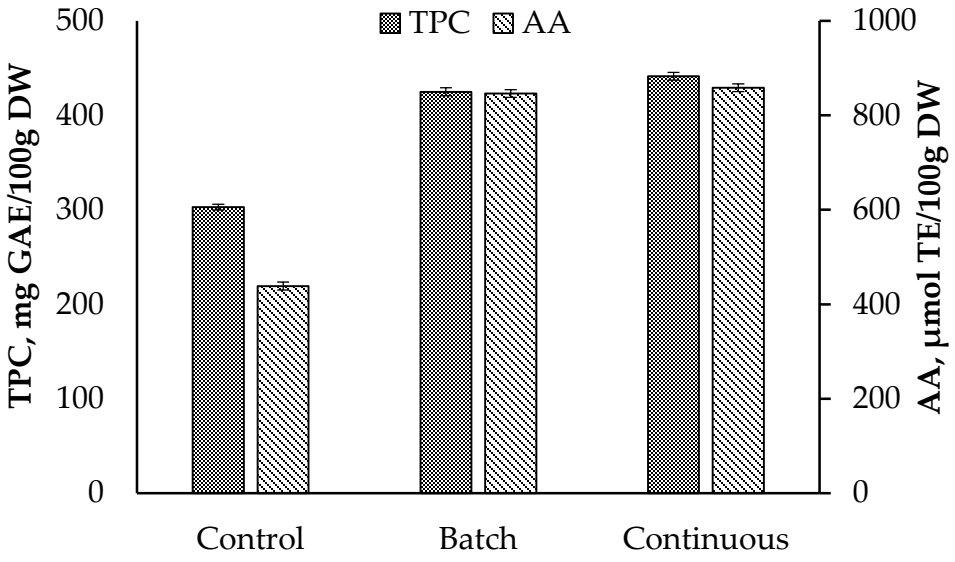
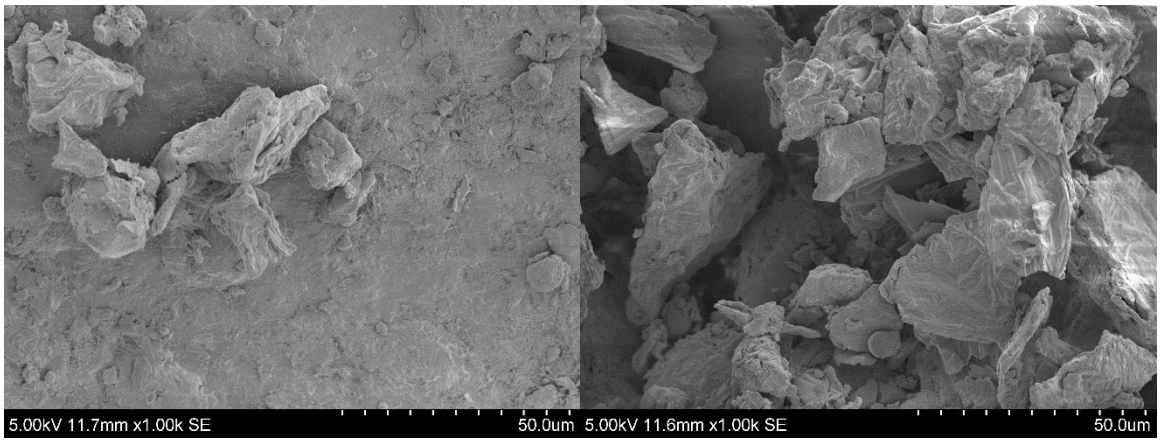


Figure 6.4 Comparison of TPC and AA of control apple pomace with that of pretreated at optimum conditions (24 h fermentation time FT, 5% (w/v) flour to water ratio FWR, 37 W/cm^2 ultrasonication intensity UI, and 90 W microwave power MWP) of batch ultrasonication and continuous ultrasonication (5% (w/v) FWR, 20 W/cm^2 UI, and 120 s ultrasonication time UT)



a **b**

Figure 6.5 Scanning electron micrographs (×1000 magnification) of (a) control, and (b) batch ultrasonicated apple pomace (24 h fermentation time, 5% (w/v) flour to water ratio, 37 W/cm^2 ultrasonication intensity, and 90 W microwave power)

(a) (b)

Figure 6.6 Images of extrudates from (a) control and (b) batch ultrasonicated apple pomace

CHAPTER 7

Application of pulsed electric field to release bound phenolics in sorghum flour and apple pomace[6]

7.1 Abstract

Mild intensity pulsed electric field (PEF) was studied to release the bound phenolics in sorghum flour (SF) and apple pomace (AP). In this study, SF and AP, naturally fermented at optimized conditions, were treated at different flour to water ratio (FWR) of 10, 27.5, 45% (w/v) and 5, 8.75, 12.5% (w/v), respectively. In addition, three levels of PEF electric field intensity (EFI) as 1, 2 and 3 kV/cm were used to treat SF and AP for the treatment time of 500, 875 and 1250 µs. Both the treated samples were analyzed for total phenolic content (TPC), antioxidant activity (AA) and phenolic characterization. For SF, optimized conditions were determined as 45% (w/v) FWR, 2 kV/cm EFI and 875 µs treatment time, while these values were 12.5% (w/v) FWR, 2 kV/cm EFI and 500 µs for AP. At these conditions, TPC and AA of SF were 24.8% and 33.9%, respectively higher than the control SF, while for AP, these numbers were observed as 37.4% and 86%, respectively. The study suggests that PEF treated SF and AP may be very useful for the preparation of processed foods with increased levels of phenolic antioxidants.

7.2 Introduction

In last few years, researches have got momentum to incorporate underutilized crops and industrial food by-products in human diet as they especially contain phenolic

[6] Lohani, U. C. and Muthukumarappan, K. 2016. Application of the pulsed electric field to release bound phenolics in sorghum flour and apple pomace. Innovative Food Science & Emerging Technology, 35: 29-35.

compound, i.e. phenolic acids, flavonoids and flavanols which possess antioxidant, anti-cancerogenic properties and health benefits (Amić et al., 2003, Oboh, 2005a).

Sorghum is one of the crops that contains phenolic compounds mainly in the forms of phenolic acids and flavonoids (Hahn et al., 1984). Sorghum utilization can be improved by incorporating it into mainstream human diet in different innovative ways such as extrusion and baking. Apple pomace (AP), byproduct from juice and cider processing industries is a rich source of polyphenolic compounds. Most of the phenolic compounds in plant are present in the bound form with the carbohydrates, lignin, pectin and proteins (Acosta-Estrada et al., 2014). This bound nature of phenolics as glycosides reduces their ability to function as good antioxidants. Therefore, by liberating these bound phenolics using some pretreatments, the antioxidants rich sorghum flour and apple pomace can be introduced to the human diet.

In past decade, microbial fermentation has been used for improving the antioxidant properties in sorghum (Svensson et al., 2010) and apple pomace (Ajila et al., 2011, Gassara et al., 2012). However, during microbial fermentation, polyphenol oxidase activity degrades the phenolics, and also acidic environment during fermentation causes the abstraction of hydride ions and rearrangement of phenolic structures (Towo et al., 2006). Furthermore, the literature related to effect of natural fermentation on AP phenolics are lacking. Therefore, natural fermentation was opted in this study as a pretreatment of sorghum flour and apple pomace prior to pulsed electric field treatment.

In recent years, application of non-thermal technologies in food processing area in order to extract the bioactive compounds and to enhance the nutritional and functional

properties in foods has been extensively used (Alka et al., 2012, Lohani and Muthukumarappan, 2015, Pranoto et al., 2013).

Pulsed electric field (PEF), as a non-thermal processing technology, involves the application of short duration electric field pulses of high intensity to materials located between two electrodes. PEF induces a transmembrane potential difference across the cell membrane. At a critical value of potential difference, electrical breakdown can result in electroporation of the cell membrane. This process increase the permeability of the cytoplasmatic membranes that results in easier release of the intracellular contents (Knorr and Angersbach, 1998). The degree of electroporation depends on product constituents, electric field intensity, type of pulse waveform, treatment time, and the pulse number (Jeyamkondan et al., 1999).

Moderate intensity pulsed electric fields may cause lethal damage to cells by permeabilizing tissue structures, thus improving intracellular metabolite extraction (Soliva-Fortuny et al., 2009). Critical electric field strength to induce membrane permeabilization is dependent on cell geometry and size (Heinz et al., 2001). Low and medium intensity fields (0.6–2.6 V/cm, 5–100 pulses, short treatment time within 0.1–10 ms; 1 Hz) have been successfully used for enhancing mass transfer in solid foods (Corrales et al., 2008).

PEF has been studied broadly for extracting bioactive compounds from raw material (Barba et al., 2015, Fincan et al., 2004, Luengo et al., 2013, Pourzaki et al., 2012, Puértolas et al., 2010), oil extraction (Guderjan et al., 2005, Puértolas and Martínez de Marañón, 2015) and solute aqueous extraction (El-belghiti and Vorobiev, 2005).

Application of PEF in order to release bound phenolics in sorghum flour (SF) and apple pomace (AP) is lacking. Therefore, in this study, PEF was applied to improve the phenolic antioxidants in sorghum flour and apple pomace. The objective was to study the effect of flour to water ratio, electrical field intensity and treatment time on total phenolic content (TPC) and antioxidant activity (AA) of SF and AP.

7.3 Materials and Methods

Sorghum flour and apple pomace powder provided by ADM Milling Co. (Overland Park, KS), and Tree Top, Inc. (Selah, WA), respectively were stored at -20°C before experimental utilization. The fermentation of SF (45% w/v) and AP (12.5% w/v) was carried out in a controlled environment with temperature 30±1°C for 12 h and 24 h, respectively. Fermented slurry of SF and AP was further diluted to keep different flour to water ratio (FWR) as one of the variables for further treatment with pulsed electric field.

7.3.1 Pulsed electric field treatment

PEF treatment of SF and AP samples was carried out using an exponential decay PEF generator. The generator contained a DC power supply (CF60/25-12C, Hipotronics, Inc., Brewster, NY, USA), a capacitor (General Atomics Electronic systems, San Diego, CA, USA) and a spark gap switch (Figure 7.1). The energy stored in the 1 µF capacitor was discharged through a parallel plate treatment chamber made from two stainless steel electrodes separated at 2 cm by an insulation material. The area between the electrodes was 30 cm^2.The voltage applied to the chamber was measured with a voltage probe (Model, P6015A, Tektronix, Inc., Beaverton, OR, USA). The pulse width of SF and AP samples at 1 Hz frequency varied from 20-30 µs depending on samples and their concentrations. Therefore, total treatment time was selected as one of the independent

variables. Higher concentration of samples initially increases the electrical conductivity of solution which further increase during PEF treatment. Gradual increase in electrical conductivity of solution causes the saturation level of electroporation that results no further release of intracellular contents (Wiktor et al., 2015). Therefore, concentration or flour to water ratio was considered as one of the independent variables in this study. FWR, electric field intensity (EFI) and treatment time (t) as independent parameters are described in Table 7.1. Specific energy intake W_S, in kJ/kg, was calculated according to the following equation Eq. (7.1) (Zhang et al., 2012):

$$W_S = \frac{1}{2}\frac{CV^2 n}{m} \tag{7.1}$$

where, C is the capacitance (F), V is the voltage (kV), n is number of pulses and m is mass of sample (kg). The specific energy intake of sorghum flour and apple pomace samples varied from 0.44-22.4 kJ/kg and 0.44-17.0 kJ/kg, respectively throughout the PEF treatment.

Samples were treated at ambient temperature (25 ºC) and the increase in temperature was observed less than 2 ºC after treatment. PEF treated AP and SF samples were microwave (Lohani and Muthukumarappan, 2015) and oven dried, respectively and stored at -20°C for further analysis.

7.3.2 Extraction of samples

The extraction of sample for determining TPC, AA and phenolic characterization was done using the method described by Khan et al. (2013). For determining TPC and AA, 1 g of SF or AP sample 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°C for the analysis of TPC and AA.

Free phenolic acid extraction was performed by adding 10 ml of 80% (v/v) aqueous methanol into 2 g of SF or AP sample. Mixture was shaken in a shaking water bath for 1 h at 25°C. After centrifugation at 3000×*g* for 20 min, the supernatant was decanted and the extraction was repeated as described above. The two supernatants were combined, evaporated to near dryness and reconstituted with methanol to a final volume of 10 ml.

7.3.2.1 Total phenolic content (TPC)

TPC of SF was determined using Folin–Ciocalteu method (Singleton et al., 1999) with some modification. 50 µl methanol extract of sample was added with 3.5 ml distilled water and 150 µl Folin-Ciocalteu reagent. The solution was vortexed and incubated for 30 min. Thereafter, absorbance of solution was measured at 760 nm against blank. Blank solution contained all the components that were present in the sample except the methanol extract. Gallic acid was used as positive control (standard) and linear regression curve between absorbance and concentration was drawn for the standard. This standard curve was used for calculating the concentration of sample and data was expressed in mg Gallic acid equivalent (GAE)/100 g dry weight (DW). This analysis was done in six replications.

7.3.2.2 Antioxidant activity (AA)

Extinction of DPPH is a free radical scavenging activity which was measured using spectrophotometric method described by Brand-Williams et al. (1995). 2,2-diphenyl-1-picrylhydrazyl (DPPH) 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 for 30 min. Thereafter, 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 weight (DW). Samples were analyzed in six replications.

7.3.2.3 Free phenolic acid characterization

Analysis of sample extracts was carried out using Thermo Scientific, Dionex Ultimate 3000 UHPLC system (Bannockburn, IL, United States) equipped with diode-array detector (DAD) and C_{18} column (150 mm × 4.6 mm) packed with 5 μm particles. The samples were injected with a mobile-phase flow rate of 800 μl/min. Gradient elution was carried out with a solvent system of water/acetic acid (99.8:0.2 v/v) as mobile phase A and acetonitrile/acetic acid (99.8:0.2 v/v) as mobile phase B. The total run time was 12 min, and the gradient elution was as follows: 0.0−3.0 min, B 10-25%; 3.0−4.5 min, B 25−45%; 4.5−6.5 min, B 45−65%; 6.5−8.0 min, B 65−85%; 8.0−9.0 min, B 85−100%.; 9.0−12.0 min, B 100−10%. All the solvents were filtered through 0.22 μm PTFE filters prior to inject. The column was maintained at 30°C while the autosampler was thermostated at 4°C. The system was controlled by Thermo Scientific Dionex Chromeleon 7 software. Benzoic acid and cinnamic acid derivatives were detected at 280 nm and 320 nm, respectively. The concentrations of phenolic acids were calculated from peak areas in comparison to calibration curves of the respective standards and were expressed as μg/g DW.

7.3.3 Moisture content

Moisture content of sample was determined by air oven standard methods recommended by AOAC (1980). Initially 5 g of sample in triplicate was dried in hot air

oven at 130-133°C for 2 h. After drying, dried sample was again weighed. Following formula is used for calculating the MC.

$$\text{MC (\%wb)} = \frac{W_i - W_f}{W_i} \times 100 \quad (7.2)$$

W_i= initial weight of sample (5 g),

W_f= weight of sample after drying, g

7.3.4 Microstructure Evaluation

The microstructure of control and PEF treated sorghum flour and apple pomace powder were examined using a scanning electron microscope (SEM) (Hitachi-S3400 N, Tokyo, Japan). Small amounts of samples were mounted on SEM specimen stubs by using double-sided adhesive tape. Each powder sample was coated with 10 Å thick layer of gold in a sputter coater before being scanned and photographed at 1000× magnification.

7.3.5 Statistical analysis

Full factorial design was used for experimental plan and results were compared by analysis of variance (ANOVA) using SPSS (16.0) statistical software. Independent variables were optimized for all responses by general factorial design using design expert 9 software (State-Ease, Inc., Minneapolis, MN). All data were reported as mean ± standard deviation of replicates. Tukey's tests were used to compare the significant differences of the mean values with the family error rate held at 0.05.

7.4 Results and Discussion

7.4.1 Effect of FWR on TPC and AA of sorghum flour and apple pomace

It is described from ANOVA that FWR and its interaction with EFI and time had significant ($p<0.05$) effect on TPC and AA of SF (Table 7.2). With increase in FWR from

10% (w/v) to 45% (w/v), TPC and AA of SF increased significantly ($p<0.05$) by 22.9% and 21.8%, respectively (Figure 7.2). With increase in solid concentration from 10% to 45% (w/v), the measured electrical conductivity of SF solution increased from 770 µS/cm to 1560 µS/cm. The higher electrical conductivity induced more electroporation phenomenon and liberation of the phenolics into the sample. These results were accordance with the findings of Teh et al. (2015), Wang et al. (2014a) and Wang et al. (2012) for canola seed cake, glutathione and polypeptides, respectively. The average specific energy intake increased from 3.67 kJ/kg to 8.12 kJ/kg with an increase in FWR from 10% to 45% (w/v) which also stimulated higher electroporation and more liberation of phenolics at higher concentration of sample. Products with high electrical conductivity reduce the resistance of the chamber and require more energy to achieve a specific electrical field in consequence (Yan et al., 2015). TPC and AA of sorghum flour were determined as 74.7 mg GAE/100g DW and 169.1 µmol TE/100g DW, respectively on average at 45% (w/v) flour to water ratio (Figure 7.2).

For apple pomace, TPC and AA were significantly ($p<0.05$) influenced by FWR and its interaction with EFI and time (Table 7.3). TPC and AA of AP increased significantly ($p<0.05$) by 15.1% and 6.9%, respectively with increase in FWR from 5% (w/v) to 12.5% (w/v) (Figure 7.2). Increase in electrical conductivity from 750 µS/cm to 1435 µS/cm with increase in solid concentration of AP resulted in higher liberation of phenolic compounds as described earlier. Also, the average specific energy intake of AP sample increased from 3.67 kJ/kg to 6.16 kJ/kg with increase in FWR, which induced more electroporation resulted in higher release of phenolics at higher sample concentration. TPC and AA of AP were observed as 402.7 mg GAE/100g DW and 799.3

μmol TE/100g DW, respectively on average at 12.5% (w/v) flour to water ratio (Figure 7.2).

7.4.2 Effect of pulsed electric field intensity on TPC and AA of SF and AP

It is depicted from ANOVA that EFI and its interaction with time significantly ($p<0.05$) influenced the TPC and AA of SF (Table 7.2). TPC and AA of SF significantly ($p<0.05$) increased by 8.2% and 11%, respectively with increase in EFI from 1 kV/cm to 2 kV/cm (Figure 7.3). The average specific energy intake increased from 1.19 kJ/kg to 4.79 kJ/kg with increase in EFI improved the permeabilization effect facilitating the release of the phenolics inside the cells. However, a significant ($p<0.05$) drop was observed in TPC and AA when EFI increased further up to 3 kV/cm. PEF treated SF didn't exhibit any significant ($p>0.05$) difference in TPC and AA at 1 kV/cm and 3 kV/cm. The higher energy input (10.8 kJ/kg) at 3 kV/cm intensity might cause lethal damage to cells due to irreversible loss of cell membrane permeability properties resulted in degradation of phenolics as well. Vallverdú-Queralt et al. (2012) also reported a decrease in total polyphenol content of tomato fruit at higher intensity field strength. In addition, these results may be because of polarization effect of starch molecules and other polarized structures which trend to attract phenolic groups by electrostatic forces and subdued their release in sample (Wang et al., 2014a). Sorghum flour had TPC and AA as 71.5 mg GAE/100g DW and 165.5 μmol TE/100g DW, respectively on average at 2 kV/cm electric field intensity (Figure 7.3).

EFI and its interaction with time showed significant ($p<0.05$) effect on TPC and AA of AP (Table 7.3). A significant ($p<0.05$) increase of 4.8% TPC and 4.4% AA of AP was observed with increase in EFI from 1 kV/cm to 3 kV/cm (Figure 7.3). Increase in the

average specific energy intake of samples from 0.99 kJ/kg to 3.99 kJ/kg with increase in EFI from 1 kV/cm to 2 kV/cm caused more electroporation that led to the higher release of bound phenolics. Nevertheless, no significant ($p>0.05$) improvement was observed in TPC and AA when EFI increased from 2 kV/cm to 3 kV/cm. Though, the higher average specific energy of 8.99 kJ/kg was transmitted during PEF treatment at 3kV/cm EFI, the minified energy efficiency due to more disintegration in tissues with increased electrical conductivity (2023 µS/cm) resulted no further release of phenolics. Increase in phenolics and antioxidant activity with increase in EFI was also reported by Boussetta et al. (2014), Luengo et al. (2013) and Xue and Farid (2015) for flaxseed, orange peel and button mushroom, respectively. TPC and AA of apple pomace were determined as 385.4 mg GAE/100g DW and 780.6 µmol TE/100g DW, respectively on average at 2 kV/cm intensity (Figure 7.3).

7.4.3 Effect of treatment time on TPC and AA of SF and AP

It is explicated from ANOVA that treatment time had significant ($p<0.05$) effect on TPC and AA (Table 7.2). Sorghum flour showed significant ($p<0.05$) increase of 5.7% and 6.3% in TPC and AA, respectively when PEF treatment time enhanced from 500 µs to 875µs (Figure 7.4). Further increase in time up to 1250 µs didn't exhibit any significant ($p>0.05$) change in both TPC and AA of SF. As treatment time increased, the number of pulses applied to the sample increased. Number of pulses corresponding to 875 µs treatment time induced metabolic response by opening the pores in the cell membrane and consequently an efflux and influx of phenolics. Further increment in pulses to achieve the 1250 µs treatment time might break the cell wall irreversibly and destroy the membrane completely resulted in no further release of phenolics in sample. In

agreement with the present study (Gachovska et al., 2010) reported no further damage of cabbage tissues to release anthocyanins after 750 µs. TPC and AA of SF were observed as 69.4 mg GAE/100g DW and 159.6 µmol TE/100g DW, respectively on average after 875 µs treatment time (Figure 7.4).

For apple pomace, TPC and AA were significantly ($p<0.05$) influenced by treatment time (Table 7.3). A significant ($p<0.05$) drop of 3.8% and 2.6% was observed in TPC and AA of AP sample, respectively with increase in treatment time from 500 µs to 875 µs followed by no significant ($p>0.05$) change in both TPC and AA with further increase in treatment time up to 1250 µs (Figure 7.4). The antioxidant potential of apple tissue is strongly contributed by inactivation of polyphenol oxidase enzyme (Wiktor et al., 2015). Perhaps, the electrical energy applied to the sample after 500 µs was not sufficient to deactivate the enzyme that resulted in degradation of phenolics. Vallverdú-Queralt et al. (2012) also reported a decrease in antioxidant activity of tomato fruit at higher pulse number. Apple pomace had TPC and AA as 390.1 mg GAE/100g DW and 793.9 µmol TE/100g DW, respectively on average after 500 µs treatment time (Figure 7.4).

7.4.4 Optimum conditions for PEF treatment

Optimum condition for TPC and AA of sorghum flour was observed as 45% (w/v) flour to water ratio, 2 kV/cm electric field intensity and 875 µs treatment time. TPC and AA of control sorghum were 63.9 mg GAE/100g DW and 133.5 µmol TE/100g DW, respectively and were increased by 24.8% and 33.9%, respectively when treated at optimum conditions (Figure 7.5). The specific energy input for SF sample was observed as 6.96 kJ/kg at the optimum conditions. For apple pomace, optimum condition of PEF

treatment was found as 12.5% (w/v) flour to water ratio, 2 kV/cm electric field intensity and 500 µs treatment time with a specific energy input of 3.0 kJ/kg. Control AP had 302.9 mg GAE/100g DW TPC and 438.6 µmol TE/100g DW AA and those were increased by 37.4% and 86%, respectively when treated at optimum conditions (Figure 7.5). In agreement with the present study, Fincan (2015), Luengo et al. (2013), Vallverdú-Queralt et al. (2012) and Wiktor et al. (2015) reported that mild intensity pulsed electric field was used to improve or retain the bioactive compounds in grape by-products, spearmint, red cabbage, orange peel, tomato fruit and plant tissues, respectively.

Above data were supported by the phenolic quantification of all samples (Table 7.4). Control and cavitated sorghum flour were found rich in salicylic acid followed by ferulic, *p*-hydroxybenzoic and caffeic acid. Increase in TPC and AA of PEF treated AP was dominantly influenced by protocatechuic acid followed by chlorogenic acid and salicylic acid (Table 7.4).

7.4.5 Microstructure analysis of pretreated sorghum flour and apple pomace

The changes in physical structure of control and PEF treated samples of sorghum flour and apple pomace at optimum conditions were imaged by scanning electron microscope (Figure 7.6). Control sorghum flour showed the less porous and compact structure. Starch granules were embedded into the structural matrix (Figure 7.6a). The SEM images revealed that the electroporation resulted an increase in porosity of the cellular membrane as starch granules were appeared in a loosen structure (Figure 7.6b). Granules of PEF treated sample were more disrupted than that of control sample which

caused increase in surface area of granules and thus releasing more phenolics which were bound with protein-carbohydrate matrix.

The effect of the fermentation and PEF treatment on the microstructure of AP powder carried out by SEM is shown in Figure 7.6. The structure of control AP powder was observed as highly impact and nonporous (Figure 7.6c). The impact of fermentation followed by electroporation is illustrated in Figure 7.6d which confirmed irregular shape particles, most of which were in the size range 10-500 μm. The fibrous, laminar structure of the particles is consistent with cellulosic insoluble fiber. It can be concluded that the structure of fiber was appealing to crack during PEF processing and disrupted fibrous structure helped in releasing more phenolics from bound matrix.

7.5 Conclusions

Fermented sorghum flour and apple pomace were treated with pulsed electric field to successfully release the bound phenolics in order to enhance the TPC and AA in both the samples. Flour to water ratio, electric field intensity and treatment time had significant ($p<0.05$) effect on TPC and AA of SF and AP. The optimum values of TPC and AA for SF and AP were observed as 79.7 mg GAE/100g DW, 178.8 μmol TE/100g DW and 416.3 mg GAE/100g DW, 815.8 μmol TE/100g DW, respectively. Electroporation of both the samples were dominantly influenced by the flour to water ratio followed by the electric field intensity and treatment time. Phenolic acids derived from protocatechuic, cholorogenic and salicylic acid were found in higher concentrations in PEF treated AP, while treated SF showed the higher concentration of salicylic, ferulic, p-hydroxybenzoic and caffeic acids. From the microstructural analysis, fermentation and electroporation caused more cell collapse and cell disruption resulting in release of

phenolics from the bound structure. The study revealed that the mild intensity pulsed electric field treatment can be successfully used to produce sorghum flour and apple pomace with better phenolic composition and antioxidant activity though the specific energy input for sorghum (starchy material) was observed to be higher than the apple pomace (fibrous material).

Table 7.1 Experimental plan for pulsed electric field treatment of sorghum flour and apple pomace

Parameters		**Levels**	**Values**
Flour to water ratio, % w/v	Sorghum flour	3	10, 27.5, 45
	Apple pomace	3	5, 8.75, 12.5
Electric field intensity, kV/cm		3	1, 2, 3
Treatment time, μs		3	500, 875, 1250

Table 7.2 Variance analysis for total phenolic content (TPC) and antioxidant activity (AA) of sorghum flour

Sources	**df**		***SS***	**MS**	**F-value**	**p-value**
FWR	2	TPC	2654.0	1327.0	2099.3	0.000*
		AA	12925.9	6462.9	455.0	0.000*
EFI	2	TPC	442.1	221.1	349.7	0.000*
		AA	3974.3	1987.2	139.9	0.000*
t	2	TPC	282.6	141.3	223.5	0.000*
		AA	1382.2	691.1	48.6	0.000*
FWR*EFI	4	TPC	382.2	95.6	151.2	0.000*
		AA	2773.8	693.5	48.8	0.000*
FWR*t	4	TPC	461.3	115.3	182.4	0.000*
		AA	539.8	134.9	9.50	0.000*
EFI*t	4	TPC	192.3	48.1	76.1	0.000*
		AA	439.5	109.9	7.74	0.000*
FWR*EFI*t	8	TPC	312.4	39.05	61.8	0.000*
		AA	1624.4	203.1	14.3	0.000*

*significant at 5% level of significance, df: degree of freedom, EFI: electrical field intensity, FWR: flour to water ratio, MS: mean square, *SS*: sum of squares, t: treatment time

Table 7.3 Variance analysis for total phenolic content (TPC) and antioxidant activity (AA) of apple pomace

Sources	**df**		***SS***	**MS**	**F-value**	**p-value**
FWR	2	TPC	39910.2	19955.1	30446.4	0.000*
		AA	37938.5	18969.2	1309.9	0.000*
EFI	2	TPC	5308.8	2654.4	4049.9	0.000*
		AA	15655.7	7827.8	540.6	0.000*
T	2	TPC	4044.3	2022.2	3085.3	0.000*
		AA	12594.8	6297.4	434.9	0.000*
FWR*EFI	4	TPC	932.3	233.1	355.6	0.000*
		AA	15672.8	3918.2	270.6	0.000*
FWR*t	4	TPC	2773.6	639.4	1057.9	0.000*
		AA	255.4	63.8	4.41	0.004*
EFI*t	4	TPC	1142.5	285.6	435.8	0.000*
		AA	612.0	153.0	10.6	0.000*
FWR*EFI*t	8	TPC	3307.23	413.4	630.7	0.000*
		AA	1200.4	150.0	10.4	0.000*

*significant at 5% level of significance, df: degree of freedom, EFI: electrical field intensity, FWR: flour to water ratio, MS: mean square, *SS*: sum of squares, t: treatment time

Table 7.4 Phenolic profile of control and pulsed electric field (PEF) treated sorghum flours (SF) and apple pomace (AP) (μg/g DW)

Compounds	Control SF	PEF treated SF	Control AP	PEF treated AP
Benzoic acids				
Protocatechuic acid	6.18±0.1[a]	7.63±0.1[b]	392.6±7.7[a]	681.4±4.7[b]
p-Hydroxybenzoic acid	13.3±0.3[a]	16.7±0.2[b]	-	-
Cinnamic acids				
Chlorogenic acid	-	-	72.5±2.4[a]	98.9±3.1[b]
Caffeic acid	10.2±0.2[a]	14.9±0.2[b]	130.8±3.2[a]	141.1±4.2[b]
p-coumaric acid	4.87±0.3[a]	6.29±0.1[b]	24.5±1.3[a]	34.3±1.8[b]
Ferulic acid	13.4±0.3[a]	17.7±0.2[b]	64.4±2.9[a]	70.1±2.3[b]
Salicylic acid	22.8±0.2[a]	26.9±0.3[b]	131.9±2.7[a]	170.2±2.9[b]

Means±SD in the same row for SF and AP with different letters are significantly different ($p<0.05$)

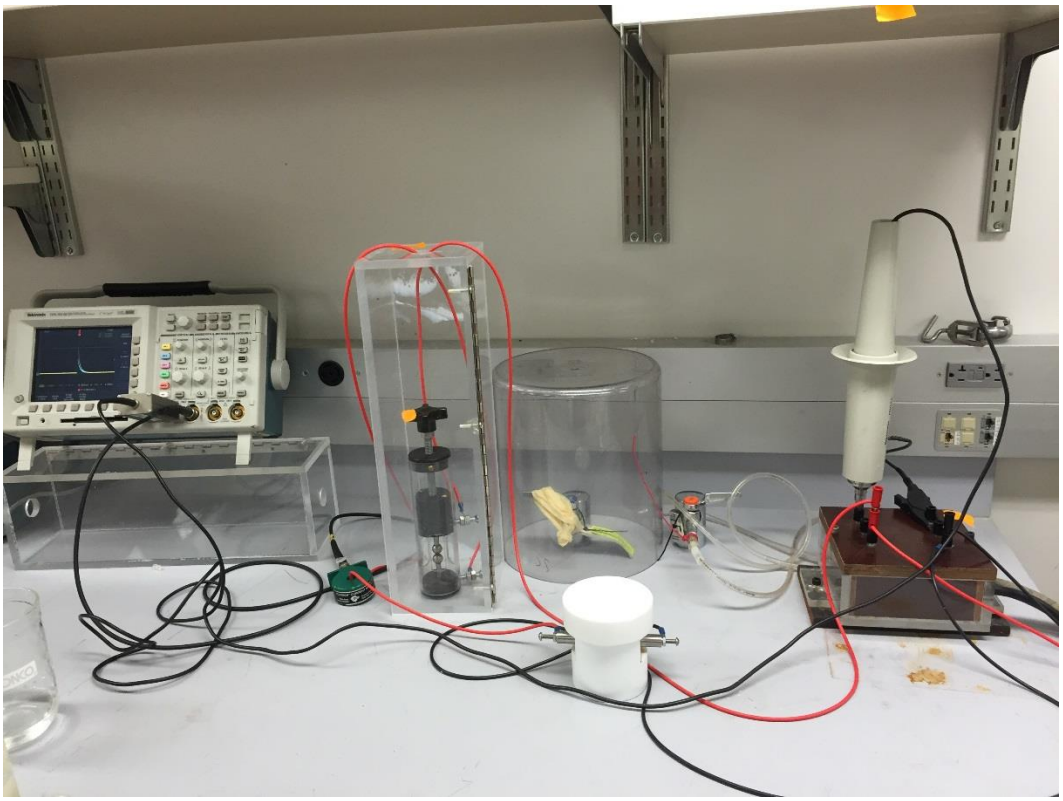


Figure 7.1 Pulsed electric field apparatus used in the study

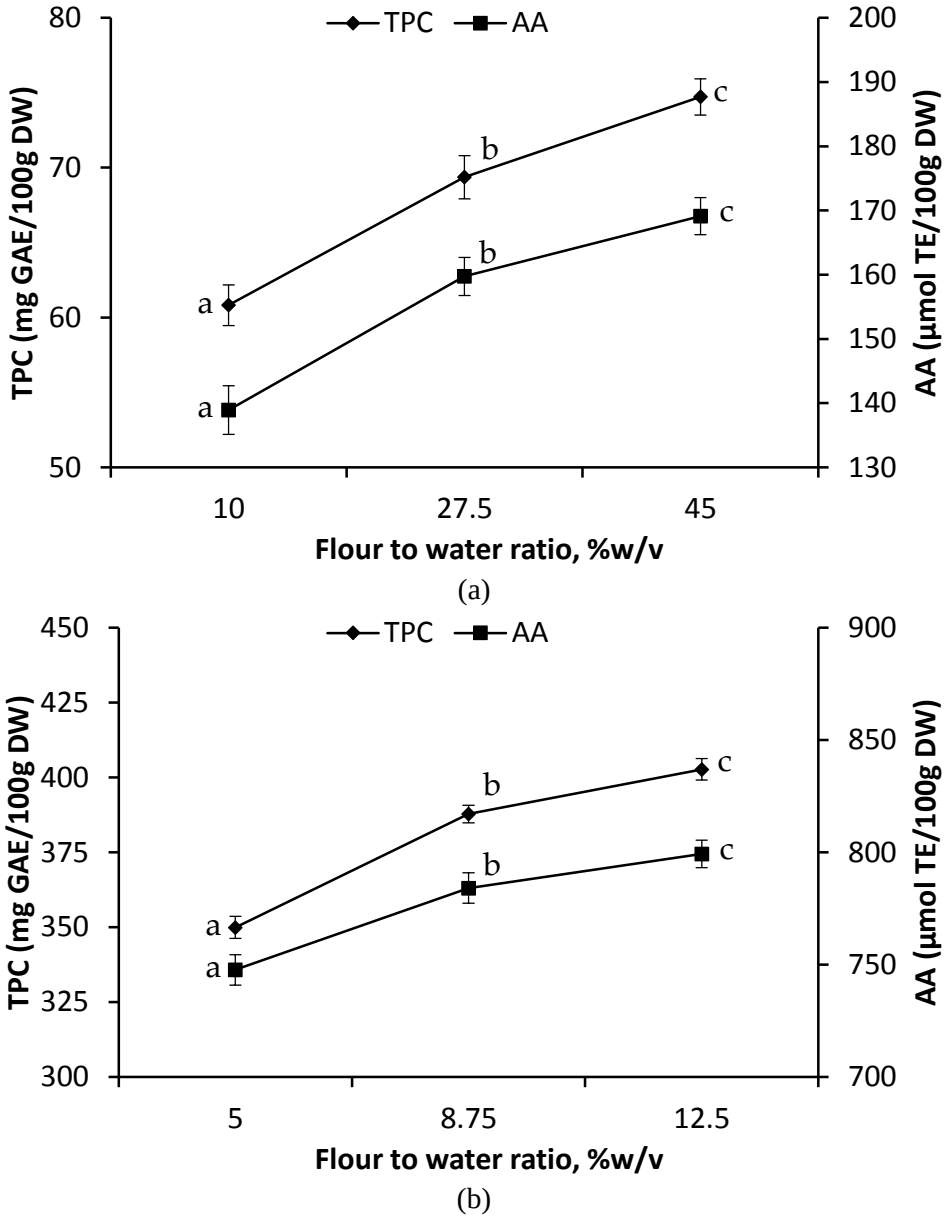


Figure 7.2 Main effect of flour to water ratio on total phenolic content (TPC) and antioxidant activity (AA) of (a) sorghum flour and (b) apple pomace. Values with the different letters at different points in the same line are significantly ($p < 0.05$) different

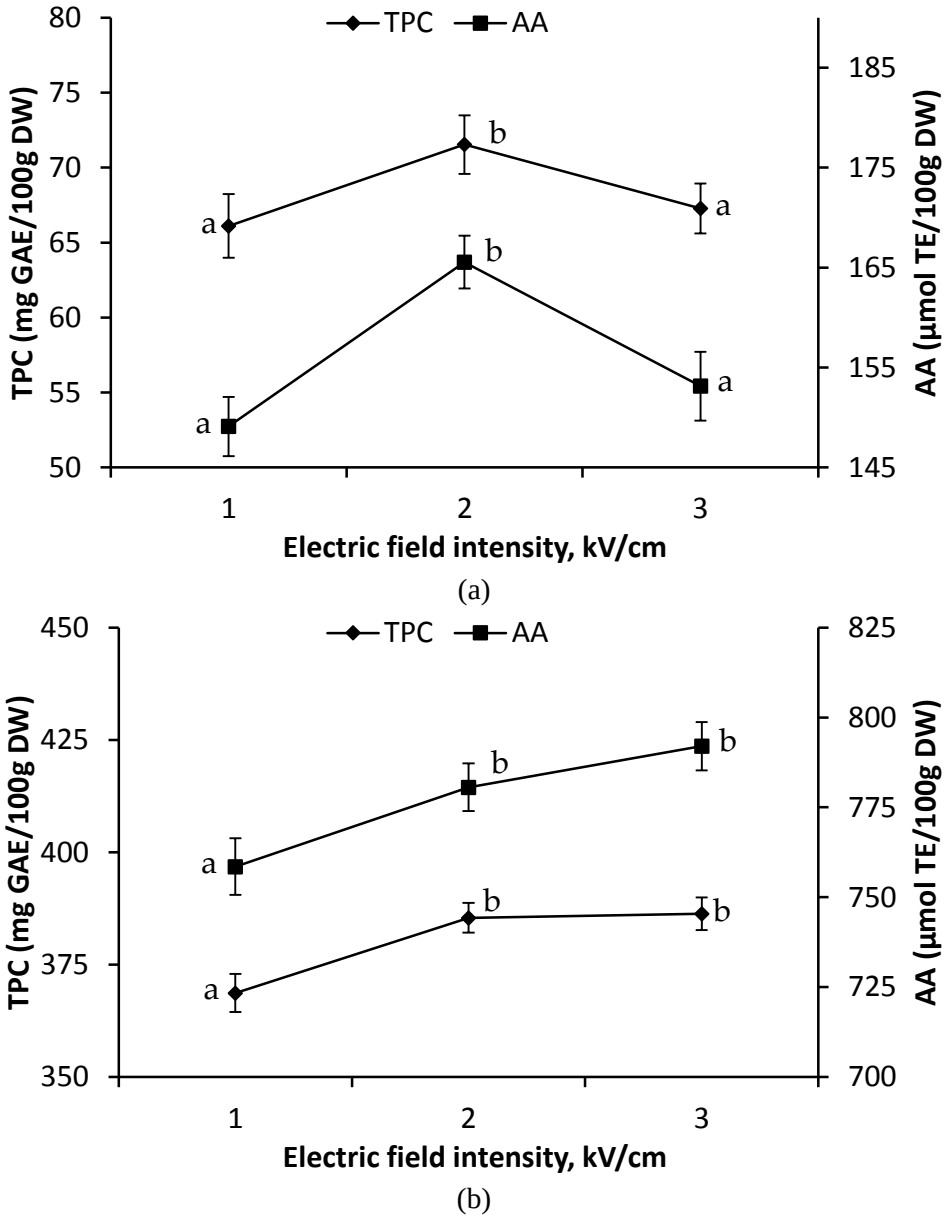
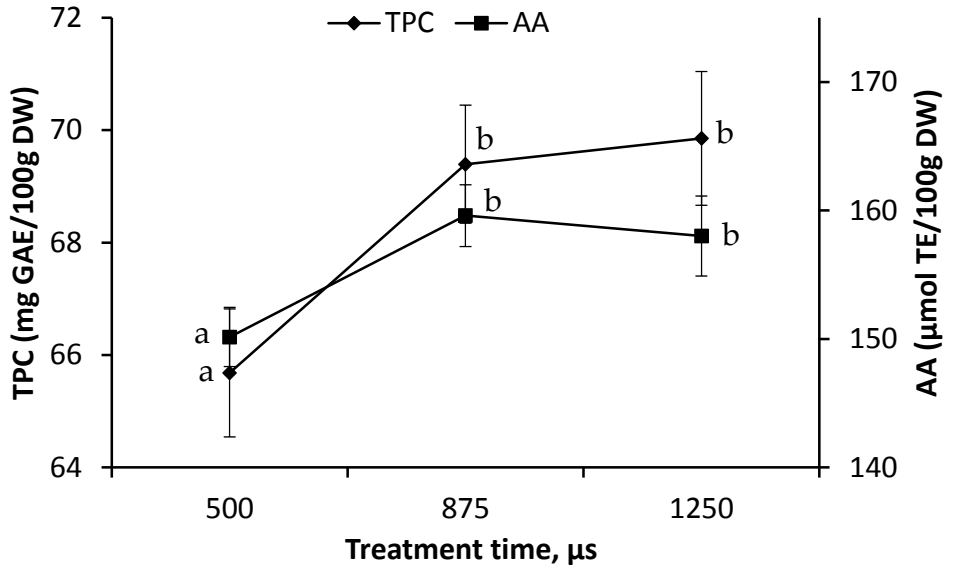
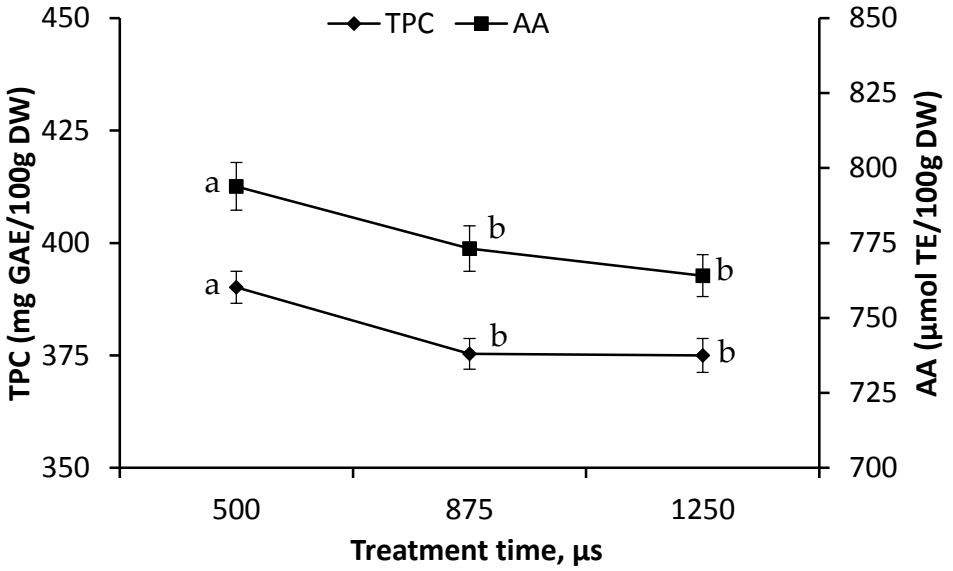


Figure 7.3 Main effect of electric field intensity on total phenolic content (TPC) and antioxidant activity (AA) of (a) sorghum flour and (b) apple pomace. Values with the different letters at different points in the same line are significantly ($p < 0.05$) different



(a)



(b)

Figure 7.4 Main effect of treatment time on total phenolic content (TPC) and antioxidant activity (AA) of (a) sorghum flour and (b) apple pomace. Values with the different letters at different points in the same line are significantly ($p < 0.05$) different

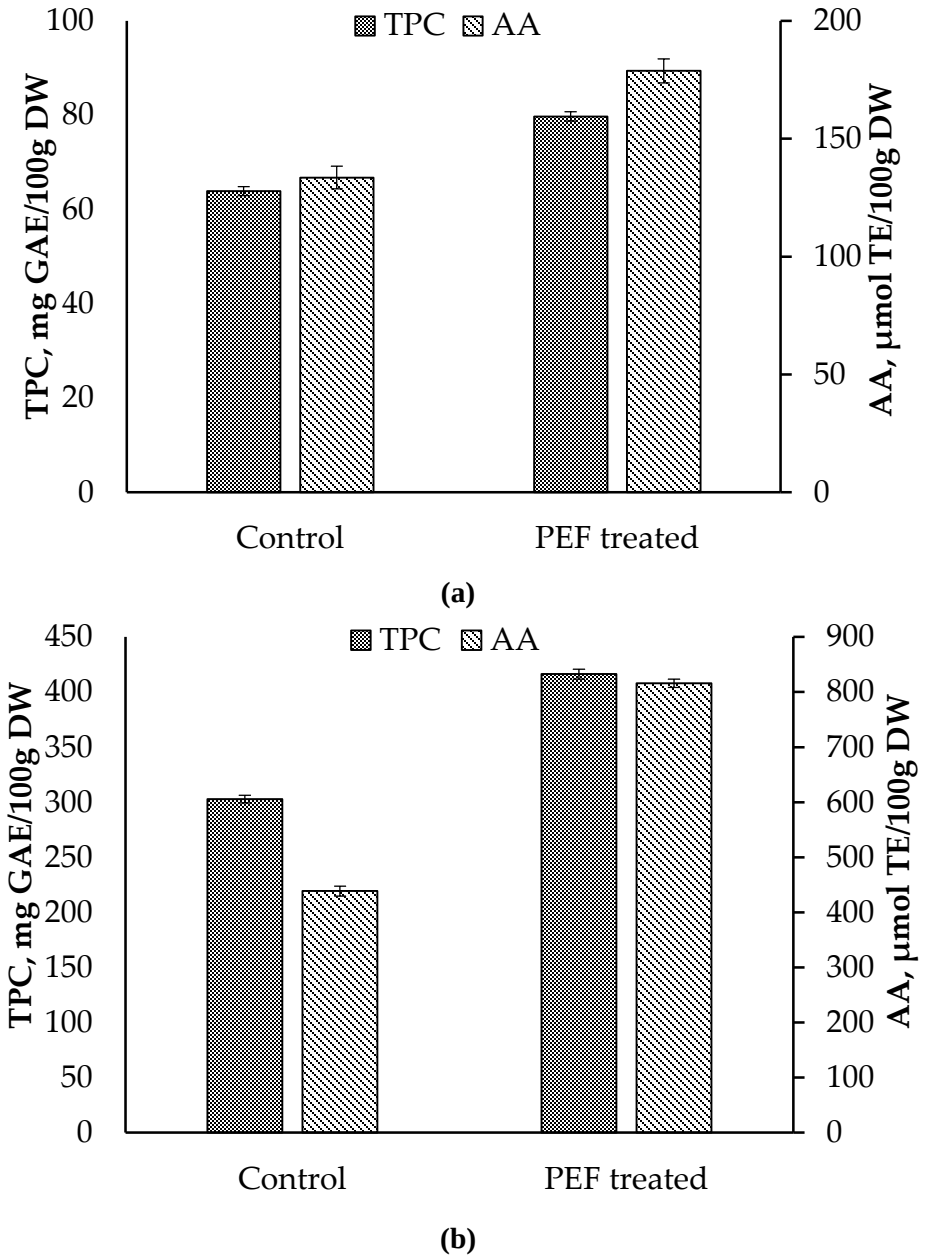


Figure 7.5 Comparison of total phenolic content (TPC) and antioxidant activity (AA) of control (a) sorghum flour with that of pretreated at optimum conditions (45% (w/v) flour to water ratio (FWR), 2 kV/cm electric field intensity (EFI), and 875 µs treatment time) and (b) apple pomace (12.5% (w/v) FWR, 2 kV/cm EFI, and 500 µs treatment time)

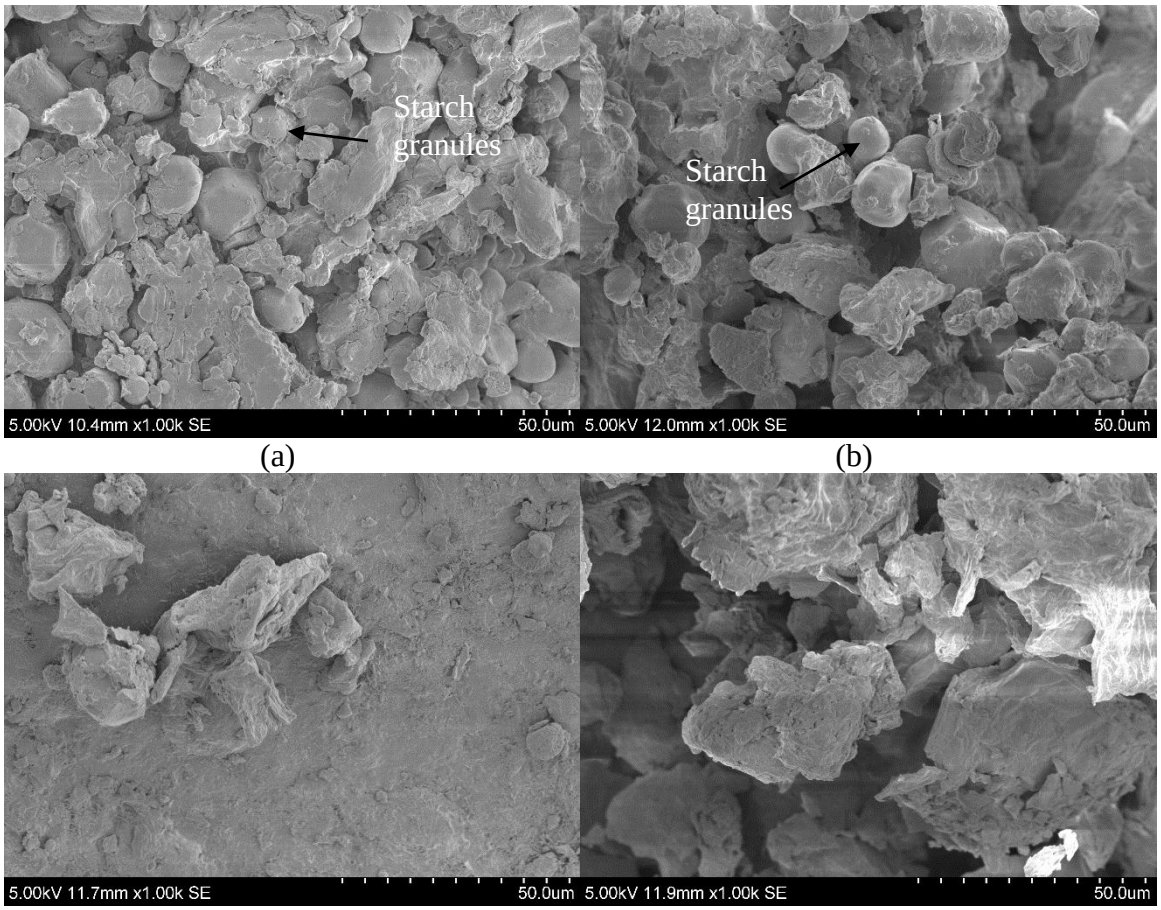


Figure 7.6 Scanning electron micrographs (×1000 magnification) of (a) control sorghum flour (SF), (b) pulsed electric field (PEF) treated SF, (c) control apple pomace (AP) and (d) PEF treated AP

CHAPTER 8

Application of hydrodynamic cavitation to improve antioxidant activity in sorghum flour and apple pomace[7]

8.1 Abstract

As a non-thermal process, ultrasonication has been recently studied to release bioactive compounds from different food commodities. But energy inefficiency and scale-up issues are the main drawbacks of ultrasonication process. To overcome this problem, hydrodynamic cavitation was studied to release the bound phenolics in sorghum flour (SF) and apple pomace (AP) to enhance their antioxidant activity eventually. In this study, SF and AP, naturally fermented at optimized conditions, were hydrodynamic cavitated at different flour to water (FWR) of 10, 27.5, 45% (w/v) and 5, 8.75, 12.5% (w/v), respectively. In addition, three types of cavitators with 2, 3 and 4 rows of holes rotor were used to cavitate SF and AP for the cavitation temperatures as 30, 35, 40°C and 40, 45, 50°C, respectively. Both the treated samples were analyzed for total phenolic content (TPC), antioxidant activity (AA) and phenolic characterization. In-vitro starch digestibility (IVSD) and total dietary fiber (TDF) were also determined for SF and AP, respectively. For SF and AP, optimized conditions were determined as 10% and 8.75% (w/v) FWR, 3 and 4 holes cavitator, 35°C and 45°C cavitation temperature, respectively. At these conditions, TPC and AA of SF were 39.5% and 38.6%, respectively higher than the control SF, while for AP, these numbers were observed as 42% and 97%, respectively. IVSD in SF showed 4.7% increase, whereas AP exhibited 7.6% increment

[7] Lohani, U. C. and Muthukumarappan, K. 2016. Application of hydrodynamic cavitation to improve antioxidant activity in sorghum flour and apple pomace. Food and Bioproducts Processing. *Under review*.

in TDF as compared to that of control samples. The study suggests that these SF and AP may be very useful for the preparation of processed foods with increased levels of phenolic antioxidants.

8.2 Introduction

In recent years, different cereals have been identified and accepted as functional foods and nutraceuticals because of good sources of dietary fibers, proteins, minerals, vitamins, and antioxidants required for human health (Charalampopoulos et al., 2002). Sorghum is one of the crops that contains phenolic compounds mainly in the forms of phenolic acids and flavonoids (Hahn et al., 1984). These compounds have potentiality to impact positively on human health because of their antioxidant and antiradical properties (Awika and Rooney, 2004). Sorghum utilization can be improved by incorporating it into mainstream human diet in different innovative ways such as extrusion and baking. Most of the phenolic compounds in plant are present in the bound form with the carbohydrates, lignin, pectin and proteins (Acosta-Estrada et al., 2014, Ajila et al., 2011). This bound nature of phenolics as glycosides reduces their ability to function as good antioxidants. Therefore, by liberating these bound phenolics using some pretreatments, the antioxidants rich sorghum flour can be introduced to the human diet.

Apple pomace (AP), byproduct from juice and cider processing industries is mostly used for direct disposal to soil in a landfill, and for pectin recovery usage (gelling agent, stabilizer and source of dietary fiber). Despite of that, tons of AP remains unutilized and causes serious environmental threats. Therefore, studies have got momentum to valorize the AP for other purposes also. AP as a rich source of antioxidant compounds could be used for increasing the stability of foods by preventing lipid

peroxidation and also for protecting oxidative damage in living systems by scavenging oxygen radicals. Apple pomace has potential source of polyphenolic compounds and most of these compounds are present in bound forms with carbohydrates, lignin, pectin and proteins (Acosta-Estrada et al., 2014, Ajila et al., 2011) that reduces their antioxidant efficacy. Therefore, release of these bound phenolics can improve their health functionality.

In recent years, acoustic or ultrasonic cavitation (UC) has been used to release the bound phenolics that resulted in enhance in antioxidant activity in sorghum (Zhao et al., 2008) and apple pomace (Ajila et al., 2011, Lohani and Muthukumarappan, 2015). There are several processes that enhance the liberation of bound phenolics. Germination, malting, fermentation and thermo-mechanical processes such as extrusion cooking and alkaline hydrolysis are most popular (Acosta-Estrada et al., 2014). Fermentation can be used in combination with the cavitation. Fermentation improves the functional properties, i.e. starch-protein digestibility, water absorption index and water solubility index (Pranoto et al., 2013, Alka et al., 2012).

In terms of energy efficiency and scale-up capability, hydrodynamic cavitation (HC) is a possible alternative to ultrasonication. Cavitation is a combined phenomenon of formation, growth and collapse of microbubbles occurring in milliseconds. It provides a high energy densities locally resulting in high pressure and temperature in a range of 100-5000 bars and 1000-10000 K, respectively at millions of locations (Kim et al., 2015). HC can be simply generated by the passage of liquid through a constriction resulting in increase in velocity at the expense of local pressure. Cavities are generated when the pressure falls below than the vapor pressure of medium at the operating temperature.

Subsequently, the pressure recovers at liquid jet expansion resulting in collapse of the cavities. The cavitation in the liquid depends on size, shape, location of the body, its surface condition, microbubble dimensions, solid particles constituting the cavitation nucleus, Cavitation, Reynolds and Weber numbers characteristic (Cai et al., 2009).

As a dimensionless parameter characterizing the cavitation conditions in hydraulic systems, the cavitation number *K* has generally been used to relate the flow conditions with the cavitation intensity (Save et al., 1997). It is defined in the following form:

$$K = \frac{P - P_v}{\frac{1}{2}\rho v_0^2} \tag{8.1}$$

where P is fully recovered downstream pressure, P_v is the vapor pressure of the liquid, ρ is the density of liquid and v_0 is the velocity of the liquid at the constriction.

Though, cavitation can be achieved even at higher cavitation numbers, for maximum benefit from the reactor, the flow conditions and the geometry should be adjusted in such a way that the cavitation number lies in the range of 0.1 to 1. For smaller cavitation numbers (*K*), the number of bubbles produced per unit time increases as well as the intensity of the cavitation process (Ozonek and Lenik, 2011).

In past years, HC has been used for sterilization of food (Milly et al., 2007), microbial cell disruption (Balasundaram and Harrison, 2006a, Balasundaram and Harrison, 2006b, Balasundaram and Pandit, 2001, Save et al., 1997), water disinfection (Arrojo et al., 2008, Jyoti and Pandit, 2001, Jyoti and Pandit, 2003, Mezule et al., 2009), wastewater treatment (Pradhan and Gogate, 2010, Sivakumar and Pandit, 2002, Wang and Zhang, 2009), and enzymatic hydrolysis of oil (Sainte Beuve and Morison, 2010). Application of HC in the area of food processing in order to increase in nutritional or

functional values is lacking. Therefore, in this study, HC was applied to release the bound phenolics from sorghum flour (SF) and apple pomace (AP) to enhance the antioxidant activity. The objective of this study was to investigate the effect of flour to water ratio, cavitator holes and cavitation temperature on total phenolic content (TPC), antioxidant activity (AA), total dietary fiber (TDF) and in-vitro starch digestibility (IVSD) of SF and AP.

8.3 Materials and Methods

Sorghum flour and apple pomace provided by ADM Milling Co. (Overland Park, KS), and Tree Top, Inc. (Selah, WA), respectively were stored at -20°C before experimental utilization. The fermentation of SF (45% w/v) and AP (12.5% w/v) was carried out in a controlled environment with temperature 30±1°C for 12 h and 24 h, respectively. Fermented slurry of SF and AP was further diluted to keep different flour to water ratio (FWR) as one of the variables for further hydrodynamic cavitation.

8.3.1 Hydrodynamic cavitation

APV hydrodynamic cavitator (215 TC, SPX flow technology, Pasteursvej, Silkeborg, Denmark) was used for the cavitation of SF and AP (Figure 8.1). This cavitator had specially designed rotors with surface indentations that influenced the flow trajectory of the samples inside the cavitator. The flow trajectory and the variable pressure zones created due to the flow pattern lead to the formation, expansion and collapsing of the microscopic bubbles giving off shockwaves into the liquid medium. The high shear and heat generated by shockwaves and internal liquid friction, respectively creates temperature uniformly throughout the entire liquid without having hot and cold space. There were a total of 44, 66 and 88 indentations placed equidistant from each other

on the 8" rotors with 2, 3 and 4 rows of holes, respectively (Figure 8.1). The speed of the rotor which determines the extent of cavitation was controlled with a variable frequency drive. The gap between the rotor and stator for 2 and 3 holes rotor was 3 mm while for the rotor with 4 holes, it was 6 mm. The speed of the rotor which determines the extent of cavitation was controlled with a variable frequency drive attached to a 7.5 kW motor. Cold water as a coolant was used to subdue the heat generated by motor. From preliminary trials, rotor speed of 3490 rpm corresponding to frequency of 60 Hz was used and the downstream pressure was maintained at 100 kPa with the help of backpressure valve to generate the phenomenon of cavitation during the sample flow through the device. Sample of 10 liters was fed into the cavitator through a positive displacement pump. Sample was recirculated till it reached desire experimental cavitation temperature.

Cavitation temperature range for SF and AP was decided from preliminary trails. SF and AP samples were cavitated at two initial temperatures, i.e. 10°C and 25°C and re-circulated till the final temperature of 40°C reached. The total time of cavitation taken with the former pair of temperature (10°C-40°C) was higher than the time taken with latter pair of temperature (25°C-40°C). Both the samples of SF and AP had insignificant ($p>0.05$) difference in their TPC and AA indicating that cavitation time didn't have any significant effect during cavitation (data not shown). Therefore, cavitation temperature was opted as one of the independent variables for this study instead of cavitation time. Flour to water ratio (FWR), cavitator holes (CH) and cavitation temperature (T) as independent parameters are described in Table 8.1. AP and SF samples were dried and stored at -20°C for further analysis.

8.3.2 Extraction of samples

The extraction of sample for determining TPC, AA and phenolic characterization was done using the method described by Khan et al. (2013). For determining TPC and AA, 1 g of SF or AP sample 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°C for the analysis of TPC and AA.

Free phenolic acid extraction was performed by adding 10 ml of 80% (v/v) aqueous methanol into 2 g of SF or AP sample. Mixture was shaken in a shaking water bath for 1 h at 25°C. After centrifugation at 3000×*g* for 20 min, the supernatant was decanted and the extraction was repeated as described above. The two supernatants were combined, evaporated to near dryness and reconstituted with methanol to a final volume of 10 ml.

8.3.2.1 Total phenolic content (TPC)

TPC of SF was determined using Folin–Ciocalteu method (Singleton et al., 1999) with some modification. 50 µl methanol extract of sample was added with 3.5 ml distilled water and 150 µl Folin-Ciocalteu reagent. The solution was vortexed and incubated for 30 min. Thereafter, absorbance of solution was measured at 760 nm against blank. Blank solution contained all the components that were present in the sample except the methanol extract. Gallic acid was used as positive control (standard) and linear regression curve between absorbance and concentration was drawn for the standard. This standard curve was used for calculating the concentration of sample and data was expressed in mg

Gallic acid equivalent (GAE)/100 g dry weight (DW). This analysis was done in six replications.

8.3.2.2 Antioxidant activity (AA)

Extinction of DPPH is a free radical scavenging activity which was measured using spectrophotometric method described by Brand-Williams et al. (1995). 2,2-diphenyl-1-picrylhydrazyl (DPPH) 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 for 30 min. Thereafter, 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 weight (DW). Samples were analyzed in six replications.

8.3.2.3 Free phenolic acid characterization

Analysis of sample extracts was carried out using Thermo Scientific, Dionex Ultimate 3000 UHPLC system (Bannockburn, IL, United States) equipped with diode-array detector (DAD) and C_{18} column (150 mm × 4.6 mm) packed with 5 µm particles. The samples were injected with a mobile-phase flow rate of 800 µl/min. Gradient elution was carried out with a solvent system of water/acetic acid (99.8:0.2 v/v) as mobile phase A and acetonitrile/acetic acid (99.8:0.2 v/v) as mobile phase B. The total run time was 12 min, and the gradient elution was as follows: 0.0−3.0 min, B 10-25%; 3.0−4.5 min, B 25−45%; 4.5−6.5 min, B 45−65%; 6.5−8.0 min, B 65−85%; 8.0−9.0 min, B 85−100%.; 9.0−12.0 min, B 100−10%. All the solvents were filtered through 0.22 µm PTFE filters prior to inject. The column was maintained at 30°C while the autosampler was thermostated at 4°C. The system was controlled by Thermo Scientific Dionex

Chromeleon 7 software. Benzoic acid and cinnamic acid derivatives were detected at 280 nm and 320 nm, respectively. The concentrations of phenolic acids were calculated from peak areas in comparison to calibration curves of the respective standards and were expressed as μg/g DW.

8.3.3 Moisture content, total dietary fiber (TDF), total starch and in-vitro starch digestibility (IVSD)

Moisture content of SF powder was determined by air oven standard methods recommended by AOAC (1980). Initially 5 g of sample in triplicate was dried in hot air oven at 130-133°C for 2 h. After drying, dried sample was again weighed. Following formula is used for calculating the moisture content (MC).

$$\text{MC (\%wb)} = \frac{W_i - W_f}{W_i} \times 100 \qquad (8.2)$$

W_i= initial weight of sample (5 g),

W_f= weight of sample after drying, g

Total dietary fiber in all AP samples was measured in the laboratory by the AOAC approved method 991.43 (AOAC, 1992). Duplicate samples of milled SF and AP samples were suspended in MES/TRIS buffer (0.05 M, pH 8.2 at 24°C) and incubated sequentially with heat-stable α-amylase (95-100°C, 30 min) to give gelatinization, hydrolysis and de-polymerization of starch, protease (60°C, 30 min) to solubilize and depolymerize proteins, and amyloglucosidase (60°C, 30 min, pH 4.5) to hydrolyse starch fragments to glucose. The enzyme digestate was then treated with four volumes of 95% ethanol (1 h) to precipitate soluble fiber. The alcohol-treated digestate was filtered through borosilicate sintered glass crucibles (40-90 μm) that had previously been matted with celite, dried, and weighed. The total dietary fiber residue present in the crucible was

washed with alcohol and acetone, dried overnight (103°C), and weighed. One duplicate from each sample was used for ash determination (525°C muffle furnace) and the other for protein determination. Total dietary fiber percent (TDF, g/100g DW) was calculated as

$$\text{TDF}=\frac{\frac{R_1+R_2}{2}-p-A-B}{\frac{m_1+m_2}{2}}\times\frac{100}{DW}\times 100 \tag{8.3}$$

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 R_2, B is the blank and DW is percentage dry weight (g) of sample.

$$B=\frac{BR_1+BR_2}{2}-BP-BA \tag{8.4}$$

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.

Total starch of SF was determined by AOAC approved method 996.11 (AOAC, 1996). The 100 mg sample and 0.2 ml, 80% (v/v) ethanol were added to a glass test tube and mixed on a vortex mixer. A magnetic stirring rod was added to each tube, and the tubes were placed in an ice water bath before 2 ml, 2 M KOH was added to each tube with stirring. The tubes were removed after 20 min, and 8 ml, 2 M sodium acetate buffer was added to each tube with stirring. Thereafter, 0.1 ml, 3000 U/ml thermostable α-amylase and 0.1 ml, 3300 U/ml amyloglucosidase (Megazyme) were added to each tube. The tubes were incubated in a 50°C water bath for 30 min with intermittent stirring on a vortex mixer. Samples were diluted to 100 ml with distilled water, centrifuged, and the supernatant was retained for analysis. The K-GLUC (GOPOD format) assay kit from

Megazyme was used for the determination of D-glucose using glucose oxidase based on absorbance at 510 nm as read on the UV−vis spectrophotometer. The glucose was converted into starch by multiplying a factor 0.9.

The in vitro starch digestion of SF samples was determined according to the method of Goñi et al. (1997). 10 ml of HCI-KCI buffer (pH l.5) was added to 50 mg sample (pH was adjusted). Then 0.2 ml of a solution containing 1 g of pepsin in 10 ml of HCI-KCI buffer was added to sample and incubated at 40°C for 1 hour in a shaking water bath. Volume was completed to 25 ml with Tris-Maleate buffer (pH 6.9). 5 ml solution of α-amylase in Tris-Maleate buffer containing 2.6 IU were added to each sample. Samples were then incubated at 37°C in a shaking water bath for 3 h. 1ml aliquot sample was placed in a tube at 100°C and was energically shaken for 5 min to inactivate the enzyme. Then 3 ml of 0.4M Sodium acetate buffer (pH 4.75) were added to aliquot, and 60 µl of amyloglucosidase was used to hydrolyze the digested starch into glucose after 45 min at 60°C in a shaking water bath. Volume was adjusted to 100 ml with distilled water. Triplicated aliquots of 0.5 ml were incubated with the K-GLUC (GOPOD format) assay kit (Megazyme) for the determination of D-glucose using glucose oxidase based on absorbance at 510 nm as read on the UV−vis spectrophotometer. The glucose was converted into starch by multiplying a factor 0.9. Results were expressed on a total starch basis.

8.3.4 Microstructure Evaluation

The microstructure of control and cavitated sorghum flour and apple pomace powder were examined using a scanning electron microscope (SEM) (Hitachi-S3400 N, Tokyo, Japan). Small amounts of samples were mounted on SEM specimen stubs by

using double-sided adhesive tape. Each powder sample was coated with 10 Å thick layer of gold in a sputter coater before being scanned and photographed at 1000× magnification.

8.3.5 Statistical analysis

Full factorial design was used for experimental plan and results were compared by analysis of variance (ANOVA) using SPSS (16.0) statistical software. Independent variables were optimized for all responses by general factorial design using design expert 9 software (State-Ease, Inc., Minneapolis, MN). All data were reported as mean ± standard deviation of replicates. Tukey's tests were used to compare the significant differences of the mean values with the family error rate held at 0.05.

8.4 Results and Discussion

8.4.1 Effect of FWR on TPC and AA of sorghum flour and apple pomace

It is depicted from analysis of variance (ANOVA) that FWR and its interaction with cavitator holes (CH) and cavitation temperature (T) had significant ($p<0.05$) effect on TPC, AA and IVSD of SF, however combined effect of FWR and T didn't show any significant ($p>0.05$) influence on AA (Table 8.2). TPC and AA of SF significantly ($p<0.05$) decreased by 36% and 9%, respectively with increase in FWR from 10% (w/v) to 45% (w/v) (Figure 8.2). With increase in solid concentration, density of the liquid increased from 1.09 to 1.23 kg/m^3 which resulted in higher Weber number (W_e). Increasing the W_e is usually accompanied by an increase in the cavitation number and thus resulting in less cavitation intensity (Ozonek and Lenik, 2011). In addition, the inlet pressure ranged from 33 kPa to 47 kPa with increase in FWR from 10 to 45% (w/v) didn't induce sufficient enough pressure drop across the surface of the rotor and

indentation for cavitation to occur to release the phenolics in SF slurry. Therefore, density of sample played a predominant role over the inlet pressure to decide the cavitation in the SF sample. IVSD increased significantly ($p<0.05$) by 6.4% with increase in FWR from 10 to 27.5% (w/v) followed by no significant ($p>0.05$) change with further increase in FWR (Figure 8.3). TPC, AA and IVSD of sorghum flour were determined as 79.7 mg GAE/100g DW, 175.3 µmol TE/100g DW and 58.6 g/100g DW, respectively on average at 10% (w/v) flour to water ratio.

For apple pomace, TPC, AA and TDF were significantly ($p<0.05$) influenced by FWR and its interaction with CH and T (Table 8.3). There was no significant ($p>0.05$) influence of temperature on TDF. With increase in FWR from 5% (w/v) to 8.75% (w/v), TPC and AA of AP increased significantly ($p<0.05$) by 11.2% and 9.9%, respectively followed by noticeable significant ($p<0.05$) drop in both the values when FWR further increased to 12.5% (w/v) (Figure 8.2). With increase in solid concentration from 5% (w/v) to 8.75% (w/v), density of the AP slurry increased from 1.05 to 1.06 kg/m^3 which was not enough to affect the cavitation in AP slurry. On the other hand, the inlet pressure increased form 64 kPa to 71 kPa developed a significant pressure drop across the rotor surface and indentation resulted in increased cavitation intensity due to prevailing of total collapse pressure over the cavitaion number. Further increase in FWR up to 12.5% (w/v) increased the inlet pressure 79 kPa that reduced the total collapse pressure as a result of increase in cavitation number and decrease in collapse pressure of single cavity. Also at higher concentration, fiber present in AP imbibed the water and consequently reduced the ratio of water in sample which affected the bubble formation for the cavitation. It is obvious that the cavitation in AP slurry prevailed by inlet pressure over the density of

medium unlike the SF slurry. TDF of AP was observed significantly ($p<0.05$) 4% higher at 45% (w/v) with compare to other two concentration levels (Figure 8.3). TPC, AA and TDF of AP were observed as 408.7 mg GAE/100g DW, 812.9 µmol TE/100g DW and 40.5 g/100g DW, respectively on average at 8.75% (w/v) flour to water ratio.

8.4.2 Effect of cavitator holes on TPC and AA of SF and AP

It is obvious from ANOVA that CH and its interaction with T significantly ($p<0.05$) influenced the TPC, AA and IVSD of SF (Table 8.2). TPC and AA of SF significantly ($p<0.05$) increased by 7.2% and 4.4%, respectively with increase in CH from 2 to 3 (Figure 8.4). The intensity of the cavitation significantly depends on the geometry of the component of cavitator. The geometric parameter α is defined as a ratio of the total circumference of the holes in the rotor to the sum of the areas of holes on the rotor. The value β, often referred to as the flow number, is the ratio of the sum of the areas of holes on the rotor to the section area of the pipeline feeding the hydrodynamic cavitator. Its magnitude significantly affects the cavitation number and thus determines the intensity of the resulting cavitation. The cavitator having more numbers of holes represented higher values of α and β. Increase in both the parameters lowered the cavitation number and in turn increased the cavitational intensity (Ozonek and Lenik, 2011). Higher cavitation of sample resulted in more release of bound phenolics. Further increase in CH produced SF with significantly ($p<0.05$) less TPC and AA. For a particular FWR and cavitation temperature, the apparent viscosity of SF slurry significantly decreased due to its shear thinning behavior. For instance, the viscosity of SF slurry at 150 s^{-1} shear rate shear rate for 10% (w/v) FWR and 35°C cavitation temperature decreased from 47.3 cP to 27.8 cP with increase in cavitator holes from 2 to

4. Due to decrease in viscosity, the Reynolds number (R_e) increased. Increasing the R_e is usually accompanied by an increase in the Cavitation number (K) (Ozonek and Lenik, 2011). Although the cavitation number decreased due to increase in cavitator holes as described earlier, increase in cavitation number due to increase in R_e would have dominant effect over the former phenomena which resulted an overall increase in cavitation number and in turn reduced the cavitation intensity. SF cavitated with 3 holes exhibited significantly ($p<0.05$) 5% higher IVSD than that of cavitated with 2 and 4 holes (Figure 8.3). Sorghum flour had TPC, AA and IVSD as 72.8 mg GAE/100g DW, 175.3 µmol TE/100g DW and 62.8 g/100g DW, respectively on average for 3 holes cavitator.

CH and its interaction with T showed significant ($p<0.05$) effect on TPC, AA and TDF of AP (Table 8.3). A significant ($p<0.05$) increase of 5.2% TPC and 5.7% AA of AP was observed with increase in CH from 2 to 4 (Figure 8.4). Cavitator with 4 holes caused increase in values of α and β that subsequently reduced the cavitation number resulted in higher cavitation intensity and more releasing of bound phenolics. On the other hand, the apparent viscosity of AP slurry increased significantly with increase in number of cavitator holes due to its shear thickening behavior. For 8.75% (w/v) and 45°C cavitation temperature, the viscosity of AP slurry increased from 215.9 cP to 1193.4 cP at 150 s^{-1} shear rate. Increase in viscosity drastically decreased the R_e and K resulted in higher cavitation intensity. With increase in CH from 2 to 3, no significant ($p>0.05$) change was found in TDF, however cavitating the AP with 4 holes exhibited significant ($p<0.05$) 6.8% increase in TDF (Figure 8.3). TPC, AA and TDF of apple pomace were determined as 393.8 mg GAE/100g DW, 800.5 µmol TE/100g DW and 42.4 g/100g DW, respectively on average for 4 holes cavitator.

8.4.3 Effect of temperature on TPC and AA of SF and AP

It is explicated from ANOVA that temperature had significant ($p<0.05$) effect on TPC, AA and IVSD of sorghum flour (Table 8.2). Sorghum flour cavitated at 45°C had significantly ($p<0.05$) 8.4% and 6.9% lower TPC and AA, respectively with compare to that of cavitated at 35°C. However, there was no significant ($p>0.05$) difference in TPC and AA of the SF samples cavitated at 35°C and 40°C (Figure 8.5). At higher temperature, the vapor pressure of solvent (water) increased. Increasing the vapor pressure inside the bubbles undermined the efficacy of cavitational collapse (Suslick et al., 1997). In addition to this, higher solubility of starch molecules in water at higher temperature reduced the bubble cavitation. With increase in temperature up to 40°C, IVSD significantly ($p<0.05$) increased by 6.7%, however no significant ($p>0.05$) change was observed further when SF was cavitated at 45°C (Figure 8.3). TPC, AA and IVSD of SF were observed as 68.6 mg GAE/100g DW, 172.7 µmol TE/100g DW and 58.3 g/100g DW, respectively on average at 35°C cavitation temperature.

For apple pomace, TPC and AA were significantly ($p<0.05$) influenced by temperature and their interactions (Table 8.3), whereas AP didn't exhibit any significant ($p>0.05$) change in TDF with increase in cavitation temperature. AP sample cavitated at 45°C was found significantly ($p<0.05$) higher by 2.4% and 2.5% in TPC and AA, respectively with compare to that of cavitated at 40°C (Figure 8.5). Density and surface tension of solution decreased with increase in temperature. Weber number is directly proportional to the ratio of density to the surface tension. During increase in temperature from 40°C to 45°C, the ratio may be less than that when temperature raised form 45°C to 50°C. Thus the less W_e resulted in lower K and higher cavitation efficiency in former

elevation of temperature. Further increase in temperature up to 50°C, degradation of phenolic acids also caused significant ($p<0.05$) reduction in TPC and AA of AP. Apple pomace had TPC, AA and TDF as 392.2 mg GAE/100g DW, 792.7 µmol TE/100g DW and 41.1 g/100g DW, respectively on average at 45°C cavitation temperature.

8.4.4 Optimum conditions for hydrodynamic cavitation

Optimum condition for TPC and AA of sorghum flour was observed as 10% (w/v) flour to water ratio, 3 cavitator holes and 35°C cavitation temperature. As a control, SF at optimum conditions of FWR and temperature was fed to the cavitator without running the rotor to see the effect of turbulence. TPC and AA of control sorghum were 62.6 mg GAE/100g DW and 132.3 µmol TE/100g DW, respectively and were increased by 42.3% and 39.8%, respectively when cavitated at optimum conditions (Figure 8.6). For apple pomace, optimum condition of cavitation was found as 8.75% (w/v) flour to water ratio, 4 cavitator holes and 45°C cavitation temperature. Similar to SF, control AP was fed to the cavitator at the optimum conditions but without rotor running. Control AP had 304.1 mg GAE/100g DW TPC and 440.2 µmol TE/100g DW AA those were increased by 41.5% and 96.3%, respectively when cavitated at optimum conditions (Figure 8.6). TPC and AA of raw SF and AP were 63.9 mg GAE/100g DW, 133.5 µmol TE/100g DW and 302.9 mg GAE/100g DW TPC, 438.6 µmol TE/100g DW, respectively. It was observed that the TPC and AA in control SF and AP were insignificantly ($p<0.05$) differ than that of raw SF and AP which indicated that cavitation played a prominent role for increasing TPC and AA of SF and AP.

Above data were supported by the phenolic quantification of all samples (Table 8.4). Control and cavitated sorghum flour were found rich in salicylic acid followed by

ferulic, *p*-hydroxybenzoic and caffeic acid. Increase in TPC and AA of cavitated AP was dominantly influenced by protocatechuic acid followed by chlorogenic acid and salicylic acid (Table 8.4).

IVSD of SF at optimum conditions of hydrodynamic cavitation was observed as 56.7 g/100g DW which was 4.7% higher than that of control SF. TDF of AP determined at optimum cavitation condition was also 7.6% more than that of control AP (Figure 8.7). Error bars shown in Figure 8.6 and Figure 8.7 represented standard deviations from mean values.

8.4.5 Microstructure analysis of cavitated sorghum flour and apple pomace

The changes in physical structure of control and cavitated samples of sorghum flour at optimum conditions were imaged by scanning electron microscope. Figure 8.8a shows the structure of control sorghum flour, which is a less porous and compact structure. The SEM images disclosed that the cavitation resulted in modification of the cellular structure. Fermentation and cavitation damaged the plant cell structure and caused more porosity (Figure 8.8b). Similar results were found by Karki et al. (2010) and Yan et al. (2011) for soy flakes and *Tremella mesenterica*, respectively for ultrasonic cavitation. Starch granules of cavitated sample were more disrupted than that of control sample which caused increase in surface area and thus releasing more phenolics which were bound with protein-carbohydrate matrix.

The effect of the fermentation and cavitation on the microstructure of cavitated AP powder carried out by SEM is shown in Figure 8.8. The structure of control AP powder was observed as highly impact and nonporous (Figure 8.8a). Fermentation

followed by cavitation ruptured the plant tissues and resulted porous structure (Figure 8.8b). Disrupted fibrous structure helped in releasing more phenolics from bound matrix.

8.5 Conclusions

Natural fermentation followed by hydrodynamic cavitation of sorghum flour and apple pomace successfully released the bound phenolics in order to enhance the TPC and AA in both the samples. Flour to water ratio, cavitator holes and cavitation temperature had significant ($p<0.05$) effect on TPC and AA of SF and AP. IVSD of sorghum flour and TDF of apple pomace also significantly ($p<0.05$) influenced by experimental variables. However, cavitation temperature didn't exhibit any significant ($p>0.05$) effect on TDF. The optimum values of TPC and AA for SF and AP were observed as 89.1 mg GAE/100g DW, 185.0 µmol TE/100g DW and 430.4 mg GAE/100g DW, 864.1 µmol TE/100g DW, respectively. Phenolic acids derived from protocatechuic, cholorogenic and salicylic acid were found in higher concentrations in cavitated AP, while treated SF showed the higher concentration of salicylic, ferulic, p-hydroxybenzoic and caffeic acids. Density for the SF (starchy sample), whereas inlet pressure for AP (fibrous material) prevailed for the cavitation efficiency. SF showed the shear thinning, however AP exhibited shear thickening behavior during the cavitation. From the microstructural analysis, fermentation and hydrodynamic cavitation caused more cell collapse and cell disruption resulting in release of phenolics from the bound structure. The study revealed that hydrodynamic cavitation can be an alternative to ultrasonic cavitation in terms of scale up capability in order to produce antioxidant rich food materials to be incorporated in further processing.

Table 8.1 Experimental plan for hydrodynamic cavitation of sorghum flour and apple pomace

Parameters		**Levels**	**Values**
Flour to water ratio, % w/v	Sorghum flour	3	10, 27.5, 45
	Apple pomace	3	5, 8.75, 12.5
Cavitator holes		3	2, 3, 4
Temperature, °C	Sorghum flour	3	35, 40, 45
	Apple pomace	3	40, 45, 50

Table 8.2 Variance analysis for total phenolic content (TPC) and antioxidant activity (AA) and in-vitro starch digestibility (IVSD) of sorghum flour

Sources	**df**		***SS***	**MS**	**F-value**	**p-value**
FWR	2	TPC	6185.88	3092.94	4465.19	0.000*
		AA	8613.59	4306.80	266.95	0.000*
		IVSD	239.19	119.59	171.02	0.000*
CH	2	TPC	2208.34	1104.17	1594.24	0.000*
		AA	2569.01	1284.50	79.62	0.000*
		IVSD	30.74	15.37	21.98	0.000*
T	2	TPC	108.58	54.29	78.38	0.000*
		AA	235.77	117.89	7.31	0.002*
		IVSD	294.89	147.45	210.84	0.000*
FWR*CH	4	TPC	1422.19	355.55	513.32	0.000*
		AA	3227.39	806.85	50.01	0.000*
		IVSD	256.33	64.08	91.64	0.000*
FWR*T	4	TPC	218.72	54.68	78.94	0.000*
		AA	145.98	36.49	2.26	0.074
		IVSD	104.67	26.17	37.42	0.000*
CH*T	4	TPC	644.38	161.09	232.58	0.000*
		AA	641.21	160.30	9.94	0.000*
		IVSD	168.07	42.02	60.08	0.000*
FWR*CH*T	8	TPC	738.43	92.30	133.26	0.000*
		AA	1011.87	126.48	7.84	0.000*
		IVSD	504.60	63.07	90.19	0.000*

*significant at 5% level of significance, CH: cavitator hole, df: degree of freedom, FWR: flour to water ratio, MS: mean square, *SS*: sum of squares, T: cavitation temperature

Table 8.3 Variance analysis for total phenolic content (TPC) and antioxidant activity (AA) and total dietary fiber (TDF) of apple pomace

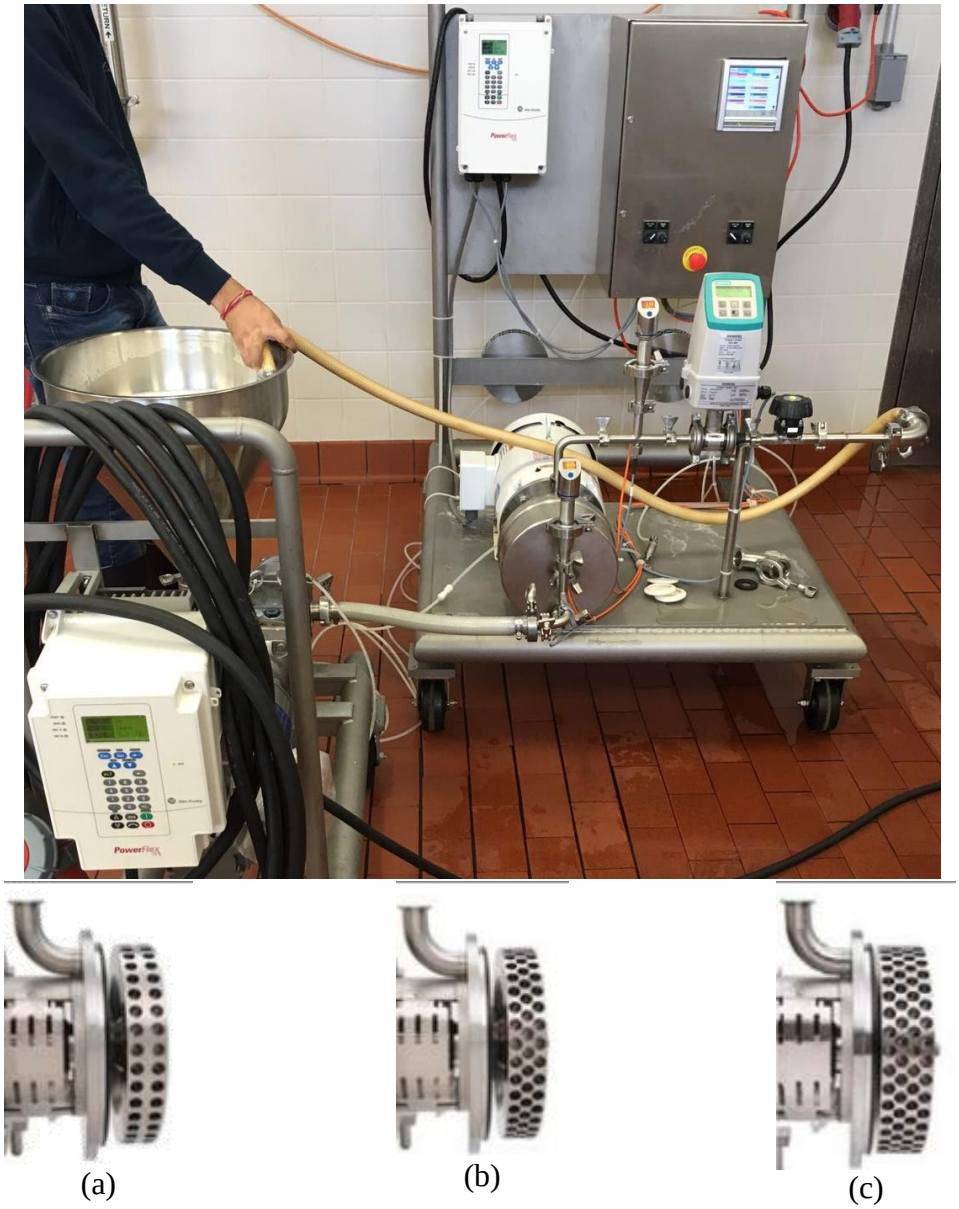
Sources	**df**		***SS***	**MS**	**F-value**	**p-value**
FWR	2	TPC	28800.2	14400.1	23340.0	0.000*
		AA	90439.3	45219.7	2410.0	0.000*
		TDF	40.9	20.5	34.2	0.000*
CH	2	TPC	5052.6	2526.3	4094.0	0.000*
		AA	25467.9	12733.9	678.6	0.000*
		TDF	9.37	4.69	7.81	0.000*
T	2	TPC	3257.0	1628.5	2639.0	0.000*
		AA	10705.5	5352.7	285.3	0.000*
		TDF	3.46	1.73	2.88	0.069
FWR*CH	4	TPC	250.5	62.6	101.5	0.000*
		AA	4992.6	1248.1	66.5	0.000*
		TDF	18.5	4.62	7.70	0.000*
FWR*T	4	TPC	1377.0	344.3	557.9	0.000*
		AA	2729.7	682.4	36.4	0.000*
		TDF	9.69	2.42	4.04	0.007*
CH*T	4	TPC	865.2	216.3	350.6	0.000*
		AA	5382.2	1345.6	71.7	0.000*
		TDF	10.55	2.64	4.39	0.003*
FWR*CH*T	8	TPC	593.7	74.2	120.3	0.000*
		AA	5592.5	699.1	37.3	0.000*
		TDF	11.3	1.41	2.34	0.026*

*significant at 5% level of significance, CH: cavitator hole, df: degree of freedom, FWR: flour to water ratio, MS: mean square, *SS*: sum of squares, T: cavitation temperature

Table 8.4 Phenolic profile of control and cavitated sorghum flours (SF) and apple pomace (AP) (μg/g DW)

Compounds	Control SF	Cavitated SF	Control AP	Cavitated AP
Benzoic acids				
Protocatechuic acid	6.16±0.1[a]	8.41±0.2[b]	394.1±6.9[a]	717.7±5.3[b]
p-Hydroxybenzoic acid	13.1±0.3[a]	18.0±0.3[b]	-	-
Cinnamic acids				
Chlorogenic acid	-	-	73.3±2.2[a]	104.5±3.9[b]
Caffeic acid	10.2±0.2[a]	16.9±0.2[b]	131.1±2.9[a]	144.6±3.2[b]
p-coumaric acid	4.81±0.2[a]	7.51±0.2[b]	24.7±1.1[a]	36.6±2.9[b]
Ferulic acid	12.9±0.3[a]	20.9±0.2[b]	64.8±2.3[a]	72.2±1.8[b]
Salicylic acid	21.6±0.2[a]	30.4±0.3[b]	132.7±2.3[a]	175.3±3.1[b]

Means±SD in the same row for SF and AP with different letters are significantly different ($p<0.05$)



(a) (b) (c)

Figure 8.1 Hydrodynamic cavitator with (a) 2 holes rotor, (b) 3 holes rotor and (c) 4 holes rotor

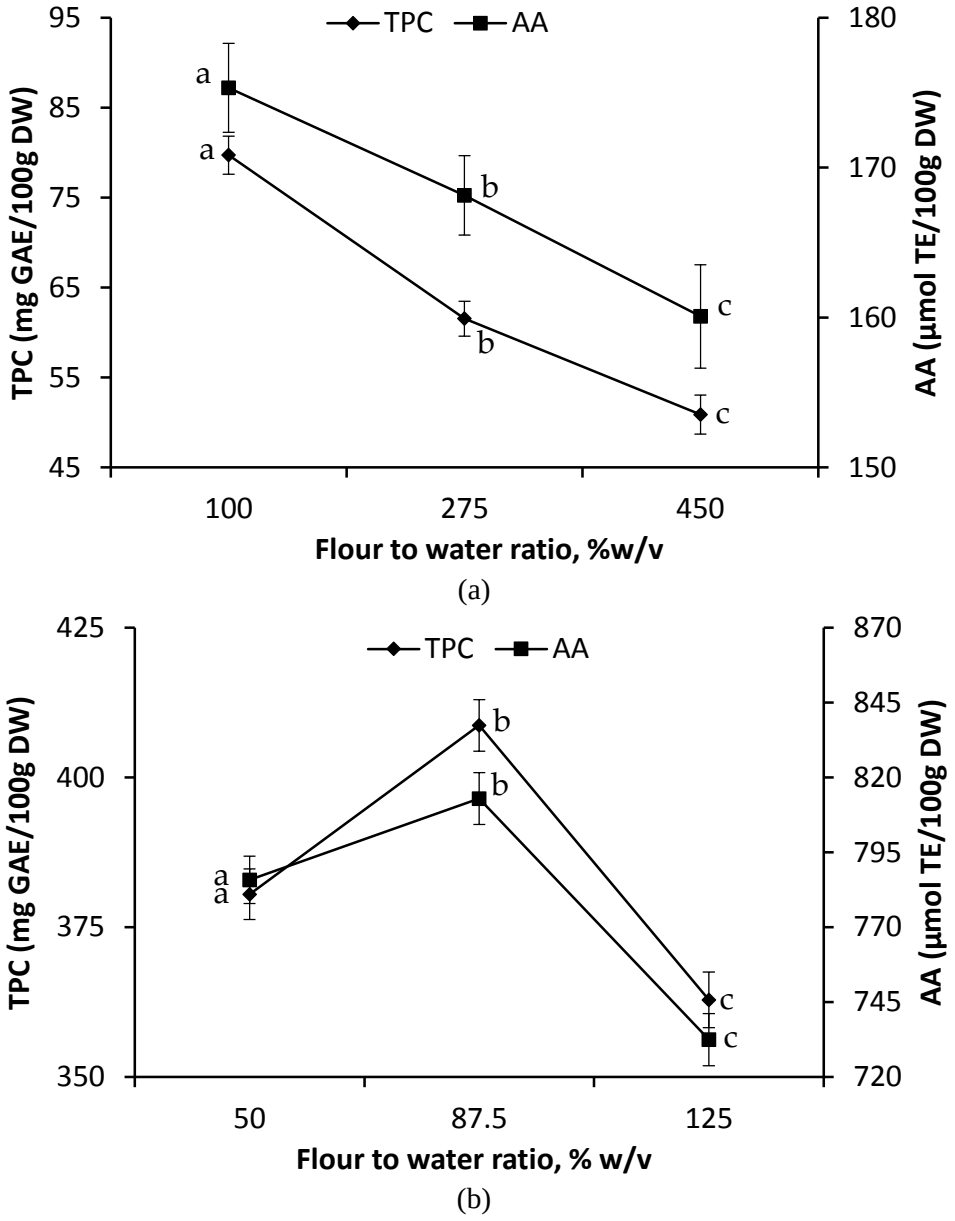


Figure 8.2 Main effect of flour to water ratio on total phenolic content (TPC) and antioxidant activity (AA) of (a) sorghum flour and (b) apple pomace. Values with the different letters at different points in the same line are significantly ($p < 0.05$) different

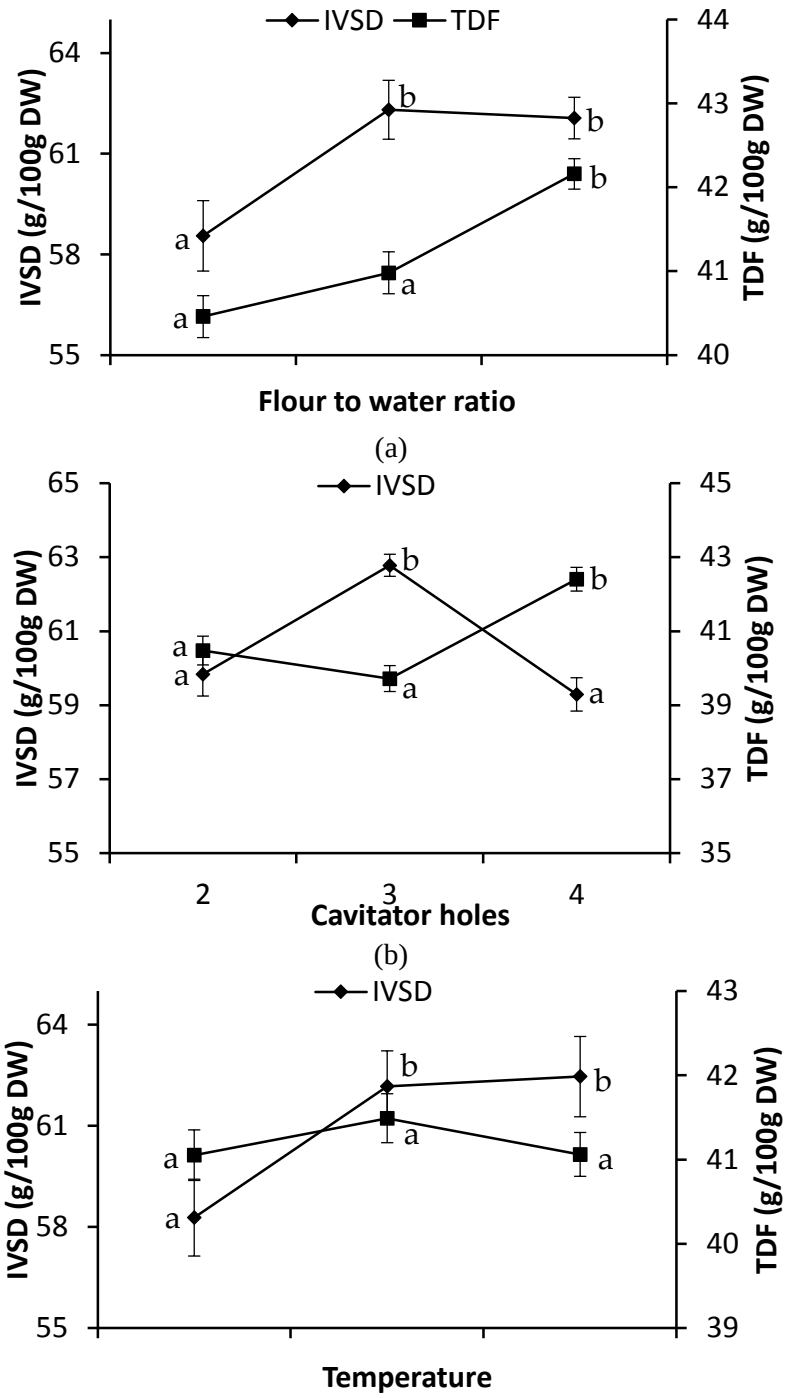


Figure 8.3 Main effect of flour to water ratio (a), cavitator holes (b) and cavitation temperature (c) on in-vitro starch digestibility (IVSD) of sorghum flour and total dietary fiber (TDF) of apple pomace. Values with the different letters at different points in the same line are significantly ($p < 0.05$) different

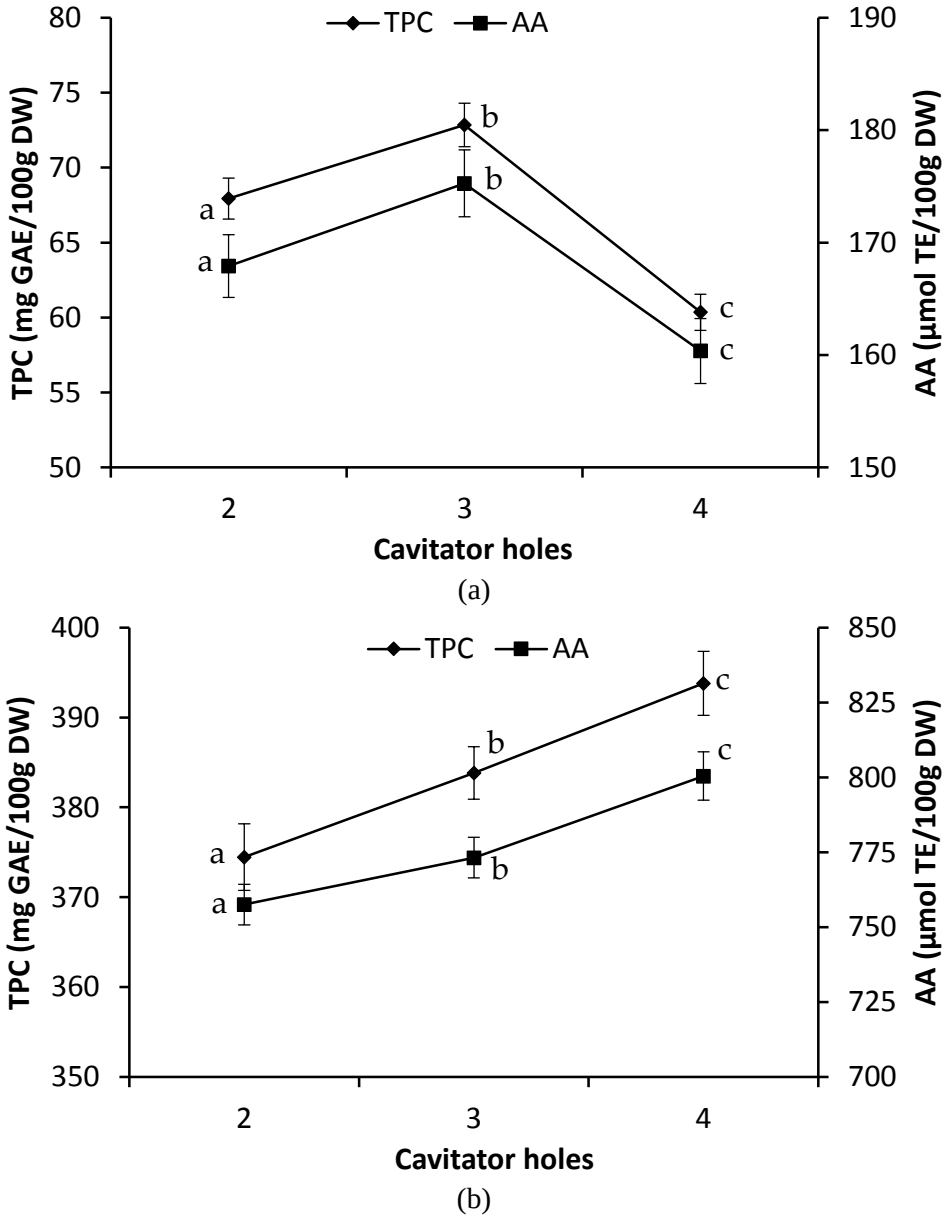


Figure 8.4 Main effect of number of rotor holes on TPC and AA of (a) sorghum flour and (b) apple pomace. Values with the different letters at different points in the same line are significantly ($p < 0.05$) different

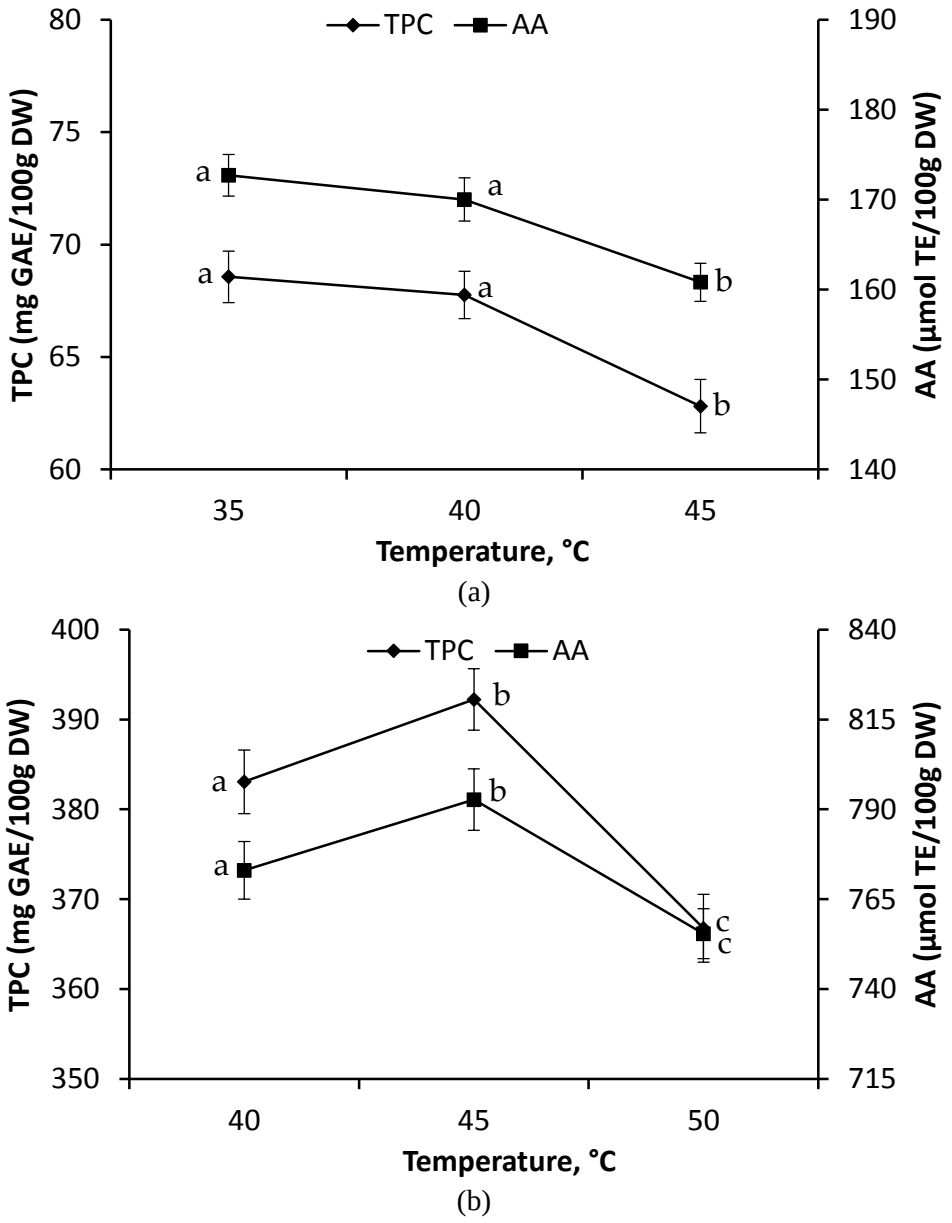


Figure 8.5 Main effect of outlet temperature on total phenolic content (TPC) and antioxidant activity (AA) of (a) sorghum flour and (b) apple pomace. Values with the different letters at different points in the same line are significantly ($p < 0.05$) different

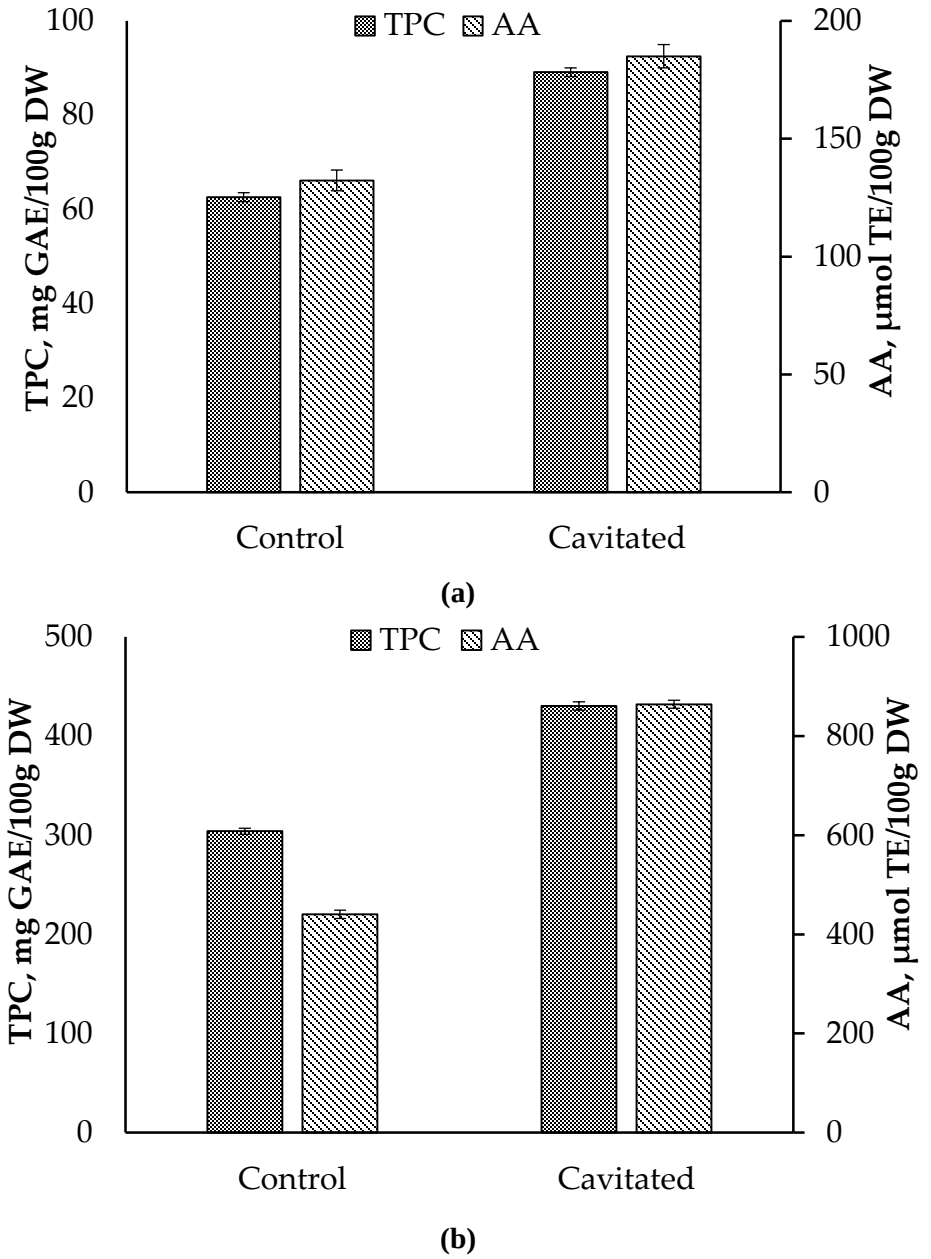


Figure 8.6 Comparison of total phenolic content (TPC) and antioxidant activity (AA) of control (a) sorghum flour with that of pretreated at optimum conditions (10%w/v) flour to water ratio (FWR), 3 cavitator holes, and 35°C temperature) and (b) apple pomace (8.75%w/v) FWR, 4 cavitator holes, and 45°C temperature)

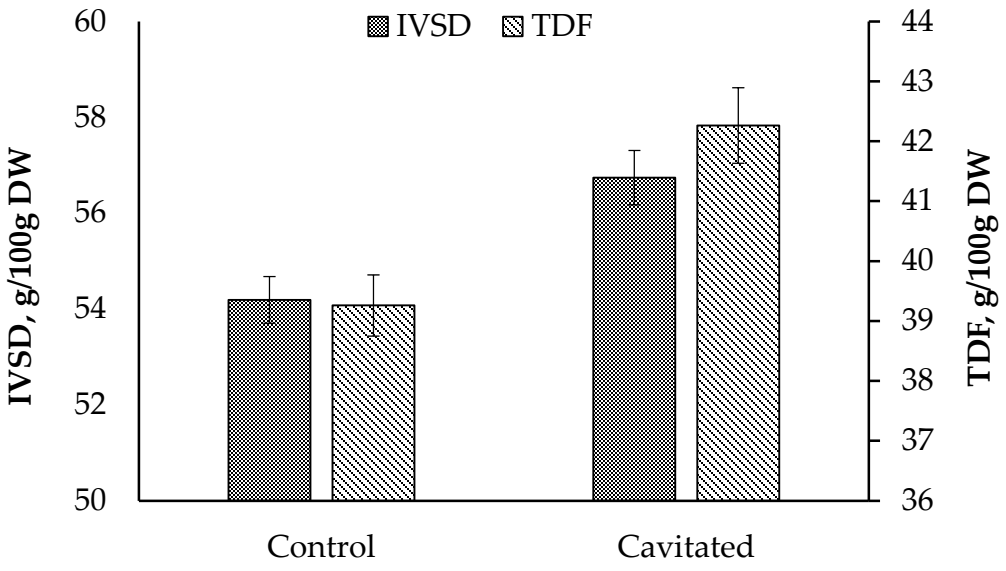


Figure 8.7 Comparison of in-vitro starch digestibility (IVSD) of sorghum flour and total dietary fiber (TDF) of apple pomace with that of cavitated at respective optimum conditions

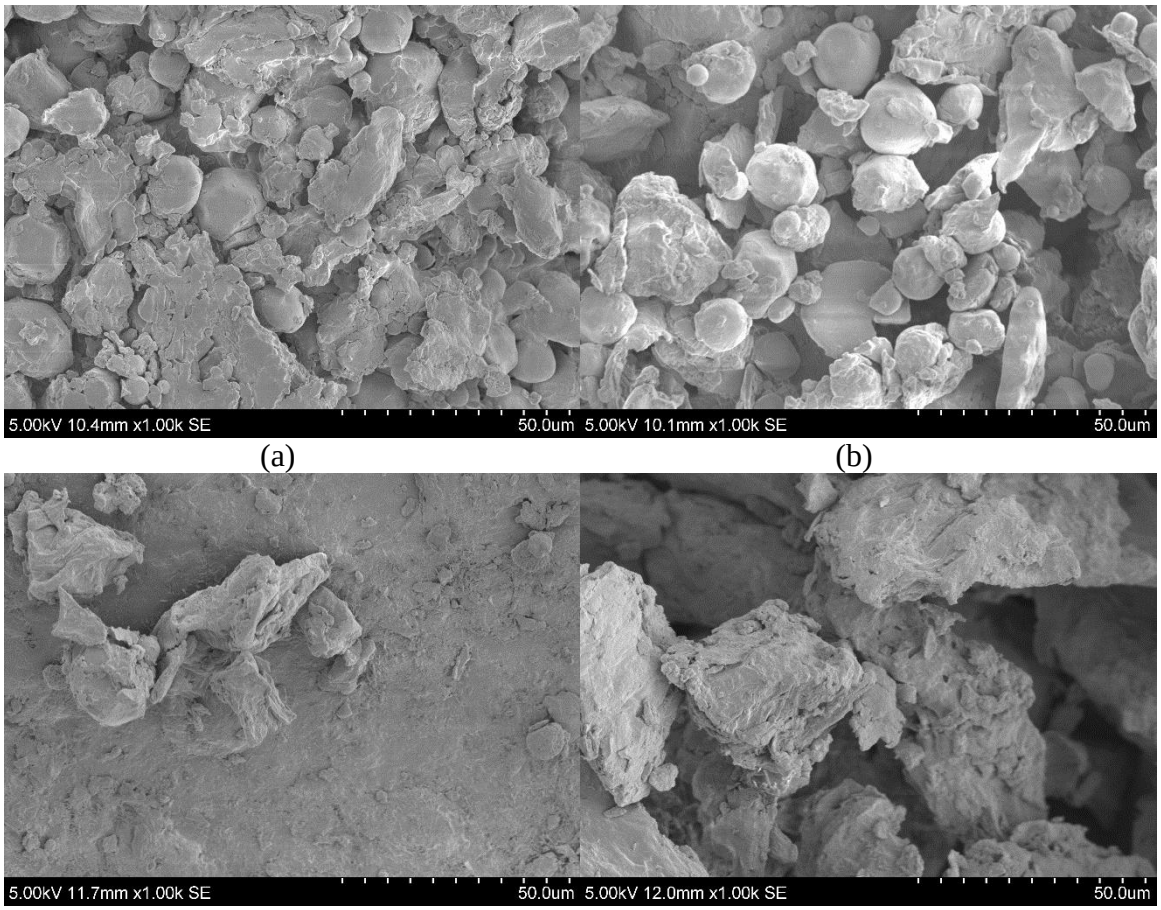


Figure 8.8 Scanning electron micrographs (×1000 magnification) of (a) control sorghum flour (SF), (b) cavitated SF, (c) control apple pomace (AP) and (d) cavitated AP

CHAPTER 9

Effect of extrusion processing parameters on antioxidant, textural and functional properties of hydrodynamic cavitated corn flour, sorghum flour and apple pomace based extrudates[8]

9.1 Abstract

Corn flour and hydrodynamic cavitated sorghum flour, apple pomace blend was extruded to investigate the effect of extrusion processing on total phenolic content (TPC), antioxidant activity (AA) along with some selected textural and functional properties. Box-Behnken design was applied for three levels of apple pomace ratio APR (10%, 20%, 30%), feed moisture FM (25%, 30%, 35%wb), extrusion die temperature T (80, 110, 140ºC) and screw speed SS (100, 150, 200 rpm) as extrusion parameters. TPC and AA of the extrudates increased during extrusion cooking with the increase in APR and decrease in T and SS. Expansion ratio, brittleness, crispness and water solubility index of extrudates were increased while hardness and water absorption index showed the deduction when compared to that of the control extrudates. Starch digestibility and dietary fiber were also observed to be increased after extrusion. At optimum conditions of 30% APR, 25%wb FM, 132ºC T and 108 rpm SS, TPC and AA of extruded products were 120.1 mg GAE/100g DW and 308.7 µmol TE/100g DW, respectively whereas these numbers found in control blend were 123.2 mg GAE/100g DW and 258.9 µmol TE/100g DW, respectively. Major phenolic acids in control and extruded products were derived by caffeic acid followed by salicylic acid and ferulic acid.

[8] Lohani, U. C. and Muthukumarappan, K. 2016. Effect of extrusion processing parameters on antioxidant, textural and functional properties of hydrodynamic cavitated corn flour, sorghum flour and apple pomace based extrudates. Journal of Food Process Engineering. *Under review*.

9.2 Introduction

In recent years, the research has got momentum to valorize the incorporation of underutilized crops, i.e. millet, sorghum and industrial food by-products, i.e. fruit pomace into main stream of human diet due to their enrichment in nutritional as well as bioactive compounds like phenolic and flavonoid antioxidants. Sorghum (*Sorghum bicolor* L.) is a gluten-free cereal that has the highest content of phenolic compounds among cereals (Cardoso et al., 2015). Moreover, it has several health benefits due to its phenolic compounds and antioxidant capacity (de MoraisCardoso et al., 2015). Apple pomace (AP), byproduct from juice and cider processing industries is a rich source of numerous phytochemicals in the form of simple sugars, pectin, and natural antioxidants (Bhushan et al., 2008) and is also composed of dietary fibers and carbohydrates (Sudha et al., 2007). Ready to eat snacks and instant cereals, which are improved by addition of functional components, such as fiber and antioxidants can be developed through the use of low-cost, versatile technologies, such as extrusion.

The extrusion process has wide applications in the production of snacks, breakfast cereals, textured vegetable proteins and animal feeds. Extrusion is a continuous process in which feed materials is subjected to mixing, shearing, heating, cooking and shaping. Extrusion cooking is an attractive process in food industry with the advantages of versatility, high productivity, quality, low cost, energy efficiency, absence of effluents and possibility of product design (Tumuluru et al., 2013).

Extrusion cooking may influence dietary fiber, protein, vitamins, and other nutrients both positively and negatively in the food (Singh et al., 2007). Similarly, it can change phenolic content in food products with two opposite effects. First, decomposition

of heat-labile phenolic compounds and polymerization of some phenolic compounds, incline to decrease the extractable phenolic content. Secondly, due to disruption of cell wall matrices and breaking of high molecular weight complexed polyphenols during extrusion, extractability of phenolic compounds is improved (Wang et al., 2014b). The net effect of extrusion on total phenolic content depends on which effect is predominant. Furthermore, extrusion cooking induces alteration in physicochemical, functional properties, polyphenolic compounds and their antioxidant activity, which also depend on raw material and extrusion process variables such as feed moisture, screw speed and configuration, die geometry, temperature and time (Sarawong et al., 2014).

Several studies have shown the positive impact of extrusion on total phenolic content (TPC) and antioxidant activity (AA) of starchy-fiber food extrudates (Bisharat et al., 2015, Hirth et al., 2014, Morales et al., 2015, Sarawong et al., 2014, Sharma et al., 2012). On the other hand, studies demonstrated that extrusion decreased the TPC and AA of extrudates (Cardoso et al., 2015, Gujral et al., 2012, Leyva-Corral et al., 2016, Ti et al., 2015, Wani and Kumar, 2015, Korkerd et al., 2016). In our preliminary trials on extrusion of corn flour (CF), sorghum flour (SF) and apple pomace blend revealed 40% and 30% reduction in TPC and AA, respectively of final extrudates. Therefore, sorghum flour and apple pomace were pretreated with hydrodynamic cavitation (HC) to improve the TPC and AA in both the materials in order to compensate the loss during extrusion. In a different study conducted by Lohani and Muthukumarappan (Chapter 8), HC not only enhanced TPC, AA in SF and AP but also improved total dietary fiber (TDF) in AP and in-vitro starch digestibility (IVSD) in SF. Therefore, it is important to investigating the

further effect of extrusion on aforementioned properties of CF, SF and AP blend extrudates.

Moisture content in the raw materials governs gelatinization reactions which in turn influence the extrudate's physical properties and nutritional composition (Thymi et al., 2005). Although high temperature and feed moisture were reported to stimulate the phenolic compound polymerization affecting their extractibility and antioxidant activity (Leyva-Corral et al., 2016), higher moisture content up to some extent have protective effect on the bioactive compounds (Brennan et al., 2011). Much lower feed moisture induced the drier extrusion conditions in which shearing effects would be more destructive on the phenolic compounds (Ozer et al., 2006).

Therefore, keeping in view the above facts, the objective of this study was to investigate the influence of apple pomace inclusion and extrusion process variable (feed moisture, barrel temperature and screw speed) on antioxidant properties (total phenolic content, antioxidant activity), textural properties (hardness, brittleness, crispness) and functional properties (water absorption index, water solubility index, total dietary fiber, in-vitro starch digestibility) of extruded products.

9.3 Materials and Methods

Sorghum flour (SF) and apple pomace (AP) provided by ADM Milling Co. (Overland Park, KS), and Tree Top, Inc. (Selah, WA), respectively were fermented followed by hydrodynamic cavitation (HC) and stored at -20°C before experimental utilization. Therefore, the blend and extrudates prepared by treated SF and AP are refereed as HC blend and HC extrudates, respectively in this study. Corn flour was donated by Cargill (Paris, IL). The chemical compositions of all the ingredients are given

in Table 9.1. Blends were formulated with corn flour, sorghum flour and apple pomace in the ratios of 10:90:10, 10:80:20 and 10:70:30, respectively.

9.3.1 Extrusion process

The extrusion experiments were carried out on a single screw extruder (Brabender Plasti-corder, model PL 2000, South Hackensack, NJ). The extruder had a screw diameter of 19 mm; a length to diameter ratio of 20:1; nominal compression ratio of 3:1; and a die opening of 3 mm. The inner barrel had a grooved surface to ensure zero slip at the wall. The barrel was divided into independent electrically heated zones (feed zone and central zone and die) cooled by air. Feed zone temperature was kept constant as 60°C throughout the experiments. The extrusion parameters comprising the independent variables were temperatures at the central and die end zone of the barrel (80, 110 and 140°C), screw speed (100, 150 and 200 rpm) and feed moisture content (25, 30 and 35% wb).

Before extrusion, experimental blends were brought to room temperature (25°C) to ensure uniform moisture distribution. The order of processing was chosen by randomizing apple pomace inclusion ratio, feed moisture levels, barrel temperatures and screw speed. Each extrusion run was brought to steady state as indicated by constant torque and melt temperatures before sampling and data collection. All extrudates were stored at -20°C for further analysis.

9.3.2 Extraction of samples

The extraction of sample for determining TPC, AA and phenolic characterization was done using the method described by Khan et al. (2013). For determining TPC and AA, 1 g sample 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°C for the analysis of TPC and AA.

Free phenolic acid extraction was performed by adding 10 ml of 80% (v/v) aqueous methanol into 2 g of sample. Mixture was shaken in a shaking water bath for 1 h at 25°C. After centrifugation at 3000×*g* for 20 min, the supernatant was decanted and the extraction was repeated as described above. The two supernatants were combined, evaporated to near dryness and reconstituted with methanol to a final volume of 10 ml.

9.3.2.1 Total phenolic content (TPC)

TPC of sample was determined using Folin–Ciocalteu method (Singleton et al., 1999) with some modification. 50 µl methanol extract of sample was added with 3.5 ml distilled water and 150 µl Folin-Ciocalteu reagent. The solution was vortexed and incubated for 30 min. Thereafter, absorbance of solution was measured at 760 nm against blank. Blank solution contained all the components that were present in the sample except the methanol extract. Gallic acid was used as positive control (standard) and linear regression curve between absorbance and concentration was drawn for the standard. This standard curve was used for calculating the concentration of sample and data was expressed in mg Gallic acid equivalent (GAE)/100 g dry weight (DW). This analysis was done in six replications.

9.3.2.2 Antioxidant activity (AA)

Extinction of DPPH is a free radical scavenging activity which was measured using spectrophotometric method described by Brand-Williams et al. (1995). 2,2-diphenyl-1-picrylhydrazyl (DPPH) 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 for 30 min. Thereafter, 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 weight (DW). Samples were analyzed in six replications.

9.3.2.3 Free phenolic acid characterization

Analysis of sample extracts was carried out using Thermo Scientific, Dionex Ultimate 3000 UHPLC system (Bannockburn, IL, United States) equipped with diode-array detector (DAD) and C_{18} column (150 mm × 4.6 mm) packed with 5 μm particles. The samples were injected with a mobile-phase flow rate of 800 μl/min. Gradient elution was carried out with a solvent system of water/acetic acid (99.8:0.2 v/v) as mobile phase A and acetonitrile/acetic acid (99.8:0.2 v/v) as mobile phase B. The total run time was 12 min, and the gradient elution was as follows: 0.0−3.0 min, B 10-25%; 3.0−4.5 min, B 25−45%; 4.5−6.5 min, B 45−65%; 6.5−8.0 min, B 65−85%; 8.0−9.0 min, B 85−100%.; 9.0−12.0 min, B 100−10%. All the solvents were filtered through 0.22 μm PTFE filters prior to inject. The column was maintained at 30°C while the autosampler was thermostated at 4°C. The system was controlled by Thermo Scientific Dionex Chromeleon 7 software. Benzoic acid and cinnamic acid derivatives were detected at 280 nm and 320 nm, respectively. The concentrations of phenolic acids were calculated from peak areas in comparison to calibration curves of the respective standards and were expressed as μg/g DW.

9.3.3 Moisture content, total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF)

Moisture content of samples was determined by air oven standard methods recommended by AOAC (1980). Initially 5 g of sample in triplicate was dried in hot air oven at 130-133°C for 2 h. After drying, dried sample was again weighed. Following formula is used for calculating the moisture content (MC).

$$\text{MC (\%wb)} = \frac{W_i - W_f}{W_i} \times 100 \tag{9.1}$$

W_i= initial weight of sample (5 g),

W_f= weight of sample after drying, g

Total dietary fiber in all extrudate samples was measured in the laboratory by the AOAC approved method 991.43 (AOAC, 1992). Duplicate samples of milled samples were suspended in MES/TRIS buffer (0.05 M, pH 8.2 at 24°C) and incubated sequentially with heat-stable α-amylase (95-100°C, 30 min) to give gelatinization, hydrolysis and de-polymerization of starch, protease (60°C, 30 min) to solubilize and depolymerize proteins, and amyloglucosidase (60°C, 30 min, pH 4.5) to hydrolyse starch fragments to glucose. The enzyme digestate was then treated with four volumes of 95% ethanol (1 h) to precipitate soluble fiber. The alcohol-treated digestate was filtered through borosilicate sintered glass crucibles (40-90 μm) that had previously been matted with celite, dried, and weighed. The total dietary fiber residue present in the crucible was washed with alcohol and acetone, dried overnight (103°C), and weighed. One duplicate from each sample was used for ash determination (525°C muffle furnace) and the other for protein determination. Total dietary fiber percent (TDF, g/100g DW) was calculated as

$$\text{TDF}=\frac{\frac{R_1+R_2}{2}-p-A-B}{\frac{m_1+m_2}{2}}\times\frac{100}{DW}\times 100 \quad (9.2)$$

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 R_2, B is the blank and DW is percentage dry weight (g) of sample.

$$B=\frac{BR_1+BR_2}{2}-BP-BA \quad (9.3)$$

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 Insoluble dietary fiber (IDF) and soluble dietary fiber (SDF), 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 Eq (9.2) and (9.3).

9.3.4 Total starch and in-vitro starch digestibility (IVSD)

Total starch of samples was determined by AOAC approved method 996.11 (AOAC, 1996). The 100 mg sample and 0.2 ml, 80% (v/v) ethanol were added to a glass test tube and mixed on a vortex mixer. A magnetic stirring rod was added to each tube, and the tubes were placed in an ice water bath before 2 ml, 2 M KOH was added to each tube with stirring. The tubes were removed after 20 min, and 8 ml, 2 M sodium acetate buffer was added to each tube with stirring. Thereafter, 0.1 ml, 3000 U/ml thermostable α-amylase and 0.1 ml, 3300 U/ml amyloglucosidase (Megazyme) were added to each

tube. The tubes were incubated in a 50°C water bath for 30 min with intermittent stirring on a vortex mixer. Samples were diluted to 100 ml with distilled water, centrifuged, and the supernatant was retained for analysis. The K-GLUC (GOPOD format) assay kit from Megazyme was used for the determination of D-glucose using glucose oxidase based on absorbance at 510 nm as read on the UV−vis spectrophotometer. The glucose was converted into starch by multiplying a factor 0.9.

The in vitro starch digestion of samples was determined according to the method of Goñi et al. (1997). 10 ml of HCI-KCI buffer (pH l.5) was added to 50 mg sample (pH was adjusted). Then 0.2 ml of a solution containing 1 g of pepsin in 10 ml of HCI-KCI buffer was added to sample and incubated at 40°C for 1 hour in a shaking water bath. Volume was completed to 25 ml with Tris-Maleate buffer (pH 6.9). 5 ml solution of α-amylase in Tris-Maleate buffer containing 2.6 UI were added to each sample. Samples were then incubated at 37°C in a shaking water bath for 3 h. 1mL aliquot sample was placed in a tube at 100°C and was energically shaken for 5 min to inactivate the enzyme. Then 3 ml of 0.4M Sodium acetate buffer (pH 4.75) were added to aliquot, and 60 µL of amyloglucosidase was used to hydrolyze the digested starch into glucose after 45 min at 60°C in a shaking water bath. Volume was adjusted to 100 ml with distilled water. Triplicated aliquots of 0.5 ml were incubated with the K-GLUC (GOPOD format) assay kit (Megazyme) for the determination of D-glucose using glucose oxidase based on absorbance at 510 nm as read on the UV−vis spectrophotometer. The glucose was converted into starch by multiplying a factor 0.9. Results were expressed on a total starch basis.

9.3.5 Texture analysis

Extrudates were subjected to hardness, brittleness and crispness using TA-XTPlus Texture Analyzer (Texture Technologies Corp., Hamilton, MA) as per method described by Altan et al. (2008b). The peak force as an indication of hardness was measured with a TA-XTPlus Texture Analyzer (Texture Technologies Corp., Hamilton, MA) using 3-point bend test. The test speed was 2 mm/s and the distance between two supports was kept as 22 mm. The curve between force vs distance was plotted and analyzed by Texture Exponent 32 software program (version 3.0). The slope (N/mm) and distance (mm) at which a product breaks were measured from force–distance curve and evaluated as crispness and brittleness, respectively (Texture Technologies, a). Both the sample extrudates were replicated 10 times. The radial expansion ratio of the extrudates was measured as the ratio of the diameter of the extrudates to the diameter of the die orifice.

9.3.6 Water absorption index (WAI) and water solubility index (WSI)

To determine the WAI and WSI of extrudates, the methodology proposed by Anderson (1982) was followed. Ground extrudates (2.5 g) was suspended in distilled water (30 ml) in a tarred 60 ml centrifuge tube. The suspension was stirred intermittently and centrifuged at 3000×*g* for 10 min. The supernatant was decanted into a tarred aluminums cup and dried at 135°C for 2 h. The weight of the gel remaining in the centrifuge tube was measured. The WAI and WSI were calculated by

$$\text{WAI}=\frac{W_g}{W_{ds}}\times 100 \tag{9.4}$$

where, WAI is water absorption index, W_g is the weight of gel (g), and W_{ds} is the weight of dry sample (g).

$$\text{WSI}=\frac{W_{ss}}{W_{ds}}\times 100 \tag{9.5}$$

where, WSI is the water solubility index (%), W_{ss} is the weight of dry solids of supernatant (g), and W_{ds} is the weight of dry sample (g).

9.3.7 Cross-sectional microstructure analysis

The microstructure of control and HC extrudates were examined using a scanning electron microscope (SEM) (Hitachi-S3400 N, Tokyo, Japan). Small amounts of samples were mounted on SEM specimen stubs by using double-sided adhesive tape. Each powder sample was coated with 10 Å thick layer of gold in a sputter coater before being scanned and photographed at 32× magnification.

9.3.8 Experimental design

A Box-Behnken design was applied for extrusion experiments to determine the effects and the optimum levels of the experimental parameters. The effects were studied at three experimental levels –1, 0, and +1. A total of 29 experiments were required with 5 center points as described in Table 9.2. The experimental data were analyzed by the response surface regression procedure and the parameters obtained from the response surface methodology (RSM) analysis were substituted into the following second-order polynomial model equation.

$$Y_i = \beta_0 + \sum_{i=1}^{k}\beta_i X_i + \sum_{i=1}^{k}\beta_{ii} X_{ii}^2 + \sum_{i=1}^{k}\sum_{j=i+1}^{k}\beta_{ij} X_i X_j \tag{9.6}$$

where Y_i is the predicted response; β_0 is the interception coefficient; β_i, β_{ii}, and β_{ij} are coefficients of the linear, quadratic, and interaction terms; X_i and X_j are the variables; and k is the number of independent parameters (k= 4).

9.3.9 Statistical analysis

Design expert 9 statistical software package (Stat-Ease Inc., USA) was used to analyze the experimental data. Multiple regression analysis and analysis of variance (ANOVA) were used to evaluate the experimental data. The modeling was started with a quadratic model including linear, squared, interaction terms. Significant terms in the model for each response were found by ANOVA. The adequacy and quality of the models were examined by evaluating the lack of fit (LOF), the coefficient of determination R^2 and the p-value obtained from the ANOVA. Derringer's desired function methodology was used to generate optimal conditions for extrusion process variables on all the properties of extruded snacks.

9.4 Results and Discussion

9.4.1 Model fitting

A response surface methodology was used to analyze the effect of extrusion parameters on antioxidant, textural and functional properties of extruded products. The results of the analysis of variance (ANOVA) are summarized to show the significance of the regression coefficients and the goodness of fit of the models (Table 9.3). It is obvious from Table 9.3 that p-values of lack of fit were not significant ($p>0.05$) for all the responses for extrusion process which indicated their suitability to predict the variations within the system. Higher coefficient of determination ($R^2 >0.82$) exhibited goodness of fit for all mathematical models. Furthermore, the experimental data showed a good fit with Eq. (1), since all regression models were statistically significant ($p < 0.05$).

The regression coefficients of mathematical model were also analyzed by RSM describing all the above mentioned responses of extruded products as a function of apple

pomace ratio APR (X_1), feed moisture FM (X_2), extrusion temperature T (X_3), and screw speed SS (X_4) for extrusion process is depicted in Table 9.4. The values of the regression coefficients presented were used in the final predictive model equations after discarding the non-significant terms. Thus, these equations were assumed to best describe the relationships between the experimental variables and the response factors in the present study.

9.4.2 Interpretation of response surface model and contour plots

Three-dimensional response surface plots and two-dimensional contour plots were obtained on the basis of the model equations mentioned above to explicate the correlation between independent and dependent variables studied in this study. Both types of plots presented the effects of two independent variables on the response factor, keeping others at level-coded zero.

9.4.2.1 Effect of extrusion variables on antioxidant properties of extruded products

TPC was significantly ($p<0.05$) influenced by linear and quadratic effects of APR and SS. Moreover, significant linear effect of T ($p<0.05$) were also observed on TPC of extruded products (Table 9.3). On the other hand, AA of extruded products were significantly ($p<0.05$) effected by linear effect of APR and T. Feed moisture within its experimental range didn't exhibit any significant ($p>0.05$) influence on TPC and AA of extrudates. Hirth et al. (2014) and Stojceska et al. (2009) also reported that an increment in feed moisture from 18-28% and 2-17%, respectively didn't affect the TPC and AA of starch based extrudates. On the contrary, Bisharat et al. (2015), Leyva-Corral et al. (2016), Obiang-Obounou and Ryu (2013), Sarawong et al. (2014) and Yagci and Gogus (2009) reported a significant drop in phenolic contents of final extrudates with increase

in feed moisture content from 14-19%, 25-28%, 23-29%, 20-50% and 12-18%, respectively. Furthermore, Ozer et al. (2006) found an increase in AA of corn, oat, chickpea, carrot, hazelnut based extrudates with increase in feed moisture from 11-15%. The determined values of TPC and AA were found in range of 57.6-126.3 mg GAE/100g DW and 77.2-325.5 µmol TE/100g DW, respectively.

Response surface plots show the effects of APR, T and SS on TPC and AA of extrudates (Figure 9.1). With regard to the combined effect by APR and T (Figure 9.1a), at low APR (10%), TPC of extrudates decreased by 14% with increase in temperature from 80ºC to 140ºC, whereas, the influence of temperature on TPC at higher APR (30%) was less significant. TPC significantly ($p<0.05$) increased by 57% with increase in APR from 10% to 30% regardless the temperature range. Similar results were observed for AA for the combined effect of APR and T (Figure 9.1d). AA of extrudates was increased by 56% when APR increased with in experimental range. This result was attributed to the initial higher TPC and AA in the raw material. At higher temperature, phenolics could be modified due to alteration in their functional group properties and solubility leading to reduced chemical reactivity. Also at the higher temperature, phenolic acids are susceptible to oxidation resulted in decreased TPC and AA. Negative effect of temperature on TPC and AA was also reported by Obiang-Obounou and Ryu (2013), Sarawong et al. (2014), Sharma et al. (2012) and Ti et al. (2015) for chestnut, banana flour, barley and black rice, respectively during extrusion.

The results concerning the effect of both APR and SS suggested that increase in SS from 100 to 150 rpm caused more significant ($p<0.05$) drop of 20% on TPC at low APR (10%) compared to that at higher level of APR (30%) (Figure 9.1b). On the other

hand, TPC increase significantly ($p<0.05$) by 47% with increment in APR from 10 to 30% regardless the SS experimental levels. Although, higher screw speed led to a decrease of torque development due to reduced filled length and residence time, increasing mechanical energy input to the system with increasing shear rate and thermal stresses had the dominant destructive effect on TPC of extrudates. These results were in the agreement with the findings of Bisharat et al. (2014) and Ozer et al. (2006) for corn flour and oat flour based extrudates, respectively.

Figure 9.1c shows the combined effect of SS and T on TPC of extrudates. As observed, gradual increase in temperature from 80ºC to 140ºC at low SS (100 rpm) decreased the TPC by 11% whereas this number was determined as 15% at higher SS (200 rpm). Similar effects were observed on TPC by varying SS from 100 to 200 rpm at both the levels of T (80ºC and 140ºC). Higher temperature and screw speed in combination had more detrimental effects on TPC of extrudates because of more thermal stresses leading decomposition or alteration of the molecular structure of phenolic compounds. These results were in accordance with the findings of Sarawong et al. (2014) for the extrusion of green banana flour.

9.4.2.2 Effect of extrusion variables on textural properties of extruded products

ANOVA shows that expansion ratio of extruded products was significantly ($p<0.05$) effected by linear terms APR, FM, T, SS and interaction term of APR, SS (Table 9.3). Significant ($p<0.05$) influence of FM and T was observed on hardness. Furthermore, brittleness was significantly ($p<0.05$) influenced by linear effect of T and interactive effect of FM and T, whereas linear terms of FM, SS and quadratic term of T had significant ($p<0.05$) effect on crispness of extrudates (Table 9.3). Expansion ratio,

hardness, brittleness and crispness varied from 0.91-1.59, 1.94-11.8 N, 2.90-7.62 mm and 1.32-5.70 N/mm, respectively within the experimental range of extrusion variables.

With regard to the combined effect by APR and FM (Figure 9.2a), ER increased by 9% with increase in APR from 10% to 30% regardless the FM. However, with increment of FM from 25% to 35%, extrudates expansion reduced by 8% regardless the APR. Higher soluble dietary fiber in raw material indicated higher expansion of extruded products (Table 9.1). Korkerd et al. (2016) reported the similar results for extrudates when mango peel fiber was added to the blend. Increasing the moisture content during extrusion reduced the elasticity of dough resulted in a lower degree of starch gelatinization through plasticization of melt and lower expansion. At low moisture, the melt had higher viscosity which related directly to shear stress of the plasticized material and the increased degree of gelatinization. Similar effect of FM (15-17%) on expansion of extrudates was observed by Korkerd et al. (2016).

The trend observed for ER upon simultaneous variation of APR and T is exhibited in Figure 9.2b. With increase in temperature from 80°C to 140°C at low APR (10%), ER increased by 21%. This increment was observed as 40% at higher pomace inclusion (30%). On the other hand, when APR increased from 10% to 30% at low temperature (80°C), no significant ($p>0.05$) change was observed in ER. Nevertheless, at higher temperature (140°C), ER increased by 17% with increased APR in experimental range. Higher temperature increased the degree of gelatinization and extent of superheated steam that caused more expansion. These results were in accordance with Gujral et al. (2012) and Korkerd et al. (2016) who reported higher expansion of extrudates at higher temperature.

As explicated from response surface plot (Figure 9.2c), no significant ($p>0.05$) change was observed in ER when SS increased from 100 rpm to 200 rpm at low APR (10%) and vice-versa, i.e. at low SS and with increment in APR from 10% to 30%. Nonetheless, at higher APR (30%) and SS (200 rpm), an increment of 40% was observed in ER of extruded products. Increasing screw speed increased the shear rate and thus the temperature of melt which led to a higher expansion ratio. This result was in accordance with the findings of Liu et al. (2000) and Altan et al. (2008a) for the oat-corn and barley-tomato pomace based extrudates, respectively.

The response surface of the effect of FM and T on hardness of extrudates (Figure 9.2d) explicated that the increment of temperature from 80°C to 100°C increased the hardness of extrudates followed by reduction in hardness by 60% when temperature further increased up to 140ºC for all experimental FM. With increase in FM from 25% to 35%, hardness of extrudates decreased insignificantly ($p>0.05$) at lower temperature (80°C), however a reduction by 33% was observed for hardness at higher temperature (140°C). Higher temperature and moisture decreased melt viscosity and increased the water vapor pressure. This favored the bubble growth which is the driving force for expansion that produced more expanded products with more crispness and softer texture. Similar results were obtained by Bisharat et al. (2015) for corn based extrudates.

The distance to break the extrudate was considered to be an indication of brittleness, with the shortest distance being the most brittle product. The interactive effect of FM and T on brittleness of extruded products is shown in response surface plot (Figure 9.3a). It was observed that brittleness decreased by 27% with increase in FM from 25% to 35% at low temperature (80°C). However, at higher temperature (140°C), no

significant ($p>0.05$) change was found in brittleness with an increment in FM within experimental range. On the other hand, brittleness increased by 33% with increase in temperature from 80°C to 140°C at low FM (25%). Furthermore, this number was increased up to 43% at higher FM (35%) when temperature elevated within its experimental range. Low hardness value with increased temperature and decreased FM would produce lower distance to break and thus extrudates with higher brittleness. This result was consistent with the findings of Altan et al. (2008b) for barley-grape pomace extrudates.

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. The lower the slope, the crisper the product is considered. The combined effect of FM and T on crispness represented by surface plot (Figure 9.3b) depicted that with increase in temperature from 80°C to 100°C, the crispness of extrudates decreased followed by significant ($p<0.05$) increase in crispness by 50% with further increase in temperature up to 140ºC regardless the FM. With increase in temperature, the degree of superheating of water increased before melt passed through the die. This in turn increased the bubble radius which caused increase in matrix radius and decrease in shell wall thickness. The thinner shell wall was responsible for crispness of the extrudates. Bisharat et al. (2015) also reported the increase in crispness of corn based extrudates with increase in temperature from 140ºC to 180ºC. As FM increased from 25% to 35%, the crispness of extrudates increased by 27% regardless the temperature. This result was attributed to the decrease in hardness with FM.

Figure 9.3c shows the combined effect of FM and SS on crispness of extrudates. As observed, with increase in SS from 100 rpm to 200 rpm, crispness of extrudates decreased by 19% at low FM (25%). Moreover, at higher FM (35%), increment in crispness was observed as 16% with increase in SS within experimental range. Increased shear rate at higher SS raised the temperature of melt which caused more expansion and crispness eventually.

9.4.2.3 Effect of extrusion variables on functional properties of extruded products

It is explicated from ANOVA (Table 9.3) that WAI was significantly ($p<0.05$) influenced by the temperature whereas, linear effects of APR, SS and interactive effects of APR, T and SS, T significantly ($p<0.05$) influenced the WSI. Extruded products exhibited the significant ($p<0.05$) linear effects of APR, SS and combined effects of APR, T; FM, SS and SS, T on IVSD. TDF of extrudates was significantly ($p<0.05$) influenced by APR. WAI, WSI, IVSD and TDF functional properties of extrudates were observed in the range of 5.06-6.65 g/g DW, 10.6-16.4 g/100g DW, 46.6-83.6 g/100g DW and 11.3-20.3 g/100g DW, respectively.

Water absorption has been generally attributed to the dispersion of starch in excess water, and the dispersion is increased by the degree of starch damage due to gelatinization and extrusion-induced fragmentation, that is, molecular weight reduction of amylose and amylopectin molecules. Figure 9.4a shows the combined effect of APR and T on WAI of extruded products. With gradual increase in temperature from 80°C to 140°C at low APR (10%), WAI increased by 19%. However, this increment was relatively less at higher APR (30%). This increasing trend was due to greater heat generation and disruption of starch molecule which favors starch gelatinization. These

results are in agreement with Alam et al. (2015), Gujral et al. (2012) and Seth et al. (2015) who observed the increase in WAI of extruded rice-carrot pomace, brown rice and yam-corn-rice with increase in temperature from 120°C to 180°C, 100ºC to 120ºC and 100ºC to 140ºC, respectively.

Water solubility index, often used as an indicator of degradation of molecular components measures the degree of starch conversion during extrusion which is the amount of soluble polysaccharide released from the starch component after extrusion. With regard to the interactive effect of APR and T on WSI (Figure 9.4b), it was observed that with increase in APR from 10% to 30%, WSI of extrudates increased by 36% at low temperature (80°C). What's more, WSI increased only by 17% at higher temperature (140°C) when inclusion of AP increased within experimental range. It could be attributed to the presence of low molecular weight compounds i.e. soluble fiber presence in pomace caused increase in WSI of extrudates. This result was in agreement with Altan et al. (2009) for barley and grape-tomato pomace based extrudates.

Response surface plot describing the combined effect of SS and T on WSI is shown in Figure 9.4c. At low SS (100 rpm), WSI decreased by 10% with increment in T from 80°C to 140°C. However, extrudates showed insignificant ($p>0.05$) change in WSI with increase in T at higher SS (200 rpm). WSI decreased by 13% with increase in SS from 100 rpm to 200 rpm at low temperature (80°C). At higher temperature (140°C), no significant ($p>0.05$) effect of SS on WSI was observed. Lower WSI at higher T and SS could be attributed to the molecular interaction between degraded starch, fiber and protein causing an increase in molecular weight resulted in a decrease in solubility. Altan et al. (2008a) and Kumar et al. (2010) also reported the similar trend of WSI with

temperature for barley-tomato pomace and rice-carrot pomace based extrudates, respectively.

The results concerning the effect of APR and T on IVSD (Figure 9.5a) indicated that IVSD increased by 36% when APR increased from 10% to 30% at low temperature (80°C), whereas increment of APR at higher temperature (140°C) decreased the IVSD slightly. Increasing APR increased the amount of soluble fiber in blend which increased the elastic properties of melt resulted in higher degree of gelatinization and increase in IVSD. At higher temperature, dextrinization of starch induced low IVSD. AT low APR (10%), gradual increase in T from 80°C to 140°C increased the IVSD of extrudates by 27%, however a drop of 13% was observed in IVSD when temperature increased in experimental range at higher APR (30%). At low APR, higher starch present in blend lost its structural integrity and increased enzyme susceptibility with the effect of temperature. Higher APR level increased the fiber amount in blend which might cause formation of protein-fiber-starch complex that reduced the susceptibility of starch to enzyme hydrolysis. Altan et al. (2009) also reported the decrease in IVSD with increase in temperature for barley-grape pomace extrudates.

The combined effect of FM and SS on IVSD (Figure 9.5b) explicated that at low SS (100 rpm), IVSD decreased by 12.5% with increase in FM from 25% to 35%, however a slight increment in IVSD was observed with increase in FM at higher SS (200 rpm). With the increase of SS from 100 rpm to 200 rpm at low FM (25%), IVSD showed a trend of decreasing by 18.8%, whereas, an increase was observed in IVSD at higher FM (35%) with increase in SS within experimental range. The intermediate levels of FM and SS didn’t influence IVSD of extrudates significantly ($p>0.05$). Higher SS imparted higher

shear resulted in disruption of the starch molecules which imbibed more water particularly at higher FM and thus improved gelatinization of starch.

The interactive effect of T and SS on IVSD of extruded products is shown in response surface plot (Figure 9.5c). At low SS (100 rpm), IVSD increased by 17% with increase in T from 80°C to 100°C followed by insignificant ($p>0.05$) change in IVSD with further increment of temperature. However, IVSD decreased by 14% at higher SS (200 rpm) when temperature increased from 80°C to 140°C. As effected by SS in the range of 100-200 rpm at low temperature (80°C), IVSD increased by 15.5%, whereas IVSD decreased by 14.5% at higher temperature (140°C) with increment of SS in experimental range. At higher T and SS, dextrinization of starch resulted in decrease in IVSD of extrudates. At low temperature, with increase in SS, relatively higher starch molecules disruption due to higher shear rate produced the extrudates with higher IVSD. Sarawong et al. (2014) found the similar results for extruded green banana flour.

With regard to the interactive effect of APR and FM on TDF (Figure 9.5d), it was observed that TDF increased by 29% with increase in APR from 10% to 30% regardless the FM. This result was harnessed by initial TDF available in raw material.

9.4.3 Optimization of the extrusion process and validation

Optimal process conditions were investigated for extrusion process variables by maximizing the TPC, AA, ER, brittleness, crispness, WSI, IVSD, TDF and minimizing the hardness and WAI. Table 9.5 indicates the optimum conditions for extrusion process along with predicted and experimental response variables values. The predicted results matched well with the experimental results which validated the RSM model, indicating Box-Behnken design could be effectively used to optimize the process parameters for

extrusion processes on antioxidant, textural and functional properties of corn, SF and AP based extrudates.

Control extruded products were produced at optimum conditions using CF, SF and AP without hydrodynamic cavitation treatment. It is explicated from Table 9.5 that TPC and AA retained in HC extrudates were 97.5% and 119.2%, respectively with compared to control blend. Furthermore, TPC and AA observed in HC extrudates were 62.5% and 67.3%, respectively higher than that of control extrudates. Moreover, textural and functional properties of HC extrudates were improved. Hardness of HC extrudates were 11.6% less than that of control extrudates, while, the expansion ratio, brittleness and crispness of HC extrudates were 13.1%, 18.8% and 19.6% more with compared to control extrudates. Macroscopic and microscopic structures of control and HC extrudates exhibiting their expansions are shown in Figure 9.6. WSI, IVSD, TDF and SDF were increased by 39.8%, 8.2%, 22.9% and 83.6%, respectively for HC extrudates whereas, WAI showed 1.9% reduction in HC extrudates with compared to that of control extrudates. Although extrusion process increased the TDF and SDF in the HC extrudates with compared to that of control blend, majority of TDF was presented mainly by the insoluble fraction (Table 9.5). This result was in accordance with Huang and Ma (2016), Korkerd et al. (2016) and Sarawong et al. (2014).

Table 9.6 depicts that HC extrudates had significantly ($p<0.05$) more benzoic acid and cinnamic acid derived phenolic acids than that of control extrudates. Both the extrudates were found rich in caffeic acid followed by salicylic acid and ferulic acid. Except protocatechuic acid, rest of the phenolic acids were not significantly ($p>0.05$) differ for control blend and extrudate samples.

9.5 Conclusions

Extrusion of corn flour and hydrodynamic cavitated sorghum flour, apple pomace blend exhibited not only the higher retention of TPC and AA in extrudates but also an improvement in textural and functional properties of extrudates. Effect of pomace inclusion on TPC and AA was dominant followed by the effect of extrusion temperature. Inclusion of pomace in the blend increased the brittleness and crispness while decreased the hardness of extrudates showing the potential of this by-product in the food. Higher expansion ratio was found for higher pomace ratio and screw speed. Higher temperature facilitate the gelatinization of starch regardless the pomace inclusion ratio and thus diminished the water absorption. Higher solubility of extrudates was observed at lower temperature and screw speed. Extrudates also showed the increment in IVSD and TDF at higher APR due to increase in fraction of SDF. Extruded products were found rich in caffeic acid followed by salicylic acid and ferulic acid. Improved antioxidant, textural and functional properties of extrudates from cavitated blend make them of interest in utilizing as functional ingredients in a variety of gluten free snack foods in the future.

Table 9.1 Chemical composition of the raw materials before the extrusion process

Component, g/100g	**Corn flour**	**Sorghum flour**	**Apple pomace**
Moisture content	10.3±0.23	9.31±0.31	9.75±0.12
Ash	0.93±0.06	1.21±0.10	1.67±0.05
Fat	1.20±0.09	3.19±0.12	2.02±0.08
Protein	6.18±0.77	9.63±0.64	5.11±0.43
Starch	84.7±2.46	64.7±1.62	8.74±0.09
Total dietary fiber	2.57±0.01	10.6±0.03	43.9±0.05
Soluble	0.91±0.01	2.67±0.01	11.9±0.02
Insoluble	1.66±0.01	7.89±0.02	32.0±0.03

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

Table 9.2 Box-Behnken experimental design for extrusion process

Run	APR	FM	T	SS	Run	APR	FM	T	SS
1	-1	-1	0	0	16	0	0	+1	+1
2	+1	0	0	-1	17	0	0	-1	+1
3	+1	0	+1	0	18	0	0	0	0
4	0	+1	0	-1	19	0	+1	0	+1
5	-1	0	0	-1	20	0	-1	-1	0
6	0	0	+1	-1	21	+1	0	0	+1
7	+1	0	-1	0	22	-1	+1	0	0
8	0	0	0	0	23	0	-1	0	-1
9	-1	0	0	+1	24	0	-1	+1	0
10	-1	0	+1	0	25	+1	+1	0	0
11	0	0	0	0	26	0	+1	-1	0
12	-1	0	-1	0	27	+1	-1	0	0
13	0	0	0	0	28	0	-1	0	+1
14	0	0	-1	-1	29	0	0	0	0
15	0	+1	+1	0					

Variables	**Levels**		
	-1	**0**	**+1**
AP ratio (APR)	10	20	30
Feed moisture (FM, % wb)	25	30	35
Extrusion temperature (T, ºC)	80	110	140
Screw speed (SS, rpm)	100	150	200

APR: apple pomace ratio, FM: feed moisture, SS: screw speed, T: extrusion temperature

Table 9.3 ANOVA and statistical parameters describing the effect of the independent variables on antioxidant, textural and functional properties of extruded products

Source	df	Mean squares									
		TPC	**AA**	**ER**	**Hardness**	**Brittleness**	**Crispness**	**WAI**	**WSI**	**IVSD**	**TDF**
Model	14	558.2^{a}	2304.6^{a}	0.06^{a}	12.8^{b}	2.11^{a}	2.24^{a}	0.26^{b}	3.72^{a}	135.9^{a}	8.11^{b}
X_1	1	5716.3^{a}	25016.3^{a}	0.06^{b}	0.75	0.91	0.33	0.17	37.90^{a}	174.4^{b}	103.4^{a}
X_2	1	189.9	690.6	0.08^{b}	11.9^{b}	0.93	7.12^{a}	0.18	0.52	19.5	1.76
X_3	1	552.3^{b}	1274.7^{b}	0.41^{a}	44.8^{a}	25.0^{a}	0.11	2.42^{a}	0.02	1.13	2.19
X_4	1	384.4^{b}	767.2	0.15^{a}	1.31	0.05	1.61^{b}	0.11	3.68^{b}	163.1^{b}	0.36
X_{12}	1	104.1	750.6	0.001	6.66	0.85	1.09	0.09	0.43	1.35	0.09
X_{13}	1	8.89	145.7	0.002	2.12	0.01	0.63	0.05	1.32^{b}	282.1^{b}	0.78
X_{14}	1	0.60	2.96	0.09^{b}	3.38	0.04	1.28	0.01	0.43	74.5	0.02
X_{23}	1	5.53	200.0	0.01	0.47	1.60^{b}	0.08	0.09	0.03	19.9	0.29
X_{24}	1	60.3	72.7	2.78×10^{-6}	0.20	1.78×10^{-4}	0.07	0.08	0.04	138.1^{b}	0.65
X_{34}	1	2.88	1.45	0.02	0.66	0.02	0.01	0.16	1.70^{b}	332.8^{b}	0.06
X_{11}	1	635.7^{b}	2461.7^{b}	0.01	0.37	0.003	0.07	0.01	0.05	3.22	0.53
X_{22}	1	65.3	113.3	1.13×10^{-6}	0.63	0.02	0.09	0.01	0.04	40.3	0.39
X_{33}	1	0.002	74.3	0.01	103.3^{a}	0.14	17.2^{a}	0.21	4.73^{a}	521.6^{a}	0.37
X_{44}	1	218.2^{b}	848.5	0.01	0.15	1.57×10^{-5}	0.02	0.003	1.93^{b}	94.2	3.80
Residual	14	42.1	207.7	0.01	2.56	0.21	0.33	0.06	0.22	22.4	1.63
LoF	10	43.1	247.3	0.01	2.53	0.25	0.37	0.07	0.28	22.9	1.34
Pure error	4	39.7	108.9	0.002	2.64	0.09	0.23	0.02	0.06	20.9	2.35
R^2		0.93	0.91	0.88	0.83	0.91	0.87	0.82	0.94	0.86	0.83
R^2_{adj}		0.86	0.83	0.75	0.66	0.82	0.74	0.63	0.89	0.72	0.67
R^2_{pred}		0.67	0.58	0.33	0.25	0.53	0.37	0.01	0.70	0.34	0.33

Significant at $p<0.001$a, $p<0.05$b, LoF: lack of fit, adj: adjusted, pred: predicted, AA: antioxidant activity, df: degree of freedom, ER: expansion ratio, IVSD: in-vitro starch digestibility, TDF: total dietary fiber, TPC: total phenolic content, WAI: water absorption index, WSI: water solubility index

Table 9.4 Regression coefficients describing the relationship between responses and independent variables for extruded products

Coeff.	TPC	AA	ER	Hardness	Brittleness	Crispness	WAI	WSI	IVSD	TDF
β_0	129.8	63.9	0.99	-35.9	-3.52	-2.13	6.42	36.5	-106.2	42.6
β_1	1.71^{a}	7.46^{a}	-0.06^{b}	-1.19	-0.26	-0.60	0.14	0.45^{a}	4.68^{b}	0.42^{a}
β_2	-3.18	1.67	0.09^{b}	0.41^{b}	0.47	-0.46^{a}	-0.05	-0.23	-8.35	-1.04
β_3	0.19^{b}	2.36^{b}	-1.08×10^{-3a}	0.97^{a}	0.11^{a}	0.39	-0.03^{a}	-0.26	4.10	-0.11
β_4	-0.29^{b}	-0.94	-0.01^{a}	0.04	-6.35×10^{-3}	-8.43×10^{-3b}	-5.27×10^{-3}	-0.15^{b}	0.52^{b}	-0.15
β_{12}	-0.10	-0.27	3.67×10^{-4}	0.03	9.20×10^{-3}	0.01^{c}	-3.10×10^{-3}	-6.59×10^{-3}	0.01	-3.14×10^{-3}
β_{13}	-4.97×10^{-3}	-0.02	7.68×10^{-5}	2.43×10^{-3}	-2.0×10^{-4}	1.32×10^{-3}	-3.81×10^{-4}	-1.91×10^{-3b}	-0.03^{b}	-1.48×10^{-3}
β_{14}	7.76×10^{-4}	-1.72×10^{-3}	2.97×10^{-4b}	1.84×10^{-3}	1.87×10^{-4}	1.13×10^{-3}	1.07×10^{-4}	6.52×10^{-4}	-8.62×10^{-3}	1.23×10^{-4}
β_{23}	-7.84×10^{-3}	-0.05	-3.16×10^{-4}	-2.28×10^{-3}	-4.22×10^{-3b}	-9.55×10^{-4}	9.75×10^{-4}	6.03×10^{-4}	-0.01	1.79×10^{-3}
β_{24}	-0.02	-0.02	-3.33×10^{-6}	-8.88×10^{-4}	-2.67×10^{-5}	-5.42×10^{-4}	5.61×10^{-4}	3.73×10^{-4}	0.02^{b}	1.61×10^{-3}
β_{34}	-5.65×10^{-4}	-4.01×10^{-4}	4.06×10^{-5}	-2.71×10^{-4}	4.44×10^{-5}	2.92×10^{-5}	-1.34×10^{-4}	4.35×10^{-4b}	-6.08×10^{-3b}	7.80×10^{-5}
β_{11}	0.09^{b}	0.19^{b}	-4.17×10^{-6}	-2.38×10^{-3}	2.17×10^{-4}	-1.01×10^{-3}	-3.22×10^{-4}	9.13×10^{-4}	-7.05×10^{-3}	2.87×10^{-3}
β_{22}	0.13	0.17	-1.25×10^{-3}	-0.01	-2.19×10^{-3}	4.72×10^{-3}	-1.66×10^{-3}	3.24×10^{-3}	0.09	9.75×10^{-3}
β_{33}	2.09×10^{-5}	-3.76×10^{-3}	3.16×10^{-5}	-4.43×10^{-3a}	-1.61×10^{-4}	-1.81×10^{-3a}	1.99×10^{-4}	9.49×10^{-4a}	-9.96×10^{-3a}	2.64×10^{-4}
β_{44}	2.32×10^{-3b}	4.57×10^{-3}	1.89×10^{-5}	-6.17×10^{-5}	-6.22×10^{-7}	2.05×10^{-5}	9.73×10^{-6}	2.18×10^{-4b}	-1.52×10^{-3}	3.06×10^{-4}

Significant at p<0.001a, p<0.05b, AA: antioxidant activity, ER: expansion ratio, IVSD: in-vitro starch digestibility, TDF: total dietary fiber, TPC: total phenolic content, WAI: water absorption index, WSI: water solubility index

Table 9.5 Estimated optimum conditions, predicted and experimental values of the responses for extrusion process

Samples	Optimum conditions				Antioxidant properties			
	APR (%)	FM (%wb)	T (°C)	SS (rpm)	TPC, mg GAE/100g DW		AA, µmol TE/100g DW	
					Predicted	Actual*	Predicted	Actual*
HC extrudates	30	25	132	108	120.9	120.1±1.72	309.2	308.7±3.71
HC blend						171.6±2.03		381.8±3.94
Control extrudates						73.9±0.82		184.5±3.19
Control blend						123.2±1.61		258.9±3.59

	Textural properties							
	ER		Hardness, N		Brittleness, mm		Crispness, N/mm	
	Predicted	Actual*	Predicted	Actual*	Predicted	Actual*	Predicted	Actual*
HC extrudates	1.22	1.21±0.01	4.91	4.88±0.22	3.73	3.77±0.14	2.46	2.51±0.19
Control extrudates		1.09±0.01		5.52±0.43		4.64±0.37		3.12±0.31

	Functional properties							
	WAI, g/g DW		WSI, g/100g DW		IVSD, g/100g DW		TDF, g/100g DW	
	Predicted	Actual*	Predicted	Actual*	Predicted	Actual*	Predicted	Actual*
HC extrudates	6.37	6.36±0.05	15.3	15.1±0.13	83.6	83.1±0.69	19.2	19.3±0.07
Control extrudates		6.48±0.07		10.8±0.21		76.8±0.78		15.7±0.03

	IDF, g/100g DW	SDF, g/100g DW
	Actual*	Actual*
HC extrudates	14.7±0.04	4.59±0.05
Control extrudates	13.2±0.02	2.50±0.01
HC blend	14.6±0.06	5.15±0.01
Control blend	14.4±0.04	4.09±0.02

Response experimental results are reported as mean ± standard deviation. *mean values in column are significantly different at 5% level of significance, AA: antioxidant activity, APR: apple pomace ratio, ER: expansion ratio, FM: feed moisture, GAE: gallic acid equivalent, HC: hydrodynamic cavitated, IDF: insoluble dietary fiber, IVSD: in-vitro starch digestibility, SDF: soluble dietary fiber, SS: screw speed, T: extrusion temperature, TDF: total dietary fiber, TE: trolox equivalent, TPC: total phenolic content, WAI: water absorption index, WSI: water solubility index

Table 9.6 Phenolic profile of blends and extrudate samples (μg/g DW)

Compounds	Control blend	Control extrudates	HC Blend	HC Extrudates
Benzoic acids				
Protocatechuic acid	17.8±0.6[a]	1.74±0.1[b]	52.9±2.9[c]	15.6±0.4[d]
p-Hydroxybenzoic acid	16.9±0.9[a]	10.7±0.8[b]	23.1±1.2[c]	16.5±0.8[a]
Cinnamic acids				
Chlorogenic acid	3.17±0.2[a]	0.47±0.01[b]	7.67±0.2[c]	2.89±0.3[a]
Caffeic acid	49.6±2.8[a]	41.8±1.2[b]	57.4±2.1[c]	49.1±1.8[a]
p-coumaric acid	9.82±0.7[a]	4.54±0.3[b]	15.1±1.0[c]	9.49±0.2[a]
Ferulic acid	35.6±1.3[a]	25.4±1.1[b]	45.7±1.7[c]	34.9±1.2[a]
Salicylic acid	50.7±2.1[a]	34.1±1.3[b]	67.5±3.1[c]	49.7±2.1[a]

Means±SD in the same row with different letters are significantly different ($p<0.05$), HC: hydrodynamic cavitated

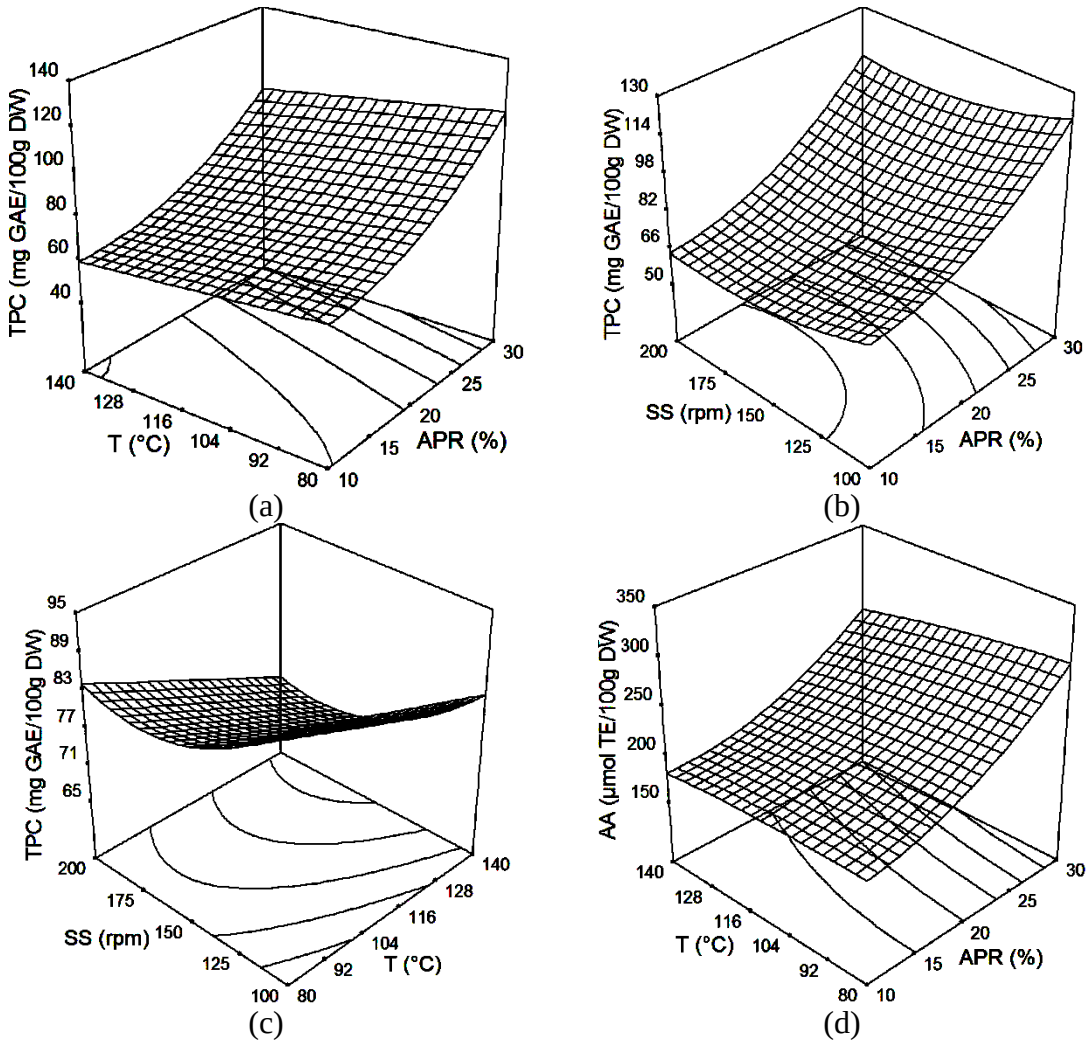


Figure 9.1 Response surface plots of total phenolic content (TPC) (a, b, c) and antioxidant activity (AA) (d) as affected by apple pomace ration (APR), extrusion temperature (T), and screw speed (SS) at corresponding 0 coded level of other two variables

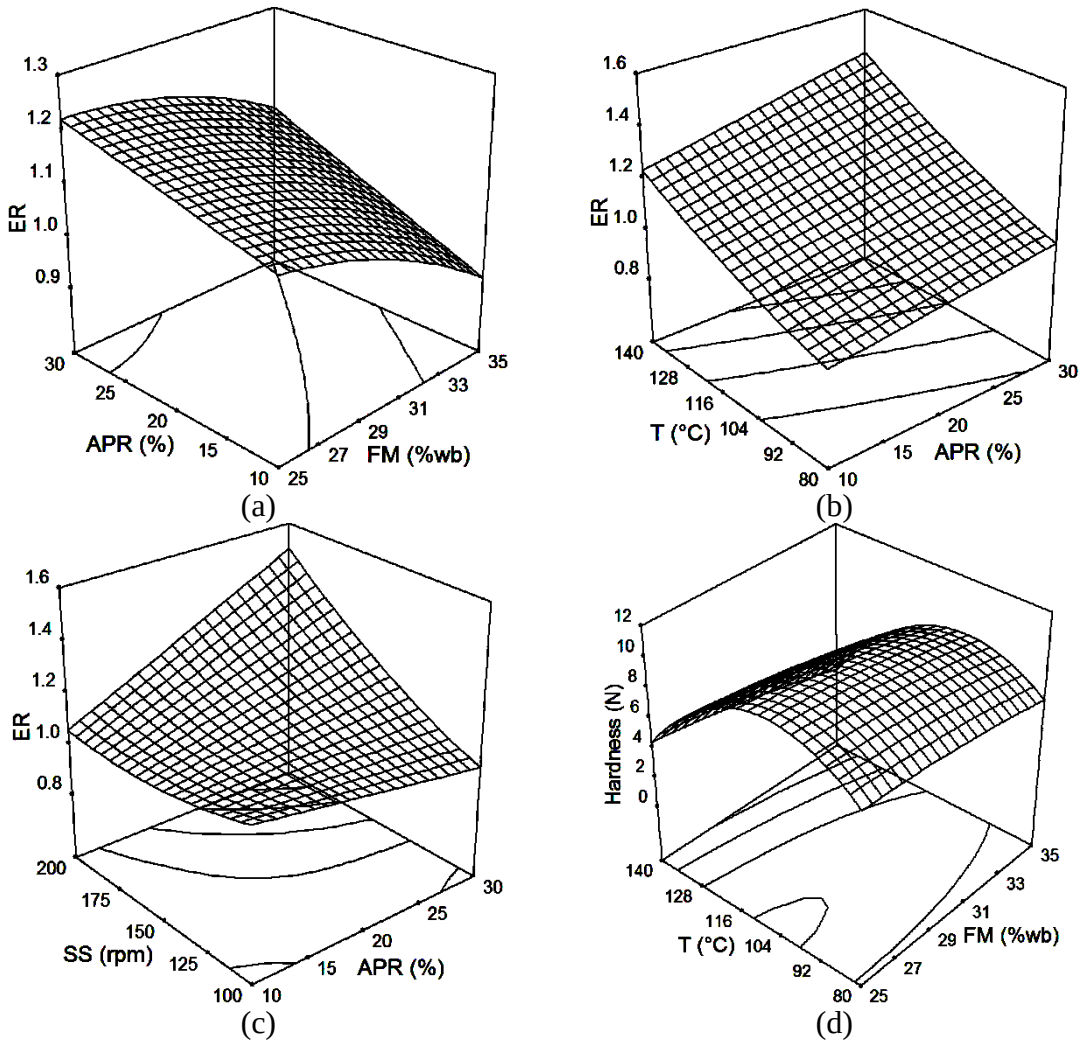


Figure 9.2 Response surface plots of expansion ratio (ER) (a, b, c) and hardness (d) as affected by apple pomace ratio (APR), feed moisture (FM), extrusion temperature (T), and screw speed (SS) at corresponding 0 coded level of other two variables

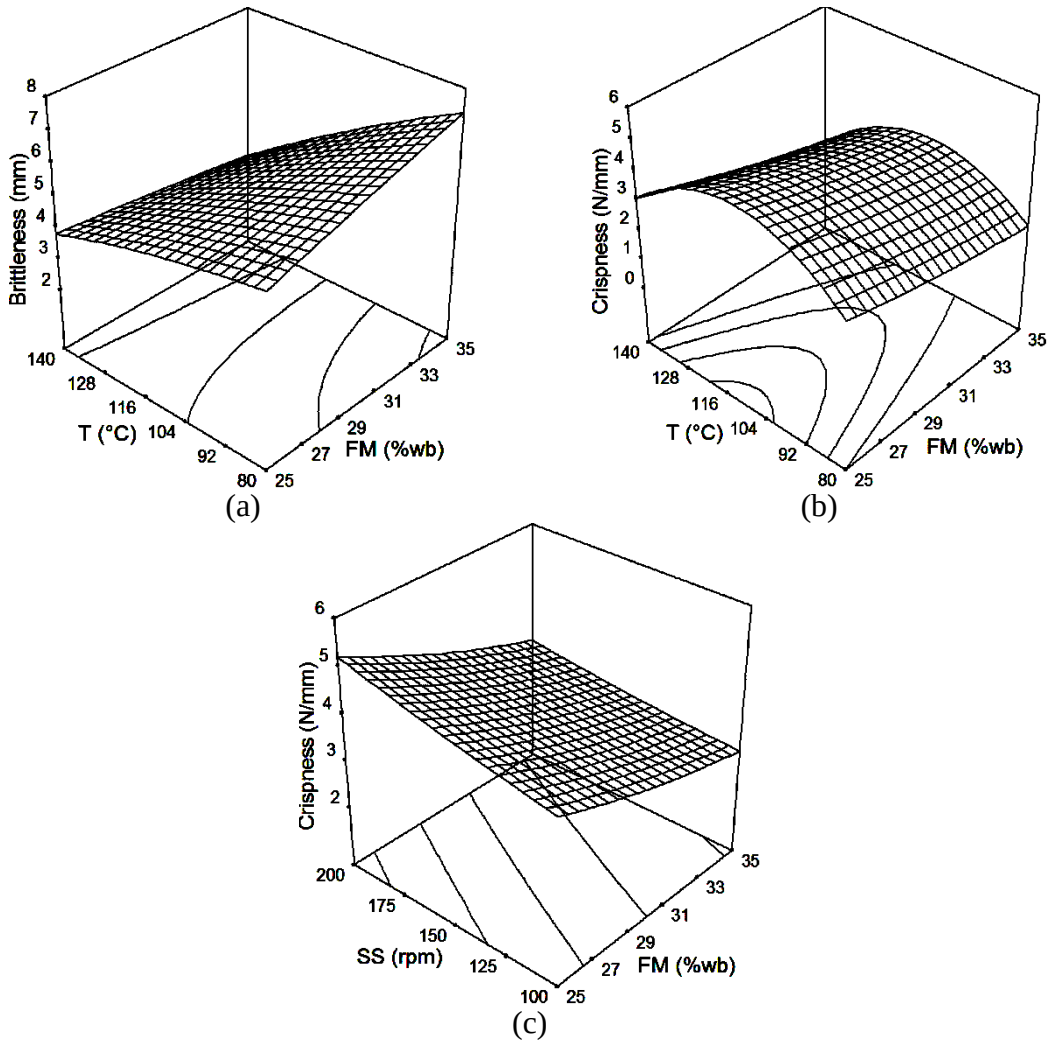


Figure 9.3 Response surface plots of brittleness (a) and crispness (b, c) as affected by feed moisture (FM), extrusion temperature (T), and screw speed (SS) at corresponding 0 coded level of other two variables

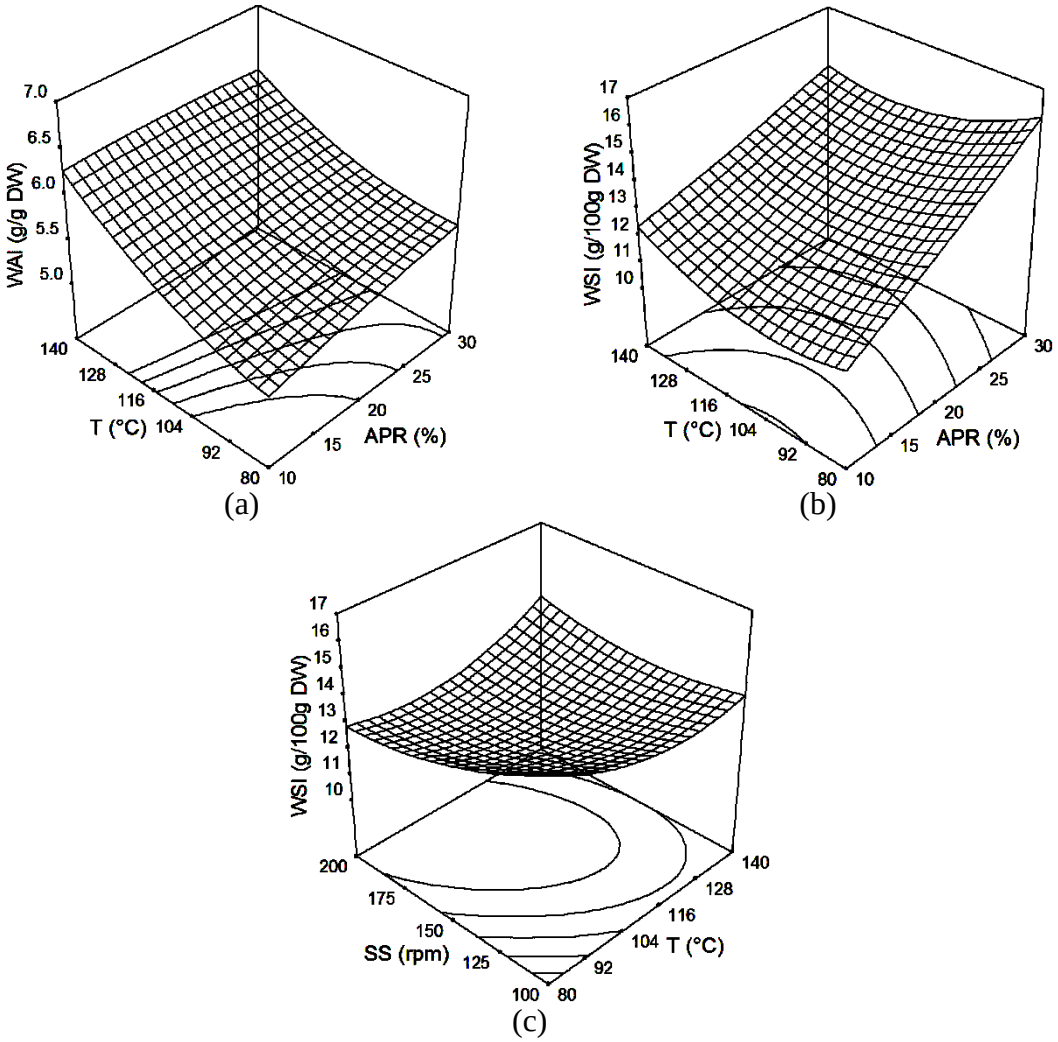


Figure 9.4 Response surface plots of water absorption index (WAI) (a) and water solubility index (WSI) (b, c) as affected by apple pomace ratio (APR), extrusion temperature (T), and screw speed (SS) at corresponding 0 coded level of other two variables

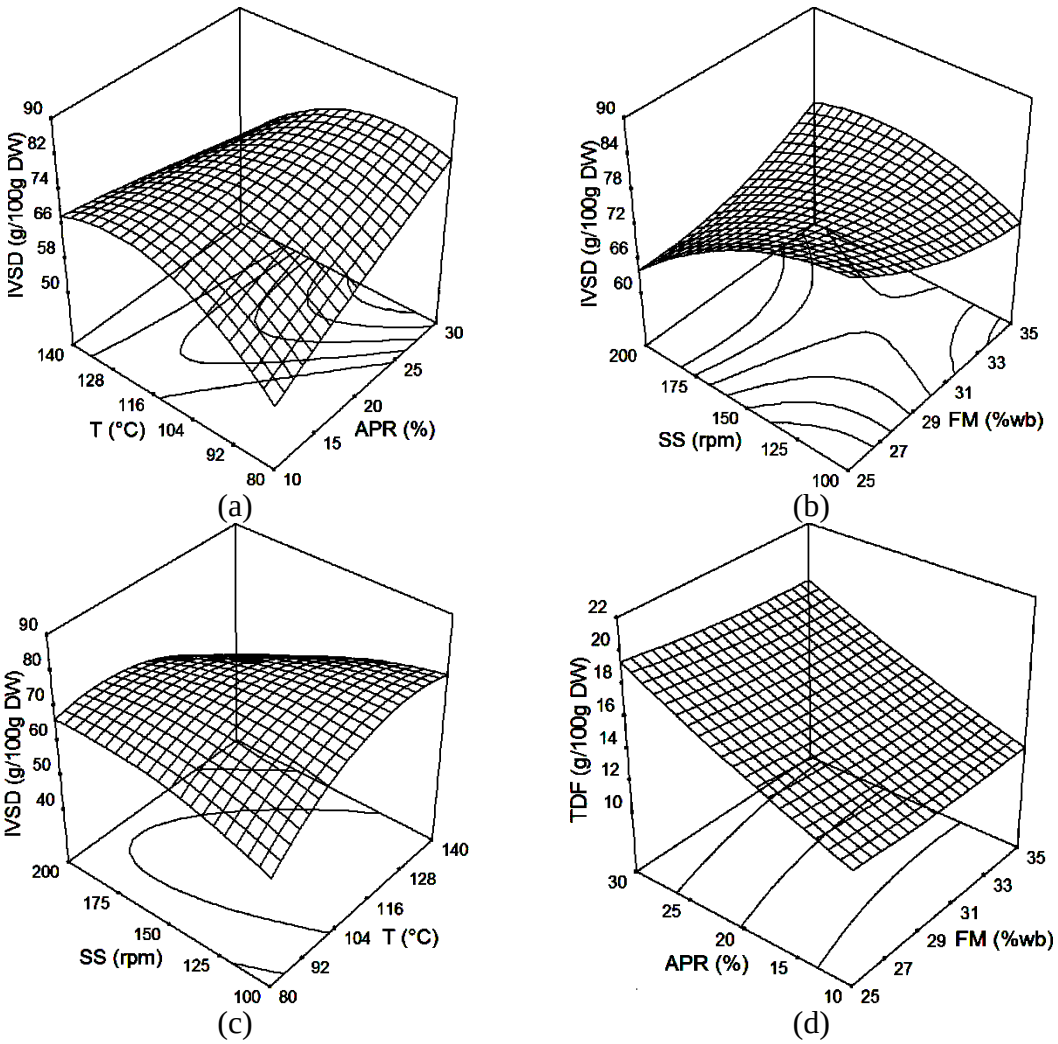


Figure 9.5 Response surface plots of in-vitro starch digestibility (IVSD) (a, b, c) and total dietary fiber (TDF) (d) as affected by apple pomace ratio (APR), feed moisture (FM), extrusion temperature (T), and screw speed (SS) at corresponding 0 coded level of other two variables

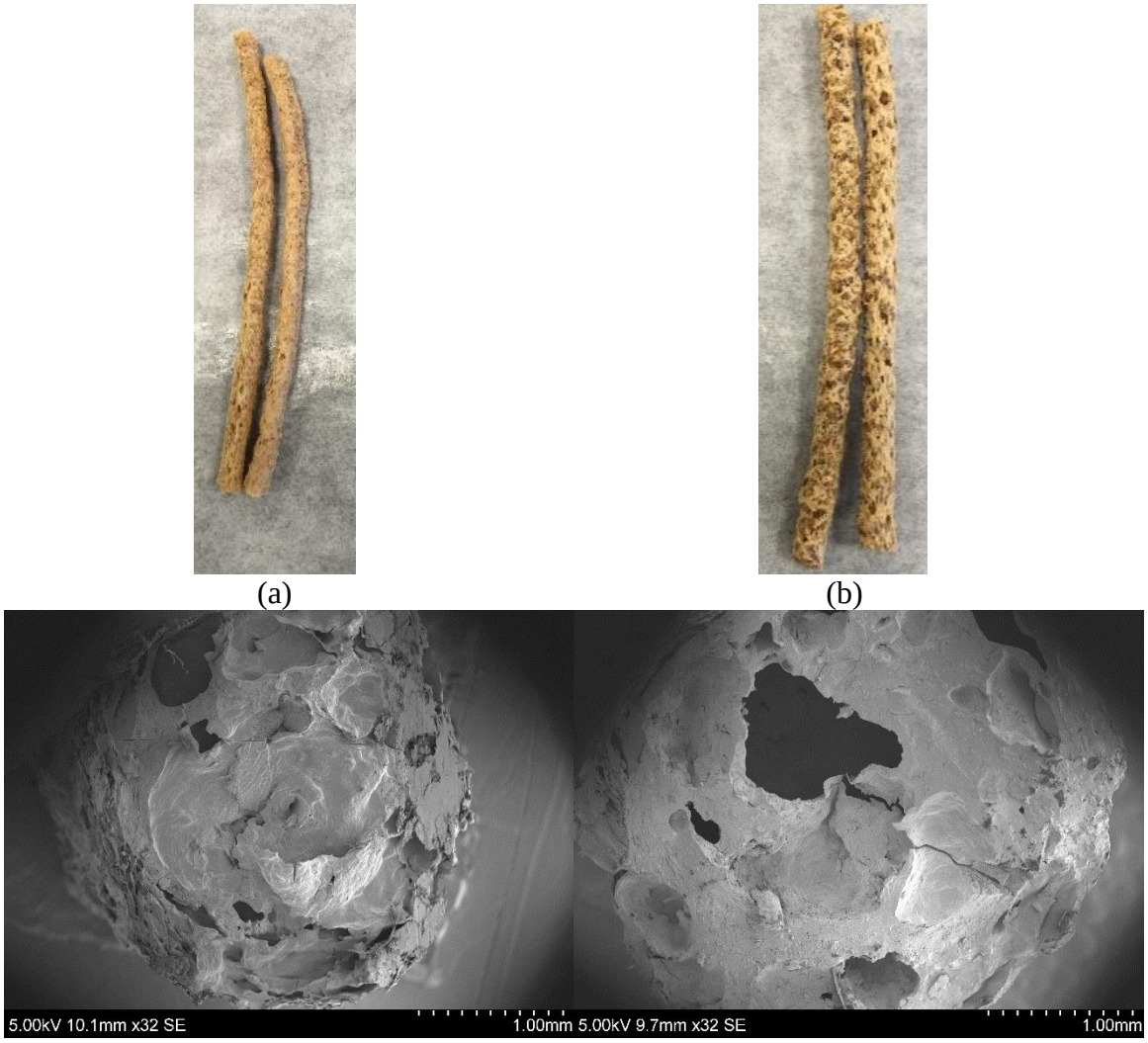


Figure 9.6 Macroscopic and cross-sectional microstructure of (a, c) control and (b, d) hydrodynamic cavitated (HC) extrudates, respectively

CHAPTER 10

Effect of liquid CO_2 assisted extrusion process on antioxidant, textural and functional properties of hydrodynamic cavitated corn flour, sorghum flour and apple pomace based extrudates[9]

10.1 Abstract

Corn flour and hydrodynamic cavitated sorghum flour, apple pomace blend was extruded to investigate the effect of CO_2 injection on the total phenolic content (TPC), antioxidant activity (AA) and some selected textural and functional properties. Box-Behnken design was applied for the three levels of apple pomace ratio APR (10%, 20%, 30%), feed moisture FM (15%, 20%, 25%wb), extrusion temperature T (80, 120, 160ºC) and screw speed SS (100, 150, 200 rpm) as extrusion parameters. Higher APR, FM and low T favored the TPC and AA of the extrudates. Expansion ratio and hardness decreased whereas brittleness and crispness increased at high temperature and low FM. Water absorption index (WAI) was affected by higher FM, T and low SS, while water solubility index (WSI) increased at higher APR, T and low FM, SS. Extrudates exhibited higher in-vitro starch digestibility (IVSD) at higher APR and T. At the optimum conditions of 30% APR, 25%wb FM, 97ºC T and 100 rpm SS, TPC and AA of the CO_2 extruded products were 130.3 mg GAE/100g DW and 326.8 µmol TE/100g DW, respectively whereas, these numbers found in the control extrudates were 116.3 mg GAE/100g DW and 305.5 µmol TE/100g DW, respectively. Though expansion ratio decreased after CO_2 extrusion, 59% less hardness and 70% more crispness were observed in the CO_2 extrudates with

[9] Lohani, U. C. and Muthukumarappan, K. 2016. Effect of CO_2 Assisted Extrusion Process on Antioxidant, Textural and Functional Properties of Hydrodynamic Cavitated Corn Flour, Sorghum Flour and Apple Pomace Based Extrudates. International Journal of Food Properties. *Under review*.

compared to that of the control extrudates. Low WAI and higher WSI were observed in the CO_2 extrudates, however extrusion with CO_2 lowered the IVSD and the total dietary fiber by 3% and 4%, respectively. The color (L*, a*, b*) results proved that the rate of Maillard's reaction rates decreased when CO_2 injected during the extrusion process. The higher inclusion of AP using CO_2 injection produced extrudates with improved antioxidant, textural and functional properties below 100°C extrusion temperature.

10.2 Introduction

Sorghum and apple pomace as an underutilized crop and industrial waste, respectively have been concerned to incorporate them in to human diet as both have health benefit due to their phenolic compounds and antioxidant capacity (de MoraisCardoso et al., 2015, Reis et al., 2014). Apple pomace is also a rich source of dietary fiber (Yan and Kerr, 2013) and ready to eat snacks and instant cereals rich in fiber and antioxidants can be developed through the use of low-cost, versatile technologies, such as extrusion.

Extrusion process is an important technology for processing of grain-based products like snacks and breakfast cereals with immense change in the texture. Extrusion is a continuous process consisted of mixing, shearing, heating, cooking and shaping of the feed material. Extrusion cooking is an attractive process in the food industry with the advantages of versatility, high productivity, quality, low cost, energy efficiency, absence of effluents and possibility of the product design (Tumuluru et al., 2013).

Extrusion cooking may influence the phenolic content in food products with two opposite effects. First, decomposition of heat-labile phenolic compounds and polymerization of some phenolic compounds, incline to decrease the extractable phenolic

content. Secondly, due to disruption of cell wall matrices and breaking of high molecular weight complexed polyphenols during extrusion, extractability of the phenolic compounds is improved (Wang et al., 2014b). The net effect of extrusion on the total phenolic content depends on which effect is predominant. Furthermore, extrusion cooking induces alteration in the physicochemical and functional properties which also depend on the raw material and extrusion process variables such as feed moisture, screw speed and configuration, die geometry, temperature and time (Sarawong et al., 2014).

In recent years, a new extrusion process involving the injection of carbon dioxide into the extruder barrel as the blowing agent instead of steam has been introduced. This is also called as cold-extrusion technique and the temperatures are maintained below 100ºC. However, the higher moisture content used in this process for keeping the product temperature below 100ºC and preventing formation of steam, resulted in the shrinkage of the extrudates (Ondo et al., 2013). In traditional extrusion process, high temperatures and low moisture contents cause the loss in the nutritional value and formation of variety of Maillard's reaction products with the potential harmful effects (Masatcioglu et al., 2013). The most significant advantage of the cold extrusion is to prevent the nutrients in foods from the effect of high temperature (Bilgi Boyaci et al., 2012). In addition, CO_2 injection reduces melt viscosity and promote small cells in the extrudates to improve texture and taste (Singkhornart et al., 2014). Another advantage of CO_2 injection is its function as plasticizer, which allows the processing of molecules which would otherwise not withstand the mechanical stresses and the operating temperatures of a standard extrusion process. Furthermore, the dissolved CO_2 acts as foaming agent during expansion through

the die. It is, therefore, to control pore generation and growth by controlling the operating conditions (Myat, 2013).

Previously, many researchers demonstrated the potential use of CO_2 injection to improve the structure formation (Bilgi Boyaci et al., 2012, Ferdinand et al., 1992, Ferdinand et al., 1990), expansion (Jeong and Toledo, 2004, Ondo et al., 2013, Singkhornart et al., 2014), appearance (Wang and Ryu, 2013a) and physicochemical properties (Singkhornart et al., 2013) of extrudates. However, little attention has been devoted to the impact of CO_2 injection on antioxidant properties of extruded products (Wang and Ryu, 2013b).

Furthermore, our preliminary trials on the extrusion of corn flour (CF), sorghum flour (SF) and apple pomace (AP) blend revealed 40% and 30% reduction in TPC and AA, respectively of the final extrudates. Therefore, sorghum flour and apple pomace were pretreated with hydrodynamic cavitation (HC) to improve the TPC and AA in both the materials in order to compensate the loss during extrusion. In a different study conducted by Lohani and Muthukumarappan (Chapter 8), HC not only enhanced TPC, AA in SF and AP but also improved the total dietary fiber (TDF) in AP and in-vitro starch digestibility (IVSD) in SF. Although CO_2 could reportedly act as a good blowing agent, the fiber-rich extrudate was rarely produced by the CO_2 injection extrusion in the past. Investigating the effect of CO_2 assisted extrusion on aforementioned properties of CF, SF and AP blend extrudates will be challenging.

Therefore, keeping in view the above facts, the objective of the study was to investigate the influence of CO_2 injection, apple pomace inclusion and extrusion process variable (feed moisture, barrel temperature and screw speed) on antioxidant properties

(total phenolic content, antioxidant activity), textural properties (hardness, brittleness, crispness) and functional properties (water absorption index, water solubility index, total dietary fiber, in-vitro starch digestibility) of extruded products.

10.3 Materials and Methods

Sorghum flour and apple pomace provided by ADM Milling Co. (Overland Park, KS), and Tree Top, Inc. (Selah, WA), respectively were fermented followed by hydrodynamic cavitation (HC) and stored at -20 °C before experimental utilization. Therefore, the blend and extrudates prepared by treated SF and AP are refereed as HC blend and HC extrudates, respectively in this study. Corn flour was donated by Cargill (Paris, IL). The chemical compositions of all the ingredients are given in Table 10.1. Blends were formulated with corn flour, sorghum flour and apple pomace in the ratios of 10:90:10, 10:80:20 and 10:70:30, respectively.

10.3.1 Extrusion process

The extrusion experiments were carried out on a single screw extruder (Brabender Plasti-corder, model PL 2000, South Hackensack, NJ). The extruder had a screw diameter of 19 mm; a length to diameter ratio of 30:1; nominal compression ratio of 3:1; and a die opening of 3 mm. Feed zone temperature (T_1) was kept constant as 50ºC throughout the experiments. The temperature at the second zone (T_2) where CO_2 injected was controlled at 20ºC with the compressed air cooling. The extrusion parameters comprising the independent variables were temperatures at the third zone (T_3) and die end zone of the barrel (80, 120 and 160ºC), screw speed (100, 150 and 200 rpm) and feed moisture content (15, 20 and 25%wb). Carbon dioxide was pressurized and injected into the barrel using a syringe pump (1000D ISCO, Lincoln, NE, USA) at 56 bar and 20ºC from a gas

cylinder. Flow rate of CO_2 was not controlled. Carbon dioxide was injected in the barrel through an injection port at 18D (Figure 10.1).

Before extrusion, experimental blends were brought to room temperature (25ºC) to ensure uniform moisture distribution. The order of processing was chosen by randomizing apple pomace inclusion ratio, feed moisture levels, barrel temperatures and screw speed. Each extrusion run was brought to steady state as indicated by constant torque and melt temperatures before sampling and data collection. All extrudates were stored in a conditioned room prior to further analysis.

10.3.2 Extraction of samples

The extraction of sample for determining TPC, AA and the phenolic characterization was done using the method described by Khan et al. (2013). For determining TPC and AA, 1 g of sample 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 °C for the analysis of TPC and AA.

Free phenolic acid extraction was performed by adding 10 ml of 80% (v/v) aqueous methanol into 2 g of sample. Mixture was shaken in a shaking water bath for 1 h at 25°C. After centrifugation at 3000×*g* for 20 min, the supernatant was decanted and the extraction was repeated as described above. The two supernatants were combined, evaporated to near dryness and reconstituted with methanol to a final volume of 10 ml.

10.3.2.1 Total phenolic content (TPC)

TPC of sample was determined using Folin–Ciocalteu method (Singleton et al., 1999) with some modification. 50 µl methanol extract of sample was added with 3.5 ml

distilled water and 150 µl Folin-Ciocalteu reagent. The solution was vortexed and incubated for 30 min. Thereafter, absorbance of solution was measured at 760 nm against blank. Blank solution contained all the components that were present in the sample except the methanol extract. Gallic acid was used as positive control (standard) and linear regression curve between absorbance and concentration was drawn for the standard. This standard curve was used for calculating the concentration of sample and data was expressed in mg Gallic acid equivalent (GAE)/100 g dry weight (DW). This analysis was done in six replications.

10.3.2.2 Antioxidant activity (AA)

Extinction of DPPH is a free radical scavenging activity which was measured using spectrophotometric method described by Brand-Williams et al. (1995). 2,2-diphenyl-1-picrylhydrazyl (DPPH) 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 for 30 min. Thereafter, 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 weight (DW). Samples were analyzed in six replications.

10.3.2.3 Free phenolic acid characterization

Analysis of sample extracts was carried out using Thermo Scientific, Dionex Ultimate 3000 UHPLC system (Bannockburn, IL, United States) equipped with diode-array detector (DAD) and C_{18} column (150 mm × 4.6 mm) packed with 5 µm particles. The samples were injected with a mobile-phase flow rate of 800 µl/min. Gradient elution was carried out with a solvent system of water/acetic acid (99.8:0.2 v/v) as mobile phase

A and acetonitrile/acetic acid (99.8:0.2 v/v) as mobile phase B. The total run time was 12 min, and the gradient elution was as follows: 0.0−3.0 min, B 10-25%; 3.0−4.5 min, B 25−45%; 4.5−6.5 min, B 45−65%; 6.5−8.0 min, B 65−85%; 8.0−9.0 min, B 85−100%.; 9.0−12.0 min, B 100−10%. All the solvents were filtered through 0.22 μm PTFE filters prior to inject. The column was maintained at 30°C while the autosampler was thermostated at 4°C. The system was controlled by Thermo Scientific Dionex Chromeleon 7 software. Benzoic acid and cinnamic acid derivatives were detected at 280 nm and 320 nm, respectively. The concentrations of phenolic acids were calculated from peak areas in comparison to calibration curves of the respective standards and were expressed as μg/g DW.

10.3.3 Moisture content, total dietary fiber (TDF), soluble dietary fiber (SDF), insoluble dietary fiber (IDF)

Moisture content of samples was determined by air oven standard methods recommended by AOAC (1980). Initially 5 g of sample in triplicate was dried in hot air oven at 130-133 °C for 2 h. After drying, dried sample was again weighed. Following formula is used for calculating the moisture content (MC).

$$\text{MC (\%wb)} = \frac{W_i - W_f}{W_i} \times 100 \qquad (10.1)$$

W_i= initial weight of sample (5 g),

W_f= weight of sample after drying, g

Total dietary fiber in all extrudate samples was measured in the laboratory by the AOAC approved method 991.43 (AOAC, 1992). Duplicate samples of milled samples were suspended in MES/TRIS buffer (0.05 M, pH 8.2 at 24°C) and incubated sequentially with heat-stable α-amylase (95-100 °C, 30 min) to give gelatinization,

hydrolysis and de-polymerization of starch, protease (60°C, 30 min) to solubilize and depolymerize proteins, and amyloglucosidase (60 °C, 30 min, pH 4.5) to hydrolyse starch fragments to glucose. The enzyme digestate was then treated with four volumes of 95% ethanol (1 h) to precipitate soluble fiber. The alcohol-treated digestate was filtered through borosilicate sintered glass crucibles (40-90 μm) that had previously been matted with celite, dried, and weighed. The total dietary fiber residue present in the crucible was washed with alcohol and acetone, dried overnight (103°C), and weighed. One duplicate from each sample was used for ash determination (525°C muffle furnace) and the other for protein determination. Total dietary fiber percent (TDF, g/100g DW) was calculated as

$$\text{TDF}=\frac{\frac{R_1+R_2}{2}-p-A-B}{\frac{m_1+m_2}{2}}\times\frac{100}{DW}\times 100 \tag{10.2}$$

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 R_2, B is the blank and DW is percentage dry weight (g) of sample.

$$B=\frac{BR_1+BR_2}{2}-BP-BA \tag{10.3}$$

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 Insoluble dietary fiber (IDF) and soluble dietary fiber (SDF), 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 Eq (10.2) and (10.3).

10.3.4 Total starch and in-vitro starch digestibility (IVSD)

Total starch of samples was determined by AOAC approved method 996.11 (AOAC, 1996). The 100 mg sample and 0.2 ml, 80% (v/v) ethanol were added to a glass test tube and mixed on a vortex mixer. A magnetic stirring rod was added to each tube, and the tubes were placed in an ice water bath before 2 ml, 2 M KOH was added to each tube with stirring. The tubes were removed after 20 min, and 8 ml, 2 M sodium acetate buffer was added to each tube with stirring. Thereafter, 0.1 ml, 3000 U/ml thermostable α-amylase and 0.1 ml, 3300 U/mL amyloglucosidase (Megazyme) were added to each tube. The tubes were incubated in a 50 °C water bath for 30 min with intermittent stirring on a vortex mixer. Samples were diluted to 100 ml with distilled water, centrifuged, and the supernatant was retained for analysis. The K-GLUC (GOPOD format) assay kit from Megazyme was used for the determination of D-glucose using glucose oxidase based on absorbance at 510 nm as read on the UV−vis spectrophotometer. The glucose was converted into starch by multiplying a factor 0.9.

The in vitro starch digestion of extrudate samples was determined according to the method of Goñi et al. (1997). 10 ml of HCI-KCI buffer (pH l.5) was added to 50 mg sample (pH was adjusted). Then 0.2 ml of a solution containing 1 g of pepsin in 10ml of HCI-KCI buffer was added to sample and incubated at 40°C for 1 hour in a shaking water bath. Volume was completed to 25ml with Tris-Maleate buffer (pH 6.9). 5 ml solution of α-amylase in Tris-Maleate buffer containing 2.6 UI were added to each sample. Samples were then incubated at 37°C in a shaking water bath for 3 h. 1mL aliquot sample was

placed in a tube at 100°C and was energically shaken for 5 min to inactivate the enzyme. Then 3 ml of 0.4M Sodium acetate buffer (pH 4.75) were added to aliquot, and 60 µL of amyloglucosidase was used to hydrolyze the digested starch into glucose after 45 min at 60°C in a shaking water bath. Volume was adjusted to 100 ml with distilled water. Triplicated aliquots of 0.5 ml were incubated with the K-GLUC (GOPOD format) assay kit (Megazyme) for the determination of D-glucose using glucose oxidase based on absorbance at 510 nm as read on the UV−vis spectrophotometer. The glucose was converted into starch by multiplying a factor 0.9. Results were expressed on a total starch basis.

10.3.5 Texture analysis

Extrudates were subjected to hardness, brittleness and crispness using TA-XTPlus Texture Analyzer (Texture Technologies Corp., Hamilton, MA) as per method described by Altan et al. (2008b). The peak force as an indication of hardness was measured with a TA-XTPlus Texture Analyzer (Texture Technologies Corp., Hamilton, MA) using 3-point bend test. The test speed was 2 mm/s and the distance between two supports was kept as 22 mm. The curve between force vs distance was plotted and analyzed by Texture Exponent 32 software program (version 3.0). The slope (N/mm) and distance (mm) at which a product breaks were measured from force–distance curve and evaluated as crispness and brittleness, respectively (Texture Technologies, a). Both the sample extrudates were replicated 10 times. The radial expansion ratio of the extrudates was measured as the ratio of the diameter of the extrudates to the diameter of the die orifice.

10.3.6 Water absorption index (WAI) and water solubility index (WSI)

To determine the WAI and WSI of extrudates, the methodology proposed by Anderson (1982) was followed. Ground extrudates (2.5 g) was suspended in distilled water (30 ml) in a tarred 60 ml centrifuge tube. The suspension was stirred intermittently and centrifuged at 3000×g for 10 min. The supernatant was decanted into a tarred aluminums cup and dried at 135 °C for 2 h. The weight of the gel remaining in the centrifuge tube was measured. The WAI and WSI were calculated by

$$\text{WAI}=\frac{W_g}{W_{ds}}\times 100 \tag{10.4}$$

where, WAI is water absorption index, W_g is the weight of gel (g), and W_{ds} is the weight of dry sample (g).

$$\text{WSI}=\frac{W_{ss}}{W_{ds}}\times 100 \tag{10.5}$$

where, WSI is the water solubility index (%), W_{ss} is the weight of dry solids of supernatant (g), and W_{ds} is the weight of dry sample (g).

10.3.7 Color

The color of ground extrudate powders was determined by using a Colorimeter (Minolta JP/CM-2500d, Japan). In the colorimeter, the sample color is denoted by the three dimensions L*, a* and b*. The L* value accounts for a measurement of the lightness of the product color from 100 (white) to 0 (black). The redness/greenness and yellowness/blueness are denoted by the a* and b* values, respectively.

10.3.8 Cross-sectional microstructure analysis

The microstructure of control and CO_2 extrudates were examined using a scanning electron microscope (SEM) (Hitachi-S3400 N, Tokyo, Japan). Small amounts

of samples were mounted on SEM specimen stubs by using double-sided adhesive tape. Each powder sample was coated with 10 Å thick layer of gold in a sputter coater before being scanned and photographed at 32× magnification.

10.3.9 Experimental design

A Box-Behnken design was applied for extrusion experiments to determine the effects and the optimum levels of the experimental parameters. The effects were studied at three experimental levels –1, 0, and +1. A total of 29 experiments were required with 5 center points as described in Table 10.2. The experimental data were analyzed by the response surface regression procedure and the parameters obtained from the response surface methodology (RSM) analysis were substituted into the following second-order polynomial model equation.

$$Y_i = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_{ii}^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \qquad (10.6)$$

where Y_i is the predicted response; β_0 is the interception coefficient; β_i, β_{ii}, and β_{ij} are coefficients of the linear, quadratic, and interaction terms; X_i and X_j are the variables; and k is the number of independent parameters (k= 4).

10.3.10 Statistical analysis

Design expert 9 statistical software package (Stat-Ease Inc., USA) was used to analyze the experimental data. Multiple regression analysis and analysis of variance (ANOVA) were used to evaluate the experimental data. The modeling was started with a quadratic model including linear, squared, interaction terms. Significant terms in the model for each response were found by ANOVA. The adequacy and quality of the models were examined by evaluating the lack of fit (LOF), the coefficient of determination R^2 and the p-value obtained from the ANOVA. Derringer's desired

function methodology was used to generate optimal conditions for extrusion process variables on all the properties of extruded snacks.

10.4 Results and Discussion

10.4.1 Model fitting

A response surface methodology was used to analyze the effect of extrusion parameters on antioxidant, textural and functional properties of extruded products. The experimental data was analyzed by analysis of variance (ANOVA) and the significance of the regression coefficients evaluated by their corresponding *p*-values and the goodness of fit of the models is presented in Table 10.3.

It is obvious from Table 10.3 that the mathematical models for ER and IVSD of extruded products were not fitted ($p>0.05$). Higher coefficient of determination (R^2 >0.77) exhibited goodness of fit for all other mathematical models. For all the responses for extrusion process, p-values of lack of fit were not significant ($p>0.05$) which indicated their suitability to predict the variations within the system. Furthermore, the experimental data showed a good fit with Eq. (1), since all regression models except for ER and IVSD were statistically significant ($p < 0.05$).

The regression coefficients of mathematical models describing all the experimental responses as a function of apple pomace ratio APR (X_1), feed moisture FM (X_2), extrusion temperature T (X_3), and screw speed SS (X_4) for extrusion process are depicted in Table 10.4. The values of the regression coefficients presented in Table 10.4 were used in the final predictive model equations. Thus, these equations were assumed to best describe the relationships between the experimental variables and the response factors in the present study.

10.4.2 Interpretation of response surface model and contour plots

Three-dimensional response surface plots and two-dimensional contour plots were obtained on the basis of the model equations mentioned above to explicate the correlation between independent and dependent variables studied in this study. Both types of plots presented the effects of two independent variables on the response factor, keeping others at level-coded zero.

10.4.2.1 Effect of extrusion variables on antioxidant properties of extruded products

Table 10.3 explicated that linear and quadratic terms of APR and T significantly ($p<0.05$) influenced the TPC and AA. In addition, significant quadratic effect of FM ($p<0.05$) and combined effect of FM-T were also observed on TPC and AA of extruded products (Table 10.3). Feed moisture within its experimental range didn't exhibit any significant ($p>0.05$) influence on TPC and AA of extrudates. This result was in accordance with Hirth et al. (2014) and Stojceska et al. (2009) who reported that an increment in feed moisture from 18-28% and 2-17%, respectively didn't affect the TPC and AA of starch based extrudates. Perversely, Bisharat et al. (2015), Leyva-Corral et al. (2016) and Sarawong et al. (2014) reported a significant drop in phenolic contents of final extrudates with increase in feed moisture content. The determined values of TPC and AA were found in range of 66.3-137.5 mg GAE/100g DW and 124.2-336.8 µmol TE/100g DW, respectively.

Figure 10.2 represents the response surface plots showing the effects of APR, FM and T on TPC of extrudates. Plots for AA were not shown as they followed the similar trend as TPC. This indicated that the majority of AA in the extruded products was due to presence of phenolic acids. This also diluted the possibility of production of Maillard's

reaction products during extrusion that could enhance the antioxidant activity in the extrudates. Singkhornart et al. (2013) reported inhibition of Maillard's reaction due to lowering the pH in the presence of CO_2. The trend observed for TPC upon simultaneous variation of APR and T is exhibited in Figure 10.2a. TPC of extrudates decreased by 29% with increase in the temperature from 80ºC to 160ºC regardless the APR level. TPC significantly ($p<0.05$) increased by 41% with increase in APR from 10% to 30% at low temperature (80ºC), while an increment of 31% was recorded for TPC with increase in APR at higher temperature (160ºC). Similar results were observed for AA for the combined effect of APR and T (plot not shown). AA of extrudates was increased by 62% when APR increased with in experimental range at low temperature (80ºC), while this number was observed as 33% at higher temperature (160ºC). Higher TPC and AA at higher APR was attributed to the initial higher TPC and AA in the raw material. At higher temperature, phenolics could be modified due to alteration in their functional group properties and solubility leading to reduced chemical reactivity. Also at the higher temperature, phenolic acids are susceptible to oxidation resulted in decreased TPC and AA. Negative effect of temperature on TPC and AA was also reported by Obiang-Obounou and Ryu (2013), Sarawong et al. (2014), Sharma et al. (2012) and Ti et al. (2015) for chestnut, banana flour, barley and black rice, respectively during extrusion.

With regard to the effect of both FM and T on TPC (Figure 10.2b) suggested that increase in FM from 15 to 25%wb caused a significant ($p<0.05$) increase of 20% TPC at low T (80ºC), however TPC decreased by 20% when FM increased within experimental level at higher temperature (160ºC). On the other hand, no significant ($p>0.05$) change was observed in TPC when T increased from 80ºC to 160ºC at low FM (15%wb),

whereas, a significant ($p<0.05$) drop of 33% was recorded with increase in T at higher FM (25%wb). Similar trend was noticed for AA for the combined effect of FM and T (graph not shown). AT low temperature, the presence of a greater amount of moisture along with CO_2 in the sample led to a gentler processing of feed in the extruder barrel subsiding the shearing effect that would be more destructive on phenolic compounds at drier extrusion condition (low feed moisture). Higher FM and T promoted phenolic polymerization that reduced the TPC and AA of extrudates. Bisharat et al. (2014) and Ozer et al. (2006) found the similar results for corn flour and oat flour based extrudates, respectively.

10.4.2.2 Effect of extrusion variables on textural properties of extruded products

ANOVA shows that hardness and brittleness of extruded products was significantly ($p<0.05$) effected by the linear term of temperature (Table 10.3). Furthermore, crispness of extrudates was significantly ($p<0.05$) influenced by the linear effect of FM, T, SS and quadratic effect of T (Table 10.3). Expansion ratio, hardness, brittleness and crispness varied from 0.95-1.32, 0.5-6.63 N, 2.16-5.33 mm and 0.44-3.61 N/mm, respectively within the experimental range of extrusion variables.

Although ER didn't exhibit the goodness of fit for the model, the combined effect of FM and T is presented in Figure 10.2c. AT low temperature (80°C), ER increased by 5% with increase in FM from 15% to 25%wb. However, with increment of FM from 15% to 25%, extrudates expansion reduced by 10% at higher temperature (160°C). On the other hand, ER increased by 14% when temperature gradually increased from 80°C to 160°C at low FM (15%wb), though this number was reduced to 7% at higher FM (25%wb). Increasing the moisture content during extrusion reduced the elasticity of

dough resulted in a lower degree of starch gelatinization through plasticization of melt and lower expansion. At low moisture, the melt had higher viscosity which related directly to shear stress of the plasticized material and the increased degree of gelatinization. Similar effect of FM (15-17%) on expansion of extrudates was observed by Korkerd et al. (2016). Higher temperature increased the degree of gelatinization and extent of superheated steam that caused more expansion. These results were in accordance with Gujral et al. (2012) and Korkerd et al. (2016) who reported higher expansion of extrudates at higher temperature.

With regard to the combined effect of FM and T (Figure 10.2d), hardness of extruded products decreased significantly ($p<0.05$) by 83% when temperature elevated from 80°C to 160°C at low FM (15%wb). At higher FM (25%wb), when T increased in experimental range, hardness increased by 75%. Higher temperature decreased melt viscosity and increased the water vapor pressure. This favored the bubble growth which is the driving force for expansion that produced more expanded products with more crispness and softer texture. Similar results were obtained by Bisharat et al. (2015) for corn based extrudates.

The distance to break the extrudate was considered to be an indication of brittleness, with the shortest distance being the most brittle product. As explicated from response surface plot (Figure 10.3a), brittleness increased by 40% with increase in extrusion temperature from 80°C to 160°C regardless the SS levels. Low hardness value with increased temperature would produce lower distance to break and thus extrudates with higher brittleness. This result was consistent with the findings of Altan et al. (2008b) for barley-grape pomace extrudates.

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. The lower the slope, the crisper the product is considered. The combined effect of FM and T on crispness represented by surface plot (Figure 10.3b) depicted that with increase in temperature from 80°C to 160°C, the crispness of extrudates increased by 67% at low FM (15%wb). Furthermore, an increment of 50% was observed in crispness when T increased within experimental range at higher FM (25%wb). With regard to the effect of FM when increased from 15% to 25%wb, crispness of extrudates increased by 33% at low temperature (80°C), however no significant change was observed in crispness with the effect of variation in FM within experimental range at higher temperature (160°C). With increase in temperature, the degree of superheating of water increased before melt passed through the die. This in turn increased the bubble radius which caused increase in matrix radius and decrease in shell wall thickness. The thinner shell wall was responsible for crispness of the extrudates. Bisharat et al. (2015) also reported the increase in crispness of corn based extrudates with increase in temperature from 140ºC to 180ºC.

Figure 10.3c shows the combined effect of T and SS on crispness of extrudates. As observed, with increase in SS from 100 rpm to 200 rpm, crispness of extrudates decreased by 25% at low temperature (80ºC). However, at higher T (160ºC), extrudates didn't show any significant change in crispness with increase in SS. Increased shear rate at higher SS raised the temperature of melt which caused more expansion and crispness eventually. Increase in crispness with increase in T from 80ºC to 160ºC at higher SS (200 rpm) was observed more with compared to that at low SS (100 rpm).

10.4.2.3 Effect of extrusion variables on functional properties of extruded products

It is explicated from ANOVA (Table 10.3) that WAI was significantly ($p<0.05$) influenced by the linear terms of FM, T, SS and interaction of FM-T. Extruded products exhibited the significant ($p<0.05$) linear effects of APR, FM, T, SS, combined effect of FM-T and quadratic effect of T on WSI. TDF of extrudates was significantly ($p<0.05$) effected by linear term of APR and quadratic term of T. WAI, WSI, IVSD and TDF functional properties of extrudates were observed in the range of 2.05-4.76 g/g DW, 9.28-22.8 g/100g DW, 55.9-83.5 g/100g DW and 10.1-18.6 g/100g DW, respectively.

Water absorption has been generally attributed to the dispersion of starch in excess water, and the dispersion is increased by the degree of starch damage due to gelatinization and extrusion-induced fragmentation, that is, molecular weight reduction of amylose and amylopectin molecules. Figure 10.4a shows the combined effect of FM and T on WAI of extruded products. With gradual increase in temperature from 80°C to 140°C at low FM (10%), no significant ($p>0.05$) change was noticed in WAI. Similar trend was obtained when FM increased within experimental range at lower T (80ºC). However, increment of temperature at higher FM increase the WAI of extrudates by 33%. Likewise, WAI increased by 33% when FM increased from 15% to 25%wb at higher T (160ºC). This increasing trend was due to greater heat generation and disruption of starch molecule which favors starch gelatinization. These results are in agreement with Alam et al. (2015), Gujral et al. (2012) and Seth et al. (2015) who observed the increase in WAI of extruded rice-carrot pomace, brown rice and yam-corn-rice with increase in temperature from 120ºC to 180ºC, 100ºC to 120ºC and 100ºC to 140ºC, respectively.

The results concerning the effect of T and SS on WAI (Figure 10.4b) indicated that as SS increased from 100 to 200 rpm at low T (80ºC), WAI increased by 33%. However at higher T (160ºC), no significant ($p>0.05$) change in WAI was observed with increment of SS. The similar result was obtained by varying the temperature from 80ºC to 160ºC at higher SS (200 rpm), whereas WAI increased by 33% with increment in T at low SS (100 rpm). Low T and higher SS or low SS and higher T, disrupted the starch molecules leading to more water bound to the starch molecules which increased the WAI of extruded product. Higher T and SS damaged the starch granules and decreased their water absorption capacity.

Water solubility index, often used as an indicator of degradation of molecular components measures the degree of starch conversion during extrusion which is the amount of soluble polysaccharide released from the starch component after extrusion. With regard to the interactive effect of APR and SS on WSI (Figure 10.4c), it was observed that with increase in APR from 10% to 30%, WSI of extrudates increased by 28% at low SS (100 rpm). Furthermore, WSI increased by 60% at higher SS (200 rpm) when inclusion of AP increased within experimental range. Higher WSI at higher APR could be attributed to the presence of low molecular weight compounds i.e. soluble fiber presence in pomace caused increase in WSI of extrudates. This result was in agreement with Altan et al. (2009) for barley and grape-tomato pomace based extrudates. On the other hand, increment of SS from 100 rpm to 200 rpm decreased the WSI by 28% at low APR (10%). What's more the decrease in WSI was less significant at higher APR (30%) with increment in SS. Low WSI at low APR and higher SS could be attributed to the

molecular interaction between degraded starch, fiber and protein causing an increase in molecular weight resulted in a decrease in solubility.

Response surface plot describing the combined effect of FM and T on WSI is shown in Figure 10.4d. At low T (80ºC), extrudates didn't exhibit any significant ($p>0.05$) change in WSI with increment in FM from 15% to 25%wb. However, WSI decreased by 27% with increase in FM at higher T (160ºC). With the effect of increase in temperature from 80ºC to 160ºC, WSI increased by 37.5% at low FM (15%wb), whereas no significant ($p>0.05$) change was observed in WAI at higher FM (25%wb) with increment in temperature. Higher FM acted as a plasticizer during extrusion that reduced the degradation of starch granules and decreased WSI. On the other hand, WSI increased with increasing T at low FM due to dextrinization or starch melting that prevailed over the gelatinization phenomena. These results were in accordance with the findings of Sarawong et al. (2014) and Selani et al. (2014) for the extrusion of green banana flour and corn-pomace, respectively.

Though the model didn't show the goodness of fit ($p>0.05$) for IVSD, the combined effect of APR and T (Figure 10.5a) explicated that at low APR (10%), no significant ($p>0.05$) change was observed for IVSD when T increased from 80ºC to 135ºC followed by 7% depletion in IVSD when T increased further up to 160ºC. At higher temperature, dextrinization of starch induced low IVSD. However, IVSD increased by 23% with increase in T from 80ºC to 160ºC at higher APR (30%). Having said that, increment of APR from 10% to 30% caused a drop of 7% at low T (80ºC), whereas an increment of 23% was observed in IVSD when APR increased with in experimental range at higher temperature (160ºC). Higher APR increased the amount of

soluble fiber in the blend and when temperature increased, the increased elastic properties of melt resulted in higher degree of gelatinization and increased IVSD. Higher APR increased the fiber amount in blend which might cause formation of protein-fiber-starch complex at low temperature that reduced the susceptibility of starch to enzyme hydrolysis.

The results concerning the effect of APR and T on TDF (Figure 10.5b), it was observed that TDF increased by 33% with increase in APR from 10% to 30% regardless the FM. This result was harnessed by initial TDF available in raw material.

10.4.3 Optimization of the extrusion process and validation

Optimal process conditions were investigated for extrusion process variables by maximizing the TPC, AA, brittleness, crispness, WSI, TDF and minimizing the hardness and WAI. Table 10.5 indicates the optimized conditions for extrusion process along with predicted and experimental response variables. The predicted results well coped with the experimental results which validated the RSM model, indicating Box-Behnken design could be effectively used to optimize the process parameters for extrusion processes on antioxidant, textural and functional properties of corn, SF and AP based extrudates.

The extruded products at optimum extrusion conditions without CO_2 injection are referred as control extrudates. It is explicated from Table 10.5 that TPC and AA of extrudates produced with CO_2, referred as CO_2 extrudates hereafter, exhibited 12% and 7% more TPC and AA, respectively with compared to that of control extrudates that may be attributed to the cooling effect of the CO_2. However, in accordance with Leyva-Corral et al. (2016), Ti et al. (2015) and Wani and Kumar (2015), the extrusion process caused 24% and 14% reduction of TPC and AA, respectively in CO_2 extrudates with compared

to that of blend. Similar effects of CO_2 on TPC and AA of tomato-green tea-corn and corn-fiber extrudates were reported by Masatcioglu et al. (2013) and Wang and Ryu (2013b).

The hardness, brittleness and crispness of CO_2 extrudates were observed to be 59%, 7% and 70% more than those of control extruded products. Although, CO_2 extrudates exhibited 3.5% reduction in ER, these samples typically had a more uniform expansion with compared to that of control extrudates (Figure 10.6). High diffusivity of CO_2 causing its rapid escape through the thin walls could explain the decrease in ER. Lower expansion was also reported by earlier researchers (Bilgi Boyaci et al., 2012, Ferdinand et al., 1990, Masatcioglu et al., 2013, Singkhornart et al., 2013).

WAI of CO_2 extrudates was 59% less than that of control extrudates, whereas an increase of 37% was observed in WSI of CO_2 extrudates with compared to the control. WAI is a function of the internal voids in the sample flour and the thickness of the cell walls of the voids (Myat, 2013). Adding the CO_2 altered the structure of voids in the blend and thus might affect the WAI of final extrudates. CO_2 injection pressure caused more starch degradation during extrusion process resulted in more WSI of extrudates. These results were in accordance with Myat (2013), Schmid et al. (2005), Singkhornart et al. (2014) and Jeong and Toledo (2004) for corn starch, wheat flour, germinated wheat flour and rice flour, respectively.

TDF, IDF and IVSD of CO_2 extrudates were found 3%, 7% and 4% less than those of control extrudates. No significant ($p>0.05$) difference was observed in IDF of both the extrudates. However, CO_2 extrudates exhibited 9% less SDF with compared to control extrudates which might result from the effective lower temperature due to cooling

effect of CO_2. These results were in accordance with the findings of Wang and Ryu (2013b) for corn grits-corn fiber based extrudates. Although CO_2 assisted extrusion process decreased the TDF and IDF in the extrudates with compared to that of blend, majority of TDF was presented mainly by the insoluble fraction (Table 10.5).

The L*, a* and b* values of control extrudates were 55.9±0.91, 9.99±0.13 and 26.7±0.19, respectively and these values for CO_2 extrudates were observed as 65.9±0.70, 8.55±0.13 and 27.95±0.23, respectively. Higher lightness, less redness and more yellowness in CO_2 extrudates were because of reduction in Maillard's reaction due to low pH induced by CO_2 (Figure 10.6). These results were consistent with (Singkhornart et al., 2014).

Table 10.6 depicts that the TPC and AA in CO_2 extrudates were significantly contributed by caffeic acid followed by salicylic acid and ferulic acid. CO_2 extrudates had significantly ($p<0.05$) more benzoic acid and cinnamic acid derived phenolic acids than that of control extrudates.

10.5 Conclusions

Extruded products developed from CO_2 assisted extrusion of corn flour and hydrodynamic cavitated sorghum flour, apple pomace blend exhibited higher retention of TPC and AA. Extrusion with CO_2 improved the textural and functional properties of extrudates as well. TPC and AA of extrudates were dominantly effected by APR and T. As obvious, APR had positive whereas temperature showed negative impact on TPC and AA of extrudates. Temperature also played a prevalent role in influencing the textural properties by decreasing hardness and increasing ER, brittleness and crispness of extrudates. Lower FM, T and SS favored the low WAI, whereas WSI increased with

increase in APR, T and decrease in FM, SS. Extrudates showed the increment in IVSD and TDF at higher APR and T due to increase in fraction of SDF. As compared to the control extrudates, CO_2 extruded products were significantly ($p<0.05$) higher in TPC and AA due to cooling effect of CO_2. Although CO_2 extrudates had low expansion ratio, more brittleness, crispness and less hardness were obtained when CO_2 was injected during extrusion. Lower WAI and higher WSI of extrudates were attributed to the degradation of starch due to CO_2 injection pressure during extrusion process. Though CO_2 assisted extrusion diminished the IVSD, TDF and IDF of extrudates, no significant change in SDF was observed in extrudates. CO_2 extrudates had more lightness and less redness indicating inhibition of Maillard's reaction during extrusion. Extruded products were found rich in caffeic acid followed by salicylic acid and ferulic acid. Owing to their improved antioxidant, textural and functional properties, inclusion of CO_2 during extrusion can be of interest in developing a variety of gluten free snacks foods in the future.

Table 10.1 Chemical composition of the raw materials before the extrusion process

Component, g/100g	**Corn flour**	**Sorghum flour**	**Apple pomace**
Moisture content	10.3±0.23	9.31±0.31	9.75±0.12
Ash	0.93±0.06	1.21±0.10	1.67±0.05
Fat	1.20±0.09	3.19±0.12	2.02±0.08
Protein	6.18±0.77	9.63±0.64	5.11±0.43
Starch	84.7±2.46	64.7±1.62	8.74±0.09
Total dietary fiber	2.57±0.01	10.6±0.03	43.9±0.05
Soluble	0.91±0.01	2.67±0.01	11.9±0.02
Insoluble	1.66±0.01	7.89±0.02	32.0±0.03

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

Table 10.2 Box-Behnken experimental design for extrusion process

Run	APR	FM	T	SS	Run	APR	FM	T	SS
1	-1	-1	0	0	16	0	0	+1	+1
2	+1	0	0	-1	17	0	0	-1	+1
3	+1	0	+1	0	18	0	0	0	0
4	0	+1	0	-1	19	0	+1	0	+1
5	-1	0	0	-1	20	0	-1	-1	0
6	0	0	+1	-1	21	+1	0	0	+1
7	+1	0	-1	0	22	-1	+1	0	0
8	0	0	0	0	23	0	-1	0	-1
9	-1	0	0	+1	24	0	-1	+1	0
10	-1	0	+1	0	25	+1	+1	0	0
11	0	0	0	0	26	0	+1	-1	0
12	-1	0	-1	0	27	+1	-1	0	0
13	0	0	0	0	28	0	-1	0	+1
14	0	0	-1	-1	29	0	0	0	0
15	0	+1	+1	0					

Variables	**Levels**		
	-1	**0**	**+1**
AP ratio (APR)	10	20	30
Feed moisture (FM, % wb)	10	15	15
Extrusion temperature (T, ºC)	80	120	160
Screw speed (SS, rpm)	100	150	200

APR: apple pomace ratio, FM: feed moisture, SS: screw speed, T: extrusion temperature

Table 10.3 ANOVA and statistical parameters describing the effect of the independent variables on antioxidant, textural and functional properties of extruded products

Source	df	Mean squares									
		TPC	**AA**	**ER**	**Hardness**	**Brittleness**	**Crispness**	**WAI**	**WSI**	**IVSD**	**TDF**
Model	14	466.9[a]	4980.3[a]	0.01	5.48[b]	1.04[b]	0.91[b]	0.47[b]	22.5[b]	62.3	7.54[b]
X_1	1	1476.6[a]	18869.3[a]	2.94×10^{-3}	2.28	0.18	0.02	0.16	109.7[a]	62.3	65.7[a]
X_2	1	48.4	518.55	0.03[b]	3.92	0.05	1.63[b]	2.27[a]	23.8[b]	68.1	0.03
X_3	1	1976.3[a]	23714.5[a]	0.06[b]	61.8[a]	12.76[a]	7.20[a]	1.29[b]	37.4[b]	32.0	0.56
X_4	1	57.7	716.2	9.01×10^{-3}	2.40	0.21	0.87[b]	1.03[b]	40.0[b]	0.24	0.05
X_{12}	1	12.5	4.16	7.90×10^{-5}	0.01	0.17	0.06	0.24	3.84	118.6	0.85
X_{13}	1	0.77	3.41	6.05×10^{-3}	0.32	0.17	0.07	0.21	1.66	167.0[b]	2.15
X_{14}	1	1.02	36.9	6.25×10^{-4}	0.30	6.40×10^{-3}	0.16	0.20	0.99	124.7	0.62
X_{23}	1	777.7[b]	5132.9[b]	4.90×10^{-3}	3.55	0.06	0.62	0.64[b]	28.31[b]	31.9	5.48
X_{24}	1	215.6	1433.8	1.93×10^{-4}	0.16	0.04	0.06	0.06	2.12	36.8	2.65
X_{34}	1	9.57	18.7	0.03	0.03	0.10	0.09	0.14	2.24	44.8	0.29
X_{11}	1	654.1[b]	9986.3[a]	5.11×10^{-3}	0.25	0.22	0.11	2.23×10^{-3}	0.12	1.71	2.92
X_{22}	1	642.8[b]	2284.9[b]	6.09×10^{-5}	0.10	0.01	0.02	0.24	10.3	26.7	5.66
X_{33}	1	340.7[b]	5559.4[b]	0.01	1.90	0.55	1.95[b]	0.03	59.3[b]	158.9	23.1[b]
X_{44}	1	3.17	343.5	0.03	3.98×10^{-3}	0.04	0.18	0.05	0.62	3.58	0.01
Residual	14	58.3	438.9	6.87×10^{-3}	1.66	0.26	0.18	0.12	3.93	36.0	2.26
LoF	10	74.4	556.4	5.45×10^{-3}	1.49	0.24	0.07	0.15	5.11	41.9	2.88
Pure error	4	18.0	145.1	0.01	2.09	0.30	0.44	0.03	0.85	21.3	0.72
R^2		0.89	0.92	0.66	0.77	0.80	0.84	0.80	0.70	0.63	0.77
R^2_{adj}		0.78	0.84	0.33	0.54	0.60	0.67	0.60	0.19	0.28	0.54
R^2_{pred}		0.40	0.57	-0.32	0.01	0.14	0.54	-0.08	0.70	-0.85	-0.24

Significant at $p<0.001$a, $p<0.05$b, LoF: lack of fit, adj: adjusted, pred: predicted, AA: antioxidant activity, df: degree of freedom, ER: expansion ratio, IVSD: in-vitro starch digestibility, TDF: total dietary fiber, TPC: total phenolic content, WAI: water absorption index, WSI: water solubility index

Table 10.4 Regression coefficients describing the relationship between responses and independent variables for extruded products

Coeff.	TPC	AA	ER	Hardness	Brittleness	Crispness	WAI	WSI	IVSD	TDF
β_0	-106.1	-485.7	0.29	19.5	10.4	2.08	1.12	32.3	-91.6	19.2
β_1	5.81[a]	18.6[a]	8.48×10^{-4}	-0.06	-0.07	-0.09	0.21	-0.49[a]	1.94	-0.11[a]
β_2	-2.85	1.10	7.80×10^{-3b}	-0.62	-0.13	-0.19[b]	-0.43[a]	-0.65[b]	5.78	-0.49
β_3	2.29[b]	7.07[b]	7.64×10^{-3b}	-0.08[a]	-0.02[a]	0.03[a]	4.29×10^{-3b}	-0.12[b]	0.99	-0.11
β_4	0.69[b]	2.24	4.02×10^{-3}	-0.01	-0.03	0.04[b]	0.03[b]	9.47×10^{-3b}	0.27	0.06
β_{12}	-0.04	0.02	8.89×10^{-5}	1.05×10^{-3}	4.07×10^{-3}	2.44×10^{-3}	-4.92×10^{-3}	0.02	-0.11	9.23×10^{-3}
β_{13}	-1.09×10^{-3}	-2.31×10^{-3}	-9.72×10^{-5}	7.03×10^{-4}	5.17×10^{-4}	3.18×10^{-4}	-5.68×10^{-4}	1.61×10^{-3}	0.02[b]	-1.83×10^{-3}
β_{14}	1.01×10^{-3}	6.07×10^{-3}	-2.50×10^{-5}	5.46×10^{-4}	8.00×10^{-5}	4.06×10^{-4}	-4.43×10^{-4}	9.95×10^{-4}	-0.01	7.89×10^{-4}
β_{23}	-0.07	-0.18	-1.75×10^{-4}	4.71×10^{-3}	6.33×10^{-4}	1.97×10^{-3}	2.00×10^{-3b}	-0.01[b]	-0.01	-5.85×10^{-3}
β_{24}	-0.03	-0.08	-2.78×10^{-5}	8.03×10^{-4}	4.00×10^{-4}	-4.68×10^{-4}	4.98×10^{-4}	-2.91×10^{-3}	0.01	-3.26×10^{-3}
β_{34}	-7.73×10^{-4}	-1.08×10^{-3}	4.22×10^{-5}	-4.14×10^{-5}	8.00×10^{-5}	-7.74×10^{-5}	-9.39×10^{-5}	-3.74×10^{-4}	-1.67×10^{-3}	-1.34×10^{-4}
β_{11}	-0.10[b]	-0.39[b]	2.81×10^{-4}	-1.94×10^{-3}	-1.84×10^{-3}	-1.28×10^{-3}	1.86×10^{-4}	1.36×10^{-3}	5.14×10^{-3}	6.71×10^{-3}
β_{22}	0.39	0.75	1.23×10^{-4}	-5.01×10^{-3}	-1.88×10^{-3}	-2.25×10^{-3}	7.70×10^{-3}	0.05	-0.08	0.04
β_{33}	-4.53×10^{-3}	-0.02	-2.83×10^{-5}	-3.39×10^{-4}	-1.83×10^{-4}	-3.43×10^{-4b}	-4.48×10^{-5}	1.89×10^{-3b}	-3.09×10^{-3}	1.18×10^{-3b}
β_{44}	-2.79×10^{-4}	-2.91×10^{-3}	-2.49×10^{-5}	-9.90×10^{-6}	3.11×10^{-5}	-6.65×10^{-5}	-3.42×10^{-5}	1.24×10^{-4}	-2.97×10^{-4}	1.38×10^{-5}

Significant at p<0.001a, p<0.05b, AA: antioxidant activity, ER: expansion ratio, IVSD: in-vitro starch digestibility, TDF: total dietary fiber, TPC: total phenolic content, WAI: water absorption index, WSI: water solubility index

Table 10.5 Estimated optimum conditions, predicted and experimental values of the responses for extrusion process

Samples	Optimum conditions				Antioxidant properties			
	APR (%)	FM (%wb)	T (°C)	SS (rpm)	TPC, mg GAE/100g DW		AA, µmol TE/100g DW	
					Predicted	Actual*	Predicted	Actual*
CO_2 extrudates	30	25	97	100	131.4	130.3±1.31	327.4	326.8±3.59
Control extrudates						116.3±0.97		305.5±3.87
Blend						171.6±2.03		381.8±3.94
	Textural properties							
	Hardness, N		Brittleness, mm		Crispness, N/mm			
	Predicted	Actual*	Predicted	Actual*	Predicted	Actual*		
CO_2 extrudates	2.84	2.88±0.13	4.33	4.35±0.11	1.11	1.12±0.02		
Control extrudates		7.10±0.97		4.66±0.28		3.71±0.46		
	Functional properties							
	WAI, g/g DW		WSI, g/100g DW		TDF, g/100g DW			
	Predicted	Actual*	Predicted	Actual*	Predicted	Actual*		
CO_2 extrudates	2.48	2.50±0.02	21.6	21.1±0.23	18.6	18.6±0.01		
Control extrudates		6.18±0.12		15.4±0.39		19.2±0.02		
	ER		IVSD, g/100g DW		IDF, g/100g DW		SDF, g/100g DW	
	Actual*		Actual*		Actual*		Actual*	
CO_2 extrudates	1.09±0.01		75.1±0.53		13.5±0.05		5.14±0.05	
Control extrudates	1.13±0.02		78.2±0.92		13.5±0.03		5.66±0.01	
Blend					14.6±0.06		5.15±0.01	

Response experimental results are reported as mean ± standard deviation. *mean values in column are significantly different at 5% level of significance, AA: antioxidant activity, APR: apple pomace ratio, ER: expansion ratio, FM: feed moisture, GAE: gallic acid equivalent, IDF: insoluble dietary fiber, IVSD: in-vitro starch digestibility, SDF: soluble dietary fiber, SS: screw speed, T: extrusion temperature, TDF: total dietary fiber, TE: trolox equivalent, TPC: total phenolic content, WAI: water absorption index, WSI: water solubility index

Table 10.6 Phenolic profile of blends and extrudate samples (μg/g DW)

Compounds	Blend	Control extrudates	CO_2 Extrudates
Benzoic acids			
Protocatechuic acid	52.9±2.9[a]	12.9±0.2[b]	22.7±0.5[c]
p-Hydroxybenzoic acid	23.1±1.2[a]	16.2±0.3[b]	17.8±0.7[c]
Cinnamic acids			
Chlorogenic acid	7.67±0.2[a]	2.53±0.1[b]	3.83±0.1[c]
Caffeic acid	57.4±2.1[a]	48.5±1.4[b]	51.0±1.2[c]
p-coumaric acid	15.1±1.0[a]	9.08±0.2[b]	10.7±0.3[c]
Ferulic acid	45.7±1.7[a]	34.3±1.3[b]	37.1±1.3[c]
Salicylic acid	67.5±3.1[a]	48.4±1.2[b]	53.2±1.9[c]

Means±SD in the same row with different letters are significantly different ($p<0.05$)

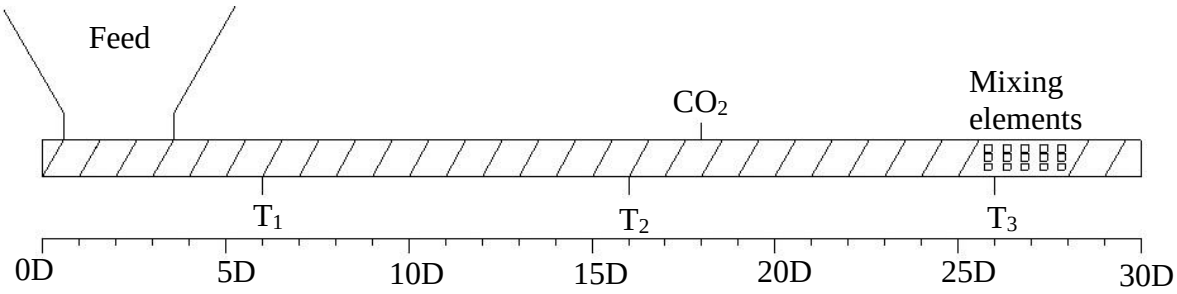


Figure 10.1 Flow diagram of the extruder with CO_2 injection

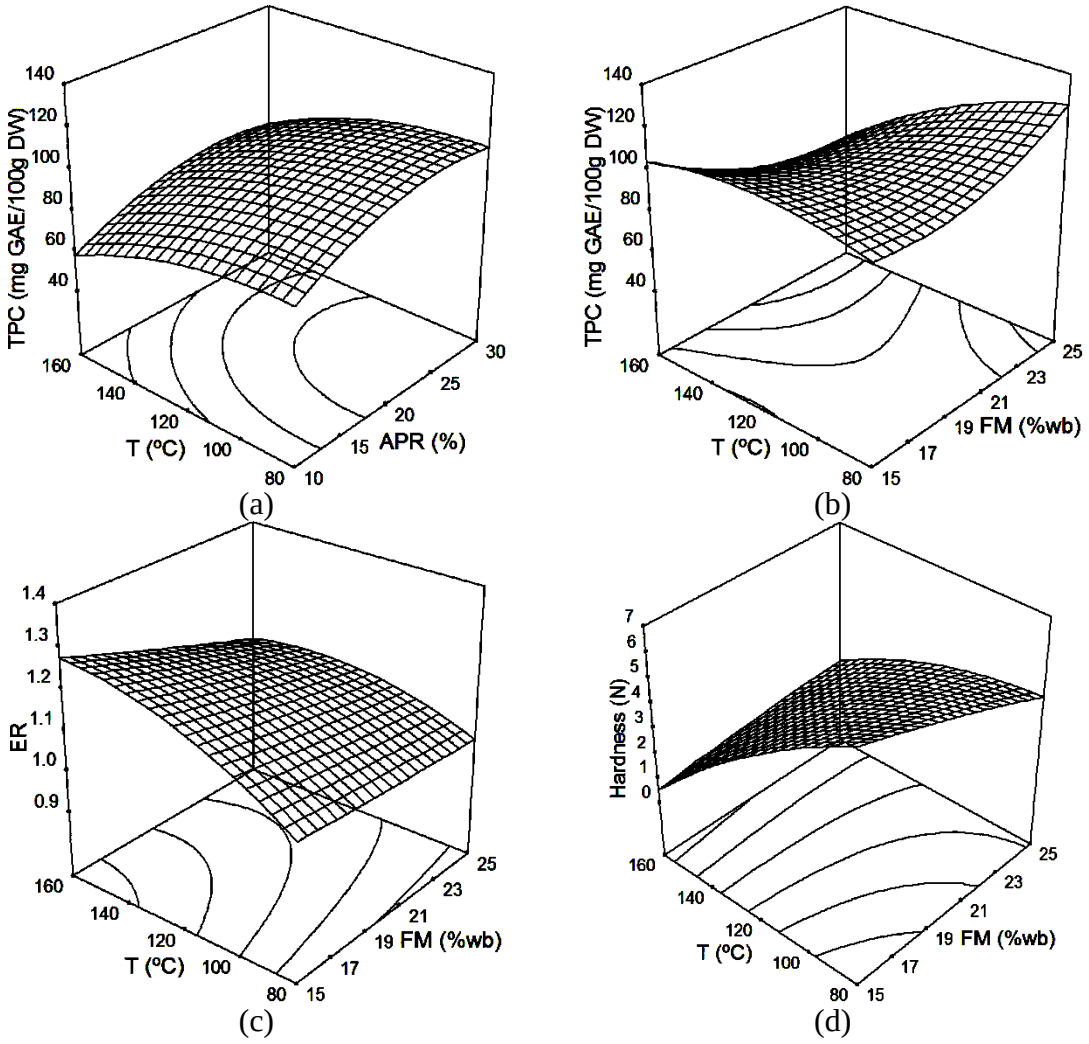


Figure 10.2 Response surface plots of total phenolic content (TPC) (a, b), expansion ratio (ER) (c) and hardness (d) as affected by apple pomace ratio (APR), feed moisture (FM) and extrusion temperature (T) at corresponding 0 coded level of other two variables

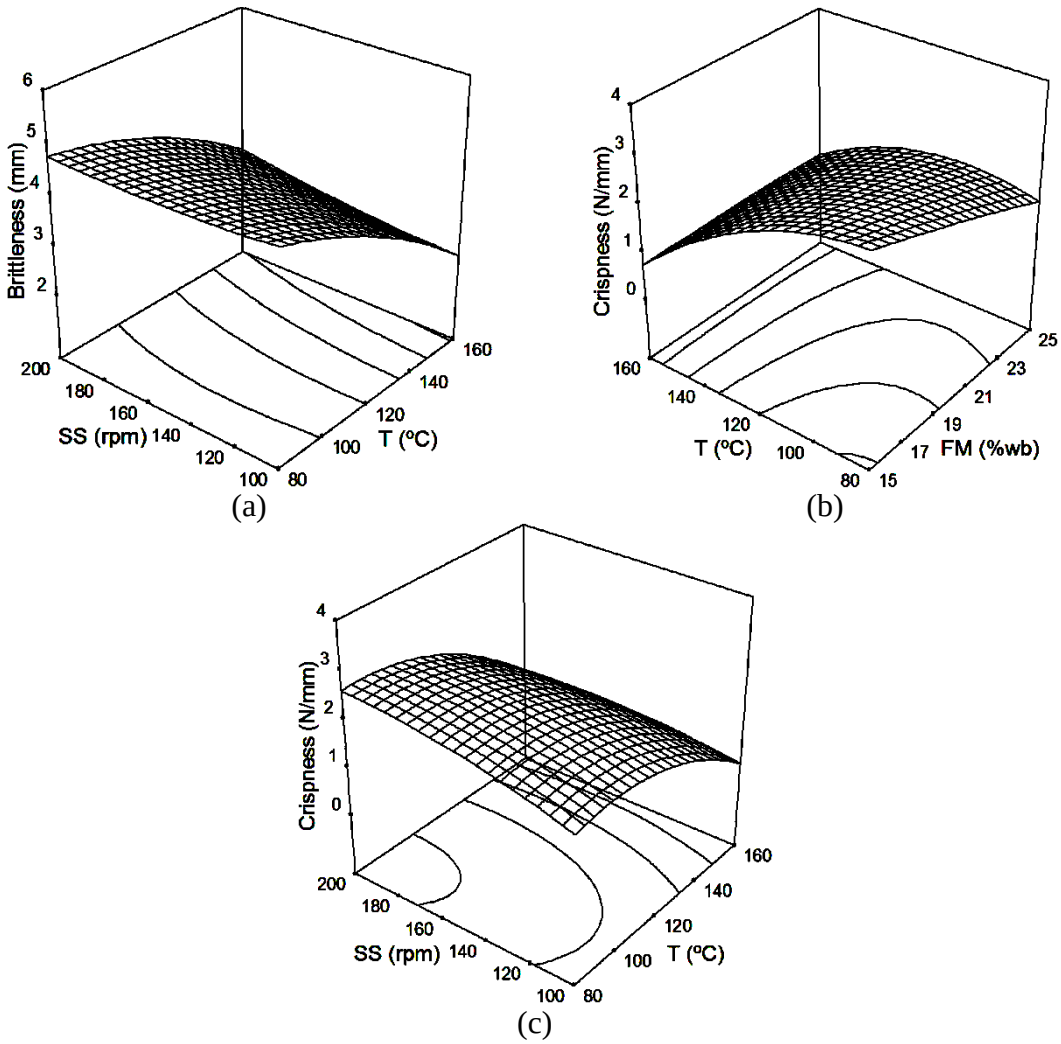


Figure 10.3 Response surface plots of brittleness (a) and crispness (b, c) as affected by feed moisture (FM) and extrusion temperature (T) and screw speed (SS) at corresponding 0 coded level of other two variables

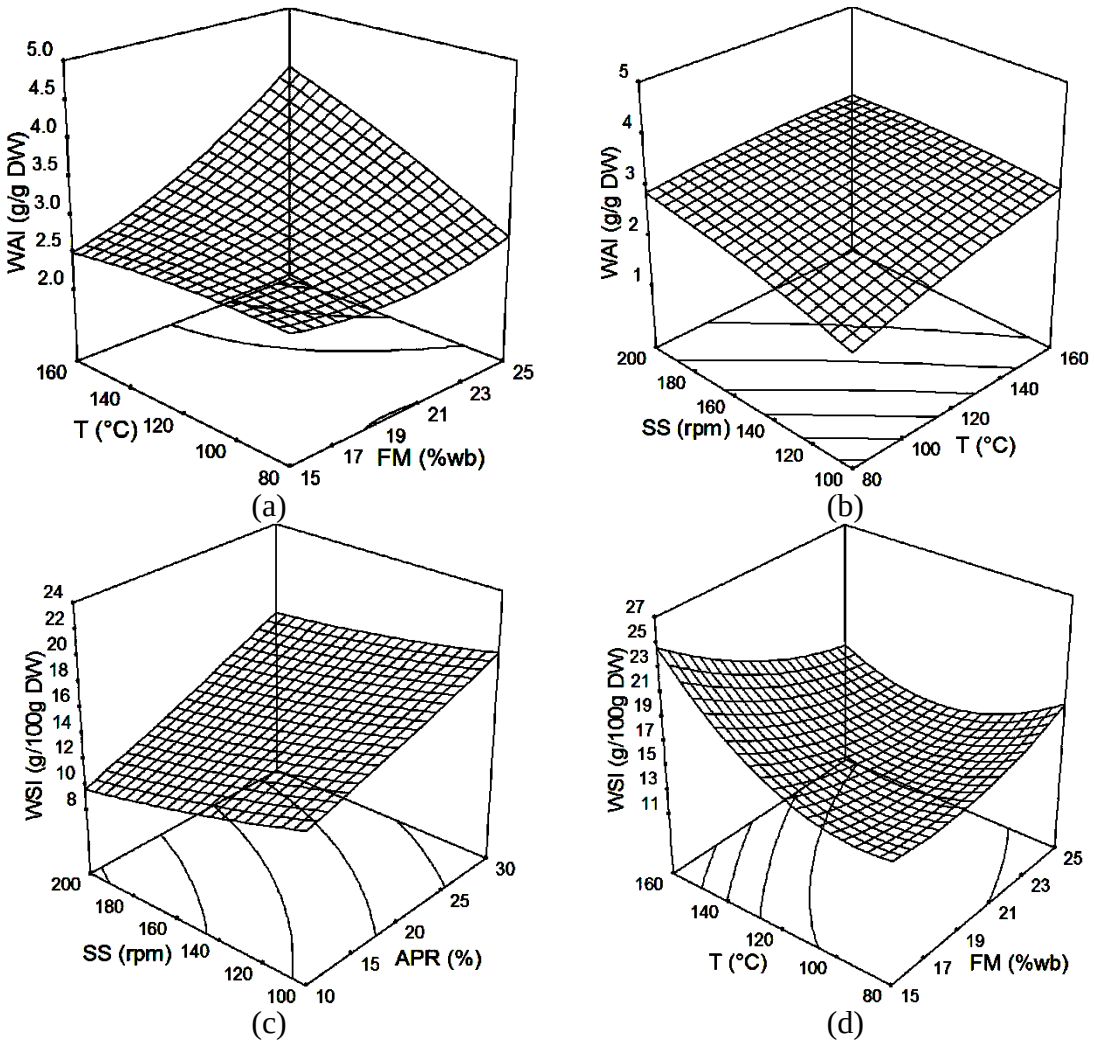


Figure 10.4 Response surface plots of water absorption index (WAI) (a, b) and water solubility index (WSI) (c, d) as affected by apple pomace ratio (APR), feed moisture (FM) and extrusion temperature (T) and screw speed (SS) at corresponding 0 coded level of other two variables

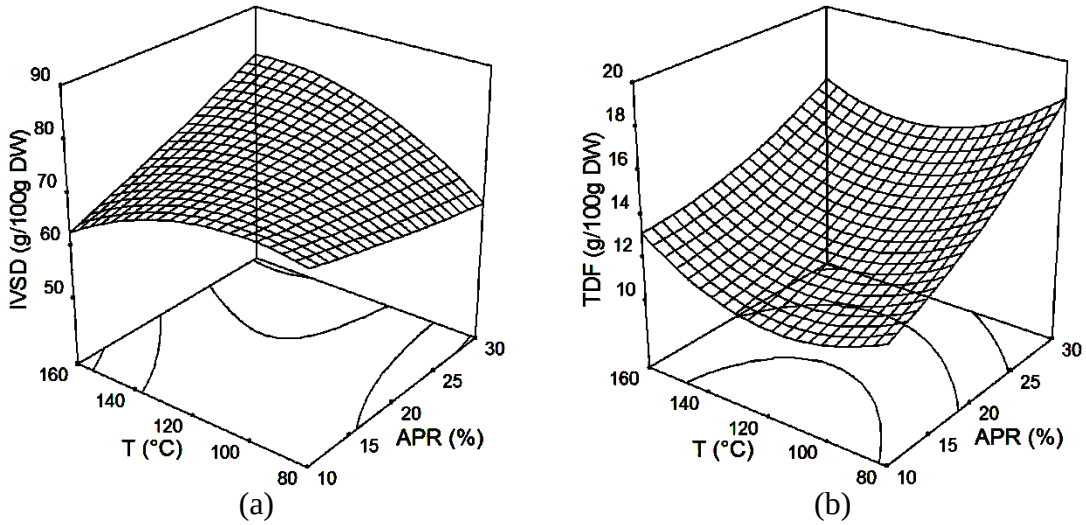


Figure 10.5 Response surface plots of in-vitro starch digestibility (IVSD) (a) and total dietary fiber (TDF) (b) as affected by apple pomace ratio (APR) and extrusion temperature (T) at corresponding 0 coded level of other two variables

Figure 10.6 Macroscopic and cross-sectional microstructure of (a, c) control and (b, d) CO_2 extrudates, respectively

CHAPTER 11

Conclusions

The primary objective of this investigation was to understand the effect of different non-thermal technologies as pretreatment, i.e. fermentation followed by batch & continuous ultrasonication or pulsed electric field or hydrodynamic cavitation on releasing of bound phenolics in sorghum flour and apple pomace in order to enhance their total content in raw materials. The secondary goal of this study was to retain maximum phenolics in processed corn flour, sorghum flour and apple pomace based extrudates using moderate moisture extrusion and CO_2 assisted extrusion technology. Although combination of fermentation with all mentioned non-thermal processes demonstrated a positive impact on total phenolic content (TPC) and antioxidant activity (AA) of both sorghum flour and apple pomace, hydrodynamic cavitation was selected due to its viable adoption at commercial scale. Among the two extrusion processes, CO_2 assisted extrusion exhibit a promising results in terms of higher antioxidant, textural, nutritional and functional properties in final extrudates.

The specific conclusions of this study are the following:

1. In the batch ultrasonication, the TPC and AA of the sorghum flour at optimum conditions of 12 h fermentation time (FT), 10 % (w/v) flour to water ratio (FWR), 30 W/cm^2 ultrasonication intensity (UI), and 3 min ultrasonication time (UT) were found as 77.8 mg GAE/ 100g DW, and 178.8 µmol TE/100g DW, respectively, whereas these values for the control sorghum flour were 63.9 mg GAE/100g DW and 133.5 µmol TE/100g DW, respectively.

2. Increasing the FT, FWR and UI during ultrasonication resulted in decreasing the TPC and AA significantly, however the UT did not have any significant effect on the TPC and AA of the sorghum flour.
3. TPC and AA of the extrudates from the ultrasonicated sorghum flour were observed 60.3% and 45.8% higher than that of the control extrudates. Extrudates from the ultrasonicated sorghum flour had improved textural (hardness, brittleness, crispness) and functional properties (water absorption index, water solubility index). Ultrasonication also reduced crystalline fraction amount and stability of starch crystallites in the sorghum flour.
4. Continuous ultrasonication with compared to the batch process enhanced the TPC and AA further by 15.8% and 6.3%, respectively with 50% less UI and UT.
5. Mild intensity pulsed electric field (PEF) at its optimized conditions of 45% (w/v) FWR, 2 kV/cm electric field intensity (EFI) and 875 µs treatment time increased the TPC and AA in the sorghum flour by 24.8% and 33.9%, respectively.
6. Hydrodynamic cavitation increased the TPC and AA in the sorghum flour by 39.5% and 38.6%, respectively at the optimized conditions of 10% (w/v) FWR, 3 rotor holes and 35°C cavitation temperature. The in-vitro starch digestibility (IVSD) of the sorghum flour also improved by 4.7%.
7. At the optimum conditions of the batch ultrasonication, i.e. 24 h FT, 5 % (w/v) pomace to water ratio (PWR), 37 W/cm^2 UI, and 90 W microwave power (MWP), TPC and AA of the apple pomace were observed as 424.98 mg GAE/ 100g DW, and 846.38 µmol TE/ 100g DW, respectively, whereas for the control apple pomace these values were 302.9 mg GAE/100g DW TPC and 438.6 µmol TE/100g DW,

respectively. The microwave drying following the fermentation and ultrasonication increased the TPC and AA in the apple pomace by 20.1% and 47.9%, respectively with compared to that of the oven drying. With the increase in the FT, PWR and MWP, TPC and AA of the apple pomace were significantly decreased.

8. TPC and AA of the extrudates from the ultrasonicated apple pomace were observed 53.8% and 94.5% higher than that of the control extrudates. The ultrasonicated apple pomace exhibited more brittleness, crispness and less hardness with compared to that of the control apple pomace extrudates.
9. With compared to the batch ultrasonication, the continuous process further improved the TPC and AA in the apple pomace by 3.8% and 1.4%, respectively using 45.9% and 50% less UI and UT, respectively.
10. At the optimized conditions of the PEF treatment (12.5% (w/v) FWR, 2 kV/cm EFI and 500 μs treatment time), the TPC and AA were enhanced by 37.4% and 86%, respectively in the apple pomace. Electroporation of the sorghum flour and the apple pomace was dominantly influenced by the flour to water ratio followed by the electric field intensity and the treatment time.
11. TPC and AA of the apple pomace during hydrodynamic cavitation increased by 42% and 97%, respectively at the optimized conditions of 8.75% (w/v) FWR, 4 rotor holes and 45°C cavitation temperature. The apple pomace also had improved total dietary fiber (TDF) improved by 7.6%.
12. Sorghum flour, after all three non-thermal treatment, showed the higher concentration of the phenolic acids derived from salicylic, ferulic, p-hydroxybenzoic and caffeic

acids, while the apple pomace had protocatechuic, cholorogenic and salicylic acids in majority.

13. Among all the three non-thermal processes, hydrodynamic cavitation exhibited its potential utilization in order to enhance the total content of phenolic compounds in the sorghum flour and apple pomace and also can be successfully employed to the pilot scale or commercial scale.
14. Corn flour and hydrodynamic cavitated sorghum flour, apple pomace blend extruded using 30% apple pomace ratio (APR), 25%wb feed moisture (FM), 132ºC temperature (T) and 108 rpm screw speed (SS) had 120.1 mg GAE/100g DW TPC and 308.7 µmol TE/100g DW AA, whereas TPC and AA of the control blend (without cavitation) were 123.2 mg GAE/100g DW and 258.9 µmol TE/100g DW, respectively.
15. Effect of the pomace inclusion on TPC and AA was dominant over the extrusion temperature. Higher expansion ratio, brittleness, crispness, IVSD, TDF and less hardness of extrudates with the higher pomace inclusion were the promising results. Improved water absorption index (WAI) and water solubility index (WSI) were also observed in the extrudates.
16. CO_2 assisted extrusion of the corn flour and hydrodynamic cavitated sorghum flour, apple pomace blend at the optimized conditions of 30% APR, 25%wb FM, 97ºC T and 100 rpm SS produced the extrudates with 130.3 mg GAE/100g DW TPC and 326.8 µmol TE/100g DW AA while, these numbers found in control extrudates (without CO_2) were 116.3 mg GAE/100g DW and 305.5 µmol TE/100g DW,

respectively. TPC and AA of the final extrudates were 5.8% and 26.2% higher than that of the control (untreated) blend of corn flour, sorghum flour and apple pomace.

17. Although CO_2 injected extrudates had the low expansion ratio, increased brittleness, crispness and decreased hardness were obtained during CO_2 assisted extrusion. Low WAI and higher WSI were observed in the CO_2 extrudates, however extrusion with CO_2 lowered the IVSD and the total dietary fiber.
18. CO_2 extrudates had more lightness and less redness indicating inhibition of the Maillard's reaction during extrusion. Extruded products were found rich in caffeic acid followed by salicylic acid and ferulic acid.

Overall, the natural fermentation followed by the hydrodynamic cavitation and CO_2 assisted extrusion produced the corn flour, sorghum flour and apple pomace based extrudates with improved TPC, AA, textural, nutritional and functional properties.

CHAPTER 12

Overall Summary

The objective of the research was to release the bound phenolics in sorghum flour and apple pomace to enhance their phenolic content and antioxidant activity during primary processing (hydrodynamic cavitation) followed by the secondary processing, i.e. liquid CO_2 assisted extrusion cooking to retain maximum total phenolic content and antioxidant activity in order to produce corn flour, sorghum flour and apple pomace based antioxidant rich extrudates with improved textural and functional properties. The significant outcomes of this research was summarize in Figure 12.1.

Sorghum flour and apple pomace were naturally fermented with 45% and 12.5% (w/v) flour to water ratio for 12 h and 24 h, respectively at 30°C. Fermented slurries of both sorghum flour and apple pomace were further hydrodynamic cavitated. Sorghum flour cavitated with 10% (w/v) flour to water ratio using rotor with 3 rows of holes (66 indentations) at 35°C cavitation temperature exhibited 39% significant ($p<0.05$) increase in total phenolic content and antioxidant activity in sorghum flour. The total phenolic content and antioxidant activity of apple pomace increased significantly ($p<0.05$) by 42% and 97%, respectively when cavitated with 8.75% (w/v) powder to water ratio using rotor with 4 rows of holes (88 indentations) at 45°C cavitation temperature.

The blend of cavitated sorghum flour, apple pomace and native corn flour in the ratio of 60:30:10 with 25%wb moisture content was cooked through liquid CO_2 assisted single screw extrusion to get the final product. The total phenolic content and antioxidant activity of CO_2 assisted extrudates at 97°C extrusion temperature and 100 rpm screw speed were found 67% significantly ($p<0.05$) higher when compared with that of control

extrudates which were extruded using the blend of native sorghum flour, apple pomace and corn flour at the similar extrusion conditions. Also, the total phenolic content and antioxidant activity of CO_2 assisted extrudates were observed 6% and 26%, respectively higher ($p<0.05$) when compared with that of blend of native corn flour, sorghum flour and apple pomace.

Textural and functional properties of extrudates were also improved. CO_2 assisted extrudates had 64% and 63% less ($p<0.05$) hardness and water absorption index, respectively when compared with that of the control extrudates. On the other hand, brittleness, crispness, expansion ratio, water solubility index, total dietary fiber and in-vitro starch digestibility were found 23%, 71%, 9%, 91%, 12.5% and 9%, respectively higher ($p<0.05$) when compared with those of the control extrudates.

It was concluded that the developed process at optimized conditions produced corn flour, sorghum flour and apple pomace based extrudates with significantly improved antioxidant, textural and functional properties.

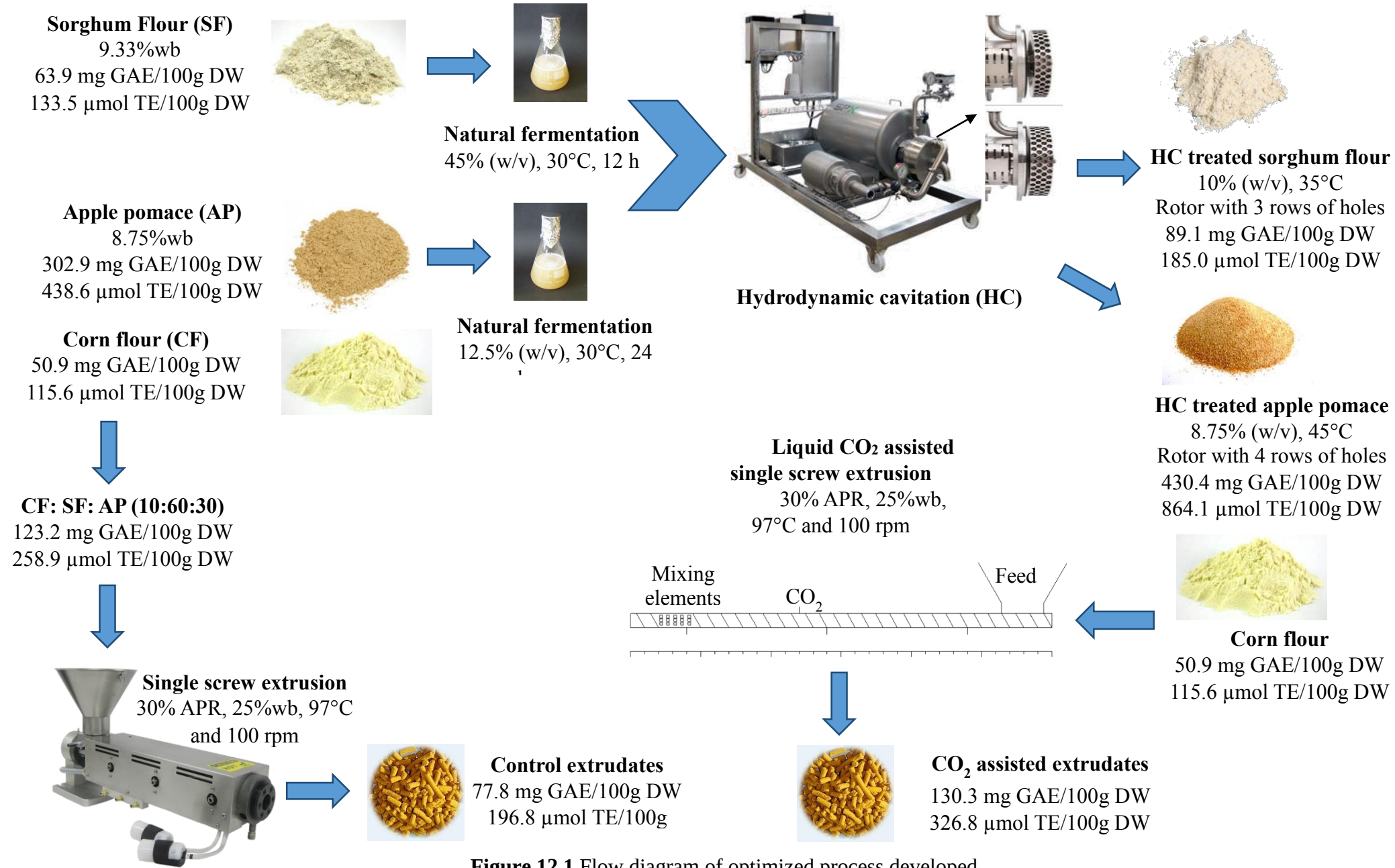


Figure 12.1 Flow diagram of optimized process developed

CHAPTER 13

Recommendations for Future Research

The present research was carried out to investigate the influence of the natural fermentation followed by either of three different non-thermal technologies, i.e. ultrasound cavitation, pulsed electric field and hydrodynamic cavitation on release of the phenolic acids in the sorghum flour and apple pomace. Further naturally fermented and the hydrodynamic cavitated sorghum flour and apple pomace were blended with the corn flour in a specific ratio and the blend was subjected to the CO_2 assisted extrusion to retain the maximum phenolics along with improved textural, nutritional and functional properties in the final extrudates. Although the developed process had potential impact on phenolic compounds of the sorghum flour (starchy) and the apple pomace (fibrous), the following investigations can be carried out as important research investigations in the near future.

1. Design and simulation study of the hydrodynamic cavitator rotor to proliferate the cavitation efficiency in the higher concentrated samples of the sorghum flour and apple pomace
2. Optimization of the hydrodynamic cavitation conditions for the blend of two or more materials for maximum antioxidant and functional properties
3. Application of the Fourier transform infrared spectroscopy and near-infrared spectroscopy to investigate the interaction between starch and fiber present in the blend for their structural modification and its effect on the phenolic modification
4. Impact of continuous process of pulsed electric field on total phenolic content and antioxidant activity of sorghum flour and apple pomace

5. Impact of the CO_2 assisted extrusion and higher moisture (>30%) feed on antioxidant, textural, nutritional and functional properties of the extrudates in a twin screw extruder and its comparison with single screw extruder
6. Design and the development of the screw profile for scale up of mixing and heat transfer on the basis of 3D numerical simulations for the twin screw extruder

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