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M.Sc. in Food Engineering

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REPUBLIC OF TURKEY

GAZİANTEP UNIVERSITY

GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES

SEARCH ON THE PRODUCTION TECHNIQUES OF WHEAT AND RED LENTIL BRANS LOW CALORIE DIETARY FIBER

M.Sc. THESIS

IN

FOOD ENGINEERING

BY

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Supervisor

Prof. Dr. Mustafa BAYRAM

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This is to certify that we have read this thesis and that in our consensus opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

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Ateka Ahmed ELMUSLI

ABSTRACT

SEARCH ON THE PRODUCTION TECHNIQUES OF WHEAT AND RED LENTIL BRANS LOW CALORIE DIETARY FIBER

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M.Sc. in Food Engineering
Supervisor: Prof. Dr. Mustafa BAYRAM
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Awareness of the importance of healthy and low calorie diets is increasing overall the world due to its nutritional and functional effects on the healthy body. Enrichment dietary products with dietary fibers become common a lot nowadays. In this study, physical and chemical changes of wheat and red lentil dietary fiber were determined for soaking, cooking, microwave and ultrasound treatments. The moisture (%, w.b.), protein (%, d.b.), ash (%, d.b.), fat (%, d.b.), starch (%, d.b.), sugar (%, d.b.), carbohydrates (%, d.b.), total dietary fiber (%, d.b.), calorie and color values (L*, a*, b* and YI) were determined. It was found that the chemical properties of wheat and red lentil brans significantly ($p \leq 0.05$) changed based on production methods, soaking, cooking, microwave and ultrasound. According to the results of analyses, the fat content of wheat and red lentil dietary fibers did not change ($p > 0.05$) in all treatments. The dietary fibers calorie content decreased ($p \leq 0.05$) in all different treatments and the dietary fiber content increased ($p \leq 0.05$) in all different treatments except soaked wheat bran.

Key Words: Wheat Bran, Red Lentil bran, Soaking, Cooking, Microwave, Ultrasound, Physical and Chemical Properties, Dietary Fibers.

ÖZET

DÜŞÜK KALORİ DİYET LİF ÜRETİMİ TEKNİKLERİ ÜZERİNE ARAŞTIRMA SİMÜLASYONU

ELMUSLİ, Ateka Ahmed
Yüksek Lisans Tezi, Gıda Mühendisliği
Danışman: Prof. Dr. Mustafa BAYRAM
Mart 2020
113 sayfa

Sağlıklı ve düşük kalorili diyetlerin önemi, sağlıklı beslenme için dünyada artmaktadır. Diyet ürünlerinin diyet lifi ile zenginleştirilmesi günümüzde çok yaygınlaşmaktadır. Bu çalışmada, ıslatma, pişirme, mikrodalga ve ultrason uygulamalarından sonra buğday ve kırmızı mercimek kepeğinin fiziksel ve kimyasal değişimleri belirlenmiştir. Nem (%, wb), protein (%, db), kül (%, db), yağ (%, db), nişasta (%, db), şeker (%, db), karbonhidratlar (%, db), toplam diyet lifi (%, db), kalori içeriği ve renk değerleri (L *, a *, b * ve YI) belirlenmiştir. Buğday ve mercimek kepeğinin kimyasal özelliklerinin ıslatma, pişirme, mikrodalga ve ultrason işlemleri uygulandıktan sonra önemli ölçüde değiştiği ($p \leq 0.05$) bulunmuştur. Analiz sonuçlarına göre, buğday ve mercimek diyet liflerinin yağ içeriği tüm işlemlerde değişmedi, bütün uygulamalarda kalori içeriği azaldı ve ıslatılmış buğday kepeği hariç tüm farklı işlemlerde diyet lif içeriği artmıştır.

Anahtar Kelimeler: Buğday Kepeği, Kırmızı Mercimek Kepeği, Islatma, Pişirme, Mikrodalga, Ultrason, Fiziksel ve Kimyasal Özellikler, Diyet Lifi.

TO MY FAMILY

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Firstly, I would like to express my sincere gratitude to my supervisor Prof. Dr. Mustafa BAYRAM for his guidance, support, patience and encouragement. I received from him at all steps of my research. I feel very lucky to have an opportunity to work with him.

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The last but never least, I want to send all my love to my family: my parents, my husband, my mother in law, my sisters, my brother and my uncles and aunts for their endless support. This thesis would not have been possible without their support, motivation, patience, encouragement. I would like to dedicate my thesis to my lovely small family my husband Muhammed Hani and my children Sena and Yezen.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

AI	Adequate intake
ANOVA	Analysis of variance
AOAC	Association Of Analytical Communities
CHD	Coronary heart disease
cm	Centimeter
CODEX	Alimentarius collection of food standards
d.b.	Dry base
DNSA	3, 5-Dinitrosalicylic acid
DP	Degree of polymerization
DSC	Differential scanning calorimeter
e.g	Exempli gratia
EtOH	Ethanol
FDA	Food and Drug Administration
FOS	Fructooligosaccharides
FTIR	Fourier Transform Infrared Spectroscopy
FOSS	Full Option Science System
g	Gram
GOS	Galactooligosaccharides
h	Hour
HMWDF	High molecular weight dietary fiber
HCl	HydroChloric Acid
HPLC	High performance liquid chromatography
IDF	Insoluble Dietary Fiber
KBr	Potassium bromide
kcal	kilocalorie
kHz	kilohertz
L	Liter
RLB	Red lentil bran

LDL	Low density lipoprotein
LMWDF	Low molecular weight dietary fiber
m^3	Cubic meter
mg	Miligram
min	Minute
ml	Mililiter
MSA	Microscopic Structural Analysis
NaOH	Sodium hydroxide
NDO	Non digestible oligosaccharides
nm	Nanometer
NSP	Non starch polysaccharides
PLM	Polarized light microscope
RMD	Resistant maltodextrins
RH	Relative Humidity
RS	Resistant starch
S	Second
SCFAs	Short chain fatty acids
SDF	Soluble dietary fiber
SDFP	Dietary fiber soluble in water and precipitated by 78% ethanol
SDFS	Dietary fiber soluble in water and soluble in 78% ethanol.
SPSS	Statistical Package for the Social Sciences
SSF	Simulated saliva fluids
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
TDF	Total dietary fiber
USDA	United States Department of Agriculture
UV	Ultraviolet
Vis	Visible
W	Watt
WB	Wheat bran
w.b.	Wet base
w/v	Weight per volume
w/w	Volume per volume
YI	Yellowness index

CHAPTER 1

INTRODUCTION

Because of increased nutritional awareness nowadays, people tend to choose healthy food. Most of the people interest in low-calorie and healthy food, so people are going to diets rich in dietary fiber. One of the best ways to control body weight is increasing dietary fiber intake in line with the recommended dietary intake because it gives a sense of satiety despite the consumption of low calorie intake.

1.1 Dietary Fiber

There are many definitions of dietary fiber, but the physiological description is the dietary components that cannot break down enzymatically into digestible units in the stomach and small intestine (Howarth et al., 2001). Indigestible carbohydrates were termed unavailable carbohydrates (McCance and Lawrence, 1929). Hipsley (1953) was the first to use dietary fiber as a new term in the same meaning. Dietary fiber is a non-digestible part of plant that includes polysaccharides and lignin. In recent times, this definition has been extended to include oligosaccharides, such as inulin and resistant starches (Anderson et al., 2009).

The definition of dietary fiber expanded to include, indigested protein, fat and inorganic components of the cell wall in addition to polysaccharides and lignin (Asp, 1987). Therefore, the definition made by Trowell (1972) includes all components of the plant cell wall that are expected to distribute to the colon. After that, this definition was revised to include only non-digestible carbohydrates and lignin, but on the other hand, it also expanded to include non-digestible polysaccharides that are not derived from plant cell walls (Trowell et al., 1976). The addition of these non-cell wall polysaccharides in the concept of dietary fiber results from that they are may be behaving similarly to the behavior of polysaccharides in the cell wall in the digestive tract. On the other hand, they cannot be analytically distinguished from

polysaccharides in the cell wall. The indigested protein exclusion of the dietary fiber concept was accepted by most workers, although some scientists accept indigestible protein to be a part of dietary fiber (Saunders and Betschart, 1980). Because of the heat treatment effects on the protein and lipid digestion and the absence of accurate methods to measure protein and lipid digestion in vitro, lipids and proteins were excluded from dietary fiber concept (Asp, 1987). Food fibers are often known as "non-starchy carbohydrates and lignin" (Dahl et al., 2005). However, many studies have shown complete non-digestion of all starch and some other studies indicate poor absorption of large starch of white bread (Levitt, 1983). Therefore, the final accepted concept of dietary fiber includes polysaccharides, oligosaccharides, such as inulin, lignin and resistant starches.

Dietary fiber is divided into two groups, soluble fibers such as pectin and beta-glycan found in oats and fruits and insoluble ones such as cellulose in whole grains and nuts. Both types of dietary fiber can be found in fiber-rich foods. Nevertheless, one third of dietary fiber found in food is soluble and the rest is insoluble (Lattimer and Haub, 2010). Researches have shown that a diet high in fiber can help to increase the good bacteria in the gut that feed on these fibers to produce substances thought to be protective of diseases and cancers such as short-chain fatty acids.

Dietary fiber is not applicable widely in the food industry because of its undesirable texture and unaccepted mouth feel in informal food products, which creates a big challenge in the dietary application of dietary fiber (Guo et al., 2018).

1.1.1 Types of Dietary Fibers:

Dietary fiber is the indigestible part of plants and consists of two main types:

Soluble dietary fiber

Soluble dietary fiber dissolves in water and gastrointestinal fluids when it passes through the gastrointestinal tract. It is transformed into a gel-like substance, which is fermented by bacteria in the large intestine, releasing gases and a bit of calorie (Salmas et al., 2017).

There is more than one class for soluble fiber (Esposito et al., 2005):

Soluble only in hot water includes agars, algins and amyloses.

Soluble in room temperature water includes curdlan, hydroxypropyl celluloses, hydroxypropylmethylcelluloses and methylcelluloses.

Soluble in room temperature and hot water includes alginates, amylopectins, carboxymethylcelluloses, and dextrins (Jones, 2004).

Insoluble dietary fiber

Insoluble dietary fiber does not dissolve in water and gastrointestinal fluids, it remains as it is when passing through the gastrointestinal tract and treated with digestive enzymes, so that insoluble fiber does not provide the body with calories. Both two types of fiber have plentiful health benefits (Salmas et al., 2017).

IDF can turn to SDF by chemical treatment, which affects the sensory properties of the product. A newer approach is the treatment of a cereal product with a tailored preparation of a Trichoderma enzyme (Napolitano et al., 2006). Scientists and researchers have proved this method because it increases the soluble dietary fiber (SDF) amount without any effect on total dietary fiber (TDF) (Napolitano et al., 2006).

1.1.2 Constituents of Dietary Fiber

Celluloses

The composition of dietary fiber affects by age, species and the anatomical structure of plant material. The most available molecule in nature is cellulose, a linear glucose D polymer with beta residue l, 4-1 sugar, is a beta isomer of starch up to 10,000 per molecule. A fine crystalline microfibril structure of cellulose comes from the hydrogen bonding of sugar residues in adjacent chains (Kay, 1982) (Figure1.1).

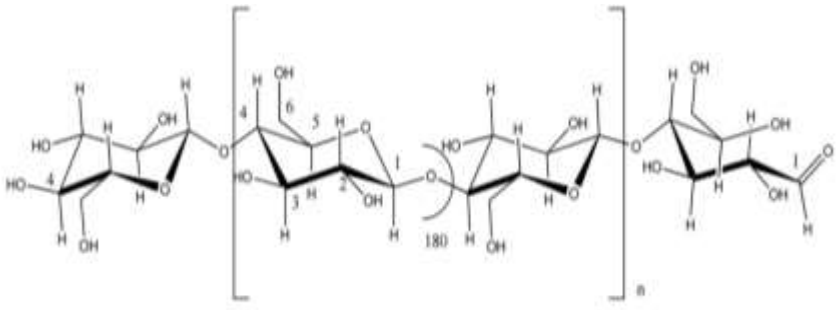


Figure 1.1 The chemical structure of cellulose polymer (Granström, 2009).

According to this structure, the cellulose molecules placed together closely, linked through the hydrogen bonds. This is the principle of the formation of microfibril and illustrates the being of cellulose insoluble in a lot of solvents even in strong alkali (Căpriţă et al., 2010). But it can be dissolved in strong acidic conditions (Alves et al., 2016). In mixed-linkage beta-glucans, containing beta-l,3-and beta-l,4-links simultaneously, this derangement in cellulose structure leads to high solubility in water.

Hemicelluloses

Hemicelluloses are the polysaccharides present in the plant cell walls dissolved by aqueous alkaline after the removal of water soluble polysaccharides and pectic polysaccharides (Hoch, 2007).

Hemicelluloses are low molecular weight polymers insoluble in water but soluble in alkaline solutions, due to its receptivity to chemical degradation, more than cellulose. It composes of a variety of sugar units that have a range of structural linkages, i.e., α- or β-(1 → 2, 1 → 3, 1 → 4, 1 → 6) α- or β-(1 → 2, 1 → 3, 1 → 4, 1 → 6). Its basic structure is made up of β-(1, 4)-linked pyranoside sugars (Figure 1.2).

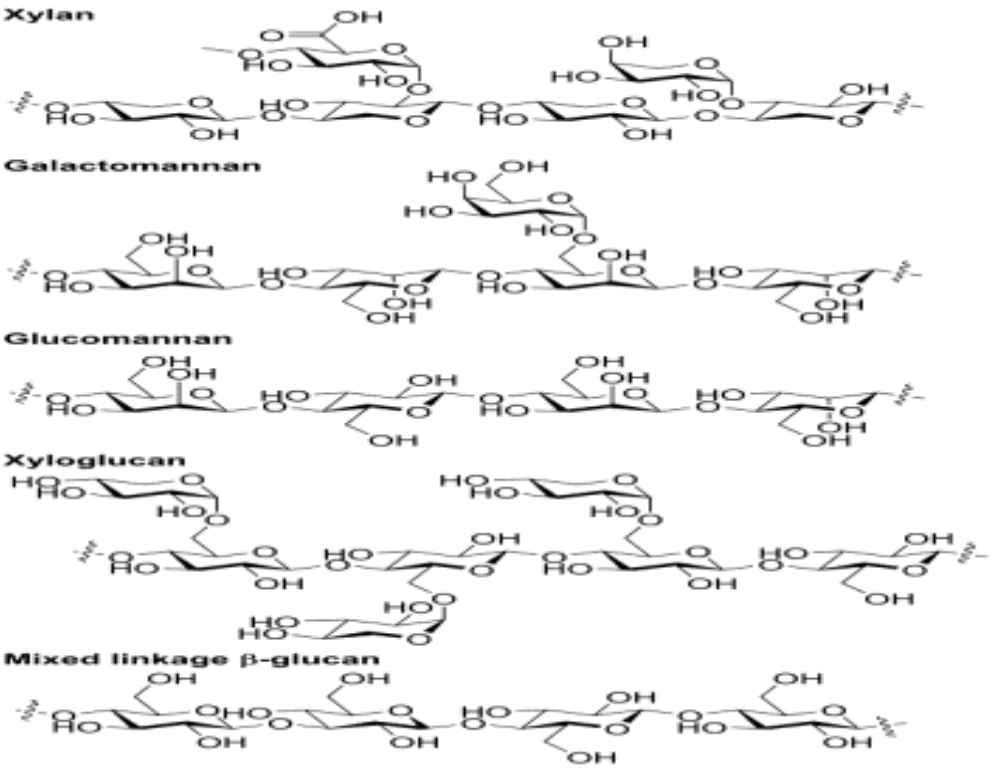


Figure 1.2 Structure of different types of hemicelluloses (Slavin, 2005).

There are three differences between cellulose and hemicellulose, hemicelluloses smaller in size (often less than 200 sugar residues), contain a variety of sugars, and are usually branched (Holm et al., 1983).

Pectins

Pectin is a natural component found in plant cell walls (Figure1.3). Natural pectin materials are part of the cell wall. They are present along with cellulose and form an adjacent layer in the center of the cell, allowing the cells of the plant tissue to adhere tightly. Natural pectin substances are widely found in fruits, roots and plant leaves in the form of protopectin, pectin and pectic acid.

The principal constituent of pectin is D-galacturonic acid. Pectin molecule is found in specific regions in the cell as α-(1→2) rhamnopyranose units from which side chains of galactose, mannose, glucose and rarely xylose (Mudgil, 2017). Part of galloronic acids present as the methyl ester. Pectin can be produced from aqueous extraction of edible plant material, mainly from the citrus peel or apple pomace, followed by selective precipitation using alcohol or salts.

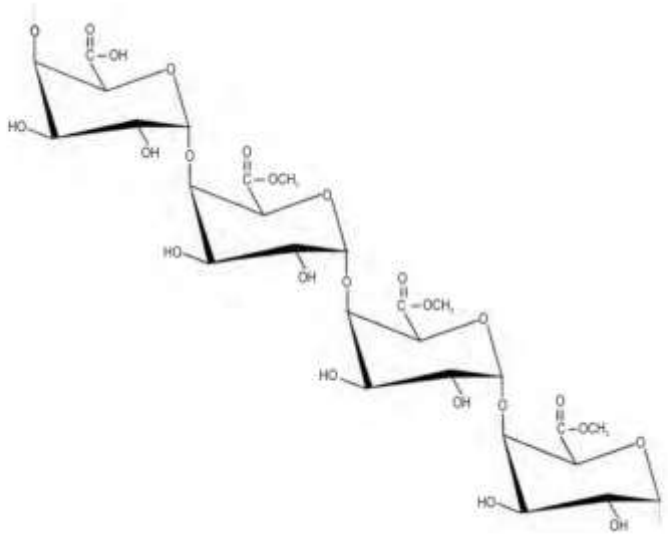


Figure 1.3 Segment of the pectin molecule (Yujaroen et al., 2008)**.**

Some of the pectins contain neutral sugars linked to each other by covalent bonds as a side chain, especially arabinose, galactose and less frequently to a xylose, rhminose and glucose. Galactic acids contain a carboxylic groups that are partially methylated and hydroxyl group secondary can be acetylated (Căpriţă et al., 2010).

Mucilage

Gum cells in the outer layer of the banana seed family contain gummy mucilage that has a high ability to bind water. Mucilage is defined as highly branched polymeric structures where the basic structure of mucilage is formed from beta-L and D-xylose residues and side chains of arabinose, rhamnose and galacturonic acid (Figure1.4). About 30-35 monomer residues are found in one molecular of mucilage (Cho et al., 1997).

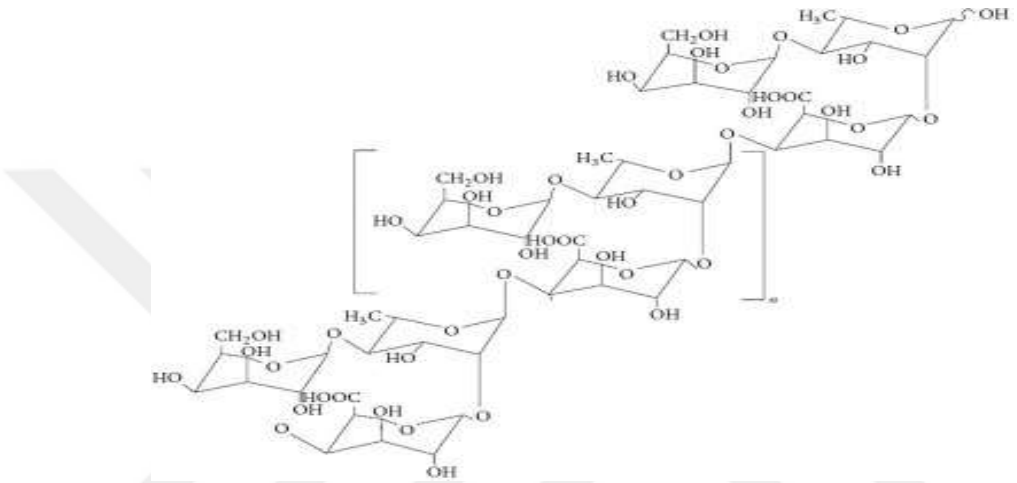


Figure 1.4 Molecular structure of okra mucilage (Mohammadi et al., 2018)**.**

Resistant starch

There is a type of starch molecules that resist digestion so that is known as resistant starch (Warshaw, 2007). It is digested and absorbed partially, the intestinal microorganisms convert it into short chain fatty acids esters (SCFAs) such as acetate, butyrate, and propionate that can be absorbed into the body or cannot be absorbed. However, it produces energy by coliform bacteria. The raw foods contain more resistant starch because the heat treatments of food gelatinize the starch and make it easy to digest.

There are several types of resistant starch

Type 1: Physically inaccessible

Physically, inaccessible starch cannot decompose by digestive enzymes. Found in; legumes completely and partially milled grains and seeds.

Type 2: Resistant granules

Resistant granules contain a huge amount of amylose. It resists the digestion considerably. They are found in fruits, potatoes, maize, corn and some pulses.

Type 3: Retrograded

When the starch rich food is cooked and cooled immediately, the structure of starch changes and becomes harder to digest which means the increase of resistance to digestion. Found in cooked/cooled foods like potatoes, bread, rice and cornflakes.

Type 4: Chemically modified

Starch chemically modified by some food producers where they extract resistant starch from some food sources often from corn and then added it to the processed food such as bread and crackers. Found in synthetic and commercialized resistant starch products, such as “Hi-Maize Resistant Starch”(Nilsson et al., 2008).

Oligosaccharides

Oligosaccharides are indigestible polysaccharide, but it is characterized by being of lower molecular weight than polysaccharides. Oligosaccharides include oligomers of fructose, i.e. inulin. In a chemical side, inulin is a heterogeneous substance of fructose polymers (Figure 1.5). It consists of chain-terminating glucosyl moieties and a repetitive fructuously moiety, which are linked by β(2,1) bonds (Ye et al., 2018). Its solubility differs according to the temperature and the degree of polymerization (DP) so that it can be included in both types of dietary fiber. Chicory roots are a very good source of inulin-type fructans [β-(2,1)-fructans], which produce lactic acid and SCFAs after fermentation in the colon (Chawla and Patil, 2010).

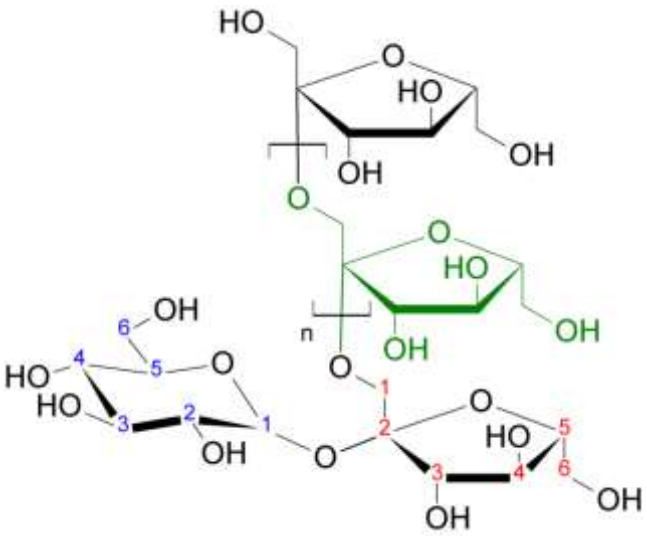


Figure 1.5 The structural chemical formula of the inulin polymer (Öztürk and Serdaroğlu, 2017).

Lignin

Lignin is not considered as a polysaccharide but it is found adjacent to hemicellulose in the plant cell wall (Figure 1.6) (Mehta et al., 2015). Lignin does not play any serious role as a single component. However, it acts with hemicelluloses simultaneously to form the cellulose microfibrials, which are corresponded to form crystalline regions. The reasons to include it the concept of dietary fibers are, strong structural adhesion between it and dietary fiber (covalent bond between lignin and Hemicellulose) in addition to its high resistance to digestion even in strong acids (Asp, 1987).

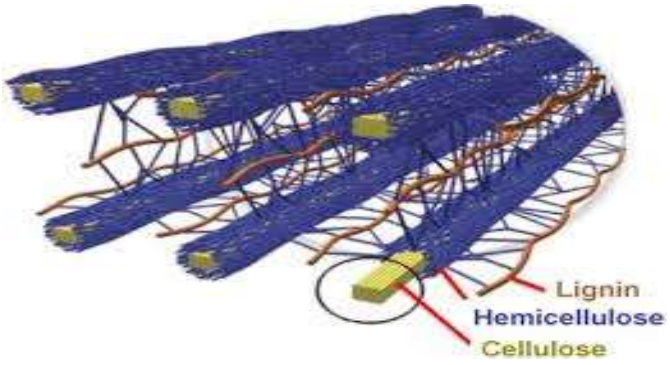


Figure 1.6 Cellulose strands surrounded by hemicellulose and lignin (Doherty et al., 2011).

1.1.3 Sources of Dietary Fiber

Different plant foods contain different quantities of fiber and the ratio differs according to maturity and plant part. The good sources of insoluble fiber are whole grains, wheat cereals, cereals bran and vegetables such as carrots, celery and tomatoes. Soluble fiber is found in barley, oatmeal, beans, nuts and fruits such as apples, berries, citrus fruits and pears. Vegetables, legumes, and whole grains are the most important sources of fiber (S. Liu et al., 2003).

- Cellulose and hemicellulose amount is high in cereals, grains and strawberries.
- Peaches are rich in lignin, high amounts of pectin are found in citrus fruits.
- Root vegetables, leafy vegetables, legumes or pulses and certain fruits such as pears and apples are the top source of cellulose (Krasteva and Kirilova, 2015).

There are many dietary fiber sources. But, the best one is whole grain foods (7-12 g/100 g). Fresh fruits and vegetables; peas and cowpeas (approximately 5 g/100 g), green vegetables (3-4 g/100 g) and nuts (approximately 11 g/100 g). Eating whole fruits (1-10 g/100 g) instead of fruit juice (0.1-0.5 g/100 g) is recommended due to the higher amount of dietary fiber is available on it. For example, one medium apple contains about 4.4 g of fiber if eaten without peeling whereas applesauce contains 2.8 g of fiber and there is no fiber in apple juices (Krasteva and Kirilova, 2015).

1.1.4 Measurement Technique

The enzymatic gravimetric method (Figure 1.7) is the best technique applied in vitro to determine dietary fiber amount in different samples. Briefly, the first step is the sample defatting and then the sample is treated with enzymes that mimic the digestive process in the human small intestine. Absorbable carbohydrates are broken down into simple sugars and later removed from the sample by precipitation and filtration as the body absorbs these components. The residual indigestible precipitate contains the dietary fiber, contains protein and inorganic material. So that protein and inorganic material must determine previously and bring out the weight (Champ et al., 2003).

Most methods for dietary fiber determination exclude solubilization step, thereby resistant starch will be found in the assay of dietary fiber (Nilsson et al., 2008).

Overall total dietary fiber was separated into different components in a variety of methods are used in dietary fiber measurement.

These components are:

HMWDF; high molecular weight dietary fiber (HMWDF = IDF + SDFP) such as cellulose, resistant starch.

IDF; dietary fiber insoluble in water such as cellulose, resistant starch and xylans.

SDFP; dietary fiber soluble in water and precipitated by 78% ethanol such as cereal β-glucan, guar gum and certain xylans.

SDFS; dietary fiber soluble in water and soluble in 78% ethanol. This is also known as low molecular weight dietary fiber (LMWDF) or non-digestible oligosaccharides (NDO) for example Fructooligosaccharides (FOS), galactooligosaccharides (GOS) and a portion of polydextrose, inulin and resistant maltodextrins (RMD) (McCleary and Prosky, 2001).

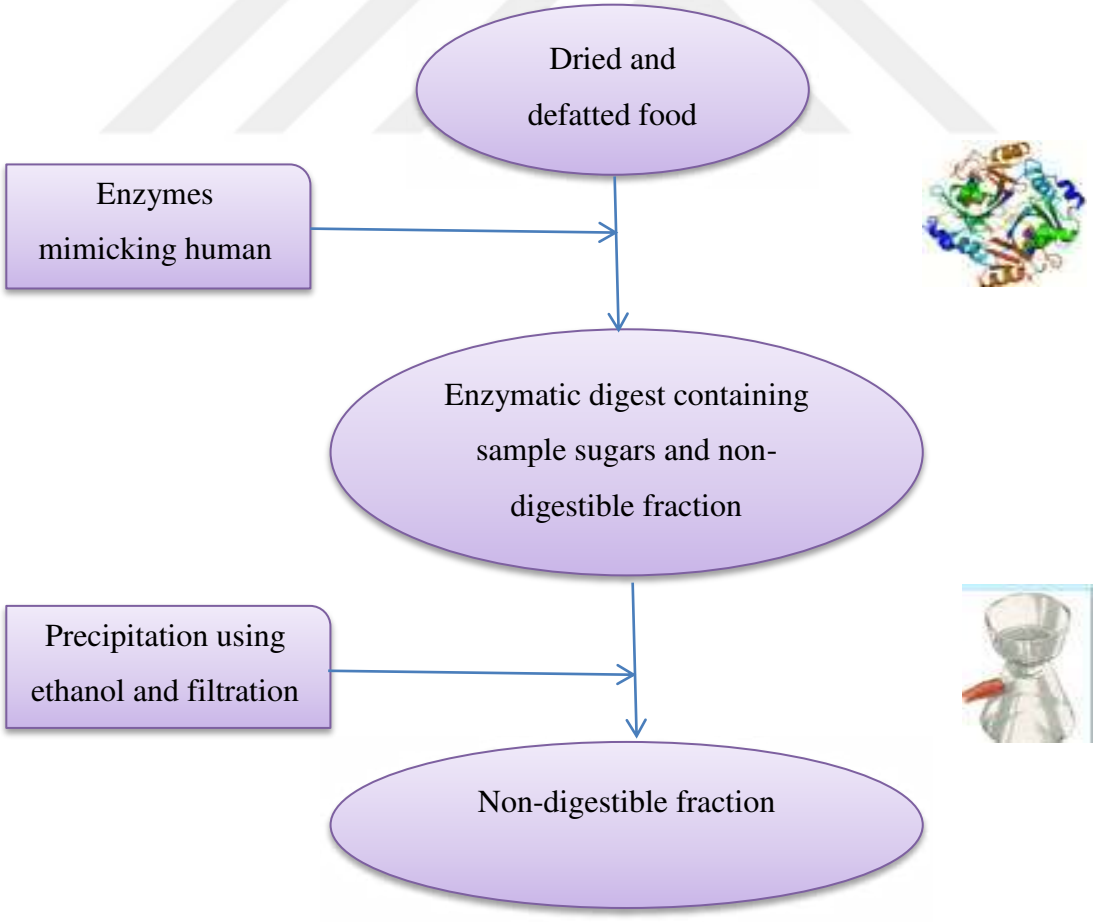


Figure 1.7 Enzymatic gravimetric method steps (Champ et al., 2003).

The enzymatic gravimetric methods for determining dietary fiber are detailed below:

1) Prosky method (AOAC 985.29)

It is used to determine high molecular weight dietary fiber (HMDF). It was presented in 1985. It is based on the usage of bacteria α-amylase and harsh conditions (pH 8.2, 100 ℃) for enzymatic incubation procedure. This method does not determine all dietary fiber components as currently defined by the CODEX Alimentarius. Most resistant starch and all non-digestible sugars are not included, which reduces the correct value of total dietary fiber present in the sample (Figure 1.8).

This method limitations are, it does not have an accurate measure of resistant starch. It estimates RS1, RS2 and RS3 less than it actually is and estimates RS4 higher than it actually is and does not measure SDFS (Prosky et al., 1985).

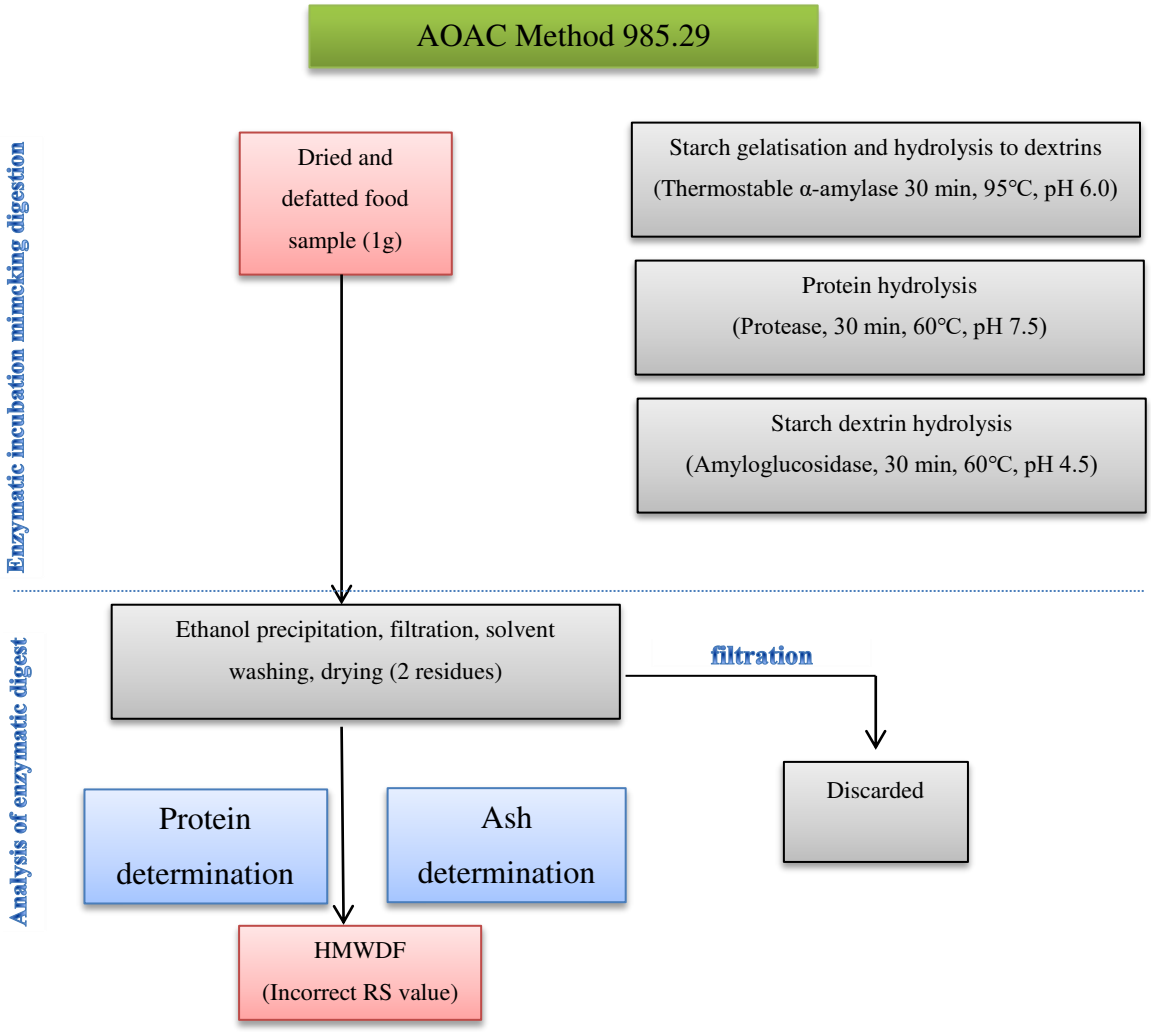


Figure 1.8 Prosky method (Prosky et al., 1985).

2) Lee Method (AOAC 991.43)

It is used for IDF and SDFP measurement. limits of this method are, the measuring of resistant starch measured values are inaccurate, underestimating RS1, RS2 and RS3, Overestimating of RS4 and does not measure SDFS (Lee and Prosky, 1995).

Lee method (Lee and Prosky, 1995) procedures are described below (Figure 1.9);

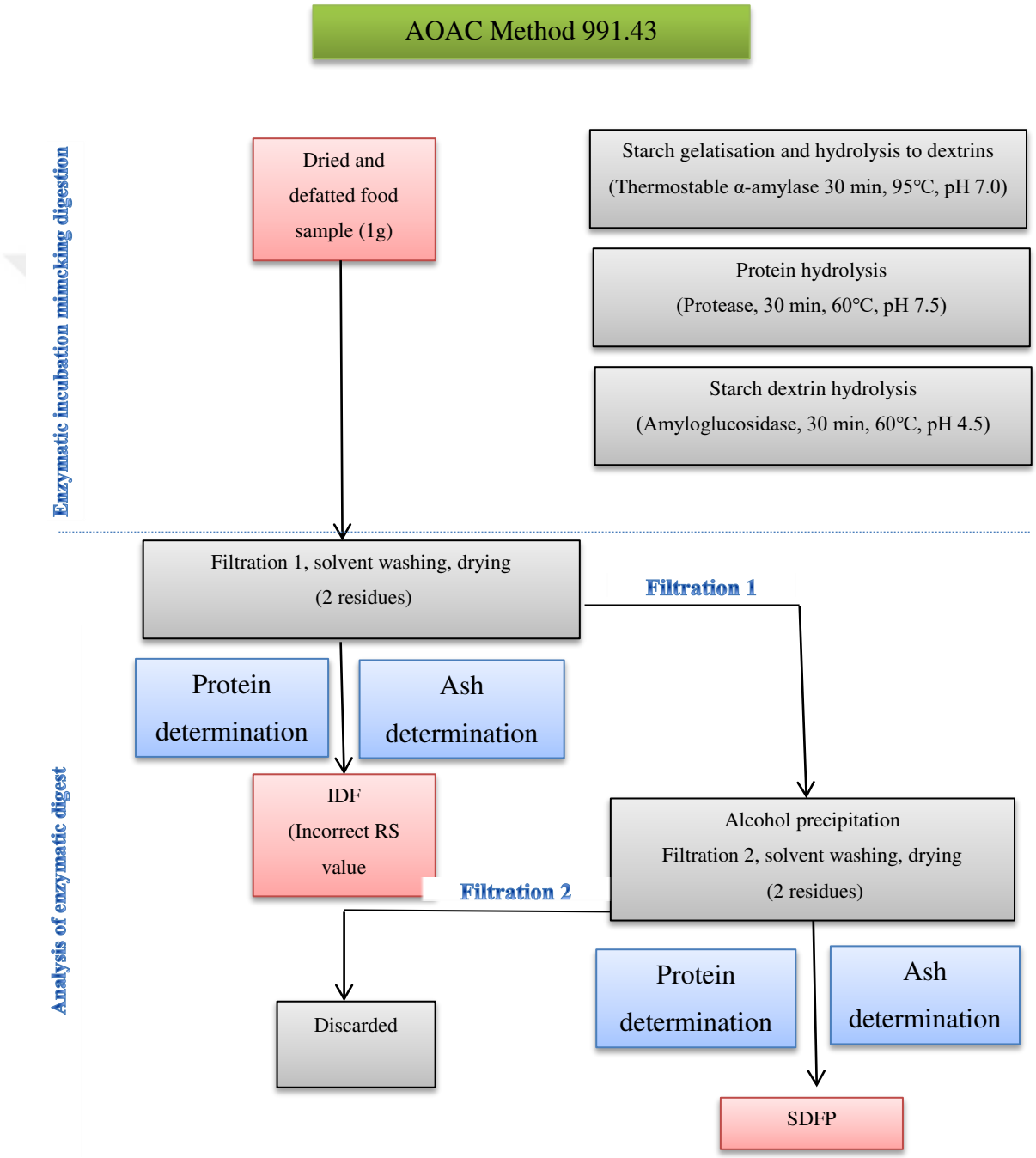


Figure 1.9 Lee Method (Lee and Prosky, 1995).

In spite of the huge approval for both of Prosky and Lee's methods for the determination of dietary fiber in the past. They do not measure all constituents of dietary fiber as because:

a) A large quantity of resistant starch (RS1, RS2 and RS3) hydrolysis during the a-amylase incubation at 95ºC, which leads to an underestimation of these RS components.

b) The SDFS components are neglected so non-digestible oligosaccharides (NDOs) are not accounted for.

c) Synthetic phosphate cross-linked resistant starch (RS4) is estimated higher than the actual value by this method. RS4 resists hydrolysis at a given incubation temperature (The ~100ºC) because of unsuitable physiological conditions (37ºC) (McCleary et al., 2013). The Prosky and Lee methods can be considered an accurate measure for the HMWDF content of samples not containing RS.

3) Matsutani Method

It is used for high molecular weight dietary fiber (HMWDF) and resistant maltodextrin (RMD) determination. It introduced in 2001, this method includes all the steps that have been implemented in a Prosky method but in addition to the HPLC analysis step to capture the resistant maltodextrin (RMD) component of dietary fiber that was soluble in 78% ethanol. This method was developed for the correct determination of dietary fiber content in the commercial product, Fibersol, which consists of ~ 90% RMD (Gordon and Okuma, 2002)**.**

The limits of this method are:

Like the previous methods, the Matsutani method is not accurate for resistant starch determining underestimates RS1, RS2 and RS3 and overestimates RS4.

The assay procedures are summarized below (Figure 1.10);

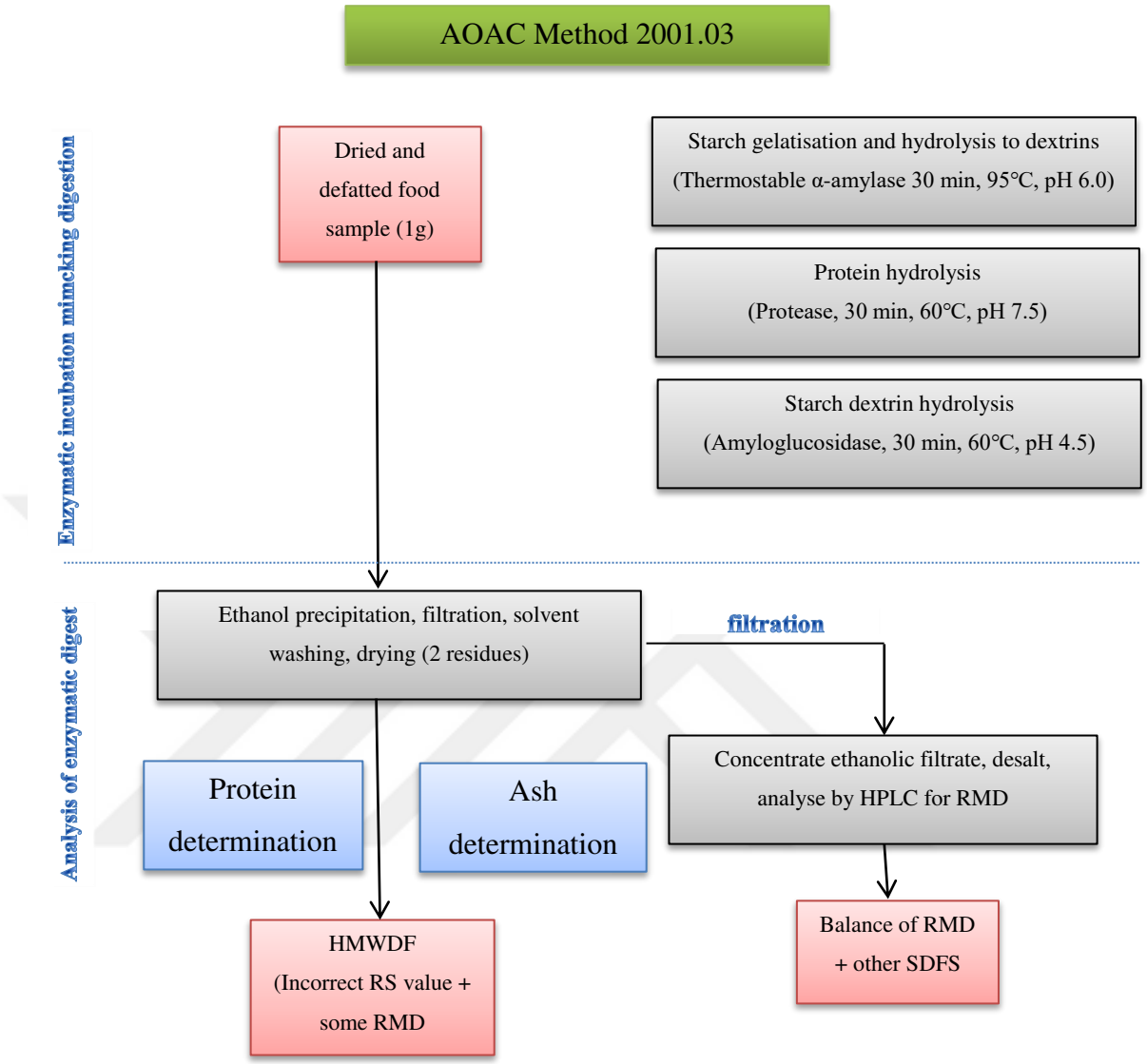


Figure 1.10 Matsutani Method (Gordon and Okuma, 2002)**.**

CODEX Alimentarius, is a collection of international criteria, codes of dealing, guidelines and other recommendations on food, production and safety. At that period Prosky, Lee and Matsutani's methods were available which could not be used to determine overall dietary fiber amount as mentioned in the new definition. The new component was added to the definition is (resistant starch) was partially estimated by Prosky, Lee and Matsutani methods

4) McCleary Method for determination of high molecular weight dietary fiber (HMWDF) and dietary fiber soluble in water and soluble in 78% ethanol SDFS.

Method limitations are:

-Resistant starch (RS2) and RS4 estimation are less than actual values.

-Modification for fructotriose measurement is required in this method.

The details of McCleary method are mentioned in the diagram below:

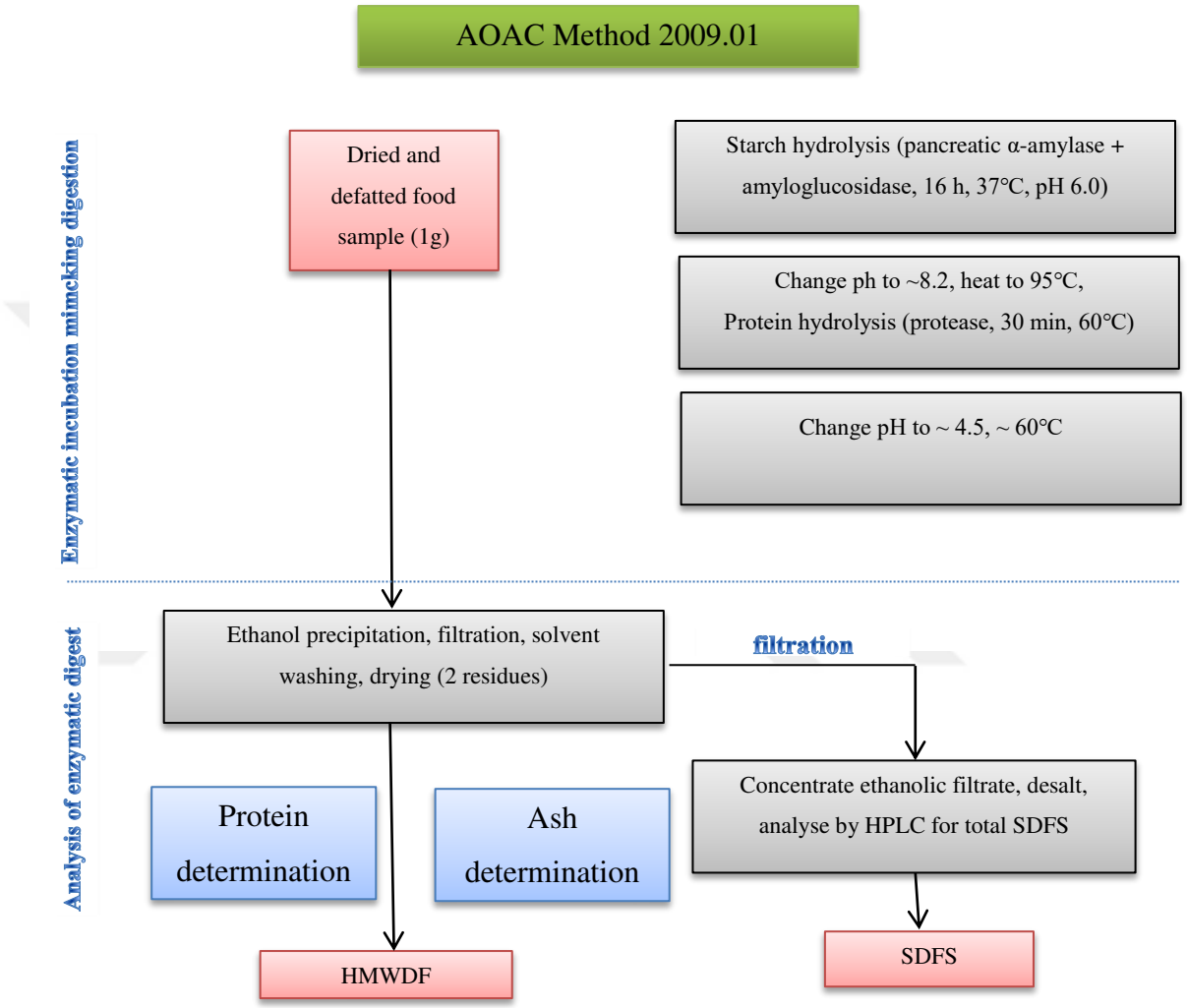


Figure 1.11 McCleary Method (HMWDF + SDFS) determination (McCleary, 2007).

5) McCleary Method for determination of IDF, SDFP and SDFS.

Method limitations:

A) RS2 and RS4 values are determined slightly less than the actual value

B) This method has to be adjusted for fructotriose, a minor constituent in FOS measurement.

The modifying of the original McCleary included an additional filtration achieved by the enzymatic digestion before the precipitation step with ethanol, received AOAC validation in 2011(McCleary et al., 2012).

Considering procedures as in AOAC 2009.01. The method overview is described here:

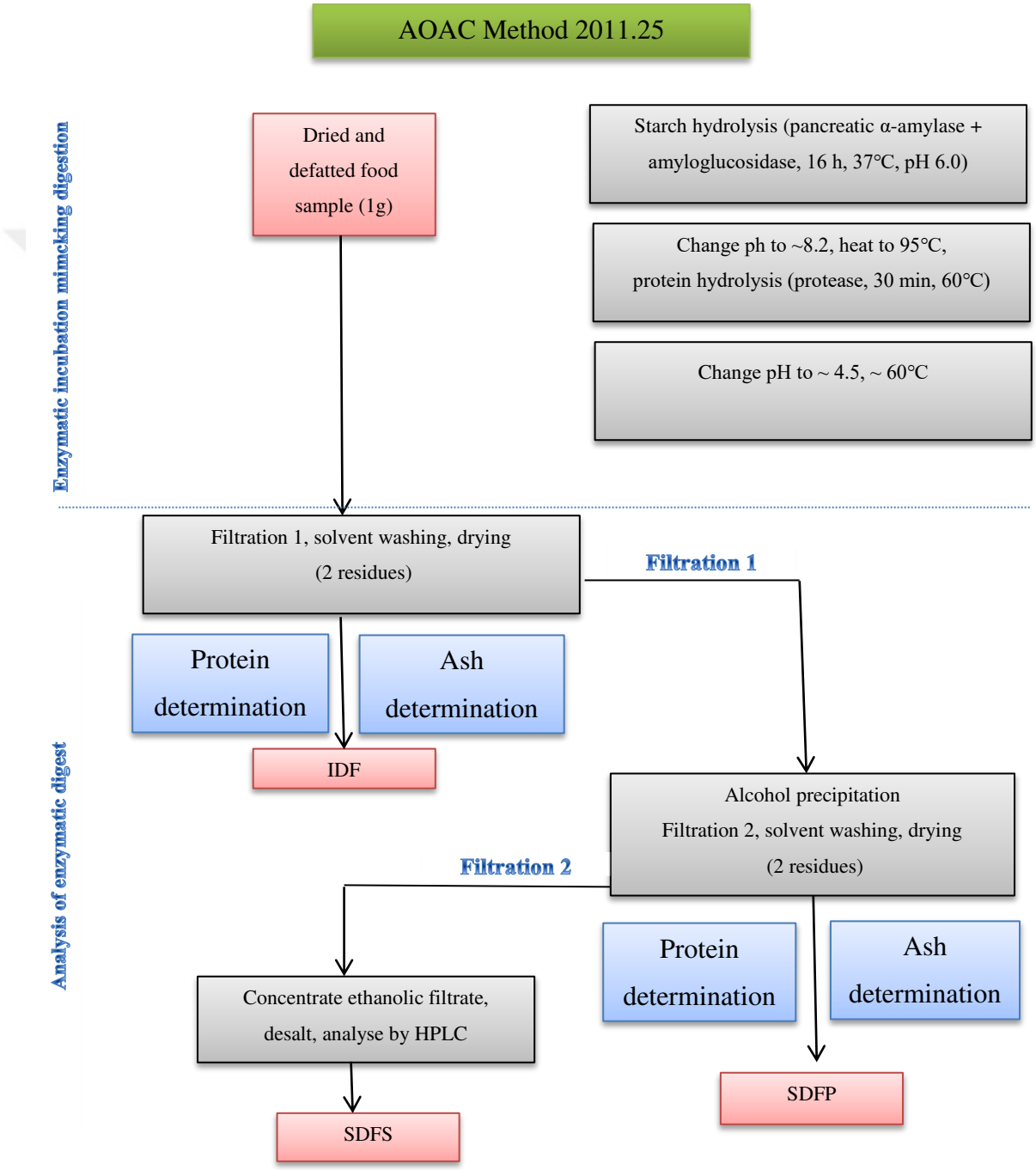


Figure 1.12 McCleary Method (IDF, SDFP and SDFS) (McCleary et al., 2012).

6) Rapid integrated total dietary fiber

For determining of HMWDF and SDFS, the method given in Figure 1.14

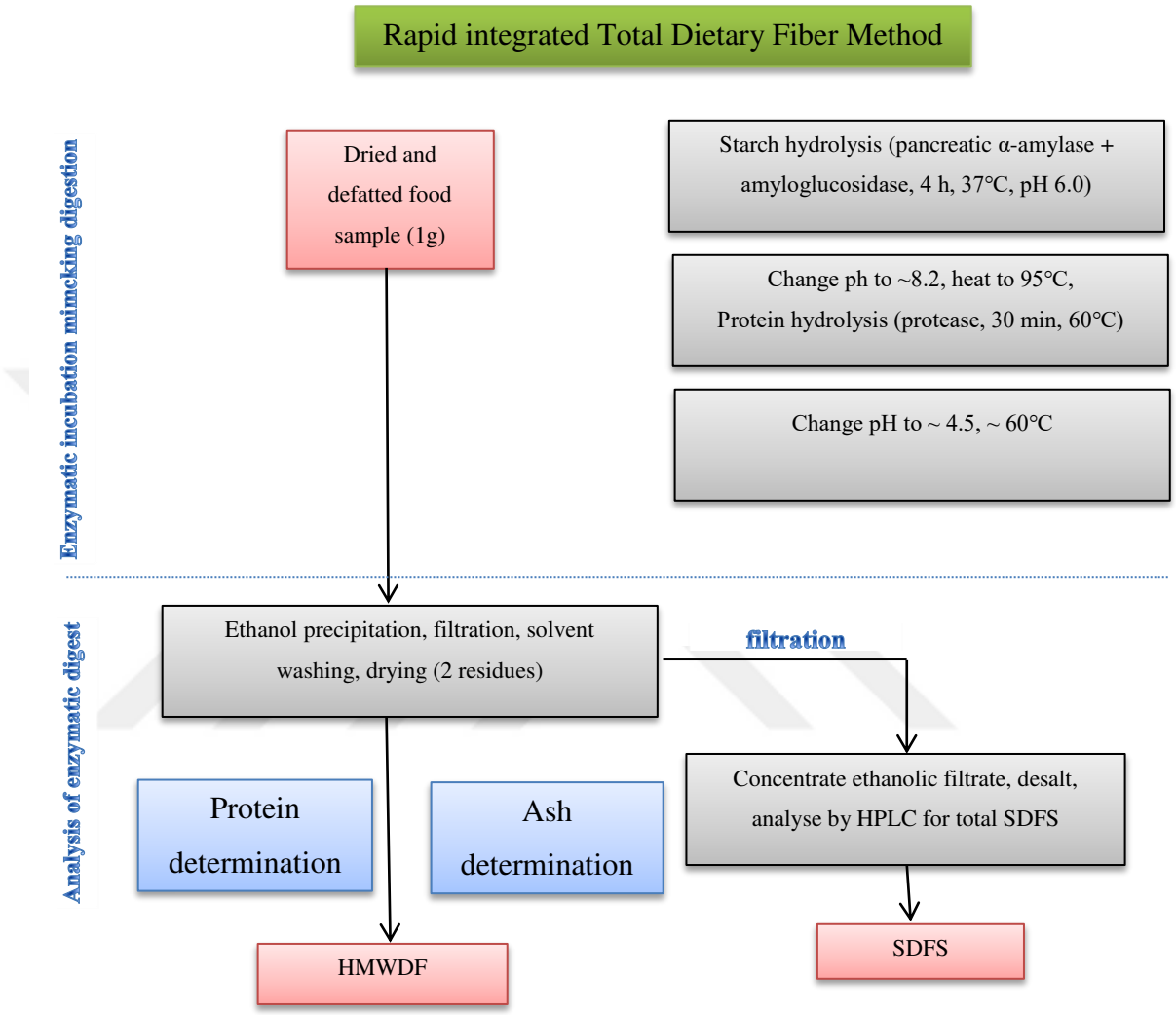


Figure 1.13 Rapid integrated total dietary fiber determination (McCleary et al.,2015).

Because of continuous efforts and researches to develop the existing methods for dietary fiber analysis this method was introduced, currently this method undergoing Vitro evaluation.

The most critical advantages of this method are:

• The reduction of incubation time from 16 hours to 4 hours because of increasing the amount of a-amylase and amyloglucosidase used in the assay led to more physiologically relevant results for samples containing resistant starch.

• The non-formation of the resistant maltodextrins, which result from the enzymatic incubation introduced in AOAC 2009.01/2011.25 during the new enzymatic incubation conditions (McCleary et al., 2015).

1.1.5 Health and Dietary Fiber

The consumption of recommended daily intake of dietary fiber provides body with a lot of health benefits, it reduces the risk for developing heart diseases (S. Liu et al., 1999), strokes (Steffen et al., 2003), high blood pressure (Whelton et al., 2005) and improves blood glucose adjustment in diabetics (Montonen et al., 2003). It also regulates cholesterol levels (Brown et al., 1999) and helps to lose weight (Lairon et al., 2005). In addition to the many benefits related to the digestive system such as esophageal ulcers, duodenal ulcers, constipation and hemorrhoids (Petruzziello et al., 2006), moreover, its role in promoting immunity in the human body (Watzl et al., 2005). Unfortunately, most people eat less than the recommendation of dietary fiber due to low intake of whole grains, vegetables, fruits, legumes and nuts

The recommended AIs for dietary fiber intake would be as follows for different age groups at a specific energy intake (Pietinen et al., 1996).

1–3 years, 19 g/d	4–8 years, 25 g/d
9–13-year-old boys, 31 g/d	9–13-year-old girls, 26 g/d
14–18-year-old boys, 38 g/d	14–18-year-old girls, 26 g/d

Cardiovascular and dietary fiber

The highest levels of fiber related to reduction prevalence rates for stroke and coronary heart disease (CHD) (Merchant et al., 2003).

The influence of dietary fiber consumption is summarized as follows; it decreases serum LDL-cholesterol values while soluble fiber affects positively in systolic blood pressure and total cholesterol and triglyceride values (Anderson et al., 2009)..

Psyllium and oat are the most widely used sources of soluble fiber and have been approved for health claims related to protection from CHD by the FDA (Anderson et al., 2009).

Diabetes prevention and dietary fiber

Almost recent studies clearly indicated that increasing dietary fiber consumption for patients with type 1 or type 2 diabetes were associated with noticeable improvements in glycemic control and reductions in the use of oral medicines and insulin doses (Anderson and Ward, 1978; Petruzziello et al., 2006).
The dietary fiber intake decreases blood glucose after meal eating and enhances insulin sensitivity (Lu et al., 2000; Weickert et al., 2006).

Obesity management and dietary fiber

All previous studies have indicated that high-fiber foods give satiety feel higher than low-fiber foods in spite of its same energy intake, for instance, a whole apple that is rich in fiber significantly more satiating than fiber free apple juice, although both of them provided the body with 60 calories. Fiber-rich food is lower in energy intake, often has a lower fat content, is larger in volume and is full of micronutrients. This larger mass of food takes longer to eat and delay gastric emptying that gives a feeling of satiety for a long (Rolls et al., 1999).

Gastrointestinal diseases and dietary fiber

Dietary fibers provide the gastrointestinal tract in human body with important benefits, in mouth dietary fiber increases chewing and saliva. In stomach increases distention and slows down emptying. In small intestine slows time of absorption of nutrients down and alters the gut hormones. In large intestine dietary fiber is fermented to short-chain fatty acids lead to increased fecal fat excretion (Howarth et al., 2001).

Fiber rich food develops a reduction in gastrointestinal disorders. It may decrease the prevalence of esophageal cancer, gastric cancer (Wu et al., 2007), peptic ulcer disease (Aldoori et al., 1997), gallbladder disease (Tsai et al., 2004), diverticular disease (Aldoori, 1997), constipation and hemorrhoids (Spiller, 2001).

Dietary fiber and the immunity

The gastrointestinal tract is one of the most important immune organs in the human body. About 60% of all lymphocytes in the human body are found in the gut-associated lymphoid tissue body**.** Many studies showed that inulin and other oligofructoses have an important role in human immunity (Vos et al., 2007). Because of the generation of small molecular fatty acids by useful bacteria and lactobacilli, this considered a health-promoting bacteria.

1.1.6 Dietary Fiber and Cereals

Lambo et al. (2005) notified that cereals are one of the most important sources of dietary fiber, providing about half of the total recommended daily fiber intake. Oats, barley and rye are good sources for soluble fibers. Most cereals contain insoluble fiber, especially wheat bran, rice and maize (Table 1.1).

Table 1.1 Dietary fiber content (% of edible weight) of different cereals as reported by USDA National Nutrient Database (USDA, 2010).

Cereal	**Dietary Fiber %**
Dehulled barley	10.1%
Brown Rice	4.6%
Corn	7.3%
Dehulled oats	10.6%
Rye	15.1 %
Wheat	12.2%
Triticale	14.6%
Millet	8.5%
White sorghum	6.3%

Wheat

Wheat is the second major human food crop after rice. The wheat grain ‘caryopsis’ consists of the germ (or embryo); the endosperm, mainly consist of starch grains, it supplies energy for germination; the thick cell-wall aleuronic layer surrounding the endosperm; and the pericarp. The bran fractions composed of the pericarp, testa, hyaline and aleurone layers (Brouns et al., 2012).

The milling process of wheat grains is based on the separation of the endosperm from the bran layers and embryo. The aleurone layers, bran layers and the embryo, are brought out the final product. Some times this process is important for the palatableness by consumers (Figure 1.15) (Rosa-Sibakov et al., 2015).

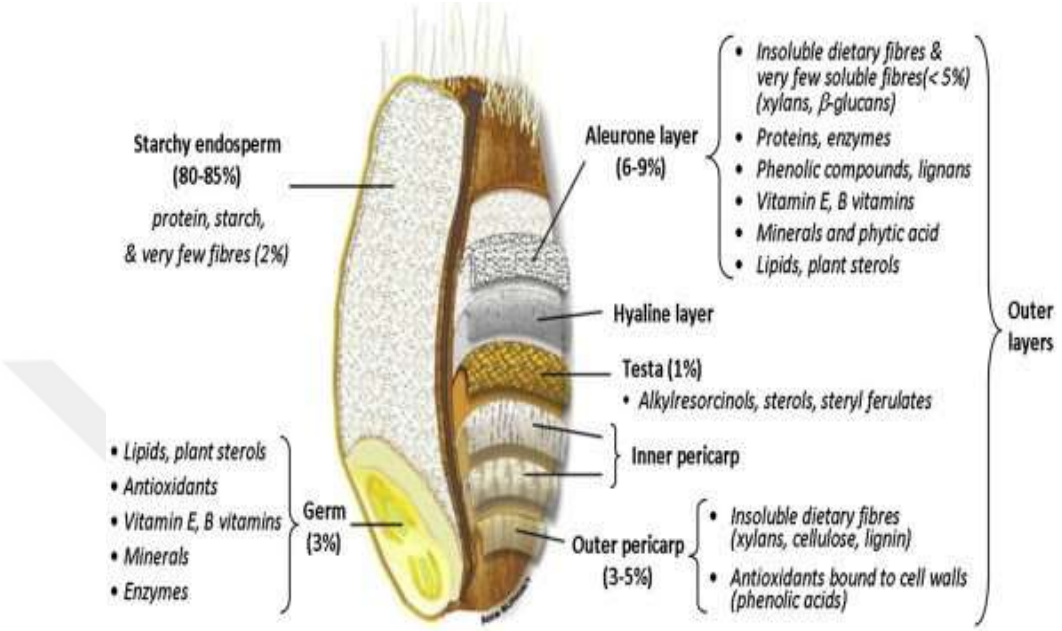


Figure 1.14 Wheat grain structure (Brouns et al., 2012).

During wheat milling process bran fractions are produced which are a very good source for fibers, minerals and vitamins such as vitamin B6, thiamine, folate and vitamin E and some phytochemicals, especially antioxidants such as phenolic compounds (Shewry, 2009). Mateo Anson et al., (2008) and Vaher et al., (2010) reported that wheat bran part has the highest antioxidant availability between wheat fractions it contains for up to 60% of this antioxidant capacity.

Wheat bran has three main effects, mechanical (affect the gastrointestinal tract movement), nutritional effects (it is an excellent source for many important nutrients for human) and antioxidant effects (result from the presence of phytonutrients).

1.1.7 Legumes and Dietary Fiber

Legumes are one of the most important resources for all components of dietary fiber, soluble, insoluble fiber and resistant starch (Figure 1.16).

According to Khan et al. (2007) study the total dietary fiber (TDF) content of legume seeds varied from 11.5 to 33.2%. This study also indicated that the fiber of legume seeds mainly consists of insoluble fiber like; cellulose, hemicellulose and lignin. Hughes and Swanson (1989) notified that beans contained 14 to 19% TDF.

Red lentil is a rich source of carbohydrates, protein and dietary fiber, vitamins, minerals, high unsaturated fatty acids and high energetic value (de Almeida Costa et al., 2006). Legumes should be processed before consumption because of their content of anti-nutrients (N. Wang et al., 2008).

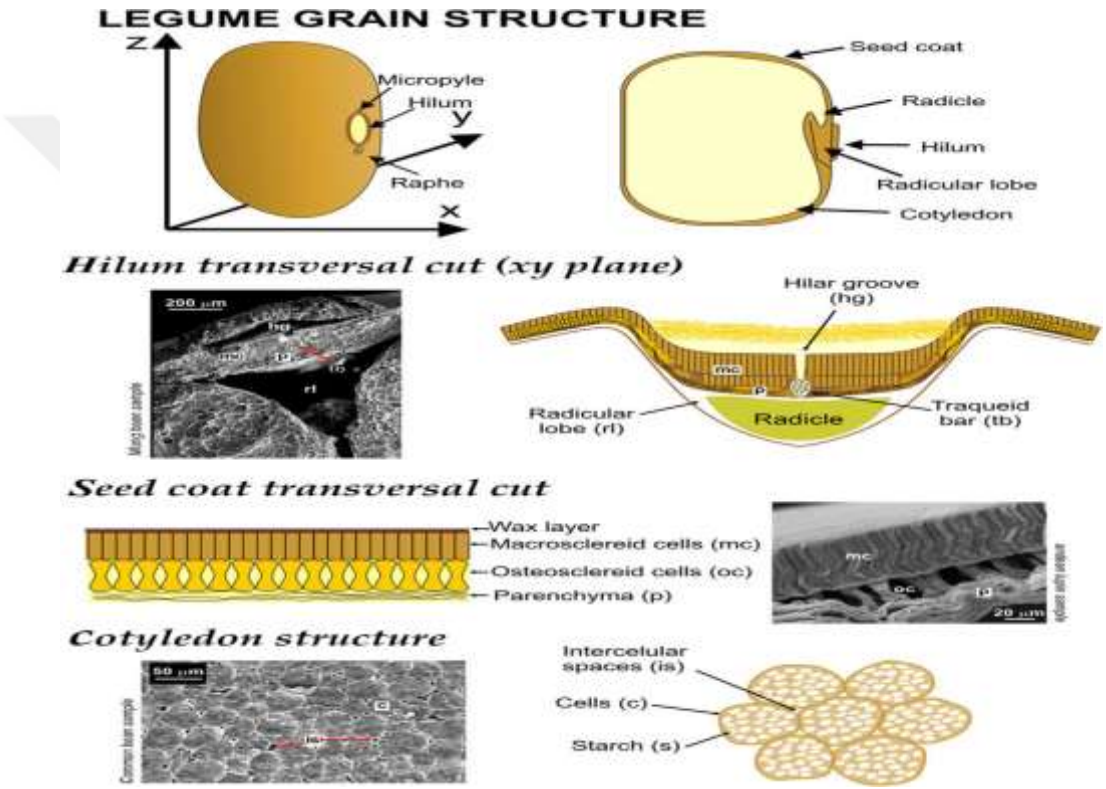


Figure 1.15 Structure of legumes grain (Miano and Augusto, 2018)

1.1.8 Dietary Fiber Production Methods

1.1.8.1 Soaking

The submerging of hard food in liquid, often water, for hours is known as soaking. Soaking is applied for softening food in addition to enhancing nutritional value as well as flavor of soaked food.

According to a previous study on cereals and legumes, the soaking treatment of both of them led to a considerable reduction in phytic acid content in addition to a

considerable decreasing in zinc and iron content, and hence soaking does not improve the bioavailability of these minerals (Lestienne et al., 2005).

Another study was applied on soaked beans, which resulted in reduction of amino acids, soluble sugars and minerals by diffusion in water thereby reduction in ash content, while the content of soluble fibers increased significantly in soaked beans. Despite the increase of starch content in the soaked bean, the starch digestibility decreased in it (Barampama and Simard, 1995).

1.1.8.2 Cooking

Applying heat to food known as cooking, whether it is roasted, boiled, fried, grilled, immersed in hot water or baked. There are several methods of cooking, including traditional methods such as cooking on the fire flame and modern techniques such as microwave oven, toaster or Stovetop.

Scientifically cooking is the transfer of thermal energy from a heat source to the food, which leads to change its physical and chemical properties, for example when cooking cereals and legumes in hot water gelatinization of starch has occurred this process is useful because it increases the digestibility of cooked food.

The more food is uncooked, the more RS is found, heat treatment leads to easier digestion due to gelatinization of starch (Nilsson et al., 2008). In contrast, excessive cooking leads to adverse results due to the retrogradation and destruction of the structure of the nutrients. Choosing the optimal cooking method is significantly maintain the quality of the final product (Beckles and Thitisaksakul, 2014).

According to previous research about dietary fiber content in apple fiber and oat bran, the total amount of dietary fiber and the amount of insoluble dietary fiber decreased after heat processing, while the amount of soluble dietary fiber increased after heat treatment. But in corn fiber total, soluble and insoluble dietary fiber decreased after heating (Chang, 1989).

According to another study, the insoluble dietary fiber components were reduced by the cooking water process in presoaked legumes. However, pressure-cooking showed a more considerable impact on the reduction of these insoluble dietary fiber components than ordinary and microwave cooking methods. Pressure cooking

reduced cellulose (17.0–35.8) and hemicellulose (37.5–42.4%) were reduced in pressure cooking whereas lignin content (15.2–27.8%) was increased in this process (Rehinan et al., 2004).

1.1.8.3 Microwave

Microwave heating is one of the most important techniques in the thermal processing of food. Because of technological development and increased awareness about the nutritional importance of food. Microwave heating is favorable due to its safety and the ability to retain nutrients with minimum losses of nutrients such as vitamins B and C, food antioxidants, phenols and carotenoids.

Recently, the microwave oven has become one of the most popular household appliances for food processing applications. Microwave heating is a volumetric heating process. In microwave heating, heat is supplied gradually throughout the entire volume of the food. This is due to the full interaction between microwave and polar water molecules and charged ions in food. Microwave energy is selectively absorbed by areas with higher moister content with more consistent temperatures and moisture content. Microwave technology has big popularity because it has advantages more than traditional heating methods. It has been applied in many food industries such as cooking, pasteurization, sterilization, melting, baking, bleaching and food drying (Venkatesh and Raghavan, 2004).

Microwave treatment is one of the most effective methods in inhibition of the rancid development of whole wheat flour. Rancidification is occurred during the storage and lead to reduce the nutritional value of food (Ertaş, 2016) and causes some quality changes, involving flavor, texture, and appearance (Eriksson, 1987). Due to the occurrence of rapid rancidity, there is a necessity for stabilization. Microwave, treatment is applied to prevent the rancidity. According to Sampathkumar (2011) research, a serious increase in total phenolic content was found in legumes when exposed to microwave heating. Khatoon and Prakash (2006) reported that cooking by microwave brought a slight decrease in starch content, ash content and protein digestibility and increased the total dietary fiber in microwaved samples. The increment in total dietary fibers of microwaved legumes could result from resistant starch that accumulated and isolated as dietary fiber components (Khatoon and Prakash, 2004).

1.1.8.4 Ultrasound

One of the most effective techniques in food processing is ultrasound processing, due to its considerable advantages in food processing. It has a lot of advantages such as fast energy and mass transfer, which lowering thermal and concentration gradients, reduces temperature, selective extraction, reduces an equipment size, rapid response to control the extraction process, fast start-up, increases production, and eliminates process steps.

Ultrasound applications based on three different methods:

The high-intensity ultrasound is applied frequently in food processing, it is used in cooking, freezing, crystallization, drying pickling/marinating in meat and vegetables, degassing in chocolate filtration in liquids, demoulding in cooked products, defoaming in Carbonated drink, emulsification in emulsions, oxidation in alcohols and cutting in fragile products (Sampathkumar, 2011).

Ultrasound-assisted enzymatic extraction technology, this application was first employed to extract arabinoxylan from wheat bran (Wang et al., 2014). Ultrasound increased the efficiency of enzymatic treatment with a higher extraction yield (Tao and Sun, 2015).

Kaya et al. (2017) reported an important decrease in total and insoluble dietary fiber in pulses with ultrasound processing. Li et al. (2014) reported that ultrasound-assisted extraction resulted in higher soluble dietary fibers yield in apple pomace. Besides, ultrasound treatment did not affect significantly on phytic acid content of the pulse hulls.

1.2 Stomach Test Theory

In some cases, food analysis cannot be applied to humans either because of high costs or due to ethical reasons. For these reasons, the so called artificial digestive digestion was introduced by simulated gastrointestinal fluids as a practical alternative widely used in many scientific, food and pharmaceutical fields. This technique is used in the laboratory to find new theories about the mechanism of digestion of macronutrients such as sugars, proteins, fats and micronutrients such as minerals and vitamins in the gastrointestinal tract of humans.

Where in the gastrointestinal artificial digestion take into account the application of physiological and physical conditions similar to those in the gastrointestinal tract in humans. The simulated gastrointestinal fluids include saliva (SSF), gastric fluid (SGF) and intestinal fluid (SIF). Minekus et al. (2014) described the formulation of simulated digestion fluids by using HCl and NaOH the pH of SSF, SGF and SIF were adjusted (SSF at 7.0, SGF at 2.0/3.0/4.0, SIF at 7.0) and each of this have to be pre-warmed at 37 C before use.

1.1.3 Aim of This Study

Wheat bran (WB) is a concentrated source of dietary fiber especially insoluble dietary fiber.

Red lentil bran is the outer envelopes of red lentils resulting from dehulling operations and is an excellent source of dietary fiber. It is obtained as a by-product of red lentil preparation for human consumption.

The common belief that bran is free calorie is false. Different amount of macronutrients are available in bran, these components provide body with calorie. Nowadays almost people tend to consume food with the least value of calories. Increasing daily intake of dietary fiber is the best way to control body weight because it gives a sense of satiety with low-calorie consumption.

The objective of this study is the production of low-calorie wheat and red lentil bran by using different techniques, such as soaking, cooking, microwave and ultrasound.

CHAPTER 2

MATERIALS AND METHODS

2.1 Material

2.1.1 Bran Samples

Wheat bran was obtained from a flour plant in Gaziantep-Turkey (the harvest year 2018). Red lentil bran was also obtained from a red lentil plant in Gaziantep-Turkey (the harvest year 2018). All analyses (moisture, protein, sugars, carbohydrates, ash, fat, total dietary fiber, starch and calorie contents) were applied on raw and treated brans. Additionally FTIR, DSC, structural analysis under the microscope, artificial stomach analysis and statistical analysis were made. The bran samples were kept in plastic jars and stored in a dry and cold room at 4C until analysis

2.1.2 Experimental Set-up

The experimental set-up is given in Figure 2.1. Wheat and red lentil brans were treated by the different techniques. These treatment techniques were soaking, cooking, microwave and ultrasound. The treatments were applied for 10, 20 and 30 min, except microwave processing, which was applied for 2, 4 and 6 min. After the treatment of wheat and red lentil brans with mentioned processes, the samples were dried in a tray dryer at 77.3 C, 5.3% RH and 0.04 m^3/s for 5 hours.

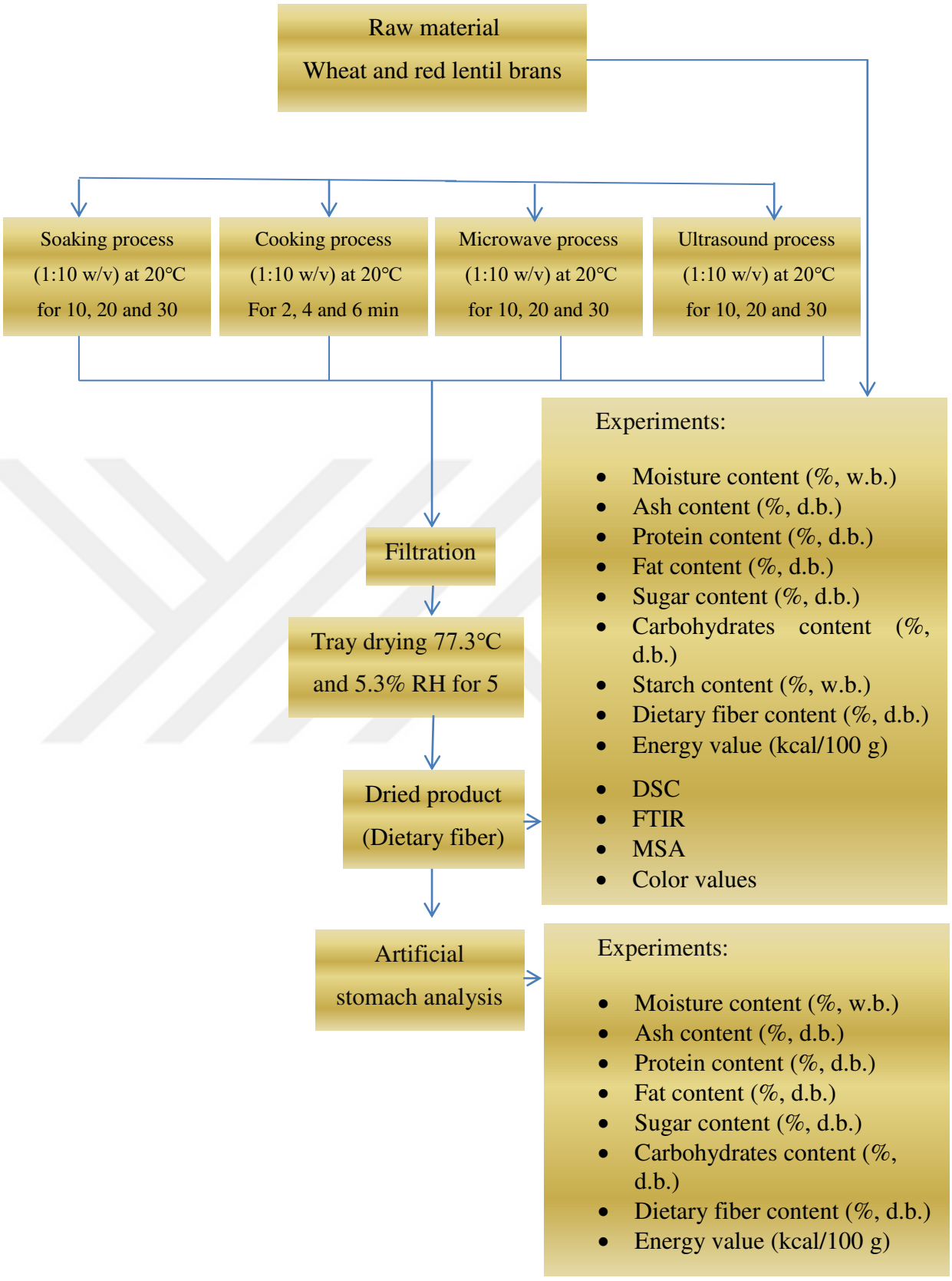


Figure 2.1 Experimental setup

2.2 Methods

2.2.1 Processes to Produce Dietary Fiber

i. Soaking Process

In order to determine the effect of the soaking process on the dietary fiber of wheat and red lentil brans, 20 g of the samples were soaked in 200 ml distilled water for 10, 20 and 30 min at 20C (Figure 2.2).

Figure 2.2 Soaking process for bran samples.

ii. Cooking Process

The cooking process was made by using 20 g of wheat and red lentil brans with 200 ml hot distilled water (1:10 w/v) at 96C for 10, 20 and 30 min to determine the effect of the cooking process on dietary fiber.

iii. Microwave

The effect of the microwave process on the dietary fiber was determined by using 20 g of bran samples (wheat and red lentil brans) with 200 ml of distilled water (1:10 w/v) for 2, 4 and 6 min at 900 W microwave power (HMT84G421, Bosch, Germany).

iv. Ultrasound

In order to determine the effect of the ultrasound process on the dietary fiber of wheat and red lentil brans, 20 g of the samples were mixed with 200 ml of distilled water, then they were treated with ultrasound process for 10, 20 and 30 min at 25 kHz and 150 W (Intersonik MIN 4, 25kHz, 150 W, Turkey) (Figure 2.3).

Figure 2.3 Ultrasonication process

2.2.2. Drying Operation

The treated samples individually (soaked, cooked, microwave treated and ultrasound) were filtered and then the paste of dietary fiber was dried in a tray drier (Armfield Tray Drier, UOP8, ENGLAND) at 77.3 C, 5.3%RH, 0.04 m^3/s airflow for 5 hours and then the samples were stored at -18C for further analysis (Figure 2.4).

Figure 2.4 Tray drying process

2.2.3. Experimental

All chemicals used in the study were analytical grade.

2.2.3.1 Determination of Moisture Content

Moisture content (%, w.b.) was determined at 105°C until constant weight reached by using the method of Association of Official Analytical Chemists (AOAC, 1990).

2.2.3.2 Determination of Ash

The ash content in the wheat and red lentil brans (%, d.b.) was determined by using AOAC (1990).

2.2.3.3 Determination of Fat Content

The Soxhlet method (AOAC Method 920.39C,1990) was applied to determine the fat content in the wheat and lentil bran samples. About 2 g of the predried sample was put into a predried extraction thimble and then full thimble covered with glass wool. Hexan was put in a boiling flask. Boiling flask and extraction thimble and condenser were assembled. Finally, the extraction in a Soxhlet extractor at a rate of five or six drops per second by condensation for about 4 h by heating solvent in boiling flask. Then the boiling flask was dried with extracted fat in an air oven at 100 for 30 min, cooled in a desiccator and weighed. The experiments were made by using Soxhlet machine (Gerhardt, SE – 416, Germany).

2.2.3.4 Determination of Protein Content

The protein content was determined by the Kjeldahl method (N*5.7) for WB and (N*3.63) for red lentil bran (RLB) by using the method AOAC (1990) with digestion block (Gerhardt, KJELDATHERM® KT-L 20, Germany) and the fully automatic distillation unit (FOSS, 2200 KT, Sweden).

2.2.3.5 Determination of Sugar Content

The method mentioned by Garriga et al. (2017) was used. One g dry weight of the sample was hydrolyzed in 50 ml of 0.25 N sulfuric acids in a thermostated bath at 50 °C for 1 hour. For both glucose solutions and bran extracts, the procedures were the same; first, in tubes of 10 ml 1 ml of bran extract, it was placed and 1 ml of 3, 5-dinitrosalicylic acid (DNSA). Then, the pH was brought to pH 10. After that, the tubes were taken to a bath thermostated at 100 °C for 5 minutes. Then, the tubes were cooled to room temperature. The volume was completed with 8 ml of distilled water, homogenized and read at 540 nm in a spectrophotometer (Biochrom Ltd, NovaspecII, Science Park, U.K.). The reading is compared against a reagent blank solution, in which the bran extract is replaced by distilled water and steps 2 to 6 are repeated.

The method demonstrated linearity in a range of 0.28 to 1.00 g/L. For the realization of the calibration curve, it was worked with concentration values lower than 1 g/L. With the absorbance values obtained, the correlation coefficient was calculated as $R^2 = 0.928$. Figure 2.5 shows the calibration curve for the determination of reducing sugars in extracts of wheat or red lentil bran by UV-Vis spectrophotometry at 540 nm. The slope of Figure 2.5 was used to calculate glucose concentration in wheat and red lentil bran samples.

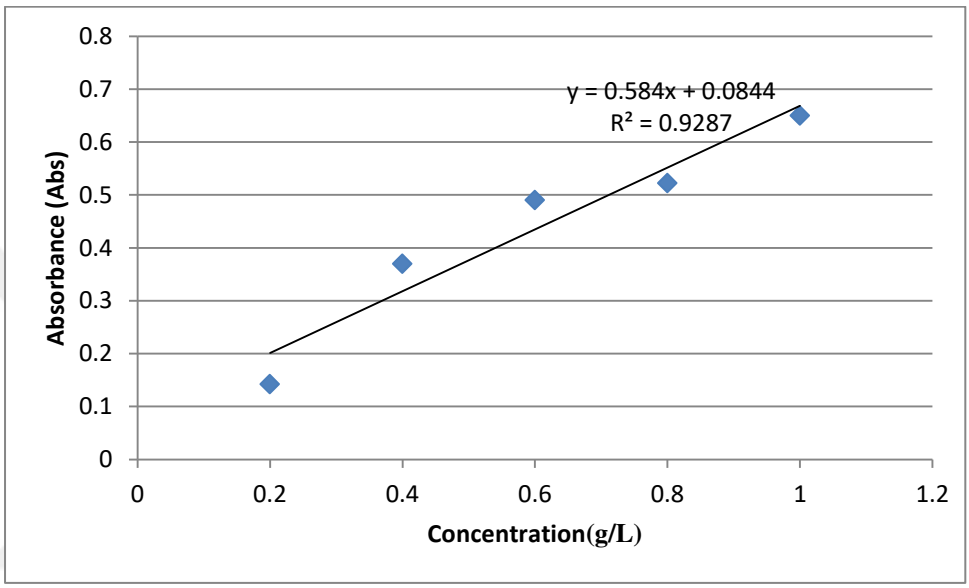


Figure 2.5 Calibration curve for the determination of sugars in wheat and red lentil by UV-Visible spectrophotometry.

2.2.3.6 Determination of Starch Content

The starch content of the samples was determined by hydrolyzing starch with HCl, then the starch value was measured with a polarimeter (PolAAr3000, Optical Activity Ltd, England). 1.25 g of sample was treated with 12.5 ml of 1% HCl in a 25 ml flask and shaken. The flask was placed in a water bath for 15 min with continuous shaking at 95-100 C. Then 7.5 ml of distilled water was added and cooled until room temperature. After that 2.5 ml of Carrez solution I (21 g of zinc acetate and 3 g of glacial acetic acid was dissolved in water, then diluted to 100 ml with water) was added to the flask and shaken for 30 sec. Then 2.5 ml of Carrez solution II (10.6 g of potassium ferrocyanide was dissolved in water, after that diluted to 10 ml with water) and shaken again for 30 sec. Finally, the mixture was filtered. The extract was poured into a 2 cm polarization tube the polarization value was determined by a polarimeter. The calculation was made using Equation 2.1.

Calculation:

$$\% \text{ Starch} = \frac{V_{TS} \times a_p}{A_D^{20} \times L_p \times W_s} \quad (2.1)$$

where:

% Starch content: (g/g, %, w.b.)

V_{TS}: total volume of sample (ml)

a_p: polarization value

A_D^{20}: specific rotation of Ewer'method for starch (182.7(wheat starch), 184.0(red lenti starch) / (dm×g/ml))

L_p: length of polarization tube (dm)

W_s: the weight of the sample (g)

2.2.3.7 Determination of Dietary Fiber

The dietary fiber determination experiments were carried out by using the Full Option Science System (FOSS) (Fibertec™ 1023, Hoganas, Sweden). The Total Dietary Fibre (TDF) content of samples was analyzed before and after different treatments; cooking, ultrasound and soaking for 10, 20 and 30 min and microwave for 2, 4, 6 min and after exposure to Simulated Gastric Fluids process (SGF).

Total dietary fiber (TDF) in wheat and red lentil bran samples was determined by an enzymatic gravimetric method (AOAC Method 985.29-1986, 2003) using Total Dietary Fibre Assay Kit (Megazyme, Ireland) by FOSS Analytical Fibertec system (Fibertec™ 1023, Hoganas, Sweden) (Figure 2.6).

Figure 2.6 FOSS Analytical Fibertec E 1023 System

The samples were dried and weighed. After weighing, each sample was enzymatically digested with α-amylase (Megazyme, Ireland) and incubated at 100 °C for 30 min, and then the samples were digested with protease (Megazyme, Ireland) and amyloglucosidase (Megazyme, Ireland) and were incubated at 60 °C. The determination procedure of TDF is shown in Figure 2.7 after digestion, the total fiber content was precipitated by adding 95% ethanol. Then, the solution was filtered. In the filtration step, first, the crucible containing Celite was taring to the nearest 0.1 mg. Then the bed of Celite in the crucible was wetted using 15 ml of 78% ethanol for the total dietary fiber determination. After that, the filtration residue was washed three times with 20 mL portions of 78% ethanol, twice with 10 ml portions of 95% ethanol and twice with 10 ml portions of acetone. After filtration crucible containing fiber residue was dried overnight in 105C in an oven then cooled in a desiccator and weighed to the nearest 0.1 mg. The protein and ash content were determined to correct any of these substances which might remain in the fiber. The dietary fiber content was calculated by using Equation 2.2 (Prosky, 1990);

Content of fiber (mg) = Residue weight (mg) – Weight of (protein+ash) (mg) (2.2)

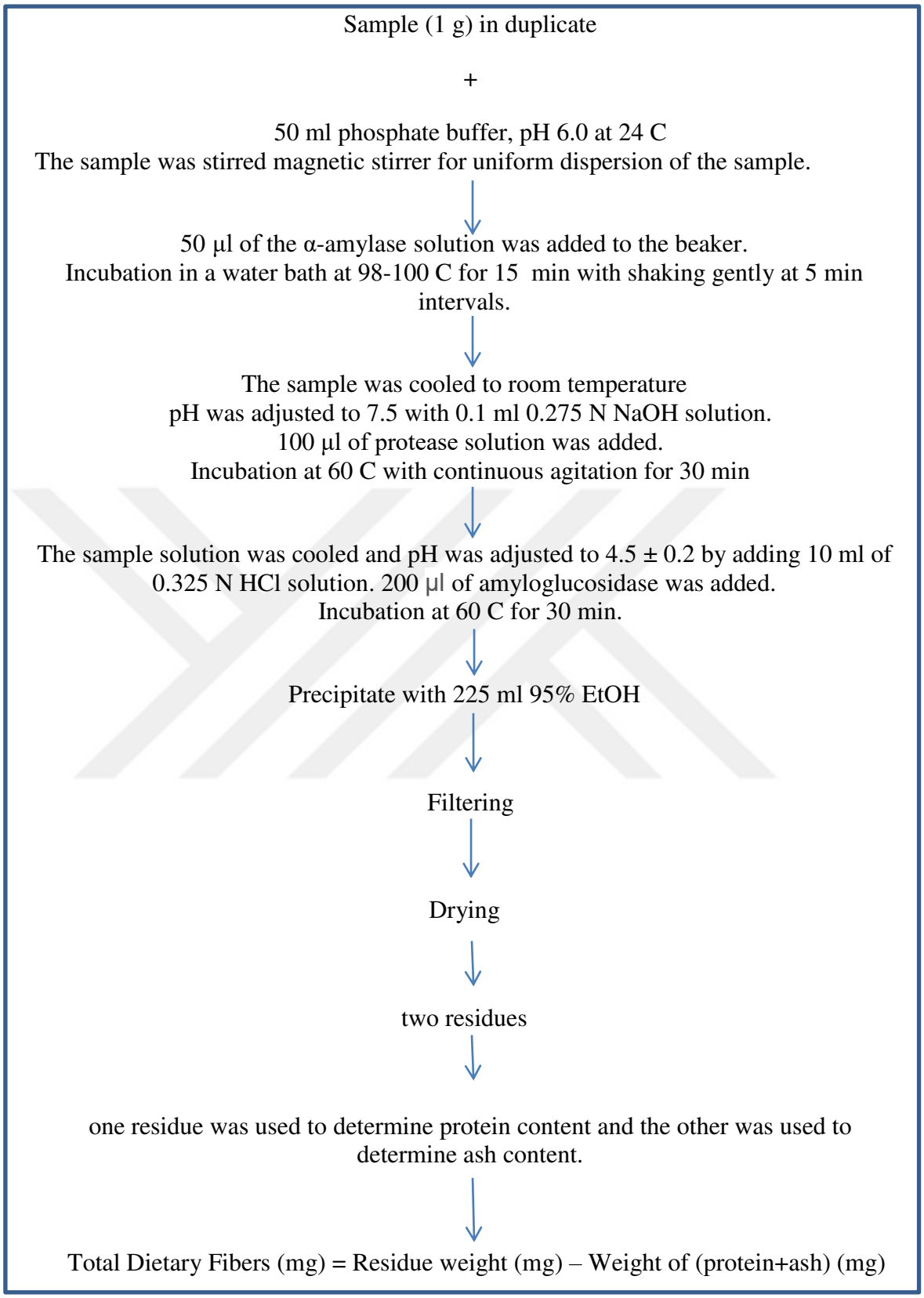


Figure 2.7 Analytical scheme for the total dietary fiber determination procedure.

2.2.3.8 Determination of Carbohydrate Content

The carbohydrate value was determined by subtracting all the percentages of moisture, fat, crude protein, ash and crude fibre from 100 (Ijarotimi and Keshinro, 2012) (Equation 2.3).

% carbohydrate = 100 – (moisture (%, w.b.) + fat (%, d.b.) + ash (%, d.b.) + crude fiber (%, d.b.) + crude protein (%, d.b.)).

2.2.3.9 Calorie Determination

The calorie content of the samples was calculated (kcal/g) by multiplying the percentages of crude protein, crude lipid and carbohydrate with the factors (2.44, 8.37 and 3.57 respectively) according to Martin and Coolidge (1978).

2.2.3.10 Measurement of Color Values

The color of wheat and red lentil bran samples were determined by measuring the CIE L* (100=white; 0=black), a* (+: red; -: green) and b* (+: yellow; -: blue) and YI (Yellowness index) values using with illuminate D65/10 as reference. HunterLab ColorFlex, (Model No. 45/0, USA) was used to measure the color values of the mentioned samples**.**

2.2.3.11 FT-IR (Fourier Transform Infrared Spectroscopy) Analysis

The spectra were obtained using an FT-IR analyzer (Perkin Elmer, Ins. Waltham, MA 02451 USA). The FT-IR spectra were recorded in transmission mode from 4000 to 400 cm^{-1} (mid-infrared region) the background value from pure potassium bromide (KBr) was applied before the scanning of the samples.

2.2.3.12 Structural Analysis Under the Microscope

The micrographs of samples were captured by a Microscopy (OLYMPUS, BX51TF, Tokyo, Japan). Wheat and red lentil bran were applied on an aluminum stub after putting it between double sided adhesive tape. In this study, magnification range 10x was used for wheat and red lentil brans.

2.2.3.13 Thermal Properties by DSC (Differential Scanning Calorimetry) Analysis

The thermal analysis by Differential Scanning Calorimetry (DSC) was conducted according to the modified method of Zhang and Wang (2013). For DSC analysis, the

wheat and bran samples were ground until a fine powder was obtained, and tightly packed into plastic bags. Approximately 15 mg of samples were weighed in an aluminum sample pan (Perkin Elmer, Pan no. B016-9321, 50 µl). The sample pan was hermetically sealed and then heated from 20 to 550 °C at a rate of 10 °C/min flushing (75 cm^3/min). The measurements were carried out with a Perkin Elmer differential scanning calorimeter equipped with Pyris 6 DSC software (DSC-6, Norwalk, Conn., U.S.A.) calibrated with indium and an empty Aluminum pan was used as a reference. The peak area was showed only between 140-190°C for all samples so, this range was applied for the thermal analysis of samples.

2.2.3.14 Artificial Stomach Analysis and Processes

The simulated gastric fluids (SGF) was prepared by mixing 2.0 g of sodium chloride with 3.2 g of pepsin from porcine stomach mucosa. After that, 7 ml of hydrochloric acid was added to the mixture and volume was made up to 1000 ml with water maintaining pH at 1.2 for simulating the digestion in gastric fluid, then 1.4 ml of SGF was added to 1g of the sample in a 10 ml test tube and incubated in shaken incubator at 80 rpm (WB-6, Wisd Co. Ltd., Texas, USA) for 0 and 120 min at 37◦C. The tubes were cooled down to room temperature, filtered through cellulose-acetate 0.45 µm.

Artificial stomach analysis was applied for selected the wheat bran (WB) samples such as 10 min soaked, 30 min cooked, 6 microwave and 10 min ultrasound treated. Either for red lentil bran, 30 min soaked, 10 min cooked, 2 min microwaved and 10 min ultrasound were exposed. These samples were selected because they have the lowest content of starch and carbohydrates and the highest content of dietary fiber that is the favorable for detection the effect of simulated gastric fluids (SGF) on dietary fiber content.

2.2.3.15 Statistical Analysis

One-way analysis of variance (ANOVA) was performed for all data to determine important differences at the significance level of α=0.05 by using SPSS for Windows (SPSS for Windows, 16.0, 2007, SPSS Inc., Chicago, IL, U.S.A.). Duncan's multiple range tests were used to determine the effect of parameters on all responses. All experiments were triplicated and the analyses were duplicated.

CHAPTER 3

RESULTS AND DISCUSSION

Wheat and red lentil brans are used as a source of dietary fiber. Brans are obtained during wheat and red lentils processing as a by-product. During their obtaining from the processing, some amounts of endosperm (carbohydrates and protein) and germ parts mix into bran. These parts affect the purity energy value as calorie and property of bran when it was used as a dietary fiber source.

It is too important to get information about the physical and chemical properties of dietary fiber to improve its nutritional value and decrease calorie level involved due to carbohydrates (starch), protein and other components mixed with bran. Using this information may make progress in the food industry sector concerned with dietary fiber enrichment products.

Raw and treated wheat and red lentil brans samples were analyzed to determine which treatment is the best to produce low calorie dietary fibers. The results of proximate composition and energy values of raw and processed wheat and red lentil bran samples are given in Figures A.1-A.9.

3.1 Properties of Raw Materials

3.1.1 Chemical and Physical Properties of Raw Wheat and Red Lentil Brans

In this study, firstly, raw wheat and red lentil brans samples were analyzed without any treatment. Then treated samples were analyzed to compare the different properties of wheat and red lentil brans samples before and after processing.

The initial moisture (%, w.b.), ash (%, d.b.), fat and protein contents (%, d.b.) of wheat bran and red lentil bran were measured as 12.16, 5.65, 3.97 and 12.3% for wheat bran; 10.4, 5.95, 1.17 and 13.52% for red lentil bran, respectively (Table 3.1).

The protein content in wheat bran (WB) was less than red lentil bran (RLB) in contrast to fat and moisture contents, which were higher than red lentil bran. Either to ash content, it was similar in wheat and red lentil brans. There was a critical difference ($p \leq 0.05$) in moisture content between wheat and red lentil bran. This agreed with a previous study that found a considerable difference between wheat bran and legumes bran in moisture content. Whereas the moisture content of wheat bran higher than legumes moisture content as notified by Elawad et al. (2018). The moisture content of legume bran was found to be in the range of 6.4 - 7.2% which were significantly ($p \leq 0.05$) lower than the moisture content of wheat bran (7.91%), which was higher than 7.56% as reported Almeida et al. (2010), and lower than 10.4% as reported by Rendleman (1982). He found that ash content in wheat bran ranged from 3.9 to 8.10%. According to other studies, ash was 3.4-8.1% (Babiker et al., 2009; Dornez et al., 2006; Fardet, 2010; Hemery et al., 2007). Fat content was 3.5-3.9 as reported by Babiker et al.(2009) and Hemery et al. (2007). The fatty acids available in wheat bran oil are not different from wheat germ oil fatty acids (Jung et al., 2010). The wheat bran oil is used in pharmaceutical or cosmetic applications in order to use it as an ingredient in the food and feed industry (Brandolini and Hidalgo, 2012). Linoleic acid is the most abundant fatty acid in wheat germ and wheat bran oil (57%), after that oleic acid with 15% (El-Shami et al., 2011; Jung et al., 2010). Wheat bran oil features a high content of carotenoids (39.2 mg/g wheat bran oil), therefore, it has a desirable nutritional quality (Apprich et al., 2014). The addition of 15% wheat bran to the chicken patties lowered the cholesterol content as reported by Talukder and Sharma (2010). The protein content for wheat bran tended to be similar to that reported by Curti et al. (2013) and Yan et al. (2015) whereas protein content was about 9.60–18.6%. While it was slightly lower than what was reported by Dornez et al. (2006) whereas protein was 13.2-18%. Wheat bran can be considered as a possible source for protein because it contains 13-18% protein. A chemical structure of wheat bran proteins is different from wheat flour proteins (Di Lena et al., 1997). The glutenins and gliadins are the basics of endosperm protein. While that bran proteins mainly contain albumins and globulins (Apprich et al., 2014). So that, bran proteins possess an approved biological and nutritional value (Di Lena et al., 1997) which in turn leads to use it as food ingredients or in special feeds.

There are limited studies on the use of red lentil bran in dietary fiber rich products in the literature. According to the previous study carried out by Elawad et al. (2018),

the moisture content of legume bran were notified in the range from 6.4 to 7.2% that less than 7.56% as found in Almeida et al (2010) study and so different from 10.4% as introduced by Rendleman (1982). In Bilgiçli and Levent (2014) study, moisture content, ash, fat and protein were 9.71, 2.01, 1.16 and 5.17% for lupin bran, respectively. Similar results for ash, protein, fat and mineral content of lupin bran have been noticed in other study carried out by Lampart-Szczapa et al. (2003). All of the moisture, ash, fat and protein contents in lupin bran were smaller than these values in red lentil bran. The ash content of legumes bran was reported in the range of 3.2 to 4.5%. The ash content of faba bean bran (3.2%) was the lowest. The ash content of soybean bran was 4.5% which was less than that found by Chaudhary and Weber (1990). The ash content of chickpea bran was 4.4%, which was lower than 5.7% as introduced by Dalgetty and Baik (2006). Legumes bran has a large range in fat content; it is in the range between 1.9 to 8.8%. The least content of fat was found in pigeon pea bran (1.9%) whereas soybean bran contained the highest level of fat (8.8%). The fat content of wheat bran is 5.6% almost higher than the fat content of legumes bran (Dalgetty and Baik (2006). Elawad et al. (2018) noticed that the addition of legumes bran to flour caused a considerable ($p \leq 0.05$) increase in fat compared to the control flour. The protein content of legumes bran ranges from 11.1 to 18.4%. As reported by Elawad et al. (2018) the soybean bran has the highest level of protein (18.4%), this was attributed to the high content of protein in soybean (38.2%) (McCarthy et al., 1977). However, chickpea bran has the least content of protein (11.1%). The flour supplemented with soybean bran, faba bean bran and wheat bran showed a significant ($p \leq 0.05$) increase in protein contents of the blends compared to the control (Dalgetty and Baik, 2006).

In this study, sugars, carbohydrates and starch contents and energy value were measured as 0.51, 60.76, 19.28% and 280.29 kcal for wheat bran; 0.77, 47.65, 16.66% and 212 kcal for red lentil bran, respectively. Javed et al. (2012) conducted that the total carbohydrates content in wheat bran ranges from 60.0 to 75.0%. The studies of Curti et al. (2013) and Yan et al. (2015) denoted that the starch in wheat bran in the range from 9.10 to 38.9. But in the studies of Dornez et al. (2006) and Hemery et al. (2007) the starch content in wheat bran was in the range of 14 to 25% that depending on the degree of milling. According to Oghbaei and Prakash (2016), the starch content in wheat bran was 21.91%. It was observed that the reduction of

wheat bran during milling improves starch digestibility. Oghbaei and Prakash (2016) reported that the starch digestibility in vitro in the range between 42 to 51% in whole and refined wheat flour, respectively. Because of the presence of a large amount of insoluble dietary fiber and anti-nutrients like tannin and phytate in bran, which able to bind enzymes and proteins, the activity of enzymes and proteins decreased in bran enrichment products. The carbohydrate content in legumes bran ranged from 61.9 to 75.0%. The highest content of carbohydrate in legumes was found in pigeon pea bran (75.0%). Whereas soybean bran contained the lowest content of carbohydrates (61.9%), this attributed to the low content of carbohydrate in soya bean seed in comparison to other legumes and this resulted from the high protein and oil levels in the soya bean seeds (Elawad et al., 2018). According to Segasothy and Phillips (1999) study, the total carbohydrate decreased significantly after enrichment of whole wheat flour with wheat and legume brans. Thereby the addition of wheat and legumes brans to whole-wheat flour leads to a decrease in the calorie content available in food, which in turn leads to approval of diabetic control.

In this study, total dietary fiber was measured as 39.29% in wheat bran and 23.064% in red lentil bran. There was a considerable difference ($p \leq 0.05$) between fiber content in wheat and red lentil bran. In Yan et al. (2015) study, the total dietary fiber content in wheat bran was 43.02%. Elawad et al. (2018) reported that the fiber content of legumes bran was in the range of 19.7 to 29.4%. The faba bean bran contained the largest content of total fiber (29.4%) followed by chickpea bran (28.5%), pigeon pea bran (24.6%) and the smallest content was found in soybean bran (19.7). Onipe et al. (2015) reported the undesirable changes in the quality after enrichment food products with wheat bran at a high level. Sobota et al. (2015) found that the addition of about 30% wheat bran or less in pasta yielded acceptable properties in comparison to pasta contains whole grain durum wheat. The main components responsible for the water absorption in the dough are the gluten network proteins. As reported by Prückler et al., (2013) arabinoxylan is the main component in wheat bran structure, forming 10.9-26.0% of total wheat bran based on a dry matter (Apprich et al., 2014). This is a common in bread treated with bran, the gluten network is not properly activated because of the existence of arabinoxylans bind water strongly and reduce the content of water available for the gluten network, which affects dough stability and increases the time is required for the development of the dough. Furthermore, the volume of

bread is lowerd as a result of reducing the gas retention and dough resilience which associated with the addition of wheat bran (Serna-Saldivar, 2016). This is common in bread containing bran. Generally, the addition of wheat bran to various cereal products affects the quality of the final product. In biscuit, the addition of wheat bran in the range 5–3 % resulted in reducing the dough pasting viscosity and increase in dietary fiber and protein levels thus, low gluten network led to the fragility of biscuit and content of digestible starch reduced (Sozer et al., 2014). Almeida et al. (2013) denoted that the addition of wheat bran to bread in the range 0–20 decreased crumb lucidity and specific volume, crumb moisture content became higher, specific loaf volume decreased and desirable sensory properties. Wójtowicz and Mościcki (2011) reported that the incorporation of wheat bran at a range of 5–25% of the flour weight to pasta production improves sensory properties, but if the wheat bran were above 20%, the pasta would be gummy. According to Almeida et al. (2010) bread enriched with wheat bran is the most acceptable between all types of bread which enhanced with other types of fibers. The improvement of the nutritional content of cakes was noticed by Lebesi and Tzia (2011) when 10–30% of wheat bran was added to the product. However, the concentration of bran higher than 10% affected negatively on the appearance, mouthfeel, taste and acceptance of the cakes. Kaur et al. (2012) reported a little acceptance of the quality of pasta when about 15% of wheat bran was added to it. Desirable dough functionality was achieved by a combination of fibers in the manufacturing of fiber-enriched dough in the research of Collar et al. (2007). There is no ideal fiber ingredient but is almost a mix of different fiber sources.

In this study, it was found that the color values of wheat bran and red lentil bran were 53.02, 52.20 for lightness (L*); 8.64, 8.32 for redness (a*); 25.48, 24.68 for yellowness (b*) and 73.23, 72.00 for yellowness index (YI), respectively. The color values of wheat bran and red lentil bran are presented in Table 3.1.

The addition of 8.9–9% of wheat bran decrease a* and b* values in bread which enriched with wheat bran compared to control (Pavlovich-Abril et al., 2015). When adding about 7 to 25% of wheat bran the hardness and resilience of bread increased (Kim et al., 2013). In noodles, the addition of wheat bran in the range 2–6 to dough sheet had lowered L* values in contrast a* and b* values increased and water absorption of cooked noodles raised (Song et al., 2013). In Wójtowicz and Mościcki

(2011) study, it was found that the more wheat bran concentration is added, the more dark color of the pasta is noticed. Increasing the additive amount of lupin bran decreased the lightness (L*) of cookies. In addition to the cookies that contain lupin bran were more reddish and more yellowish in comparison to control cookie. The use of lupin flour and lupin bran in cookie formulation led to harder cookie texture in comparison to compared to control cookies (Bilgiçli and Levent, 2014).

In this study, it was found that the wheat bran and red lentil bran were significantly ($p \leq 0.05$) different in the L*, b* and YI values, moisture content, fats, starch, total dietary fiber, sugars, calorie content and carbohydrate. However, no significant difference ($p > 0.05$) was found in ash and protein contents and a* value (Table 3.1).

Table 3.1 A proximate composition of raw wheat and red lentil bran

Properties	Unit	Wheat Bran	Red lentil Bran
Moisture content	(%, w.b.)	12.16±0.15	10.85±1.13
Ash content	(%, d.b.)	5.65±0.4	5.95±0.35
Protein content	(%, d.b.)	12.35±1.05	13.52±0.63
Lipid	(%, d.b.)	3.97±0.26	1.17±0.08
Starch	(%, d.b.)	19.28 ±0.33	16.66±0.7
Sugars	(%, d.b.)	0.51±0.11	0.79±0.14
Carbohydrates	(%, d.b.)	60.76±1.27	47.65±0.086
Dietary fiber	(%, d.b.)	39.29±0.86	23.06±0.41
Calorie	(kcal/100 g)	280.29±0.39	212±2.20
L*		68.23±0.02	59.45±0.35
a*		6.48±0.046	6.19±0.01
b*		25.99±0.01	15.87±0.22
YI		60.63±0.07	47.5±0.59

±: standard deviation, d.b. : dry base, w.b. : wet base

3.1.2 FTIR (Fourier Transform Infrared Spectroscopy) Analysis

The FTIR spectra of the raw wheat bran sample were recorded in the range 4,000 - 400 cm^{-1} (Figure 3.1). FTIR analysis is applied to obtain information about chemical bonds and groups in organic molecules. The peaks between 3200 - 3400, 2935 - 2915, 2865 - 2845, 1650 - 1590, 1560 - 1540, 1420 - 1300, 1210 - 1320 and 1050 - 990 cm^{-1} correspond to O–H group stretching, C–H asymmetric stretching vibrations, C–H symmetric stretching vibrations, N–H bending, the presence of aliphatic nitro compounds, C–H bending, C–O stretching, aliphatic phosphates (P–O–C) stretching mode, respectively.

The wheat and red lentil bran absorbance peaks are almost similar, which illustrated the similarity in the chemical composition between them. But, wheat bran has additional different functional groups at these wave numbers; 3410 - 3900, 2260 - 2100, 1850 -1650, 1800 - 2100, 850 - 995 and 705 - 570 cm^{-1}, which correspond to aliphatic O–H stretching, C≡C stretch, C=O group, transition metal carbonyls in simple aromatic compounds, aromatic phosphates P-O-C stretch and C-S stretching. The peaks were found in the FTIR bands range 1500 - 1750 cm^{-1} and 2800 - 3000 cm^{-1} are understood to come from protein and fat (Coates (2006).

The results were similar to that reported in insoluble dietary fiber from Canna edulis by-product (Xie et al., 2017)), oat bran soluble dietary fiber (Zhang et al., 2009) and in dietary fiber from Canna edulis ker by product and oat bran dietary fiber (Zhang and Wang, 2013). Furthermore, the chemical compositions related to the functional properties.

The four main regions can be distinguished in wheat and red lentil bran FTIR spectra:

- Fatty acids (3050-2800 cm^{-1})
- Amide I and II (1700-1500 cm^{-1})
- Polysaccharides (1200-900 cm^{-1})
- Aromatic groups (900-500 cm^{-1}) were observed only in wheat bran.

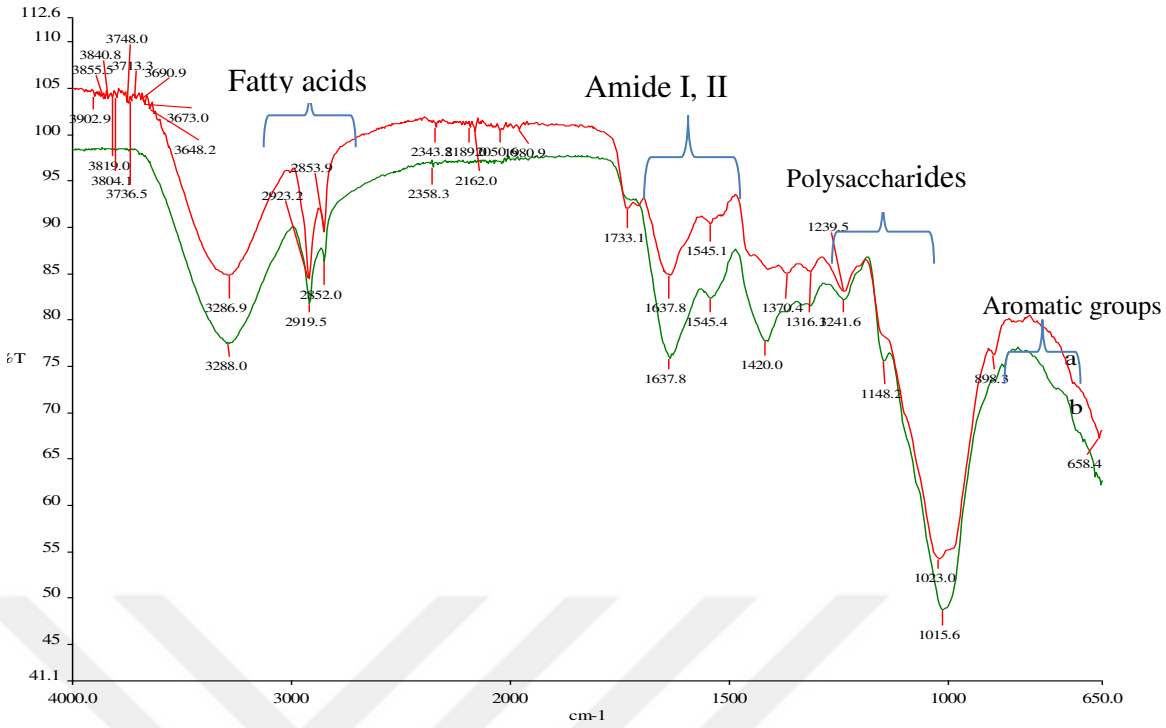


Figure 3.1 FTIR spectrum of wheat (a) and red lentil (b) bran.

3.1.3 Microscopic Structural Analysis (MSA)

Microscopic Structural Analysis (MSA) was used to characterize the surface morphology of wheat and lend bran. MSA micrographs of samples were taken at 10x magnification as presented in Figures 3.2-3.3. The surface structure of the substrate before treating was rough in texture with extensive compact and complex mesh.

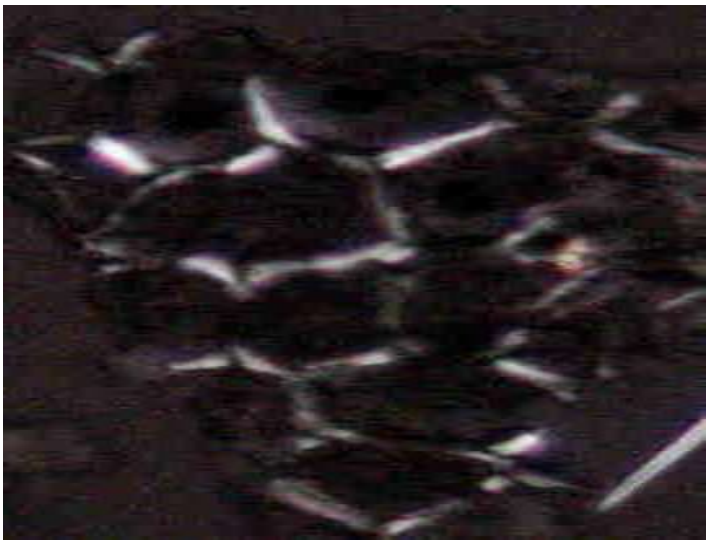


Figure 3.2 Microscopic Structural Analysis for raw wheat bran.

Figure 3.3 Microscopic Structural Analysis for raw red lentil bran.

3.1.4 Thermal Properties Analysis by DSC (Differential Scanning Calorimetry)

DSC is a technique that provides specific information about thermal properties. It provides important data related to the temperature and enthalpy associated with transitions in materials during endothermic or exothermic processes.

One endothermic peak was observed in both wheat and red lentil bran DSC thermograms that correspond to water release from wheat and red lentil bran. The peak temperature of raw wheat bran (179.79 C) was higher than the red lentil bran (174.22 C), which means wheat bran highly heat stable (Figure 3.4).

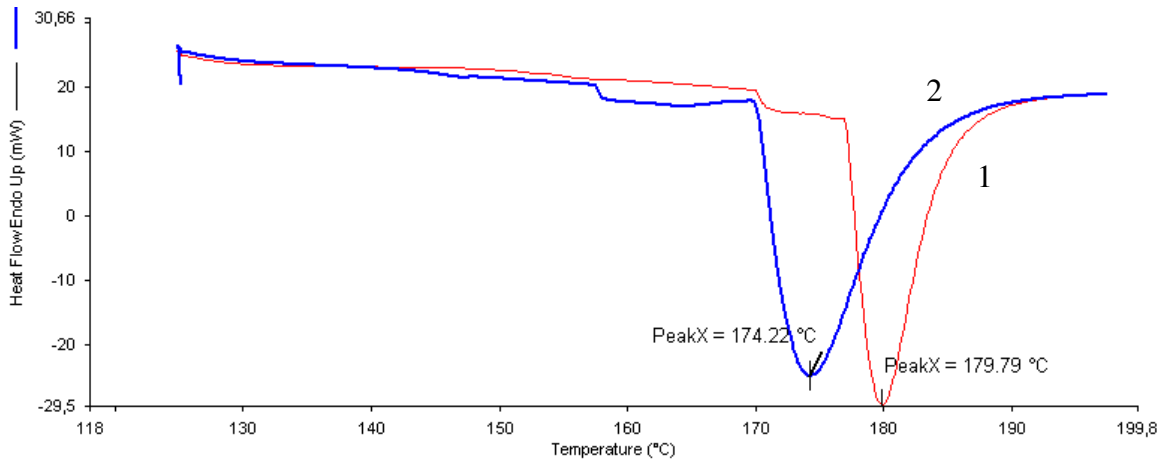


Figure 3.4 Corresponding DSC curve to (1) wheat bran and (2) red lentil bran min.

3.1.5 Effect of Simulated Gastric Fluids (SGF) on Wheat and Red lentil Bran:

In order to determine the behavior of raw wheat and red lentil bran with Simulated Gastric Fluids (SGF), SGF was used for them.

The moisture, ash, protein, fat, carbohydrate, total dietary fiber contents and energy values for raw wheat and red lentil brans that exposed to simulated gastric fluids (SGF) were as; 12.36, 11.14% for moisture content; 5.25, 5.41% for ash; 9.5, 10.27% for protein; 3.9, 1.05% for fat; 58.78, 50.32% for carbohydrates; 37.65, 21.02 for dietary fibers and 265.68, 213.5 kcal/100 g for energy values, respectively (Table 3.4).

Total dietary fiber, carbohydrate and protein contents decreased significantly ($p \leq 0.05$) in raw samples after artificial stomach analysis.

3.2 Effect of Different Treatments on Wheat Bran Properties

3.2.1 Effect of Soaking on Wheat Bran

3.2.1.1 Effect of Soaking on Chemical and Physical Properties of Wheat Bran

Proximate composition and energy values of soaked wheat bran samples (Table 3.2) showed that the moisture content (w.b.), ash (d.b.), protein (d.b.), fat (d.b.), starch (d.b.), sugar (d.b.), carbohydrates (d.b.), total dietary fiber (d.b.) and calorie values (d.b.) of soaked wheat bran were measured as 12.32, 13.61, 13.72, 3.99, 19.21, 0.48, 55.14, 37.49% and 260.57 kcal/100 g for 10 min; 3.4, 1.83, 12.64, 4.1, 20.61 0.32, 53.62, 37.07%, and 256.58 kcal/100 g for 20 min; 3.79, 2.11, 12.71 4.12, 22.53, 0.27, 53.64, 36.07%, and 256.99 kcal/100 g for 30 min, respectively. In soaked WB protein, fat and starch contents increased as long as time increased due to the degradation of fat-protein complexes and the formation of starch polymers during soaking, respectively. In contrast to moisture, ash, sugars, carbohydrates and dietary fiber content that decreased by increasing the time of soaking. Soaking was significantly affective ($p \leq 0.05$) on moisture, ash, sugars, carbohydrates and calorie values.

In comparison to raw wheat bran, all results decreased except protein, starch and fat that increased after soaking. These results are agreed with Hamad et al. (2015) who found that protein raised slightly from 14.1% to 14.4% and fat content raised from

4.8% to 5.1% in wheat bran. While ash content decreased from 4.3% to 3.4% compared to raw material. These results in agreement with Shaker et al. (1995) who reported that nutrients loss could be due to the leaching of soluble mineral and other nutrients into soaking water.

Afify et al. (2012) studied the difference between raw and soaked sorghum and found that moisture content, ash, protein and fiber decreased after soaking. In addition to a considerable decrease in phytic acids in wheat after soaking (Liu et al., 2007; Mosharraf et al., 2009; Noort et al., 2010).

There was a critical effect ($p \leq 0.05$) of soaking time on starch and sugar contents of WB in contrast to moisture, ash, carbohydrates, calorie and protein and total dietary fiber contents in wheat bran.

There was no important effect ($p > 0.05$) of soaking time on all components except the considerable difference ($p \leq 0.05$) between soaking for 10 min and soaking for 30 min in sugar content of wheat bran.

It was found that color values of soaked wheat bran were 62.67 for lightness (L*), 7.33 for redness (a*), 25.99 for yellowness (b*) and 64.07 for yellowness index (YI) for wheat bran soaked for 10 min; 60.92, 6.26, 25.82 and 66.08 for 20 min; 63.31, 7.2, 25.23 and 63.4 for 30 min, respectively. The soaking of wheat bran decreased L*, b* values and increased a* and YI values in comparison to raw wheat bran due to the leaching of soluble solids from wheat bran to water. Soaking affects significantly ($p \leq 0.05$) L*, a* and YI values. Time of soaking significantly affected ($p \leq 0.05$) L*, YI* and b* but a* showed a negligible influence by soaking. There was a significant difference ($p \leq 0.05$) in L* between wheat bran soaked for 10 min and 20 min.

3.2.1.2 FTIR (Fourier Transform Infrared Spectroscopy) Analysis and Wheat Bran

The FTIR spectra of the soaked wheat bran were recorded in the range 4,000 -400 cm^{-1}. The peaks between 3200 - 3400, 2935 - 2915, 2865 - 2845, 2100 - 2260, 1800 - 2100, 1650 - 1850, 1590 - 1650, 1540 -1560, 1210 - 1320, 820-890 and 705 - 570 cm^{-1} correspond to O–H group stretching, C–H asymmetric stretching vibrations, C–H symmetric stretching vibrations, C≡C stretch, transition metal carbonyls in simple aromatic compounds, C=O group, N–H bending, the presence of aliphatic

nitro compounds, C-O stretching mode, C–O–O– stretching and the last indicates disulfides C–S stretch, respectively.

Functional groups in soaked samples increased in comparison to raw samples. The functional group increased by increasing the soaking time. The difference in FTIR spectra between soaked samples was observed at 1154 cm^{-1} in 30 min soaked wheat bran samples that corresponds to C–N stretching in secondary amine (Figure 3.5).

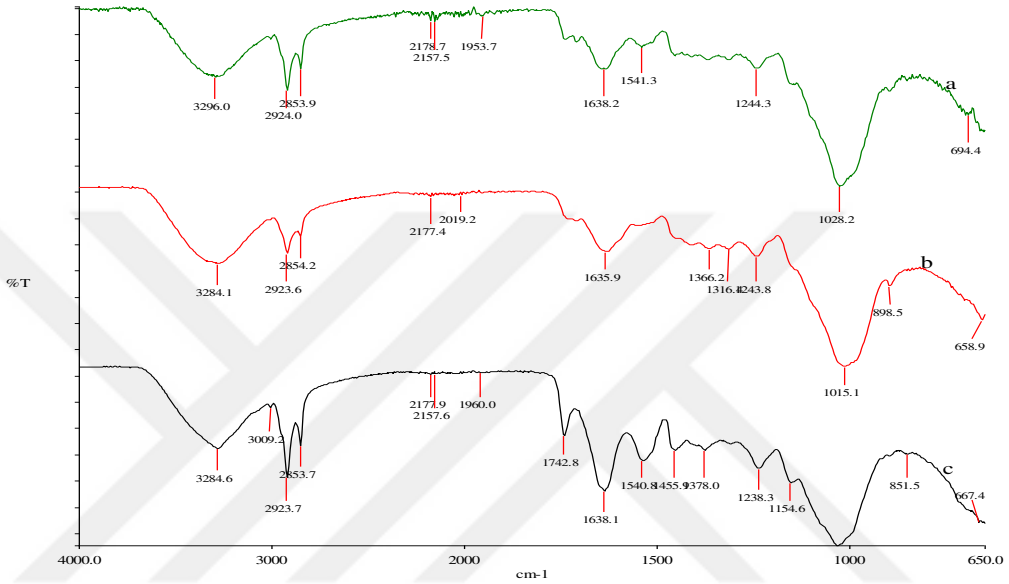
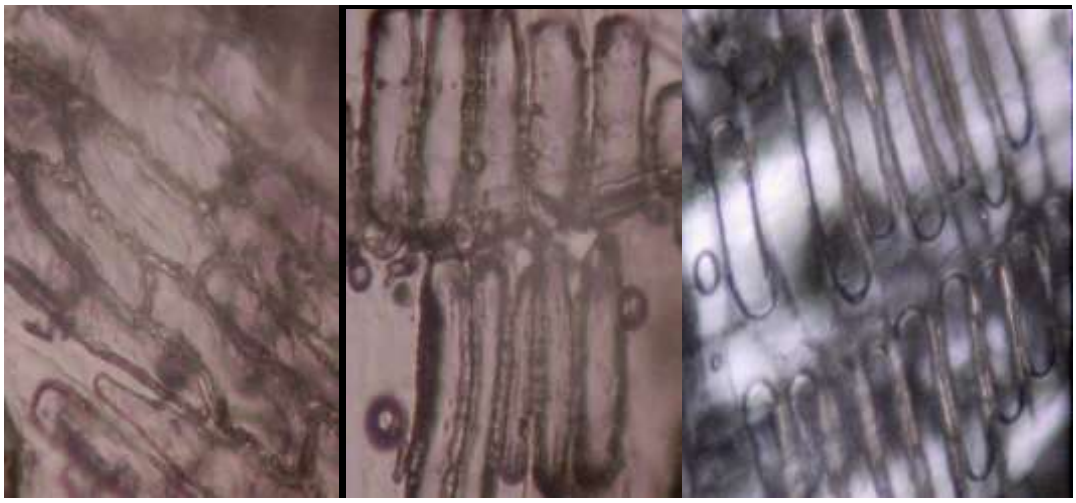


Figure 3.5 FTIR spectrum of (a) 10, (b) 20 and (c) 30 min soaked wheat bran.

3.2.1.3 Microscopic Structural Analysis

The thermal treatments of wheat bran had a rough surface with a number of pores and cavities on its surface. The surface of thermally treated wheat bran samples became comparatively smoother in comparison to soaked samples (Figure 3.6).



(a) (b) (c)

Figure 3.6 Microscopic Structural Analysis for (a) 10 min, (b) 20 min and (c) 30 min soaked wheat bran.

3.2.1.4 Thermal Properties by DSC (Differential Scanning Calorimetry)

DSC is useful in studying thermodynamic information on the gelation process of polysaccharides. The endothermic peaks during the thermal transition from DSC measurement indicate the melting of structural domains. The apparent melting enthalpy value refers to the amount of energy required for breaking the H-bonding within the junction zones (Lazaridou et al., 2004).

A large endothermic transition peak was observed in all samples at a temperature of about 160-198C that corresponding to water release from wheat bran. The peak temperature of the treated groups was higher than the untreated. This results in agreement with Zhang et al. (2011) whose concluded that the peak temperature of soluble dietary fibers from extruded oat bran was higher than that of soluble dietary fibers from untreated oat bran, because of the increase in short-chain soluble dietary fibers concentration compared with raw samples. Short-chain soluble dietary fibers have strong hydrogen bonds within its junction zones that require more energy to decompose the soluble dietary fibers crystalline structure (Li et al., 2006) (Figure 3.7).

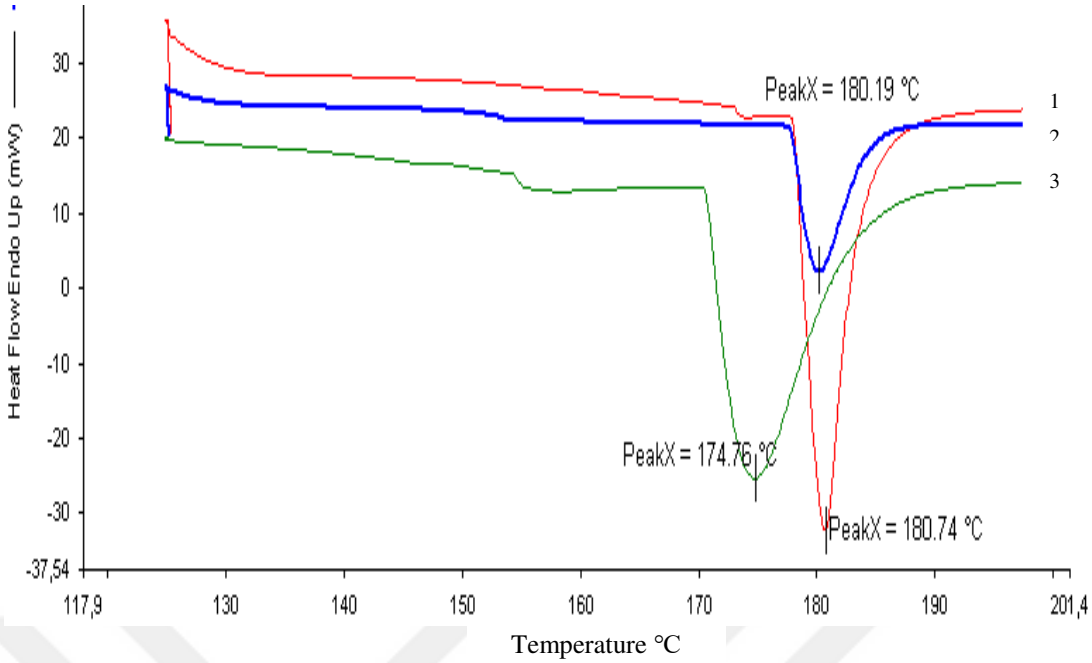


Figure 3.7 Thermal analysis of wheat bran. (1) wheat bran soaked for 10 min, (2) wheat bran soaked for 20 min and (3) wheat bran soaked for 30 min.

3.2.1.5 Effect of Simulated Gastric Fluids (SGF) on Soaked Wheat Bran:

The moisture, ash, protein, fat, carbohydrate, total dietary fiber contents and energy values for 10 min soaked wheat bran that exposed to simulated gastric fluids (SGF) were measured as; 13.01%, 1.23%, 5.43%, 3.95%, 58.28%, 34.12% for dietary fibers and 254.37 kcal/100 g, respectively (Table 3.4).

Carbohydrates, ash, TDF, protein contents and energy value significantly decreased ($p \leq 0.05$) after artificial stomach analysis.

3.2.2 Effect of Cooking on Wheat Bran

3.2.2.1 Effect of Cooking on Chemical and Physical Properties of Wheat Bran

The proximate composition and energy values of cooked wheat bran samples presented in Table 3.2. The moisture, ash, protein, fat, starch, sugar, carbohydrates, fiber and calorie contents of cooked wheat bran were measured as 12.52, 5.07, 11.05, 2.99, 18.78,0.32, 59.19, 40.16% and 263.29 kcal/100 g for wheat bran cooked for 10 min; 13.17, 5.05, 11.01, 3.03, 18.72, 0.3, 59.22, 41.33% and 263.64 kcal/100 g for 20 min; 14.01, 5.01, 10.32, 3.26, 18.42, 0.21, 60, 43.55% and 266.66 kcal/100 g for 30 min, respectively. In comparison to raw wheat bran, all results decreased except fiber content that increased significantly ($p \leq 0.05$) after cooking. Cooking was affective

($p \leq 0.05$) in energy value and the proximate compositions except starch, ash and carbohydrate contents that did not ($p > 0.05$) influence by cooking. These results tend to be similar to those reported by Ifemeje et al. (2015), whose found that cooking decreased protein, fat, ash, total carbohydrate and calorie contents in all the samples analyzed. The total dietary fiber content increased after cooking this is in agreement with Caprez et al. (1986) whose reported the increase of total and soluble dietary fibers and the decrease in protein and starch in wheat bran after cooking.

The decrease may result from the diffusion of soluble nutrients to the cooking water. The decrease in protein can be due to the denaturation of protein during heat treatment. Cooking increases the moisture content of wheat bran samples due to the gelatinization of starch due to the absorption of water (Ifemeje et al., 2015). The formation of protein-fiber complex during cooking illustrates the decrease in protein and the increase in total dietary fibers of wheat bran. The degradation of starch decreases starch content. In contrast to total fibers which increased after cooking may be due to the formation of resistant starch during cooking (Caprez et al., 1986). Wang et al. (1993) found that insoluble dietary fiber decreased in boiled wheat bran. In comparison to raw wheat bran due to the disruption of covalent bonds in the carbohydrate and protein complexes, which in turn leads to smaller and more soluble molecules. This was agreed with Lena et al. (1997), whose noticed that the soluble content of wheat bran increased enzymatically.

In cooked wheat bran moisture content, carbohydrates, calories and fibers content increased as long as time increased in contrast to protein, fat, ash, sugars, and starch content that decreased by increasing the cooking time.

The difference between cooking time was affective ($p \leq 0.05$) in moisture content, sugars and dietary fibers in wheat bran. In contrast, starch, carbohydrates, fat, ash and protein contents did not significantly ($p \leq 0.05$) influence by soaking time. There was a considerable difference in moisture content between wheat bran cooked for 10 min and 20 min. A significant difference was also noticed in ash content between wheat bran cooked for 10 min and 20 or 30 min. In addition to the important difference in sugar content between wheat bran cooked for 10 or 20 min and 30 min. Hence, no considerable difference ($p > 0.05$) was observed in sugar content between 10 min and 30 min. Dietary fiber showed a significant difference between cooking

for 10 or 20 min and 30 min. Hence, no difference in dietary fiber content between wheat bran cooked for 10 and 20 min.

It was found that color values of cooked wheat bran were 64.42 for lightness (L*), 6.16 for redness (a*), 26.35 for yellowness (b*) and 63.45 for yellowness index (YI) for wheat bran cooked for 10 min; 63, 6.43, 26.25 and 65.04 for 20 min; 58.44, 6.6, 25.23 and 66.19 for 30 min, respectively. The cooking of wheat bran decreased ($p \leq 0.05$) lightness, redness and yellowness index but yellowness increased in ($p \leq 0.05$) comparison to raw wheat bran. Nevertheless, it affected significantly ($p \leq 0.05$) lightness and yellowness index but redness and yellowness showed a negligible influence ($p > 0.05$) by cooking.

There was a significant difference ($p \leq 0.05$) in L* between wheat bran cooked for 10 min and 20 or 30 min. In addition to the important difference between cooking for 20 min and 30 min. b* showed a considerable difference between all categories of cooking time except between 10 min and 20 min. YI* was significantly different between all different periods of cooking.

3.2.2.2 FTIR (Fourier Transform Infrared Spectroscopy) Analysis

The FTIR spectra of the cooked wheat bran were recorded in the range 4,000 -400 cm^{-1}. The peaks between 3200 - 3400, 2935 - 2915, 2865 - 2845, 2100 - 2260, 1800 - 2100, 1650 - 1850, 1590 - 1650, 1540 -1560, 1210 - 1320, 1020-1090, 820-890 and 705 - 570 cm^{-1} correspond to O–H group stretching, C–H asymmetric stretching vibrations, C–H symmetric stretching vibrations, C≡C stretch, transition metal carbonyls in simple aromatic compounds, C=O group, N–H bending, the presence of aliphatic nitro compounds, C-O stretching mode, C–N stretch in primary amine, C–O–O– stretching and the last indicates disulfides C–S stretch, respectively.

The peak between 1020-1090 cm^{-1} that corresponds to C–N stretch in primary amine was observed only after the cooking process. It can be observed that the cooked wheat bran has higher absorbance by the main functional groups than the raw bran; indicating an increasing amount of these functional groups after the thermal treatment. The functional group increased by increasing the cooking time (Figure 3.8).

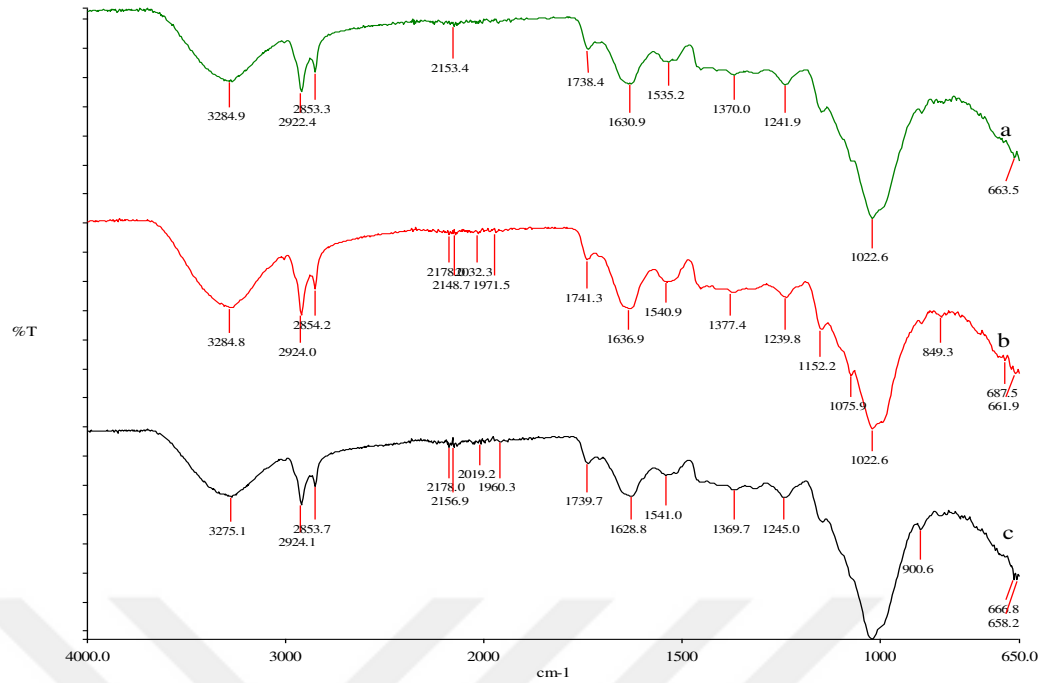
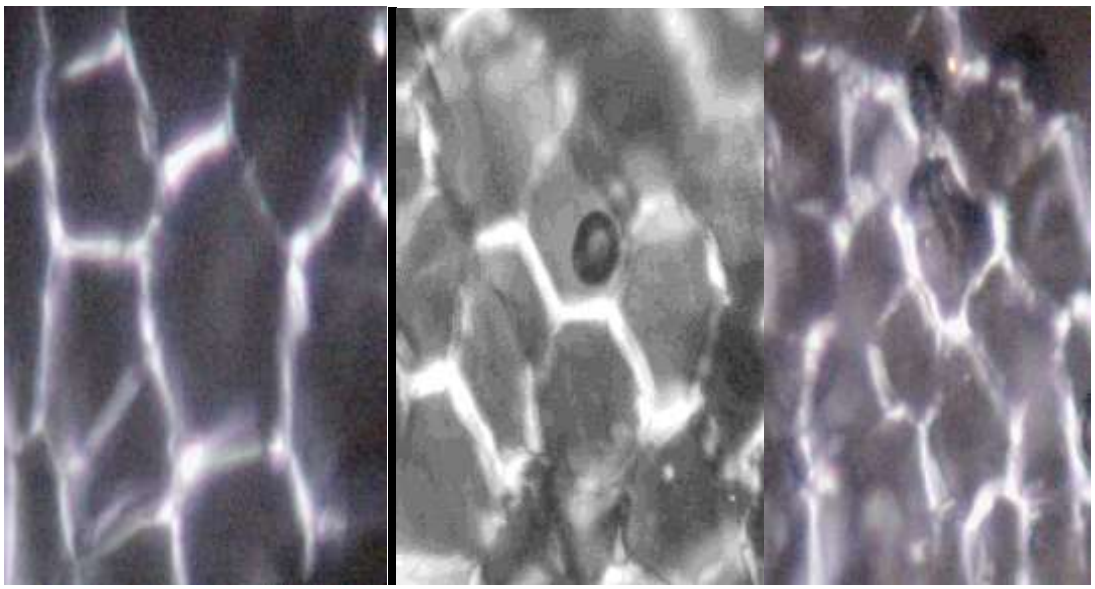


Figure 3.8 FTIR spectrum of (a) 10 min, (b) 20 min and (c) 30 min cooked wheat bran.

3.2.2.3 Microscopic Structural Analysis

The surface morphology of the cooked wheat bran that was observed by MSA was regular (Figure 3.9).



(a) (b) (c)

Figure 3.9 Microscopic Structural Analysis for (a) 10 min, (b) 20 min and (c) 30 min cooked wheat bran.

3.2.2.4 Thermal Properties by DSC (Differential Scanning Calorimetry)

A large endothermic transition peak was observed in all samples at a temperature of about 167-174C that corresponding to water release from wheat bran (Figure 3.10).

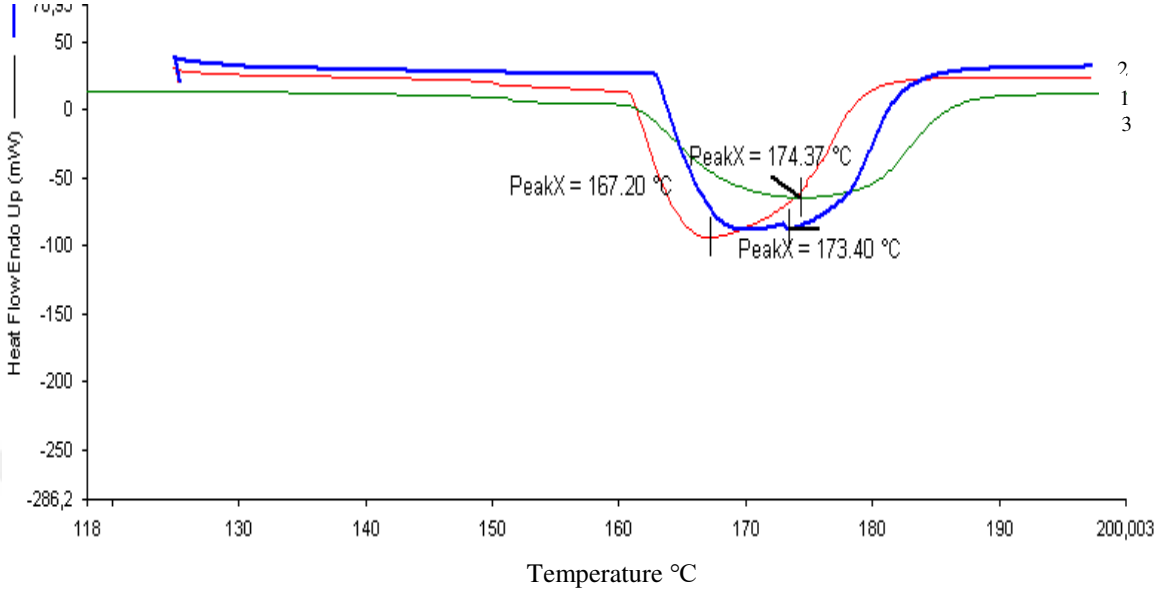


Figure 3.10 Thermal analysis of wheat bran. (1) wheat bran cooked for 10 min, (2) wheat bran cooked for 20 min and (3) wheat bran cooked for 30 min.

3.2.2.5 Effect of Simulated Gastric Fluids (SGF) on Cooked Wheat Bran:

The moisture, ash, protein, fat, carbohydrate, total dietary fiber contents and energy values for wheat bran cooked for 30 min were noted as; 14.32%, 3.31%, 3.26%, 4.58%, 56.69%, 40.1%, and 240.84 kcal/100 g, respectively.

Total dietary fiber, moisture, ash, protein contents and energy value decreased significantly ($p \leq 0.05$) after artificial stomach analysis.

3.2.3 Effect of Microwave on Wheat Bran

3.2.3.1 Effect of Microwave on Chemical and Physical Properties of Wheat Bran

In the study, moisture content, ash, protein, fat, starch, sugar, carbohydrates, fiber and energy values of microwaved wheat bran were measured as 11.05, 6.55, 10.19, 3.67, 16.03, 0.46, 58.34, 40.53% and 263.8 kcal/100 g for 2; 11.01, 6.8, 11.1, 3.66, 9.85, 0.4, 57.23, 42.35% and 262.02 kcal/100 g for 4 min; 10.32, 7.37, 10.93, 3.57, 8.56, 0.38, 57.6, 43.96% and 262.19 kcal/100 g for 6 min, respectively. In comparison to raw wheat bran, all values decreased in contrast to fiber and ash content. Microwave treatment was effective ($p \leq 0.05$) in all mentioned contents

except fat content. The increase in ash is similar to other studies carried out the wheat germ and wheat bran, several authors reported that the ash content increases with microwave and autoclave treatments (Nandeesh et al., 2011; Porres et al., 2003; Srivastava et al., 2007).

The decrease of sugars may have resulted from the diffusion of soluble nutrients to water. The decrease of protein can be due to the denaturation of protein during heat treatment. The formation of protein fiber complex during cooking illustrates the decrease in protein and the increase in total dietary fibers in WB. However, because of starch degradation, the starch content decreased. In contrast, total dietary fibers increased after heat treatment may be due to the formation of resistant starch during cooking (Caprez et al., 1986).

The increase in ash content could be due to the loss of phytic acids by treatment with microwave, which in turn leads to an increase in the bioavailability of minerals.

In microwaved wheat bran, time was affective on sugar and starch contents. There was a considerable difference ($p \leq 0.05$) in fiber content between wheat bran microwaved for 2 and 6 min. A significant difference was noticed in sugar content between wheat bran microwaved for 2 and 4 or 6 min. In addition to the important difference in starch content between wheat bran microwaved for 2 and 4 min or 6 min. Hence no significant difference ($P > 0.05$) was observed in sugar and starch content between 4 and 6 min.

It was found that color values of 2 min microwaved wheat bran were 65.45 for L*, 6.93 for a*, 25.21 for b* and 61.33 for YI; 62.37, 6.63, 25.34 and 64.26 for 4 min microwaved wheat bran; 62.4, 6.84, 25.79 and 64.23 for 6 min microwaved wheat bran, respectively.

Processing of wheat bran with microwave decreased L* and b*, in contrast to a* and YI values increased in comparison to raw wheat bran. Microwave treatment affected significantly ($p \leq 0.05$) on the values of L* and YI, but a* and b*. There was a significant difference ($p \leq 0.05$) in L* and YI between wheat bran microwaved for 2 min and 4 or 6 min. Microwave treatment showed lower redness, yellowness in wheat bran (Ertaş, 2016). In microwaved whole wheat the redness (a*) values of bread crumb and crust color increased in comparison to control samples (Demir and

Elgün, 2014). Yadav et al. (2010) found that microwave treatments on whole wheat flour increase the dry matter more than the other stabilization processes. S. Zhao et al. (2007) reported that microwave treatment increased the dry matter and also increased the ash content in processed samples. Microwave treatment is one of the most important treatments in the dephytinisation of high dietary fiber products (Ertaş, 2016).

3.2.3.2 FTIR (Fourier Transform Infrared Spectroscopy) Analysis

The FTIR spectra of the microwaved wheat bran samples were recorded in the range 4,000 -400 cm^{-1}. The peaks between 3200 - 3400, 2935 - 2915, 2865 - 2845, 2100 - 2260, 1800 - 2100, 1650 - 1850, 1590 - 1650, 1540 -1560, 1210 - 1320, 820-890 and 705 - 570 cm^{-1} correspond to O–H group stretching, C–H asymmetric stretching vibrations, C–H symmetric stretching vibrations, C≡C stretch, transition metal carbonyls in simple aromatic compounds, C=O group, N–H bending, the presence of aliphatic nitro compounds, C-O stretching mode, C–O–O– stretching and the last indicates disulfides C–S stretch, respectively.

Functional groups increased after microwave treatment. The functional group increased by increasing the treatment time. The difference in FTIR spectra between microwaved samples was observed at the peaks between 820-890 cm^{-1} in 30 min microwaved samples that correspond to C–O–O– stretching. It can be observed that the microwaved wheat bran has higher absorbance by the main functional groups than the raw bran; indicating an increasing amount of these functional groups after the thermal treatment. No difference was observed between different times of treatments (Figure 3.11).

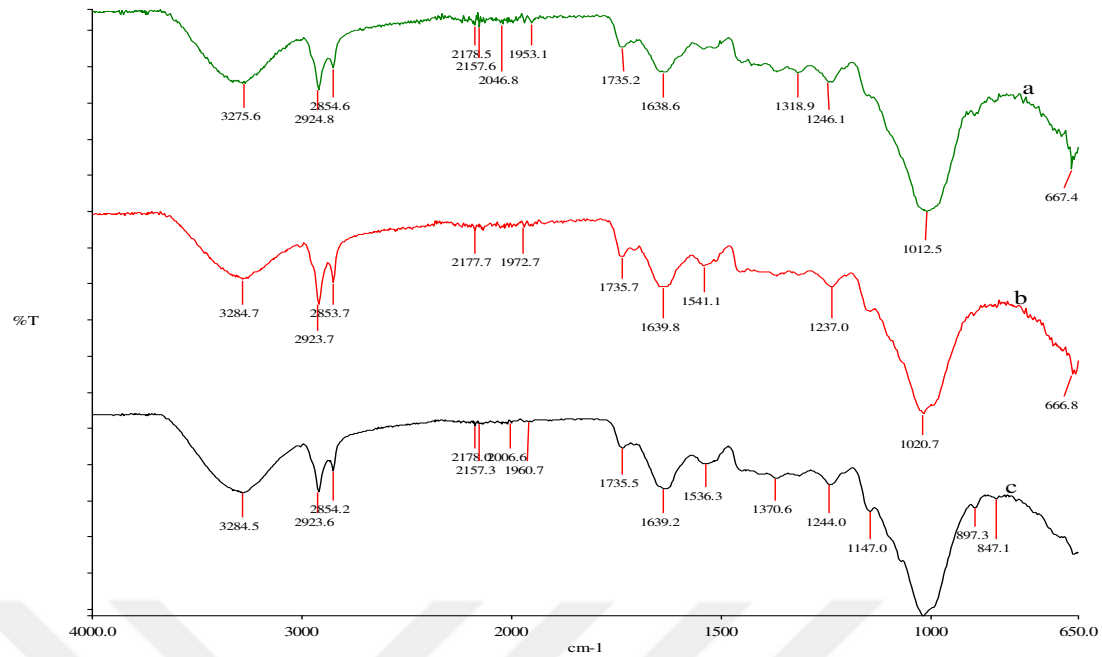
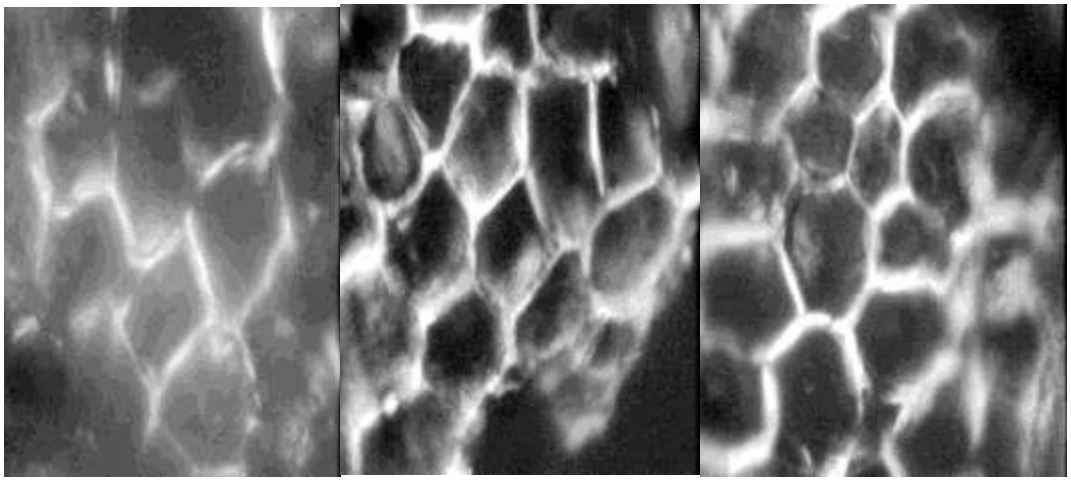


Figure 3.11 FTIR spectrum of (a) 2, (b) 4 and (c) 6 min microwaved wheat bran.

3.2.3.3 Microscopic Structural Analysis (MSA)

The thermally treated wheat bran had a rough surface with a number of pores and cavities on its surface. The surface of microwaved wheat bran samples became comparatively smoother in comparison to soaked and ultrasound treated samples (Figure 3.12).



(a) (b) (c)

Figure 3.12 Microscopic Structural Analysis for (a) 2 min, (b) 4 min and (c) 6 min microwaved wheat bran.

3.2.3.4 Thermal Properties by DSC (Differential Scanning Calorimetry)

A large endothermic transition peak can be observed in all samples at a temperature of about 161-198C that corresponding to water release from wheat bran. The peak temperature of the treated groups was higher than the raw state. The results were similar to the study of M. Zhang et al. (2011) (Figure 3.13).

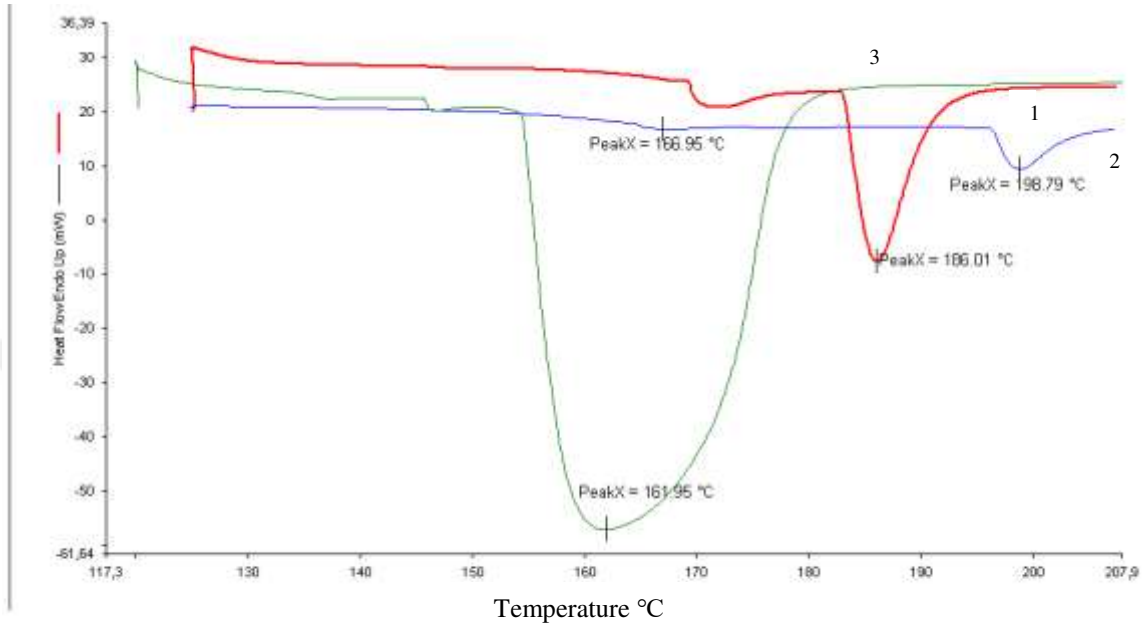


Figure 3.13 Thermal analysis of wheat bran (1) wheat bran microwaved for 2 min, (2) wheat bran microwaved for 4 min and (3) wheat bran microwaved for 6 min.

3.2.3.5 Effect of Simulated Gastric Fluids (SGF) on Microwaved Wheat Bran

The moisture, ash, protein, fat, carbohydrate, total dietary fiber contents and energy values for wheat bran microwaved for 6 min were measured as; 10.8%, 6.82%, 3.53%, 4.12%, 56.93%, 41.04% and 242.83 kcal/100 g, respectively.

Total dietary fiber, moisture, ash, protein contents and energy value decreased significantly ($p \leq 0.05$) after artificial stomach analysis.

3.2.4 Effect of Ultrasound on Wheat Bran

3.2.4.1 Effect of Ultrasound on Chemical and Physical Properties of Wheat Bran

A proximate composition and energy values of wheat bran samples treated with ultrasound (Table 3.2) showed that the moisture content, ash, protein, fat, starch,

sugar, carbohydrates, total dietary fiber and calorie contents of wheat bran treated with ultrasound were measured as 12.35, 5.03, 10.03, 3.35, 16.32,0.48, 59.04, 37.19% and 263.28 kcal/100 g for 10 min; 13.63, 4.98, 10.28, 3.19, 16.15, 0.42, 57.72, 36.99% and 257.84 kcal/100 g for 20 min; 14.29, 4.52, 10.02, 3.94, 15.23, 0.39, 56.85, 35.84% and 260.82 kcal/100 g for 30 min, respectively. In comparison to raw wheat bran, all values decreased except the moisture content. The ultrasonication of wheat bran was affective on the starch, carbohydrates, fiber, protein, moisture, ash contents and energy value. The effect of ultrasound on fibers available in wheat bran was similar to the study of Kaya et al. (2017) reported the significant increase in total and soluble dietary fiber in pulses treated with ultrasound.

According to Zhao et al. (2014) study, about goat milk when 20 kHz, 800W, ultrasound applied for 0–20 min at 38 °C, a considerable increase was observed in whey protein denaturation, soluble calcium and phosphorus, water-holding capacity and cross-linking. In contrast to McDonnell et al. (2014), who conducted that no effect on water-holding capacity and structure of meat was treated with ultrasound.

Ultrasonication in a bath reduced the protein content of the mung bean and maize starches, suggesting the enhanced enzyme susceptibility of starch through the ultrasonication process (Chan et al., 2010). In starch isolation from maize flour and hominy feed at 20 kHz, 10% slurry, 100% amplitude, the recovery of starch was increased by ultrasonication. This, in turn, decreased protein and lipid contents. However, lightness increased and yellowness decreased (Zhang et al., 2005).

Ultrasound treatment time was effective ($p \leq 0.05$) on moisture, starch and carbohydrate contents.

It was found that the color values of ultrasound treated wheat bran were 64.76 for L*, 7.51 for a*, 25.8 for b* and 63.13 for (YI) for 10 min; 63.80, 7.6, 25.4 and 63.63 for 20 min; 65.37, 6.68, 25.35 and 61.29 for 30 min, respectively.

The lightness (L*) and yellowness decreased but (b*) redness (a*) and (YI) increased in comparison to raw wheat bran.

Ultrasonication affected significantly ($p \leq 0.05$) lightness (L*), yellowness index (YI) and redness (a*) but yellowness (b*) was not affected by ultrasonication. There was a significant difference ($p \leq 0.05$) in the yellowness index (YI) between wheat bran treated with ultrasound for 10 min and 20 or 30 min. A considerable difference in yellowness index (YI) between samples treated with ultrasound for 20 min and wheat bran samples treated with ultrasound for 30 min was observed.

3.2.4.2 FTIR (Fourier Transform Infrared Spectroscopy) Analysis

The FTIR spectra of the ultrasound treated wheat bran were recorded in the range 4,000 - 400 cm^{-1}. The peaks between 3200 - 3400, 2935 - 2915, 2865 - 2845, 2100 - 2260, 1800 - 2100, 1650 - 1850, 1590 - 1650, 1540 -1560 and 1210 – 1320 cm^{-1} correspond to O–H group stretching, C–H asymmetric stretching vibrations, C–H symmetric stretching vibrations, C≡C stretch, transition metal carbonyls in simple aromatic compounds, C=O group, N–H bending, the presence of aliphatic nitro compounds and C-O stretching mode, respectively.

The peaks between 820 – 890 cm^{-1} and 705 – 570 cm^{-1} that correspond to C–O–O– stretching and C–S stretching mode, respectively were lost after treatment with ultrasound. Ultrasound treated samples hed similar functionnal groups those found after soaking treatments. No difference was observed between different times of treatments (Figure 3.14).

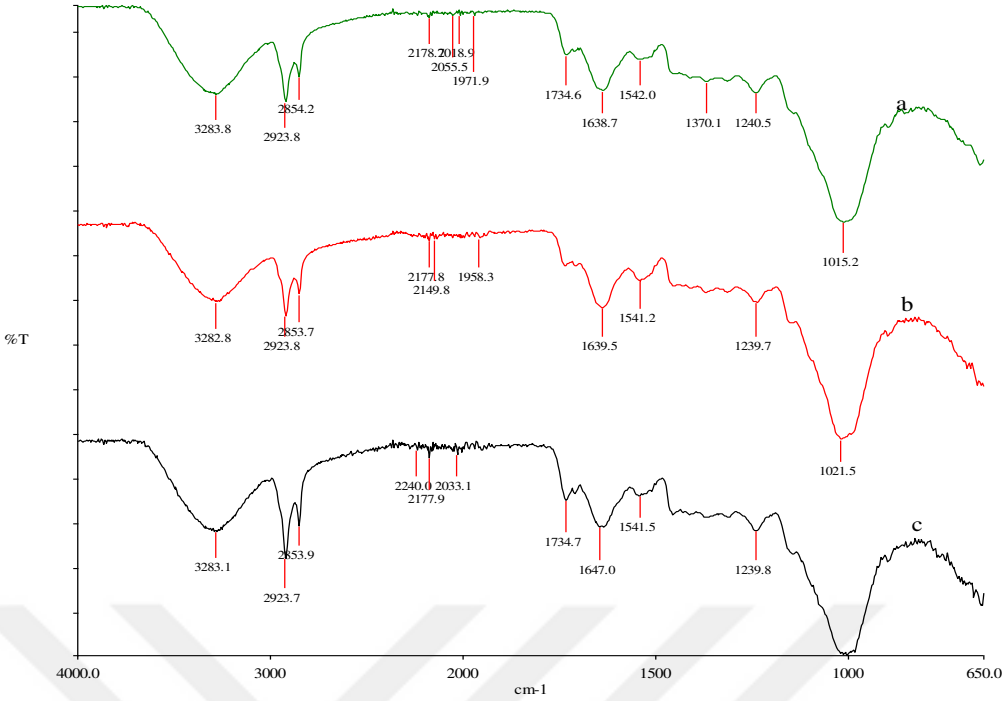
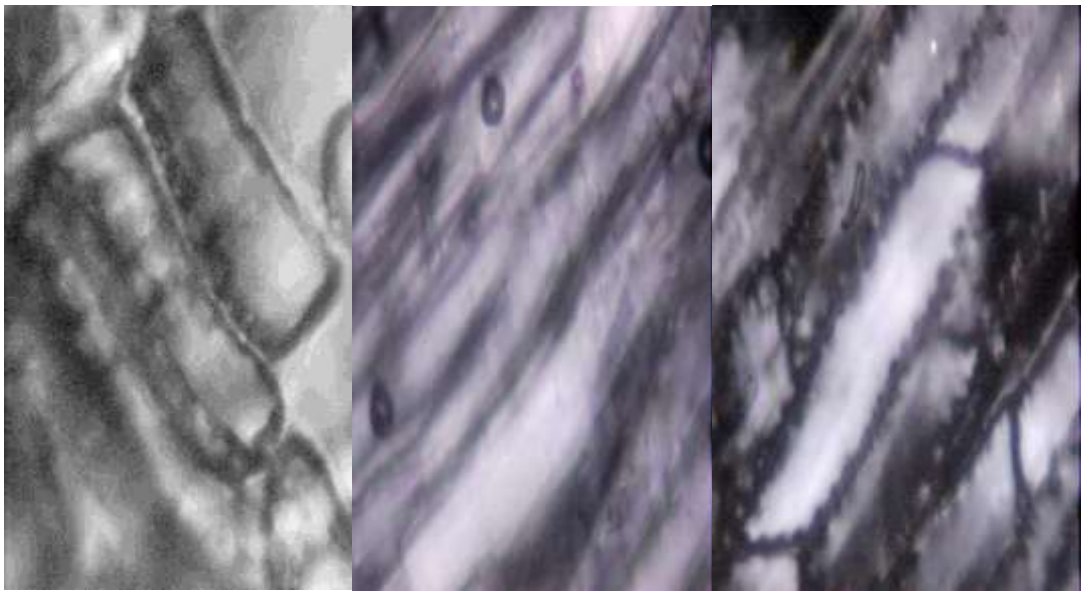


Figure 3.14 FTIR spectrum of (a) 10, (b) 20 and (c) 30 min ultrasound treated wheat bran.

3.2.4.3 Microscopic Structural Analysis (MSA)

The surface morphology of the ultrasound treated dietary fiber was observed by MSA was more irregular than samples exposed to heat treatment such as microwave and cooking (Figure 3.15). The irregular, hollow and pores morphology may be attributed to some functional properties of the insoluble dietary fibers, including water, toxic ions, fat, bile acid salts, and glucose adsorptions as well as α-amylase inhibition (Wang et al., 2015).



(a) (b) (c)

Figure 3.15 Microscopic Structural Analysis for (a) 10 min, (b) 20 min and (c) 30 min ultrasound treated wheat bran.

3.2.4.4 Thermal Properties by DSC (Differential Scanning Calorimetry)

A large endothermic transition peak can be observed in all samples at a temperature of about 160-192C that corresponding to water release from wheat bran. According to this study, the most heat stable samples between all treatments that treated with ultrasound for 30 min, in contrast, samples treated with ultrasound for 10 min showed the lowest endotherm peak temperature (Figure 3.16).

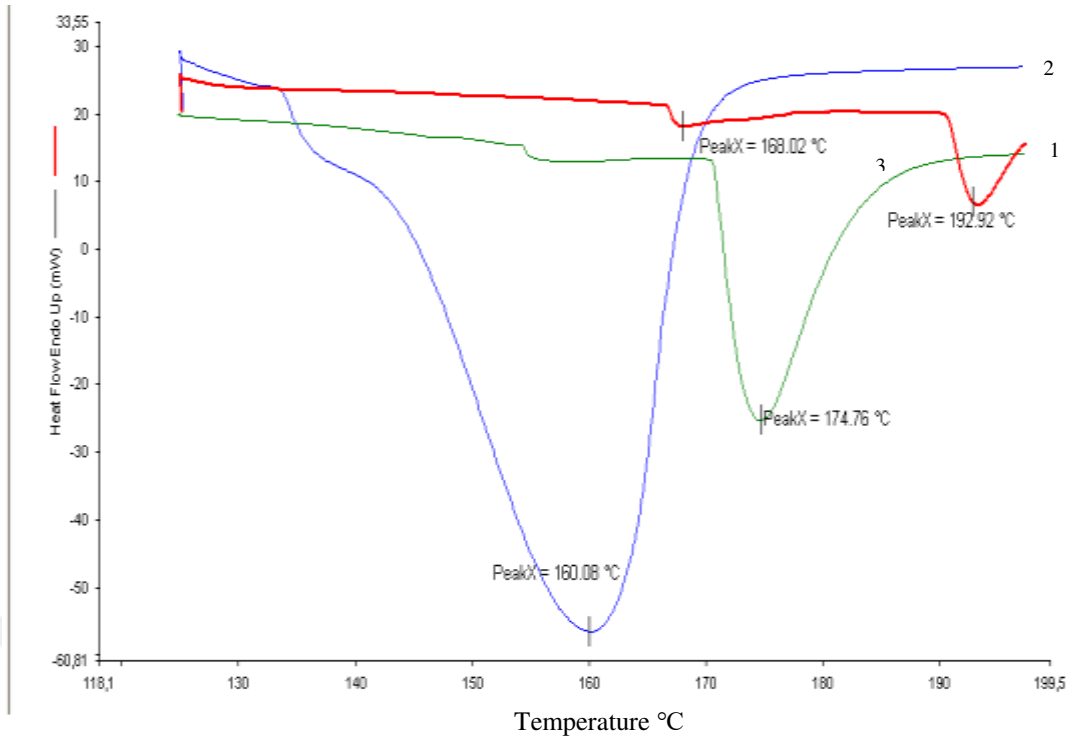


Figure 3.16 Thermal analysis of wheat bran treated with ultrasound for (1) 10 min, (2) 20 min and (3) 30 min.

3.3.4.5 Effect of Simulated Gastric Fluids (SGF) on Ultrasound Treated Wheat Bran:

The moisture, ash, protein, fat, carbohydrate, total dietary fiber contents and energy values for wheat bran treated with ultrasound for 10 min, were measured as; 12.47%,4.57%, 35.9%, 5.71%, 58.72%, 35.99% and 251.6/100 g, respectively.

Protein, dietary fiber contents and energy value decreased significantly ($p \leq 0.05$) after artificial stomach treatment.

Table 3.2 A proximate composition of raw and processed wheat bran

Treatment	Moisture content (%, w.b.)	Ash (%, d.b.)	Protein (%, d.b.)	Fat (%, d.b.)	Starch (%, d.b.)	Sugars (%, d.b.)	Carbohydrates (%, d.b.)	Total dietary fibers (%, d.b.)	Calorie (kcal/100 g)	L*	a*	b*	YI
Control	12.16 ±0.15^{a}	5.65 ±0.41^{a}	12.35 ±1.06^{a}	3.97 ±0.19^{a}	19.28 ±0.33^{a}	0.51 ±0.11^{a}	60.7 6±1.27^{a}	39.29 ±0.86^{a}	280.29 ±14.88^{a}	68.23 ±0.02^{a}	6.48 ±0.046^{a}	25.99 ±0.01^{a}	60.63 ±0.07^{a}
Soaking 10 min	12.32 ±0.33^{a}	1.92 ±0.53^{b}	12.43 ±0.55^{a}	3.99 ±0.14^{a}	19.21 ±0.65^{a}	0.48 ±0.07^{a}	55.14 ±1.88^{b}	37.49 ±0.169^{a}	260.57 ±5.33^{a}	62.67 ±0.7^{b}	7.33 ±0.21^{b}	25.31 ±0.35^{a}	64.07 ±0.06^{b}
Soaking 20 min	13.61 ±0.52^{b}	1.83 ±0.52^{b}	12.64 ±0.74^{a}	4.1 ±0.41^{a}	20.61 ±1.35^{ab}	0.32 ±0.28^{b}	53.62 ±0.67^{b}	37.07 ±0.03^{a}	256.58 ±2.94^{b}	60.92 ±0.67^{b}	7.26 ±0.11^{b}	25.82 ±0.05^{a}	66.08 ±0.028^{c}
Soaking 30 min	13.72 ±0.42^{b}	2.11 ±0.33^{b}	12.71 ±1.11^{a}	4.12 ±0.35^{a}	22.53 ±1.01^{b}	0.27 ±0.05^{b}	53.64 ±2.16^{b}	36.07 ±0.73^{a}	256.99 ±2.15^{b}	63.13 ±0.12^{b}	7.2 ±0.1^{b}	25.23 ±0.12^{a}	63.49 ±0.09^{d}
Control	12.16 ±0.15^{a}	5.65 ±0.41^{a}	12.35 ±1.06^{a}	3.97 ±0.19^{a}	19.28 ±0.33^{a}	0.51 ±0.11^{a}	60.7 6±1.27^{a}	39.29 ±0.86^{a}	280.29 ±14.88^{a}	68.23 ±0.02^{a}	6.48 ±0.046^{a}	25.99 ±0.01^{a}	60.63 ±0.07^{a}
Cooking 10 min	12.52 ±0.53^{a}	5.07 ±0.55^{a}	11.05 ±0.48^{b}	2.99 ±0.02^{a}	18.78 ±0.24^{a}	0.32 ±0.28^{b}	59.19 ±0.7^{a}	40.16 ±0.86^{a}	263.29 ±4.14^{b}	64.42 ±0.1^{b}	6.16 ±0.12^{a}	26.35 ±0.05^{a}	63.45 ±0.19^{b}
Cooking 20 min	13.17 ±0.14^{ab}	5.05 ±0.16^{a}	11.01 ±0.2^{b}	3.03 ±0.21^{a}	18.72 ±0.45^{a}	0.3 ±0.04^{b}	59.22 ±0.51^{a}	41.18 ±1.14^{ab}	263.64 ±0.87^{b}	63 ±0.1^{c}	6.43 ±0.11^{a}	26.25 ±0.3^{a}	65.04 ±0.04^{c}
Cooking 30 min	14.01 ±0.57^{b}	5.01 ±0.22^{a}	10.32 ±0.43^{b}	3.26 ±0.33^{a}	18.42 ±0.45^{a}	0.21 ±0.04^{b}	60 ±1.17^{a}	43.55 ±0.32^{b}	266.66 ±2.92^{b}	58.44 ±0.48^{d}	6.6 ±0.26^{a}	25.23 ±0.31^{b}	66.19 ±1.19^{d}
Control	12.16 ±0.15^{a}	5.65 ±0.41^{a}	12.35 ±1.06^{a}	3.97 ±0.19^{a}	19.28 ±0.33^{a}	0.51 ±0.11^{a}	60.7 6±1.27^{a}	39.29 ±0.86^{a}	280.29 ±14.88^{a}	68.23 ±0.02^{a}	6.48 ±0.046^{a}	25.99 ±0.01^{a}	60.63 ±0.07^{a}
Microwave 2 min	11.05 ±0.48^{b}	6.55 ±0.52^{a}	10.19 ±0.71^{b}	3.67 ±0.41^{a}	16.03 ±0.89^{b}	0.46 ±0.028^{ab}	58.34 ±0.48^{a}	40.53 ±1.73^{b}	263.85 ±2.47^{b}	65.47 ±1.15^{b}	6.93 ±0.99^{a}	25.2 ±1.03^{a}	61.33 ±0.9^{a}
Microwave 4 min	11.01 ±0.2^{b}	6.8 ±1^{a}	11.1 ±0.84^{ab}	3.66 ±0.56^{a}	9.85 ±0.8^{c}	0.4 ±0.02^{b}	57.23 ±2.2^{a}	42.35 ±1.31^{b}	262.02 ±1.99^{b}	62.37 ±0.99^{c}	6.63 ±1.06^{a}	25.34 ±1.15^{a}	64.26 ±1.04^{b}
Microwave 6 min	10.32 ±0.43^{b}	7.37 ±0.43^{a}	10.93 ±0.24^{ab}	3.57 ±0.51^{a}	8.56 ±0.48^{c}	0.38 ±0.02^{bc}	57.6 ±0.72^{a}	43.96 ±1.79^{b}	262.19 ±2.6^{b}	62.4 ±0.8^{c}	6.84 ±0.94^{a}	25.79 ±1.11^{a}	64.23 ±1.15^{b}
Control	12.16 ±0.15^{a}	5.65 ±0.41^{a}	12.35 ±1.06^{a}	3.97 ±0.19^{a}	19.28 ±0.33^{a}	0.51 ±0.11^{a}	60.7 6±1.27^{a}	39.29 ±0.86^{a}	280.29 ±14.88^{a}	68.23 ±0.02^{a}	6.48 ±0.046^{a}	25.99 ±0.01^{a}	60.63 ±0.07^{a}
Ultrasound 10 min	12.35 ±0.55^{a}	5.03 ±0.19^{ab}	10.03 ±0.75^{b}	3.35 ±0.46^{a}	16.32 ±0.44^{b}	0.48 ±0.08^{a}	59.04 ±0.97^{ab}	37.19 ±0.67^{b}	263.28 ±1.88^{b}	64.76 ±0.81^{bc}	7.51 ±1.07^{a}	25.8 ±0.84^{a}	63.13 ±0.99^{b}
Ultrasound 20 min	13.63 ±.56^{ab}	4.98 ±0.12^{ab}	10.28 ±0.42^{b}	3.19 ±0.56^{a}	16.15 ±0.08^{bc}	0.42 ±.03^{a}	57.72 ±0.43^{b}	36.99 ±0.65^{b}	257.84 ±3.93^{b}	63.80 ±0.65^{b}	7.60 ±1.06^{a}	25.4 ±0.76^{a}	63.63 ±0.80^{b}
Ultrasound 30 min	14.29 ±0.63^{b}	4.52 ±0.38^{b}	10.20 ±0.33^{b}	3.94 ±0.2^{a}	15.23 ±0.34^{c}	0.39 ±0.04^{a}	56.85 ±0.49^{bc}	35.84 ±0.95^{b}	260.82 ±3.88^{b}	65.37 ±0.36^{c}	6.68 ±0.62^{a}	25.35 ±0.78^{a}	61.29 ±.05^{ab}

Different letters denote significantly different in each treatment at α=0.05.

±: standard deviation, d.b. : dry base, w.b. : wet base.

3.3 Effect of Different Treatments on Red lentil Bran

3.3.1 Effect of Soaking on Red lentil Bran

3.3.1.1 Effect of Soaking on Chemical and Physical Properties of Red Lentil Bran

In the study the moisture, ash, protein, fat, starch, sugar, carbohydrates total dietary fibers and energy values were measured as 11.02, 4.81, 13.6, 1.19, 17.99, 0.59, 47.58, 27.52% and 213 kcal/100 g for red lentil bran was soaked for 10 min; 11.21, 4.27, 13.68, 1.21, 20.52, 0.53, 47.83, 27.7% and 214.26 kcal/100 g for 20 min; 11.9, 4.15, 13.94, 1.27, 22.23, 0.32, 47.44, 28.03% and 214 kcal/100 g for 30 min, respectively. All results are shown in Table 3.3.

The soaking of red lentil bran caused an increase ($p \leq 0.05$) in the moisture, protein, fat, starch, carbohydrates and calorie contents whereas ash, sugars and total dietary fiber contents decreased ($p \leq 0.05$). Soaking was significantly affective ($p \leq 0.05$) on the ash, fiber, starch, sugars and calorie values.

These results agreed with Barampama and Simard (1995) found an increase in starch, protein, fat, carbohydrates and fiber in beans after soaking in contrast to ash and soluble sugars. They were decreased ($p \leq 0.05$) after beans soaking, besides to decrease toxins and starch digestibility in beans. The fat decrease could be due to the formation of fat protein complexes. The decrease in ash and sugars resulted from the diffusion of minerals and sugars in water. The digestibility of starch decreased because starch tends to form polymers.

Aguilera et al. (2009) studied protein and total fiber contents of chickpea, white bean and pink mottled cream bean after soaking and dehydration they reported that soaked chickpea had 22.3% lower protein content than raw grains. The soluble fiber content of all soaked legumes was higher than control samples whereas insoluble fiber decreased. In Egounlety and Aworh (2003) study, it was found that the level of oligosaccharides was affected significantly after soaking. About 50% of raffinose and more than 55–60% of sucrose and stachyose lost, which in turn shows the importance of soaking in bean processing.

Doss et al. (2011) reported that soaking processing reduced various antinutritional compounds such as total free phenolic significantly.

Either to Desalegn (2015) both fat and carbohydrates increased in contrast to ash, fiber and protein that are showed lower values after soaking chickpea flours.

Khattab and Arntfield (2009) reported an increase in vitro protein digestibility of all soaked legumes was noticed. Whereas a slight difference was observed in percent in vitro protein digestibility of soaked faba beans, peas, chickpea, and kidney beans in El-Hady and Habiba (2003) study. There was a significant effect of soaking time on starch and sugar contents in red lentil bran in contrast to moisture, ash, fat, carbohydrates, fibers, calorie and protein contents in red lentil bran.

In soaked red lentil bran, all values of chemical properties increased by increasing with soaking time except moisture, ash and sugar contents. Both moisture content, fats, carbohydrates, ash, protein calorie and total fiber contents did not influence significantly ($p>0.05$) by changing the soaking period. There was a significant difference ($p\leq0.05$) between soaking for 10 min and soaking for 20 or 30 min in starch content of red lentil bran. While there was no significant difference ($p>0.05$) was observed between soaking for 20 and 30 min. No considerable effect difference ($p>0.05$) in sugar content was noticed between red lentil bran soaked for 10 and 20 min. Whereas there was a significant difference ($p\leq0.05$) in sugar content between red lentil bran soaked for 10 and 30 min in addition to the significant difference ($p\leq0.05$) in sugar content between red lentil bran soaked for 20 and 30 min.

It was found that the color values of soaked red lentil bran were 39.78 for L*, 8.16 for a*, 16.1 for b* and 65.23 for (YI) for 10 min; 41.29, 8.15, 17.1 and 66.14 for 20 min 40.52, 8.08, 16.56 and 65.72 for 30 min, respectively.

The redness (a*), yellowness index (YI) and yellowness (b*) increased and lightness (L*) of red lentil bran decreased ($p\leq0.05$) due to soaking. According to Bayram et al. (2004) soaking increased L* and b* and decreased a* and YI of soybean.

Soaking significantly affected ($p\leq0.05$) the L*, YI and b* values while a* value was not influenced by soaking. Soaking time affected significantly ($p\leq0.05$) the L*, a* and b* values. There was a significant difference ($p\leq0.05$) in L* between red lentil

bran soaked for 10 and 20 min. Either to b* the difference occured between samples soaked for 10 and 20 min or 30 min and between samples soaked for 20 and 30 min.

3.3.1.2 FTIR (Fourier Transform Infrared Spectroscopy) Analysis

The peaks between 3200-3400, 2935–2915, 1900-2300, 1650–1590, 1500-1600, 1560–1540, 1420-1300, 1210-1320, 1050–990, 820- 890 and 705–570 cm^{-1} correspond to O–H group stretching, C–H asymmetric stretching vibrations, the presence of cyano compounds, C–H symmetric stretching vibrations, aromatic ring bands, NH bending, C–H bending in CH, CH2 and CH3 compounds, C–O stretching mode, aliphatic phosphates (POC stretch), C–O–O stretching and the last indicates the presence of C–S stretching, respectively.

The peaks between 2865–2845 were lost after soaking. (Figure 3.17).

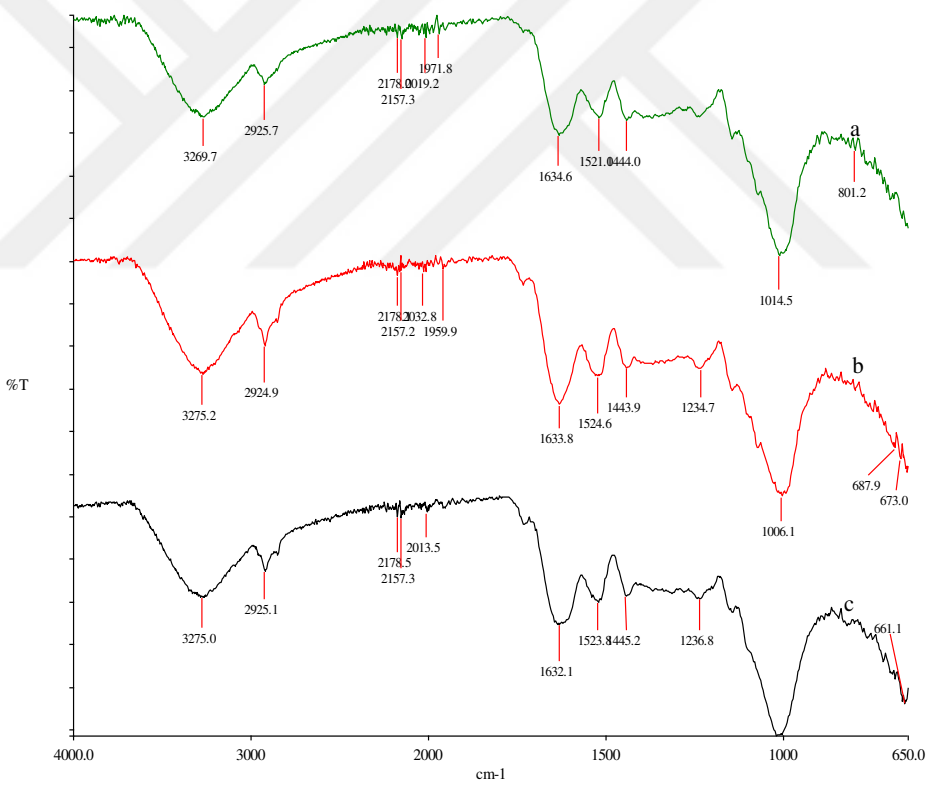
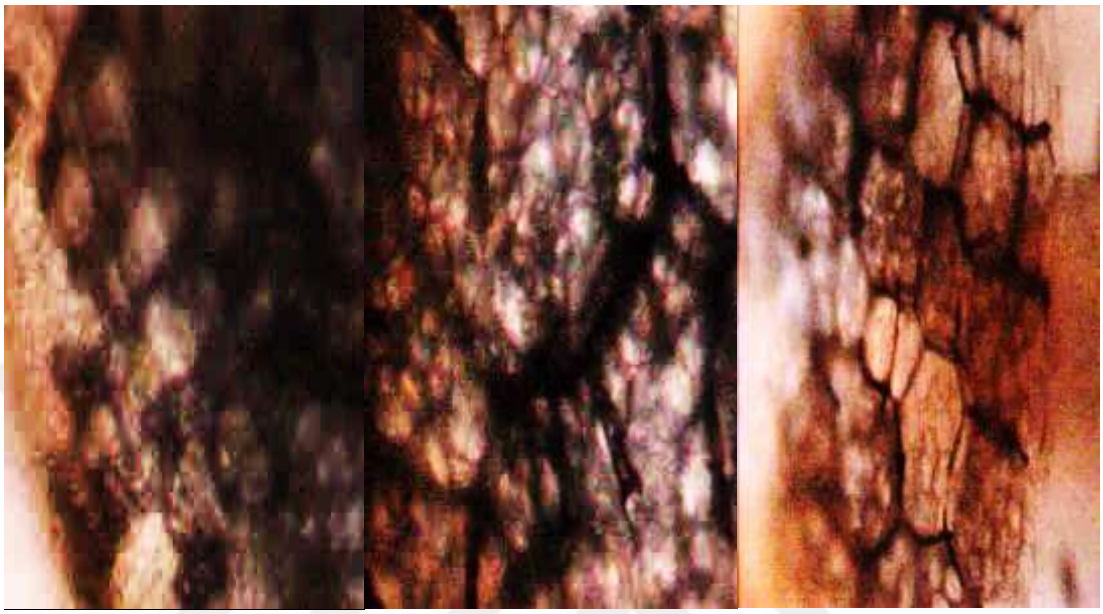


Figure 3.17 FTIR spectrum of (a) 10, (b) 20 and (c) 30 min soaked red lentil bran.

3.3.1.3 Microscopic Structural Analysis (MSA)

MSA was used to characterize the surface morphology of the red lentil bran. MSA of red lentil bran before and after treatment was taken at 10x magnifications (Figures 3.18).

The red lentil bran had a rough surface with a number of pores and cavities on its surface. The surface of the soaked red lentil bran was relatively intact. The irregular cavities and porous morphology could be accounted for some functional properties of dietary fibers.



(a) **(b)** **(c)**

Figure 3.18 Microscopic Structural Analysis for (a) 10 min, (b) 20 min and (c) 30 min soaked red lentil bran.

3.3.1.4 Thermal Properties by DSC (Differential Scanning Calorimetry) Analysis

The endothermic peaks during the thermal transition from DSC measurements referred to the melting of structural domains. One major endothermic peak was observed in soaked samples at a temperature about 166 - 177 C that corresponding to water evaporation in red lentil bran. The peak temperature of the treated groups was lower than in untreated samples. This indicates that more energy is required to pick up water from soluble dietary fibers available in red lentil bran samples as was observed in the root of Angelica keiskei (Xie et al., 2016).

The decrease of red lentil bran thermal stability after treatment was conducted from DSC thermograms in contrast to wheat bran. According to this study, the samples were soaked for 30 min were the most heat stable (Figure 3.19).

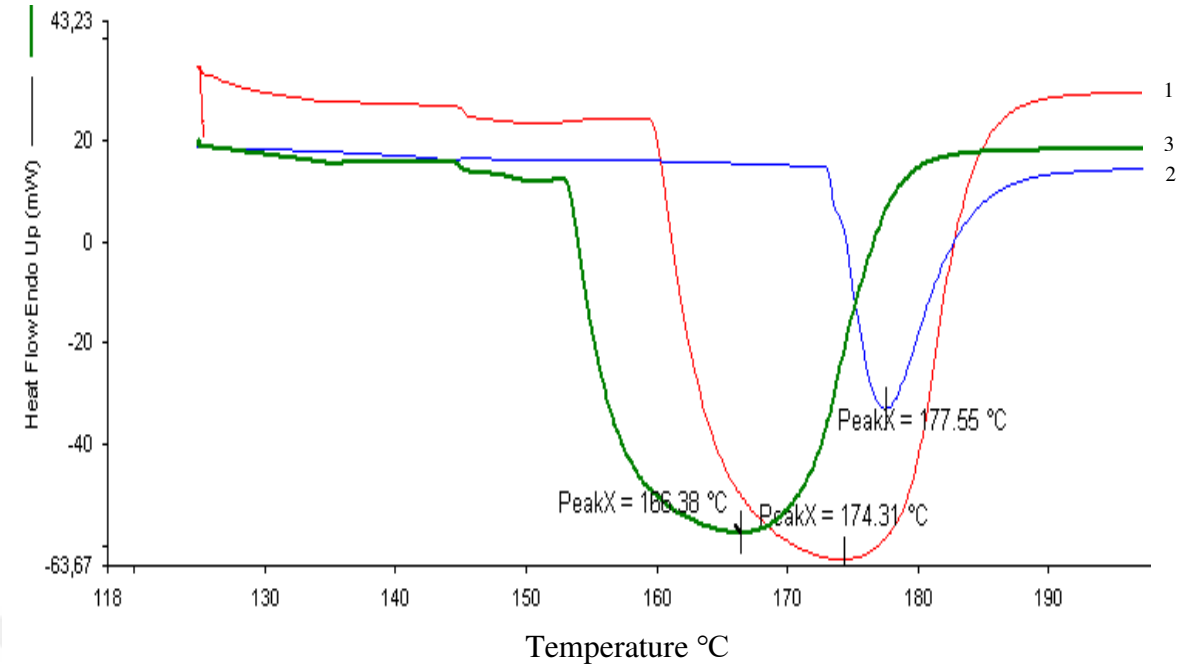


Figure 3.19 Thermal analysis of red lentil bran. (1) red lentil bran soaked for 10 min, (2) red lentil bran soaked for 10 min and (3) red lentil bran soaked for 10 min.

3.3.1.5 Effect of Simulated Gastric Fluids (SGF) on Soaked Red lentil Bran:

The moisture, ash, protein, fat, carbohydrate, total dietary fiber contents and energy values for 30 min soaked red lentil bran were as; 10.25% 3.75% 10.54% 1.28% 52.38%, 35.03% 223.42 kcal/100 g, respectively.

Total dietary fiber, ash, moisture and carbohydrate contents affected significantly ($p \leq 0.05$) after artificial stomach analysis.

3.3.2 Effect of Cooking on Red lentil Bran

3.3.2.1 Effect of Cooking on Chemical and Physical Properties of Red lentil Bran

In the study, the initial moisture, ash, protein, fat, starch, sugar, carbohydrates, total dietary fiber and calorie contents of cooked bran were measured as 12.14, 4.2, 13.71, 1.17, 7.13,0.78, 46.89, 28.39% and 210.66 kcal/100 for 10 min; 12.19, 4.17, 13.93, 2.02, 7.31, 0.83, 45.89, 28.83% and 214.72 kcal/100 g 20 min; 12.34, 3.91, 14.13, 2.25, 7.13, 0.87, 45.57, 29.15% and 215.99 kcal/100 g for 30 min, respectively (Table 3.3).

In comparison with raw red lentil bran, cooking was effective on the moisture ash, starch, total dietary fiber contents and energy values that increased. The total dietary fiber and energy values showed a significant increase ($p \leq 0.05$). In contrast, the moisture content, ash and starch contents decreased significantly ($p \leq 0.05$) after cooking. There was no significant effect ($p > 0.05$) of cooking on protein, fat, sugars and carbohydrate in red lentil bran. These results are similar to the study of Barampama and Simard (1995) that cooking increased the sugars and total dietary fiber contents and decreased ash content due to the liberation of minerals in cooking water but did not affect a protein content. The significant increase in total dietary fiber was also observed by Barampama and Simard (1995) due to the formation of resistant starch whereas starch in cooked samples had become indigestible by amylopectin enzymes hence resistant starch was the main reason for insoluble dietary fiber increasing and protein fiber complex occurrence during cooking illustrate the increasing of total dietary fibers in lentil bran (Bressani, 1993). Whilst Kutoš et al. (2003) studied the effect of thermal processing on kidney beans and conducted that the polysaccharides solubilization reduces total fiber content as a result of losing soluble fiber. The starch reduction resulted from starch solubilization (Bishnoi and Khetarpaul, 1993; Nnanna and Phillips, 1990), which in turn increased soluble sugars increased (Buckle and Sambudi, 1990).

In cooked red lentil bran sugars, carbohydrates, calorie and total dietary fiber contents increased as long as time increased in contrast to moisture content, ash and starch content that decreased by increasing the time of soaking.

It was found that color values of cooked red lentil bran were 35.13 for L*, 6.68 for a*, 12.17 for b* and 58.71 for (YI) for 10 min; 63.80, 7.6, 25.4 and 63.63 for 20 min; 65.37, 6.68, 25.35 and 61.29 for 30 min, respectively.

During the cooking of red lentil bran, redness (a*), yellowness index (YI) and yellowness (b*) increased in contrast to lightness (L*) that decreased after cooking.

The difference between cooking times was effective on sugars in red lentil bran in contrast to starch, carbohydrates, fats, total dietary fibers and protein.

There was a significant difference ($p \leq 0.05$) in moisture content between 10, 20 and 30 min. A significant difference ($p \leq 0.05$) was also noticed in sugars content between red lentil bran cooked for 10, 20 and 30 min.

Cooking affected significantly ($p \leq 0.05$) lightness (L*) yellowness index (YI), and yellowness (b*) but redness (a*) showed a negligible difference after cooking. Time was effective only on L* value.

3.3.2.2 FTIR (Fourier Transform Infrared Spectroscopy) Analysis

The peaks between 3200-3400, 2935–2915, 1900-2300, 1650–1590, 1500-1600, 1560–1540, 1420-1300, 1210-1320, 1050–990, 820- 890 and 705–570 cm^{-1} correspond to O–H group stretching, C–H asymmetric stretching vibrations, the presence of cyano compounds, C–H symmetric stretching vibrations, aromatic ring bands, NH bending, C–H bending in CH, CH2 and CH3 compounds, C–O stretching mode, aliphatic phosphates (POC stretch), C–O–O stretching and the last indicates the presence of C–S stretching, respectively.

Many new functional groups were observed after cooking such as 1000-1300,1300-1500,1590-1650 cm^{-1} which correspond to C–C stretch, C–H bending and N–H bending in primary amine, respectively (Figure 3.20).

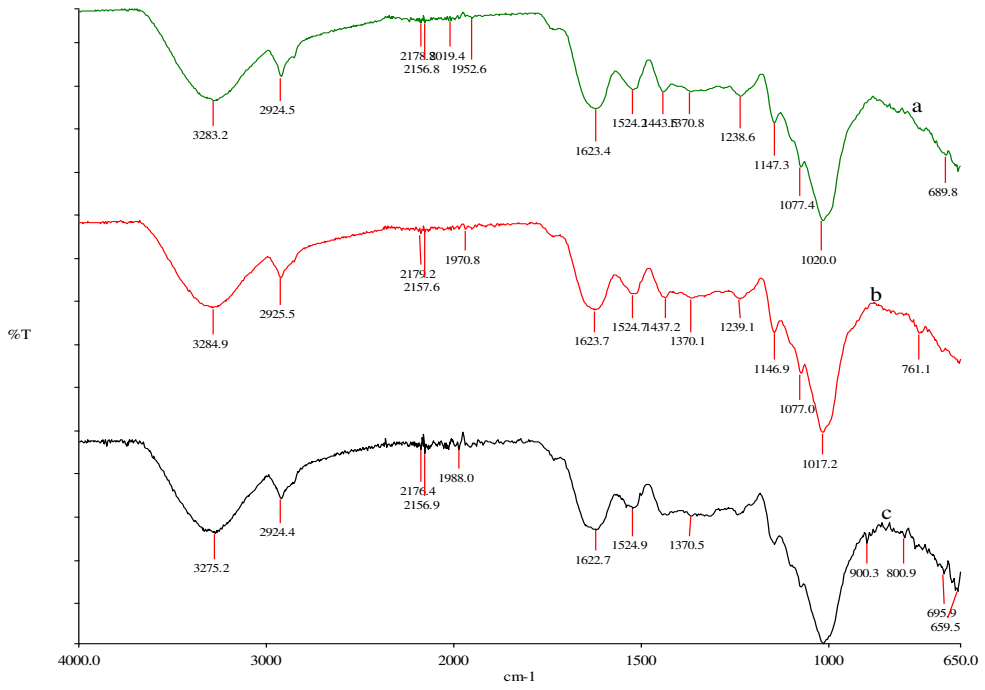
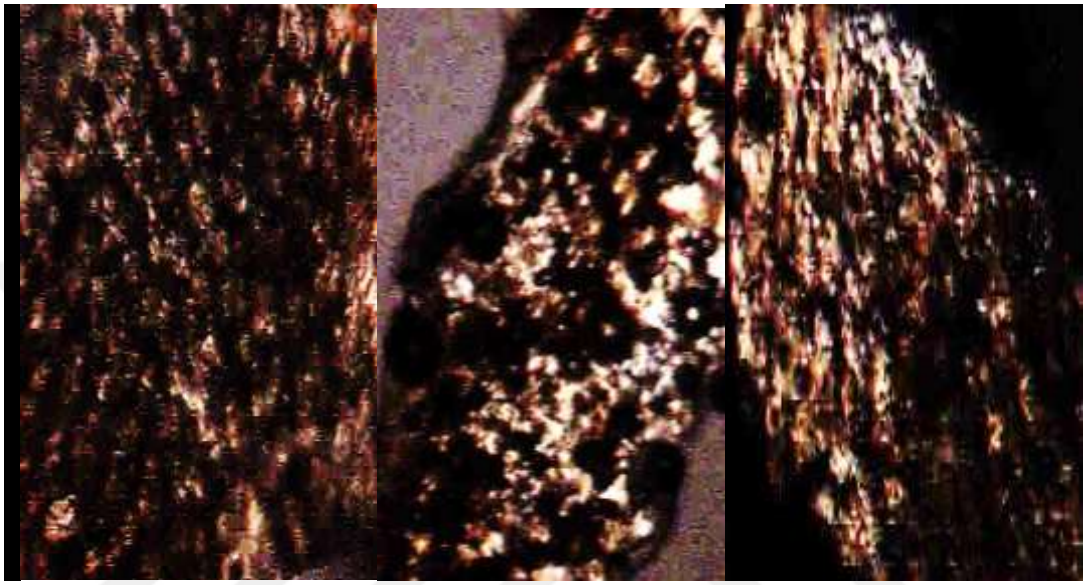


Figure 3.20 FTIR spectrum of (a) 10, (b) 20 and (c) 30 min cooked red lentil bran.

3.3.2.3 Microscopic Structural Analysis (MSA)

MSA was used to characterize the surface morphology of the red lentil bran. MSA of red lentil bran before and after treatment was taken at 10x magnifications (Figure 3.21). The red lentil bran had a rough surface with a number of pores and cavities on its surface. The surfaces of cooked samples were partially disintegrated, whereas the surface was more compact.



(a) **(b)** **(c)**

Figure 3.21 Microscopic structural analysis for (a) 10 min, (b) 20 min and (c) 30 min cooked red lentil bran.

3.3.2.4 Thermal Properties by DSC (Differential Scanning Calorimetry) Analysis

The endothermic peaks during the thermal transition from DSC measurements referred to the melting of structural domains. One major endothermic peak was observed in all samples at a temperature about 170 - 174 C that corresponding to water evaporation in red lentil bran. The peak temperature of the treated groups was lower than in untreated samples. This could indicate that more energy is required to release water from soluble dietary fibers available in raw red lentil bran samples. The decrease of red lentil bran thermal stability after treatment was conducted from DSC thermograms in contrast to wheat bran. These endothermic peaks could correspond to the melting point of resistant starch type 3 as reported by Sievert and Pomeranz (1990), whereas starch exhibited a transition from 132 to 163 C this thermal effect

has been attributed to the melting of recrystallized amylose and was also observed in resistant starch fractions from amylomaize. According to that study, the observed peaks refer to soluble dietary fiber degradation (Figure 3.22).

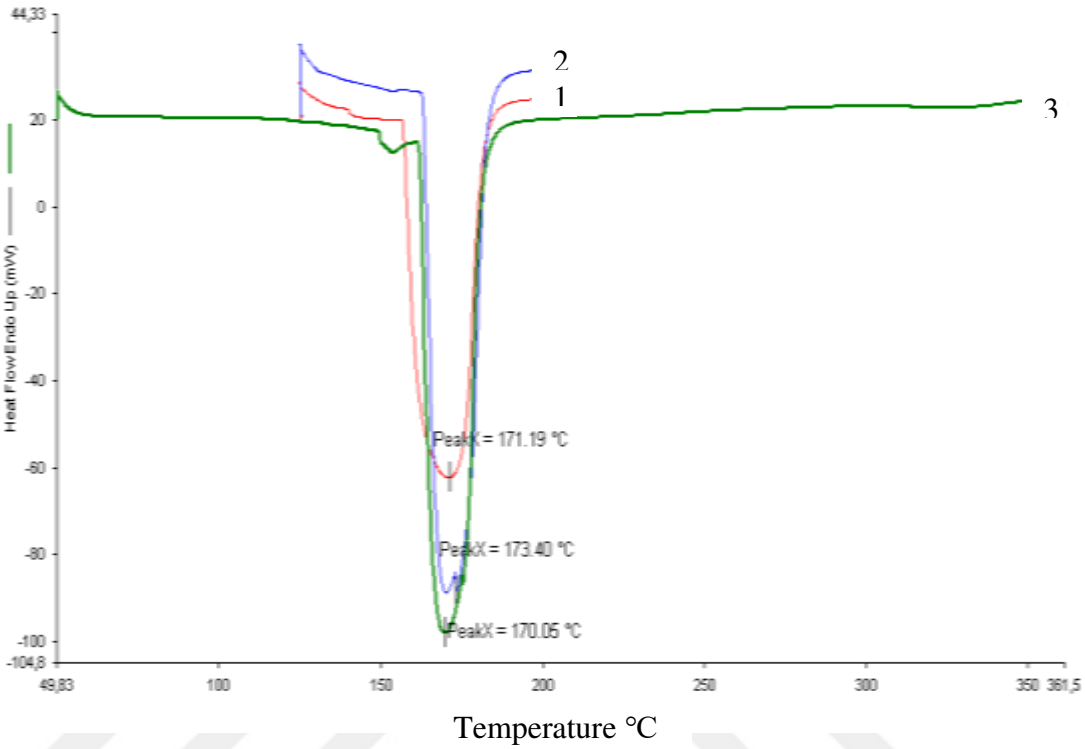


Figure 3.22 Thermal analysis of red lentil bran. (1) red lentil bran cooked for 10 min, (2) red lentil bran cooked for 20 min and (3) red lentil bran cooked for 30 min.

3.3.2.5 Effect of Simulated Gastric Fluids (SGF) on Cooked Red lentil Bran:

The moisture, ash, protein, fat, carbohydrate, total dietary fiber contents and energy values for red lentil bran cooked for 10 min were noted as 12.43%, 4.01%, 1.1%, 10.01%, 46.61% 32.34% and 200.02 kcal/100 g, respectively.

Moisture, carbohydrate, total dietary fiber, ash contents and energy value decreased significantly ($p \leq 0.05$) after artificial stomach analysis.

3.3.3 Effect of Microwave on Red lentil Bran

3.3.3.1 Effect of Microwave on Chemical and Physical Properties of Red lentil Bran

A proximate composition and energy values of microwaved red lentil bran samples (Table 3.3) showed that the moisture content, ash, protein, fat, starch, sugar,

carbohydrates, total dietary fiber and calorie contents of microwaved red lentil bran were measured as 11.29, 3.76, 14.58, 1.18, 7.45, 0.52, 25.8% and 214.63 kcal/100 g for 2 min; 1183, 3.75, 14.13, 1.63, 7.13, 0.63, 46.85, 26.8% and 215.44 kcal/100 g for 4 min; 12.54, 3.63, 13.7, 1.77, 7.4, 0.78, 47.18, 36.9% and 215.2 kcal/100 g for 6 min, respectively. In comparison to raw red lentil bran, ash, starch and carbohydrate contents decreased by microwave treatment in contrast to total dietary fiber, moisture, protein, fat sugars and ash contents. Microwave treatment was significantly effective ($p \leq 0.05$) on the starch, ash, fiber and moisture contents.

Microwave treatment brought a decrease in starch and ash content and did not show a significant effect ($p > 0.05$) on protein and fat content that agreed with other studies carried out legumes. Khatoon and Prakash (2006) reported that the cooking by microwave brought a slight decrease in starch content, this may be attributed to the loss of solids in the cooking water. The decrease in the ash content of microwaved samples that could be due to the diffusion of minerals in the water. Khatoon and Prakash (2006) conducted a decrease in protein digestibility after microwave that could be attributed to the Maillard reaction during heating. The same study found an increase in the starch digestibility of microwaved legume starch as a result of swelling and rupture of starch granules, which in turn facilitates the role of amylase in hydrolysis also disrupts the inhibitors of amylase and phytate (Jood et al., 1998). The increment in total dietary fibers of microwaved legumes could result from resistant starch that accumulated and isolated as dietary fiber components (Khatoon and Prakash, 2004).

In microwaved red lentil bran, time was affective in sugar and protein content. There was a significant difference ($p \leq 0.05$) in sugars content between red lentil bran microwaved 2 and 6 min. A significant difference was noticed in protein content between red lentil bran microwaved for 2, 4 and 6 min.

It was found that the color values of microwaved red lentil bran were 42.6 for L*, 8.21 for a*, 15.58 for b* and 61.83 for (YI) for 2 min; 38.64, 8.08, 15.43 and 64.82 for 4 min; 37.29, 7.15, 14.57 and 61.78 for 30 min, respectively.

YI, a* and L* were affected significantly ($p \leq 0.05$) by microwave treatment in contrast to b* that was not affected significantly by microwave treatment. There was

a significant difference in L* values in all times of microwave treatment. YI value showed a significant difference between 2 and 4 min, also a significant difference was observed between 4 and 6 min. A significant difference in a* was noticed between red lentil bran microwaved for 2 and 4 min. Finally, yellowness b* showed a considerable difference between 2 and 4 min.

3.3.3.2 FTIR (Fourier Transform Infrared Spectroscopy) Analysis

The peaks between 3200-3400, 2935–2915, 1900-2300, 1650–1590, 1500-1600, 1560–1540, 1420-1300, 1210-1320, 1050–990, 820- 890 and 705–570 cm^{-1} correspond to O–H group stretching, C–H asymmetric stretching vibrations, the presence of cyano compounds, C–H symmetric stretching vibrations, aromatic ring bands, NH bending, C–H bending in CH, CH2 and CH3 compounds, C–O stretching mode, aliphatic phosphates (POC stretch), C–O–O stretching and the last indicates the presence of C–S stretching, respectively.

Many new functional groups were observed after microwave treatment such as 1000-1300, 1300-1500, 1590-1650 cm^{-1} which correspond to C–C stretch, C–H bending and N–H bending in primary amine, respectively (Figure 3.23).

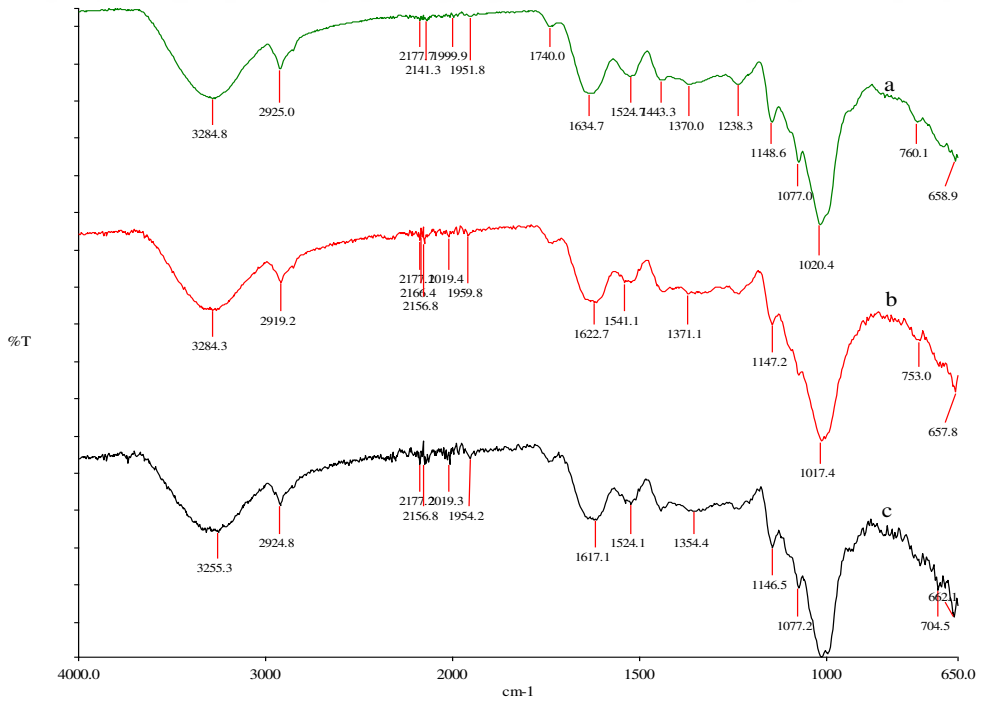
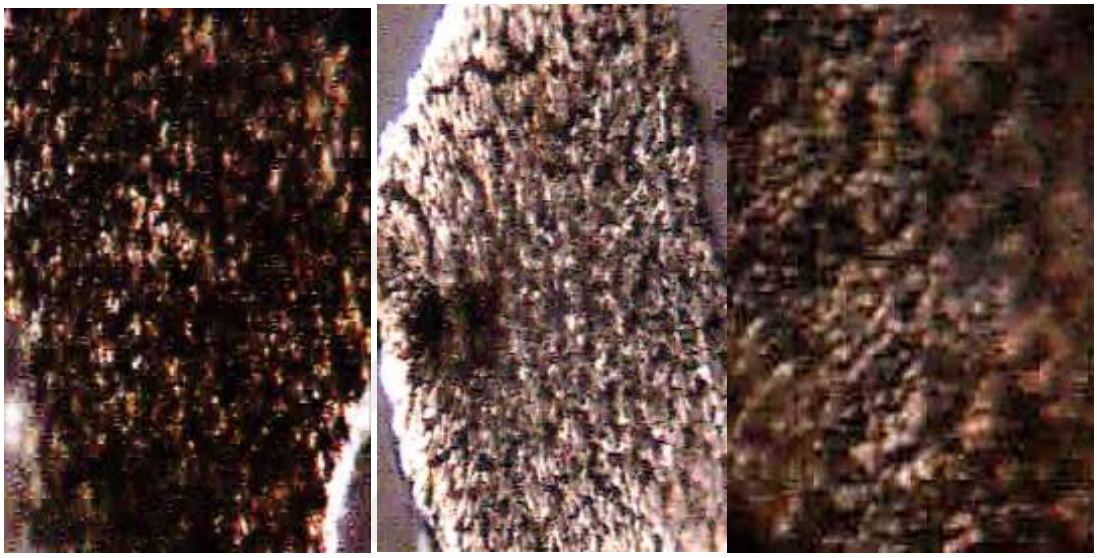


Figure 3.23 FTIR spectrum of (a) 2, (b) 4 and (c) 6 min microwaved red lentil bran.

3.3.3.3 Microscopic Structural Analysis (MSA)

MSA was used to characterize the surface morphology of the red lentil bran. MSA of red lentil bran before and after treatment was taken at 10x magnifications (Figure 3.24). The red lentil bran had a rough surface with a number of pores and cavities on its surface.

The surfaces of microwaved samples were partially disintegrated, whereas the surface was more compact.



(a) **(b)** **(c)**

Figure 3.24 Microscopic Structural Analysis for (a) 2 min, (b) 4 min and (c) 6 min microwaved red lentil bran.

3.3.3.4 Thermal Properties by DSC (Differential Scanning Calorimetry) Analysis

The endothermic peaks during the thermal transition from DSC measurements referred to the melting of structural domains. One major endothermic peak was observed in all samples at a temperature about 164 - 171 C. These endothermic peaks could correspond to the melting point of resistant starch type 3 as reported by Sievert and Pomeranz (1990), whereas starch exhibited a transition from 132 to 163 C this thermal effect has been attributed to the melting of recrystallized amylose and was also observed in RS fractions from amylomaize. According to the observed peaks refers to soluble dietary fiber degradation (Figure 3.25).

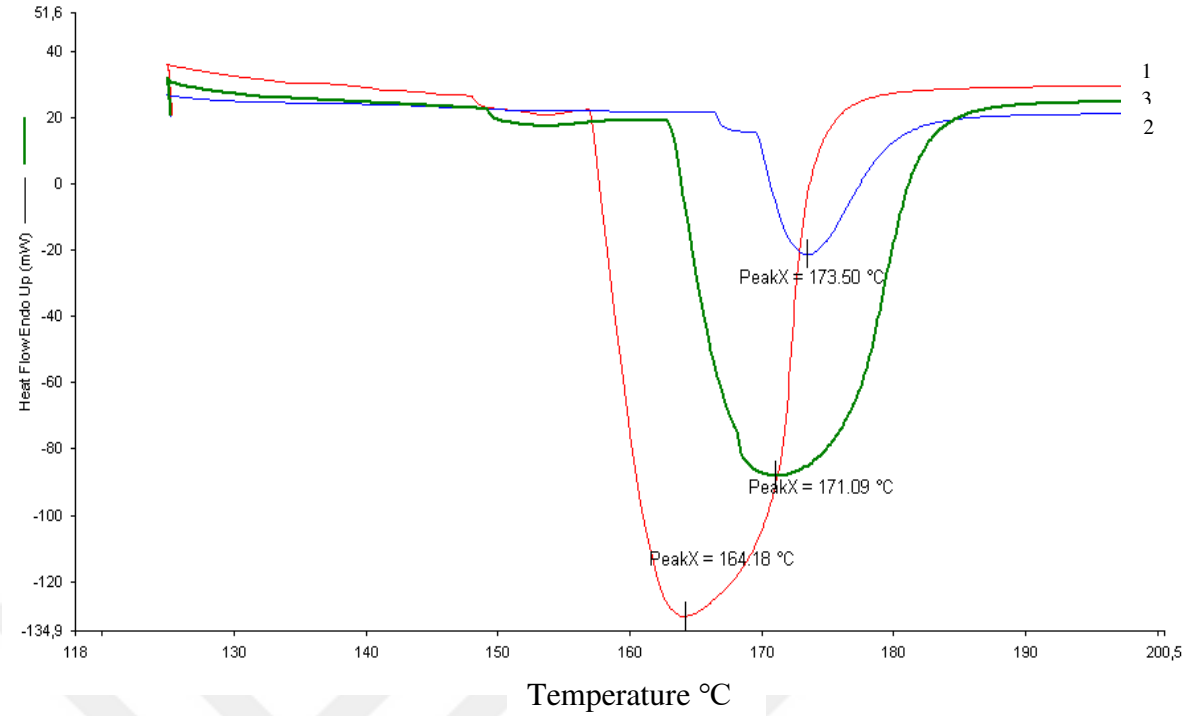


Figure 3.25 Thermal analysis of microwaved red lentil bran for (1) 2 min, (2) red 4 min and (3) 6 min.

3.3.3.5 Effect of Simulated Gastric Fluids (SGF) on Microwaved Red lentil Bran

The moisture, ash, protein, fat, carbohydrate, total dietary fiber contents and energy values for red lentil bran treated with microwave for 10 min were as; 11.59%, 3.29%, 1.56%, 10.29%, 47.12%, 39.06% and 213.98 kcal/100 g, respectively.

Total dietary fiber, ash, carbohydrate contents decreased significantly ($p \leq 0.05$) after artificial stomach analysis due to the diffusion of soluble dietary fiber into SGF and the degradation of protein by the digestive enzymes.

3.3.4 Effect of Ultrasound on Red lentil Bran

3.3.4.1 Effect of Ultrasound on Chemical and Physical Properties of Red lentil Bran

A proximate composition and energy values of red lentil bran samples treated with ultrasound (Table 3.3) showed that the moisture content, ash, protein, fat, starch, sugars, carbohydrates, total dietary fiber and calorie contents of ultrasound treated samples were measured as 11.85, 5.47, 12.02, 0.89, 11.9, 0.56, 47.96, 22.22% and 208 kcal/100 g for 10 min ultrasound processed red lentil bran; 12.19, 5.19, 12.09, 0.82, 11.89, 0.31, 47.91, 20.7% and 207.4 kcal/100 g for red lentil bran was treated

with ultrasound for 20 min 12.08, 4.71, 11.62, 0.78, 11.01, 0.25, 49, 20.63% and 209.83 kcal/100 g for lentil bran that was treated with ultrasound for 30 min, respectively.

All values decreased ($p \leq 0.05$) during the ultrasound application except moisture content. Ultrasound treatment was significantly effective ($p \leq 0.05$) on the starch, fiber, protein, moisture, ash, sugar and calorie values. The effect of ultrasound on total dietary fibers available in red lentil bran in agreement with Kaya et al. (2017) reported a significant decrease in total and insoluble dietary fiber in pulses with ultrasound processing. Li et al. (2014) reported that ultrasound assisted extraction resulted in higher soluble dietary fibers yield in apple pomace. Besides, ultrasound treatment did not affect significantly on phytic acid content of the pulse hulls.

A modification of the secondary and tertiary structure of protein resulted from the breakdown of hydrogen bonding or Van der Walls interaction in the polypeptide chains was observed by Ercan and Soysal (2013). The effect of ultrasound was significant for water absorption due to water filling into free capillary intermolecular voids. In starch isolation from maize flour and hominy feed, at 20 kHz, 10% slurry, 100% amplitude, increased the recovery of starch. The recovered starch had decreased protein and lipid contents (Zhang et al., 2005).

It was found that color values of ultrasound treated red lentil bran were 39.52 for L*, 7.49 for a*, 17.27 for b* and 67.06 for YI for 10 min; 42.12, 8.1, 17.45 and 65.45 for 20 min; 30.98, 8.28, 17.62 and 64.39 for 30 min, respectively. During the ultrasonication, a*, YI and yellowness b* increased but L* of red lentil bran decreased.

Ultrasonication had a significant ($p \leq 0.05$) effect on the L* YI, a* and b* values. Ultrasonication time did not affect color values significantly ($p > 0.05$). It was effective only on sugar content. In the literature starch isolation from yellow dent maize at 20 kHz, ultrasonication was applied at various stages during processing, ultrasonication increased the starch yield and purity, increased the whiteness and decreased the yellowness of starch (Zhang et al., 2005). In this study, the time of ultrasonication affected significantly ($p \leq 0.05$) a redness and yellowness index. There

was a significant effect in a* between 10 min and 20 min as for YI, there was a significant effect ($p \leq 0.05$) between all times.

3.3.4.2 FTIR (Fourier Transform Infrared Spectroscopy) Analysis

The peaks between 3200-3400, 2935–2915, 1900-2300, 1650–1590, 1500-1600, 1560–1540, 1420-1300, 1210-1320, 1050–990, 820- 890 and 705–570 cm^{-1} correspond to O–H group stretching, C–H asymmetric stretching vibrations, the presence of cyano compounds, C–H symmetric stretching vibrations, aromatic ring bands, NH bending, C–H bending in CH, CH2 and CH3 compounds, C–O stretching mode, aliphatic phosphates (POC stretch), C–O–O stretching and the last indicates the presence of C–S stretching, respectively.

Ultrasound treated samples show functional groups similar to those found after soaking treatments (Figure 3.26).

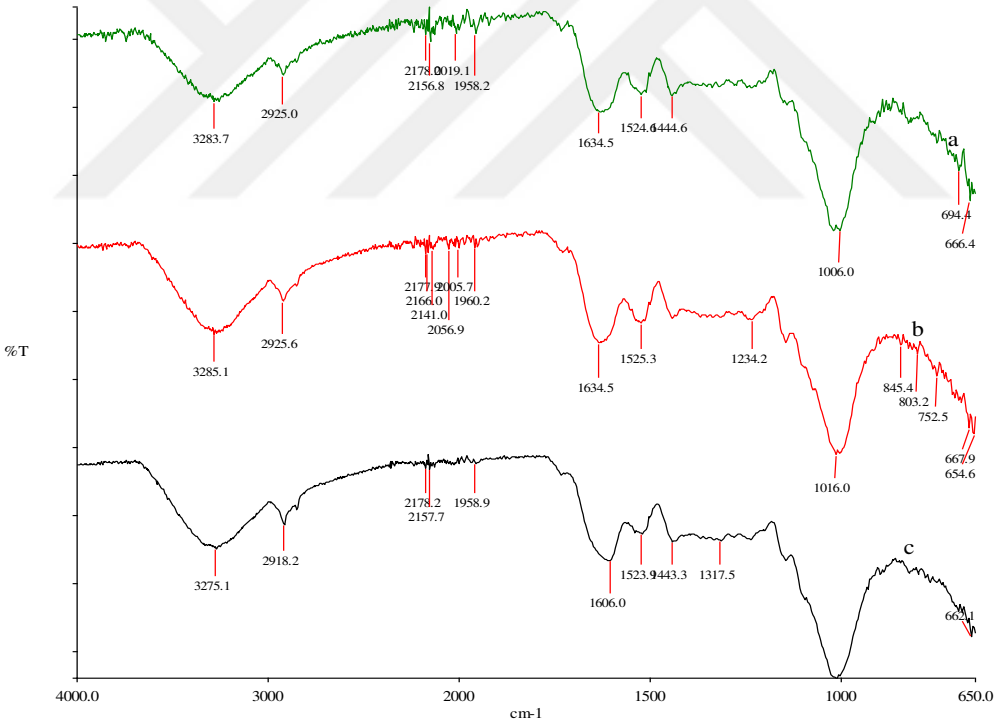


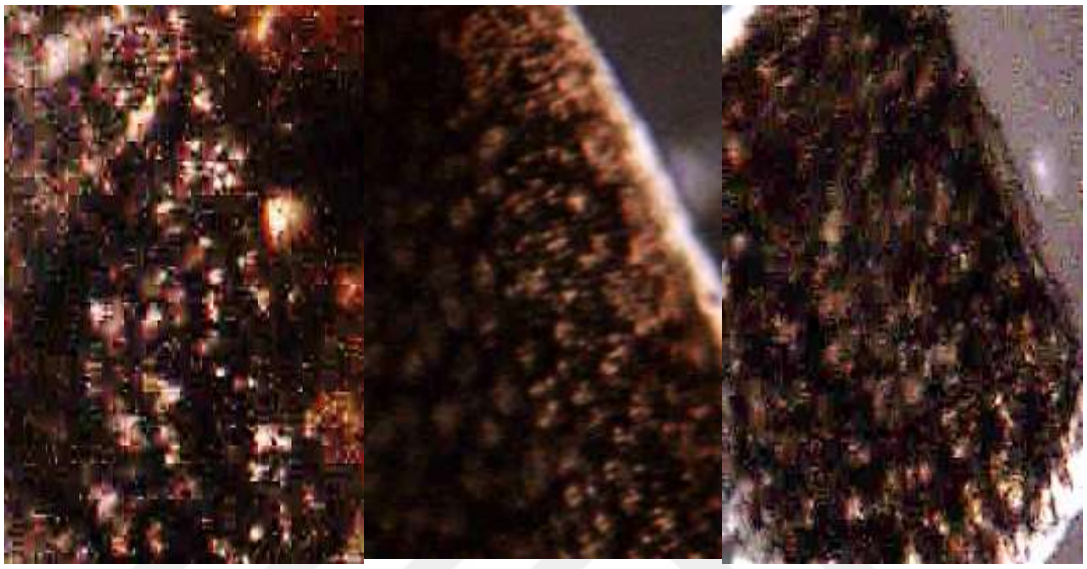
Figure 3.26 FTIR spectrum of (a) 10, (b) 20 and (c) 30 min ultrasound treated red lentil bran.

3.3.4.3 Microscopic Structural Analysis (MSA)

MSA was used to characterize the surface morphology of the red lentil bran. MSA of red lentil bran before and after treatment was taken at 10x magnifications (Figure

3.27). The red lentil bran had a rough surface with a number of pores and cavities on its surface.

The surface of the ultrasound treated red lentil bran was partially disintegrated, whereas the surface was more compact. The irregular cavities and porous morphology could be accounted for some functional properties of dietary fibers.



(a) **(b)** **(c)**

Figure 3.27 Microscopic Structural Analysis for (a) 10 min, (b) 20 min and (c) 30 min ultrasound treated red lentil bran.

3.3.4.4 Thermal Properties by DSC (Differential Scanning Calorimetry) Analysis

The endothermic peaks during the thermal transition from DSC measurements referred to the melting of structural domains. One major endothermic peak was observed in all samples at a temperature about 160 - 174 C that corresponding to water evaporation in red lentil bran (Figure 3.28).

These endothermic peaks could correspond to the melting point of resistant starch type 3 as reported by Sievert and Pomeranz (1990).

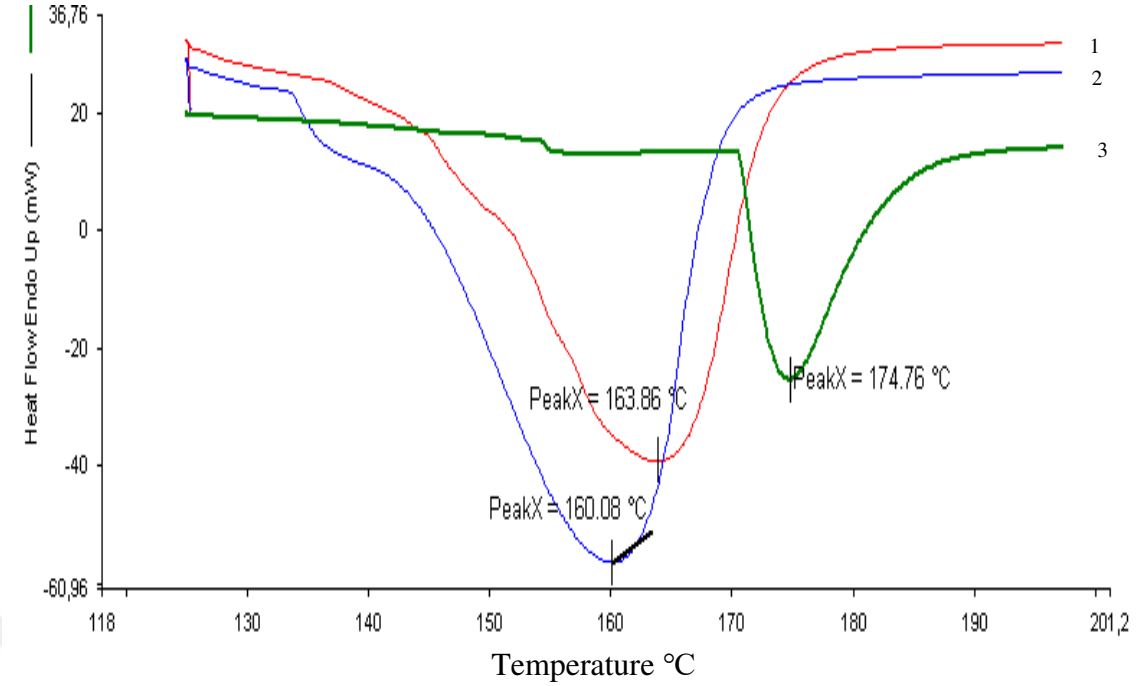


Figure 3.28 Thermal analysis of ultrasound treated red lentil bran (1) red lentil bran treated for 10 min, (2) red lentil bran treated for 20 min and (3) red lentil bran treated for 30 min.

3.3.4.5 Effect of Simulated Gastric Fluids (SGF) on Red lentil Bran Treated with Ultrasound

The moisture, ash, protein, fat, carbohydrate, total dietary fiber contents and energy values for red lentil bran treated with ultrasound for 10 min, were measured as; 12.38%, 4.1%, 0.88%, 8.92%, 49.25%, 36.7 % and 195.67 kcal/100 g, respectively.

Ash, moisture contnt, total dietary fiber, protein contents and energy value decreased significantly ($p \leq 0.05$) after artificial stomach analysis.

Table 3.3 A proximate composition of raw and processed red lentil bran

Treatment	Moisture content (%, w.b.)	Ash (%, d.b.)	Protein (%, d.b.)	Fat (%, d.b.)	Starch (%, d.b.)	Sugars (%, d.b.)	Carbohydrates (%, d.b.)	Total dietary fibers (%, d.b.)	Calorie (kcal/100 g)	L*	a*	b*	YI
Raw	10.85 ±0.52^{a}	5.95 ±0.35^{a}	13.52 ±0.56^{a}	1.17 ±0.42^{a}	16.66 ±0.5^{a}	0.79 ±0.07^{a}	47.65 ±0.03^{a}	23.06 ±0.83^{a}	212 ±18.05^{a}	59.45 ±0.35^{a}	6.19 ± 0.01^{a}	15.87 ±0.22^{a}	47.5 ±0.59^{a}
Soaking 10 min	11.02 ±0.3^{3a}	4.81 ±0.59^{ab}	13.60 ±1.21^{a}	1.19 ±0.26^{a}	17.99 ±0.57^{ab}	0.59 ±0.07^{ab}	47.58 ±1.18^{a}	27.52 ±0.94^{b}	213 ±2.19^{a}	39.78 ±0.7^{b}	8.16 ±0.05^{b}	16.15 ±0.07^{a}	65.23 ±1.76^{b}
Soaking 20 min	11.21 ±0.38^{a}	4.27 ±0.46^{b}	13.68 ±1.22^{a}	1.21 ±0.12^{a}	20.52 ±1.34^{bc}	0.53 ±0.07^{bc}	47.83 ±1.5^{a}	27.7 ±0.8^{b}	214.26 ±4.19^{a}	41.29 ±0.29^{c}	8.15 ±0.055^{b}	17.17 ±0.06^{b}	66.14 ±0.19^{b}
Soaking 30 min	11.4 ±0.41^{a}	4.15 ±0.48^{bc}	13.94 ±0.96^{a}	1.27 ±0.24^{a}	22.23 ±1.25^{c}	0.32 ±0.02^{c}	47.44 ±0.86^{a}	28.03 ±1.49^{b}	214 ±3.32^{a}	40.52 ±0.5^{bc}	8.08 ±0.017^{b}	16.56 ±0.14^{c}	65.72 ±1.49^{b}
Raw	10.85 ±0.52^{a}	5.95 ±0.35^{a}	13.52 ±0.56^{a}	1.17 ±0.42^{a}	16.66 ±0.5^{a}	0.79 ±0.07^{a}	47.65 ±0.03^{a}	23.06 ±0.83^{a}	212 ±18.05^{a}	59.45 ±0.35^{a}	6.19 ± 0.01^{a}	15.87 ±0.22^{a}	47.5 ±0.59^{a}
Cooking 10 min	12.14 ±0.4^{b}	4.28 ±0.5^{b}	13.71 ±0.41^{a}	1.17 ±0.36^{a}	7.51 ±0.41^{b}	0.78 ±.02^{a}	46.89 ±1.42^{a}	28.39 ±0.55^{b}	210.66 ±1.63^{a}	35.13 ±1.13^{b}	6.68 ±0.42^{a}	12.17±1.86^{b}	58.7 ±1.76^{b}
Cooking 20 min	12.19 ±.49^{b}	4.17 ±0.52^{b}	13.93 ±0.99^{a}	1.02 ±0.12^{a}	7.31 ±0.26^{b}	0.91 ±0.03^{ab}	45.89 ±1.99^{a}	28.83 ±0.77^{b}	214.72 ±3.97^{a}	34.59 ±0.89^{b}	6.77 ±1.01^{a}	11.83 ±1.43^{b}	56.04 ±2.05^{b}
Cooking 30 min	12.34 ±.36^{b}	3.91 ±0.82^{b}	14.13 ±0.35^{a}	0.9 ±0.04^{a}	7.13 ±0.17^{b}	1.02 ±0.08^{b}	45.57 ±1.42^{a}	29.15 ±0.21^{b}	215.99 ±1.49^{a}	28.97 ±1.23^{c}	6.21 ±0.32^{a}	10.48 ±1.04^{b}	55.63 ±2.2^{b}
Raw	10.85 ±0.52^{a}	5.95 ±0.35^{a}	13.52 ±0.56^{a}	1.17 ±0.42^{a}	16.66 ±0.5^{a}	0.79 ±0.07^{a}	47.65 ±0.03^{a}	23.06 ±0.83^{a}	212 ±18.05^{a}	59.45 ±0.35^{a}	6.19 ± 0.01^{a}	15.87 ±0.22^{a}	47.5 ±0.59^{a}
Microwave 2 min	11.29 ±0.57^{ab}	3.76 ±0.18^{b}	14.58 ±0.55^{a}	1.18 ±0.2^{a}	7.45 ±0.46^{b}	0.52 ±0.11^{b}	47.39 ±0.74^{a}	25.8 ±0.8^{b}	214.63 ±1.76^{a}	42.6 ±0.45^{b}	8.21 ±0.16^{b}	15.58 ±0.44^{a}	61.83 ±0.06^{b}
Microwave 4 min	11.83 ±0.73^{ab}	3.75 ±0.17^{b}	14.13 ±0.14^{ab}	1.63 ±0.5^{a}	7.13 ±0.62^{b}	0.63 ±0.09^{ab}	46.85 ±1.25^{a}	26.8 ±1.1^{b}	215.44 ±0.82^{a}	38.64 ±0.55^{c}	8.08 ±0.07^{b}	15.43 ±0.02^{a}	64.82 ±0.1^{c}
Microwave 6 min	12.54 ±0.48^{b}	3.63 ±0.51^{b}	13.07 ±0.67^{b}	1.77 ±0.19^{a}	7.04 ±.55^{b}	0.78 ±.05^{a}	47.18 ±0.73^{a}	26.98 ±0.19^{b}	215.2 ±1.88^{a}	37.29 ±0.56^{a}	7.15 ±0.19^{c}	14.57 ±0.16^{b}	61.78 ±0.55^{b}
Raw	10.85 ±0.52^{a}	5.95 ±0.35^{a}	13.52 ±0.56^{a}	1.17 ±0.42^{a}	16.66 ±0.5^{a}	0.79 ±0.07^{a}	47.65 ±0.03^{a}	23.06 ±0.83^{a}	212 ±18.05^{a}	59.45 ±0.35^{a}	6.19 ± 0.01^{a}	15.87 ±0.22^{a}	47.5 ±0.59^{a}
Ultrasound 10 min	11.85 ±1.45^{a}	5.47 ±0.39^{a}	12.02 ±0.77^{ab}	0.89 ±0.14^{a}	11.9 ±0.63^{b}	0.56 ±0.04^{b}	47.96 ±2.53^{a}	22.22 ±3.05^{a}	208 ±6.65^{a}	39.52 ±0.38^{a}	7.49 ±0.43^{b}	17.27 ±0.14^{b}	67.06 ±0.19^{b}
Ultrasound 20 min	12.19 ±0.93^{a}	5.19 ±0.53^{a}	12.09 ±0.5^{ab}	0.82 ±0.12^{a}	11.89 ±0.63^{b}	0.31 ±0.05^{c}	47.91 ±1.85^{a}	20.7 ±0.2^{a}	207.4 ±5.31^{a}	42.12 ±0.53^{a}	8.1 ±0.17^{b}	17.45 ±0.34^{b}	65.45 ±0.42^{c}
Ultasound 30 min	12.08 ±0.80^{a}	4.71 ±0.55^{a}	11.62 ±0.72^{b}	0.78 ±0.08^{a}	11.01 ±0.65^{b}	0.25 ±00.05^{c}	49.00 ±0.84^{a}	20.63 ±1.99^{a}	209.83 ±1.59^{a}	30.98 ±2.67^{a}	8.28 ±0.31^{b}	17.62 ±0.2^{b}	64.39 ±0.46^{c}

Different letters denote significantly different in each treatment at α=0.05.

±: standard deviation, d.b. : dry base, w.b. : wet base.

3.4 Simulated Gastric Fluids Analysis and Processes (Artificial Stomach Analysis)

Artificial stomach analysis was applied for selected wheat bran samples such as 10 min soaked, 30 min cooked, 6 microwave and 10 min ultrasound treated. Either for red lentil bran, 30 min soaked 10min cooked, 2 min microwaved and 10 min ultrasound were exposed to SGF. These samples were selected because they had the lowest content of starch and carbohydrates and the highest content of dietary fiber that are the favorable for detection the effect of simulated gastric fluids (SGF) on dietary fiber content.

Moisture content of cooked and microwaved WB and cooked and ultrasound treated RLB affected significantly by SGF. The artificial stomach analysis showed a significant decrease ($p \leq 0.05$) in ash contents of all WB and RLB samples axcept microwaved wheat bran. Total dietary fibers decreased significantly ($p \leq 0.05$) in all wheat bran samples except wheat bran treated with ultrasound and soaking. In addition to a significant increase in TDF in soaked and ultrasound treated WB and in all RLB samples. Fat content wasnot effected ($p \leq 0.05$) in all treatments. Protein decreased significantly in all WB samples. Carbohydrates decreased in soaked wheat bran and in cooked red lentil bran. Energy values affected significantly ($p \leq 0.05$) in all treated wheat and red lentil treated bran samples except soaked and microwaved RLB.

Kiers et al. (2000) noticed that the improvement of legumes digestibility during the cooking of soybean and cowpea. Total digestibility was changed from 36.5 to 44.8% and from 15.4 to 40.9%, respectively. The significant increase ($p \leq 0.05$) in the digestibility of maize and cowpea is related to the gelatinization of the high quantity of starch available in soybean and cowpea, which in turn raise the moisture content and makes starch more easily available for the enzymatic degradation.

Barampama and Simard (1995) denoted the decrease in starch digestibility of soaked beans because starch tends to form polymers. Khattab and Arntfield (2009) reported an increase in vitro protein digestibility of all soaked legumes that analyzed. Torres et al. (2016) found an increase in vitro protein digestibility in soaked Lablab purpurens and a red variety of Vigna unguiculata and a considerable decrease in protein digestibility in Canavalia brasiliensis and pink and white variety of Vigna

unguiculata. Whereas a slight difference was observed in percent in vitro protein digestibility of soaked faba beans, peas, chickpea, and kidney beans in El-Hady and Habiba (2003) study. Khatoon and Prakash (2006) conducted a decrease in protein digestibility after microwave treatment that could be attributed to the Maillard reaction during heating. The same study found an increase in the starch digestibility of microwaved legume starch because of swelling and rupture of starch granules.

The decrease in ash content is attributed to the diffusion of soluble solids into Simulated Gastric Fluids. The impact of SGF on total dietary fiber depends on the type of dietary fiber involved, generally, bran contains 92% insoluble dietary fiber and 8% soluble fibers. The influence of simulated gastric fluids in samples exposed to heat treatment was higher than counterpart in the raw state due to the solubilization of soluble dietary fiber after heat treatment which in turn increases the permeability of the cell wall resulting in increasing the cell wall pore size, hence the digestibility of bran increased. Ribas-Agustí et al. (2014) conducted that permeability of the carrot cell wall towards molecules is enhanced after heat treatment as a result of the solubilization of native pectin. An increment of the digest viscosity because of the dietary fiber solubilization reduces the bioavailability of the lipophilic compound. There are many factors affect the viscosity of the digest. The first is the chemical structure of the dietary fiber. Generally, rigid polymers have a higher viscosity than branched polymers (Belitz et al., 2009). For the same reason, high molecular weight polysaccharides produce more viscous. Hydrolysis of soluble dietary fiber during thermal treatments decrease the viscosity (Izydorczyk et al., 2000). Different production procedures of dietary fiber affect viscosity which is related to their molecular weight and to the degree of branching (Ragaee et al., 2001).

Table3.4 Chemical properties of wheat and bran samples after artificial stomach analysis

		Moisture content (%, w.b.)	Ash (%, d.b.)	Total dietary fiber (%, d.b.)	Carbohydrates (%, d.b.)	Calories (kcal/100 g)	Fat (%, d.b.)	Protein (%, d.b.)
Wheat bran	Raw	12.36±0.27[a]	5.25±0.11[a]	37.65±0.29[a]	58.78±0.16[a]	265.68±1.97[a]	3.9±0.3[a]	9.5±0.23[a]
	Soaked (10 min)	13.01±0.36[a]	1.23±0.7[b]	34.12±0.36[b]	58.28±0.57[a]	254.37±5.3[b]	3.95±0.21[a]	5.43±0.35[b]
	Raw	12.36±0.27[a]	5.25±0.11[a]	37.65±0.29[a]	58.78±0.16[a]	265.68±1.97[a]	3.9±0.3[a]	9.5±0.23[a]
	Cooked (30 min)	14.32±0.49[b]	3.31±0.44[b]	40.1±0.68[b]	56.69±0.44[a]	240.845±5.51[b]	3.26± .45[a]	4.58±0.43[b]
	Raw	12.36±0.27[a]	5.25±0.11[a]	37.65±0.29[a]	58.78±0.16[a]	265.68±1.97[a]	3.9±0.3[a]	9.5±0.23[a]
	Microwaved (30 min)	10.85±0.13[b]	6.82±0.72[b]	41.04±0.46[b]	56.93±0.66[a]	242.839±6.44[b]	3.53±0.26[a]	4.12±0.16[b]
	Raw	12.36±0.27[a]	5.25±0.11[a]	37.65±0.29[a]	58.78±0.16[a]	265.68±1.97[a]	3.9±0.3[a]	9.5±0.23[a]
	Ultrasound (10 min)	12.47±0.55[a]	4.57±0.36[a]	35.9±0.59[b]	58.72±0.26[a]	251.602±8.03[b]	3.35±0.44[a]	5.71±0.38[b]
Lentil bran	Raw	11.14±0.05[a]	5.41±0.01[a]	21.02±0.2[a]	50.32±=.55[a]	213.5±0.95[a]	1.05±0.04[a]	10.27±0.45[a]
	Soaked (30 min)	10.25±0.58[a]	3.75±0.59[b]	35.03±1.04[b]	52.38±0.54[b]	223.428±25.42[a]	1.28±0.33[a]	10.54±0.47[a]
	Raw	11.14±0.05[a]	5.41±0.01[a]	21.02±0.2[a]	50.32±=.55[a]	213.5±0.95[a]	1.05±0.04[a]	10.27±0.45[a]
	Cooked (10 min)	12.43±0.31[b]	4.01±0.43[b]	32.34±0.4[b]	46.61±0.83[b]	200.029±9.66[b]	1.1±0.19[a]	10.01±0.18[a]
	Raw	11.14±0.05[a]	5.41±0.01[a]	21.02±0.2[a]	50.32±=.55[a]	213.5±0.95[a]	1.05±0.04[a]	10.27±0.45[a]
	Microwaved (10 min)	11.59±0.19[a]	3.29±0.43[b]	39.06±0.43[b]	47.12±0.32[b]	213.987±22.23[a]	1.56±0.48[a]	10.29±0.4[a]
	Raw	11.14±0.05[a]	5.41±0.01[a]	21.02±0.2[a]	50.32±=.55[a]	213.5±0.95[a]	1.05±0.04[a]	10.27±0.45[a]
	Ultrasound red lentil bran (10 min)	12.38±42[b]	4.1±0.21[b]	36.7±0.95[b]	49.25±0.5[a]	195.675±24.01[b]	0.88±0.09[a]	8.92±14[b]

Different letters denote significantly different in each treatment at α=0.05 level.

±: standard deviation, d.b. : dry base, w.b. : wet base.

CHAPTER 4

CONCLUSION

In this study, it was concluded that;

- The source of bran was important which resulted in considerable differences in a proximate composition.
- The most of the chemical properties significantly changed after processing with different treatments.
- Moisture content and calorie value in wheat bran affected by all treatments.
- Ash, total dietary fibers and starch contents in red lentil bran affected by most treatments.
- Fat content in wheat bran affected only by cooking.
- Carbohydrates and fat contents in red lentil bran did not affect by any processing.
- The sugar, carbohydrates and calorie contents of wheat bran decreased continuously in all processes.
- The ash content of the treated red lentil bran decreased continuously after the red lentil bran processing in contrast to, moisture content that increased after different processes.
- Soaking was the most important process to produce low calorie dietary fiber in wheat bran.
- The simulated gastric fluids decreased calorie content in all treated wheat and red lentil bran samples except soaked red lentil bran.

- The differences between this study and previous studies may be attributed to cultivar differences, climatic and environmental conditions of growing the cereals and legumes.

- Wheat and red lentil bran are by-products of industry. During the production of these brans, some endosperm or other parts of seeds are contaminated which leads to inaccurate results obtained by different analyses.

- For the future studies, a specific parts of bran such as the pericarp, testa, hyaline or aleurone layers can be studied to obtain information about a proximate composition that contain before and after processing with different treatments.

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APPENDIX

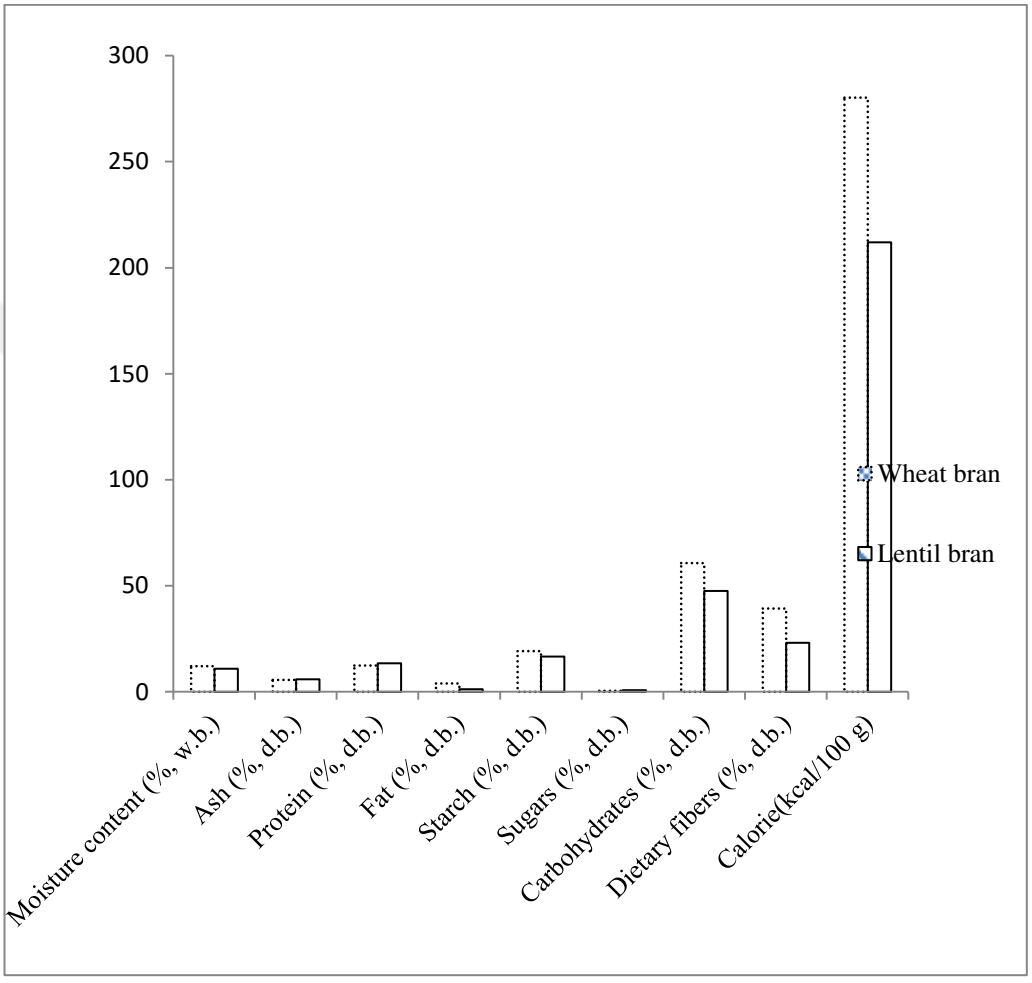

Figure A.1 Chemical properties of raw wheat bran and lentil bran

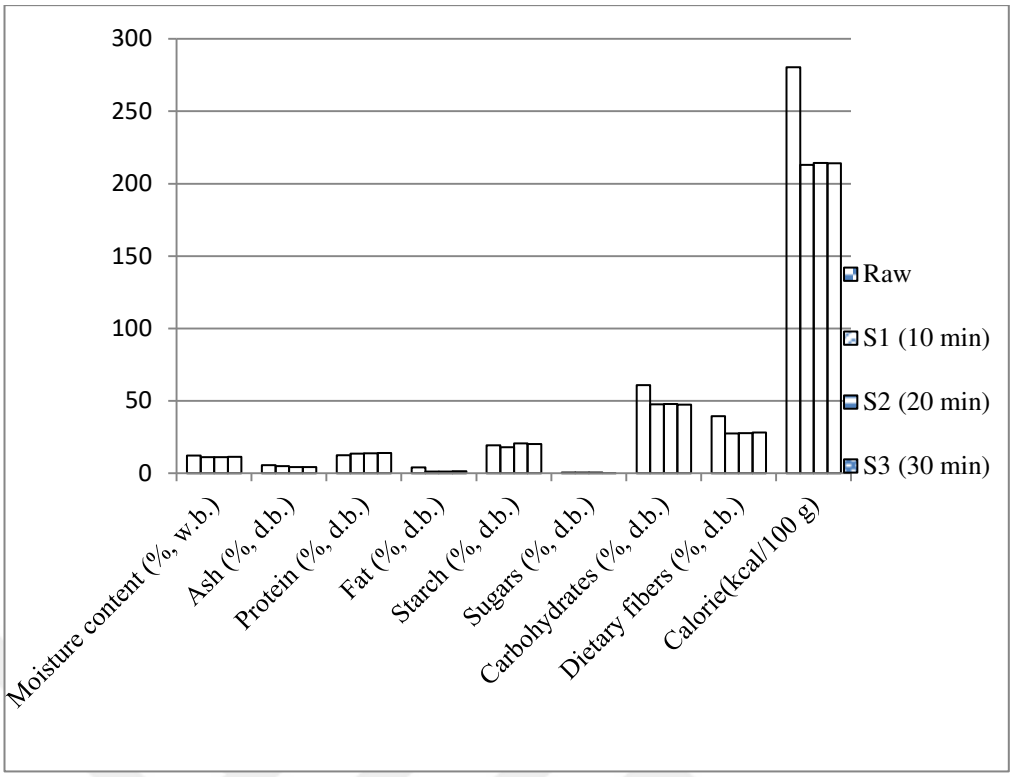

Figure A.2 Chemical properties of soaked wheat bran

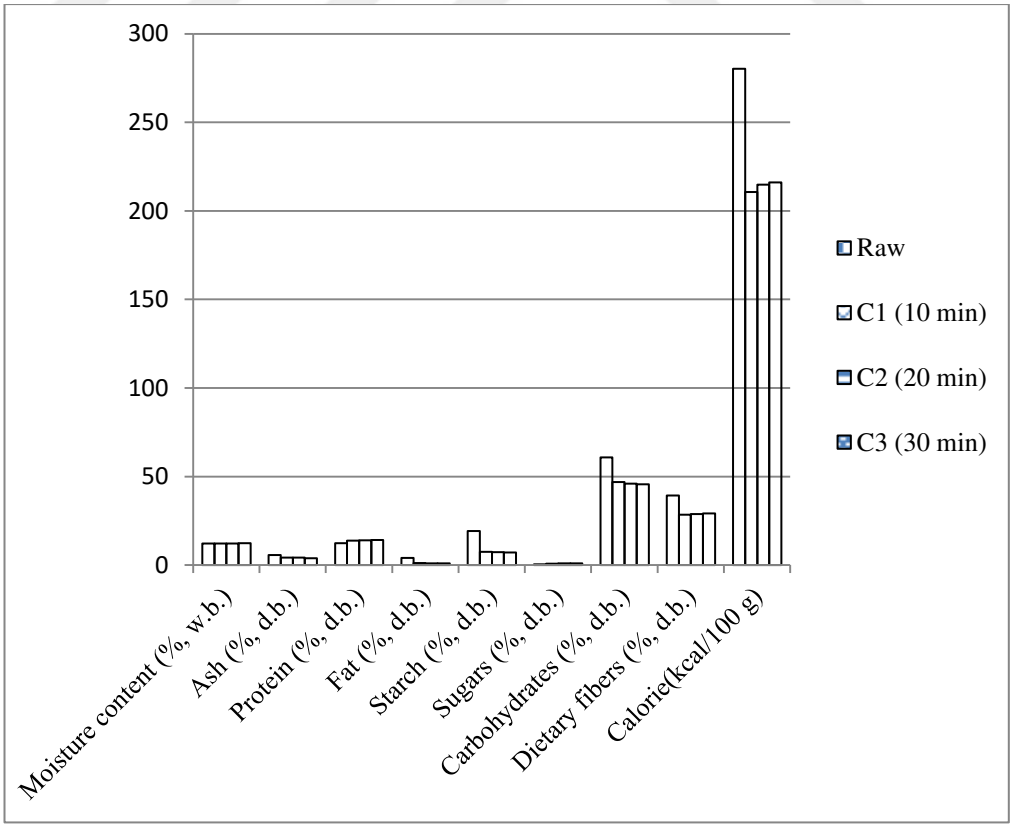

Figure A.3 Chemical properties of cooked wheat bran

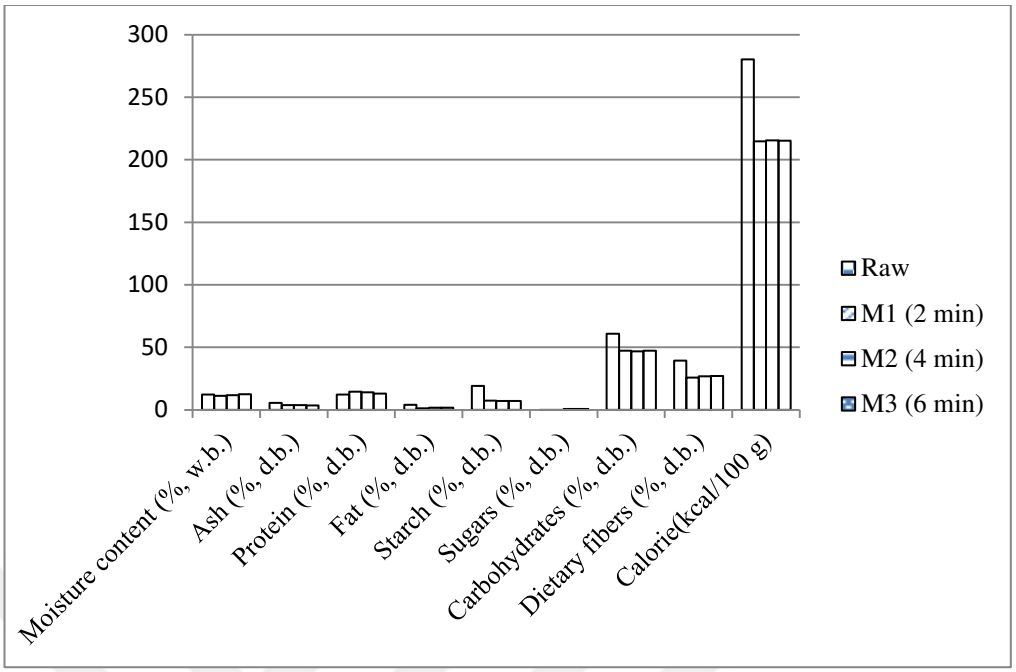

Figure A.4 Chemical properties of microwaved wheat bran

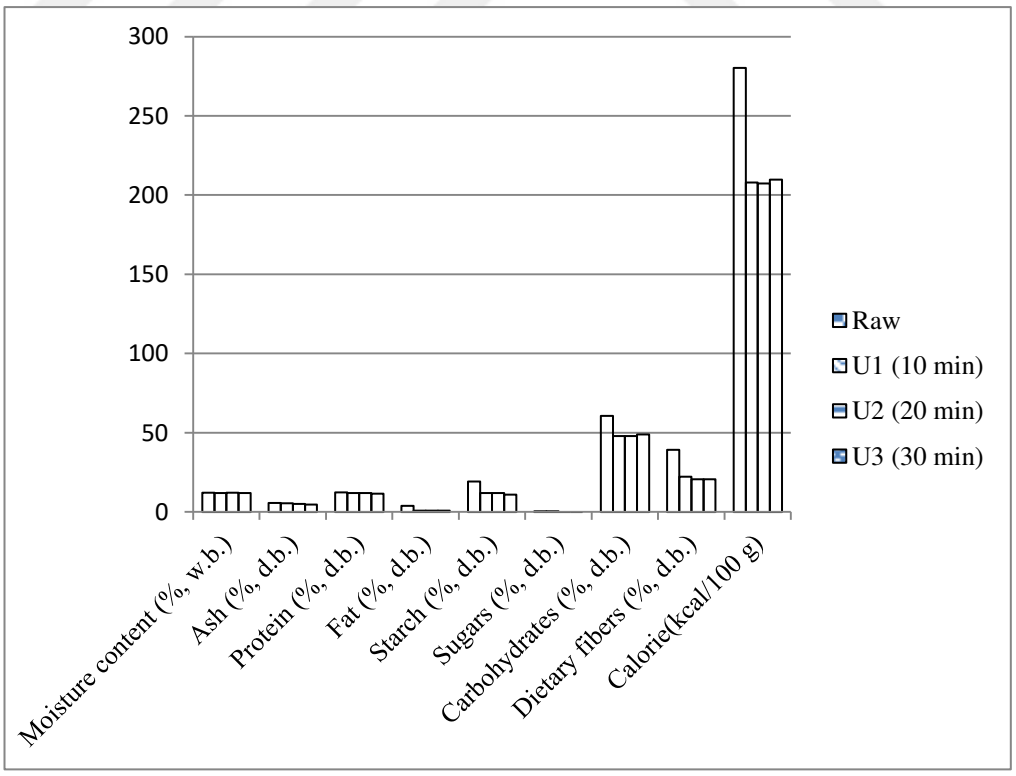

Figure A.5 Chemical properties of ultrasound treated wheat bran

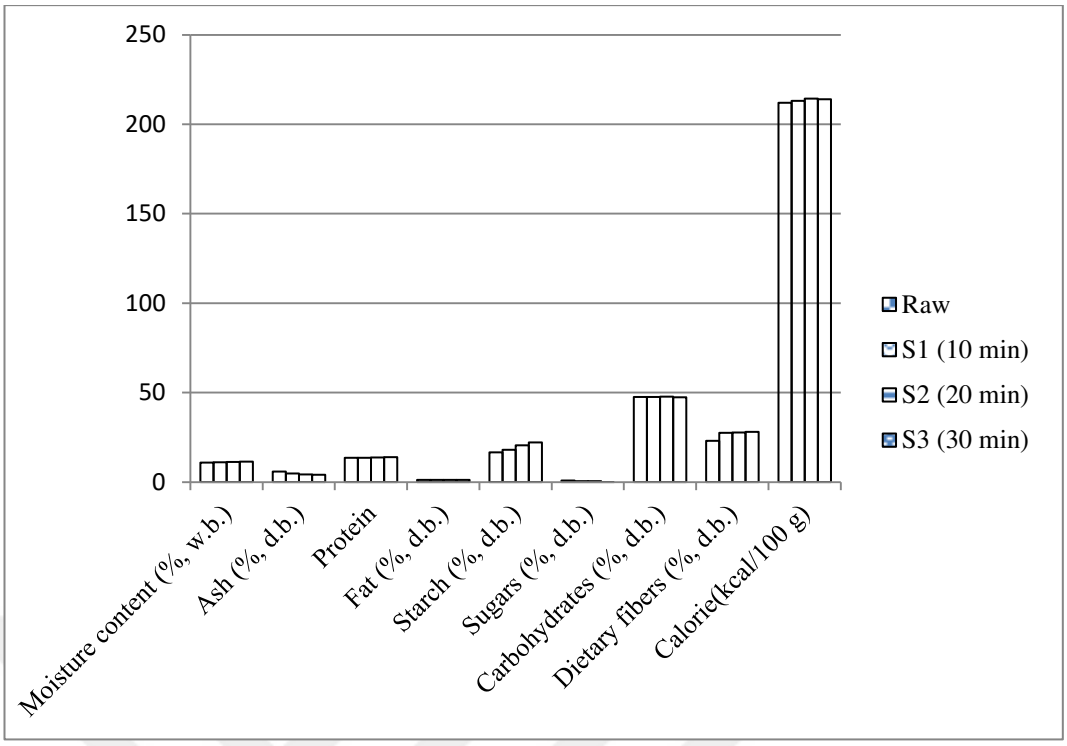

Figure A.6 Chemical properties of soaked lentil bran

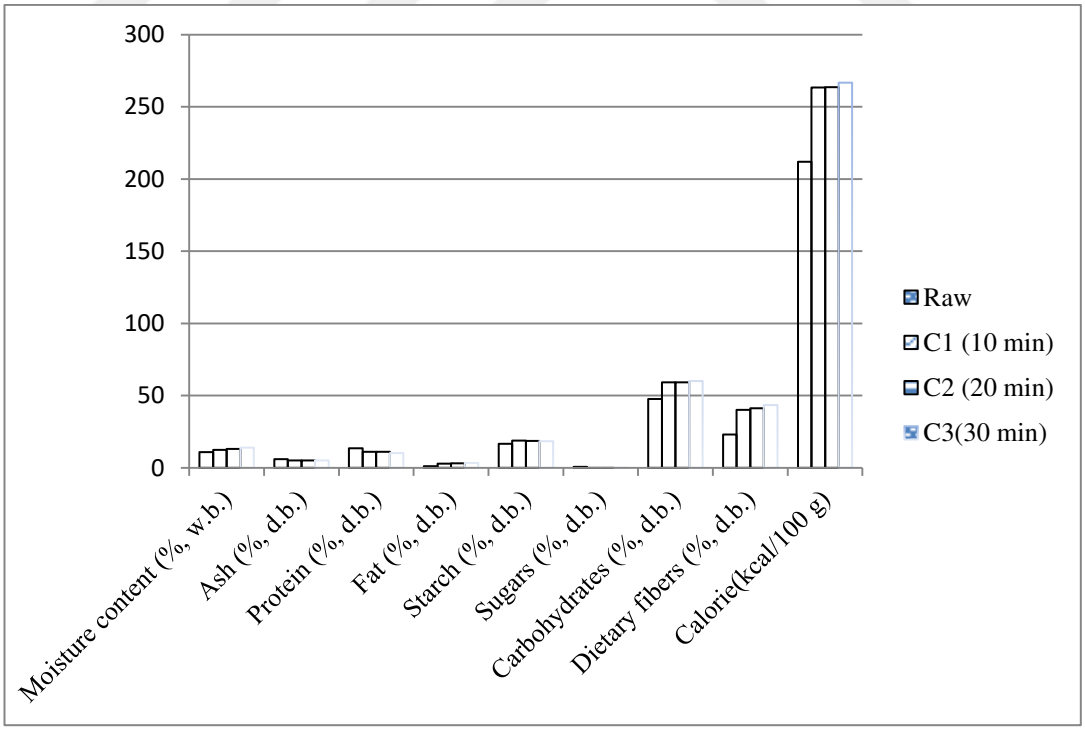

Figure A.7 Chemical properties of cooked lentil bran

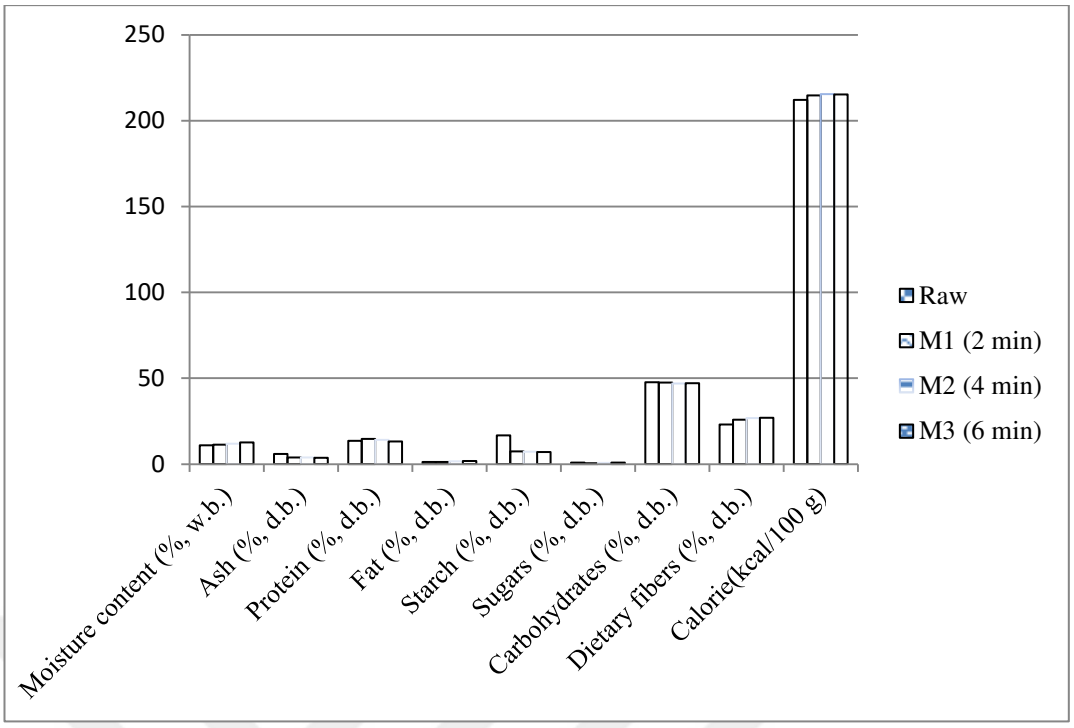

Figure A.8 Chemical properties of microwaved lentil bran

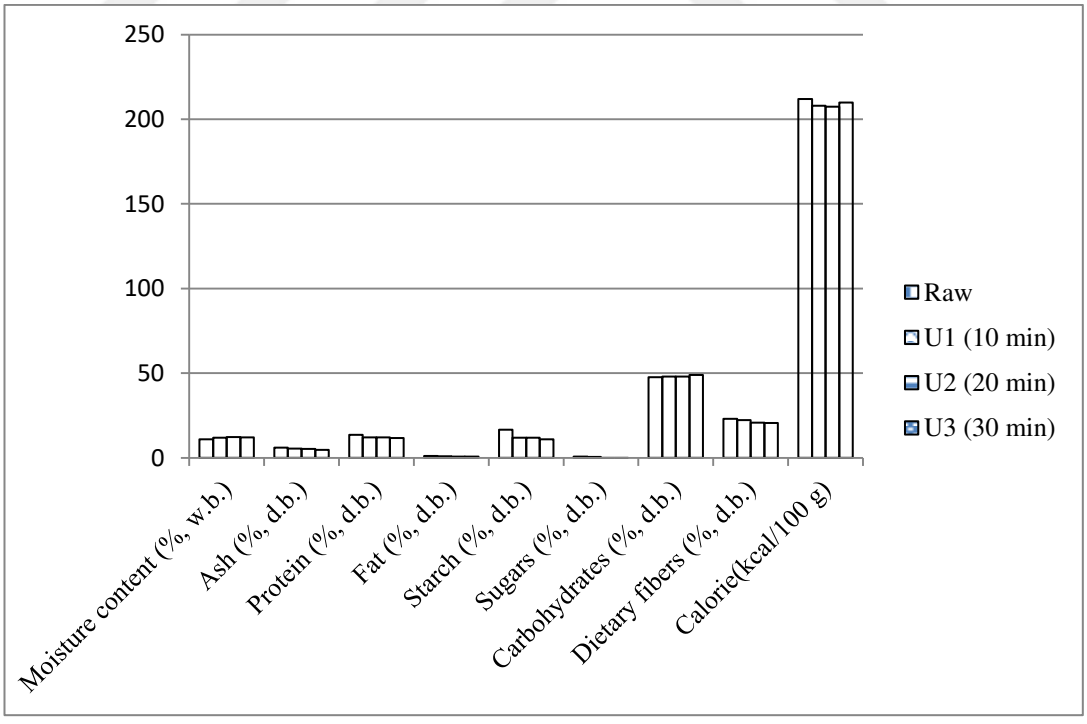

Figure A.9 Chemical properties of ultrasound treated lentil bran