The effect of fermentation, spent coffee grounds and juices of lemon fruits and rosemary leaves on quality of whole wheat bread

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DECLARATION

This thesis is my original work and has not been presented for a degree award in any other University.

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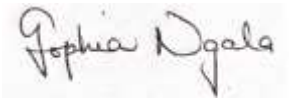
Signature…  ……Date…5th November, 2021

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DEDICATION

I dedicate this PhD thesis to my parents, the late Mr. Faustin Nzitabakuze and Mrs. Françoise Nyiramajeri, for their love, care and prayers throughout my educational journey. This thesis is also dedicated to my wife, Mrs. Angelique Uwamahoro, to my children, Mr. Rodrigue Mugisha, Mr. Roger Mahirwe and Rolda Hirwa, for their invaluable love and patience. Equally, I didicate this thesis to the families of Mr. Bertin Niyikiza and Mr. J. Bosco Munyaribanje, whose outderstanding support and encouragement enabled me to successfully complete this work. Finally, I dedicate this Ph. D thesis to The Almighty God who has strengthened my life throughout my studies.

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GENERAL ABSTRACT

Whole wheat bread is increasingly consumed in Rwanda. Bread wheat is among the top priority crops that Rwanda has promoted to boost the national economy. However, bread made from locally produced wheat varieties has been claimed to be of low acceptance. The objective of this study was to evaluate the effect of fermentation, spent coffee grounds and juices of lemon fruits and roemary leaves on quality of whole wheat bread. Whole Wheat flour **(W)** from wheat varieties (Gihundo, Kibatsi, Nyaruka and Reberaho) grown in Musanze, Kinigi, Rwanda, spent coffee grounds **(SCG)**, juice from lemon fruit **(L),** juice from rosemary leaves **(R)** and fermentation duration were assessed for their effect on the physical and sensory characteristics, proximate composition, acrylamide content and microbial contamination of bread during 7 days of storage at room temperature (17-23^{o}C). The mixture of whole wheat flour from the four wheat varieties, spent coffee grounds, juice from lemon fruit, juice from rosemary leaves, salt and water were fermented. Instant dry yeast was used as a raising agent at 34^{o}C, 60% relative humidity for 60 min and 39^{o}C, 85% relative humidity for 120 min. The dough was baked at 180^{o}C for 20 min.

Whole wheat flour from Gihundo variety had the highest values of ash content (1.47 %), total dietary fiber (15.97 %), water absorption capacity (89.00 %), dough development time (7.62 min) and brightness (84.67 %). Whole wheat flour from Nyaruka variety showed the lowest values of water absorption capacity (80.00 %), dough development time (6.33 min) and brightness (80.33). While whole wheat flour from Reberaho variety exhibited the lowest values of ash content (0.98 %) and total dietary fiber (12.44 %). There was no significant difference ($p > 0.05$) in protein content (10.00 % and 10.85 %) among whole wheat flours from the four wheat varieties. Whole flour from Gihundo wheat variety exhibited high values for most of the physicochemical characteristics determined in comparison to the other three varieties. Thus, the wheat flours from all the varieties could possibly be categorized as all-purpose since their protein contents were between 9% and 12%. It is important to select grains or flour from the wheat varieties based on the individual cultivar because their derivative products could have a more desired quality.

The control breads (W breads) were made from whole wheat flour dough fermented for 60 min without incorporation of SCG and LR. The control bread samples were firmer than the breads

containing SCG and SCG+LR with the same time of fermentation. The long fermentation (120 min) and the inclusion of spent coffee grounds, and spent coffee grounds + juices of lemon fruits and rosemary leaves in doughs, caused the supplemented breads to have lower L*, a* and b* values than control breads. Low L*, a* and b* values indicate minimum darkness, redness and yellowness of bread. Aroma, taste and appearance of spent coffee grounds+juices of lemon fruit and rosemary leaves breads from doughs fermented for 120 min were the most liked. All whole wheat breads supplemented with spent coffee grounds, and spent coffee grounds + juices of lemon fruits and rosemary leaves, satisfied consumers' preferences. Whole wheat breads supplemented with spent coffee grounds, and spent coffee grounds+juices of lemon fruit and rosemary leaves were low in protein, fat and ash contents and higher in dietary fiber than control breads. Control breads from Gihundo variety had the highest acrylamide content (47.00 µg/kg). Addition of spent coffee grounds + juices of lemon fruit and rosemary leaves in dough fermented for 60 min reduced significantly ($p \leq 0.05$) the acrylamide formation in control breads from 47.00 µgkg^{-1} to 10.50 µg kg^{-1}.

Total viable counts (TVC) and total molds and yeasts (TMY) significantly ($p \leq 0.05$) increased with the storage time of 7 days. Whole wheat bread with added spent coffee grounds + juices of lemon fruit and rosemary leaves (dough fermented for 60 min) from Nyaruka variety and whole wheat bread containing spent coffee grounds (dough fermented for 120 min) from Gihundo variety had the lowest and the highest TVC, respectively, on 7th day of storage. While whole wheat bread supplemenred with spent coffee grounds + juices of lemon fruit and rosemary leaves (dough fermented for 60 min) from Kibatsi variety and whole wheat bread containing spent coffee grounds (dough fermented for 120 min fermentation) from Reberaho wheat variety showed the lowest and highest TMY, respectively, on 7th day of storage. All whole wheat breads from dough fermented for 120 min had lower TVC than those from dough fermented for 60 min, whereas it was the opposite for TMY. TVC for all breads up to 5th and on 7th day of storage was within the satisfactory and borderline ranges which are <4 log cfu g^{-1}, and between 4 log cfu g^{-1} and 6 log cfu g^{-1}, respectively. On the 7th day, whole wheat bread supplimented with spent coffee grounds + juices of lemon fruit and rosemary leaves followed by whole wheat bread containing spent coffee grounds, showed lower TVC than control breads. The application of spent coffee grounds and juices of lemon fruit and rosemary leaves as generally recognized as safe natural ingredients can be exploited by bakeries to improve the sensory attributes and shelf stability of whole wheat bread.

CHAPTER ONE: GENERAL INTRODUCTION

1.1.Background information

Wheat is one of the cereals with high production worldwide. Bread wheat variety (genus *Triticum* and species *aestivum*) is the most cultivated, accounting for over 90% of the total wheat production worldwide (Gooding, 2009). The growing of wheat started in 10,000 B.C, from where whole wheat grain was consumed as bread. In that time, wheat grains were stone milled into flour. In 3,000 BC, Egyptians started making leavened breads by using oven baking. Afterwards, milling of wheat grains was improved from watermills (85 B.C.) to windmills (1190 A.D.) and to current mills (from 1873 A.D.) with rollers. The wheat grains have been milled into refined flour for making different food products since the development of roller mills as they have been able to separate bran and germ from the endosperm part (Anson, 2010).

Later in 1970s with some publications on the health benefits of dietary fiber, consumption of whole grain started rising slowly (Trowell, 1972). Further studies were conducted and published in 1980 and 1990 years on health benefits of consuming whole grain wheat products (Anson, 2010). This has led to the growing availability of whole wheat grain products on the marketplace (Whitney, 2013). Whole grains are defined as grains consisted of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components (the starchy endosperm, germ, and bran) are present in the same relative proportions as they exist in the intact caryopsis (AACC, 1999).

A lot of researches have showed that whole grain is a rich source of strong health promoting compounds (Phytochemicals) such as lignans, tocotrienols, phenolic compounds, and antinutrients including phytic acid, tannins and enzyme inhibitors (Slavin, 2004) However, the overall acceptability of whole wheat grain products has been low, mostly due to their undesired physical and sensory characteristics.

Low loaf volume, dense crumb, grainy, nutty and bitter flavors, darker crumb and crust of the whole wheat breads have been always linked with their low liking by consumers (De Kock *et al*., 1999; Lebesi & Tzia, 2011).

Wheat is among the top priority crops that Rwanda has promoted to boost the economy (Newtimes, 2017; Newtimes, 2014). In 2017 years, Rwanda Agriculture Board (RAB) launched ten wheat varieties in attempt to increase the production through yield and resistance to diseases (MINAGRI, 2017; Newtimes, 2017). The new varieties comprising Nyaruka (EAGLE10), Cyumba (KINGBIRD), Majyambere (HAWK120), Mizero (WREN), Rengerabana (ROBIN), Reberaho (KORONGO), Keza (SUNBIRD) and Gihundo (TAI) were introduced in 2012 in Rwanda and others such as Kibatsi (EN161) and Nyangufi (EN48) were introduced in 2007 and 2013, respectively (RAB, 2017; MINAGRI, 2017).

All of them were released in January, 2017 after being developed and evaluated for agronomic performance in research centres of Rwerere, Kinigi and Nyamagabe and in several farmers' fields across the country (RAB, 2017). All of the new wheat varieties released are bakers' choice (MINAGRI, 2017). These wheat varieties have improved the access to and availability of whole wheat grain breads of which consumption has increased in Rwanda due to awareness being done on health promoting compounds of whole grains. These compounds are mainly concentrated in the outer portions (bran) and germ of the grain (Onipe *et al.*, 2015).

It is in this regard the present study was conducted to evaluate the physico-chemical, sensory and safety (acrylamide and microbiological) characteristics of the resultant whole baked breads made from some of these wheat grain varieties released in Rwanda in 2017. Acrylamide is a chemical compound formed during maillard reaction and was reported to cause possible carcinogenicity, neurotoxicity, reproductive and developmental toxicity effects to consumers.

Maillard reaction occurs between amino compounds (principally amino acids) and carbohydrates during heating (baking, frying, roasting, grilling) above 100^{o}C (Becalski *et al*., 2003; Matthäus *et al.*, 2004). The level of acrylamide formation is favored by the heating temperature above 100^{o}C and time, type and concentrations of sugars, amino acids, temperature, time, pH, water activity, leavening agents and antioxidants (Keramat *et al.*, 2011). Acrylamide formation in food is the glucoconjugate of the asparagine with the presence of reducing sugar or a compound with carbonyl group at high temperature and low moisture (Blank *et al.*,2005). Specifically, asparagine and reducing sugars are the limiting precursors for acrylamide formation in cereal and potato products, respectively (Krishnakumar & Visvanathan, 2014; Amrein *et al*., 2004). Acrylamide has been placed in 2A carcinogen by the International Agency for Research on Cancer and a Category 2

carcinogen and Category 2 mutagen by the European Union (Ibrahim *et al*., 2019). The variation in acrylamide content in breads is mainly caused by the quality of raw material, formulation and processing methods.The researchers have found more acrylamide content in whole wheat bread than that found in refined or wheat bran breads (Boyaci Gündüz & Cengiz, 2015). Over half of acrylamide exposure is found in the consumption of bread, highly heated coffee and potatoes (Freisling *et al*., 2013).

The mean and highest acrylamide levels in breads were reported as 30 and 425µg kg^{-1}, respectively, in the European Food Safety Authority's (EFSA) monitoring report about acrylamide levels in food from 2007 to 2010 in Europe (EFSA, 2011). Arisseto *et al*. (2009) found the acrylamide levels in breads in Brazil in the range from<20 to 71µg kg^{-1}. Similar results showed the mean and highest acrylamide levels in breads as 25 and 70µg kg^{-1}, respectively, and were reported from the Netherlands (Boon *et al*., 2005). Svensson *et al.*, (2003) reported the mean and highest acrylamide levels in breads in Sweden as 50 and 160µg kg^{-1}, respectively. Mean acrylamide levels determined in breads in Egypt and Belgium were 64 and 30µgkg^{-1}, respectively (Matthys *et al.*, 2005; Saleh & El-Okazy, 2007). In another study, mean acrylamide level in Chinese foods was 38µg$kg^{-1}$and the range was 10–133µg kg^{-1} (Chen *et al.*, 2008). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) estimation for the mean dietary acrylamide intake for general population including children, is between 1 and 4 µg kg^{-1} body weight/day (FAO/WHO, 2005). Tolerable daily intake (TDI) for neurotoxicity from acrylamide was estimated to be 40 µg kg^{-1} body weight/day; TDIs for cancer were estimated to be 2.6 and 16 µg kg-1 body weight/day based on acrylamide or its metabolite glycidamide, respectively (Tardif *et al.*, 2010). The level of daily acrylamide consumption varies from country to another due to the different dietary habits and the way how foods are treated and processed.

So far, a number of mitigation strategies to reduce the acrylamide content in foods have been proposed and tested at laboratory and pilot plant scales. This includes the selection of foods such as raw material low in acrylamide precursors, processing temperature/time, addition of some amino acids like glycine, cysteine, organic acids and acidulants, calcium ions, cyclodextrin, natural antioxidants or antioxidant extracts. The use of asparaginase and replacement of reducing sugars with sucrose are also listed among reduction strategies (Acrylamide toolbox, 2019).

However, it has been difficult to put all these mitigations in practice at industry level due to many reasons, including the access, availability, cost and safety regulations.

Regarding the safety in terms of microbial harm, bread as other foods are easily contaminated with microbes such as bacteria and moulds, mostly when they are stored at uncontrolled environment conditions like temperature, air, water activity, pH, food surface contact, equipments, food handler, etc. According to Fik (2004), bread is placed among the foods with a very short shelf life about 6 days. In contrast to spoilage of bread by moulds, breads are spoilt by yeast after baking by practices such as poor hygiene handling of bread coolers, racks, conveyor belts, slicing machines, etc. (Saranraj & Geetha, 2012).

Mode of product preparation, season and product type affect the growth of moulds in most products and these account for a percentage loss between 1 and 5% in bakery products globally (Butt *et al.*, 2004). According to Tarar *et al.* (2010), bread starts spoiling after 48 hours being outside of the oven.

The growing awareness about health issues arising from food consumption preserved with chemicals has led to improving food safety by using natural preservatives. Spices and herbs are one of the most used preservative agents in food. Some natural ingredients such as rosemary and lemon extracts have been reported to act as antimicrobial agents against molds, bacteria and yeasts. This was often revealed in some previous experiments that rosemary and lemon extracts possess diverse biological activities, including antioxidant and antimicrobial activity from their non- nutrient secondary metabolites like phenolic compounds, etc. Antioxidants present in spent coffee grounds were assumed to lower the maillard reaction for the acrylamide formation in biscuits (Martinez-Saez *et al.*,2017; Fernandez-Gomez *et al.*, 2015; Mesías *et al.*, 2014). Studies that evaluated the effect of prooxidants and antioxidants on the formation of acrylamide in the bread, showed that there was in substantial reduction in acrylamide levels depending on the nature of the natural extract (Hedegaard *et al.*, 2008; Zhang & Zhang, 2007; Zhang *et al*., 2008). Therefore, the combination of natural ingredients with other preservative processing conditions can help to produce safer foods.

As the quality and safety of baked cereal products are concerned, spent coffee grounds, the juice of rosemary leaves, the juice of lemon fruit and prolongation of dough fermentation duration

have been suggested to be used to evaluate their effects on the physical and sensory quality, contents of acrylamide, molds, bacteria and yeasts of whole wheat breads.

1.2. Statement of the problem

Wheat is among the top priority crops that Rwanda has promoted to boost the national economy (Newtimes, 2014, 2017). In 2017 years, Rwanda Agriculture Board (RAB) launched ten wheat varieties in attempt to increase the production through yield and resistance to diseases (MINAGRI, 2017; Newtimes, 2017).

These wheat varieties have improved the access to and availability of whole wheat grain breads of which consumption has increased in Rwanda due to awareness being done on health promoting compounds of whole grains. However, there is still limited information on the processing quality of these new varieties. This lack of enough information can lead to the rejection of these wheats by farmers and processors. Therefore, more information for some of the physicochemical characteristics of the wheats for their processing into whole bread is needed.

In addition, whole breads from wheat grains have been reported to contain high amount of acrylamide. Acrylamide is a chemical compound formed from maillard reaction during baking and was reported to cause possible carcinogenicity, neurotoxicity, reproductive and developmental toxicity effects to consumers. During storage, the shelf life of whole wheat bread is affected by microbial and chemical degradation (oxidation and staling), resulting to off-flavor, rancidity, and other deterioration. Consequently, this leads to the low acceptance and economic loss of whole wheat bread. Spent coffee grounds, juice of rosemary leaves and the juice of lemon fruit due to their antioxidant and antimicrobial activity, have been reported to tackle acrylamide formation and slow down both oxidative and microbiological spoilage of whole wheat bread.

1.3.Justification

In Rwanda like in other parts of the world, the production and consumption of whole wheat bread have steadily increased due to increasing awareness on its positive health effects to consumers. The variety of wheat is among other major factors which influence the quality of this type of bread. However, there is still lack of enough information on the quality of whole bread from the new wheat varieties released in Rwanda in 2017. In this regard, there is a need to evaluate the physico-chemical characteristics of these wheat varieties, responsible of major effects on the overall acceptance of the whole wheat bread.

Similarly, for the safety, whole wheat bread is exposed to formation of acrylamide and microbial contamination during baking and storage, respectively. To tackle these problems, researchers have found many technological and chemical strategies for controlling acrylamide formation and microbial contamination, but for their use in breads at the factory level, they are rarely available, when available, most of them are costly, and when costly, a big number of them is regarded as unsafe by consumers.

Therefore, this research shows that spent coffee grounds and juices of lemon fruit and rosemary leaves as generally recognized as safe natural ingredients can be exploited by bakeries to improve the sensory attributes and shelf stability of whole wheat bread. It infoms wheat companies in general about the differences in the physicochemical characteristics of Gihundo, Kibatsi Nyaruka Reberaho wheat varieties, and hence ultimately enables them to easily decide on which variety to choose for various reasons. The present work is a basis for further studies by research institutions in other aspects of safety and quality of whole wheat bread.

1.4 Objectives of the study

The overall objective

The overall objective was to determine the effect of fermentation, spent coffee grounds and juices of lemon fruit and rosemary leaves on quality of whole wheat bread.

The specific objectives were:

i. To determine the physicochemical characteristics of whole wheat flour from selected Rwandan wheat varieties;
ii. To evaluate the effect of dough fermentation, spent coffee grounds and juices from lemon fruit and rosemary leaves on physical and sensory characteristics of whole wheat bread;
iii. To evaluate the effect of dough fermentation, spent coffee grounds and juices from lemon fruit and rosemary leaves on chemical characteristics of whole wheat bread;
iv. To assess effect of dough fermentation, spent coffee grounds and juices from lemon fruit and rosemary leaves on the shelf stability of whole wheat bread.

1.5. Hypotheses

i. There is no difference in the physicochemical characteristics of whole wheat flour from selected Rwandan wheat varieties;
ii. There is no effect of dough fermentation, spent coffee grounds and juices from lemon fruit and rosemary leaves on physical and sensory characteristics of whole wheat bread;
iii. Dough fermentation, spent coffee grounds and juices from lemon fruit and rosemary leaves do not affect chemical characteristics of whole wheat bread;
iv. Dough fermentation, spent coffee grounds and juices from lemon fruit and rosemary leaves do not affect the shelf stability of whole wheat bread.

CHAPTER TWO: LITERATURE REVIEW

2.1. Wheat grain history

Wheat is one of the cereals with high production worldwide. Bread wheat variety (genus *Triticum* and species *aestivum*) is the most cultivated, accounting for over 90% of the total wheat production worldwide (Gooding, 2009). The growing of wheat started in 10,000 B.C, from where whole wheat grain was consumed as bread. In that time, wheat grains were stone milled into flour. In 3,000 BC, Egyptians started making leavened breads by using oven baking. Afterwards, milling of wheat grains was improved from watermills (85 B.C.) to windmills (1190 A.D.) and to current mills (from 1873 A.D.) with rollers. The wheat grains have been milled into refined flour for making different food products since the development of roller mills as they have been able to separate bran and germ from the endosperm part (Anson, 2010). Later in 1970 years with some publications on the health benefits of dietary fiber, consumption of whole grain started rising slowly (Trowell, 1972). Further studies were conducted and published in 1980 and 1990 years on health benefits of consuming whole grain wheat products (Anson, 2010). This has led to the growing availability of whole wheat grain products on the marketplace (Whitney, 2013). Whole grains are defined as grains consisted of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components (the starchy endosperm, germ, and bran) are present in the same relative proportions as they exist in the intact caryopsis (AACCI, 1999).

A lot of researches have showed that whole grain is a rich source of strong health promoting compounds (Phytochemicals) such as lignans, tocotrienols, phenolic compounds, and antinutrients including phytic acid, tannins and enzyme inhibitors (Slavin, 2004). However, the overall acceptability of whole wheat grain products has been low, mostly due to their undesired physical and sensory characteristics.

Low loaf volume, dense crumb, grainy, nutty and bitter flavors, darker crumb and crust color of the whole wheat breads have been always linked with their low liking by consumers (De Kock *et al*., 1999; Lebesi & Tzia, 2011; Lebesi & Tzia, 2011).

2.2. Species, quality and baked products of wheat grains

2.2.1. Quality characteristics of the major wheat classes and baked products

Wheat species are *Triticum aestivum* and *Triticum turgidum var. durum* known as bread wheat and pasta wheat, respectively. Other species are grown on small land or expanding the market of healthy foods. These are *Triticum monococcum var. monococcum* (einkorn), *Triticum turgidum var. dicoccum* (emmer), and *Triticum aestivum var. spelta* (spelt). The glumes of emmer, spelta, and most of einkom, are not removed from the grain during threshing.

Major wheat classes are hard red spring wheat, hard red winter wheat, hard white wheat, soft wheat and durum wheat. Hard red spring wheats have a hard red kernel, high protein content (12- 15%), high water absorption, and strong gluten properties. They are characterized by high flour yield with minimal losses of protein and ash contents. Hard red spring wheats produce breads with high volume and they are used in making of pan breads, hearth breads, rolls, croissants, bagels, hamburger buns, and pizza crust (Table 2.1).

Hard red winter wheats have a medium hard kernel with good milling, and produce flour with medium strong, mellow gluten properties and good baking performance. Their protein content is between 9.5 and 13.5%. They are used to make of pan bread, hearth bread, rolls, all-purpose flour, and pastry products (Table 2.1). Hard white wheats are similar to hard red wheats in their milling and baking characteristics. Their protein content is between 10-15%. They find their applications in production of pan breads, rolls, tortillas, asian noodles, and whole wheat flour (Table 2.1).

Soft wheat can be spring or winter, red or white. Their endosperm and gluten are soft and weak, respectively, with protein content ranging between 8 and 11%. The bakery products for soft wheats are fit breads, cakes, pastries and crackers, pretzels, cookies, quick breads, muffins, noodles, and snack foods (Table 2.1). Durum wheats are known as the hardest of all cultivated wheats, and have a vitreous amber- colored kernels with protein content from 11 to 15%. Their endosperm is yellow and the gluten is yellow, strong and elastic. Pasta products are made from this wheat (Table 2.1).

Table 2. 1. Wheat classes, their general characteristics and uses

Class	General characteristics	General uses
Hard red winter (HRW)	High protein, strong gluten, high water absorption	Bread and related products
Soft red winter (SRW)	Low protein, weak gluten, low water absorption	Cakes, cookies, pastries, pie crusts, crackers, biscuits
Hard red spring (HRS)	Very high protein, strong gluten, high water absorption	Bread, bagels, pretzels and related products
Hard white	High protein, strong gluten, high water absorption, bran lacks pigment	Bread and related products
Soft white	High protein, strong gluten, high water absorption	Noodles, crackers, wafers and other products in which specks are undesirable
Durum	High protein, strong gluten, high water absorption	Pasta

Source: Atwell (2001)

2.3. Wheat production in Rwanda

Wheat is one of the cereals with high production worldwide. Bread wheat variety (genus Triticum and species aestivum) is the most cultivated, accounting for over 90% of the total wheat production worldwide (Gooding, 2009). Spring wheat is grown mainly in hilly areas of Rwanda (Newtimes, 2017; Newtimes, 2014). It is in the 3rd place after Kenya and Tanzania in Easter Africa (Newtimes, 2014). Most of the wheat used by several wheat milling factories in Rwanda are imported; which presents business opportunities for any investors who may venture into Rwanda's wheat industry. Over 80 % of the wheat consumed is imported, costing the country about $13 million annually (Newtimes, 2014). Rwanda Agriculture Board (RAB) launched ten wheat varieties in attempt to increase the production through yield and resistance to diseases (MINAGRI, 2017; RAB, 2017; Newtimes, 2017). The new varieties comprising Nyaruka (EAGLE10), Cyumba (KINGBIRD), Majyambere (HAWK120), Mizero (WREN), Rengerabana (ROBIN), Reberaho (KORONGO), Keza (SUNBIRD) and Gihundo (TAI) were introduced in Rwanda in 2012 and others such as Kibatsi (EN161) and Nyangufi (EN48) were introduced in 2007 and 2013, respectively. All of them were released after being developed and evaluated for agronomic performance in research centres of Rwerere, Kinigi and Nyamagabe and in several farmers' fields across the country (RAB, 2017). These wheat varieties are bakers' choice on both national and international markets (MINAGRI, 2017).

2.4. Selection of wheat grains based on chemical parameters

The contents of moisture, protein and ash are criteria used to select wheat grains. High moisture content, while increasing the microbial risks, it is good for the miller and not preferred by the baker. Low moisture content increases the damage of endosperm, thus affecting bread consistency. The ideal moisture content is between 14.5 and 15.5% for water activity of 0.65. The ash content is used to check milling performance. High ash content indicates high flour yield, and increases the revenue for miller, but not preferred by the baker. Flour with low and high contents of protein are used to make un/fermented wheat products, respectively.

2.5. Selection of wheat flour for bread making

Flour proteins from the mill are the contributor for the formation of gluten, an essential component of bread making processes. Wheat variety, soil type, growing practices and seasons affect the content and quality of gluten forming proteins. The wheat grain flour is composed of the endosperm, comprising mainly starch and protein, the bran, comprising mainly protein and fibre, the germ, comprising protein, fibre, minerals and vitamins.

Whole grain flour is supposed generally to contain the equal amounts of the endosperm, bran and germ to these found in the original grain before milled. For some countries in Europe and America, they consider whole grain flour as flour with greater than 50% of endosperm, bran and germ, compared to intact grain.The refined (white) grain flour, mainly endosperm fractions, is produced by separating bran and germ from the endosperm (Catterall, 1998).

The protein content of the flour can be adjusted by the miller. The protein content of the flour indicates the protein content of the grain, i.e., the higher protein content of the flour, the higher protein content of the grain. High protein content of flour causes high volume of gas trapped in the dough, and subsequently, gives a final product with high volume. The supplementation of flour protein can be done with external protein by using a dried vital gluten source (Chamberlain, 1984). The latter can be incorporated in dough to make a whole grain bread to increase its volume and other textural characteristics. Due to their functional properties, the proteins contained in endosperm part are best suited in bread making rather than proteins found in brans and germ which weaken the dough network (Cauvain, 1987). The measure of bran amount in flour can be measured by “Grade Colour Figure (GCF)”. High GCF indicates low volume bread with a darker crumb because of dilution effect of functional protein from endosperm.

Falling number is determined to check the status of the flour in relation to amylases. The higher falling number, the higher α-amylase. Amylases are enzymes which are synthetized during the growth cycle of plant. They reach their peak at the wet season of harvesting. α-amylase breaks down the damaged starch into dextrins. The latter with the help of β-amylase, are converted into maltose. If high amount of dextrins have not been converted into maltose, they accumulate on the slicer blades, enabling them to cut efficiently. Thus, bread loaves can be crushed. Millers adjust

flour to low falling number by using grist. Flour with damaged endosperm absorbs more water than that with undamaged endosperm (Stauffer, 1998).

2.6. Wheat grain chemical composition

2.6.1. Protein

The protein content for wheat grain is generally between 10 and 15% for dry weight of cultivars cultivated under fields (Snape *et al.*, 1993).The variation in protein content is caused by genetic and environmental factors, in particular, the availability of nitrogen from fertilization. Refine flour is about 2% protein content lower than whole grain flour (Jensen & Martens, 1983).

The protein quality is indicated by the fractions of essential amino acids. If one essential amino acid is absent, the others break down and excreted. This results in restricted growth of consumer. The essential amino acids are 10: lysine, isoleucine, leucine, phenylalanine, tyrosine, threonine, tryptophan, valine, histidine, and methionine. Cysteine as it is only produced from methionine, is often included in the list. Refined flour has lower amount of lysine than whole grain flour due to high amount of lysine-poor prolamin storage proteins (gluten proteins), about 80% of the total proteins, concentrated in the starchy endosperm (Table 2.2). Other parts of grain are richer in lysine (Shewry & Halford, 2002).

Table 2. 2. Minimum physiological requirements for essential amino acid for adults and ranges of total protein and essential amino acid compositions for wholemeal and white wheat flour

	WHO Adult intake ($g100g^{-1}$ protein)	Sample	Range		Mean ($g100g^{-1}$protein)
			Min ($g100g^{-1}$protein	Max ($g100g^{-1}$protein)	
Total Protein (%N × 5.7)		Whole meal	7.7	17.20	14.35
		White flour	7.5	15.80	13.09
Tryptophan	0.6	White flour	0.68	1.01	0.85
Threonine	2.3	Whole meal	1.74	3.10	2.54
		White flour	1.60	2.80	2.24
Isoleucine	3.0	Whole meal	2.05	4.00	3.14
		White flour	2.13	4.30	3.09
Leucine	5.9	Whole meal	3.79	7.10	5.94
		White flour	3.93	7.00	5.65
Lysine	4.5	Whole meal	2.50	3.82	2.88
		White flour	1.7	2.90	2.22
Methionine	1.6	Whole meal	1.0	1.40	1.20
		White flour	0.83	1.50	1.13
Cysteine	0.6	Whole meal	2.1	2.80	2.43
		White flour	1.4	3.30	2.17
Phenylalanine	3.8	Whole meal	1.79	4.90	3.90
		White flour	1.87	5.00	3.75
Tyrosine		Whole meal	1.1	1.90	1.54
		White flour	1.02	1.80	1.39
Valine	3.9	Whole meal	2.56	4.80	3.88
		White flour	2.34	4.40	3.54
Histidine	1.5	Whole meal	2.2	3.66	2.66
		White flour	1.90	3.71	2.69

Source: WHO/FAO/UNU Expert Consultation (2002)

2.6.2. Carbohydrates

Monosaccharides, disaccharides and oligosaccharides

The carbohydrate content of wheat grain is about 85%, where 80% is starch concentrated in endosperm. Starch and dietary fiber (DF) play role in providing energy and in promoting human health, respectively. DF influences grain processing and end quality of the final product (Stone & Morell, 2009).

Glucose and fructose are monosaccharides of carbohydrate, accounting between 0.03– 0.09% (dw) and between 0.06–0.08% (dw), respectively (Lineback & Rasper, 1988**).** Sucrose (comprising glucose and fructose units) and maltose (two glucose units) are disaccharides, raffinose (galactose, glucose, and fructose units) is the trisaccharide of carbohydrate, accounting between 0.54–1.55% (dw), 0.05–0.18% (dw), and between 0.19–0.68% (dw), respectively, (Lineback and Rasper, 1988). For oligosaccharides, the polymers of fructose, most notably fructo-oligosaccharides and fructans are abundant in wheat grain. Fructans were reported to vary between 4.0-3.4% in bran, 2.5-1.7%) in germ and between 1.7-1.4% in flour (Haska *et a*l., 2008).

Starch

Starch is about 60–70% of the wheat grain mass. Refined/white flour accounts for 70–85% of the total starch (Toepfer *et al*., 1972). Starch is made up of 2 glucose polymers: amylose, which comprises single unbranched (1→4) α-linked chains of up to several thousand glucose units and amylopectin which is highly branched (with (1→6) α-linkages as well as (1→4) α-linkages) and may comprise over 100,000 glucose unit residues. In most species, including wheat, amylose and amylopectin occur in a ratio of 1:3 amylose: amylopectin.

Cell wall polysaccharides

The cell wall polysaccharide of the grain (Table 2.3) is composed of arabinoxylan (AX) and (1→3, 1→4)-β-D-glucan (β glucan), with smaller amounts of cellulose (1→4)-β-D-glucan) and

glucomannan (Table 2.4). AX comprises a backbone of β-D-xylopyranosyl (xylose) residues linked through (1→4) glycosidic linkages with some residues being substituted with α-L-arabinofuranosyl (arabinose) residues at either one or two positions. Some arabinose residues present as single substitutions on xylose may also be substituted with ferulic acid at the five position, allowing the oxidation of ferulate present on adjacent AX chains to give dehydrodimers (diferulate cross-links). The extent of diferulate cross- linking is important as it affects the physio-chemical properties (notably solubility and viscosity) of AX and hence the behavior in food processing and also probably the health benefits. Wheat β- glucan is poorly soluble, where 10–15% of the total in whole grain samples is soluble in hot water (Nemeth *et al.*, 2010). The cell walls of the starchy endosperm (i.e., white flour) (Table 2.3) account for about 2–3% of the dry weight and comprise about 70% AX and 20% β- glucan, with 2% cellulose and 7% glucomannan (Mares & Stone, 1973)**.** Starchy endosperm AX contains only low levels of ferulic acid: 0.2–0.4% (w/w) of extractable (WE- AX) and 0.6–0.9% (w/w) of or unextractable (WU- AX) (Bonnin *et al*., 1998) (Table 2.4). The aleurone AX (Table 2.3) are highly esterified and cross- linked with about 3.2% of the AX dw being ferulic acid and 0.45% being diferulic acid (Antoine *et al.*, 2003; Parker *et al.*, 2005).

Table 2. 3. Contents and composition of cell wall in wheat grain tissues

Tissue	Cell walls (% dw)	Components				
		Cellulose	Lignin	Xylan	β- glucan	Glucomannan
Starchy endosperm	2–3	2	0	70	20	7
Bran		29	8	64	6	–
Aleurone	40	2–4	0	62–65	29–34	–
Outer pericarp		30	12	60	–	–

Calculated based on % dw

Source: Shewry *et al.* (2010)

Table 2. 4. Ranges of cell wall polysaccharides in wholegrain wheat and white flour

Components (g100g^{-1}dw)	Sample	No. samples	Range		Mean
			Min	Max	
Total dietary fiber	Wholemeal	138	10.26	15.5	13.39
	White flour	10	1.94	6.27	3.52
Total AX	Wholemeal	173	5.53	8.88	6.60
	White flour	110	1.88	3.58	2.64
WE- AX	Wholemeal	166	0.29	1.62	0.57
	White flour	110	0.30	0.91	0.58
WU- AX	Wholemeal	20	5.87	8.16	6.61
	White flour	90	1.52	2.93	2.10
β- glucan	Wholemeal	166	0.29	1.10	0.81
	White flour	–	–	–	–

Note: AX: arabinoxylan**, WE-AX:** extractable- arabinoxylan**, WU-AX:** unextractable- arabinoxylan

Source: Shewry and Hey (2015b)

2.6.3. Antioxidant properties and health benefits of phenolic acids

Phenolic acids of phenolic compounds are the main group of phytochemicals (Table 2.5) in wheat grain. They exist as free, soluble bound to low molecular weight compounds such as sugars, and as bound forms linked to cell wall polysaccharides, arabinoxylan in particular, by ester bonds.

Phenolic acids possess strong antioxidant activity (Adom *et al.*, 2003; Beta *et al.*, 2005). Phenolic acids, mainly ferulic acid have been severely reported to improve vascular function in humans (Katz *et al.*, 2001; Katz *et al.*, 2004; Vauzour *et al.*, 2010; Rodriguez-Mateos *et al.*, 2013).

Table 2. 5**.**Variation in the contents of phytochemicals (phenolics and terpenoids), in wholegrain of wheat and white flour

Component	Fraction	Range	Fold variation	Mean
Phenolics				
Total phenolic acids	Whole grain	326–1171 μgg^{-1} dm	3.4	657 μgg^{-1} dm
Total phenolic acids	White flour	171–190 μgg^{-1} dm	1.1	180 μgg^{-1} dm
Free phenolic acids	Whole grain	3–30 μgg^{-1} dm	10	10.6 μgg^{-1} dm
Conjugated phenolic acids	Whole grain	76–297 μgg^{-1} dm	3.9	162.5 μgg^{-1} dm
Bound phenolic acids	Whole grain	208–878 μgg^{-1} dm	4.2	484.9 μgg^{-1} dm
Bound ferulic acid	Whole grain	162–721 μgg^{-1} dm	4.5	367.4 μgg^{-1} dm
Alkylresorcinols	Whole grain	241–677 μgg^{-1} dm	2.8	432 μgg^{-1} dm
Lignans	Whole grain	3.4–22.70 μgg^{-1} dm	6.7	10.5 μgg^{-1} dm
Terpenoids				
Total tocols	Whole grain	27.6–79.7 μgg^{-1} dm	2.9	49.9 μgg^{-1} dm
Tocopherols	Whole grain	12.3–33.2 μgg^{-1} dm	2.7	19.9 μgg^{-1} dm
α- Tocopherol (vitamin E)	Whole grain	9.1–19.9 μgg^{-1} dm	2.2	13.6 μgg^{-1} dm

Source: Shewry and Hey (2015b)

Table 2. 5: Cont.

Component	Fraction	Range	Fold variation	Mean
α- Tocopherol (vitamin E)	Whole grain	9.1–19.9 μgg^{-1} dm	2.2	13.6 μgg^{-1} dm
Tocotrienols	Whole grain	12.5–52.0 μgg^{-1} dm	4.2	30.02 μgg^{-1} dm
Total sterols (inc stanols)	Whole grain	670–959 μgg^{-1} dm	1.43	844 μgg^{-1} dm
% stanols	Whole grain	11–29%	2.6	23.9%
β- carotene	Whole grain	0.11–0.24 μgg^{-1} dm	2.1	0.19 μgg^{-1} dm
Lutein	Whole grain	0.93–1.3 μgg^{-1} dm	1.4	1.14 μgg^{-1} dm
Zeaxanthin	Whole grain	0.23–0.44 μgg^{-1} dm	1.9	0.33 μgg^{-1} dm

A moisture content of 14% was assumed to convert to dry matter basis (dm). Stanols are expressed as % total sterols+stanols.

Source: Shewry and Hey (2015b)

Phenolic acid, tocopherol and carotenoid compositions in acetone extract of wheat bran have displayed antioxidant functions such as scavenging of hydroxyl radical, 2,2-diphenyl-1-picryhydrazyl radical and superoxide radical anion, 2,2'-azinobis (3-ethylbenzothiazoline-6 -sulfonic acid, oxygen radical absorbing capacity and chelating capacities against Cu^{2+} and Fe^{2+} (Zhou *et al.*, 2005). Antioxidant properties of ferulic acid have been associated with health beneficial effects against cancer, cardiovascular disease, diabetes and Alzheimer's disease (Zhao & Moghadasian, 2008). Extracts of wheat bran have been reported to show antioxidant potentials against human low-density lipoprotein (LDL) oxidation and free radicals (Yu *et al*., 2005).

Studies reported that phytonutrients found in wholegrain could have bioactivities such as antimutagenic, anticarcinogenic, antioxidant, antimicrobial, and anti-inflammatory properties Okarter & Liu, 2010; Lillioja *et al.*, 2013; Jideani *et al.*, 2014). For example, flavonoids in wholegrain have been reported to act as antioxidant and anticancer compounds (Adom & Liu, 2002). Anthocyanin also has been demonstrated to reduce the risk of colon cancer by inhibiting cancer cell production in the colon (Žilić *et al.*, 2012). Ferulic acid and diferulates as phytonutrients are uniquely found in wholegrain compared to fruits and vegetables (Adom & Liu, 2002). Antioxidants have shown to act at low concentration by reducing the risks of chronic diseases (Abdel-Aal & Rabalski, 2008). Phenolic compounds, most notably phenolic acids, are

the major antioxidant contributors in whole-grain products (Abdel-Aal *et al.*, 2012; Abdel-Monem *et al.*, 2013).

Flours made from hard wheat was reported to exhibit higher radical scavenging capacity, as determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), than flours from soft wheat (Ragaee *et al.*, 2006). Moore *et al.*(2009) reported a link between the levels of free phenolic acids of wheat bran and antioxidant capacity. By the time antioxidant capacity was great, the release of the phenolic acids was also great. In addition, Adom *et al*. (2003) showed that the radical scavenging capacity was related to the level of phenolic acids in white spring durum cultivar. In a study done on wheat varieties where the radical scavenging capacity was determined by ABTS, DPPH, and oxygen radical absorbance capacity (ORAC) Iqbal *et al.* (2007) found that the antioxidant capacity was correlated with the levels of total phenol and anthocyanin in wheat.

2.7. Promotion of whole grain consumption

The scientific evidences and increasing campaigns on health promoting compounds in whole grain have gained the attention of consumers for the consumption of whole grain foods. Health claims for whole grain foods was firstly approved and amended by American FDA in 1999 and in 2003, respectively (van der Kamp *et al.*, 2014**).** In US and UK, whole grain food is defined as food containing 51% weight of whole grain and 50% in Sweden. For the definition given in 1999 by American Association of Cereal Chemists, a whole grain is: ‘a grain consisted of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components – the starchy endosperm, germ and bran – are present in the same relative proportions as they exist in the intact caryopsis’(van der Kamp *et al.*, 2014).

The US dietary guidelines for Americans explicitly recommends adults to consume at least three ounce-equivalents of whole grain per day, and that at least half of the grains consumed should be whole grains (van der Kamp *et al.*, 2014). Also, in Switzerland as well in Australia, there are recommendations on consumption of more whole grain foods.

2.8. Whole wheat bread and white wheat bread

Bread is the most consumed bakery product (Aini & Maimon, 1996). It is basically made from flour, water, yeast and salt, followed by a series of processes involving mixing, kneading, proofing, shaping and baking (Dewettinck *et al.*, 2008; Banu *et al.*, 2012). The processing of bread from refined whole grain flour contributes to the substantial reduction of some nutritional and health promoting compounds in white bread (Maneju *et al.*, 2011). The increasing awareness on consuming functional foods, foods which provide additional health benefits to those coming from normal nutrients, have led the bakeries to produce breads from whole wheat grains as well as white breads (Ndife & Abbo, 2009).

Studies have shown that whole grain bread contains more fiber, minerals, essential amino acids, phytochemicals than white/refined bread (Dewettinck *et al.*, 2008). This is because these phytonutrients are highly concentrated in bran and germ that constitute whole bread compared to white/refined bread made only from endosperm (Catterall, 1998). Thus, whole wheat flour gives bread richer in nutritional value and health benefits than white bread from refined flour (Dewettinck *et al.*, 2008)**.** However, consumers like white breads more than whole breads due to less appealing organoleptic and textural characteristics of this bread made from whole grain flour (Maneju *et al.*, 2011). There must be a regular campaign for bakeries to produce by using improved processing technologies more whole grain breads desired by consumers (Banu *et al.*, 2012). The trend towards increasing consumption of wholegrain foods poses a risk of acrylamide exposure because with no mitigation, high consumption of whole bread could possibly result in high intake of acrylamide. Fortunately, there is a possibility of balancing risks associated with acrylamide consumption against health benefits of whole grain bread consumption.

2.8.1. Physical and sensory characteristics of whole bread

Loaf volume, specific volume, crumb firmness, color, weight, height, shape, and flavor are the major physical and sensory characteristics of bread for a consumer (Katina *et al*., 2006). Bread made from wholegrain wheat flour often is characterized by lower loaf volume, firmer and denser crumb, and darker crumb and crust than bread made from refined wheat flour (Cai *et al.*, 2014).Thus, the addition of bran or fiber into a starchy endosperm dough for the purpose of making a bread with improved health benefits, could give a bread with low quality of some physical and sensory attributes (Katina *et al.*, 2006)**.**

The low loaf volume of a whole bread is caused by the dilution of gluten by high bran/fiber concentration in a dough. The inference of insoluble fiber against the formation of gluten network, thus, there is a rupture of gas cell (Courtin & Delcour, 2002)**.** The interaction between starch and gluten of flour becomes week (Oates, 2001). Insufficient water for the development of gluten network due to high water absorption capacity of both insoluble and soluble fibers (Gill *et al.*, 2002). It causes high mixing tolerance, tenacity, and small extensibility. The expansion of cell is poor due to less gas retention (Gómez *et al.*, 2003)**.**

Low specific volume of whole grain bread is directly related to its low loaf volume (Lee *et al*., 2001). The weight of the whole grain bread is always higher than that of refined/white bread. This is due to low loaf volume and specific volume of whole bread, compared to these of white bread. The crust color of whole bread has been scored poor in many sensory reports to be not attractive, as it should be smooth and golden brown (Sanful, 2011).

Temperature and time during baking, fibres and starch in dough and the quantity of absorbed water in dough mixing, contribute to the crumb texture (Gómez *et al.*, 2003). Whole bread is characterized by coarse structure and the size of crumb pore is large. Crumb with a big number of small size pore is desirable (Banu *et al.*, 2012). Low number of pores in a bread is caused by the release of gas during proofing, due to disruption of gluten network in a dough (Sullivan *et al*., 2011). Taste is the basis for generally liking the food product. The taste for a bread refers to sweetness The overall acceptability for a whole grain bread has been scored low in several sensory reports due to that the taste was less sweet.

2.9. Effect of bread-making on phenolic compounds and antioxidants

Dough making starts with mixing, where ingredients, water and flour are blended to develop a dough. Oxidation of phytonutrients by enzymes available in flour is initiated by the water added. Phytonutrients such as carotenoids, tocols, phenols are affected (Maraschin *et al.*, 2008; Ktenioudaki *et al.*, 2014). In the study done by Vogrincic *et al.*(2010), bread from tartary buckwheat flour, they found a decrease in flavonoid rutin in dough after mixing and proofing; and was undetectable after baking. For french bread made from wholegrain and white flour, carotenoid level in dough also decreased after mixing (Leenhardt *et al*., 2006b). The study found the correlation with carotenoid decrease and lipoxygenase activity.

In a study done by Moore *et al.*(2009) on antioxidant properties of pizza dough made from whole grain flour, they found that the contents of free and bound ferulic acids significantly increased and decreased after 18 18 and 48 hours of fermentation under 4°C refrigeration, respectively. Maillard reaction has been reported be a source of acrylamide formation in bread and to highly increase antioxidant capacity of bread crust in comparison to crumb because the crust is exposed to a higher temperature in oven during baking (Lindenmeier & Hofmann, 2004). In experiment done about the changes on free and bound phenolic acids during the baking of whole grain dough, the authors reported that heating increased and decreased free and bound phenolic acids in the bread, respectively (Vogrincic *et al.*, 2010; Ktenioudaki *et al.*, 2014; Abdel-Aal & Rabalski, 2013).

However, the increase of phenolic compounds was observed only in white bread, which is bran-free, and not in wholegrain bread. (Gelinas & McKinnon, 2006) found that flour phenolic acids increased after baking white bread dough, not whole one. The level of flavonoid compunds was also shown to reduce significantly after baking (Alvarez-Jubete *et al*., 2010)**.** Carotenoid content was reported to decrease higher in the bread crust than in crumb (Hidalgo *et al.*, 2010). Whole breadmaking process reduces phenolic compounds mainly concentrated in the outer layers of grain, but the bread still has more health benefits for consumers than refined bread. It is recommended to use ingredients and processing methods that are able to significantly reduce the losses of phytonutrients and antioxidants for the production of healthier breads.

2.10. Discovery of acrylamide in food

The acrylamide was discovered for the first time in workers who were constructing a railway tunnel in Hallandsås mountain in Sweden in 1997. The workers were found to be exposed to 0.07-17.7 nanomol acrylamide/g Hb, the levels that went beyond the No Observed Adverse Effect Level (NOAEL). The source of the acrylamide which was believed to come from the tunnel, was investigated for its link with the cancer. The acrylamide had previously been considered as a 'probable human carcinogen' (IARC, 1994). The evaluation of acrylamide consumption and the cancer risks in the railway tunnel workers found that other sources of acrylamide were possible (Hedegaard *et al.*, 2008; Törnqvist, 2005)**,** because the workers were daily consuming 100 µg of acrylamide. One of the other sources of acrylamide was assumed to be foods (Tareke *et al.*, 2002; Granvogl *et al.*, 2007)**.** Tareke *et al.* (2002) found that feeding rats with fried feed exhibited a ten-fold increase in haemoglobin (Hb) adduct contents of AA in comparison with the control samples. It was reported that the levels of AA in the fried feeds corresponded with that increase in adduct levels of AA in the rats fed with these feeds (Tareke *et al.*, 2000).

In 2000 years, the same people conducted research and found the heated carbohydrate and protein rich foods had the highest levels of acrylamide ranging between 50-4000 $\mu g kg^{-1}$, and 5-50 $\mu g kg^{1}$, respectively (Tareke *et al.*, 2002) and these findings were announced in the press conference by Swedish National Food Administration and Stockholm University. Since this public announcement, researches on acrylamide formation in foods and its mitigations strategies have multiplied. In subsequent studies in 2002 years, it was revealed that the formation of acrylamide in foods is caused by Maillard reaction in which free asparagine and reducing sugars take part under elevated temperature (Mottram *et al.*, 2002; Stadler *et al.*, 2002). After these scientific reports, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the data on the presence of acrylamide in foods from many countries, mainly in Europe and North America. The result showed that high acrylamide levels are found in potato-based products, cereal-based products and coffee (JECFA, 2005) (Table 2.6, Figure 2.2). In Germany, the main source of acrylamide is bread with about 18-46% of acrylamide intake (Hilbig *et al.*, 2004). While in Sweden, the major source of acrylamide intake is potato-based products, with about 45%, 22% comes from coffee intake and 33% from cereal-based products, where breads (soft and crisp)

intake contribute to 22% (Figure 2.1, Figure 2.2). Consumer's exposure to acrylamide by age group in different countries is shown in Table 2.7.

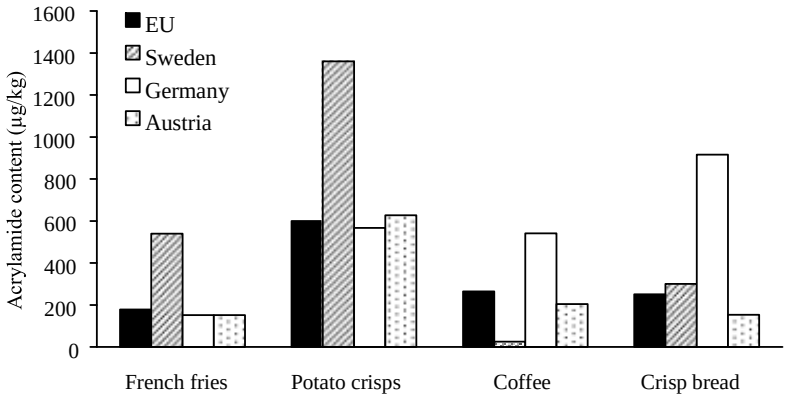


Figure 2. 1. Acrylamide content in the most common food products in some european countries

Source: Jecfa (2005); Arwa (2008)

Table 2. 6. Content of acrylamide in different food items

Food item	Number of samples*	Mean content ($\mu g kg^{-1}$)
Cereals and cereals-based products (collective)	11 327	366
Breads and rolls	5 145	446
Pastry and biscuits	4 980	350
Breakfast cereals	1 130	96
Pizza	85	33
Fish and seafood	107	25
Meat and offal	325	19
Milk and milk products	147	5.8
Nuts and oilseeds	203	84
Potato Products (collective)	10 077	477
Potato baked	99	169
Potato crisps	3 555	752
Potato chips	6 309	334

* The total numbers of individual samples, allowing for the number of samples blended into composites

Source: JECFA (2005); Arwa (2008)

Table 2.6: Cont.

Food item	Number of samples*	Mean content ($\mu g kg^{-1}$)
Potato Products (collective)	10 077	477
Potato baked	99	169
Potato crisps	3 555	752
Potato chips	6 309	334
Coffee and Tea	1 455	509
Coffee (ready to drink)	93	13
Coffee (ground, instant or roasted)	709	288
Coffee decaffeinated	34	688
Coffee extracts	119	1 100
Coffee substitutes	368	845
Green tea (roasted)	101	306
Cocoa products	23	220
Sugars and honey	133	24
Vegetables	193	17
Fruits dried and processed	49	131
Alcoholic beverages	99	6.6
Infant formula	117	< 5
Baby food (dry powder)	24	16
Baby food (biscuits, etc.)	32	181
Dried food	13	121

* The total numbers of individual samples, allowing for the number of samples blended into composites

Source: JECFA (2005); Arwa (2008)

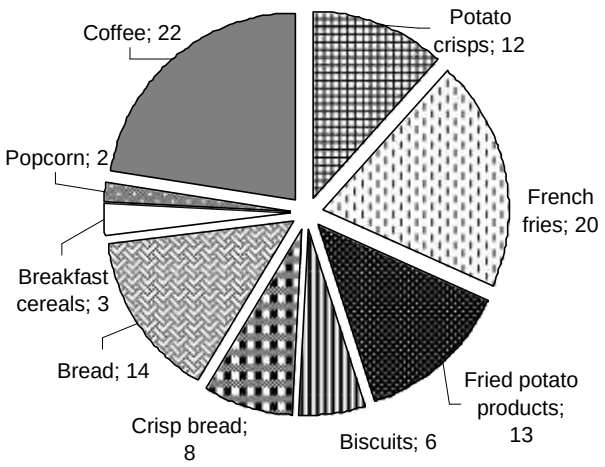


Figure 2. 2. Percentage contribution of different products to acrylamide intake in Sweden.

Source: Arwa (2008)

Table 2. 7. Acrylamide exposure estimates

	Mean daily intake (μgkg^{-1} /day)	Age group
Sweden	0.45	18 – 74
Norway	0.51	16 – 30
Netherlands	0.48	1 – 97
Germany	1.1	15 – 18
Switzerland	0.28	16 – 57
France	1.4	2 – 14

Data taken between 2002 – 2004 years

Source: Arwa (2008)

2.11. Chemistry of acrylamide

Acrylamide is a hydrophilic solid molecule (Anon, 1991) with no odor and white. Acrylamide generally forms from the hydration of acrylonitrile with sulphuric acid between 90-100°C or by catalytic hydration using a copper catalyst. It is soluble in acetone, acetonitrile and water. It is polymerized when heated, which limits the determination of its boiling point at ambient pressure. Its boiling temperature is at 125°C under 3.34 kPa (25 mm Hg), and is a thermally unstable molecule (Table 2.8).

Table 2.8. Physical parameters of acrylamide

Parameter	Specification
Chemical formula	C_3H_5NO
Molecular weight	71.08 $gmol^{-1}$
Melting point	84 – 85°C
Solubility	216g100 g^{-1} water at 30°C
Boiling point	125 °C at 3.34 kPa
Vapour pressure	0.007 mm Hg at 20°C
Vapour density (Air = 1)	2.4 at 175°C
Specific gravity	1.1222 $kgdm^{-3}$ at 30°C

Source: Arwa (2008)

Due to its two functional groups, an amide group and the electron-deficient vinylic double bond (Figure 2.3), acrylamide takes part in a large range of reactions, including nucleophilic and Diel-Alder additions and radical reactions. The amide group makes acrylamide to involve in hydrolysis, dehydration, alcoholysis and condensation with aldehydes, while the vinylic double bond reacts with ammonia, aliphatic amines, phosphines, chlorine, bromine, bisulphite and dithiocarbamates, as well as proteins (Friedman, 2003; Girma *et al*., 2005)**.**

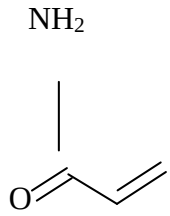


Figure 2. 3. Chemical structure of the acrylamide molecule

Source: Arwa (2008)

Acrylamide is a biodegradable substance used to purify wastes and drinking water. It can also be applied as a flocculent and in the synthesis of polymers and gels (Bologna *et al*., 1999; Smith *et al.*,1996). Acrylamide is used to prepare polyacrylamide for gels for electrophoresis in laboratories. Acrylamide as a weak acidic and basic conjugated amide, has the ability to react with metal ions. The reaction occurs either at the organic or the amide groups (Girma *et al*., 2005).

2.12. Pathways of acrylamide formation

The first researches on the mechanistic pathways for the formation of acrylamide in food suggested the Maillard reaction as the major pathway, most notably where the free amino acid asparagine (Asn) and with reducing sugars are involved as the major precursors in the reaction (Mottram *et al*., 2002; Stadler, *et al*., 2002). Afterwards, many researches proposed different pathways for the Maillard reaction (Figure 2.4.). The first step is the reaction between free asparagine and a carbonyl source (amino-carbonyl reaction), giving in N-glycosyl asparagine, which in turn is hydrolysed, resulting in the stable Shiff base (Blank *et al*., 2005; Stadler *et al.*, 2002). The Shiff base then is decarboxyled that produces in a decarboxylated Schiff base, which after tautomerisation gives decarboxylated amadori compound. From this intermediate acrylamide is produced, along with an aminoketone via β-elimination reaction and cleavage of the carbon-nitrogen covalent bond (Becalski *et al.*, 2003; Mottram *et al*., 2002; Stadler *et al.*, 2004).

The decarboxylated Shiff base may decarboxylate, producing 3-aminopropionamide (3-APA), which in turn gives acrylamide with the elimination of ammonia (Granvogl *et al.*, 2004; Granvogl & Schieberle, 2006; Zyzak *et al.*, 2003). It has been proposed that 3-APA is produced during asparagine heat degradation and acrylamide is produced then after. Granvogl *et al.* (2004) revealed that reducing sugars are not always required for the formation of acrylamide. At temperatures (100-180°C) asparagine is decarboxylated, giving 3-APA and then acrylamide. A further research in foods reported that 3-APA is a transient intermediate of acrylamide production in food processing (Granvogl & Schieberle, 2006). Another alternative pathway suggested is the production of pyrolytic acrylamide from wheat gluten, with protein-bound alanine as the major amino acid (Table 2.9). In a model experiment, heat treatment of gluten produced high levels of acrylamide (Claus *et al*., 2006b). The addition of gluten to the dough increased acrylamide content in the order of 20%.

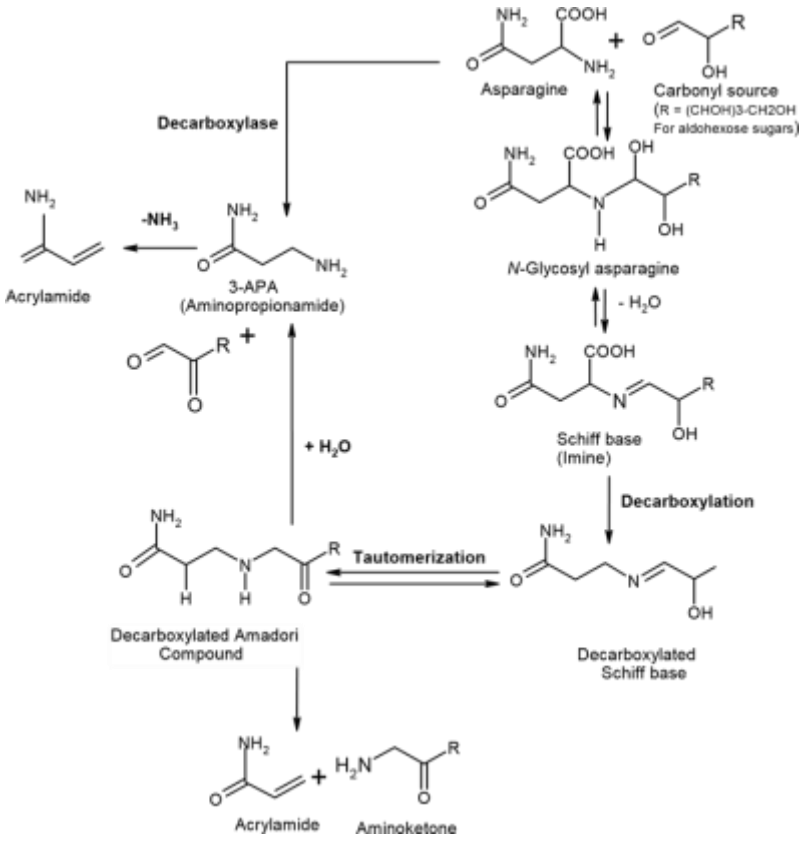


Figure 2. 4. Some suggested pathways for acrylamide formation from asparagine

Source: Arwa (2008)

2.13. Effect of reaction parameters on the formation of acrylamide from asparagine

2.13.1. Water content

Water content favors the formation of acrylamide as it is shown in Figure 2.5, where it may affect the reaction temperature and heat transfer. In the presence of fructose in the reaction, the formation of acrylamide increased when water content was up to 200µL, and declined afterwards. Regarding glucose addition in the system, the formation of acrylamide started decreasing when water content was over 50 µL. But it increased once again at 1000µL water. The physical state of the reaction system changed from solid to suspension when water was between 50-200 µL and become a solution at 500 µL water.

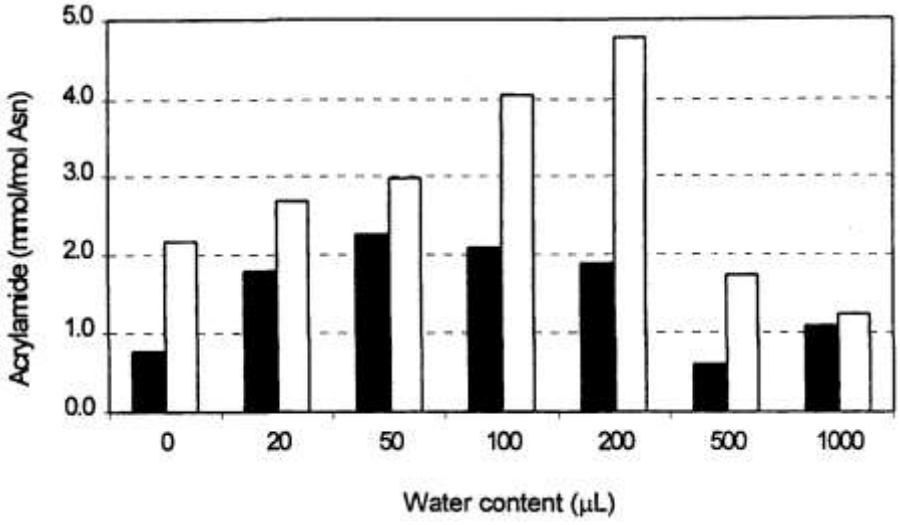


Figure 2. 5. Formation of acrylamide in binary mixtures of asparagine and glucose as function of moisture. Asparagine and glucose (black bars) or fructose (white bars) as function of moisture (180°C, 5 min).

Source: Blank *et al.* (2005)

2.13.2. pH

Some reports has shown that acrylamide formation in some foods can be controlled by a low pH (Jung *et al*., 2003). This proposition is based on the fact that the initial amino-carbonyl reaction is prevented due to protonation of the amino group at low pH. The reaction series when fructose was present, produced significantly low amount of acrylamide at pH 3 (2.2 mmol/mol), while at pH 8, it formed more acrylamide (3.3 mmol/mol). Other reports also showed that the optimum formation of acrylamide was at around pH 8 (Rydberg *et al*., 2003). The reaction formed higher amounts of acrylamide when fructose was added in comparison to glucose addition (Figure 2.6). When fructose was in the sample with no water, the reaction produces more acrylamide levels than glucose in the similar sample (Figure 2.6), even though glucose as an aldohexose, is considered to more react chemically, since the hydration of the aldehyde group is not done. Therefore, other factors such as physical state of the chemical reaction may be taken into consideration to explain the formation of acrylamide (Robert *et al.*, 2004).

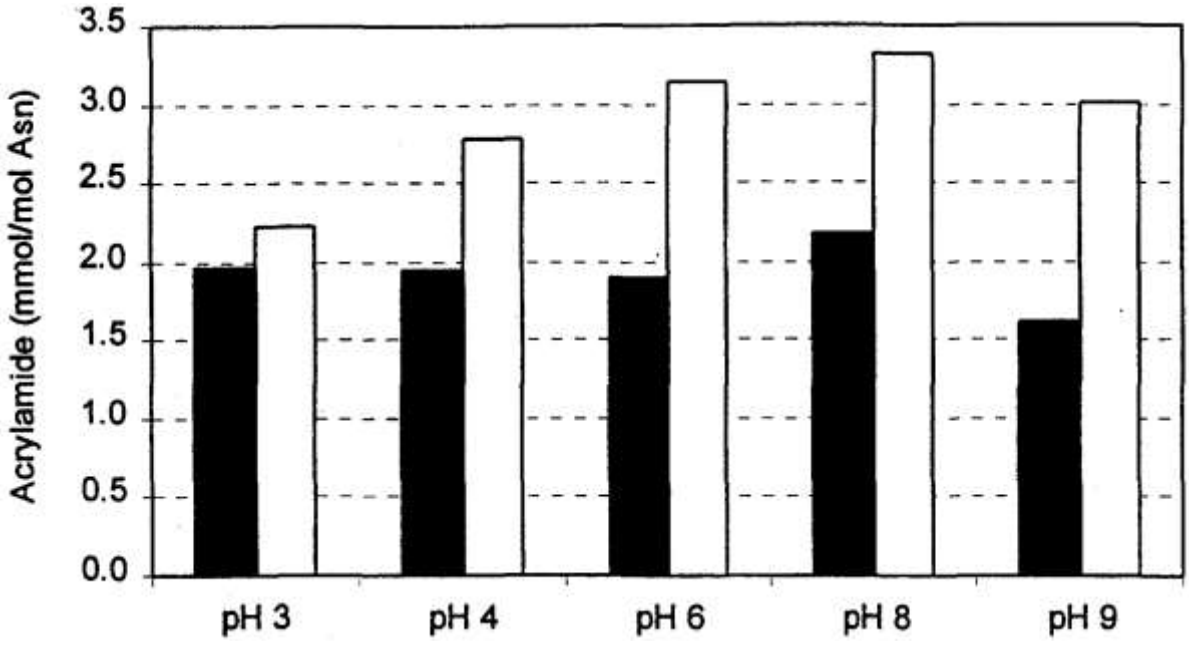


Figure 2.6. Formation of acrylamide in binary mixtures of asparagine and glucose as function of pH. Asparagine and glucose (black bars) or fructose (white bars) as function of pH (180°C, 5 min, 20µL water).

Source: Blank *et al.* (2005)

2.13.3. Temperature

The reaction temperature and time have been shown to influence the formation of acrylamide. In a study done on the pyrolysis of glucose and asparagine for either 5 or 60 min at different temperatures, it was revealed that the formation of acrylamide was higher at 120°C for long time and the highest at 160°C for short time pyrolysis (Figure 2.7). The decrease in acrylamide formation was likely caused by polymerization of the molecule (Stadler *et al.,* 2004). Therefore, the heat load (temperature vs time) in food processing should be taken into account for the control of acrylamide formation.

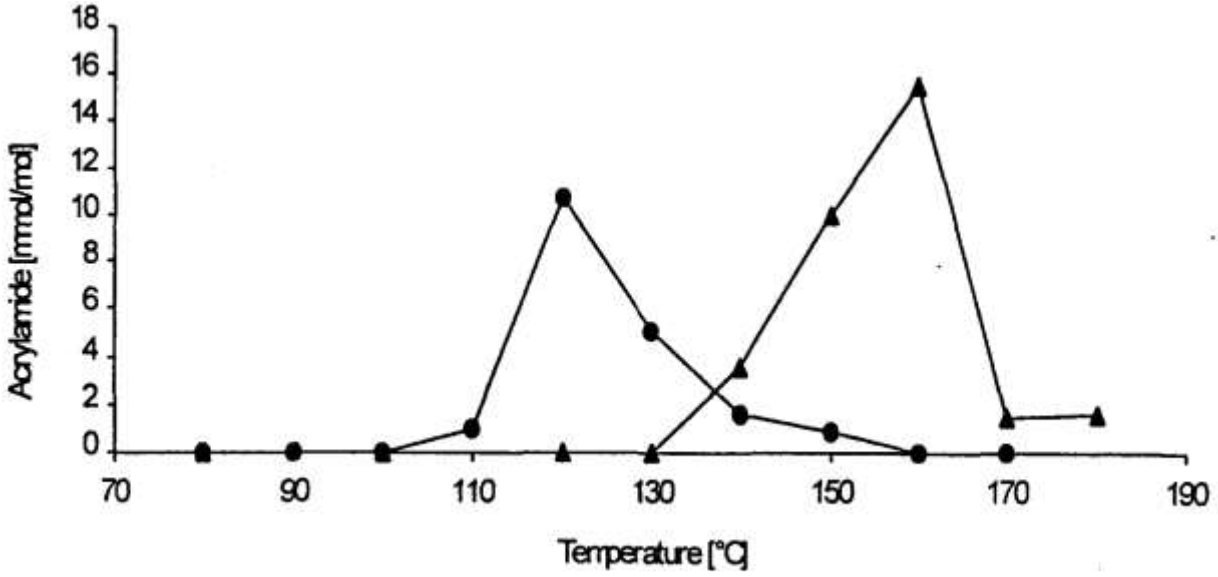


Figure 2.7. Formation of acrylamide by heating a binary equimolar mixture of asparagine and glucose. 5 (Δ) and 60 (•) minutes

Source: Blank *et al.* (2005)

2.14. Pre- and post-harvest effects on acrylamide precursors in cereals

For commodity, an approach is needed to obtain desired levels of the focus compound.
A CIAA (CIAA, 2009) showed the concentrations of free asparagine in wheat bran of 113mgkg^{-1}, and between 267 and 434mgkg^{-1} in wheat flour. In pre-harvest period, there is interaction of genetic diversity (different cultivars) with the physiological age of the storage organ (tubers and grains), environment (climate) and crop management (water and nutrient supply).
In post-harvest period, duration and conditions at storage also influence concentrations of reducing sugars and asparagine. Mitigation strategies to reduce reducing sugars and asparagine may affect yield and other quality attributes which may have financial and/or environmental repercussions.

2.14.1. Genetic resource

There is variation in the concentration of asparagine in cereal species and varieties within a species. A study done on 28 wheat samples, reported a variation in the concentrations of asparagine between 150 and 450mgkg^{-1}. Another one reported an increase of 5 times range among 31 wheat varieties (Taeymans *et al.*, 2004), where for instance, the concentrations of asparagine were between 74 (variety Vault) and 664mgkg^{-1} (variety Abbot).
It was found that 4 wheat varieties containing 267, 321, 390 and 434mgkg^{-1} asparagine, respectively, with almost the same concentrations of the reducing sugars produced bread with 656, 667, 923 and 874mgkg^{-1}, respectively. According to the regression coefficient (R^2) for the correlation which was 0.79, it was suggested that almost 80% of the variation in acrylamide levels was due to variations in asparagine contents of the wheat flour. Table 2.11 also shows why in cereals, where sugars are abundant, asparagine (8% of the molar concentration of reducing sugars in rye and 3 in wheat) is the limiting factor for acrylamide formation in baked cereals, despite its largely low concentration compared to the contents of sugars, whereby asparagine is 8% of the content of reducing sugars for rye and 3% for wheat. Whereas, reducing sugars remain the limiting factor for the formation of acrylamide in heated potato products regardless the content of asparagine in fresh potatoes which is higher as double than that of total reducing sugars (Table 2.11). In cereals, the level of reducing sugars can be 10–50 folds higher than that of asparagine,

depending on cereal species (Table 2.9). It is possible to breed wheat varieties characterized by low asparagine, but it has been reported that to know the trait stability, very little is known about the interaction of the variety with the environment. Genetic modification by preventing the build up of asparagine by changing the expression of asparaginase has been reported by a researchers in the UK, (Elmore *et al*., 2005).

Table 2.9. Chemical composition of rye flour, whole wheat flour and potato flake

	Rye flour	Potato flake	Whole wheat flour
Asparagine (µmolkg^{-1})	4800	27 000	1300
Total free amino acids (µmolkg^{-1})	18 400	70 300	8500
Total reducing sugars (µmolkg^{-1})	57 700	53 200	49 300

Source: Elmore *et al.* (2005); Seal *et al.* (2008)

2.14.2. Environment

The levels of asparagine and acrylamide in wheat flour and heated wheat products vary depending on the growing sites of the wheats. CIAA, (2009) showed that a processor who baked wheat flour from four different regions in the same industrial process recorded varied levels of acrylamide ranging from 13 and 796 mgkg^{-1}. Taeymans *et al.*(2004) obtained different concentrations of asparagine in flour, varying from 329 and to 664 mgkg^{-1} from variety *Abbot* grown at five different sites. Three samples of the wheat variety *Claire* had between 163 and 232mgkg^{-1} and five *Rialto* ones had from 178 to 286 mgkg^{-1} of asparagine contents. It was reported that these levels of asparagine increased with the increase in saline concentrations of the soil, more particularly in sensitive wheat varieties (Lea *et al.*, 2007).

In the contract, the levels of asparagine decreased with increase in the concentration of substrate or soil sulfur (Muttucumaru *et al.*, 2006). In field trials done, sulphur fertilization showed significant effect on the levels of free amino acids in wheat and correlated with baking characteristics, 3-aminopropionamide and formation of acrylamide (Granvogl *et al*., 2007).

2.14.3. Crop management

Soil preparation, irrigation, crop protection and fertilization may have effect on asparagine concentrations. Nitrogen fertilization to increase grain yield has been reported as well to largely influence by increasing protein and asparagine concentrations (Figure 2.8) (Confederation of the Food and Drink Industries of the EU & CIAA, 2004).

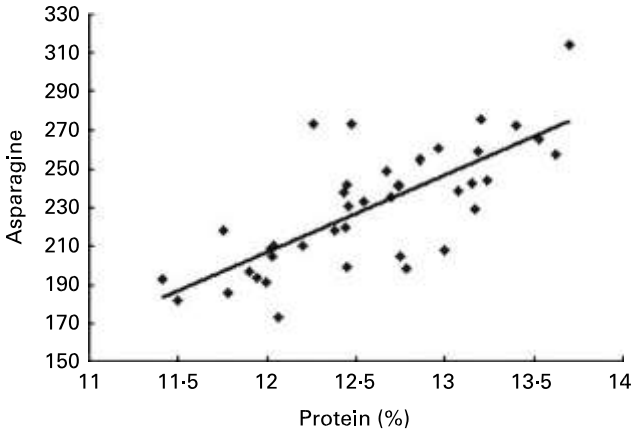


Figure 2.8. Correlation of asparagine level ($mgkg^{-1}$) with protein content for wheat grown over a range of nitrogen fertiliser regimes

Source: Seal *et al.* (2008)

It has been proposed that in order to have a cereal containing desired less amount of asparagine and subsequently reduced formation of acrylamide during processing, the best choice is to search for genotypes which have low amounts of asparagine, to grow them under a regime of moderate nitrogen at the sites that do not allow buildup of asparagine.

2.15. Natural antimicrobial preservatives property of herbs and spices

The use of chemical preservatives in foods has grown concern about their safety. Negative effects to consumers have been related to the intake of many of these preservatives once applied in food. Several studies have done on the properties of herbs and spices as natural preservatives to control

food borne pathogens and spoilage microbes (Dengate and Ruben, 2002; Parke & Lewis, 1992; Ring *et al.*, 2001). Microbial contamination is a big concern for breads to be kept at room temperature for long time. The determination of pH and water activity (a_w) can reveal the type of microbial spoilage for bread. The a_w of wheat bread is generally higher than 0.96 (Puhr & D'Appolonia, 1992; Smith *et al*., 2004) and the pH is higher than 5.5 (8). The shelf-life of breads is mostly endangered by mold growth, in which *Penicillium* spp. and *Aspergillus* spp. are the major mold species (Legan, 1993). Molds are followed by *Bacillus subtilis* as bacterium which forms rope on breads. *Bacillus subtilis* is found in raw bakery materials and their spores can resist baking temperature (Suhr & Nielsen, 2004). Bread is also spoilt by chalk yeasts which form dust-type spots (Nikolajeva *et al.*, 2015). *Zygosaccharomyces bailli* and *Saccharomyces cereviseae* are considered as major species. Breads can also be spoilt by non-chalk yeasts, e.g. *Wickerhamomyces anomalus. W. anomalus* and *S. fibuligera.*

There are many technological measures and organic acids for controlling microbial contamination in baked foods, but few of them, are commercially used in breads (Smith *et al.*, 2004). The main organic acids used in bread are propionic acid and its salts. Packaging and storage can be used to encounter the growth of microbes in breads after baking. Modified atmosphere packaging and storage of breads at created cooling environment might be also a healthier alternative solution against using chemical preservatives in breads. However, at industrial scale, these solutions require huge cost that might not be covered to the extent the bakery wants to get benefits.

The strategies which are not technological and not chemical have been suggested and are still a very interesting subject of development. The use of essential oils (EOs) from spices and herbs is among these strategies. EOs can been applied as natural preservatives to control microbial contamination of foods (Degirmencioglu *et al.*, 2011). Spices and herbs have been used for many years as natural antimicrobial substances to preserve foods and to fortify their aroma and flavor. Spices and herbs can encounter the growth of both Gram–positive and Gram– negative food borne pathogens or spoilage bacteria, yeast, and molds (Juneja *et al.*, 2012). It has been reported that 60% of bread spoilage is due to mold contamination, most notably, *Penicillium species and Aspergillus niger*. Molds also produce off flavor, mycotoxins and allergenic compounds in breads. Spices and herbs as plants own substances such as alkaloids, glycosides, etc., with antimicrobial activity. The classification of these secondary metabolite compounds is categorized as following: 1. phytoanticipins which are inner components in a plant; 2. inducible components and; 3.

phytoalexins which are induced inhibitory compounds when a plant gets attacked by a pathogen (Gould, 1996; Sanchez Maldonado *et al.,* 2015). The ability of EOs against fungal and bacterial growth has been known since the Middle Ages (Bakkali *et al.,* 2008). Their application in foods may require quite high concentrations in order to act efficiently. Furthermore, their isolation or synthesis are costly.

Among 300 of around 3 000 EOs discovered have been used in the food industry, pharmaceutical, agronomic and cosmetic industries (Burt, 2004). Presently, the growing awareness for green consumption and growing negative perception of synthetic/chemical preservatives have interested scientists and industries to research and to optimize applications of EOs in foods.

2.16. Antimicrobial and antioxidant activity of rosemary extract

Rosemary (*Rosmarinus officinalis*, L.), has been grown worldwide as flavoring and medicinal herbs. Furthermore, it is used due to its antimicrobial and antioxidant characteristics to enhance shelf-life of foods. It can be applied either as leaves or leaves extracts. To obtain extracts, leaves are dried, ground and extraction by solvents such as ethanol, methanol, acetone, hexane, and water or using a mixture of solvents, follows (Senanayake, 2013).

The antioxidant substances such as phenolic diterpenes, carnosic acid and carnosol, flavonoids such as flavones and flavonols, and phenolic acids such as ferulic, rosmarinic, and chlorogenic and caffeic acids make over 90% of the antioxidant activity of rosemary extract with strong effect (Campo *et al.*, 2000; Chen *et al.*, 1996; Cuvelier *et al.*, 1996; Frankel *et al.*, 1996; Huang *et al*., 1997; Moreno *et al*., 2006; Pen˜ uelas & Munne´-Bosch, 2005; Richheimer *et al*., 1996; Wellwood and Cole, 2004).

Rosemary phenolic extracts have been reported to possess antimicrobial and antioxidant activities, and have been introduced as preservatives in the food industry (Frankel *et al*., 1996; Cowan, 1999; Smith-Palmer *et al.*, 1998; Venturini *et al*., 2008; Zaika, 1988; Zink, 1997). Food products can be prevented from growth of undesirable microorganisms, spoilage, off-flavor, rancidity, and deterioration and thus improved for stability and loner shelf life, by phenolic extracts which show a large scale of antimicrobial and antioxidant activities as contamination or spoilage are rarely caused by one factor. Antimicrobial and antioxidant activities of plant is favored by its growth and

harvest conditions, as well as extraction methods. Rosemary extract formulations are sold in an oil-soluble form, as a dry powder, and in water-dispersible or water-miscible formulations (Aguilar *et al.*, 2008; Bozˇin *et al.*, 2007).

2.17. Effect of antioxidant phytochemicals on acrylamide content in food

Phytoceuticals like polyphenols or plant antioxidants among other chemicals are believed to act outstandingly through free radical scavenging activity against acrylamide formation in foods (Jin *et al.*, 2013; Kalita *et al.*, 2013;Salazar, Arámbula-Villa, Hidalgo, *et al.*, 2012; Zhu *et al.*, 2010). Several research findings have related active antioxidant substances such as p-coumaric acid, gallic acid, ferulic acid and caffeic acid with substantial reduction of acrylamide concentration in foods close to half or even lower (Abdel-Monem et al., 2013; Kotsiou *et al*., 2011; Zhu *et al.*, 2009). Several hypotheses have stated that antioxidant substances can reduce acrylamide formation in foods. However, there still exist a dispute about effect of antioxidant activity on acrylamide content and antioxidant activity in certain cases. As acrylamide is formed via several sequenced reactions between asparagine and a carbonyl compound with generation of different intermediates, antioxidant compounds can intervene in reactions through these steps according to their molecular structure and functional groups, to increase or reduce acrylamide formation regardless of antioxidant activity (Jin *et al*., 2013). For instance, curcumin has been reported to increase acrylamide level, due to its specific functional groups, not to its antioxidant activity (Hamzalioglu *et al.*, 2013). Similarly, some phenolic acids like chlorogenic acid was shown to convert sucrose into more reactive intermediates like 5-Hydroxymethylfurfural which reacted possibly with asparagine, and increased acrylamide content (Kocadagl *et al*., 2012). On the contrary, some flavonoids like naringenin or epicatechin were reported to decrease acrylamide concentration by reacting with and trapping different intermediates of Maillard reaction (Cheng *et al.*, 2009; Totlani & Peterson, 2005). Jin *et al.* (2013) revealed that antioxidants can interfere in 4 parts of maillard reaction: 1- reactive carbonyl pool as substrate, 2- asparagine as substrate, 3- intermediates and 4- acrylamide as product, to reduce or increase reaction between 2 substrates by activating or deactivating them. Furthermore, these authors reported that antioxidants can change intermediates or final compound to finally change acrylamide formation.

Various studies exhibited that increase in concentration of antioxidant failed to slow down the production of acrylamide in all experiments, instead, there was an increase in acrylamide (Kotsiou *et al.,* 2010; Yuan *et al.*, 2011; Zhu *et al.,* 2010). Comparatively to antioxidants, other conditions such as heating temperature and time, pH and moisture content significantly can change the concentration of acrylamide (Jin *et al.*, 2013). It was reported that crude aqueous extracts in some experiments inhibited the production of acrylamide at high level in comparison to their pure components, most notably, poly phenols. This is the reason why in addition to poly phenolic compounds, other phytochemicals can also mitigate acrylamide formation. These phytochemicals can do it either by free radical scavenging activity or by other ways including trapping carbonyl moiety or/and precipitating asparagine and components such as proteins, peptides, saccharides and monovalent/divalent cations (Oral *et al.*, 2014; Cheng *et al.*, 2009; Zhu *et al*. 2009; Zhu *et al.* 2010).

CHAPTER THREE: THE PHYSICOCHEMICAL CHARACTERISTICS OF WHOLE WHEAT FLOUR FROM SELECTED RWANDAN WHEAT VARIETIES

Abstract

The present study aimed at determining the physicochemical characteristics of whole wheat flour from selected Rwandan wheat varieties in order to know their potentials for processing. Four varieties namely Gihundo, Kibatsi, Nyaruka, and Reberaho were selected. These wheat varieties were grown at the same location and under the same agro ecological and cultural conditions. The grains from these varieties were milled into flour containing all bran and germ. Gihundo wheat variety had the highest values for ash content (1.47 %) and total dietary fiber content (15.97 %), water absorption capacity (89.00 %), dough development time (7.62 min) and brightness (84.67 %). For the same physicochemical characteristics, whole flour from Nyaruka wheat variety showed the lowest values for water absorption capacity (80.00 %), dough development time (6.33 min) and brightness (80.33), while whole flour from Reberaho wheat variety exhibited the lowest values for the contents of ash (0.98 %) and total dietary fiber (12.44 %). The protein content ranged between 10.00 % and 10.85 % for whole flours from all wheat varieties. The results showed that whole flour from Gihundo wheat grain variety exhibited high values for most of the physicochemical characteristics determined in comparison to the other three varieties. It is important to select grains or flour from these wheat varieties newly introduced in Rwanda based on the individual cultivar because their derivative products could have a more desired quality.

3.1. Introduction

Whole wheat flours have been gaining an increase in demand and utilization for their nutritive value and health benefits in different food products for human consumption worldwide. Whole grain flour as flour consists of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components (the starchy endosperm, germ, and bran) are present in the same relative proportions as they exist in the intact caryopsis. Whole grain flour contains more vitamins, minerals, antioxidants, bioactive phytochemicals and dietary fiber than refined flour (Chung *et al.*, 2009; Adom and Sorrells, 2005; Slavin, 2004). Due to health promoting effects of phytochemicals and dietary fiber, an increased consumption of whole grains is recommended (Seal, 2015). While the extraction yield affects the (dough) farinograph test properties of whole wheat flours, these phytochemical compounds can also affect the quality of the end-use products such as low loaf volume and dense crumb structure, grainy, nutty and bitter flavors (Heinio, 2009; Chang and Chambers, 1992), and darker crumb and crust color (Lebesi and Tzia, 2011).

Among the phytochemicals, carotenoids and phenolic compounds such as anthocyanins can act as antioxidants and impact the color and flavor of the food product (Rao and Rao, 2007). On average, the total carotenoid content was 2.57 mg kg^{-1} for the whole wheat grain, 1.92 mg kg^{-1} for the endosperm, 9.11mg kg^{-1} for the germ and 0.74 mg kg^{-1} for the bran. Those values for the endosperm, the germ and the bran were calculated based on seed fraction mass proportions to whole grain (Ndolo and Beta, 2013). Therefore, depending on the varieties, the physicochemical characteristics of whole wheat grains would significantly differ and influence the processing and quality of end-products. Some physicochemical attributes of the grains and flours of these new wheat varieties released
in Rwanda in 2017 have not yet been determined so far in order to provide industries and consumers wheat with preferred specific quality traits and functionality (MINAGRI, 2017; Newtimes, 2017; RAB, 2017). This may often result in promising wheat cultivars that can be rejected by a farmer, seller, processor or consumer (Battenfield *et al.*, 2016). With regard to this, the present study aimed to determine the physicochemical characteristics of whole wheat grains which can influence the quality of their based products.

3.2. Materials and methods

3.2.1 Collection of the samples

Four (4) dry wheat grain varieties namely TAI, EN161, Eagle10 and Korongo with their local names Gihundo, Kibatsi, Nyaruka, and Reberaho, respectively, were collected from the stores of Rwanda Agriculture and Animal Resources Development Board (RAB) located at Kinigi, Musanze district, Rwanda. These wheat varieties were grown in RAB farms at the temperature varying between at 16.6 and 21.5 ^{o}C, at the same location and under the same agro ecological and cultural conditions in the crop year 2018. The wheats were grown in volcanic soil and fertilized with urea and Diammonium phosphate (DAP). The wheat grains were sampled in the same year, packed in high density polyethylene bags and stored at room temperature prior to milling.

3.2.2 Preparation of the raw samples

3.2.2.1 Wheat grains milling

Before milling, the wheat grains were conditioned to 15.5 % moisture content by the addition of distilled water and were left for at least 24 hr at ambient conditions in a closed plastic container for the absorption of the moisture (Mishra, 2016). The wheat grains were conditioned to get fine whole wheat flour. AACC (2003) International Approved Method 26-95.01 was used to calculate the amount of water to be added for wheat grains tempering:

$$ml = \left(\left(\frac{100 - \%\, moisture}{100 - 15.5\,\%}\right) - 1\right) x\ grams\ of\ wheat\ grains$$

The conditioned wheat grains of each variety were wholly milled for 15 min by using a laboratory hammer mill (CM 1090 Cemotec, 2009, China). All bran and germ were mixed with the flour. The flour was packaged in high density polyethylene envelop and stored at 5^{o}C in a fridge (SM302NW, SM302NW1014009, 2010/08, China) prior to analysis and baking.

3.2.3 Determination of physicochemical characteristics

3.2.3.1 Moisture content of whole wheat flour

The whole wheat flour sample (0.5g) was used for the determination of moisture content by using moisture analyser (Model: HE 53/01, Mettler Toledo, 2017, China).

3.2.3.2. Ash content of whole wheat flour

The sample was prepared and ash content was determined as described by AACC (1999) International Methods (62-05.01) and (08-01.01), respectively, by using a muffle furnace (Lindberg/Blue 1100°C Box furnace BF 51800 series Ashville, NC). Whole wheat flour (5 g) were put into ashing dish that has been ignited, cooled in desiccator, and weighed soon after attaining room temperature. The samples were placed in muffle furnace at 575°C and incinerated until light gray ash was obtained or to constant weight. The samples were cooled in desiccator and weighed soon after room temperature was attained. The ash content was calculated as below:

$$\% \text{ Ash content} = \frac{\text{Weight of residue}}{\text{Sample weight}} \text{ X } 100$$

3.2.3.3. Total fat content of whole wheat flour

Total fat content was determined by AACC (1999) International Method 30-10.01. with HCl acid hydrolysis. Five grams of whole wheat flour were weighed into extraction thimbles and fixed into extraction flask of known weight. Extraction was carried out using petroleum ether on electro-thermal model equipment for eight hours. At the completion of the extraction, the petroleum ether was removed by evaporation on an electrical bath and the remaining fat in the flask was heated to constant weight at 100°C, cooled for 15 minutes and weighed. The fat content was calculated as follows:

$$\% \text{ Fat content} = \frac{\text{Weight of fat (Corrected for blank)}}{\text{Sample weight}} \text{ x } 100$$

3.2.3.4. Total protein content of whole wheat flour

The determination of total protein content was done by AACC (1999) International Kjeldhal Method 46-12.01. Digester (Heating Digester, DK,Velp Scientifica) and Distillation unit (Semi-automatic, UDK 139, Velp Scientifica) were used. The samples (1g) of whole wheat flour were placed in digestion flask. Polyethylene packet of catalyst, and 25 ml concentrated H_2SO_4 (0.1N) were added to flask. Flask containing 50 ml boric acid-methyl redmethylene blue indicator solution was placed under condenser tube. Concentrated NaOH (50 ml) was added and boiled until all ammonia distilled (at least 150 ml of distillate). The protein content was calculated as below:

$$\% \text{ protein } = \frac{\text{ml standard H2SO4 x 1.4007 x N of H2SO4 x 5.7}}{\text{Weight of sample(g)}}$$

3.2.3.5. Total dietary fiber content of whole wheat flour

Dietary fiber content was determined as per AACC (1999) International Method 32-07.01.
The samples (1g) of whole wheat flour were put into beakers. MES-TRIS (40 ml) blend buffer solution (pH 8.2) was added to each beaker. Heat-stable α-amylase solution (200 µl) was added and incubated for 35 min at 95°C with continuous agitation. Protease solution (100 µl) was added to each sample and incubated in shaking water bath at 60°C, with continuous agitation for 30 min. To each sample, 225 ml 95% EtOH preheated to 60°C was added. Residues were formed at room temperature for 60 min.

Total dietary fiber content was calculated as follows (AACC, 1999) International Method 32-07.01):

$$\text{Total dietary fiber (\%)} = \frac{\frac{R_1 + R_2}{2} - p - A - B}{\frac{m_1 + m_2}{2}} \times 100$$

where R_1 = residue weight 1 from m1, R_2 = residue weight 2 from m_2, m_1 = sample weight 1, m_2 = sample weight 2, A = ash weight from R_1, p = protein weight from R_2, and

$$B = \text{blank} = \frac{BR_1 + BR_2}{2} - BP - BA$$

where BR = blank residue, BP = blank protein from BR_1, and BA = blank ash from BR_2.

3.2.3.6. Total carbohydrate content of whole wheat flour

Total carbohydrate content was calculated as described by FAO (2003).

Total carbohydrate content = 100 – (moisture % – protein % – fat % – ash %).

3.2.3.7 Bulk density of whole wheat flour

The bulk density was determined as described by Khalid *et al.* (2017). The flour samples were gently filled into 10 ml graduated plastic cylinders. The bottom of the cylinder was gently tapped on a laboratory bench covered with foam several times until there was no further diminution of the sample level. The mass of the sample was calculated and the bulk density was calculated as mass of sample per unit volume of sample (g^{-ml}).

3.2.3.8 Oil absorption capacity of whole wheat flour

The oil absorption capacity was determined as described by Khalid *et al.* (2017). Each sample of 0.5 g was mixed with 6 ml of mustard oil in pre-weighed centrifuge tubes. The contents were vortexed for 1 min to disperse the sample in the oil. The samples were then kept for 30 min in vertical positions and then subjected to centrifugation of 3000 rpm for 15 min (3-18KS, Sigma Laborzentrifugen GmbH, Germany). The layer of the oil was removed by pipette and the tubes were kept in inverted position for 10 min to drain the oil before reweighing. The gain in mass was expressed as grams of oil absorbed per gram of flour.

3.2.3.9. Wheat grain hardness

The wheat hardness was determined as described by Manley (1995). The ground grain samples were obtained by passing the whole wheat grain (15.5 % moisture content) through a Model 3100 hammer mill (Falling Number AB, Huddinge, Sweden) equipped with 1mm screen. The wheat hardness was calculated as the % of ground wheat grain (10 g) forcing through 75 micrometer air jet sieve in 90 s. The mass less than 4.0 g indicated a hard wheat, while 4.0 g or more indicated a soft wheat.

3.2.3.10. Rheological characteristics of whole wheat flour

The determined rheological parameters were the water absorption, dough development time, dough stability time and mixing tolerance index. The parameters were determined by using Brabender Dvisburg, 800101, Germany. AACC (2000) method (54-21.02) was used. The flour sample (300 g) was placed into a mixing bowl and distilled water was added by burette-titration up to the optimum dough consistency. The curve was centered on the 500-Barbender Unit (BU) line ±20 BU by adding the appropriate amount of water until the curve left the 500-BU line.

3.2.3.11. Determination of least gelation concentration

Least gelation concentration was determined according to method described by Benelhadj *et al*. (2016). The sample of flour was mixed with 5 mL of distilled water in a centrifuge tube to obtain 2; 4; 6; 8; 10; 12; 14; 16; 18 or 20% (w/w) concentrations. The centrifuge tubes were heated for 1 h in a boiling water bath, cooled rapidly at 20^{o}C and further cooled for 2 h in a refrigerator at 4^{o}C. The least gelation concentration was regarded as the concentration at which the sample from the inverted tube did not fall or slip.

3.2.3.12. Color of whole wheat flour

The color was determined by a color reader (CR-10, Konica Minolta, inc. Japan) on the basis of L* (100 for white and 0 for black), a* (Positive values for red color and negative values for green color) and b* values (Positive values for yellow color and negative values for blue color). Before measuring, the instrument was standardized by white tile (L* = 89.5, a* = -0.7, b* = 1.8).

3.2.4. Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using SAS System for Windows (version 9.3, SAS Institute, Cary, NC). Treatment means were separated using the Tukey test and least significant difference was accepted at $p \leq 0.05$.

3.3. Results and discussion

3.3.1. The proximate composition of whole wheat flours

The proximate composition of whole wheat flour from selected Rwandan wheat varieties is shown in Figure. 3.1.

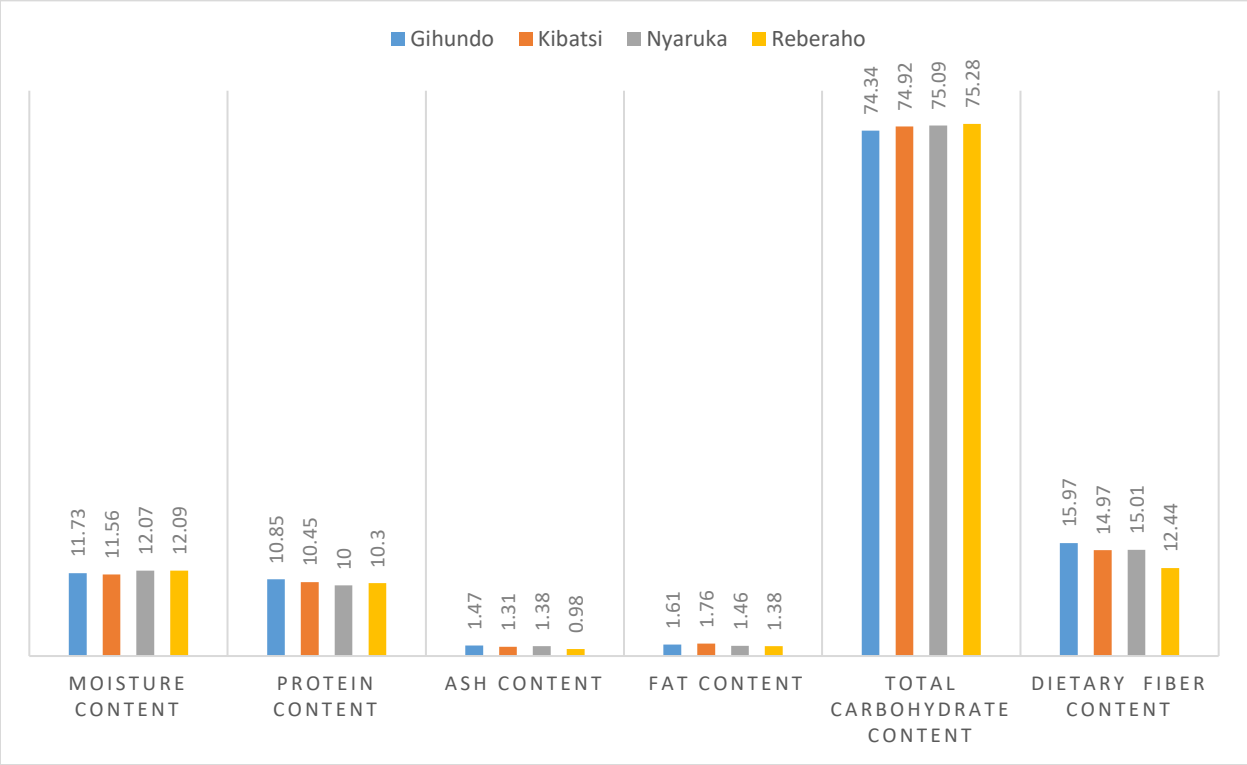


Figure. 3.1. The proximate composition of whole wheat flours from Gihundo, Kibatsi, Nyaruka and Reberaho wheat varieties (Dry weight basis except for moisture content)

Values are means ±SD of 3 replications and are in %. Treatment means are significantly different at $p \leq 0.05$.

Moisture content ranged from 11.56 % to 12.09 %. The moisture contents of the wheat flours from Nyaruka and Reberaho varieties were not significantly different ($p > 0.05$) and were higher than those from the other two wheat varieties. Increase in moisture content could have been associated

with increase in fibre (Maneju and Udobi, 2011). Excess moisture content above 12 % may be detrimental as it can lead to mould growth, toxin formation, insect infestation, sprouting in storage (Ezeama, 2007) and has the effect of reducing the protein content as a percentage of total mass. On the other hand, grain with excessively low moisture would result in a hard grain with low flour yield (Mishra, 2016).

The results for protein content were between 10.00 % and 10.85 %. There was no significant difference in protein content ($p > 0.05$) among whole wheat flours from the four wheat varieties, may be because they were grown at the same location and under the same agro ecological and cultural conditions. The wheat flours from all the varieties could possibly be categorized as all-purpose since their protein contents were between 9% and 12% (Chu, 2004).

The content for ash was between 0.98 % and 1.47 % and the content for dietary fiber was from 12.44 % to 15.97 %. The ash content results were in line with 0.42 % found by Khalid (2017) and from 1.46 to 1.56 % reported by Mishra (2016). Khalid *et al.* (2017) worked on the proximate composition of the native and irradiated whole wheat flour from wheat grains var. WH-1021, while Mishra (2016) compared the proximate composition of whole wheat flours and wheat flours made from five wheat cultivars (Advance, Prevail, Select, Brick and Forefront). The results for dietary fiber levels were similar to those found by Bressiani *et al.* (2016), where they were between 12.45 % and 15.95 % in the study done on the effect of particle size on the damaged starch content, proximate composition, gluten content, phenolic content and free sulfhydryl groups of fine whole wheat flour, medium whole wheat flour and coarse whole wheat flour from BRS Guabiju, wheat variety. There was a significant difference ($p \leq 0.05$) of the ash and dietary fiber contents among whole wheat flours from all wheat varieties. The ash content may have differed due to mineral content which varied according to wheat cultivar (Bressiani *et al*.,2016). Therefore, it could be the reason why the whole wheat flour from Gihundo variety had the highest ash and dietary fiber contents, while the whole wheat flour from Reberaho variety had the lowest ash and dietary fiber contents. The high ash content of the flour indicates that whole grain flour could be an important source of minerals. Increased dietary fiber content of the flour has several health benefits; it aids in the digestion of the bread in the colon and reduces constipation often associated with bread produced from white wheat flour (Jideani, 2009). Dietary fibre plays a significant role in the

prevention of several diseases such as cardiovascular diseases, diverticulosis, constipation, irritable colon, cancer and diabetes (Slavin, 2004).

The minimum and maximum total fat contents were 1.38 % and 1.76 %, respectively. Fat content differed significantly ($p \leq 0.05$) from one type of whole wheat flour to another one. Flour from Kibatsi had the highest fat content, while flour from Reberaho wheat variety had the lowest fat content. Wheat grains with a high amount of germ could have given whole wheat flours with a high amount of fat (Mishra, 2016). Fat plays a significant role in the shelf life of food products and as such relatively high fat content could be undesirable in food products. This is because fat can promote rancidity in foods, leading to development of unpleasant and odorous compounds. Bread loaf volume can increase with free polar fat contents that are naturally present in a particular cultivar, mostly when utilized samples are from pure wheat breeding lines (Chung *et al.*,1982).

In the study investigating the relationships between textural qualities of noodles and flour lipids, Qiyu and Siyuan (2009) found that as the free lipid content of the flour was increased, hardness of noodles linearly increased, reaching a maximum at a level of 1.84 g 100 g^{-1} flour, thereafter falling to a low value. The same author reported that cohesiveness of noodles was significantly ($p \leq 0.05$) decreased due to removal of free lipid while the highest cohesiveness value was obtained at a free lipid content of 1.24 g 100 g^{-1} flour. A higher free lipid content in flour would reduce cohesiveness of noodles. According to these findings, whole wheat breads made from the analysed wheat varieties would be harder and less cohesive.

Total carbohydrate content ranged between 74.34 % and 75.28 %. There was a significant difference ($p \leq 0.05$) in carbohydrate contents among whole wheat flours from all wheat varieties, where whole wheat flours from Reberaho and Gihundo varieties had the highest and the lowest carbohydrate contents, respectively. B-vitamins and minerals from the wheat bran and germ of the whole wheat breads avail carbohydrate for proper assimilation in humans (Connection, 2017). Whole wheat flour from Reberaho wheat variety could be a good source of metabolisable energy and could assist also in fat metabolism because of its high carbohydrate content (Ifie, 2011).

3.3.2. The physical properties of whole wheat flours

The physical properties of whole wheat flours from selected Rwandan wheat varieties are present in Table 3.1

Table 3. 1. The physical properties of whole wheat flours from different wheat varieties

Wheat variety grains	Grain hardness (%)	Bulk density (g ml^{-1})	Oil absorption capacity (g g^{-1})	Least gelation concentration (%)
Gihundo	30.30 ± 0.10^{a}	0.56 ± 0.13^{b}	1.45 ± 0.01^{d}	70.23 ± 0.03^{d}
Kibatsi	33.60 ± 0.01^{b}	0.35 ± 0.09^{a}	1.31 ± 0.10^{c}	56.23 ± 0.02^{c}
Nyaruka	38.60 ± 0.02^{c}	1.24 ± 0.12^{a}	0.34 ± 0.01^{a}	40.36 ± 0.15^{a}
Reberaho	38.20 ± 0.21^{c}	1.28 ± 0.01^{b}	0.32 ± 0.02^{a}	47.42 ± 0.21^{b}

Values are means ± SD of 3 replications. Treatment means followed by different superscripts in the same column are significantly different at $p \leq 0.05$.

The grain hardness was from 30.30% (3.03 g) to 38. 60 % (3.86 g). The results showed that all wheat grains were possibly hard as their results were below 4 g or 40 % (Manley ,1995). The range for oil absorption capacity was from 1.24 g g^{-1} to 1.45 g g^{-1}. These results were in line with the ones obtained by Khalid *et al.* (2017). Oil absorption capacity changed with the types of whole wheat flour, where Gihundo and Nyaruka varieties gave whole wheat flours with the highest and the lowest oil absorption capacity, respectively. The high amount of dietary fiber in Gihundo wheat flour (Table 3.1) might have been responsible for the high oil absorption capacity of the flour (Chou and Huang, 2003). The high oil absorption capacity makes the flour suitable in facilitating enhancement in flavor and mouthfeel when used in food preparations (Kaushal and and Kumar, 2012). The results for bulk density of the whole wheat flours were between 0.32 g ml^{-1} and 0.56 g ml^{-1}. These results were near to those found by Offia *et al.* (2015). The bulk density of whole wheat flours from Kibatsi, Nyaruka and Reberaho did not differ significantly (p >0.05). Flour from

Gihundo wheat variety could be denser in comparison to flours from other wheat varieties, and it meant that Gihundo grain endosperm could be filled out better than the endosperm from the other variety grains. The high bulk density of flours suggests their suitability such as thickener in food products and for use in food preparations since it helps to reduce paste thickness which is an important factor in convalescent and child feeding (Kaushal and Kumar, 2012). In contrast, low bulk density would be an advantage in the formulation of complementary foods (Akpata and Akubor, 1999).

The results for least gelation concentration (LGC) were in the range of 40.36 % and 70.23 %. Whole wheat flour from Gihundo showed high LGC value possibly due to its high total protein content (10.85%) compared to that of flour from Kibatsi (10.45%), Reberaho (10.30%) and Nyaruka (10.00%), as well as to protein quality. Fleming et al. (1975) reported that gelation capacity of legume proteins was attributable to the globulin fraction. The increasing concentration of protein enhances the interaction among the binding forces which in turn increases the gelling ability of the flour (Lawal, 2004). LGC can also vary from flour to flour depending on the relative ratios of their structural constituents like protein, carbohydrates, and lipids, suggesting that interactions between such components may also have a significant role on LGC (Abbey & Ibeh, 1988; Sathe et al., 1982). The lower the least gelation concentration, the better is the gelating ability of the protein ingredient and the swelling ability of the flour is enhanced (Kaushal and Kumar, 2012). Least gelation capacity measures the minimum amount of flour needed to form a gel in a measured volume of water. The low least gelation concentration of wheat flour may be an asset as an additive to other gel forming materials in food products.

3.3.3. The rheological properties of whole wheat flours

The rheological properties of whole wheat flours from selected Rwandan wheat varieties are shown in Figure. 3.2.

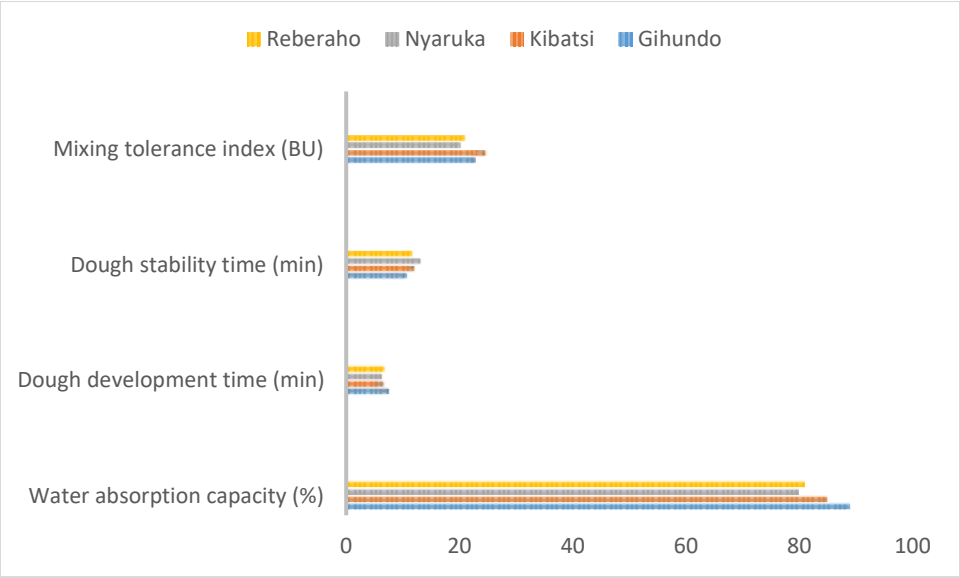


Figure. 3.2. Rheological properties of whole wheat flour from Gihundo, Kibatsi, Nyaruka and Reberaho wheat varieties.

Values are means ±SD of 3 replications. Treatment means are significantly different at $p \leq 0.05$.

The range for water absorption capacity was between 80 % and 89 %. The range for those results was compared to 0.85 g g^{-1} (85 %) found by Khalid *et al.* (2017) for wheat grains var. WH-1021 in the study on physicochemical properties of native and irradiated whole wheat flour. Gihundo and Nyaruka varieties gave whole wheat flours with high and low values of water absorption capacity, respectively in comparison to other varieties. The total fiber content in the flour might have been responsible for high and low water absorption capacity as reported by Chou and Huang (2003). In this regard, Gihundo variety which produced whole wheat flour with high water absorption (89.00%) had high total fiber content (15.97%, Fig. 3.1) and whole wheat flour from Nyaruka variety with low water absorption capacity (80.00%) showed low total fiber content

(12.44%, Fig. 3.1). Water absorption capacity is a key parameter in the purchase of flour for breadmaking (Webb and Owens, 2003).The high water absorption capacity of the flours may assure product cohesiveness (Houson and Ayenor, 2002).

The time for dough development was between 6.33 min and 7.62 min and for stability, it was between 10.72 min and 11.65 min. Gihundo variety followed by Kibatsi variety produced whole wheat flours with long development time in comparison to Nyaruka and Reberaho varieties. The last two were not significantly different ($p > 0.05$) in development time. As the dough development time is the time taken from when water is added up to when the dough reaches maximum consistency, the results indicated that whole wheat flours from Nyaruka and Reberaho varieties took short time for optimum mixing compared to flours from Gihundo and Kibatsi varieties. The stability time shows the maximum consistency for a dough and is a good indication of its strength.

Flours from Nyaruka and Gihundo varieties took the longest and the shortest time, respectively, for dough stability. Thus, the dough obtained from Nyaruka wheat variety lasted long with high consistency, while similar consistency for the dough from Gihundo wheat variety lasted a short time. The results for dough development time (DDT) and dough stability were in line with those found by Bressiani *et al*. (2016). In the evaluation of the effect of particle size on mixture properties, extensional properties and paste properties of refined flour and whole grain wheat flour from BRS Guabiju, wheat variety, those authors reported between 7.60 min and 12.93 min for DDT, between 11.6 min and 12.8 min for dough stability for fine whole grain wheat flour, medium whole grain wheat flour and coarse whole grain wheat flour. For the flours which showed low dough development and stability times, they could have been affected by weakened gluten network with fiber-rich bran particles (Bae et al., 2014).

Mixing tolerance index (MTI) was from 20.17 BU to 22.88 BU. Mixing tolerance index significantly differed ($p \leq 0.05$) in whole wheat flours obtained from all wheat varieties. Whole wheat flour from Gihundo and Kibatsi varieties showed the highest and the lowest values of mixing tolerance index. This indicated that dough from Gihundo variety whole wheat flour was hard and dough from Kibatsi variety whole wheat was soft during mixing. The hardness of dough mixing could contribute to firm product texture. Mishra (2016) who compared the rheological parameters among ground whole wheat flours and flour incorporated with treated bran, found the results in

same range, where MTI was between 18.13 BU and 27.75 BU for ground whole wheat flours from five wheat cultivars (Advance, Prevail, Select, Brick and Forefront).

3.3.4. The color of whole wheat flours

The color charactersitics of whole wheat flour from selected Rwandan wheat varieties are present in Table 3.2.

Table 3.2. The color of whole wheat flours from different wheat varieties

Wheat variety grains	L*	a*	b*
Gihundo	84.67±1.04^{c}	1.50±3.01^{a}	10.67± 3.07^{a}
Kibatsi	82.67± 2.08^{b}	1.90±0.27^{ab}	10.23± 0.54^{a}
Nyaruka	80.33±1.04^{a}	2.10± 1.04^{b}	10.47±1.50^{a}
Reberaho	80.52± 3.03^{a}	2.30±0.58^{b}	10.14±1.04^{a}

Values are means ±SD of 3 replications. Treatment means followed by different superscripts in the same column are significantly different at $p \leq 0.05$.

The color ranged between 80.33 and 84.67, 1.4 and 2.3 and 10.14 and 10.67 for L*, a* and b* values, respectively. Gihundo variety followed by Kibatsi variety produced whole wheat flours with high L*values in comparison to Nyaruka and Reberaho varieties. The last two were not significantly different ($p > 0.05$) in L* values. The results indicated that whole wheat flour from Gihundo variety was brighter than the remaining flours. The flour brightness seemed be caused by the wheat cultivar (Maghirang et al., 2006). Redness (a*) and yellowness (b*) values were not significantly different in whole wheat flours from all wheat varieties ($p > 0.05$). The beneficial

effect of wheat bran against colon cancer is attributed to the presence of a high concentration of polyphenolic compounds, which are likely released into the colon as a result of bacterial fermentation (Monica and Whole Grain Connection, 2017). The obtained L*, a* and b*values were very closed to those found by (Mishra, 2016). The author obtained 82.08, 1.71 and 10.64 for L*, a* and b* average values, respectively, for whole grain flour from five hard red spring wheat cultivars.

3.4. Conclusion

The proximate composition was significantly different among whole wheat flours from the wheat grain varieties, except protein content. The rheological parameters of the doughs were impacted by wheat varieties. The whole wheat flour brightness seemed to be caused by the wheat cultivar. Beyond the quantity and economic reasons that most of bakeries qualify wheat grains by their extraction yields, it is also important to consider other qualities such rheological characteristics, color and proximate composition to select grains or flour from these wheat varieties newly introduced in Rwanda based on the individual cultivar because their derivative products could have a more desired quality for competitive markets. For their nutritive value, whole wheat flours have been gaining an increase in demand and use in different food products for human consumption worldwide.

CHAPTER FOUR: THE EFFECT OF DOUGH FERMENTATION, SPENT COFFEE GROUNDS AND JUICES OF LEMON FRUITS AND ROSEMARY LEAVES ON PHYSICAL AND SENSORY ATTRIBUTES OF WHOLE WHEAT BREADS

Abstract

The demand for whole grain flour is increasing worldwide due to its nutritive and health- promoting attributes. Spent coffee grounds (SCG), juices of lemon fruit (L) and rosemary leaves (R), and dough fermentation were assessed for their impact on the texture profile, color and sensory attributes of bread. The mixture of 200g whole wheat flour, 4% spent coffee grounds, 1% juice of lemon fruit and 1% juice of rosemary leaves were fermented using 2% instant dry yeast at 34^{o}C, 60% relative humidity (RH) for 60 min and at 39^{o}C, 85% RH for 120 min, separately. The dough was baked at 180^{o}C for 20 min. The control breads were made for 60 min of fermentation without incorporation of SCG and LR. The values for firmness of the breads were between 1338 g and 6881g. SCG+LR bread (fermented for 120 min) from Reberaho wheat varieties had the highest firmness (1955 g) among other whole wheat breads containing SCG or SCG+LR. The springiness values of the breads were between 0.716 and 0.940 where the springiness of breads from Gihundo variety was the highest. The ranges for whole wheat bread crumb color were 61.17 and 74.37 for L* values, 7.44 and 12.633 for a* values and 12.18 and 24.37 for b*. SCG breads (fermented for 60 min) from Nyaruka variety and SCG+LR breads (fermented for 120 min) from Reberaho variety. a had the lowest L*(61.17) and a* (7.44) values, respectively. Low L*, a*, b* values indicate lower darkness, redness and yellowness, respectively. Breads substuted with SCG+LR (fermented for 120 min) scored high in texture (7), aroma (6.2), taste (5.9), appearance (5.7) and general acceptability (5.7) in comparison to the other breads. Whole wheat breads obtained, satisfied consumers' preferences. Therefore, the application of spent coffee grounds, juices of lemon fruit and rosemary leaves in bread making represents a good opportunity.

4.1. Introduction

The scientific evidences and increasing campaigns on health promoting compounds of whole grain have gained the attention of consumers for the consumption of whole grain based- foods (van der Kamp *et al.*, 2014). The United States' dietary guidelines for Americans explicitly recommend adults the consumption at least three ounce-equivalents of whole grain per day, and that at least half of the grains consumed should be whole grains (van der Kamp *et al.,* 2014). The increasing awareness on consuming functional foods, foods which provide additional health benefits to those coming from normal nutrients, has led the bakeries to produce breads from whole wheat grains as well as white breads (Ndife *et al.,* 2013). Physical, texture profile and sensory characteristics of bread are generally the basis for liking this wheat product by consumer. Physico-chemical characteristics of raw materials and ingredients, and fermentation time are among other major factors which influence the quality of bread. Demirkesen *et al.* (2010) demonstrated that the increase in high crumb hardness was associated with high amount of bran dietary fibre in whole wheat bread. High and low lightness of bread can be caused by strong and weak enzymatic browning of carotenoids, respectively, during dough fermentation (Leenhardt *et al.,* 2006b). Aroma and taste of bread are affected by aromatic compounds from the raw material and ingredients incorporated in the product formulation (Allegrone *et al.,* 2006).

Products from rosemary leaves and lemon fruit have been used in food preparation for their health promoting compounds and preservative properties (Bozˇin *et al.,* 2007; Salim-ur-Rehman *et al.,* 2007).These parts of the plants are well known to be a rich source of phytochemicals and possess anti-microbial potential (Bozˇin *et al.,* 2007; Salim-ur-Rehman *et al.,* 2007)**.** Spent coffee grounds (SCG), as a by-product from coffee brewing, has recently been tested as a new ingredient in food preparation. It was found that SCG has some chemical substances that are beneficial to consumers and was reported to be safe to be applied in food preparation (Martinez-Saez *et al.,* 2017). SCG was used in making of biscuit and bread. Few studies have worked on the application in combination of juices from rosemary leaves (R) and lemon fruit (L), and SCG in food preparation. Consumers like white wheat breads more than whole wheatbreads due to less appealing organoleptic, physical and textural characteristics of

whole bread (Maneju *et al.*, 2011). Consequently, it leads to the low acceptance and economic loss of whole wheat bread. Bakeries need to fulfil the increasing consumer's preferences of healthier and tastier whole wheat breads (Banu *et al.*, 2012). High bran dietary fibre contained in whole wheat flour was reported to increase crumb hardness (Demirkesen *et al.*, 2010) and dough fermentation duration influences enzymatic browning of carotenoids, thereby affects color of bread (Leenhardt *et al.*, 2006b). The inclusion of products from herbs in baking recipe can affect crust and crumb, taste, texture and aroma of bread (Salim-ur-Rehman *et al.*, 2007).

It is in this regard that the present study aimed furthering this research by using the same raw material, ingredients and processing conditions to evaluate their effects on physical quality of whole wheat breads in order to satisfy consumers' preferences regarding the texture, colour and sensory characteristics.

4.2. Materials and methods

4.2.1. Collection of the samples

Four dry wheat grain varieties namely TAI, EN161, Eagle10 and Korongo with the local names Gihundo, Kibatsi, Nyaruka, and Reberaho, respectively, were collected from the stores of Rwanda Agriculture and Animal Resources Development Board (RAB), located at Kinigi, Musanze district, Rwanda. These wheat varieties were grown in RAB farms at the temperature varying between at 16.6 and 21.5 ^{o}C, at the same location and under the same agro ecological and cultural conditions in the crop year 2018. The wheats were grown in volcanic soil and fertilized with urea and Diammonium phosphate (DAP). They were packaged in high density polyethylene bags and stored at room temperature prior to milling. The wastes, known as spent coffee grounds, were directly taken after brewing coffee (Coffea Arabica var. bourbon) in a coffee shop in Musanze town, Rwanda and stored in a transparent plastic bottle in a fridge (SM302NW, SM302NW1014009, 2010/08, China) at 5^{o}C prior to analysis. Fresh green lemon fruits (var. African rough lemon) and raw green rosemary leaves (Var. Arp rosemary herb) were bought from the market in Musanze town, Rwanda, packed in

plastic sachets and stored at 5ºC in refrigerator (Hisense, HBM17158SS, 2015, China) prior to processing. The coffee, lemon and rosemary were all grown in Rwanda.

4.2.2. Preparation of the raw samples

4.2.2.1. Milling of wheat grains

Before milling, the wheat grains were conditioned to 15.5 % moisture content in order to get a fine particle size whole wheat flour by the addition of distilled water and were left for at least 24 hr at ambient conditions in a closed plastic container for the absorption of the moisture (Mishra, 2016). AACC (2003) Approved Method 26-95.01 was used to calculate the amount of water to be added for wheat grains tempering:

$$\boldsymbol{m}l = \left(\left(\frac{100 - \%moisture}{100 - 15.5\%}\right) - 1\right) x\ grams\ of\ wheat\ grains$$

The conditioned wheat grains of each variety were wholly milled by using a laboratory hammer mill (CM 1090 Cemotec, 2009, China). All bran and germ were mixed with the flour. The flour was packaged in high density polyethylene envelop and stored at 5ºC in a fridge (SM302NW, SM302NW1014009, 2010/08, China) prior to analysis and baking.

4.2.2.2. Preparation of juices from lemon fruits and rosemary leaves

The lemon and rosemary juices were obtained by using a blender (Moulinex, LM241, Genuine, 2017, France). The lemon fruits (3kg) were washed and peeled with knife. The rosemary leaves were picked off the woody branch by using hand and washed. Then after, they were cut into quarters and juiced by

by using a blended (Moulinex, LM241, Genuine, 2017, France) for between 30 secs and 1 min until the extraction is complete. Rosemary leaves (5kg) were ground in the blender (Moulinex, LM241, Genuine, 2017, France) between 2 and 4 min until all leaves were thoroughly ground. To get the clear juice, lemon fruit pulp and ground rosemary leaves were separately sqeezed in a cheese cloth (grade 90, 44 × 36 weaves; 2018, Zhuojie, China) by using hand. The wastes (peels, seeds and other solid materials resulted from the extraction of juices were deposited in a bin. The clarified lemon and rosemary juices were kept in transparent plastic bottles and stored at 5^{o}C in a fridge (SM302NW, SM302NW1014009, 2010/08, China) prior to analysis and processing.

4.2.3. Baking

The dough comprising 200g whole wheat flour from each of the wheat variety grains (TAI, EN161, Eagle10 and Korongo), 2% instant dry yeast (GB Ingredients, Dordrecht, 2019, Holland), 2% sodium chloride and potable water (125 mL) (Fredriksson *et al.*, 2004). The amounts of 4% spent coffee grounds (Martinez-Saez *et al.*, 2017), 1% lemon fruit juice and 1% rosemary leaf juice were added. The electric balance (Explorer EX 223, version 2.00/2.00. SN B 333687045, IR Sensor, OHAUS) was used. The mixture was fermented and proofed in a fermenter (Manz Backtechnik GmbH, Creglingen, Germany) at 34^{o}C, 60% relative humidity for 60 min and at 39^{o}C, 85% relative humidity for 120 min after being mixed in dough mixer (Combisteel Dough Mixer Liter, 7455.1400, 2011, China) (Surdyk *et al.*, 2004). Rotation of the mixer was 54 rotations per minute (rpm) for 3 min until the dough came together and then switched to 104 rpm for 6 min. Each fermented dough was covered with a lid and baked at 180^{o}C for 20 min in an oven (Electric baking oven, Lemarkz, Model: LGO-24A, 2010, India). The control bread from each wheat variety was made for 60 min of fermentation without incorporation of SCG and LR. The loaves were depanned and cooled for 2hr, packed in unperforated low density polythene bags, closed and stored at 5^{o}C in a fridge (SM302NW, SM302NW1014009, 2010/08, China) prior to analysis.

4.2.4. Determination of weight, specific volume and density of breads

The weight was determined using electronic balance (JA2002, Yhequipment, China, 2017). Volume, specific volume and density were determined by AACC (2003) International approved method 10-05-01. Specific volume was calculated as cm^3g^{-1} by dividing the loaf volume by its weight. The bread density was calculated as its weight divided by loaf volume.

4.2.5. Assessment of color and texture profile of breads

The breads were kept at room temperature for 1 day. The color of bread crumb was measured by a color reader (CR-10, Konica Minolta, inc. 2016, Japan) on the basis of L*, a* and b* values. Before measuring, the instrument was standardized by white tile (L* = 89.5, a* = -0.7, b* = 1.8). The bread was sliced with bread sclicer (Macadams Baking systems,Model: SM 302NW0, Machine no:SM302NW1014009, 2010, China). The sliced breads were 20mm. The measurement of crumb color was done in the middle on both sides of each slice. The texture profile of bread crumb was analysed by texture analyser (TA.XT.Plus, stable micro systems, 2015, London) with compression probe(SMSP/75) with the following settings and parameters: Sequence Title: TPA1, T.A. Variable No. 1: Compression, Pre-Test Speed: 1.00mm/sec, Test speed: 5.00 mm/sec, Post-Test Speed: 5.00 mm/sec,T.A Variable No: 5:0.0g, Distance: 10.000 mm, Strain: 75.0%, Trigger type: Auto (Force), Trigger Force: 5.0 g.

4.2.6. Evaluation of the sensory characteristics of breads

The bread samples from the four wheat varieties were evaluated for sensory attributes of texture, color, taste, appearance and overall acceptability by a trained 10-member panel, using a 7-point Hedonic scale varying from 1 (dislike very much) to 7 (like very much). A score of 4 was the lower limit of acceptability (Eleazu et al., 2014; Abong et al., 2011). Sensory evaluation was carried out in a sensory evaluation laboratory under white light.

4.2.7. Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using SAS System for Windows (version 9.3, SAS Institute, Cary, NC). Treatment means were separated using the Tukey's test and least significant difference was accepted at $p \leq 0.05$.

4.3. Results and discussion

4.3.1. Texture profile of whole bread crumb supplemented with spent coffee grounds and juices of lemon fruit and rosemary leaves

Texture profile of whole bread crumb made by adding some spent coffee grounds and juices of lemon fruit and rosemary leaves is shown in Table 4.1.

Table 4. 1. Texture profile of whole of wheat bread crumb produced by incorporating spent coffee grounds and juices of lemon fruit and rosemary leaves.

Wheat variety	Ingredients	Fermentation (min)	Firmness (g)	Cohesiveness*	Springiness*	Resilience*
Gihundo	W	60	5717 ± 0.83^{k}	0.646 ± 0.70^{df}	0.843 ± 0.68^{fg}	0.253 ± 0.63^{bcd}
	W:SCG		3360 ± 0.78^{c}	0.730 ± 1.03^{h}	0.94 ± 1.93^{efg}	0.240 ± 0.73^{bcd}
	W:SCG+LR		1468 ± 0.98^{b}	0.610 ± 0.91^{c}	0.716 ± 0.29^{a}	0.276 ± 1.03^{abc}
	W	120	5706 ± 0.53^{k}	0.632 ± 1.22^{def}	0.820 ± 0.73^{de}	0.233 ± 0.23^{abc}
	W:SCG		3343 ± 1.81^{c}	0.696 ± 0.83^{g}	0.720 ± 1.30^{a}	0.230 ± 0.90^{abc}
	W:SCG+LR		1444 ± 0.69^{b}	0.620 ± 2.31^{cd}	0.793 ± 0.63^{c}	0.320 ± 1.08^{f}
Kibatsi	W	60	6651 ± 1.39^{l}	0.620 ± 0.74^{cde}	0.84 ± 0.62^{efg}	0.226 ± 0.41^{abc}
	W:SCG		3205 ± 1.22^{h}	0.720 ± 0.62^{gh}	0.890 ± 1.83^{i}	0.213 ± 0.03^{ef}
	W:SCG+LR		3012 ± 0.73^{g}	0.566 ± 0.97^{b}	0.723 ± 0.51^{a}	0.230 ± 0.77^{ab}
	W	120	6613 ± 1.38^{l}	0.513 ± 0.88^{a}	0.86 ± 0.03^{gh}	0.200 ± 0.23^{a}
	W:SCG		3105 ± 0.23^{f}	0.610 ± 1.73^{c}	0.80 ± 0.41^{cd}	0.160 ± 0.74^{bcd}
	W:SCG+LR		1518 ± 2.31^{c}	0.613 ± 1.93^{c}	0.736 ± 0.39^{ab}	0.256 ± 0.88^{bcd}
Nyaruka	W	60	6881 ± 0.91^{m}	0.610 ± 0.53^{c}	0.730 ± 0.34^{ab}	0.226 ± 1.39^{abc}
	W:SCG		5245 ± 2.20^{j}	0.730 ± 0.88^{h}	0.833 ± 0.53^{ef}	0.210 ± 0.23^{de}
	W:SCG+LR		5043 ± 2.03^{i}	0.646 ± 0.77^{def}	0.730 ± 0.87^{ab}	0.243 ± 0.81^{ef}
	W	120	6866 ± 1.81^{m}	0.500 ± 0.39^{g}	0.876 ± 0.21^{hi}	0.223 ± 0.73^{abc}
	W:SCG		5220 ± 0.97^{j}	0.720 ± 0.34^{gh}	0.783 ± 0.29^{c}	0.200 ± 0.21^{bcd}
	W:SCG+LR		1338 ± 0.63^{a}	0.646 ± 0.74^{f}	0.750 ± 0.23^{b}	0.263 ± 0.62^{cd}
Reberaho	W	60	6872 ± 0.43^{m}	0.617 ± 1.71^{c}	0.725 ± 0.87^{ab}	0.241 ± 0.74^{abc}
	W:SCG		5222 ± 2.20^{j}	0.757 ± 1.30^{h}	0.817 ± 0.93^{ef}	0.239 ± 0.34^{de}
	W:SCG+LR		2351 ± 0.90^{e}	0.637 ± 0.39^{def}	0.741 ± 0.98^{ab}	0.333 ± 0.73^{ef}
	W	120	6847 ± 1.73^{m}	0.604 ± 0.88^{g}	0.851 ± 0.78^{hi}	0.247 ± 0.97^{abc}
	W:SCG		5012 ± 0.23^{i}	0.710 ± 0.93^{gh}	0.771 ± 1.08^{c}	0.217 ± 0.69^{bcd}
	W:SCG+LR		1955 ± 0.87^{d}	0.634 ± 0.82^{f}	0.747 ± 0.70^{b}	0.351 ± 0.91^{cd}

Values are means ±SD of 3 replications. Means with different superscript within a column are significantly different at $p\leq 0.05$. W: Whole wheat flour (Control flour), W: SCG: Whole wheat flour supplemented with spent coffee grounds (SCG), W: SCG+LR: Whole wheat flour supplemented with spent coffee grounds, juice of lemon fruits (L) and juice of rosemary leaves (R).

*****Dimensionless.

The values for firmness of the breads were between 1338g and 6881g.The hardness of 100% wheat flour bread that was fermented for 60 min from Nyaruka followed by Kibatsi varieties was higher than that of breads from Gihundo and Reberaho ($p \leq 0.05$). Regarding ingredients, the control breads were firmer than breads containing SCG and SCG+LR. The substitution of whole wheat flour as a rich source of bran dietary fiber (Wang *et al.*, 2014) with SCG and SCG+LR reduced the dietary fiber content of the resultant bread compared to control bread (Table 5.1). The results are in agreement with the report of Demirkesen *et al.* (2010) who demonstrated that the increase in crumb firmnes was associated with high amount of bran dietary fibre in whole wheat bread.

The W: SCG+LR breads from the dough fermented for 120 min were lighter than those fermented for 60 min ($p \leq 0.05$). This was possibly due to long fermentation of dough with low pH (Table 5.3) that was enhanced by lemon juice addition. The latter could have lowered the pH (Table 5.3) to the optimum for yeast's activity to release more gas. With low pH values, there is a net positive charge, and proteins become more soluble (Komlenić *et al.*, 2010). Bread firmness indicates its mechanical strength and deformation behavior which in turn influence consumers' perception of texture (Gao *et al.*, 2014).

The springiness values of the breads were between 0.716 and 0.940 (Table 4.1). The springiness of breads from Gihundo variety was higher than those of the breads made from the remaining varieties. The breads from Nyaruka and Reberaho varieties had the least springiness ($p \leq 0.05$) as shown in Table 4.1. The incorporation of SCG and SCG+LR decreased and increased respectively the springiness of the control breads when fermentation of dough was extended to 120 min ($p \leq 0.05$). Springiness is a measure of how much the bread crumb springs back after being compressed once and it can be defined as the elasticity of the bread crumb. It is an important parameter to determine the staling degree of bread (Boz and Karaoğlu, 2013).

The minimum and maximum cohesiveness of the breads were 0.500 and 0.757 (Table 4.1), respectively. The control bread processed from Nyaruka variety had the lowest cohesiveness while the bread from Gihundo had the highest value ($p \leq 0.05$). The separate incorporation of SCG and SCG+LR decreased and increased respectively the cohesiveness of the control breads when fermentation duration was prolonged from 60 min to 120 min. The decrease in cohesiveness indicates increased susceptibility of the bread to fracture or crumble.

The range for resilience of the breads was 0.160 and 0.351(Table 4.1). The resilience of the breads was not significantly different ($p>0.05$) among the control breads from all the varieties. Increase and decrease in resilience were observed when whole wheat flour was mixed with SCG+LR and SCG, respectively. When fermentation duration was prolonged from 60 to 120 min, the resilience of control bread decreased with the supplementation of whole wheat flour with SCG and increased with SCG+LR incorporation ($p\leq0.05$). Resilience is correlated with the extent of staling. Typically, the fresher the bread, the higher is its resilience value, while stale bread shows little or no resilience. Staling makes the crumb structure or internal matrix stiffer or firmer and more brittle (crumbly) over time (Cauvain, 2004). The resilience of bread is directly proportional to its springiness (Zhang,1999).

4.3.2. The color characteristics of whole wheat bread crumb subsituted with spent coffee grounds and juices of lemon fruits and rosemary leaves

The color characteristics of whole bread crumb from selected Rwandan wheat varieties and produced by adding spent coffee grounds and juices of lemon fruit and rosemary leaves are present in Table 4.2.

Table 4.2. Color of whole wheat bread crumb made by incorporating spent coffee grounds and juces of lemon fruits and rosemary leaves

Wheat variety	Ingredients	Fermentation (min)	L*	a*	b*
Gihundo	W	60	71.77±0.97^{j}	7.63±0.19^{a}	21.43±0.36^{j}
	W:SCG		65.60±0.10^{e}	8.50±0.14^{ab}	18.63±0.28^{h}
	W:SCG+LR		68.23±1.34^{h}	8.03±0.08^{ab}	18.50±0.06^{h}
	W	120	74.37±0.44^{l}	8.56±0.15^{b}	24.37±0.10^{k}
	W:SCG		69.50±0.16^{i}	10.90±0.11^{c}	17.73±0.97^{g}
	W:SCG+LR		72.50±0.91^{k}	8.30±0.13^{ab}	17.73±0.28^{g}
Kibatsi	W	60	69.77±0.72^{i}	8.56±0.23^{b}	17.33±0.47^{f}
	W:SCG		65.77±0.90^{e}	8.56±0.11^{b}	17.70±0.34^{g}
	W:SCG+LR		68.40±0.25^{h}	8.30±0.97^{ab}	16.30±0.51^{d}
	W	120	67.23±1.04^{g}	12.63±0.14^{d}	20.50±0.27^{i}
	W:SCG		63.23±0.27^{c}	12.36±0.87^{d}	16.53±1.66^{de}
	W:SCG+LR		65.77±1.01^{e}	8.63±0.36^{b}	16.77±1.10^{e}
Nyaruka	W	60	65.63±0.08^{e}	8.33±0.27^{ab}	12.80±2.20^{a}
	W:SCG		61.17±2.20^{a}	8.43±0.13^{ab}	16.23±0.57^{d}
	W:SCG+LR		63.10±1.04^{c}	8.367±0.51^{ab}	17.53±0.44^{fg}
	W	120	64.83±0.27^{d}	10.40±0.97^{c}	13.83±0.10^{b}
	W:SCG		62.73±0.04^{b}	10.90±1.45^{c}	14.60±0.89^{c}
	W:SCG+LR		63.57±0.18^{c}	7.83±0.11^{ab}	16.37±1.17^{d}
Reberaho	W	60	67.12±0.13^{g}	8.79±0.18^{ab}	12.18±0.90^{a}
	W:SCG		64.20±0.28^{d}	8.11±1.34^{ab}	16.26±0.43^{d}
	W:SCG+LR		65.41±1.67^{e}	8.27±2.34^{ab}	17.33±1.34^{fg}
	W	120	66.24±0.28^{f}	10.37±0.22^{c}	13.71±0.07^{b}
	W:SCG		62.11±0.15^{b}	10.19±0.97^{c}	14.47±0.28^{c}
	W:SCG+LR		64.48±0.19^{d}	7.44±0.39^{ab}	16.14±0.10^{d}

Values are means ±SD of 3 replications. Means with different superscript within a column are significantly different at $p \leq 0.05$. W: Whole wheat flour (Control flour), W: SCG: Whole wheat flour supplemented with spent coffee grounds (SCG), W: SCG+LR: Whole wheat flour supplemented with spent coffee grounds, juice of lemon fruits (L) and juice of rosemary leaves (R).

The ranges for bread color were 61.17 and 74.37 for L* values, 7.44 and 12.633 for a* values and 12.18 and 24.37 for b* values (Table 4.2). The L* values were significantly different ($p \leq 0.05$) in the control breads from all the wheat varieties. The control breads from Gihundo and Nyaruka varieties had the highest and lowest L* values, respectively. The higher L* value means that bread from Gihundo variety was brighter than the breads from the varieties which had lower L* values. Therefore, the bread crumb brightness in this study seemed to be caused by the wheat cultivar. The control breads from all the wheat variety doughs fermented for 60 min had higher L* values of crumb color than the ones from the doughs with fermentation extended to 120 min. The a* and b* values of the bread increased when the dough fermentation time increased from 60 to 120 min. Thus, the dough fermentation had significant effect on the a* and b* colors of the breads. The inclusion of SCG and SCG+LR in doughs caused the supplemented breads to have lower L*, a* and b* values than the control breads. The W: SCG+LR breads had higher L* values than W: SCG bread. This was probably due to synergy of the pH lowering capacity of L (Table 5.3) and the antioxidant capacity of SCG, L and R (in the present study determined 25.84, 8.08 and 26.77 mg100 g^{-1}, respectively) to reduce asparagine content during fermentation and mitigate Maillard reaction during baking which could otherwise increase L*, a* and b* values of the breads as some researchers reported on it. Gökmen *et al.* (2007) found that reduced pH of the dough had effect on the color of bakery products by reducing the formation of Maillard reaction products. Martinez-Saez et al. (2017) also realized the contribution of antioxidant compounds to minimize the level of the Maillard reaction products which could favor the darkness, redness and yellowness of the breads.

4.3.3. The physical characteristics of whole bread incorporated with spent coffee grounds and juices of lemon fruits and rosemary leaves

The Physical characteristics of whole bread crumb from selected Rwandan wheat varieties and produced by adding spent coffee grounds and juices of lemon fruit and rosemary leaves are present in Table 4.3.

Table 4.3. Physical characteristics of whole wheat bread crumb made by addition of spent coffee grounds and juces of lemon fruits and rosemary leaves

Wheat variety	Ingredients	Fermentation (min)	Weight (g)	Specific volume (cm^3/g)	Density (g/cm^3)
Gihundo	W	60	140.30 ± 1.45^{ab}	3.067 ± 0.44^{abc}	0.48 ± 0.07^{a}
	W:SCG		142.00 ± 2.10^{b}	2.933 ± 0.07^{a}	0.483 ± 0.08^{a}
	W:SCG+LR		141.70 ± 0.68^{b}	2.967 ± 0.34^{ab}	0.49 ± 0.03^{a}
	W	120	138.70 ± 1.17^{ab}	3.233 ± 0.47^{abc}	0.513 ± 0.09^{a}
	W:SCG		141.30 ± 2.10^{ab}	3.167 ± 1.17^{abc}	0.513 ± 0.57^{a}
	W:SCG+LR		141.90 ± 2.17^{b}	3.00 ± 1.11^{ab}	0.516 ± 0.19^{a}
Kibatsi	W	60	139.30 ± 1.15^{ab}	2.967 ± 0.40^{ab}	0.52 ± 0.91^{a}
	W:SCG		141.00 ± 1.34^{ab}	3.00 ± 0.44^{ab}	0.523 ± 007^{a}
	W:SCG+LR		141.00 ± 0.33^{ab}	3.033 ± 0.34^{abc}	0.52 ± 0.28^{a}
	W	120	138.30 ± 0.38^{ab}	3.30 ± 0.05^{abc}	0.526 ± 0.43^{a}
	W:SCG		141.00 ± 0.09^{ab}	3.267 ± 0.08^{abc}	0.533 ± 0.78^{a}
	W:SCG+LR		141.70 ± 0.57^{b}	3.067 ± 0.68^{abc}	0.523 ± 0.25^{a}
Nyaruka	W	60	138.70 ± 0.90^{ab}	3.033 ± 0.97^{abc}	0.53 ± 0.33^{a}
	W:SCG		140.30 ± 0.51^{ab}	3.067 ± 0.40^{abc}	0.536 ± 0.28^{a}
	W:SCG+LR		140.70 ± 0.78^{ab}	3.133 ± 1.10^{abc}	0.526 ± 0.06^{a}
	W	120	137.70 ± 0.25^{a}	3.40 ± 2.10^{c}	0.533 ± 0.43^{a}
	W:SCG		140.30 ± 0.09^{ab}	3.33 ± 0.22^{bc}	0.54 ± 0.91^{a}
	W:SCG+LR		141.00 ± 0.33^{ab}	3.14 ± 0.34^{abc}	0.541 ± 0.09^{a}
Reberaho	W	60	137.70 ± 0.97^{ab}	3.05 ± 0.97^{abc}	0.522 ± 0.22^{a}
	W:SCG		142.30 ± 1.07^{ab}	3.09 ± 0.05^{abc}	0.511 ± 0.13^{a}
	W:SCG+LR		142.70 ± 0.19^{ab}	3.11 ± 0.03^{abc}	0.54 ± 0.11^{a}
	W	120	136.70 ± 2.10^{a}	3.41 ± 1.45^{c}	0.501 ± 007^{a}
	W:SCG		141.30 ± 0.97^{ab}	3.30 ± 0.18^{bc}	0.513 ± 0.91^{a}
	W:SCG+LR		142.00 ± 0.08^{ab}	3.133 ± 0.57^{abc}	0.542 ± 0.03^{a}

Values are means ±SD of 3 replications. Means with different superscript within a column are significantly different at $p\leq 0.05$. W: Whole wheat flour (Control flour), W: SCG: Whole wheat flour supplemented with spent coffee grounds (SCG), W: SCG+LR: Whole wheat flour supplemented with spent coffee grounds, juice of lemon fruits (L) and juice of rosemary leaves (R).

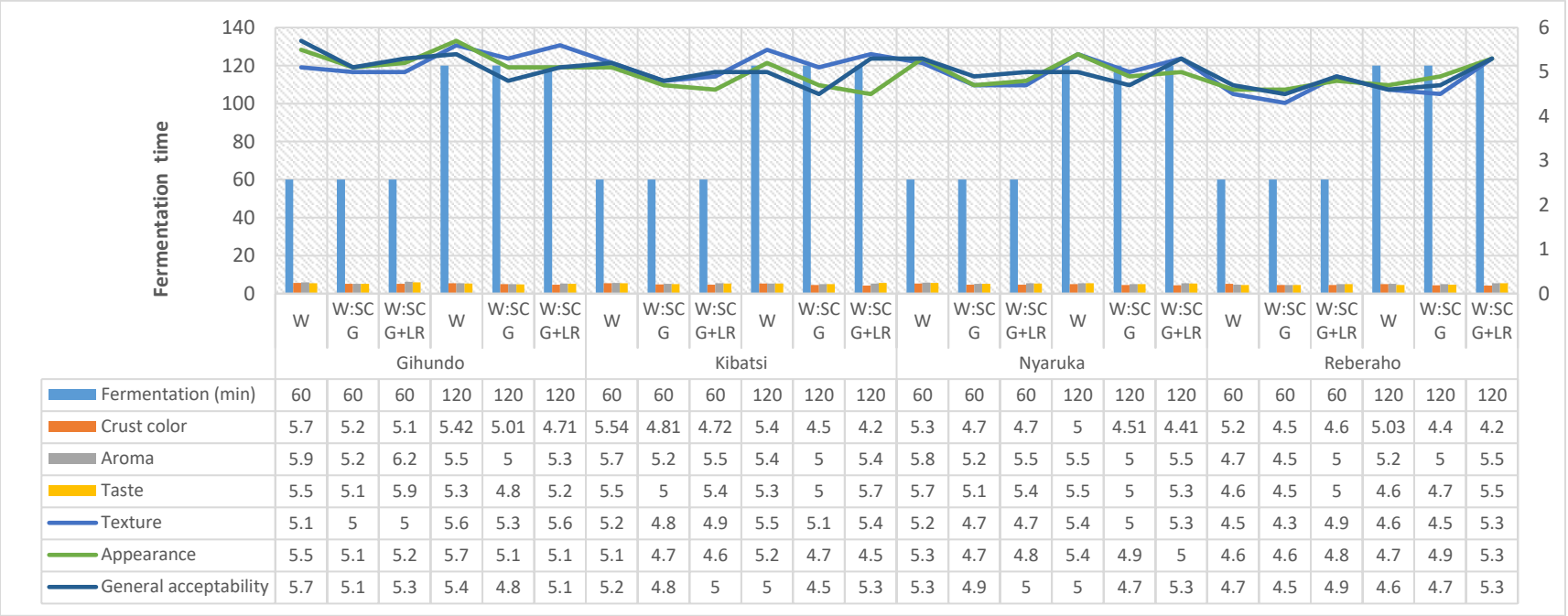
The bread weight was in the range of 136.7g and 142.7 g (Table 4.3). The weight of the breads which was not significantly different ($p>0.05$) from the control breads from Kibatsi and Nyaruka wheat varieties, was lower than the weight of the control bread from Gihundo variety. The W: SCG and W: SCG+LR breads had higher weight than the control breads. The decrease in weight may be due to increase in crude fiber content of the supplemented bread samples. The control breads from doughs fermented for 120 min had lower weight than the same breads from doughs fermented for 60 min ($p>0.05$), while fermentation duration did not significantly ($p>0.05$) affect the weight of W: SCG+LR breads.

The specific volumes ranged between 2.933 cm^3g^{-1} and 3.41 cm^3g^{-1} (Table 4.3). The specific volumes were significantly different ($p\leq0.05$) among the control breads, where breads from Gihundo and Nyaruka varieties showed the highest and lowest specific volumes, respectively. The small size bran flour may have contributed to the low specific volume as it was reported in another study that fine bran resulted in a lower specific loaf volume than bread containing coarse or medium size bran (Tamanna and Mahmood, 2015).

The control breads from all the wheat varieties had high specific volume compared to W: SCG and W: SCG+LR breads. The decrease in specific volume may have been caused by fiber from SCG which reduced the gluten network that is required for good gas-holding properties (Noort *et al.*, 2010). When dough fermentation time was increased from 60 min to 120 min, there was a higher increase in comparison to that of other breads due to the fact that fermenting yeasts were in a favorable environment of acidic conditions for longer time. In this case, the lemon fruit juice (L) addition could have reduced the dough pH to the extent the yeasts became more active. The density of the breads was in the range of 0.480 gcm^{-3} and 0.542 gcm^{-3}(Table 4.3). The density was not significantly different ($p>0.05$) between the control breads from Gihundo and Nyaruka varieties which was lower than that of the breads from the other varieties ($p\leq0.05$). The W: SCG and SCG+LR breads were less dense than the only wheat (control) breads. The addition of SCG increased the dietary fiber content of the control breads, therefore reduced its density (Park *et al.*, 1997). The dough fermentation duration did not affect the density of control breads ($p>0.05$). The W: SCG+LR breads and W: SCG breads from the doughs fermented for 60 min were denser than the similar breads from the doughs fermented for 120 min ($p\leq0.05$).

4.3.4. The sensory attributes of whole bread incorporated with spent coffee grounds and juices of lemon fruits and rosemary leaves

The organoleptic characteristics of whole bread from selected Rwandan wheat varieties and produced by adding spent coffee grounds and juices of lemon fruit and rosemary leaves are shown in Figure 4.1. A 7-point Hedonic scale was used.



	W	W:SCG	W:SCG+LR	W	W:SCG	W:SCG+LR	W	W:SCG	W:SCG+LR	W	W:SCG	W:SCG+LR	W	W:SCG	W:SCG+LR	W	W:SCG	W:SCG+LR	W	W:SCG	W:SCG+LR	W	W:SCG	W:SCG+LR
	Gihundo						Kibatsi						Nyaruka						Reberaho					
Fermentation (min)	60	60	60	120	120	120	60	60	60	120	120	120	60	60	60	120	120	120	60	60	60	120	120	120
Crust color	5.7	5.2	5.1	5.42	5.01	4.71	5.54	4.81	4.72	5.4	4.5	4.2	5.3	4.7	4.7	5	4.51	4.41	5.2	4.5	4.6	5.03	4.4	4.2
Aroma	5.9	5.2	6.2	5.5	5	5.3	5.7	5.2	5.5	5.4	5	5.4	5.8	5.2	5.5	5.5	5	5.5	4.7	4.5	5	5.2	5	5.5
Taste	5.5	5.1	5.9	5.3	4.8	5.2	5.5	5	5.4	5.3	5	5.7	5.7	5.1	5.4	5.5	5	5.3	4.6	4.5	5	4.6	4.7	5.5
Texture	5.1	5	5	5.6	5.3	5.6	5.2	4.8	4.9	5.5	5.1	5.4	5.2	4.7	4.7	5.4	5	5.3	4.5	4.3	4.9	4.6	4.5	5.3
Appearance	5.5	5.1	5.2	5.7	5.1	5.1	5.1	4.7	4.6	5.2	4.7	4.5	5.3	4.7	4.8	5.4	4.9	5	4.6	4.6	4.8	4.7	4.9	5.3
General acceptability	5.7	5.1	5.3	5.4	4.8	5.1	5.2	4.8	5	5	4.5	5.3	5.3	4.9	5	5	4.7	5.3	4.7	4.5	4.9	4.6	4.7	5.3

Figure 4.1. Sensory attributes of whole wheat bread supplemented with spent coffee grounds and juces of lemon fruits and rosemary leaves.

Values are means ±SD of 3 replications. Least significant difference was accepted at $p \leq 0.05$. W: Whole wheat flour (Control flour), W: SCG: Whole wheat flour supplemented with spent coffee grounds, W: SCG+LR: Whole wheat flour supplemented with spent coffee grounds (SCG), juices of lemon fruits (L) and juices of rosemary leaves (R).

The range of values for color, aroma, taste, texture, appearance and general acceptability were respectively between 4.2 and 5.7, 4.5 and 6.2, 4.5 and 5.9, 4.3 and 5.6, 4.5 and 5.7, and 4.5 and 5.7. The control breads from Gihundo variety had higher color and appearance scores than the other control breads ($p \leq 0.05$), (Table 4.4). The breads from Kibatsi, Nyaruka and Reberaho varieties showed non-significant difference ($p > 0.05$) in color. The control breads from Gihundo varieties were the most liked ($p \leq 0.05$) probably due their crust color which was the most brown (Table 4.2). The W: SCG and W: SCG+LR breads (Table 4.2) received a lower score for color than the control ($p \leq 0.05$), possibly because their color was not brown enough (Table 4.2). On the other hand, the aroma and taste of W:SCG+LR breads were liked the most ($p \leq 0.05$), probably due to the aromatic compounds from these ingredients (Allegrone *et al.*, 2006).The W: SCG+LR breads from doughs fermented for 120 min scored higher in texture, aroma, taste, appearance and general acceptability, but lower in color in comparison to other breads from doughs fermented either for 60 min or 120 min. This could be related to the SCG+LR incorporation in the doughs and their long fermentation that may have favored breads to become more porous and to have a pronounced flavor (Allegrone *et al.*, 2006).

4.4. Conclusion

The wheat varieties, dough fermentation and incorporation of spent coffee grounds, lemon fruit juice and juice of rosemary leaves into dough significantly affected the texture profile, color, physical characteristics and sensory attributes of whole grain breads. The control breads from doughs fermented for 60 min were harder than the supplemented breads with the same time of fermentation. The long fermentation and the inclusion of spent coffee grounds, juice of lemon fruit and juice of rosemary leaves in doughs influenced low L*, a* and b* values and high general acceptability of the resulting breads in comparison to control breads. Whole wheat breads obtained, satisfied consumers' preferences on texture profile, color and sensory attributes. Therefore, the application of spent coffee grounds, juices of lemon fruit and rosemary leaves in bread making represents a good opportunity.

CHAPTER FIVE: CHEMICAL CHARACTERISTICS OF WHOLE WHEAT BREADS INCORPORATED WITH SPENT COFFEE GROUNDS AND JUICES OF LEMON FRUITS AND ROSEMARY LEAVES

Abstract

Whole wheat bread is increasingly consumed in Rwanda. Wheat is among the top priority crops that Rwanda has promoted to boost the national economy. The objective of this study was to evaluate the effect of fermentation duration and adding spent coffee grounds and juices of lemon fruits and rosemary leaves on the chemical characteristics of whole bread. The ingredients were spent coffee grounds (SCG), juice of lemon fruit (L), juice of rosemary leaves (R). The mixture of whole wheat flour with these were fermented using instant dry yeast at 34ºC, 60% relative humidity for 60 min and at 39ºC, 85% relative humidity for 120 min, separately. The dough was baked at 180ºC for 20 min. W: SCG and W: SCG+LR breads were low in protein, fat and ash contents and higher in total dietary fiber than control breads. Control breads from Gihundo variety had the highest acrylamide content (47.00 µg/kg). Addition of SCG + LR in dough fermented for 60 min reduced significantly ($p \leq 0.05$) the acrylamide formation in control breads from 47.00 µg/ kg to 10.50 µg/kg, respectively. Acrylamide content in all breads did not decrease significantly ($p > 0.05$) during the storage time. The present study suggests the selection of wheat varieties low in asparagine concentration and fermentation duration, SCG, L and R can be exploited as generally recognized as safe and very cheap ingredients for their capacity to mitigate acrylamide formation.

5.1. Introduction

Studies were conducted and published on health benefits of consuming whole grain wheat products (Anson, 2010). This has led to the growing availability of whole wheat bread on the marketplace (Whitney, 2013). A lot of researches have showed that this kind of bread is a rich source of strong health promoting compounds such dietary fiber. These compounds are mainly concentrated in the outer portions (bran) and germ of the grain (Onipe et al., 2015). However, the researchers have

found more acrylamide content in whole wheat bread than that found in refined or wheat bran breads (Boyaci Gündüz & Cengiz, 2015). Acrylamide is a chemical compound formed during maillard reaction and was reported to cause possible carcinogenicity, neurotoxicity, reproductive and developmental toxicity effects to consumers. Specifically, asparagine and reducing sugars are the limiting precursors for acrylamide formation in cereal and potato products, respectively (Krishnakumar & Visvanathan, 2014); (Amrein et al., 2004). The growing awareness about health issues arising from food consumption preserved with chemicals has led to improving food safety by using natural preservatives. This was often revealed in some previous experiments that rosemary and lemon extracts possess diverse biological activities, including antioxidant activity from their non- nutrient secondary metabolites like phenolic compounds, etc. Antioxidants present in spent coffee grounds were assumed to lower the maillard reaction for the acrylamide formation in biscuits (Martinez-Saez et al., 2017), (Fernandez-Gomez et al., 2015); (Mesías et al., 2014). Studies that evaluated the effect of antioxidants on the formation of acrylamide in the bread, showed that there was in substantial reduction in acrylamide levels depending on the nature of the natural extract (Hedegaard et al., 2008); (Zhang & Zhang, 2007); (Zhang et al., 2008). In this regard, spent coffee grounds, the juices from rosemary leaves and lemon fruits added in dough ingredients to to evaluate their effects on proximate composition and acrylamide formation in whole wheat breads.

5.2. Materials and methods

5.2.1. Collection of the samples

Four dry wheat grain varieties namely TAI, EN161, Eagle10 and Korongo with the local names Gihundo, Kibatsi, Nyaruka, and Reberaho, respectively, were collected from the stores of Rwanda Agriculture and Animal Resources Development Board (RAB), located at Kinigi, Musanze district, Rwanda. These wheat varieties were grown in RAB farms at the temperature varying between at 16.6 and 21.5 ^{o}C, at the same location and under the same agro ecological and cultural conditions in the crop year 2018. The wheats were grown in volcanic soil and fertilized with urea and Diammonium phosphate (DAP). The wheat grains were sampled in the same year, packed in high

density polyethylene bags and stored at room temperature prior to milling. The wastes, known as spent coffee grounds, were directly taken after brewing coffee (Coffea Arabica var. bourbon) in a coffee shop in Musanze town, Rwanda and stored in a transparent plastic bottle in a freezer (SM302NW, SM302NW1014009, 2010/08, China) at -20^{o}C prior to analysis. Fresh green lemon fruits (var. African rough lemon) and raw green rosemary leaves (Var. Arp rosemary herb) were bought from the market in Musanze town, Rwanda, packed in plastic sachets and stored at 5^{o}C in refrigerator (Hisense, HBM17158SS, 2015, China) prior to processing. The coffee, lemon and rosemary were all grown in Rwanda.

5.2.2. Preparation of the raw samples

5.2.2.1. Wheat grains milling

Before milling, the wheat grains were conditioned to 15.5 % moisture content in order to get a fine particle size whole wheat flour by the addition of distilled water and were left for at least 24 hr at ambient conditions in a closed plastic container for the absorption of the moisture (Mishra, 2016). AACC Approved Method 26-95.01 (AACC, 2003) was used to calculate the amount of water to be added for wheat grains tempering:

$$\boldsymbol{m}l = \left(\left(\frac{100 - \%\, moisture}{100 - 15.5\%}\right) - 1\right) x\ grams\ of\ wheat\ grains$$

The conditioned wheat grains of each variety were wholly milled by using a laboratory hammer mill (CM 1090 Cemotec, 2009, China). All bran and germ were mixed with the flour. The flour was packed in high density polyethylene envelop and stored at -20^{o}C in a freezer (SM302NW, SM302NW1014009, 2010/08) prior to analysis and baking.

5.2.2.2. Lemon fruit and rosemary leaves juice making

The lemon and rosemary juices were obtained by using a blender (Moulinex, LM241, Genuine, 2017, France). The lemon fruits (3kg) were washed and peeled with knife. The rosemary leaves were picked off the woody branch by using hand and washed. Then after, they were cut into quarters and juiced by by using a blended (Moulinex, LM241, Genuine, 2017, France) with no water added for between 30 secs and 1 min until the extraction is complete. Rosemary leaves (5kg) were ground in the blender (Moulinex, LM241, Genuine, 2017, France) between 2 and 4 min until all leaves were thoroughly ground with no water added. To get the clear juice, lemon fruit pulp and ground rosemary leaves were separately sqeezed in a cheese cloth (grade 90, 44 × 36 weaves; 2018, Zhuojie, China) by using hand. The wastes (peels, seeds and other solid materials resulted from the extraction of juices were deposited in appropriate bin. The clarified lemon and rosemary juices were kept in transparent plastic bottles and stored at 6^{o}C in a fridge (SM302NW, SM302NW1014009, 2010/08) prior to analysis and processing.

5.2.3. Baking

The dough comprising 200g whole wheat flour from each of the wheat variety grains (TAI, EN161, Eagle10 and Korongo), 2% instant dry yeast (GB Ingredients, Dordrecht, 2019, Holland), 2% sodium chloride and potable water (125 mL) (Fredriksson *et al.*, 2004). The amounts of 4% spent coffee grounds (Martinez-Saez *et al.*, 2017), 1% lemon fruit juice and 1% rosemary leaf juice were added. The electric balance (Explorer EX 223, version 2.00/2.00. SN B 333687045, IR Sensor, OHAUS) was used. The mixture was fermented and proofed in a fermenter (Manz Backtechnik GmbH, Creglingen, Germany) at 34^{o}C, 60% relative humidity for 60 min and at 39^{o}C, 85% relative humidity for 120 min after being mixed in dough mixer (Combisteel Dough Mixer Liter, 7455.1400, 2011, China) (Surdyk *et al.*, 2004). Rotation of the mixer was 54 rotations per minute (rpm) for 3 min until the dough came together and then switched to 104 rpm for 6 min. Each fermented dough was covered with a lid and baked at 180^{o}C for 20 min in an oven (Electric baking oven, Lemarkz, Model: LGO-24A, 2010, India). The control bread from each wheat variety was made for 60 min of fermentation without incorporation of SCG and LR. The loaves were depanned and cooled for 2hr, packed in unperforated low density

polythene bags, closed and stored at -20^{o}C in a freezer (SM302NW, SM302NW1014009, 2010/08, China) prior to analysis.

5.2.4. Freeze drying of dough

Bread dough was first frozen at -20^{o}C in a freezer (SM302NW, SM302NW1014009, 2010/08). Then after, it was transferred into freeze-drier (Christ, Alpha 2-4 LS plus, SN: 24/61/11/2017, Germany) at -82^{o}C, 0.1 mbar vacuum for 24 hours and got completely dried. Dried dough was ground by using a laboratory grinder (Moulinex, LM241, Genuine, 2017, France) into fine flour for next analysis.

5.2.5. Determination of chemical parameters

5.2.5.1. Ash content for whole wheat breads

Bread sample was prepared and ash content was determined as described by AACC (1999) International Methods (62-05.01) and (08-01.01), respectively, by using a muffle furnace (Lindberg/Blue 1100°C Box furnace BF 51800 series Ashville, NC). Whole wheat bread was ground into fine flour and 3 samples (5 g each) were put into ashing dish that has been ignited, cooled in desiccator, and weighed soon after attaining room temperature. The samples were placed in muffle furnace at 575°C and incinerated until light gray ash was obtained or to constant weight. The samples were cooled in desiccator and weighed soon after room temperature was attained. The ash content was calculated as below:

$$\textbf{\% ash content} = \frac{\text{Weight of residue}}{\text{Sample weight}} \text{ X } 100$$

5.2.5.2. Total fat content for whole wheat breads

Total fat content was determined by AACC (1999) International Method 30-10.01. with HCl acid hydrolysis. Five grams of fine flour samples from ground whole wheat bread were weighed into extraction thimbles and fixed into extraction flask of known weights. Extraction was carried out using petroleum ether on electro-thermal model equipment for eight hours. At the completion of the extraction, the petroleum ether was removed by evaporation on an electrical bath and the remaining fat in the flask was heated to constant weight at 100°C, cooled for 15 minutes and weighed. The fat content was calculated as follows:

$$\% \text{ Fat content} = \frac{\text{Weight of fat (Corrected for blank)}}{\text{Sample weight}} \text{ x } 100$$

5.2.5.3. Total protein content for whole wheat bread

The determination of total protein content was done by AACC (1999) International Method 46-12.01.

Digester (Heating Digester, DK,Velp Scientifica) and Distillation unit(Semi-automatic, UDK 139, Velp Scientifica) were used. Whole wheat bread was ground into fine flour and 3 samples (1g each) were placed in digestion flask. Polyethylene packet of catalyst, and 25 ml concentrated H_2SO_4 (0.1N) were added to flask. Flask containing 50 ml boric acid-methyl redmethylene blue indicator solution was placed under condenser tube. Concentrated NaOH (50 ml) was added and boiled until all ammonia distilled (at least 150 ml of distillate). The protein content was calculated as below:

$$\% \text{ protein } = \frac{\text{ml standard H2SO4 x 1.4007 x N of H2SO4 x 5.7}}{\text{Weight of sample(g)}}$$

5.2.5.4. Total dietary fiber content of whole wheat bread

Dietary fiber content was determined as per AACC (1999) International Method 32-07.01. Whole wheat bread was ground into fine flour and 3 samples (1g each) were put into beakers. MES-TRIS (40 ml) blend buffer solution (pH 8.2) was added to each beaker. Heat-stable α-amylase solution (200 µl) was added and incubated for 35 min at 95°C with continuous agitation. Protease solution (100 µl) was added to each sample and incubated in shaking water bath at 60°C, with continuous agitation for 30 min. To each sample, 225 ml 95% EtOH preheated to 60°C was added. Resudues were formed at room temperature for 60 min. Total dietary fiber content was calculated as follows:

$$\text{Dietary fiber (\%)} = \frac{\frac{R_1 + R_2}{2} - p - A - B}{\frac{m_1 + m_2}{2}} \times 100$$

where R_1 = residue weight 1 from m1, R_2 = residue weight 2 from m_2, m_1 = sample weight 1, m_2 = sample weight 2, A = ash weight from R_1, p = protein weight from R_2, and

$$\text{B=Blank} = \frac{BR_1 + BR_2}{2} - BP - BA$$

where BR = blank residue, BP = blank protein from BR_1, and BA = blank ash from BR_2.

5.2.5.5. Total carbohydrate content of whole wheat bread

Total carbohydrate content was calculated as described by (FAO, 2003).

Total carbohydrate content = 100 – (moisture % – protein % – fat % – ash %)

5.2.5.6. Determination of free asparagine in whole wheat flour, dough, bread, spent coffee grounds, lemon fruit and rosemary leaves juices

The determination of free asparagine was done by following the method of Hamlet et al.(2008). Stock standard solutions of all amino acids were prepared at 1 mg ml^{-1} in distilled water. The preparation of mixed standards was done by blending and serially diluting the stock standards with distilled water to give calibration standards. To prepare reagent for the derivatization, ten (10) mg of *o*-phthaldialdehyde (OPA) was dissolved in 100 µl of methanol, making 1 ml with borate buffer (0.4M, pH 10.2). 20 µl of of 3-mercaptopropionic acid (3-MPA) was the added. Samples (2.5 g) were extracted with 20 ml of acetic acid and allowed to incubate. The supernatants were adjusted to 100 ml with 0.01 M acetic acid. Aliquot of 3ml of the sample, 50 µl borate buffer and 50 µl OPA reagent were added into the sample derivatization vial containing 800 µl water. The mixture of an aliquot (3ml) HCl (0.01 M, 1:1v/v) and 4 ml of the solution was prepared. Pipetting of HCl and solution was done with a pipette inserted with graduate cylinder (Eppendorf easypet 3), and pipette filler (SN: 181248, Thermoscientific, China). The eluate was added into the sample derivatization vial for analysis by using UPLC (LCMS-8050), with fluorescence detector (model RF-20AXS, CAT.No: 228-45148-48, Serial nbr:L20505000663 AE, Japan). For lemon fruit and rosemary leaves juices, juices (39 ml) was diluted with 40 ml of distilled water. All solutions were shaked (Controlled Environment Incubator shaker, New Bruswik Scientific Co., Inc., Edison, N.J, USA) at 350 rpm for 30 min at 20^{o}C, and then centrifuged by using centrifuge (5415D, Eppendorf) at 3000 rpm for 10 min.

5.2.5.7. Reducing sugars for whole wheat flour, dough, bread, spent coffee grounds, lemon fruit and rosemary leaves juices

The individual sugars were determined by using the procedure for samples containing low fat and low protein (Latimer, 2012). For whole wheat flour, dough, bread and spent coffee grounds, $CaCO_3$ (1g) was added into the sample (1g) to neutralize it. The ethanol of 25 mL 85% was also added into the sample and shaked by using water bath at 60^{o}C for 1 h. The sample was removed from water bath and immediately filtered through a filter paper. The extraction with 25 mL boiling

85% ethanol was done three times. The ethanol was evaporated on rotary evaporator at 45^{o}C until the remaining aqueous solution was approximately 3 ml. The solution was mixed with distilled water volumetric flask up to into a 10 ml mark. For lemon fruit and rosemary leaves juices, each juice (100 µl) was diluted with distilled water (1000 µl) to be vortexed by using a vortex (Model No: SI_0166, SN: 16-1114, genie touch mixer scientific industries inc., USA and filtered with syringe filter (hydrophilic Germany). By using a pipette (Petman, Gilson), the solution (1.5 ml) from whole wheat flour, dough, bread, spent coffee grounds, lemon fruit and rosemary leaves juices samples was filtered through a 0.2µm nylon filter into a HPLC vial. Each sample was analyzed by using HPLC (waters 2695, separation module) with refractive index detector (Waters, 2414).

Calculation: $\text{Total amount of each sugar}(g/100g) = \frac{\text{AsplxC xDFx100}}{\text{AstdxWx1000}}$

where:

Aspl = Area/peak height of each sugar in sample solution

Astd = Area/peak height of sugar standard

Cstd = Concentration of sugar standard (µg/ml)

DF= Total dilution factor

W= Weight of the sample in grams

100 = Conversion factor to report results in mg/100g

1000= Conversion from mg/ml to g/ml

5.2.5.8. Total antioxidant activity for whole wheat flour, dough, bread, spent coffee grounds, lemon fruit and rosemary leaves juices

The total antioxidant activity was determined using DPPH procedure (Sahli, 2015). Two grams of whole wheat flour, dough, bread and spent coffee grounds, and 200 µl of juices were mixed with 10 ml 80% methanol, separately. The mixture was shaken by using mechanical shaker (Controlled Environment Incubator shaker, New Bruswik Scientific Co., Inc., Edison, N.J, USA) at 25^{o}C for 24 hr. Afterwards, the mixture was then centrifuged at 4,000 rpm for 10min. The supernatant aliquot was taken out for determination. A working DPPH solution was prepared by diluting 200

ml of stock solution with approximately 800 ml of 50% ethanol. DPPH (50 µl) was added into supernatant aliquot sample (50 µl and was shaken by using a plate shaker (Insel Shaker, SN:95/S89/73, Hamble SO3 8DH, England). The absorbance was read at 515 nm in a Microtiter plate spectrophotometer reader (BioTek Instruments, SN: 216574, USA). The results were expressed as mg of Trolox Equivalents per 100 g sample.

5.2.5.9. Acrylamide content for whole wheat breads and spent coffee grounds

Acrylamide content was determined by using the protocol of Al-Taher (2012). All reagents namely acetonitrile, n-hexane, formic acid and water, were LC/MS grade. The acrylamide standard stock solution ($1mgml^{-1}$) was prepared by dissolving 100mg of the acrylamide in 100ml acetonitrile and stored at $4^{o}C$. The internal standard (methacrylamide) stock solution ($100.000\mu gml^{-1}$) was made up by pipetting 0.5 ml of the 1mg/ml standard into 50ml acetonitrile and stored at $4^{o}C$. All working solutions were prepared daily by serial dilution in acetonitrile. One gram from bread and spent coffee grounds samples was weighed a 50ml centrifuge tube from the agilent bond elut QuEChERS Extraction kit. The internal standard (13C3 acrylamide) was added at 500ng/g. Hexane (5ml) was added and the tube was vortexed. Water (10 ml) and acetonitrile (10ml) were added followed by the agilent bond elut QuEChERS extraction salt mixture for acrylamides (p/n 5982-5850). The sample tubes were shaken for 1min vigorously and centrifuged at 5000rpm for 5 min. Separation of the chemicals was performed on a reversed phaseC18 column (2.1mmx150mm, 3µm). The acrylamide in the sample was determined by using HPLC/LCMS-8050 (Model: RF-20AXS, SN: L20505000663 AE, Shimadzu corporation, Japan). The acrylamide content was expressed in μgkg^{-1}.

5.2.6. Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using SAS System for Windows (version 9.3, SAS Institute, Cary, NC). Treatment means were separated using the Tukey's test and least significant difference was accepted at $p \leq 0.05$.

5.3. Results and discussion

5.3.1. The proximate composition of whole breads supplemented with spent coffee grounds, juices of lemon fruits and rosemary leaves

The proximate composition of breads produced using mixture of whole wheat flour, spent coffee grounds and juices of lemon fruits and rosemary leaves is presented in Table 5.1.

Table 5. 1. Proximate composition of whole wheat breads made using spent coffee grounds and juices of lemon fruits and rosemary leaves.

Wheat variety	Ingredients	Fermentation (min)	Moisture content content (%)	Protein content (%)	Fat content (%)	Ash content (%)	Total carbohydrate content (%)	Total dietary fiber content (%)
Gihundo	W	60	23.90 ± 0.23^{c}	7.40 ± 1.17^{ef}	1.40 ± 1.17^{hi}	1.70 ± 0.11^{gh}	65.60 ± 0.47^{f}	14.40 ± 0.27^{rs}
	W:SCG		28.20 ± 0.59^{lm}	7.20 ± 0.07^{cd}	1.20 ± 1.10^{fg}	1.60 ± 1.04^{fg}	61.80 ± 0.97^{c}	14.20 ± 0.80^{pq}
	W:SCG+LR		26.80 ± 1.01^{ij}	7.10 ± 2.20^{bc}	1.10 ± 0.36^{ef}	1.40 ± 0.07^{de}	$63.60\pm.0.68^{e}$	14.10 ± 0.58^{op}
	W	120	25.50 ± 1.66^{f}	7.70 ± 0.08^{g}	1.30 ± 0.39^{gh}	1.80 ± 2.17^{h}	63.70 ± 0.05^{e}	14.50 ± 0.39^{s}
	W:SCG		30.20 ± 1.24^{pq}	7.40 ± 0.05^{ef}	1.20 ± 0.05^{fg}	1.60 ± 2.10^{fg}	59.60 ± 0.07^{b}	14.30 ± 0.07^{qr}
	W:SCG+LR		29.00 ± 0.27^{n}	7.30 ± 0.19^{de}	1.10 ± 0.07^{ef}	1.40 ± 0.87^{de}	61.20 ± 1.15^{bc}	14.20 ± 0.25^{pq}
Kibatsi	W	60	23.30 ± 0.25^{b}	7.20 ± 0.09^{cd}	1.50 ± 0.67^{i}	1.50 ± 0.09^{ef}	66.50 ± 1.07^{g}	13.10 ± 0.89^{hi}
	W:SCG		$27.60^{k}\pm0.98$	7.00 ± 0.18^{bc}	1.40 ± 0.33^{i}	1.40 ± 0.34^{de}	62.60 ± 0.16^{d}	13.0 ± 0.57^{h}
	W:SCG+LR		25.70 ± 0.23^{fg}	6.90 ± 0.08^{ab}	1.20 ± 0.43^{fg}	1.20 ± 2.34^{c}	65.00 ± 1.19^{f}	12.70 ± 0.06^{g}
	W	120	26.50 ± 0.67^{i}	7.50 ± 0.13^{f}	1.20 ± 1.45^{fg}	1.70 ± 0.97^{gh}	63.10 ± 0.47^{e}	13.40 ± 0.19^{jk}
	W:SCG		31.40 ± 0.20^{r}	7.40 ± 0.15^{ef}	1.10 ± 1.67^{ef}	1.50 ± 0.44^{ef}	58.60 ± 0.62^{a}	13.20 ± 0.07^{ij}
	W:SCG+LR		29.30 ± 0.47^{o}	7.20 ± 0.97^{cd}	1.00 ± 1.34^{de}	1.40 ± 2.10^{de}	61.10 ± 0.24^{bc}	13.10 ± 0.14^{hi}
Nyaruka	W	60	22.40 ± 0.36^{a}	7.00 ± 0.03^{bc}	1.20 ± 0.78^{fg}	1.50 ± 0.10^{ef}	67.90 ± 0.69^{h}	140.00 ± 0.27^{o}
	W:SCG		27.90 ± 0.10^{l}	6.80 ± 0.18^{ab}	1.00 ± 0.28^{de}	1.40 ± 0.90^{de}	62.20 ± 0.40^{d}	13.80 ± 0.19^{n}
	W:SCG+LR		25.00 ± 0.45^{d}	6.60 ± 0.11^{a}	0.90 ± 0.22^{cd}	1.30 ± 0.89^{cd}	66.20 ± 0.57^{n}	13.50 ± 1.11^{kl}
	W	120	23.80 ± 0.13^{c}	7.30 ± 0.91^{de}	1.00 ± 0.91^{de}	1.60 ± 0.34^{fg}	66.30 ± 1.10^{g}	14.20 ± 1.70^{pq}
	W:SCG		29.30 ± 0.55^{o}	7.10 ± 0.33^{bc}	0.80 ± 0.89^{bc}	1.50 ± 0.25^{ef}	61.30 ± 1.17^{bc}	14.00 ± 1.14^{o}
	W:SCG+LR		25.90 ± 1.11^{gh}	7.00 ± 0.51^{bc}	0.60 ± 0.34^{a}	1.30 ± 0.38^{cd}	65.20 ± 0.97^{f}	13.70 ± 0.08^{lm}
Reberaho	W	60	23.20 ± 0.48^{b}	7.10 ± 0.44^{bc}	1.10 ± 0.11^{ef}	1.20 ± 0.15^{c}	67.40 ± 1.11^{h}	11.00 ± 0.32^{bc}
	W:SCG		27.90 ± 0.29^{l}	7.00 ± 0.40^{bc}	1.00 ± 0.78^{de}	1.00 ± 0.03^{b}	63.10 ± 0.05^{e}	10.70 ± 0.17^{ab}
	W:SCG+LR		25.80 ± 0.12^{fg}	6.80 ± 0.59^{ab}	0.70 ± 0.12^{ab}	0.80 ± 0.10^{a}	65.90 ± 0.09^{f}	10.60 ± 0.11^{a}
	W	120	25.20 ± 0.27^{de}	7.40 ± 1.12^{ef}	0.80 ± 0.72^{bc}	1.50 ± 0.11^{ef}	65.10 ± 0.07^{f}	12.00 ± 0.19^{f}
	W:SCG		30.30 ± 0.19^{p}	7.20 ± 0.17^{cd}	0.90 ± 0.43^{cd}	1.40 ± 0.14^{de}	60.20 ± 0.13^{b}	11.70 ± 0.17^{de}
	W:SCG+LR		28.00 ± 0.11^{l}	7.10 ± 0.67^{bc}	0.60 ± 0.13^{a}	1.20 ± 0.17^{c}	63.10 ± 1.19^{e}	11.50 ± 0.09^{cd}

Values are means ±SD of 3 replications and are determined on dry matter basis. Means with different superscript within a column are significantly different at $p\leq 0.05$. W: Whole wheat flour (Control flour), W: SCG: Whole wheat flour supplemented with spent coffee grounds (SCG), W: SCG+LR: Whole wheat flour supplemented with spent coffee grounds, juice of lemon fruits (L) and juice of rosemary leaves (R).

The contents of total protein, total fat, ash and total dietary fiber for breads ranged from 7.7% (Bread from Gihundo variety, fermentation 120) to 6.6% (Bread SCG+LR from Nyaruka variety, fermentation 60 min), 1.5% (Bread from Kibatsi variety, fermentation 60 min) to 0.6% (SCG+LR bread from Nyaruka variety, fermentation 60 min), from 1.8% (breads from Gihundo variety, fermentation 120 min) to 0.8% (W: SCG+LR bread from Reberaho variety, fermentation 60 min) and from 14.5% (breads from Gihundo variety, fermentation 120 min) to 10.6 % W: SCG+LR bread from Reberaho variety, fermentation 60 min), respectively (Table 5.1). It shows that W: SCG and W: SCG+LR breads were low in protein, fat and ash contents and higher in dietary fiber than only wheat breads.

5.3.2. The antioxidant activity of whole bread substituted with spent coffee grounds, juices of lemon fruit and rosemary leaves

The antioxidant activity levels of breads produced from whole wheat flour, spent coffee grounds and juices of lemon fruit and rosemary leaves are shown in Figure 5.1.

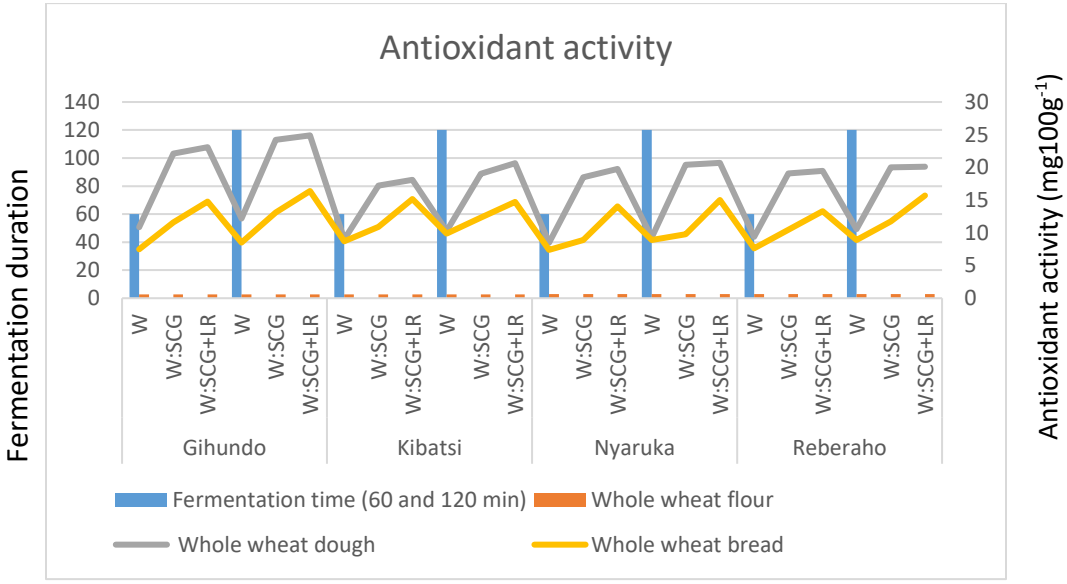


Figure 5.1. Antioxidant activity of whole wheat flour, dough and whole wheat bread added with spent coffee grounds and juices of lemon fruit and rosemary leaves

Values are means ±SD of 3 replications. Least significant difference was accepted at p≤ 0.05. W: Whole wheat flour (Control flour), W:SCG: Whole wheat flour supplemented with spent coffee grounds (SCG), W: SCG+LR: Whole wheat flour supplemented with spent coffee grounds, juice of lemon fruits (L) and juice of rosemary leaves (R).

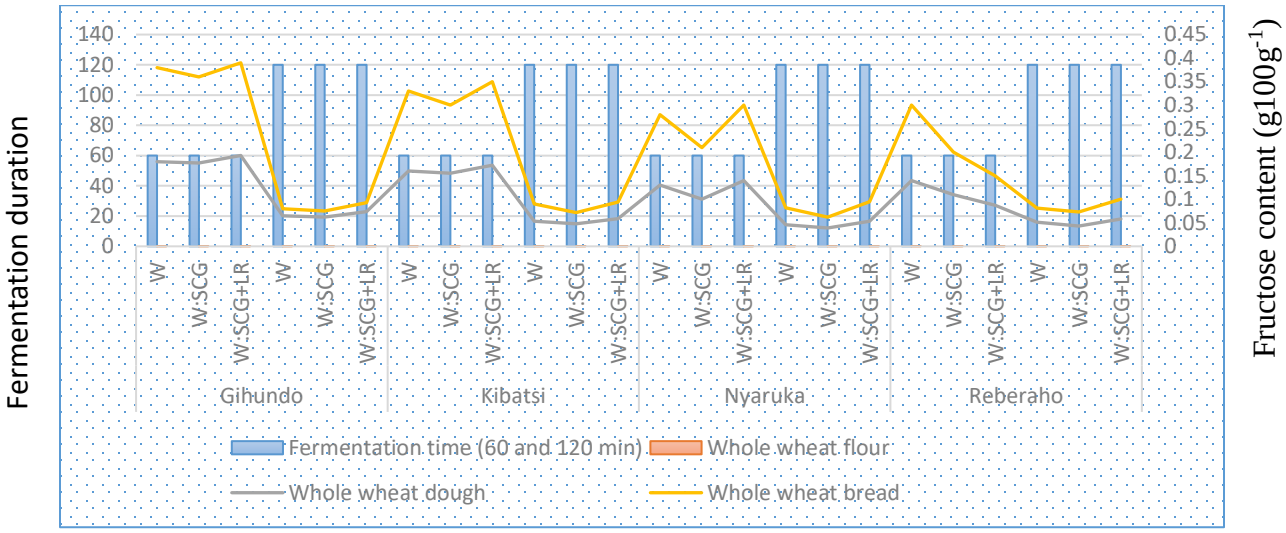
The spent coffee grounds and juices obtained from rosemary leaves and lemon fruit as an external source of antioxidants to the bread were mixed with flour to make dough. As determined in the present study, the antioxidant capacity for spent coffee grounds, juice from lemon fruits and juice from rosemary leaves were as high as 25.84, 8.08 and 26.77 mg100 g^{-1}, respectively compared to whole flours which had between 2.73 and 2.97 mg100g^{-1}. Thus, the addition of SCG and SCG+RL into flour increased the antioxidant capacity of doughs where it ranged from 8.48 to 24.9 mg100g^{-1}.

The flours from all varieties showed significant difference ($p \leq 0.05$) in antioxidant capacity where whole flours from Nyaruka and Gihundo varieties had the highest and the lowest, respectively. The whole wheat doughs were fermented for 60 and 120 min. The results showed that antioxidant capacity reduced in all breads in comparison to their respective doughs. Also Sahli (2015) reported that bread products had higher and lower scavenging capacity for DPPH radicals than wholegrain flour and straight dough, respectively. This was due that some phenolic compounds were possibly damaged by heat later during baking and consequently breads exhibited low antioxidant capacity in comparison to doughs (Sahli, 2015), (Katina et al., 2007), (Yu *et al.*, 2013).

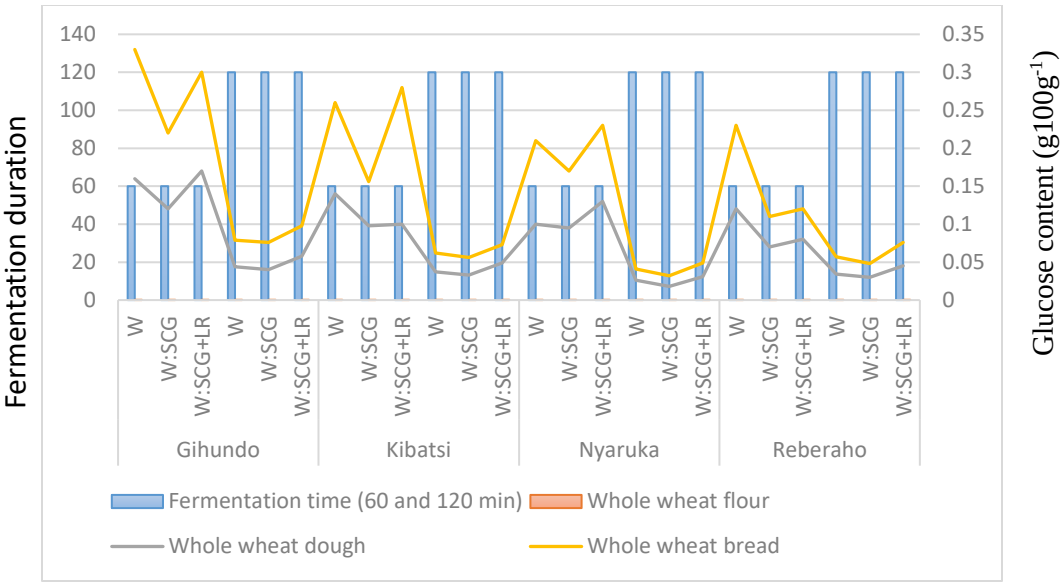
The phenolic compounds were given an attention in the present discussion because they are dominant compounds among other antioxidant phytonutrients in wheat grain (Abdel-Aal & Rabalski, 2013). Fermentation duration also affected the antioxidant capacity where dough fermented for 60 min showed lower antioxidant capacity than that the one fermented for 120 min, either mixed with or without SCG and SCG+LR, and it was the same for breads. It showed that the longer yeast fermentation, the higher antioxidant compounds were found in doughs and breads, for the reason that yeast could have caused the release of more phenolic compounds which enabled dough to show higher antioxidant capacity when fermentation was extended.

5.3.3. The content of reducing sugars of whole bread with spent coffee grounds and juices of lemon fruits and rosemary leaves added.

The concentration of reducing sugars of whole flour, dough and whole breads from selected Rwandan wheat varieties is shown in Figure 5.2. The spent coffee grounds and juices obtained from lemon fruits and rosemary leaves were applied to supplement the bread.



a. Fructose content of whole wheat flour, dough and whole wheat bread produced from Gihundo, Kibatsi, Nyaruka and Reberaho wheat varieties



b. Glucose content of whole wheat flour, dough and whole wheat bread produced from Gihundo, Kibatsi, Nyaruka and Reberaho wheat varieties

Figure 5.2. Contents of fructose (a) and glucose (b) in whole wheat flour, dough and whole wheat bread with spent coffee grounds and juices of lemon fruit and rosemary leaves incorporated.

Values are means ±SD of 3 replications. Least significant difference was accepted at $p \leq 0.05$. W: Whole wheat flour (Control flour), W: SCG: Whole wheat flour supplemented with spent coffee grounds (SCG), W: SCG+LR: Whole wheat flour supplemented with spent coffee grounds, juice of lemon fruits (L) and juice of rosemary leaves (R).

The contents for fructose and glucose were determined in whole flour, resultant doughs and breads from wheat varieties. The results for fructose (0.016g100g^{-1}-0.02g100g^{-1} and glucose (0.024 g100g^{-1}-0.037 g/100g for control whole flour were in the ranges reported by other researchers.

Hamlet *et al.* (2008) found 0.019 g100g^{-1}-0.074g100g^{-1} for fructose and 0.061g100g^{-1}-0.105 g100g^{-1} for glucose. Ecem reported 1.88 gkg^{-1} for fructose, 2.53 gkg^{-1} for glucose. Georgiana *et al.* (2013) also obtained 0.53 mgg^{-1} for fructose, 0.45 mgg^{-1} for glucose.

For control doughs, the results (0.088 g100g^{-1} for 60 min -0.13 g100g^{-1} for 120 min) for fructose and 0.075 g100g^{-1} for 60 min -0.13 g100g^{-1} for 120 min for glucose were close to the values obtained by Georgiana *et al.*, (2013) where fructose and glucose were 3.20 mgg^{-1} and 1.5 mgg^{-1} for 60 min fermentation, respectively, and 1.16 mgg^{-1} and 0.38 mgg^{-1} for 120 min fermentation,

respectively. Hamlet et al. (2008) found 0.079 $g100g^{-1}$-0.148 $g100g^{-1}$ for fructose and 0.295$g100g^{-1}$-0.164 $g100g^{-1}$ for glucose; and Ecem reported 17.33 gkg^{-1} for fructose and 14.59 gkg^{-1} for glucose.

During the dough fermentation process, the contents of fructose and glucose increase especially in the first 60 min of fermentation because amylase, an enzyme which degrades starch in flour, is constantly generating new glucose and maltose (Sahlstrom *et al.*, 2004). The fermentation of glucose and fructose occurs within the first 60 min to 120 min and the fermentation of fructose and maltose within the 120 to 180 min. Gihundo and Nyaruka varieties produced whole wheat flour, doughs and breads with the highest and lowest contents for glucose and fructose, respectively. Fructose and glucose were not detected in SCG and juice of rosemary leaves. Rather, lemon fruit juice (its fructose and glucose contents were 1.1 and 0.99 $g100g^{-1}$, respectively) increased these reducing sugars in the dough. The results showed there was an increase in fructose and glucose from dough to breads. This is to mean that formation of new fructose and glucose happened in the bread during baking. Reducing sugars such as fructose and glucose which are in many literatures believed to be also limiting factors for acrylamide formation in different foods, increased in breads instead of reducing in the present study.

Knowing that reducing sugars are normally degraded by yeast during fermentation and seeing their increase after baking, it showed that they were formed during baking (Westerlund et al., 1989). Predominant sugars in bread were, in decreasing order, maltose, fructose and glucose/maltulose (Westerlund *et al.*, 1989). The content of glucosaccharides that could not be degraded by amylolytic enzymes was significantly higher in crust than in crumb, indicating occurrence of reversion and/or transglycosidation reactions among the oligosaccharides originally present in flour or dough and those produced by thermal starch depolymerisation (Westerlund *et al.*, 1989).

5.3.4. The concentration of asparagine and pH of whole wheat bread supplemented with spent coffee grounds and juices of lemon fruits and rosemary leaves.

The content of asparagine and pH of whole flour, dough and whole breads from selected Rwandan wheat varieties are presnted in Table 5.2. and Table 5.3, respectively. The spent coffee grounds and juices extracted from lemon fruits and rosemary leaves were added in dough.

Table 5. 2. The content of asparagine of whole flour, dough and whole breads substituted with spent coffee grounds and juices of lemon fruits and rosemary leaves

Wheat variety	Ingredients	Fermentation (min)	Asparagine content (mg100g-1)			
			Whole wheat flour	Whole wheat dough		Breads
				Before fermentation	After fermentation	
Gihundo	W	60	0.399±0.06^{d}	0.49±0.02^{d}	0.097±0.004^{l}	0.050±0.001^{f}
	W:SCG			0.48±0.04^{d}	0.098±0.004^{l}	0.051±0.002^{f}
	W:SCG+LR			0.47±0.07^{d}	0.091±0.005^{k}	0.038±0.002^{d}
	W	120		0.49±0.02^{d}	0.065±0.03^{f}	0.028±0.001^{b}
	W:SCG			0.48±0.05^{d}	0.066±0.003^{f}	0.029±0.001^{b}
	W:SCG+LR			0.47±0.03^{d}	0.054±0.001^{c}	0.024±0.001^{a}
Kibatsi	W	60	0.372±0.02^{c}	0.45±0.08^{c}	0.091±0.003^{k}	0.051±0.002^{f}
	W:SCG			0.44±0.05^{c}	0.091±0.004^{k}	0.051±0.002^{f}
	W:SCG+LR			0.43±0.04^{c}	0.073±0.004^{h}	0.033±0.001^{c}
	W	120		0.45±0.06^{c}	0.062±0.003^{e}	0.029±0.001^{b}
	W:SCG			0.44±0.02^{c}	0.061±0.002^{e}	0.051±0.002^{f}
	W:SCG+LR			0.43±0.09^{c}	0.049±0.001^{b}	0.024±0.001^{a}
Nyaruka	W	60	0.331±0.04^{a}	0.39±0.04^{a}	0.078±0.004^{i}	0.049±0.002^{ef}
	W:SCG			0.39±0.03^{a}	0.077±0.003^{i}	0.048±0.002^{ef}
	W:SCG+LR			0.38±0.04^{a}	0.069±0.004^{g}	0.038±0.001^{d}
	W	120		0.39±0.08^{a}	0.057±0.004^{d}	0.028±0.001^{b}
	W:SCG			0.39±0.07^{a}	0.077±0.003^{i}	0.029±0.001^{b}
	W:SCG+LR			0.38±0.05^{a}	0.045±0.001^{a}	0.024±0.001^{a}
Reberaho	W	60	0.361±0.09^{b}	0.44±0.08^{b}	0.085±0.004^{j}	0.046±0.002^{e}
	W:SCG			0.42±0.06^{b}	0.086±0.004^{j}	0.046±0.002^{e}
	W:SCG+LR			0.43±0.07^{b}	0.073±0.003^{h}	0.038±0.001^{d}
	W	120		0.44±0.03^{b}	0.054±0.002^{c}	0.030±0.002^{bc}
	W:SCG			0.42±0.05^{b}	0.085±0.003^{j}	0.030±0.001^{bc}
	W:SCG+LR			0.43±0.02$^{\mathbf{b}}$	0.049±0.002^{b}	0.024±0.005^{a}

Values are means ±SD of 3 replications. Means with different superscript within a column are significantly different at p≤ 0.05. W: Whole wheat flour (Control flour), W: SCG: Whole wheat flour supplemented with spent coffee grounds (SCG), W: SCG+LR: Whole wheat flour supplemented with spent coffee grounds, juice of lemon fruits (L) and juice of rosemary leaves (R).

Table 5.3. pH of whole flour, dough and whole breads substituted with spent coffee grounds and juices of lemon fruits and rosemary leaves

Wheat variety	Ingredients	Fermentation (min)	pH					
			SCG	L	R	Whole wheat flour	Whole wheat dough (Before fermentation)	Whole wheat bread
Gihundo	W	60					5.83±0.12^{m}	5.81±0.37^{j}
	W:SCG						5.82±0.23^{m}	5.80±0.35^{j}
	W:SCG+LR						5.35±0.32^{d}	4.90±0.12^{d}
	W	120				6.22^{c}	5.73±0.15l^{m}	5.61±0.28^{i}
	W:SCG						5.71±0.24^{l}	5.50±0.19^{hi}
	W:SCG+LR						5.37±0.34^{d}	4.81±0.31^{d}
Kibatsi	W	60					5.45±0.19^{f}	5.42±0.29^{gh}
	W:SCG						5.41±0.27^{e}	5.40±0.11^{i}
	W:SCG+LR					5.91^{a}	5.02±0.11^{a}	4.40±0.28^{ab}
	W	120					5.24±0.18^{c}	5.20±0.21^{ef}
	W:SCG						5.36±0.29^{d}	5.32±0.27^{gh}
	W:SCG+LR						5.07±0.30^{a}	4.31±0.32^{a}
Nyaruka	W	60	6.15	2.70	5.82		5.68±0.27^{k}	5.53±0.34^{hi}
	W:SCG						5.62±0.35^{i}	5.42±0.19^{gh}
	W:SCG+LR						5.23±0.31^{c}	4.50±0.17^{bc}
	W	120				6.12^{b}	5.61±0.20^{i}	5.31±0.15^{fg}
	W:SCG						5.54±0.33^{h}	5.20±0.21^{ef}
	W:SCG+LR						5.21±0.38^{c}	4.40±0.26^{ab}
Reberaho	W	60					5.56±0.42^{hi}	5.53±0.23^{hi}
	W:SCG						5.55±0.25^{gh}	5.51±0.12^{i}
	W:SCG+LR					6.14^{b}	5.12±0.21^{b}	4.60±0.36^{c}
	W	120					5.49±0.49^{g}	5.31±0.13^{fg}
	W:SCG						5.45±0.42^{f}	5.31±0.24^{fg}
	W:SCG+LR						5.14±0.10^{b}	4.50±0.27^{bc}

Values are means ±SD of 3 replications. Means with different superscript within a column are significantly different at $p \leq 0.05$. W: Whole wheat flour (Control flour), W: SCG: Whole wheat flour supplemented with spent coffee grounds (SCG), W: SCG+LR: Whole wheat flour supplemented with spent coffee grounds, juice of lemon fruits (L) and juice of rosemary leaves (R).

Asparagine content was determined in whole wheat flour, dough (before/after fermentation) and in bread, however was not detected in SCG, L and R. Its content was significantly different ($p \leq 0.05$) among flours from all wheat varieties. This shows that asparagine concentration was dependent upon the variety since the four (4) wheat varieties were grown at the same location and under the same agro ecological conditions. It was reported that the levels of asparagine can increase with the increase in saline concentrations of the soil, more particularly in sensitive wheat varieties (Lea *et al.*, 2007). In the contract, the levels of asparagine can decrease with increase in the concentration of substrate or soil sulfur (Muttucumaru et al., 2006). Soil preparation, irrigation, crop protection and fertilization may have effect on asparagine concentrations.

Nitrogen fertilization to increase grain yield has been reported as well to largely influence by increasing protein and asparagine concentrations (Confederation of the Food and Drink Industries of the EU & (CIAA), 2004). Sprouting increases protease activity, transport of nitrogen, leading to high content of asparagine in wheat. It also accumulates during stress conditions of deficiency of minerals, drought, salt, exposure to metal toxic and attack of pathogen (Lea et al., 2007). Whole wheat flour obtained from Gihundo and Nyaruka varieties contained the highest (0.399 $mg100g^{-1}$) and the lowest (0.331$mg100g^{-1}$) amounts of asparagine, respectively.

Asparagine is the principal limiting precursor for acrylamide formation in cereal products (Arwa, 2008); (Amrein *et al.*, 2004);(Bråthen et al., 2005); (Bråthen & Knutsen, 2005) ;(Surdyk et al., 2004). Therefore, wheat varieties that show high asparagine level should have special treatment and processing conditions aiming at reducing unexpected acrylamide formation. It is possible to breed wheat varieties characterized by low asparagine, but it has been reported that to know the trait stability, very little is known about the interaction of the variety with the environment. Genetic modification by preventing the build up of asparagine by changing the expression of asparaginase has been reported by researchers in the UK, (Elmore et al., 2005).

The results of free asparagine found in whole wheat flour were in range with those obtained by (Hamlet et al., 2008). The authors reported the lowest 212 and the highest 538 mg kg^{-1} asparagine for the whole wheat flour from French and Solstice wheat varieties, respectively. Fredriksson *et al.*, (2004) found also between 0.48 and 0.51 g kg^{-1} asparagine for whole wheat flour.

Asparagine content was determined in dough (mixture of whole wheat flour, water, yeast, salt, spent coffee grounds (SCG), juices of lemon fruit and rosemary leaves) after 2 min after mixing (Dough before fermentation/Unfermented dough, Table 5.2) and in dough fermented for 60 and 120 min (Dough after fermentation, Table 5.2). For the unfermented dough, the lowest (0.39 $mg100g^{-1}$) and the highest (0.49 $mg100g^{-1}$) asparagine contents were found in the test doughs made from Nyaruka and Gihundo wheat varieties, respectively, and in their mixture with SCG. The findings were close to those reported by Fredriksson *et al.*(2004) who obtained between 0.29 and 0.34 gkg^{-1} asparagine content for whole wheat dough before fermentation.

The results showed that the incorporation of SCG did not significantly ($p>0.05$) affect the asparagine concentration in all unfermented/fermented dough samples (Table 5.2). Similarly, juices of lemon fruit and rosemary leaves when mixed with other ingredients did not significantly change the content of asparagine in the unfermented dough. The non-effect of the two ingredients was caused possibly by their low asparagine contents and low ratio compared to those of whole wheat flour. The asparagine content in whole wheat flour increased in all unfermented dough samples because of protease activity and/or its leaching in the wet dough. On the contrary, asparagine concentration in unfermented whole wheat dough samples (0.39-0.49 $mg100g^{-1}$) decreased significantly ($p\leq0.05$) in fermented whole wheat dough samples (0.025-0.081$mg100g^{-1}$). This was observed in a study done by (Fredriksson et al., 2004) in which the contents of asparagine were between 0.29 and 0.34 gkg^{-1} for unfermented whole wheat dough and 0.05 gkg^{-1} for fermented whole wheat dough. The decrease was caused by yeasts which utilized asparagine as an important source of nitrogen for their growth during fermentation (White & Munns, 1950).

Fermented dough from the 100% whole wheat flour of Nyaruka and Gihundo varieties had the lowest (0.073 $mg100g^{-1}$) and highest (0.081 $mg100g^{-1}$) asparagine contents, respectively. pH for control and W: SCG breads were not significantly different and were lower than W: SCG+LR breads. It was obvious that L which showed the lowest pH (2.70) could lower subsequently the pH of the resultant breads. Breads from dough fermented for 120 min were significantly ($p\leq0.05$) lower in pH than the breads from dough fermented for 60 min. The incorporation of RL in the recipe showed significant effect on the asparagine by decreasing its amount in fermented dough. Juice from lemon fruit as a source of organic acids, lowered pH to the extent preferred yeasts to

utilize some nutrients for their growth during fermentation. Therefore, asparagine content decreased as this compound was among other degraded nutrients. Fermentation duration showed significant change in asparagine concentration, where dough fermented for short time (60 min) had higher asparagine content than that fermented for long time (120 min). The decrease in asparagine concentration due to extension of fermentation duration was reported in the other studies (Benedito De Barber et al., 1989). The pH values of all doughs decreased with fermentation (Çelik & Gökmen, 2020). It has been reported that pH variation in fermented doughs greatly depends on both the production of organic acids or consumable acids and slightly on CO_2 dissolution in the water phase (Claus *et al.*, 2008); (Zhang *et al.*, 2006); (Palacios *et al.*, 2006). The juice from lemon fruits and fermentation duration separately or in combination could help reduce significantly asparagine concentration in dough, when acrylamide formation during baking is to be lowered. Since only the nonprotonised form of asparagine can form the Schiff base, when pH passes the isoelectric point of the asparagine amino group, the initial amino-carbonyl reaction may be hampered due to the protonation of the amino group (Lingnert *et al.*, 2002), thereby decreasing the Maillard reaction and acrylamide content in bread.

Asparagine concentration in fermented dough (0.025-0.081mg$100g^{-1}$) decreased significantly in bread (0.012-0.046 mg$100g^{-1}$) due to its reaction during heating of the dough and the bread. This was found in other work where (Granby et al., 2008) found 0.14 gkg^{-1} (dw, no addition of asparagine) in fermented dough and 0.017 gkg^{-1}(dw) in untoasted wheat bread. The reduction in asparagine in bread was in agreement with (Çelik & Gökmen, 2020) who reported about the reduction of free amino acid concentrations in bread crust-like model systems during heating at 200 °C. During heating, asparagine is the principal limiting precursor for acrylamide formation in cereal products (Arwa, 2008); (Amrein et al., 2004); (Bråthen & Knutsen, 2005); (Bråthen & Knutsen, 2005);(Surdyk et al., 2004). (Granby et al., 2008) also found the decrease in asparagine during toasting of the wheat bread. The loss of free asparagine has been reported to be higher in the crust (McDermott & Pace, 2007).

5.3.5. The acrylamide content of whole wheat bread supplemented with spent coffee grounds and juices of lemon fruits and rosemary leaves

The concentration of acrylamide of whole breads from selected Rwandan wheat varieties and the chemical characteristics of ingredients are presnted in Table 5.4 and Table 5.5, respectively. The ingredients incorporated in dough were spent coffee grounds and juices extracted from lemon fruits and rosemary leaves.

Table 5. 4. The content of acrylamide of whole wheat breads stored at room temperature

Wheat variety	Ingredients	Fermentation (min)	Acrylamide formation (μgkg^{-1})			
			Whole wheat breads			
			Storage (days)			
			1	3	5	7
Gihundo	W	60	47.23±0.92^{w}	47.23±1.77^{w}	47.23±0.72^{w}	47.21±0.81^{w}
	W:SCG		16.90±0.80^{g}	16.90±0.92^{g}	16.90±0.89^{g}	16.70±2.32^{g}
	W:SCG+LR		12.18±0.62^{b}	12.18±1.19^{b}	12.18±0.17^{b}	12.17±0.81^{b}
	W	120	44.01±2.02^{v}	44.02±1.32^{v}	44.01±2.24^{v}	43.98±1.62^{v}
	W:SCG		13.12±0.36^{d}	13.12±2.42^{d}	13.12±1.97^{d}	13.10±0.82^{d}
	W:SCG+LR		10.50±2.27^{a}	10.50±0.52^{a}	10.50±2.29^{a}	10.40±1.02^{a}
Kibatsi	W	60	31.10±1.41^{u}	31.10±0.79^{u}	31.10±1.28^{u}	31.10±2.12^{u}
	W:SCG		26.60±2.29^{p}	26.60±0.94^{p}	26.60±0.81^{p}	26.50±1.21^{p}
	W:SCG+LR		19.64±0.99^{m}	19.64±2.37^{m}	19.64±1.80^{m}	19.64±0.87^{m}
	W	120	29.50±1.17^{s}	29.50±2.27^{r}	29.50±2.74^{r}	29.50±2.65^{r}
	W:SCG		23.23±0.62^{o}	23.23±1.10^{o}	23.23±0.82^{o}	23.23±0.93^{o}
	W:SCG+LR		10.60±0.51^{a}	10.60±1.42^{a}	10.60±0.77^{a}	10.40±1.49^{a}
Nyaruka	W	60	30.30±0.82^{s}	30.30±1.02^{s}	30.30±1.19^{s}	30.10±2.02^{s}
	W:SCG		19.01±2.21^{j}	19.01±0.91^{j}	19.01±0.79^{j}	19.01±0.62^{j}
	W:SCG+LR		18.08±1.07^{i}	18.08±2.25^{i}	18.08±2.02^{i}	18.08±0.61^{i}
	W	120	27.24±2.34^{q}	27.24±1.12^{p}	27.24±1.34^{q}	27.24±1.84^{q}
	W:SCG		14.41±2.12^{e}	14.41±2.43^{e}	14.41±1.14^{e}	14.41±2.25^{e}
	W:SCG+LR		15.90±1.11^{f}	15.90±0.59^{f}	15.90±0.68^{f}	15.70±1.82^{f}
Reberaho	W	60	30.50±0.98^{t}	30.50±0.02^{u}	30.50±0.91^{t}	30.40±0.76^{u}
	W:SCG		22.90±1.27^{n}	22.90±0.66^{n}	22.90±0.66^{n}	22.90±0.42^{n}
	W:SCG+LR		19.23±1.01^{k}	19.23±0.92^{k}	19.23±0.70^{k}	19.22±1.12^{k}
	W	120	27.20±2.23^{q}	27.20±1.20^{p}	27.20±1.25^{q}	27.10±1.52^{q}
	W:SCG		12.34±1.22^{c}	12.34±0.70^{c}	12.34±1.29^{c}	12.32±2.32^{c}
	W:SCG+LR		17.09±1.15^{h}	17.08±2.82^{h}	17.09±2.22^{h}	17.09±0.87^{h}

Values are means ±SD of 3 replications. Means with different superscript within a column are significantly different at p≤ 0.05. W: Whole wheat flour (Control flour), W: SCG: Whole wheat flour supplemented with spent coffee grounds (SCG), W: SCG+LR: Whole wheat flour supplemented with spent coffee grounds, juice of lemon fruits (L) and juice of rosemary leaves (R).

Table 5.5. The chemical characteristics of ingredients added to whole wheat dough

Ingredients	Chemical characteristics					
	Antioxidant capacity (mg/100g)	Fructose content (g/100g)	Glucose content (g/100g)	Asparagine content (mg/100g)	Acrylamide content (µg/kg)	pH
Spent coffee grounds (SCG)	25.84	nd	Nd	nd	5.41	6.14
Juice of lemon fruit (L)	8.08	1.1	0.99	nd	nd	2.7
Juice of rosemary leaves (R)	26.77	nd	Nd	nd	nd	5.82

Values are means ±SD of 3 replications. **nd:** Not detected.

W bread from Gihundo variety had the highest acrylamide content (47.23 μgkg^{-1}), while W breads Nyaruka wheat variety had the lowest (30.347 μgkg^{-1}). This showed that Gihundo variety possessed the highest asparagine content (Table 5.2), the limiting precursor for acrylamide formation in cereal products (Arwa, 2008); (Amrein *et al.*, 2004);(Bråthen *et al*., 2005)*;*(Bråthen & Knutsen, 2005)*;*(Surdyk *et al.*, 2004). Therefore, wheat varieties that might possess high asparagine content should be known for special treatment and processing conditions aiming at reducing unexpected acrylamide formation. Acrylamide is a probable carcinogen and neurotoxic substance to humans.

Acrylamide content in all samples (bread crumb+crust) were between 10.5 and 47.23 μgkg^{-1}. The major part of this acrylamide was in the crust according to other studies which reported that more than 99% of the acrylamide is produced in the crust, where the temperature reaches much higher values than in the bread crumb, where the temperature does not exceed 100°C (Lingnert *et al.*, 2002; Surdyk *et al.*, 2004; Gökmen & Senyuva, 2007).

The acrylamide levels obtained in the present study were in the ranges (47.6 μgkg^{-1} bread crust) of yeast fermented bread (80 min fermentation) found by Esfahani *et al.* (2017) and 17 gkg^{-1} (dry wheat bread) found by Surdyk *et al.*, (2004) for the dough fermented for 120 min and baked at

270^{o}C for 15 min without added asparagine and fructose. The results were also in line with 33 and 4 μgkg^{-1} acrylamide (whole bread weight basis) for dough fermented for 30 min and 6 hr, respectively, reported by Fredriksson *et al.*(2004)**.** They are once again in the range reported by Arribas-Lorenzo & Morales, 2012; Serpen *et al.*, 2012; Forstova *et al.*, 2014; Negoita *et al.*, 2014); The authors highlighted that breads produced with different raw materials, manufacturing recipe, and processing conditions have acrylamide concentrations ranging from 10 to 150 μgkg^{-1}.

Addition of SCG+LR in dough fermented for 60 min reduced significantly ($p<0.05$) the acrylamide formation in W: W breads from 47.00 μgkg^{-1} to 10.5 μg kg^{-1}, approx. 85.5%. It was possibly due to high antioxidant capacity (Table 5.5) that SCG (25.84 mg100g^{-1}), L (8.08 mg100g^{-1}) and R (26.77 mg100g^{-1}) juices showed in comparison to that of whole wheat flour (2.73-2.90 mg100g^{-1}). The acrylamide of the breads was not increased by the incorporation of SCG as this ingredient showed low concentration of acrylamide (in the present study determined 5.41 μg kg^{-1}) with regard to that of control breads.

The addition of antioxidants has been found to influence the Maillard reaction and their use has been recommended for the reduction of acrylamide formation in some foods (Krishnakumar & Visvanathan, 2014; Ou *et al.*, 2010; Fernandez *et al.*, 2003; Tareke, 2003; Summa *et al.*, 2006; Zhang *et al.*, 2007; Hedegaard *et al.*, 2008; Zhu et al., 2009; Zhu *et al.*, 2010). The results are in good line with a study that showed the effect of prooxidants and antioxidants on the formation of acrylamide in the bread, in which addition of 1% of rosemary extract resulted in a 57-67% reduction in acrylamide levels depending on the nature of the extract (Hedegaard *et al.*, 2008; Zhang *et al.*, 2005; Zhang *et al.*, 2008). Also, in another study done on the use of spent coffee grounds as food ingredient in bakery products, antioxidants present in SCG were assumed to lower the maillard reaction for the acrylamide formation in biscuits (Martinez-Saez *et al.*, 2017; Fernandez-Gomez *et al.*, 2015; Mesías *et al.*, 2014). Therefore, wheat varieties and other ingredients showing higher antioxidant capacity (Table 5.5) should be selected and used for the reduction of acrylamide formation.

Additionally, L juice possibly helped SCG+R lower the pH of the dough to the optimum pH (<5.5, Table 5.3). This pH of SCG+LR dough (Table 5.3) could have enhanced yeast to degrade more free asparagine, present in the matrix, whereby acrylamide was found very low compared to W

breads and SCG breads. The acrylamide content was seen significantly higher in all breads from dough fermented for 60 min than that of the breads from dough fermented for 120 min. It may have been caused by the presence of high amount of degraded free asparagine in 120 min fermented dough in comparison to the free asparagine remained intact in 60 min fermented dough. Asparagine which is a limiting precursor towards acrylamide formation, is consumed by yeast as a nitrogen source for its metabolic activity during fermentation (Benedito De Barber *et al.*, 1989)*;* (Fredriksson *et al.*, 2004). The SCG and juices of lemon fruits (L) and rosemary leaves (R) which were incorporated in dough for their antioxidant capacity (Table 5.5) to mitigate acrylamide formation did it.

The results showed there was an increase in fructose and glucose from dough to breads. This is to mean that formation of new fructose and glucose happened in the bread during baking. Reducing sugars such as fructose and glucose which are in many literatures believed to be also limiting factors for acrylamide formation in different foods, increased in breads instead of reducing in the present study. Knowing that reducing sugars are normally degraded by yeast during fermentation and seeing their increase after baking, it showed that they were formed during baking (Westerlund *et al.*,1989). Predominant sugars in bread in decreasing order were maltose, fructose and glucose/maltulose (Westerlund et al., 1989). The content of glucosaccharides that could not be degraded by amylolytic enzymes was significantly higher in crust than in crumb, indicating occurrence of reversion and/or transglycosidation reactions among the oligosaccharides originally present in flour or dough and those produced by thermal starch depolymerisation (Westerlund et al., 1989).

Therefore, they are not necessary the limiting factors as asparagine for the acrylamide formation in yeast leavened wheat breads. This interpretation is supported by other researches which showed that the reducing sugars are the major limiting factors in potatoes (Krishnakumar & Visvanathan, 2014; Weisshaar, 2004; Noti *et al.*, 2003; De Wilde *et al.*,2004; Sanny *et al.*, 2012), while asparagine (mainly in the cereal bran) is the major limiting factors in cereal products (Krishnakumar & Visvanathan, 2014; Amrein *et al.*2004). Amrein *et al.* (2004); Amrein et al. (2003); Stadler (2006); Claus et al. (2008) reported that reducing sugars were not a limiting factor for acrylamide formation in yeast-leavened wheat bread after finding that more than 99% of the

acrylamide formed in the bread crust and high contents of reducing sugars in the bread crusts (0.40-0.64 g of fructose and 0.25-0.35 g of glucose per 100 g of dry crust (Surdyk et al., 2004). The results were supported by the confirmation saying that glycoconjugates of asparagine are the major source of acrylamide in foods under low moisture conditions at elevated temperatures in the presence of reducing sugars or a suitable carbonyl source (Blank et al., 2005).

Acrylamide content in all breads did not decrease significantly in a 7- day storage at room temperature (17-23^{o}C) as it was also reported by (Michalak *et al.*, 2016) that the storage for 15 and 30 days at 4^{o}C and 25^{o}C did not favour acrylamide formation. However, they found that acrylamide was not stable compound in some processed plant products with a long shelf-life, where it decreased particularly at higher temperatures.

5.4. Conclusion

In this study, the asparagine concentration was dependent upon the variety. The lowest and the highest asparagine contents were found in the whole flour made from Nyaruka and Gihundo wheat varieties, respectively. Reducing sugars increased after baking. Thus, they were not necessary the limiting factors as asparagine for the acrylamide formation in whole wheat bread. SCG and R showed higher antioxidant capacity than whole wheat flour. Thereby, the addition of SCG+LR in dough fermented for 60 min helped reduce significantly asparagine concentration in dough. Subsequently, the acrylamide formation in W: SCG+LR breads was significantly reduced as well. It would be important to reduce acrylamide formation in baked products using wheat cultivar, low in asparagine concentration and incorporate SCG and R, high in antioxidant capacity.

CHAPTER SIX: THE SHELF STABILITY OF WHOLE WHEAT BREAD SUPPLEMENTED WITH SPENT COFFEE GROUNDS AND JUICES OF LEMON FRUITS AND ROSEMARY LEAVES

Abstract

Local whole wheat bread is increasingly consumed in Rwanda. However, whole grain breads have been reported to undergo high losses, mainly due to microbial contamination. The objective of this study was to evaluate the effect of ingredients and fermentation on shelf stability of whole wheat breads in closed unperforated low density polythene bag during 7 days of storage at room temperature (17-23^{o}C). The bread was prepared by baking at 180^{o}C for 20 min a dough containing whole wheat flour from each wheat variety, spent coffee grounds, juice of lemon fruit, juice of rosemary leaves, salt and water. The dough was fermented by using instant dry yeast at 34^{o}C, 60% relative humidity for 60 min and at 39^{o}C, 85% relative humidity for 120 min, separately. Total viable counts (TVC) and total molds and yeasts (TMY) significantly ($p \leq 0.05$) increased with the storage time. W: SCG+LR bread (dough fermented for 60 min) from Nyaruka variety and W: SCG bread (dough fermented for 120min) from Gihundo variety had the lowest and the highest TVC, respectively, on 7th day of storage, while W: SCG+LR bread (dough fermented for 60 min) from Kibatsi variety and W: SCG bread (dough fermented for 120 min fermentation) from Reberaho wheat variety showed the lowest and highest TMY, respectively, on 7th day of storage. Breads from dough fermented for 120 min had lower TVC than those from dough fermented for 60 min, whereas it was the opposite for TMY. TVC for all breads up to 5th and on 7th day of storage was within the satisfactory and borderline ranges.

6.1. Introduction

Whole grain bread is easily contaminated with microbes such as bacteria and moulds, mostly when they are stored at uncontrolled environment like temperature, air, water activity, pH, food surface contact, equipments, food handler. According to Fik (2004), bread is placed among the foods with a very short shelf life about 6 days. In contrast to spoilage of bread by moulds, breads are spoilt by yeast after baking by practices such as poor hygiene handling of bread coolers, racks, conveyor belts, slicing machines, etc (Saranraj & Geetha, 2012). Mode of product preparation, season and product type affect the growth of moulds in most products and these account for a percentage loss between 1 and 5% in bakery products globally (Butt *et al.,* 2004). According to (Tarar *et al.,* 2010), bread starts spoiling after 48 hours being outside of the oven. The growing awareness about health issues arising from food consumption preserved with chemicals has led to improving food safety by using natural preservatives. Spices and herbs are one of the most used preservative agents in food. Some natural ingredients such as rosemary and lemon extracts have been reported to act as antimicrobial agents against molds, bacteria and yeasts. This was often revealed in some previous experiments that rosemary and lemon extracts possess diverse biological activities, including antioxidant and antimicrobial activity from their non- nutrient secondary metabolites like phenolic compounds, etc.

As the safety of baked cereal products is concerned, spent coffee grounds, juices of rosemary leaves and lemon fruit and prolongation of dough fermentation duration have been suggested to be used to evaluate their effects on the shelf sability of whole wheat breads during storage.

6.2. Materials and methods

6.2.1. Collection of the samples

Four dry wheat grain varieties namely TAI, EN161, Eagle10 and Korongo with the local names Gihundo, Kibatsi, Nyaruka, and Reberaho, respectively, were collected from the stores of Rwanda Agriculture and Animal Resources Development Board (RAB), located at Kinigi, Musanze district, Rwanda. These wheat varieties were grown in RAB farms at the temperature varying between at

16.6 and 21.5 ^{o}C, at the same location and under the same agro ecological and cultural conditions in the crop year 2018. The wheats were grown in volcanic soil and fertilized with urea and Diammonium phosphate (DAP). They were packed in high density polyethylene bags and stored at room temperature prior to milling. The wastes, known as spent coffee grounds, were directly taken after brewing coffee (Coffea Arabica var. bourbon) in a coffee shop in Musanze town, Rwanda and stored in a transparent plastic bottle at 5^{o}C in a fridge (SM302NW, SM302NW1014009, 2010/08) to analysis. Fresh green lemon fruits (var. African rough lemon) and raw green rosemary leaves (Var. Arp rosemary herb) were bought from the market in Musanze town, Rwanda, packed in plastic sachets and stored at 5^{o}C in a fridge (SM302NW, SM302NW1014009, 2010/08) prior to processing. The coffee, lemon and rosemary were all grown in Rwanda.

6.2.2. Preparation of the raw samples

6.2.2.1. Wheat grains milling

Before milling, the wheat grains were conditioned to 15.5 % moisture content in order to get a fine particle size whole wheat flour by the addition of distilled water and were left for at least 24 hr at ambient conditions in a closed plastic container for the absorption of the moisture (Mishra, 2016). AACC (2003) Approved Method 26-95.01 was used to calculate the amount of water to be added for wheat grains tempering:

$$\boldsymbol{m}l = \left(\left(\frac{100 - \%moisture}{100 - 15.5\%}\right) - 1\right) x\ grams\ of\ wheat\ grains$$

The conditioned wheat grains of each variety were wholly milled by using a laboratory hammer mill (CM 1090 Cemotec, 2009, China). All bran and germ were mixed with the flour. The flour was packaged in high density polyethylene envelop and stored at 5^{o}C in a fridge (SM302NW, SM302NW1014009, 2010/08) prior to analysis and baking.

6.2.2.2. Lemon fruit and rosemary leaves juice making

The lemon and rosemary juices were obtained by using a blender (Moulinex, LM241, Genuine, 2017, France). The lemon fruits (3kg) were washed and peeled with knife. The rosemary leaves were picked off the woody branch by using hand and washed. Then after, they were cut into quarters and juiced by by using a blended (Moulinex, LM241, Genuine, 2017, France) with no water added for between 30 secs and 1 min until the extraction is complete. Rosemary leaves (5kg) were ground in the blender (Moulinex, LM241, Genuine, 2017, France) between 2 and 4 min until all leaves were thoroughly ground with no water added. To get the clear juice, lemon fruit pulp and ground rosemary leaves were separately sqeezed in a cheese cloth (grade 90, 44 × 36 weaves; 2018, Zhuojie, China) by using hand. The wastes (peels, seeds and other solid materials resulted from the extraction of juices were deposited in a bin. The clarified lemon and rosemary juices were kept in transparent plastic bottles and stored at 5^{o}C in a fridge (SM302NW, SM302NW1014009, 2010/08) prior to analysis and processing.

6.2.3. Baking

The dough comprising 200g whole wheat flour from each of the wheat variety grains (TAI, EN161, Eagle10 and Korongo), 2% instant dry yeast (GB Ingredients, Dordrecht, 2019, Holland), 2% sodium chloride and potable water (125 mL) (Fredriksson *et al.*, 2004). The amounts of 4% spent coffee grounds (Martinez-Saez *et al.*, 2017), 1% lemon fruit juice and 1% rosemary leaf juice were added. The electric balance (Explorer EX 223, version 2.00/2.00. SN B 333687045, IR Sensor, OHAUS) was used. The mixture was fermented and proofed in a fermenter (Manz Backtechnik GmbH, Creglingen, Germany) at 34^{o}C, 60% relative humidity for 60 min and at 39^{o}C, 85% relative humidity for 120 min after being mixed in dough mixer (Combisteel Dough Mixer Liter, 7455.1400, 2011, China) (Surdyk *et al.*, 2004). Rotation of the mixer was 54 rotations per minute (rpm) for 3 min until the dough came together and then switched to 104 rpm for 6 min. Each fermented dough was covered with a lid and baked at 180^{o}C for 20 min in an oven (Electric baking oven, Lemarkz, Model: LGO-24A, 2010, India). The control bread from each wheat variety was made for 60 min of fermentation without incorporation of SCG and LR. The loaves were depanned and cooled for 2hr, packaged in unperforated low density polythene bag, closed and stored at room temperature between 17-23^{o}C prior to analysis.

6.2.4. Determination of moisture content and water activity for whole wheat breads

Moisture content (MC) of the whole wheat bread was determined using moisture analyser (Model: HE 53/01, Mettler Toledo, 2016, China). Water activity (a_W) was determined using Dew point water activity analyzer (4 TE, Aqua Lab, SN: SN 40005761, 2016, USA). Breads packaged in unperforated low density polythene bag, closed and stored at room temperature between 17-23^oC were ground by using a laboratory grinder (Moulinex, LM241, Genuine, 2017, France) into fine flour. Bread flour sample (0.5g) was used for MC and a_W determination.

6.2.5. Determination of pH for spent coffee grounds, juices of lemon fruits and rosemary leaves, whole wheat flours, whole wheat doughs and whole wheat breads

pH was determined by using pH meter (EUTECH INSTRUMENTS Ph510, cyberscan, 2016, USA). Buffers 4, 7, 10 were used to calibrate a pHmeter. Freeze-dried dough and bread were ground by using a laboratory grinder (Moulinex, LM241, Genuine, 2017, France) into fine flour. The sample (0.2 g) from SCG, whole flour, ground dough and bread was mixed in 25 ml distilled water and vortexed for 3 minutes. The mixture was held at room temperature for 1 hr and centrifuged at 3000 rpm/10min. Then, the suprenant was used to measure pH of the bread.

6.2.6. Determination of microbiological analysis for whole wheat breads

Total viable counts (TVC) and total yeasts and molds (TYM) were determined as described by (APHA (American PublicHealth Association), 2001). Bread samples in unperforated low density polythene envelopes were stored at room temperature (17-23^oC) prior to analysis. The samples were ground by using a laboratory grinder (Moulinex, LM241, Genuine, 2017, France) into fine flour. Ten grams of the ground bread was mixed with Peptone water and sub samples were serially diluted decimally 4 times and 0.1 mL aliquots were spread plated on Potato Dextrose Agar (PDA) and Nutrient Agar (NA). The NA plates were also incubated at 37^oC. The colonies for both PDA

and NA plates were counted and expressed as colony forming units per gram ($cfug^{-1}$) of samples. All counts were done in 3 replications after each 2 days for a 7-day of storage time and expressed in log $cfug^{-1}$.

6.2.7. Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using SAS System for Windows (version 9.3, SAS Institute, Cary, NC). Treatment means were separated using the Tukey's test and least significant difference was accepted at $p \leq 0.05$.

6.3. Results and discussion

6.3.1. pH of whole breads produced using spent coffee grounds and juices of lemon fruits and rosemary leaves in storage

Th pH of breads in storage made using mixture of whole wheat flours, spent coffee grounds and juices of lemon fruits and rosemary leaves is shown in Table 6.1. The whole flours were obtained from selected Rwandan wheat varieties.

Table 6.1. pH of whole wheat breads substituted with spent coffee grounds and juices of lemon fruits and rosemary leaves at room temperature.

Wheat variety	Ingredients	Fermentation (min)	pH			
			Whole wheat breads			
			Storage (days)			
			1	3	5	7
Gihundo	W	60	5.81±0.37^{j}	5.61±0.14^{kl}	5.42±0.16^{gh}	5.40±0.29^{lm}
	W:SCG		5.80±0.35^{j}	5.70±0.31^{l}	5.50±0.13^{h}	5.51±0.11^{m}
	W:SCG+LR		4.90±0.12^{d}	4.80±0.34^{e}	4.41±0.38^{d}	4.32±0.28^{e}
	W	120	5.61±0.28^{i}	5.42±0.15^{ij}	5.11±0.32^{e}	5.01±0.19^{hi}
	W:SCG		5.50±0.19^{hi}	5.33±0.12^{hi}	5.10±0.34^{e}	5.14±0.32^{ij}
	W:SCG+LR		4.81±0.31^{d}	4.60±0.22^{d}	4.23±0.31^{bc}	4.11±0.37^{cd}
Kibatsi	W	60	5.42±0.29^{gh}	5.33±0.21^{hi}	5.11±0.12^{e}	5.02±0.25^{hi}
	W:SCG		5.40±0.11^{i}	5.61±0.31^{kl}	5.42±0.23^{gh}	5.32±0.15^{kl}
	W:SCG+LR		4.40±0.28^{ab}	4.23±0.17^{ab}	4.05±0.21^{a}	4.07±0.19^{b}
	W	120	5.20±0.21^{ef}	5.11±0.15^{fg}	5.00±0.27^{e}	4.86±0.18^{g}
	W:SCG		5.32±0.27^{gh}	5.33±0.31^{hi}	5.01±0.36^{e}	5.13±0.17^{ij}
	W:SCG+LR		4.31±0.32^{a}	4.10±0.38^{a}	4.00±0.31^{a}	3.81±0.13^{a}
Nyaruka	W	60	5.53±0.34^{hi}	5.33±0.16^{hi}	5.11±0.24^{e}	4.85±0.45^{g}
	W:SCG		5.42±0.19^{gh}	5.32±0.19^{hi}	5.02±0.19^{e}	4.90±0.31^{gh}
	W:SCG+LR		4.50±0.17^{bc}	4.30±0.26^{bc}	4.05±0.24^{a}	3.73±0.27^{a}
	W	120	5.31±0.15^{fg}	5.11±0.23^{fg}	4.90±0.43^{de}	4.61±0.34^{f}
	W:SCG		5.20±0.21^{ef}	5.10±0.20^{fg}	5.02±0.32^{e}	4.85±0.22^{g}
	W:SCG+LR		4.40±0.26^{ab}	4.23±0.19^{ab}	4.04±0.31^{a}	3.72±0.23^{a}
Reberaho	W	60	5.53±0.23^{hi}	5.42±0.17^{ij}	5.10±0.19^{e}	5.01±0.18^{hi}
	W:SCG		5.51±0.12^{i}	5.50±0.32^{jk}	5.33±0.38^{fg}	5.21±0.31^{jk}
	W:SCG+LR		4.60±0.36^{c}	4.41±0.22^{c}	4.32±0.31^{cd}	4.05±0.17^{bc}
	W	120	5.31±0.13^{fg}	5.12±0.29^{fg}	5.01±0.18^{e}	4.86±0.14^{g}
	W:SCG		5.31±0.24^{fg}	5.20±0.28^{gh}	5.00±0.32^{e}	4.91±0.32^{gh}
	W:SCG+LR		4.50±0.27^{bc}	4.41±0.12^{c}	4.23±0.29^{bc}	4.24±0.34^{de}

Values are means ±SD of 3 replications. Means with different superscript within a column are significantly different at $p \leq 0.05$. W: Whole wheat flour (Control flour), W: SCG: Whole wheat flour supplemented with spent coffee grounds (SCG), W: SCG+LR: Whole wheat flour supplemented with spent coffee grounds, juice of lemon fruits (L) and juice of rosemary leaves (R).

6.3.2. Water activity of whole breads made using spent coffee grounds and juices of lemon fruits and rosemary leaves in storage

Water activity of whole wheat bread from selected Rwandan wheat varieties is presented in Table 6.2.

Table 6.2. Water activity of whole wheat breads added with spent coffee grounds and juices of lemon fruits and rosemary leaves at room temperature.

Wheat variety	Ingredients	Fermentation (min)	Water activity (a_W)			
			Whole wheat breads			
			Storage (days)			
			1	3	5	7
Gihundo	W	60	0.94±0.04^{cd}	0.92±0.03^{b}	0.92±0.05^{bc}	0.90±0.07^{bc}
	W:SCG		0.96±0.01^{e}	0.93±0.03^{bc}	0.92±0.04^{bc}	0.90±0.04^{bc}
	W:SCG+LR		0.93±0.08^{bc}	0.91±0.08^{a}	0.91±0.03^{a}	0.89±0.02^{ab}
	W	120	0.93±0.03^{bc}	0.91±0.06^{a}	0.90±0.03^{a}	0.88±0.04^{a}
	W:SCG		0.94±0.02^{cd}	0.93±0.06^{bc}	0.92±0.01^{bc}	0.90±0.04^{bc}
	W:SCG+LR		0.96±0.07^{e}	0.93±0.04^{bc}	0.93±0.04^{cd}	0.90±0.01^{bc}
Kibatsi	W	60	0.93±0.02^{bc}	0.91±0.08^{a}	0.91±0.02^{a}	0.89±0.02^{ab}
	W:SCG		0.94±0.03^{cd}	0.92±0.02^{b}	0.91±0.03^{a}	0.89±0.03^{ab}
	W:SCG+LR		0.92±0.09^{ab}	0.90±0.08^{a}	0.90±0.03^{a}	0.89±0.05^{ab}
	W	120	0.93±0.03^{bc}	0.91±0.02^{a}	0.91±0.02^{a}	0.88±0.04^{a}
	W:SCG		0.95±0.05^{de}	0.93±0.03^{bc}	0.92±0.02^{bc}	0.90±0.06^{bc}
	W:SCG+LR		0.96±0.09^{e}	0.94±0.02^{c}	0.94±0.05^{d}	0.92±0.06^{c}
Nyaruka	W	60	0.95±0.04^{de}	0.93±0.03^{bc}	0.92±0.03^{bc}	0.89±0.05^{ab}
	W:SCG		0.96±0.02^{e}	0.94±0.07^{c}	0.94±0.04^{d}	0.92±0.02^{c}
	W:SCG+LR		0.92±0.02^{ab}	0.90±0.02^{a}	0.90±0.02^{a}	0.88±0.03^{a}
	W	120	0.93±0.03^{bc}	0.91±0.01^{a}	0.91±0.01^{a}	0.89±0.06^{ab}
	W:SCG		0.94±0.09^{cd}	0.92±0.07^{b}	0.91±0.02^{a}	0.89±0.06^{ab}
	W:SCG+LR		0.94±0.07^{cd}	0.92±0.02^{b}	0.92±0.02^{bc}	0.90±0.01^{bc}
Reberaho	W	60	0.94±0.02^{cd}	0.92±0.03^{b}	0.91±0.03^{a}	0.89±0.09^{ab}
	W:SCG		0.96±0.03^{e}	0.94±0.02^{c}	0.94±0.02^{d}	0.92±0.08^{c}
	W:SCG+LR		0.91±0.07^{a}	0.90±0.03^{a}	0.90±0.02^{a}	0.88±0.04^{a}
	W	120	0.92±0.03^{ab}	0.90±0.04^{a}	0.90±0.03^{a}	0.88±0.03^{a}
	W:SCG		0.94±0.05^{cd}	0.92±0.02^{b}	0.91±0.04^{a}	0.89±0.05^{ab}
	W:SCG+LR		0.93±0.03^{bc}	0.91±0.01^{a}	0.90±0.06^{a}	0.88±0.02^{a}

Values are means ±SD of 3 replications. Means with different superscript within a column are significantly different at $p \leq 0.05$. W: Whole wheat flour (Control flour), W: SCG: Whole wheat flour supplemented with spent coffee grounds (SCG), W: SCG+LR: Whole wheat flour supplemented with spent coffee grounds, juice of lemon fruits (L) and juice of rosemary leaves (R).

6.3.3. Moisture content of whole breads supplemented with spent coffee grounds and juices of lemon fruits and rosemary leaves in storage

The moisture content of whole wheat breads in storage is shown in Table 6.3. The breads were made by mixing whole wheat flours, spent coffee grounds and juices of lemon fruits and rosemary leaves.

Table 6. 3**.** The moisture content of whole wheat breads kept at room temperature

Wheat variety	Ingredients	Fermentation (min)	Moisture content (%)			
			Whole wheat breads			
			Storage (days)			
			1	3	5	7
Gihundo	W	60	23.91±1.23^{c}	22.62±1.41^{b}	21.03±0.73^{bc}	20.31±0.98^{bc}
	W:SCG		28.22±1.56^{lm}	27.03±1.56^{ef}	25.52±1.39^{fg}	25.03±0.73^{gh}
	W:SCG+LR		26.81±0.98^{ij}	25.54±0.93^{cd}	24.03±0.91^{ef}	23.60±0.81^{ef}
	W	120	25.52±0.23^{f}	24.04±0.57^{c}	22.41±2.23^{cd}	21.72±1.03^{cd}
	W:SCG		30.24±0.73^{pq}	29.02±1.38^{gh}	27.52±1.78^{hi}	27.01±1.23^{ij}
	W:SCG+LR		29.02±1.62^{n}	28.10±0.78^{fg}	27.02±1.73^{hi}	26.30±1.81^{hi}
Kibatsi	W	60	23.34±2.03^{b}	22.02±0.90^{b}	20.50±0.69^{b}	20.04±0.83^{b}
	W:SCG		27.62±0.79^{k}	26.30±0.23^{de}	24.61±0.63^{ef}	24.02±2.20^{fg}
	W:SCG+LR		25.71±1.52^{fg}	24.41±0.29^{c}	22.80±0.93^{cd}	22.11±1.38^{de}
	W	120	26.50±1.08^{i}	25.03±2.30^{cd}	23.51±2.30^{de}	23.05±2.13^{ef}
	W:SCG		31.42±2.22^{r}	30.01±2.72^{h}	28.45±1.83^{i}	28.02±0.43^{j}
	W:SCG+LR		29.33±2.13^{o}	28.0±0.43^{fg}	26.37±0.74^{gh}	25.62±1.93^{gh}
Nyaruka	W	60	22.44±0.03^{a}	21.1±0.62^{a}	19.50±0.43^{a}	19.06±0.88^{a}
	W:SCG		27.93±0.21^{l}	26.61±0.41^{de}	25.02±1.57^{fg}	24.82±0.34^{fg}
	W:SCG+LR		25.03±1.21^{d}	24.50±2.33^{c}	22.91±1.78^{cd}	22.20±0.77^{de}
	W	120	23.83±1.69^{c}	22.51±1.53^{b}	21.04±0.39^{bc}	20.30±1.93^{bc}
	W:SCG		29.31±0.51^{o}	28.03±2.23^{fg}	26.50±0.87^{gh}	26.05±1.30^{hi}
	W:SCG+LR		25.91±1.29^{gh}	24.62±1.43^{c}	23.03±1.37^{cd}	22.61±2.73^{de}
Reberaho	W	60	23.20±0.92^{b}	22.01±1.83^{b}	20.62±1.79^{b}	20.02±0.37^{bc}
	W:SCG		27.92±2.27^{l}	26.60±2.41^{de}	25.01±0.38^{fg}	24.51±0.80^{fg}
	W:SCG+LR		25.82±2.09^{fg}	24.41±0.79^{c}	22.90±0.93^{cd}	22.40±0.99^{de}
	W	120	25.21±0.73^{de}	24.01±1.03^{c}	22.82±0.19^{cd}	22.03^{d}±0.71^{e}
	W:SCG		30.34±0.24^{p}	29.01±2.76^{gh}	27.51±0.56^{hi}	27.12±0.89^{ij}
	W:SCG+LR		28.01±0.89^{l}	27.72±1.84^{ef}	26.19±2.33^{gh}	25.81^{g}±2.93^{h}

Values are means ±SD of 3 replications. Means with different superscript within a column are significantly different at p≤ 0.05. W: Whole wheat flour (Control flour), W: SCG: Whole wheat flour supplemented with spent coffee grounds (SCG), W: SCG+LR: Whole wheat flour supplemented with spent coffee grounds, juice of lemon fruits (L) and juice of rosemary leaves (R).

pH (Table 6.1), a_W (Table 6.2), moisture content (Table 6.3) decreased with increasing storage time from 5.83, 0.96, 31.42% (Day 1) to 3.72, 0.88 and 19.06% on last day of storage (Day 7), respectively, regardless of the wheat variety, ingredients and fermentation time. W breads made from Nyaruka variety showed significantly ($p<0.05$) low moisture content (22.44%) compared to other breads from whole flours from the rest of varieties at day 1 of storage. Breads produced from whole flours from Gihundo, Kibatsi and Reberaho varieties were not significantly different in moisture content. At 7^{th} day, water activity (0.88) for the breads from W: W flours (120 min fermentation) from Gihundo, Kibatsi and Reberaho varieties was lowest and was not different.

Bread from W: W flour from Gihundo variety exhibited the highest pH (5.81) and the rest of wheat varieties gave breads which were not significantly different ($p\leq0.05$) in pH. Breads containing SCG followed by breads mixed with LR were higher in moisture content and a_W than W bread. pH for W and W: SCG breads were not significantly different and were lower than W: SCG+LR breads. It was obvious that L which showed the lowest pH could lower subsequently the pH of the resultant breads. Breads from dough fermented for 120 min were significantly ($p\leq0.05$) lower in pH than the breads from dough fermented for 60 min. Regarding pH for stored breads, the production of acidic volatile compounds acids resulting from aldehyde oxidation generally continues after the baking process, which clearly explains the gradual lowering of pH of this particular product (Jensen *et al*., 2011a).

6.2.4. Total viable counts of whole wheat bread with spent coffee grounds and juices of lemon fruits and rosemary leaves incorporated

Total viable counts for whole wheat breads in storage is shown in Table 6.4. The breads were produced by incorporating spent coffee grounds and juices of lemon fruits and rosemary leaves.

Table 6. 4. Total viable counts of whole wheat breads in storage at room temperature

Wheat variety	Ingredients	Fermentation (min)	Total viable counts (log cfu g^{-1})			
			Whole wheat breads			
			Storage (days)			
			1	3	5	7
Gihundo	W	60	2.20±0.10^{c}	3.20±0.22^{c}	5.20±0.13^{b}	5.80±0.28^{d}
	W:SCG		2.10±0.13^{b}	3.20±0.18^{c}	5.20±0.07^{b}	5.70±0.20^{c}
	W:SCG+LR		2.00±0.14^{a}	3.10±0.37^{b}	4.20±0.03^{a}	4.70±0.21^{b}
	W	120	2.10±0.08^{b}	3.20±0.13^{c}	5.20±0.20^{b}	5.70±0.29^{c}
	W:SCG		2.10±0.02^{b}	3.10±0.05^{b}	5.20±0.21^{b}	5.70±0.17^{c}
	W:SCG+LR		2.00±0.15^{a}	3.10±0.22^{b}	4.20±0.25^{a}	4.60±0.27^{a}
Kibatsi	W	60	2.20±0.02^{c}	3.20±0.21^{c}	5.20±0.22^{b}	5.80±0.13^{d}
	W:SCG		2.10±0.17^{b}	3.20±0.19^{c}	5.20±0.07^{b}	5.70±0.16^{c}
	W:SCG+LR		2.10±0.31^{b}	3.00±0.02^{a}	4.20±0.19^{a}	4.70±0.05^{b}
	W	120	2.20±0.11^{c}	3.20±0.23^{c}	5.20±0.22^{b}	5.80±0.18^{d}
	W:SCG		2.10±0.34^{b}	3.20±0.19^{c}	5.20±0.22^{b}	5.70±0.14^{c}
	W:SCG+LR		2.10±0.14^{b}	3.10±0.07^{b}	4.20±0.29^{a}	4.60±0.08^{a}
Nyaruka	W	60	2.20±0.05^{c}	3.20±0.24^{c}	5.20±0.01^{b}	5.80±0.21^{d}
	W:SCG		2.20±0.02^{c}	3.20±0.15^{c}	5.20±0.07^{b}	5.70±0.29^{c}
	W:SCG+LR		2.10±0.08^{b}	3.10±0.11^{b}	4.20±0.37^{a}	4.70±0.02^{b}
	W	120	2.20±0.29^{c}	3.20±0.03^{c}	5.20±0.34^{b}	5.70±0.19^{c}
	W:SCG		2.10±0.11^{b}	3.10±0.27^{b}	5.20±0.21^{b}	5.70±0.18^{c}
	W:SCG+LR		2.10±0.29^{b}	3.10±0.09^{b}	4.20±0.03^{a}	4.60±0.34^{a}
Reberaho	W	60	2.20±0.27^{c}	3.20±0.13^{c}	5.20±0.21^{b}	5.70±0.23^{c}
	W:SCG		2.10±0.23^{b}	3.10±0.34^{b}	5.20±0.28^{b}	5.70±0.05^{c}
	W:SCG+LR		2.10±0.13^{b}	3.10±0.13^{b}	4.20±0.24^{a}	4.70±0.12^{b}
	W	120	2.10±0.14^{b}	3.20±0.18^{c}	5.20±0.02^{b}	5.70±0.04^{c}
	W:SCG		2.10±0.02^{b}	3.10±0.27^{b}	5.20±0.13^{b}	5.70±0.06^{c}
	W:SCG+LR		2.10±0.22^{b}	3.10±0.07^{b}	4.20±0.03^{a}	4.60±0.26^{a}

Values are means ±SD of 3 replications. Means with different superscript within a column are significantly different at $p \leq 0.05$. W: Whole wheat flour (Control flour), W: SCG: Whole wheat flour supplemented with spent coffee grounds (SCG), W: SCG+LR: Whole wheat flour supplemented with spent coffee grounds, juice of lemon fruits (L) and juice of rosemary leaves (R).

6.2.5. Total molds and yeasts of whole wheat breads produced using spent coffee grounds and juices of lemon fruits and rosemary leaves

Total molds and yeasts (TMY) of whole breads in storage is shown in Table 6.5. The breads were made by adding spent coffee grounds and juices of lemon fruits and rosemary leaves in whole wheat flours.

Table 6. 5. Total molds and yeasts of whole wheat breads kept at room temperature

Wheat variety	Ingredients	Fermentation (min)	Total Molds and Yeasts (log cfu g^{-1})			
			Whole wheat breads			
			Storage (days)			
			1	3	5	7
Gihundo	W	60	nd	2.30±0.23^{c}	4.80±0.07^{c}	5.10±0.15^{d}
	W:SCG		nd	2.40±0.12^{d}	4.90±0.18^{d}	5.10±0.04^{d}
	W:SCG+LR		nd	2.10±0.30^{b}	3.60±0.21^{a}	4.70±0.26^{a}
	W	120	nd	2.30±0.11^{c}	4.80±0.29^{c}	5.20±0.17^{e}
	W:SCG		nd	2.40±0.21^{d}	4.90±0.20^{d}	5.10±0.31^{d}
	W:SCG+LR		nd	2.30±0.23^{c}	3.60±0.33^{a}	4.90±0.39^{c}
Kibatsi	W	60	nd	2.30±0.03^{c}	4.80±0.09^{c}	5.20±0.21^{e}
	W:SCG		nd	2.40±0.13^{d}	4.90±0.04^{d}	5.10±0.24^{d}
	W:SCG+LR		nd	2.10±0.09^{b}	3.70±0.18^{b}	4.80±0.05^{b}
	W	120	nd	2.40±0.19^{d}	4.80±0.13^{c}	5.20±0.14^{e}
	W:SCG		nd	2.40±0.17^{d}	4.90±0.21^{d}	5.20±0.03^{e}
	W:SCG+LR		nd	2.30±0.25^{c}	3.70±0.27^{b}	4.90±0.13^{c}
Nyaruka	W	60	nd	2.40±0.04^{d}	4.80±0.19^{c}	5.10±0.37^{d}
	W:SCG		nd	2.40±0.20^{d}	4.80±0.23^{c}	5.10±0.32^{d}
	W:SCG+LR		nd	2.00±0.33^{a}	3.70±0.11^{b}	4.80±0.11^{b}
	W	120	nd	2.40±0.31^{d}	4.80±0.17^{c}	5.20±0.17^{e}
	W:SCG		nd	2.40±0.07^{d}	4.90±0.14^{d}	5.10±0.29^{d}
	W:SCG+LR		nd	2.30±0.24^{c}	3.80±0.37^{b}	4.90±0.22^{c}
Reberaho	W	60	nd	2.30±0.37^{c}	4.80±0.22^{c}	5.20±0.13^{e}
	W:SCG		nd	2.40±0.14^{d}	4.80±0.06^{c}	5.10±0.27^{d}
	W:SCG+LR		nd	2.10±0.35^{b}	3.70±0.04^{b}	4.80±0.10^{b}
	W	120	nd	2.40±0.03^{d}	4.80±0.16^{c}	5.20±0.13^{e}
	W:SCG		nd	2.40±0.17^{d}	4.80±0.09^{c}	5.20±0.19^{e}
	W:SCG+LR		nd	2.30±0.07^{c}	3.70±0.12^{b}	4.90±0.21^{c}

Values are means ±SD of 3 replications. Means with different superscript within a column are significantly different at $p \leq 0.05$. W: Whole wheat flour (Control flour), W: SCG: Whole wheat flour supplemented with spent coffee grounds (SCG), W: SCG+LR: Whole wheat flour supplemented with spent coffee grounds, juice of lemon fruits (L) and juice of rosemary leaves (R).

nd: Not detected

Total viable count (TVC) (Table 6.4) and total molds and yeasts (TMY) (Table 6.5) significantly increased with the storage time of 7 days. For TVC, their range was from 2.00 to 2.20 log cfu g^{-1} on day 1, and from 4.60 to 5.80 log cfu g^{-1} on day 7 (last day of storage). For TMY, they were not detected on 1stday, they were between 2.00 and 2.40 log cfu g^{-1} on on 3rd day, and between 4.70 and 5.20 log cfu g^{-1} on the last day of storage.

W: SCG+LR bread (60 min fermentation) from Nyaruka variety and W: SCG bread (120 min fermentation) from Gihundo variety had the lowest and the highest TVC, respectively (Table 6.4), while W: SCG+LR bread (60 min fermentation) from Kibatsi variety and W: SCG bread (120 min fermentation) from Reberaho wheat variety showed the lowest and highest TMY, respectively, on day 7 (Table 6.5). The results showed that breads from dough fermented for 120 min had lower TVC than those from dough fermented for 60 min, whereas it was the opposite for TMY. TVC for all breads up to 5th and on 7th day of storage was within the satisfactory and borderline ranges which are < 4 log cfug^{-1}, and between 4 log cfu g^{-1} and 6 log cfu g^{-1}, respectively. On the 7th day,

SCG+LR, followed by SCG breads, showed lower TVC than wheat only breads may due to their high antimicrobial activity. W: SCG+LR breads showed the lowest TVC, possible due to the lowest pH (Table 6.1) along with their high antimicrobial activity from SCG and LR. TVC reduced in W: SCG breads with pH >5.4 and $a_W \leq 0.91$ (Table 6.2), while TVC reduced dramatically in W: SCG or W: SCG+LR breads and where pH 4.3-4.8 (Table 6.1), $a_W < 0.91$. But where $a_W > 0.91$, TVC increased in breads regardless their pH, and inclusion of SCG or SCG+LR. Breads with $a_W \geq 0.86$ showed an increase in TMY and decreased where pH was 4.3-4.8, but at $a_W \leq 0.91$. TVC or TMY increased in breads with $a_W > 0.91$ independent of pH and incorporation of SCG or SCG+LR. On the other hand, SCG or SCG+LR reduced TVC or TMY drastically in breads with pH lower than 5. SCG or SCG+LR may have acted as antimicrobial agents against TVC and TMY in breads as it was often revealed in some previous experiments to possess diverse biological activities, including antioxidant and antimicrobial activity due to its non- nutrient secondary metabolites such as the phenolic diterpenes, carnosol, carnosic acid, methyl carnosate, rosmanol, and epirosmanol, and phenolic acids such as ferulic, rosmarinic, and chlorogenic and caffeic acids (Campo *et al.*, 2000).

The results showed that to be more efficient, SCG or SCG+LR should be used with the control of pH and a_W. It is known that the water activity and pH are critical to the survival of microbes in breads (Puhr & D'Appolonia, 1992). Generally, mold growth is by far the most important shelf-life limiting factor of bread products where they are tolerant of acid conditions and favour an acidic pH (3.5-5.5). Foods with pH value <4.5 are not usually spoiled by bacteria but are more susceptible to mold spoilage. In other previous studies, inhibitory and fungicidal concentrations of the citrus juice concentrates against three fungal strains were successful (Ehigbai et al., 2016). Hence, these authors suggest that the lemon juice concentrates may have beneficial antimicrobial roles that can be exploited in controlling unwanted microbial growth. In the present study, the lemon juice which was added into dough succeeded to lower the pH of the breads where it was between 5.81 and 4.31 on day 1 of storage.

Furthermore, the effect of SCG and SCG+LR on the microbial contamination of the whole wheat breads could be explained based on the antioxidant capacity these ingredients exhibited in the final breads (Figure 5.1).The relationship between antioxidant capacity and antimicrobial activity was reported in other works where it was found that carnosic acid as the major component of total phenolic content (promising compounds for increasing antioxidant capacity) showed activity against all bacteria tested similar to that detected for pure carnosic acid, that rosemary extract formulations efficiently inhibited the growth of gram-positive bacteria (Klancnik et al., 2009). Also Moreno et al. (2006) reported that carnosic acid and rosmarinic acid were the main bioactive antimicrobial compounds present in the rosemary extracts. It is in this regard breads incorporated with SCG+LR (had the highest overall antioxidant capacity, Figure 5.1), followed by breads with SCG showed lower TVC and TMY than W breads (had the lowest overall antioxidant capacity, Figure 5.1).

6.4. Conclusion

pH, water activity, moisture content decreased with increasing storage time, respectively, regardless of the wheat variety, ingredients and fermentation time. Total viable count (TVC) and total molds and yeasts (TMY) significantly increased with the storage time as well. W: SCG+LR bread (60 min fermentation) from Nyaruka variety and W: SCG bread (120 min fermentation) from Gihundo variety had the lowest and the highest TVC, respectively, while W: SCG+LR bread (60 min fermentation) from Kibatsi variety and W: SCG bread (120 min fermentation) from Reberaho wheat variety showed the lowest and highest TMY, respectively, on day 7 of storage. TVC for all breads up to 5^{th} and on 7^{th} day of storage was within the satisfactory and borderline ranges, respectively.

CHAPTER SEVEN: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1. Discussion

Whole flours from all selected Rwandan wheat varieties could possibly be categorized as all-purpose since their protein contents were between 9% and 12%. It is important to select grains or flour from the wheat varieties based on the individual cultivar because their derivative products could have a more desired quality. The results showed that all wheat grains were possibly hard as their results were below 4 g (40 %).

The W: SCG+LR breads from the dough fermented for 120 min were lighter than those fermented for 60 min ($p \leq 0.05$), possibly due to long fermentation of dough with low pH that was enhanced by lemon juice addition. The latter could have lowered the pH to the optimum for yeast's activity to release more gas. Bread firmness indicates its mechanical strength and deformation behavior which in turn influence consumers' perception of texture. When fermentation duration was prolonged from 60 to 120 min, the resilience of control bread decreased with the supplementation of whole wheat flour with SCG and increased with SCG+LR incorporation ($p \leq 0.05$). Resilience is correlated with the extent of staling. Whole wheat breads obtained, satisfied consumers' preferences on texture profile, color and sensory attributes.

The results showed there was an increase in fructose and glucose from dough to breads. This is to mean that formation of new fructose and glucose happened in the bread during baking. The content of glucosaccharides that could not be degraded by amylolytic enzymes was significantly higher in crust than in crumb, indicating occurrence of reversion and/or transglycosidation reactions among the oligosaccharides originally present in flour or dough and those produced by thermal starch depolymerisation. Therefore, these sugars are not necessary the limiting factors as asparagine for the acrylamide formation in yeast leavened whole wheat breads. Addition of spent coffee grounds and juices of lemon fruits and rosemary leaves in dough helped reduce significantly the acrylamide formation in breads, possibly due to high antioxidant capacity that these ingredients juices showed in comparison to that whole wheat flour. pH for W and W: SCG breads were not significantly different and were lower than W: SCG+LR breads. It was obvious that L which showed the lowest pH could lower subsequently the pH of the resultant breads. The production of acidic volatile

compounds acids resulting from aldehyde oxidation generally continues after the baking process, which clearly explains the gradual lowering of pH of whole bread during storage time.

TVC for all breads up to 5^{th} and on 7^{th} day of storage is within the satisfactory and borderline ranges, respectively. SCG or SCG+LR may have acted as antimicrobial agents against TVC and TMY in breads as it was often revealed in some previous experiments to possess diverse biological activities, including antioxidant and antimicrobial activity due to their non- nutrient secondary metabolites such as the phenolic diterpenes, carnosol, carnosic acid, methyl carnosate, rosmanol, and epirosmanol, and phenolic acids such as ferulic, rosmarinic, and chlorogenic and caffeic acids. The results showed that to be more efficient, SCG or SCG+LR should be used with the control of pH and aW.

7.2. Conclusions

The proximate composition is different among whole wheat flours, except protein content. The flours from all varieties do not differ in antioxidant capacity. Whole wheat flour from Gihundo and Reberaho varieties have the highest and the lowest dietary fiber contents, respectively.

Gihundo and Nyaruka varieties give whole wheat flours with the highest and the lowest oil absorption capacity, respectively. Gihundo and Nyaruka varieties produce whole wheat flours with long dough development time and short dough stability time, respectively. Gihundo variety followed by Kibatsi variety produce whole wheat flours with high L*values in comparison to Nyaruka and Reberaho varieties.

The W: SCG+LR breads from the dough fermented for 120 min were lighter than those fermented for 60 min. The W: SCG+LR breads from doughs fermented for 120 min scores higher in general acceptability. Fructose and glucose contents increase after baking. SCG and SCG+LR doughs show higher antioxidant capacity than control dough. Addition of SCG+LR in dough fermented for 60 min reduces significantly the acrylamide formation in whole breads.

pH, aW and moisture content decreased with increasing storage time. Breads containing SCG followed by breads mixed with SCG+LR are higher in moisture content and aW than control

breads during storage. Total viable count (TVC) and total molds and yeasts (TMY) significantly increase with the storage time as well. TVC for all breads up to 5th and on 7th day of storage is within the satisfactory and borderline ranges, respectively.

7.3. Recommendations

Bakeries should be trained on using natural ingredients such as spent coffee grounds, juices of rosemary leaves and lemon fruits in attempt to improve sensory attributes and shelf stability of of whole wheat breads.

Wheat farmers and companies in general should be about checking the physicochemical characteristics of wheat varities and hence ultimately enabling them to easily decide on which variety to choose for processing.

As the data for acrylamide content in foods in African countries are limited, reseaches at national level need to be done on acrylamide occurence and its daily intake from various foods by consumers. Acrylamide was reported to be a possible carcinogen, and can cause neurotoxicity, reproductive and developmental toxicity effects to humans.

Further studies should be carried out to explore the effects of spent coffee grounds, juice of rosemary leaves, juice of lemon fruit on other quality aspects of whole wheat bread not covered in the present study.

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Appendix

Annex 1. Sensory evaluation score sheet

Date of evaluation:……

Product evaluated:……

Name of evaluator:……..

Please evaluate the food samples provided and indicate the degree of your liking for color, flavor, texture, appearance and overall acceptability. Please do not communicate or consult with anyone while scoring.

Use the numerical scores from the scoring card provided. Enter your score under the sample in the scoring sheet.

7-point Hedonic scale

Quality	**Score**
Dislike very much	1
Dislike	2
Dislike slightly	3
Fair	4
Like slightly	5
Like	6
Like very much	7

The scoring card

Sample code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Color										
Flavor										
Texture										
Appearance										
General acceptability										

Comments:……

……..