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DEVELOPMENT AND EVALUATION OF BISCUITS/COOKIES FROM SPECIAL WHOLEGRAIN CEREAL AND LEGUME FLOURS

by
Teresa Maria Ferreira Maia de Andrade e Castro

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Thesis presented to Escola Superior de Biotecnologia of Universidade Católica Portuguesa to fulfil the requirements of Master of Science degree in Food Engineering

by
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June 2022

RESUMO

O objetivo deste estudo baseou-se em analisar e comparar determinadas propriedades físico-químicas de farinhas de cereais, pseudocereais e leguminosas, formular receitas de bolachas com base nessas farinhas e avaliá-las a nível físico, químico e sensorial.

A farinha de trigo foi utilizada como referência, na proporção de 100:0. Farinha de trigo sarraceno, farinha de trigo integral, e farinha de centeio integral foram misturadas com farinha de trigo na proporção de 100:0, 50:50, 75:25, e 25:75. A farinha de trigo foi misturada com farinha de grão-de-bico em proporções de 100:0, 95:5, 90:10 e 85:15 e farinhas de trigo sarraceno e grão-de-bico numa proporção de 95:5, 90:10 e 85:15.

Do ponto de vista sensorial, os biscoitos com base em farinha de trigo e farinha de trigo integral numa proporção de 50:50, farinha de trigo sarraceno e farinha de grão-de-bico (90:10) na sua composição foram os mais satisfatórios.

Nutricionalmente, os biscoitos com farinha de trigo sarraceno e farinha de grão-de-bico numa proporção de 90:10 são uma escolha viável para consumidores que têm preferência por produtos sem glúten ou celíacos. Os biscoitos com farinha de trigo integral e farinha de centeio integral numa proporção de 25:75, os biscoitos com apenas farinha de centeio integral ou com apenas farinha de trigo integral na sua composição são também uma escolha desejável para os consumidores que desejam produtos com elevado teor de fibra.

Palavras-Chave: Farinhas de cereais, pseudocereais, leguminosas, bolachas, teor de fibra, produtos sem glúten

ABSTRACT

The aim of this study was to analyse and compare selected physicochemical properties of special flours from cereal, pseudocereal and legume, formulate recipe and chemically and sensorial evaluated baked biscuits based on special wholegrain cereal and legume flours.

Biscuit (white) wheat flour was used for the blank sample in the ratio of 100:0. Buckwheat flour, wholegrain wheat flour, and wholegrain rye flour were mixed with biscuit wheat flour in ratios of 100:0, 50:50, 75:25, and 25:75. Biscuit wheat flour was mixed with chickpea flour in ratios of 100:0, 95:5, 90:10 and 85:15 and buckwheat and chickpea flours in a ratio of 95:5, 90:10 and 85:15.

From the sensory point of view, the biscuits with biscuit wheat flour and wholegrain wheat flour in a ratio of 50:50, and buckwheat flour and chickpea flour (90:10) in their composition, were the most satisfying.

Nutritionally, biscuits with buckwheat flour and chickpea flour in a ratio of 90:10 are a viable choice for people with gluten-sensitive or celiac disease. Biscuits with biscuit wheat flour and wholegrain rye flour in a ratio of 25:75, biscuits with only wholegrain rye flour or with only wholegrain wheat flour in their composition are also a desirable choice for consumers who desires products with high content of fibre.

Keywords: Flours from cereal, pseudocereal, legume, biscuit, content of fibre, gluten free products

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisor, Professor Marcela Sluková, for her incredible support and dedication of my study, for her motivation, patience, and insightful feedbacks during all my work in the laboratory. She was an amazing teacher and also a friend to me.

I would like to thank my colleague, Remziye Köpük, for her co-working, and patience in help and teaching me all her knowledge about the theme of my thesis. She has been a huge mentor for me. Both of them, Professor Marcela and Remziye, made this stage of my life happen in the best way possible and provided me with a magnificent work environment.

Also, I am deeply grateful to the Department of Saccharides and Cereals at Faculty of Food and Biochemical Technology in Prague for welcoming me and believing in my value as a student.

Besides them, I would like to express my gratitude to Professor Maria João Monteiro and to Professor Cristina Silva for the support, and encouragement they gave me during my study. Thank for your concern and interest on my work.

Last but not the least, thank my father, my mother, and my two sisters, Ana, and Leonor and all of my friends for their support and for helping me achieve my professional goals and dreams. Their support was my biggest motivation to do my best and complete this study.

List of Abbreviations

BWF+BF - Biscuit Wheat Flour and Buckwheat Flour

BWF+CF - Biscuit Wheat Flour and Chickpea Flour

BF+CF - Buckwheat Flour and Chickpea Flour

BWF+WRF - Biscuit Wheat Flour and Wholegrain Rye Flour

BWF+WWF - Biscuit Wheat Flour and Wholegrain Wheat Flour

BD - Breakdown

CAGR - Compound Annual Growth Rate

SC-SCR - Sodium Carbonate

SU-SCR - Sucrose

SRC - Solvent Retention Capacity

SDF - Soluble Dietary Fibre

SB - SetBack

DW - Demineralized Water

FV - Final Viscosity

IDF - Insoluble Dietary Fibre

LA - Lactic Acid

PTe - Pasting Temperature

PTi - Peak Time

PV - Peak Viscosity

PCA - Principal Component Analysis

RS - Resistant Starch

RVA - Rapid Visco Analyser

TDF - Total Dietary Fibre

WAC - Water Absorption

List of Tables

List of Figures

TABLE OF CONTENTS

INTRODUCTION

Nowadays, consumers demand food with health benefits that meet beyond their basic nutritional needs (Tumbas Šaponjac et al., 2016). Thus, there has been an increasing demand for products with a nutritious component that provides benefits to consumers' health and well-being.

The formulation of biscuits is one of the products that has been evolving over the years and its market has been growing exponentially. Besides, demand for biscuits is increasing due to the innovative packaging, addition of ingredients, innovative technologies, and different combination of flavours.

Wholegrain cereal and legume flour are some of the ingredients that have been evaluated to integrate into the formulation of biscuits due to their beneficial properties to human health. The wholegrain flour is rich in fibre, minerals, vitamins, and phenolic compounds. Also, it has positive effects on human health such as the reduction of diabetes, obesity, and cancer (Bressiani et al., 2017; Wójtowicz et al., 2015).

Legumes, in turn, are rich in unsaturated fatty acids, have high protein content, are a source of polyphenols and are gluten-free. This last characteristic makes them a viable choice for people with gluten-sensitive or has celiac disease. Also, legumes reduce the risk of suffering cardiovascular disease, type II diabetes, and metabolic syndrome and they provide benefits in terms of weight control and gastrointestinal health (Garrido-Galand et al., 2021).

Thus, the present work aims to formulate and analyze physical, chemical, and sensorial, biscuits based on special wholegrain cereal and legume flours.

THEORY

Sustainability

The consumption of animal-origin products has increased in the five decades and this fact has consequences for the environment, human health, and animal welfare (Bonnet et al., 2020). According to Food and Agriculture Organization (FAO, 2017) it is expected the global population will reach nine billion and if this number of animal-origin products keep growing it would be environmental unsustainable since the production would need to rise by two hundred million tonnes and the global greenhouse gas emissions from agriculture will also increase (Bonnet et al., 2020).

Based on the fact that population continue to increase, overproduction of food culminates in food waste, a billion people worldwide are obese and chronically malnourished (Aggarwal et al., 2016; Alongi et al., 2019), and based on the fact that red and processed meat consumption is associated with all-cause mortality like colorectal, pancreatic, and prostate cancer is extremely necessary to search for alternatives and sustainable sources of protein to ensure an adequate protein intake to world population (Garrido-Galand et al., 2021).

Therefore, in this context, encouraging the production and consumption of plant-based food, like cereals, legumes and seeds is an effective way to be more sustainable concerning protein intake. Most foods, in the baking industry, made from wheat and other cereals are poor sources of protein, often resulting in poor nutritional quality. So, the integration of wholegrain cereal and legume flour in the formulation of biscuits can be an interesting alternative to the existing ones in the market, using sustainable ingredients with high nutritional value.

Trends and consumers' demand for biscuits

Nowadays, food "ready to go" is one of the most recent ongoing trends. According to the Market Intelligence Report for Biscuits and a research report published by Market Research Future, the biscuits' market is a sector that has been growing exponentially reaching a significant valuation by the end of the foresight period of 2018-2023. The global biscuits market expects to reach 126 billion euros by 2023, at a compound annual growth rate (CAGR) of 5% (GlobeNewswire, 2019; Wollongong University, n.d.).

Furthermore, consumers' demand food that meets more than only basic nutritional needs. Thus, there has been a growing demand for fortification of products with a healthy and nutritious component that provides health and wellness benefits to consumers (Njike et al., 2016).

Biscuits are one of the products that have been developed and optimized regarding their nutritional component as gluten-free, low sugar, and high content of dietary fibre and protein. According to the Verified Market Research Report, the high-fibre biscuit market is growing at a significantly fast rate over the past few years, and it is estimated that the market will continue to grow between 2021 and 2028 (GlobeNewswire, 2019; Wollongong University, n.d.).

Biscuits

Definition of biscuits

Biscuits have been made for hundreds of years. The origin of this name is from the Latin – *bis coctus* – which means twice-cooked and refers to products that were baked and then dried in a slow oven (Davidson, 2019).

Biscuits are consumed by all age groups in many countries since they are ready-to-eat, affordable, and convenient as they can be eaten right out of the package (Milkesa, 2020). Additionally, a long self-life, and the nutritional component support their consumption (Davidson, 2019).

History of biscuits

In the past, snack food, more concretely biscuits, often had an unhealthy reputation since they usually consisted only by flour, sugar, and fat. However, consumers' trends and demands are changing. They are more conscious of their health and are turning to products with functional claims such as being source or high in protein and fibre, low in sugars and in fat (Biscuit people, 2021).

Categories of biscuits

Biscuits can be subdivided into four categories – crackers, cookies, semi-sweet, and hard sweet biscuits.

Crackers are characterized by having low-fat levels, usually with no added sugar, and a high-water level, 15-25%. Also, they have low moisture content (2.5%), and their gluten is well or fully developed. The semi-sweet biscuits have attractive colour and good texture and volume. Their doughs have low fat and sugar, and their water content is around 12%. Also, they are baked to low moisture contents (3.0%), and they have developed and strong gluten that provides an elastic dough. The short doughs, as the name implies, have short doughs with low water content but higher sugar and fat contents than the semi-sweet biscuits and crackers (Zydenbos & Humphrey- Taylor, 2016). Cookies or short dough biscuits contain elevated levels of fat and sugar, and low recipe water levels (Cauvain, 2015; Davidson, 2019).

Composition of biscuits

Biscuits are made with different ingredients some to elevate the flavour and texture, but their main ingredients are flour, fat, and sugar.

Wheat flour

Wheat flour is the principal ingredient of biscuits which is composed of protein, fat, carbohydrate, fibre, vitamins, and minerals. The amount of protein (mainly gluten) determines the flour strength. When the dough is baked, if it is composed by weak flour with low-protein content produces a soft and tender cookie, and if it composed by strong flour with high protein content produces crackers and hard biscuits.

Wheat flour contains fractions of storage proteins (gliadins and glutenins) which in the presence of water combine to form gluten. As the dough is mixed, the gluten forms an elastic network during kneading which gives the dough strength and elasticity.

Wheat flour constituents

The most abundant component of wheat flour is starch (70-75% in dry matter) which represents almost all of the carbohydrate content, and it is a polysaccharide made up of glucose units linked together with amylose and amylopectin to form long chains. Amylopectin consists of chains of α-(1,4)-linked glucopyranosyl residues joined through α-(1,6)-linkages and amylose is the principal starch molecule in wheat flour, built α-(1,4)-linked glucopyranosyl units. Regarding the minor components, wheat starch is composed of proteins (<0.6%) and fats (0.8-1.2%), present on the surface and inside the granule (Manley, 2001).

Besides the starch, wheat flour is rich in proteins (8-11% in dry matter). Proteins can be divided based on their properties into gluten and non-gluten proteins. The non-gluten proteins (15-20%) contain mainly monomeric proteins - globulins and albumins. The gluten proteins are approximately 80-85% of total wheat protein mainly in the endosperm of wheat grain. Gluten proteins have elevated levels of proline and glutamine, 14% and 35% respectively and they can be divided into glutenin and gliadin. Also, wheat flour has non-starch polysaccharides which generally refer to arabinoxylan, cellulose, β-glucan, hemicelluloses, fructans, and arabinogalactan peptides. It contains small levels of lipids (2%), mono-, di-, and oligosaccharides such as fructose, glucose, sucrose, glucofructans, and raffinose. Wheat is also a reliable source of niacin, thiamine (B_1), pyridoxine (B_6), riboflavin (B_2), pantothenic acid, tocopherol, zinc, and iron (Manley, 2001).

Grain morphology of the internal structure

The caryopsis or commonly known as kernel or grain consists of a pericarp and a seed which is composed of the embryo or germ, endosperm, nucellar epidermis, and seed coat (testa).

The different tissues constituting the wheat grain, normally, are described based on their embryogenic origin and structure (Barron et al., 2007). The peripheral tissues of the grain are made up of the outer pericarp, the inner pericarp, seed coat (testa), hyaline layer, and the aleurone layer (Barron et al., 2007).

Pericarp

The pericarp involves the whole seed, 5% of the grain, and protects it from mechanical damage by being composed of several layers – the outer pericarp and inner pericarp. It contains high proportions of cellulose and lignin. Also, it is composed of 0.5% fat, 6% protein, and 2% mineral and the remaining components are non-starch polysaccharides. The outer pericarp has the "epidermis," the "hypodermis" and "remnants of thin-walled cells," the innermost layer. The inner pericarp is composed of intermediate cells, cross cells, and tube cells (Grundas & Wrigley, 2015).

Seed coat and nucellar epidermis

The seed coat is a sub-surface outer layer consisting of lignin, and hemicelluloses. It is also composed, to a lesser extent, of minerals, vitamins, pigments, alkylresorcinols, and phenolic compounds. The seed coat consists of three layers (5 to 8µm of thickness): a thick outer cuticle, a layer that contains pigment, and a thin inner cuticle. All the seed coat is firmly joined to the tube cells on their distal side and the nucellar epidermis (7µm of thickness) on its proximal side (inner). The nucellar epidermis is tightly bound to both the seed coat and the aleurone layer (Grundas & Wrigley, 2015).

Endosperm

The endosperm represents 80-85% of the wheat grain. It is formed by the outer aleurone layer and the starchy endosperm. It is composed of starch (70-80%) and 10-20% of storage proteins (general prolamins and glutelins, in wheat gliadins and glutenins).

Also, the endosperm is composed of distinct types of cells – “peripheral,” “prismatic,” and “central.” Its cell walls are composed of hemicelluloses, especially pentosans, and β-glucans (Grundas & Wrigley, 2015).

The aleurone layer

The aleurone layer (outer endosperm) surrounds all kernel, covering both the starchy endosperm and the germ. The aleurone layer is rich in nutrients and other nutritionally important substances such as minerals and contains one-third of the grain’s thiamine content.

Germ or embryo

The germ represents 2.5-3.5% of the kernel and it is composed of the embryonic axis and scutellum which its main function is storage. It is high in protein, dietary fibre, sugar (mainly sucrose and raffinose), lipids, minerals, B vitamin, and E vitamin. However, it is poor storage stability, due to a large number of fats with unsaturated fatty acids as well as the presence of hydrolytic and oxidative enzymes (lipase or lipoxygenase), which makes germ highly susceptible to rancidity (Zhou et al., 2014).

Wheat milling

Wheat milling technology

The wheat milling is a mechanical and continuous process. This process aims to separate endosperm, and recover its maximum quantity, without contamination by peripheral layers of the grain. It removes the endosperm from the crushed grain, separating it from the germ, coating layers, and scutellum. The result of this process depends on differences in mechanical and rheological properties of the grain components and different grains may be blended before milling to ensure the production of a uniform product (“gristing”) (Bechtel et al., 2009; McKevith, 2004). The equipment parameters such as the roll speed, roll fluting, and roll speed differential also has an impact on the result of the process (Dal-Pastro et al., 2016).

The milling starts with cleaning and scouring wheat grains to separate and remove non-wheat material and coarse impurities. After this, before wheat enters the mill, water is added for a few hours (24-36 h) to increase the moisture content of the grain to 14-15%, a_w 0.68-0.70 – “tempering”. This step is particularly important as it increases the plasticity of the outer layer of the grain, preventing it from fracturing during milling and ensuring its easier separation from the endosperm (Dal-Pastro et al., 2016). If heat is also applied during tempering, then the process is referred "conditioning" (hydrothermal treatment of grain) (McKevith, 2004).

The process can be divided into three parts: breakage, purifying, and reduction. During the first stage, using fluted roller mills, grain is broken and undergoes a sequence of reduction, grinding, and sifting operations separates the endosperm from outer grain layers. Outer grain layers of the grain are removed by sieving and the inner endosperm fractions are milled to produce coarse flour and then smooth or plain flour (Berghofer et al., 2003; Dal-Pastro et al., 2016).

The purifier aims to classify the endosperm, coarse flour, and bran products based on the density, shape, and surface area of their particles. Finally, the last stage – the reduction aims to reduce endosperm into flour, using rolls with a smoothed surface (Berghofer et al., 2003; Dal-Pastro et al., 2016).

Through this process, white flour is obtained when the extraction rate is 75% or less. If this percentage exceeds 80% the flour will contain coating layers, and if the flour extraction approaches 100%, obtains wholegrain flour (Aprodu et al., 2010).

Technical consequences of milling

During milling occurs several technological changes that can cause starch damage at different structural levels, including the fragmentation or rupture of granules, degradation of starch molecules, and disruption of crystalline structure (Yu et al., 2015). Mechanical changes to the starch can occur and therefore it increases the level of enzyme activity. Also, during grinding, temperatures can reach as high as 50-60ºC or even around 80ºC between the rollers, which can denature the proteins. This fact leads to a lower wet gluten yield, and it can decrease the water absorption capacity of the flour.

After milling, under normal conditions of temperature and humidity, flour is stored or aged. This step can beneficially affect the quality of the flour since the flour will change to white colour and it will develop better baking properties such as the gluten quality improves and its extensibility decrease (McKevith, 2004).

Nutritional consequences of milling

Fractionation of the grain during milling is important from a nutrition perspective. The final nutrient content depends on the extent to which the layers are removed during processing since the fibre, minerals, and vitamins tend to be concentrated in the outer bran and aleurone layer of the grain. The proportion of vitamins in the final flour would be lower if the grain is too processed. In addition, milling may decrease some of the bioactive substances such as phenolic compounds that are found in cereals.

However, starch and protein are less affected during the process as they are concentrated in the endosperm of the grain. Some of the lipids, which are mainly present in the grain and bran, are distributed during milling into other fractions and refined flours also have a higher glycemic index than wholegrain products (McKevith, 2004).

Plant-based food

Biscuits are traditionally made by using white wheat flour. However, this type of flour reduces the fibre content and the nutritional density of the final product and has lower amount of protein. Enrichment of cereal flour with legumes and wholegrain cereal improves the nutritional quality of the products since they are a reliable source of protein, carbohydrates, vitamins, and non-nutrients such as dietary fibre, some amino acids, and antioxidants (Binou et al., 2020).

Plant-based food ingestion has many positive environmental aspects such as reducing greenhouse gas emissions, increasing system productivity by helping diversify crop rotations, and restoring soil nitrogen without using fertilizers (Garrido-Galand et al., 2021). It exhibits a good nutritional profile, since it has phenolic compounds with antioxidant properties and dietary fibre. Plant based food also have some antinutritional components such as phytic acid. Phytic acid structures allow chelating with cations (magnesium, calcium, copper, iron, and potassium) to form insoluble salts – phytates (*myo*-inositol-1,2,3,4,5,6-hexakisphosphates). Due to the lack of enzyme phytase, mineral absorption is affected, forms complexes with proteins that alter their structure and decrease protein solubility, proteolytic digestibility, and enzymatic activity. It also reduces the blood glucose response (glycemic index of the product). However, the consumption of phytates also has some positive aspects on human health. It has an anticarcinogen effect, regulates insulin secretion, and prevents heart diseases, and renal stone development. Also, phytate has antioxidant properties. It can chelate free iron and inhibits iron-driven hydroxyl radical formation and suppress lipid peroxidation (Kumar et al., 2010; Madsen & Brinch-Pedersen, 2015).

Dietary fibre

According to *Codex*, dietary fibre can be defined as the union of three or more monomer units of carbohydrate polymers that are not hydrolysed by endogenous enzymes in the small intestine of humans (Zydenbos & Humphrey-Taylor, 2016).

Dietary fibre includes carbohydrates analogous to lignin, synthesized carbohydrate compounds, and resistant starches. It also includes non-starch polysaccharides and resistant oligosaccharides (Zydenbos & Humphrey-Taylor, 2016). The main polysaccharides present in the fibre fraction are arabinoxylan (65%) and β-glucans (29%) while the percentage of cellulose is much lower (Brouns et al., 2012; Zhang et al., 2018).

Dietary fibre can reduce many health hazards such as type II diabetes, gastrointestinal disorders, cardiovascular complications, and certain types of cancer. Its consumption also increases satiety and accelerates intestinal peristalsis resulting in the elimination of intestinal toxins and body weight management (Binou et al., 2020; Lin et al., 2019; Tosh & Yada, 2010).

Wholegrain wheat flour

Wholegrain flour is an important source of dietary fibre. AACCI defined it as intact caryopsis whose main components – starchy endosperm, germ, and bran – are present in the same relative proportions (AACCI, 2001). This fact implies that wholegrain wheat flour has more fibres, minerals, vitamins (particularly B group, vitamin E), and phenolic compounds than refined wheat flour.

Furthermore, wholegrain wheat flour has many positive effects on human health such as the reduction of diabetes, obesity, and cancer. Also, it is responsible for improving the metabolism, reducing the level of bad cholesterol in the blood, improving the function of the gastrointestinal tract, affecting the human cardiovascular system, and giving a satiety filling (Bressiani et al., 2017; Wójtowicz et al., 2015). However, besides the health benefits, it can cause sensory and structural changes in food, especially coarse particles of flour can disrupt the gluten network, leading to lower consumer acceptance of bread and bakery products (Bressiani et al., 2017).

Wholegrain rye flour

Wholegrain rye, like other cereals, has a caryopsis with multiple outer layers. Rye grain morphology is formed by 86.5% of endosperm, 10% of coating layers, and 3.5% of germ (Aman et al., 2010).

In the milling process, the bran and germ are separated from the endosperm, which is milled into flour. The main constituent of the composition of the rye grain is carbohydrate (non-starch polysaccharides and starch). It contains, compared to wheat and other cereal, more fibre, β-glucans and pentosans (arabinoxylans and arabinogalactans), which improve peristalsis and helps to reduce blood cholesterol levels and aid diabetes treatment, and colon cancers (Czubaszek et al., 2021a). The vitamin and mineral content and composition of rye are similar to the other cereal grains. Wholegrain rye flour contains a significant amount of vitamin E, fytosterols, riboflavin, thiamine, and α-tocopherol.

Buckwheat flour

Buckwheat is a pseudocereal and it contains a high percentage of protein (with dominance of albumins and globulins protein fractions, and minor content of prolamins and glutelins), dietary fibre, vitamins (especially B group), minerals (potassium, magnesium, calcium, iron, and sodium) and other bioactive compounds such as phenylpropanoid, and phenolic compounds (polyphenols and phenolic acids). Flavonoid rutin is the major bioactive compound of buckwheat (Wet et al., 1995; Yadav & Yadav, 2016). These phenolic compounds and antioxidant activity have a positive effect on the human body and some chronic disease as diabetes, hypertension, and other cardiovascular diseases. Also, during the processing and storage, the antioxidants inhibit lipid peroxidation (Filipčev et al., 2011; Kaur et al., 2015), and can be used by people with celiac disease since it is gluten-free (Hussain & Kaul, 2018).

Regarding milling process, buckwheat flour milling is similar to wheat and rye flour, although since the buckwheat seed is triangular and has a harder coat, so is required a deeper milling to remove its skins, and its yield is 58-75%. Compared to wheat flour, buckwheat flour has the same quantity of protein and starch, but it does not have gluten. This last property of buckwheat is particularly important because is the cause that does differ in the behaviour when cooked (Yadav & Yadav, 2016).

Legumes

Legumes are composed of high protein (>19%), high carbohydrate (>60%), and low amount of fats (<6%) (Zydenbos & Humphrey-Taylor, 2016). Also, legumes contain phytic acid, tannins, lectins, and inhibitory enzymes. It is a reliable source of iron, B-complex vitamins, zinc, magnesium, calcium, contain polyphenols, and are gluten-free. This last characteristic makes them an excellent choice for people with gluten-sensitive or who have celiac disease. Also, legumes reduce the risk of suffering cardiovascular disease, type II diabetes, and metabolic syndrome and they provide benefits in terms of weight control and gastrointestinal health (Czubaszek et al., 2021b).

Chickpea flour

The seed of chickpea is composed of embryo, coat, and cotyledons, 1.5%, 15.5%, and 83% respectively. The main constituent is a carbohydrate, 63.5% of the total seed, and is mainly starch. Regarding starch, 10% are soluble sugars, including oligosaccharides that cannot be broken by human digestive enzymes such as stachyose and raffinose. 23% are proteins that are deficient in the sulfur-containing amino acids methionine and cystine. Chickpea flour is low in antinutritional factors such as proteases and amylase inhibitors (Kninghts & Hobson, 2016).

Chickpea flour has lower content of starch, elevated level of fibre, protein, polyphenols, and minerals. Also, it is a reliable source of digestibility proteins, and essential amino acid lysine, and due to the high amount of complex carbohydrates, it has low glycemic index and has dietary fibre content almost 3 times that of wheat flour. This type of flour is rich in unsaturated fatty acids, minerals, and vitamins such as thiamine, riboflavin, and β-carotene (a precursor of vitamin A) (Dhankhar et al., 2019).

Therefore, the integration of wholegrain cereal and legume flour in the formulation of biscuits seems to be an interesting option to highlight the qualities of these ingredients and, together, improve the nutritional quality of the biscuits. Thus, in the next section, several biscuit recipes will be tested, and evaluation of the biscuits will be performed.

EXPERIMENTAL PART

In this work, biscuit (white) wheat flour, wholegrain wheat flour, wholegrain rye flour, buckwheat flour, and chickpea flour were used for the formulation of different recipes of biscuits.

Flour is the main ingredient of the formulation of biscuits, and its quality contributes to final product properties. The quality of flour can be considered as a combination of performance and consistency of the dough, which is impacted by genetics, agronomics, milling, and baking (Kweon et al., 2011).

Thus, in this section, several tests were carried out based on analytical and physical methods to analyze the characteristics of the flours and predict the quality of the dough and the final product. Tests were performed for moisture, ash, protein, and content of insoluble, soluble, and total fibre and content of total and resistant starch. The flours' retention capacity and its viscosity and pasting properties were also analysed.

After analysing all the flour samples individually that will be used to characterize their physicochemical properties and also the biscuits, some flours will be combined in different proportions. Biscuit wheat flour will be used for the blank sample in the ratio of 100:0. Buckwheat flour, wholegrain wheat flour, wholegrain rye flour wheat flours will be mixed with biscuit wheat flour in ratios of 100:0, 50:50, 75:25, and 25:75. Biscuit wheat flour will be mixed with chickpea flour in ratios of 100:0, 95:5, 90:10 and 85:15 and buckwheat flour and chickpea flour in a ratio of 95:5, 90:10 and 85:15.

Flour analysis

Moisture content

The moisture content is the flour's lost weight when dried under specific conditions. These analyses were determined according to standard ICC No. 110/1.

These data were used for other tests, so this measure is an essential first step in analysing flour quality. The moisture content of the flours is particularly important to predict their shelf life and the solid's content.

Materials used:

Common laboratory equipment, aluminium dishes with lids, dryer, desiccator, analytical balance

Workflow:

Aluminium plates were pre-heated for 5 min at 130°C before being weighed. 5.0000 ± 0.0001 g of sample was weighed on the aluminium dishes, which have been previously dried. Uncover dishes with samples and their lids were placed into a dryer for 1 h at 130ºC. Following that, dishes their lids were placed in a desiccator for 20 min to cool down to room temperature, and then weighed. All weight measurements were performed with an accuracy of 0.0001 g. Two determinations were performed for each flour sample.

Formulation of results:

The moisture content of flours was calculated using the equation (1):

$$w\ (\%) = \frac{m_0 - m_1}{m_0} \cdot 100 \qquad (1)$$

w moisture content of sample (%)

m_0 weight of sample before drying (g)

m_1 weight of residue after drying (g)

Ash content

Ash is the non-burnable inorganic component that stays after burning flour in an oven under specific conditions. The ash content of flour indicates milling performance (flour yield) by indirectly revealing the amount of coating layer/bran contamination in flour. These analyses were determined by ICC No. 104/1.

Equipment used:

Common laboratory equipment, analytical balance, muffle furnace (VEB Elektro Bad Frankenhausen), porcelain cups, desiccator

Workflow:

0.0001 g of flour was weighed to a porcelain cup in a furnace at 900ºC (± 10ºC). The organic material initially burns quite fiercely and after the flame was extinguished, the furnace was closed, and the samples of biscuit wheat flour, wholegrain wheat flour and wholegrain rye flour were burned for 2h. The other samples, buckwheat flour and chickpea flour, needed more time in the furnace, approximately 3h and 4h respectively. After this time, the cups were removed from the furnace, allowed to cool for a few minutes on a refractory plate, and transferred to a desiccator to cool to room temperature. Two determinations were performed for each flour sample.

Formulation of results:

The ash content in dry matter x was calculated according to the following equation (2):

$$x\ (\%) = m_1 \cdot \frac{100}{m_0} \cdot \frac{100}{100 - w} \quad (2)$$

m_1 weight of residue after burning (g)

m_0 weight of sample before burning (g)

w moisture content of sample (%)

Protein content

The total amount of protein has an important influence on the baking properties of all types of flour. It was measured using the Kjeldahl method (ICC No. 105/2) with boric acid modification (AACC Method 46-12.01). The organic constituents are oxidized in the presence of a catalyst. The ammonia formed after another step is distilled and titrated.

This method measure nitrogen and not protein directly so the amount of nitrogen ascertained by titration is multiplied by a factor specified for each food. In this case, the factor for converting nitrogen to protein is 5.7.

Equipment used:

Common laboratory equipment, mineralization unit Digestor 2006, temperature control Controller 2000, distillation unit Kjeltec System 1002, mineralization tubes, Erlenmeyer flasks 250 ml, burette, analytical balance

Chemicals used:

Concentrated sulfuric acid (ρ = 1.84 $g \cdot l^{-1}$), mixed selenium catalyst, hydrochloric acid (0.1 $mol \cdot l^{-1}$), sodium hydroxide (40% *w/w*), boric acid (2% *w/w*, ρ = 2.06 $g \cdot l^{-1}$)

Workflow:

1.00 g of flour sample was weighed into a mineralization tube and then 14.5 ml of 96% sulphuric acid and two catalyst tablets were also added into the same mineralization tube.

The mineralization tube was placed in a heat block without stirring, and the mixture was allowed to mineralize until obtained a translucent yellowish solution. After that, the mixture was diluted with 75 ml of distilled water and steam distillation was performed in a Kjeltec apparatus. After four minutes, the resulting ammonia was collected in 25 ml of boric acid. The resulting solution was titrated with a solution of hydrochloric acid from green to a greyish colour.

Two determinations were made for each flour sample. An approximate value of zero millilitres was assumed for the blank sample.

Formulation of results:

The nitrogen content x_N was calculated according to the following equation (3):

$$x_N = \frac{(V - V_0) \cdot c_{(HCl)} \cdot M_N}{m_s \cdot 10} \ (\%) \qquad (3)$$

V consumption of volumetric solution of hydrochloric acid in titration of sample (ml)

V_0 consumption of volumetric solution of hydrochloric acid in titration of blank sample (ml)

m_s weight of sample (g)

$c_{(HCl)}$ hydrochloric acid concentration ($mol \cdot l^{-1}$)

$M_{(N)}$ molecular weight of nitrogen ($g \cdot mol^{-1}$)

Fibre content: Insoluble, soluble, and total dietary fibre

Total dietary fibre

According to AOAC 985.26, the total dietary fibre was determined using the enzymatic-gravimetric method.

Equipment used:

Common laboratory equipment, desiccator, analytical balance, Fibretec system filtration and washing unit, electric furnace (MLW), pH meter (inoLAB), dryer, laboratory balance, thermostatic water bath

Chemicals used:

Megazyme enzyme set, ethanol (78% *v/v*), ethanol (96% *v/v*), acetone (99.5% *v/v*), aqueous sodium hydroxide solution (5% *v/v*), MES-TRIS buffer (0.005 $mol \cdot l^{-1}$, pH 8.2 at 24ºC), hydrochloric acid c = 0.561 $mol \cdot l^{-1}$, hexane (95% *v/v*), celite (filtration aid, SiO_2)

Workflow:

0.5 g of celite was weighed into the frits and at least 30 min before the start of the fibre content measurement, the water bath was turned on to 96 ± 2°C. 1.000 ± 0.005 g of flour sample was measured in 600 ml beaker lids and then was added to the flasks with 40 ml of MES-TRIS buffer and 50 µl of α-amylase. The sample was mixed and then 50 µl of α-amylase was added and the contents of the flasks were again mixed. The contents flasks were covered with aluminium foil caps and placed in a bath at 96°C for 30 min.

After 30 min, the flasks were removed from the bath and the temperature of the bath was set to 60°C. Sample residues were transferred from the flask to the suspension and the walls were rinsed with 10 ml of distilled water. 100 µl of protease was added to all flasks and mixed. The flasks were again covered with caps and placed in a pre-heated bath at 60°C for 30 min. The flasks were removed and 5 ml of 0.561 $mol \cdot l^{-1}$ hydrochloric acid was added to each. Subsequently, the pH was measured with calibrated pH meter and adjusted to between 4.1-4.8 in each flask using a 5% hydrochloric acid solution. 200 µl of amyloglucosidase was then added to the flasks and mixed. The flasks were covered with caps and placed in a bath with shaking for 30 min.

After incubation, about 225 ml of 96% ethanol heated to 60°C was added to the flasks. The amount of ethanol added was determined using the formula: $V_{ethanol}$ = (flask with filtrate - empty flask) × 4. The flasks were left in the hood at laboratory temperature for 1 h. The frits were placed in a filter apparatus and Celite in the frits was wetted with 15 ml of 78% ethanol. Subsequently, the flasks with precipitate were placed on the filter apparatus and their walls were rinsed with 78% ethanol as needed to facilitate the transfer of adhered residues back into the solution. After filtration, the flasks were transferred to the washer where they were washed twice with 15 ml of 78% ethanol, twice with 15 ml of 96% ethanol and twice with 15 ml of acetone. After washing, the frits were removed from the apparatus and placed in an oven where they were dried at 103°C overnight. On the next day, the frits were removed from the oven, placed in a desiccator to cool, and then weighed. One determination was made for each biscuit sample.

Formulation of results:

The total fibre content was calculated according to the following equation (4):

$$Total\ dietary\ fibre = \frac{R}{m} \cdot 100\ (\%) \tag{4}$$

R residue weight from sample m (g)

m sample weight (g)

Insoluble and soluble dietary fibre

The experimental method for determining the insoluble and soluble fibre of samples is similar to the total dietary fibre. Although, after the last water bath the process is different. Thus, the frits were washed with 2 portions of 10 ml of water at 70ºC (insoluble fibre) and followed the same procedure that the measuring of total dietary fibre. The samples that were to measure the soluble fibre was previously added 4 volumes of 96% EtOH at 60ºC and then rest 1 h to precipitate. After that, the procedure was the same as the other samples.

Solvent retention capacity

The solvent retention capacity (SRC) profiling can provide information of baking and processing characteristics of different type of flours (Ram et al., 2005). It was analysed according to the AACC Method 56-11.02.

This method is based on the reaction of individual flour components with aqueous lactic acid solution (5% *w/w*), demineralized water, aqueous sodium carbonate solution (5% *w/w*), and aqueous sucrose solution (50% *w/w*). The SRC is expressed as the weight of solvent retained by the flour after centrifugation of the flour suspension with the solvents previous mention.

The retention capacity of the lactic acid solution and the sodium carbonate solution is associated with the glutenin characteristics, and starch damage, respectively. The retention capacity of the sucrose solution is determined by the arabinoxylan properties, and the water retention capacity is affected by all these substances of a hydrocolloidal nature of flour mentioned.

Equipment used:

Laboratory balance, 50 ml tube with cap (Falcon to centrifuge), shaker, centrifuge (Eppendorf 5702), common laboratory equipment

Chemicals used:

Demineralized water, aqueous sucrose solution (50% *w/w*), aqueous sodium carbonate solution (5% *w/w*), aqueous lactic acid solution (5% *w/w*)

Workflow:

First, centrifuge tubes with caps were weighed, and then 5.00 ± 0.05 g of flour and 25.00 ± 0.05 g of the solvent were weighed into each tube. The resulting mixture was mixed to form a flour suspension and then the tubes were placed in a shaker and allowed to stir for 20 min. After 20 min, the suspension was centrifuged at 2.500 rpm for 15 min.

Then, the supernatant was spilled off and the open tube was allowed to dry for 10 min. After drying, the tube with the resulting gel and the appropriate cap were weighed. Two determinations were made for each flour sample.

Formulation of results:

The retention capacities of the individual components (SRC) in each solvent were converted to 14% moisture of the flour according to the following equation (5):

$$SRC = \left(\frac{weight\ of\ gel}{weight\ of\ flour} - 1\right) \cdot \left(\frac{86}{100 - \%moisture\ content}\right) \cdot 100\ (\%) \qquad (5)$$

Water absorption

This analysis was determined using a farinograph according to AACCI Method 54-21.02. Farinograph is a rheological instrument simulating the dough's behaviour during its formation and kneading. Through this equipment, it is possible to evaluate several parameters such as stability, development time, and mixing tolerance index of the dough.

The amount of water that needs to be added to the flour to balance the farinograph curve on the 600-Farinograph units (FU) line for maximum consistency characterizes the farinograph water absorption.

Equipment used:

Farinograph Brabender with thermostat, farinograph burette, laboratory balance

Chemicals used:

Distilled water

Workflow:

The first step was to turn on the farinograph instrument. According to the moisture content of each type of flour, 300.0 ± 0.5 g of flour was weighed, and then the flour was transferred to the farinograph. The farinograph burette was filled with distilled water tempered at 30 ± 5°C, and the flour was allowed to stir for 1 min. After 1 min, distilled water was added from the burette into the farinograph bowl. An amount of water was added to achieve a 500-600 FU maximum dough consistency. Then the kneading was stopped, and the kneader was emptied and cleaned.

As many measurements as necessary were made until a farinograph curve with a maximum consistency of 500-600, depending on the mixture of the flours, FU was obtained, and the result is the amount of water added in %.

Resistant starch

These experimental analyses were performed according to Resistant Starch Assay Kit from Megazyme.

Equipment used:

Laboratory balance, tubes, vortex mixer, water bath, centrifuge, spectrophotometer

Chemicals used:

Pancreatic α-amylase (10 mg/ml), ethanol (99% v/v), 2 mol·l^{-1} KOH, 1.2 mol·l^{-1} sodium acetate buffer, GOPOD reagent

Workflow:

- Hydrolysis and solubilisation of Non-Resistant Starch

100 ± 5 mg of sample were weighed into each screw cap tube. After that, was added 4.0 ml of pancreatic α-amylase (10 mg/ml) containing amyloglucosidade (3 U/ml) to each tube. The tubes were mixed on a vortex mixer and attached them horizontally in a shaking water bath, at 37°C, for 16 h. The next step was treated the contents with 4 ml of ethanol (99% v/v) with vigorous stirring on a vortex mixer and then they were centrifuged two times (without the caps) at 1,500 g for 10 min.

- Measurement of Resistant Starch

2 ml of 2 mol·l^{-1} KOH and a magnetic stirrer bar were added to the tube and suspend the pellets by stirring for 20 min in an ice bath. Then, was added 8 ml of 1.2 mol·l^{-1}

sodium acetate buffer to each tube with stirring on the magnetic stirrer. Immediately after that, was added 0.1 ml of amyloglucosidade, mixed, and placed the tubes in a water bath at 50°C. The tubes were incubated for 30 min and then centrifuged at 1.500 g for 10 min without dilution. After that, 0.1 ml aliquots (in duplicate) of either the diluted or the undiluted supernatants was transferred into glass test tubes and was added 3.0 ml of GOPOD reagent and incubated at 50°C for 20 min. The last step was measured the absorbance of each solution at 510 nm against the reagent blank.

- Measurement of Non-Resistant (Solubilised) Starch

For this measure was combined the supernatant solutions obtained on centrifugation of the initial incubation with the supernatants obtained from the subsequent two 50% ethanol washings and adjust the volume to 100 ml with 100 mmol·l^{-1} sodium acetate buffer (pH 4.5) in a volumetric flask. 0.1 ml aliquots of this solution were incubated with 10 µl of dilute amyloglucosidade solution in 100 mmol·l^{-1}sodium maleate buffer for 20 min at 50°C. After, was added 3.0 ml of GOPOD reagent and the tubes were incubated for 20 min at 50°C. The last step was again measured the absorbance at 510 nm.

Formulation of results

For the calculation of resistant starch, non-resistant starch, and total starch content (percentage on a dry weight basis) in test samples were used in following equation (6) since the samples containing less than 10% of resistant starch:

Resistant Starch (g/100g sample) = $\triangle E \times F \times \frac{10.3}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$ (6)

$$= \triangle E \times \frac{F}{W} \times 9.27$$

Total Starch = Resistant Starch + Non-Resistant Starch

Where:

△E = absorbance read against the reagent blank

F = conversion from absorbance to micrograms

100/0.1 = volume correction

1/1000 = conversion from micrograms to milligrams

W = dry weight of sample analysed

100/W = factor as a % of sample weight

162/180 factor to convert free D-glucose to anhydro-D-glucose

10.3/0.1 = volume correction for samples containing 0-10% RS

Rapid Visco Analyser

Rapid Visco Analyser is a rotational viscometer, which under specific conditions of temperature, records the viscosity of the samples.

Equipment used:

Rapid Visco Analyser 4500, computer, canister, laboratory balance, weighing vessel, paddle, common laboratory equipment

Chemicals used:

Distilled water

Workflow:

The RVA and the computer were switched on 30 min before starting the measured to allowed to warm up. The parameters - temperature, rpm, and time - were set and after that the software showed the weight of sample which is approximately 3 g according to the moisture content of the sample. Then, 25 ± 0.1 ml of distilled water was measured into a new canister and weighted the amount of flour into a weighing vessel. It was transferred into the canister with distilled water. After that, a paddle was put into the canister and vigorously jogged the blade through the sample up and down 10 times. Then, the canister was inserted into the instrument and the measurement cycle was started. After 16 min, when the test was finished, canister was removed. Two determinations were made for each sample parallels.

Baking process

Flours and their blends were used to make biscuits based on simple baking recipe (**Table 1**).

Table 1 General recipe of the biscuits.

Ingredients	Weight (g)
Pure flour or a mixture of flours	300
Sugar	60
Rapeseed oil	50
Hydrogen carbonate	3
Salt	3
Water	Based on experimental water absorption value

<u>Instruments and materials:</u>

Farinograph, analytical and digital balance, laboratory oven, container, lid roller, former, baking tray, baking paper, filter paper, stopwatches, and common laboratory materials

<u>Workflow:</u>

The first step was turned on the farinograph, heated the water to 30ºC, and weighed all ingredients in the analytical balance. After that, the mixture of flour was put into the farinograph for 3 min to homogenize it (if it was pure flour did not need the pre-mixing). Then, hydrogen carbonate, sugar, salt, and oil were added to the middle of the mixture and homogenized for 1 min more. After that, the volume of water was added and approximately 20 min were needed to develop the dough.

Subsequently, dough was taken out of the farinograph and was transferred to a covered container. The container was put in a fermenter (temp. 30ºC, RH 95-100%) for 30 min. After that, the dough was divided into two parts, and was formed round slices of 5 cm in diameter and 0.5 cm in thickness. Then, slices were transferred to a baking tray and were put in the oven at 180 ± 5ºC for 15 min. After baking, the biscuits were rested for 10 min at room temperature. 6 biscuits were selected to measure, in pairs, their weight and volume, and then they were packed in polypropylene bags and were put in the freezer for 3 weeks to analyze them.

Sensory evaluation

Sensory evaluation of biscuits was performed by a panel of 4 semi-trained panellists. Samples were rated using a five-point hedonic scale (5 = like extremely, 1 = dislike extremely) and evaluation of the biscuits was conducted 10 min after cooling.

In order to obtain more reliable results a larger and more representative tasting panel should be used (ISO 11136:2014), but technical constraints relative to panellist recruitment and panel dimension imposed the use of the above-mentioned operating conditions.

Texture analyses

Equipment used:

Computer, texturometer Texture Analyser (Stable Micro System, UK)

Workflow:

The texturometer and the computer were switched on and the parameters – force (N), distance (mm), and time (s) - were settled. After that, the sample was inserted into the instrument and the measurement started. After 32 secs, when the test was finished, the sample was removed. Four determinations were made for each sample parallels.

RESULTS AND DISCUSSIONS

This section gives the results of the flours, doughs and biscuits analysed. The results obtained are interpreted in the context of nutritional and technological quality of the raw materials and bakery products, and results are discussed and compared with those of literature sources.

Table 2 Chemical composition, in weight percentage (w/w) and dry matter of the flour, of biscuit wheat flour, wholegrain wheat and rye flour, buckwheat flour and chickpea flour.

Samples	*Moisture Content (%)*	*Protein Content (% in dry matter)*	*Ash Content (% in dry matter)*	*SRC (%)*				*Fibre Content (% in dry matter)*			*Total Starch Content (% in dry matter)*	*RS Content (%)*	*Water Absorption (%)*
				DW	*sucrose*	*soda*	*LA*	*IDF*	*SDF*	*TDF*			
Biscuit wheat flour	11.04 ± 0.10	9.54 ± 0.04	0.54 ± 0.01	63.31 ± 0.11	92.96 ± 0.30	75.94 ± 0.52	94.27 ± 0.38	0.93 ± 0.00	2.92 ± 0.01	5.03 ± 0.00	80.98 ± 1.94	0.44 ± 0.05	58.45
Wholegrain wheat flour	9.30 ± 0.03	12.56 ± 0.00	1.48 ± 0.17	79.87 ± 0.02	104.37 ± 0.52	106.89 ± 1.05	98.06 ± 0.07	6.41 ± 0.05	2.74 ± 0.00	10.23 ± 0.00	44.66 ± 0.96	0.48 ± 0.05	71.8
Buckwheat flour	11.13 ± 0.01	8.01 ± 0.09	0.94 ± 0.01	96.12 ± 0.28	144.17 ± 5.81	98.60 ± 1.12	102.58 ± 0.68	2.97 ± 1.33	2.38 ± 0.01	5.18 ± 0.03	63.76 ± 1.77	0.37 ± 0.09	61.09
Wholegrain rye flour	8.35 ± 0.05	8.43 ± 0.03	1.31 ± 0.01	129.05 ± 1.02	106.18 ± 0.26	129.02 ± 3.25	127.01 ± 2.49	9.58 ± 0.18	2.40 ± 0.79	13.45 ± 1.10	42.31 ± 0.00	0.50 ± 0.06	71.35
Chickpea	9.01 ± 0.25	19.41 ± 0.02	2.57 ± 0.02	81.02 ± 0.01	116.60 ± 7.22	80.82 ± 0.36	80.56 ± 0.82	11.50 ± 0.03	1.29 ± 0.00	12.71 ± 0.06	40.73 ± 4.12	0.91 ± 0.12	56.08

Note.: These values are the result of the experimental analyses.

DW – demineralized water, *LA* - lactic acid, *IDF* – insoluble dietary fibre, *SDF* – soluble dietary fibre, *TDF*- total dietary fibre, *RS* – resistant starch

The data, in percentage, on the chemical properties of biscuit wheat flour, wholegrain wheat and rye flours, buckwheat flour, and chickpea flour are present in **Table 2**. The results of these analyses should be evaluated separately by legumes, pseudocereal, and cereal flours.

Moisture content

During milling, the components of the grain flour are separated (Liu et al., 2015). The milling process affects the granulation of particles in their outer layers, and it influences the technological behaviour and sensory properties of flours (Skřivan et al., 2021). Wholegrain rye flour and wholegrain wheat flour have the same milling process. These two types of flours are transformed by new milling technology, which is an impact mill with a vertical axis of rotation. With this, the temperature of the grist is lower than the conventional one, and therefore, the flour is exposed to less thermal stress and starch damage is also lower (Skřivan et al., 2021).

Moreover, the moisture content in wholegrain wheat flour (9.30±0.03%) and wholegrain rye flour (8.35±0.05%) is lower than the other flours due to the fact they are suspended in the air stream throughout the disintegration, while the granulation and residence time inside are calibrated (Skřivan et al., 2021). Therefore, the moisture content of the biscuit wheat flour and the buckwheat flour are treated by conventional technology, so they have a bigger percentage, 11.04±0.10% and 11.13±0.01% respectively. Chickpea flour, as a legume, should have low moisture content. According to the research by Hamdani et al. (2020), the moisture content of chickpea flour was 10.0 ± 0.5% which is similar to the experimental value, 9.01±0.25%.

Protein content

Regarding the protein content of the flours, chickpea flour has the highest value (19.41±0.02%). This result was expected because chickpea is a legume and based on the literature is known that legume flours have a higher content of protein than cereal and pseudocereal flours. The protein source of wholegrain wheat flour is bran and endosperm, while biscuit wheat flour is only in the endosperm. Thus, the value of protein content in wholegrain wheat flour should be bigger than in biscuit wheat flour. According to the literature, the protein content of biscuit wheat flour should be lower than 10% and the experimental value was 9.54±0.04%. The value of protein content of buckwheat is the lowest one, 8.01±0.09, and the value of wholegrain rye flour is the second lowest, 8.43±0.03%. Torbica et al. (2012) also observed similar values. Based on their study, the protein content of wholegrain rye flour was 7.71% and 12.28% of buckwheat flour. These values result from the fact that they are rich in albumin and globulin, but they have a lower content of glutelin and prolamin than wheat flour (Wet et al., 1995).

Ash content

In grains, the minerals are mainly present in the centre of the endosperm. They also are in the aleurone layer, coating layers, and an amount inside of germ. Chickpea flour, as a legume, have higher ash content (2.57±0.02%) than the other flours. The biscuit wheat flour has the lowest value (0.54±0.01%) and this result is also related to the colour of the flour. The value is a result of its minerals are only present inside the endosperm. However, some of them can be through from the other layers, like the aleurone layer. Based on the literature is expected that its value is approximately T530, which means the value of minerals (ash in the dry matter of the flour) multiplied by 100. Comparing this value with the experimental one it can be concluded that the values are similar. In case of buckwheat flour and wholegrain rye, processed seeds have a hard and more cohesive coat, so it is necessary to break the seed before milling. This fact, allow some component, such as minerals (magnesium, calcium, potassium, iron, and sodium (Wet et al., 1995), through into the flour increasing their component in minerals, 0.94±0.01%, and 1.31±0.01% respectively.

Solvent retention capacity (SRC)

SRC method specifically designed for cereal flours, specifically wheat flours (baking and biscuit flours). In the case of other types of flours, their properties and composition (gluten-free flours such as buckwheat and chickpea flours) will be considered in the SRC values interpretation.

The retention capacity of the lactic acid solution and the sodium carbonate solution is associated with the glutenin characteristics, and the degree of starch damage, respectively. The retention capacity of the sucrose solution is determined by the arabinoxylan properties (content of water-extractable pentosans), and the water retention capacity is affected by all these components of flour mentioned (Ram et al., 2005).

Distilled water reflects the ability of flour to hold water (Xiao et al., 2006). It is possible do a correlation between water (DW), water absorption and fibre content. The SCR values in water correspond to a higher farinographic absorption and fibre content. As expected, the values in water in wholegrain wheat flour and wholegrain rye flour are higher than the other flours since they have more dietary fibre, and more arabinoxylans as a part of water-soluble dietary fibre. The SRC value in DW for chickpea flour can be comparable to that of value for wholegrain wheat flour. As expected, the DW values for these two flours are similar.

The sucrose values are related to the absorption capacity of water-extractable arabinoxylan and the behaviour of flour during the mixing process for production sweet biscuits. Buckwheat flour appears to be higher in comparison with the values of biscuit wheat flour and wholegrain wheat flour, as well in wholegrain rye flour. This value is difficult to interpret since buckwheat flour, when determining SCR values, behaves differently than the other flours. The SCR value in sucrose for chickpea is between buckwheat flour and wholegrain rye flour.

The SCR sodium carbonate solution values are related to the degree of starch damage. The starch damage can occur mechanically due to pressure, crash, and forces, and biochemically by enzymatic hydrolysis. Also, it can occur thermally, and chemically by acid hydrolysis. The damage starch value in wholegrain wheat flour and wholegrain rye flour should be lower since their milling process is less aggressive than the other flours and their starch granules should not be damaged. A probable reason for that is SCR sodium carbonate is related to the damaged starch but also with fractions of protein soluble in alkali solution and the fact that wholegrain flour could contain higher content if arabinoxylan from aleurone layer. According to Xiao et al. (2006), sodium carbonate is sensitive to the swelling of pentosans (arabinoxylan) in flour. On other hand, biscuit wheat flour has the lowest value of SRC in soda (75.94%) which is desirable because of the reduced browning of the surface of the biscuit during baking (damaged starch degrades more quickly into glucose, a reducing agent that readily enters the Maillard reaction).

The lactic acid values are related to the quality of glutenin (a gluten faction) and that reflects the strength of the wheat dough. Wholegrain wheat and wholegrain rye flour have a high percentage of protein in their composition, so these experimental values were expected. Buckwheat value in lactic acid should be lower because is gluten-free so it should not react with the lactic acid solution. In this case, protein fractions capable of dissolving in acidic solution were involved in the interaction and this value could be also a result of the interaction and the contribution of hydrocolloids and other components.

Fibre content

Dietary fibre (DF) is mainly present in aleurone and coating layers of the grain and its percentage present in flours depends on cereal variety and milling process (Benítez et al., 2018).

Wholegrain flour is a reliable source of dietary fibre. Both wholegrain wheat and rye flours have a high percentage of dietary fibre in their composition. The value of total dietary fibre is a sum of soluble and insoluble fibre and other components in minor amounts. Based on the experimental values, chickpea has the highest value in insoluble fibre, 11.50±0.03% and biscuit wheat flour the lowest, 0.93±0.00%. Wholegrain rye flour and chickpea flour, also have a significant amount of DF, 13.45±1.10, 12.71±0.06%, respectively.

Starch content

Enzymatic methods used to quantify the resistant and total starch are applied to wheat flours. The other flours granulation is different to the wheat flours and maybe this fact implied they react differently during the process.

Legume flours, based on literature, have high content of resistant starch. Chickpea starch has higher amylose and resistant starch, which is responsible for lower glycemic index of the flour and legume-based products (Lu et al., 2022). Wholegrain flours also have a significant amount of resistant starch due to their grain composition. Buckwheat, as a pseudocereal has the lowest value of resistant starch, 0.37%.

Water Absorption

Among dough rheology methods, farinograph is one of the rheology methods that is typically used to obtain information on a flour's water absorption behaviour and on the mixing time of a dough, as related to wheat gluten development.

Flour for biscuits production generally requires low damaged starch, minimal gluten strength, low water absorption and arabinoxylans. Damaged starch generated during flour milling, soluble and insoluble arabinoxylan influences the water absorption. Both significantly increase the water-holding capacity of flour which is a characteristic of excellent quality biscuit flours (Kweon et al., 2011).

The analyses of water absorption are used to apply to wheat flours, so the other types of flours are simulated. The experimental value of farinographic water absorption of the biscuit wheat flour was 58.45% and the theoretical one, according to the literature, is 59%. The values in water absorption in wholegrain wheat flour and wholegrain rye flour are higher than the other flours, 71.80%, and 71.35% respectively, since they have more dietary fibre, and more arabinoxylans and their presence increased water absorption. According to the study by Kaur & Singh (2005), the water absorption of chickpea should be higher due to it has more hydrophilic constituents such as polysaccharides.

Viscosity behaviour of starch

The starch pasting properties of flour, as measured by the RVA, are shown in **Figure 1** and **Table 2**. The results of this method provide a relative measure of starch gelatinization, swelling, and gelling ability (Brennan & Samyue, 2004).

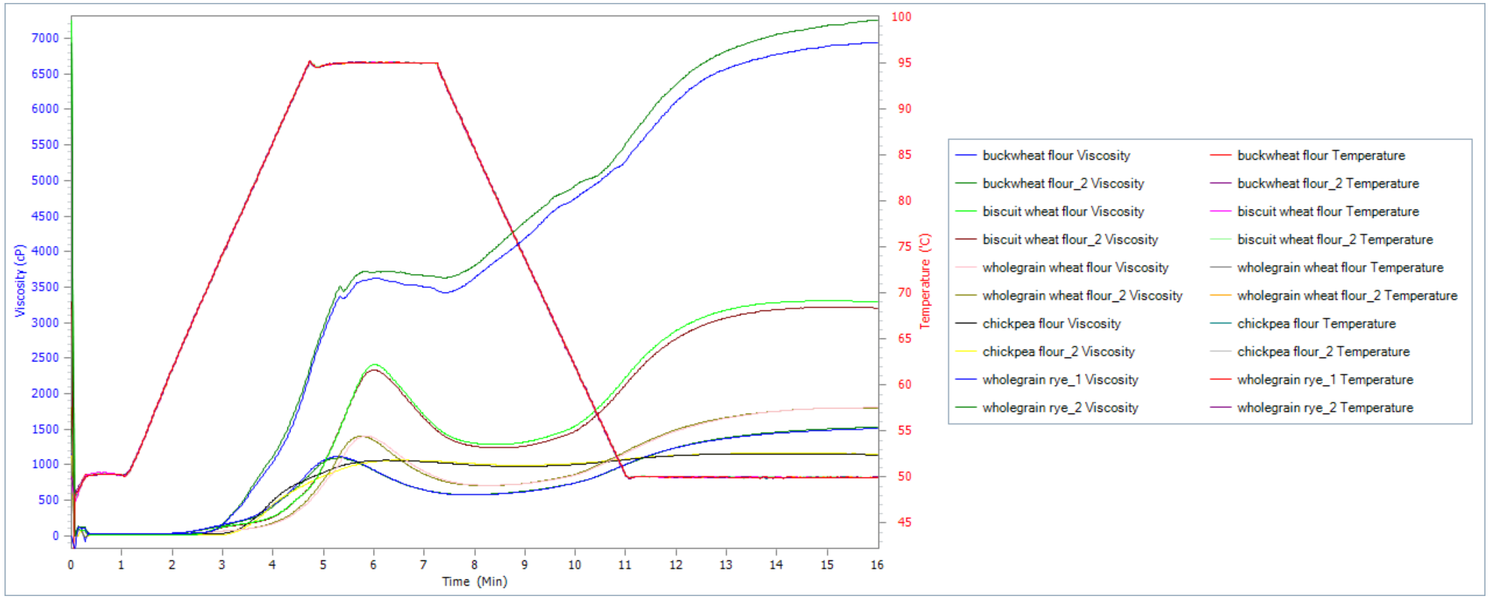


Figure 1 RVA pasting curves of cereal (biscuit wheat flour, wholegrain wheat, and rye flours), pseudocereal (buckwheat flour) and legume flours (chickpea flour).

The shape of the pasting curve differed depending on type of flour, time and temperature, and their profile reflects the interaction of the water and the starch (Ragaee & Abdel-Aal, 2006). Among the cereals, pseudocereal and legume flour, significant differences were observed and will be analysed separately. Two determinations were made for each sample parallels to obtain reliable results. In this graph (**Figure 1**) is possible to see all sample pasting curves together.

This graph (**Figure 1**) is divided into 5 distinct stages: the addition of water to the flour sample, heating, holding at a maximum temperature, cooling, and a final holding stage.

The initial stage, during the first minutes with temperatures below 50ºC, occurs mixing and hydration of the sample with distilled water.

The following phase, which resembles the cooking cycle, is heating. During this period, the viscosity increases due to the gelatinisation phenomenon. It happens when, in the presence of water, starch is heated causing its expansion and rupture. Amylose and amylopectin then leak out of granules generating a paste.

The holding stage (setback), which follows, starts to reduce viscosity at maximum temperature and enables a quick flow of water into the granule. As a result, there is less water available, and the grains begin to crash into one another.

The holding stage is followed by the cooling stage. During this stage, the viscosity increases again and retrogradation occurs. The amylopectin and amylose chains realign themselves, especially amylopectin chains because amylose recrystallize during 2 hours after cooling whereby amylopectin recrystallize for several days, to form a more crystalline structure.

The final holding period is the last stage where the temperature remains constant while the viscosity continues to increase until it stabilizes. After reaching the peak viscosity, due to disintegration of the granules, the viscosity of the starch paste reduces quickly (Balet et al., 2019).

Table 3 Pasting behaviour measured using Rapid Visco Analyser (RVA) by biscuit wheat flour, buckwheat flour, wholegrain wheat and rye flours, and chickpea flour.

	PTe (ºC)	PTi (min)	PV (cp)	Hold Time	Hold Temp	Hold Visc	BD (cp)	FV (cp)	SB (cp)
Buckwheat flour	71.75±0.0	6.17±0.1	3674±66.5	7.40±0.0	93.03±15.6	3522.5±84.1	151.5±21.5	7095.0±64.3	3572.5±0.0
Biscuit wheat flour	84.70±0.0	6.00±0.0	2368±55.2	8.47±0.2	80.10±15.4	1262.0±19.8	1106.0±58.7	3247.5±23.3	1985.5±0.0
Wholegrain wheat flour	86.78±0.6	5.80±0.1	1403±3.5	8.27±0.1	82.43±2.1	709.5±1.44	693.0±2.8	1801.0±0.7	1091.5±0.1
Chickpea flour	77.10±0.5	6.30±0.1	1063±0.7	8.80±0.1	76.10±14.1	983.0±13.4	79.5±9.2	1145.5±4.9	162.5±0.2
Wholegrain rye flour	78.63±2.8	5.30±0.0	1106±10.6	7.87±0.0	87.25±4.2	576.0±14.8	529.5±14.1	1516.0±9.9	940.0±0.0

PTe = pasting temperature; PTi = peak time; PV = peak viscosity; BD = breakdown; FV = final viscosity; SB = setback

Note.: cp = mPa · s

The RVA pasting properties of buckwheat, biscuit wheat flour, wholegrain wheat and rye flours and chickpea flour are present in **Table 3**. In all samples were not found significant differences in pasting temperatures. Wholegrain wheat flour has the highest value of pasting temperature and buckwheat has the lowest value. Regarding peak time, no significant differences were observed. These values are related to the viscosity so wholegrain rye flour has the lowest value for peak time and peak viscosity.

Chickpea flour has the lowest values along the heating-cooling cycle (peak, breakdown, and setback viscosities) and biscuit wheat flour has the highest values since biscuit wheat flour has the higher content of starch (80.98 ± 1.94%). Wholegrain wheat flour and wholegrain rye flour are rich in fibre content, and it is known, based on the literature, that fibre, especially insoluble fibre, can reduce viscosity and increases water absorption in an aqueous suspension of flour. Gómez et al. (2010) also observed the same conclusions regarding biscuit wheat flour and wholegrain wheat and rye floursFLAG.

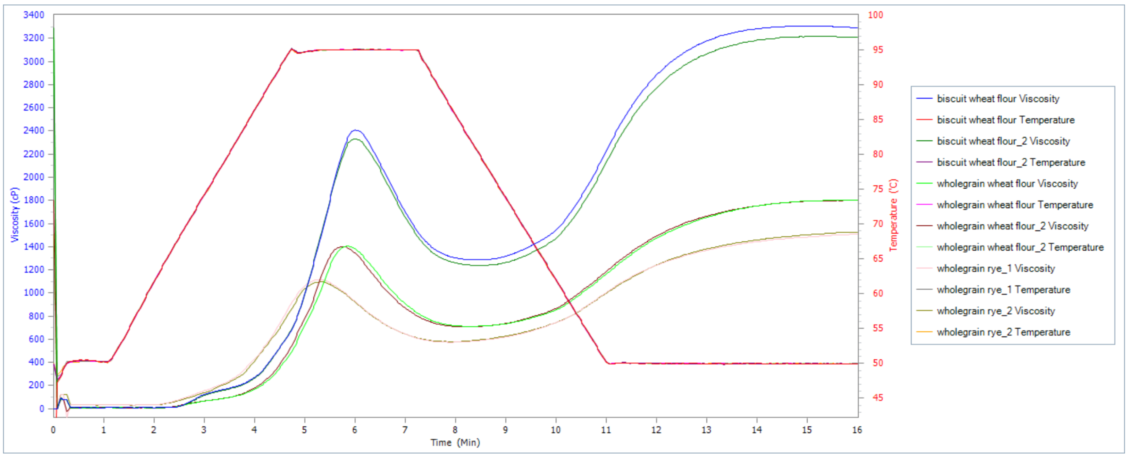


Figure 2 RVA pasting curves of cereal - biscuit wheat flour, wholegrain wheat, and rye flours.

This graph (**Figure 2**) shows the behaviour of cereal flours - biscuit wheat flour and wholegrain wheat and rye flours. In RVA pasting curves of wheat flours, a small peak often occurs before the main pasting peak which is the result of damaged starch present in the flour. Damaged starch granules usually allow faster penetration by water, and these granules absorb more water and start to swell before undamaged granules.

The pasting temperature of wholegrain wheat flour is higher than wholegrain rye flour and biscuit wheat flour which means wholegrain wheat flour needs a higher temperature to cook a given sample and it spends more energy costs. The value of peak viscosity of biscuit wheat flour is bigger than the others (2368±55.2 cp). The hold temperature is similar in all samples and the hold viscosity in wholegrain flour is lower than the value of biscuit wheat flour (1262±19.8 cp).

Thus, wheat flour, when mixed with water and during kneading, forms a three-dimensional viscoelastic matrix of the dough (Torbica et al., 2010). Wholegrain flours contain parts of the bran and parts of the coating layers in their composition and some of its constituents such as arabinogalactans, arabinoxylans, β-glucans, phytates and lipids can interact with the gluten network, delay its formation, and reduce gelling process. These structural changes decrease the elasticity and stability of the dough and reduce the gelling ability, weakening the baking quality (Bucsella et al., 2016). When added water to wholegrain rye flour, rich in fibre, soluble fibres do not dissolve immediately, which delays the achieving of viscosity (Villemejane et al., 2016).

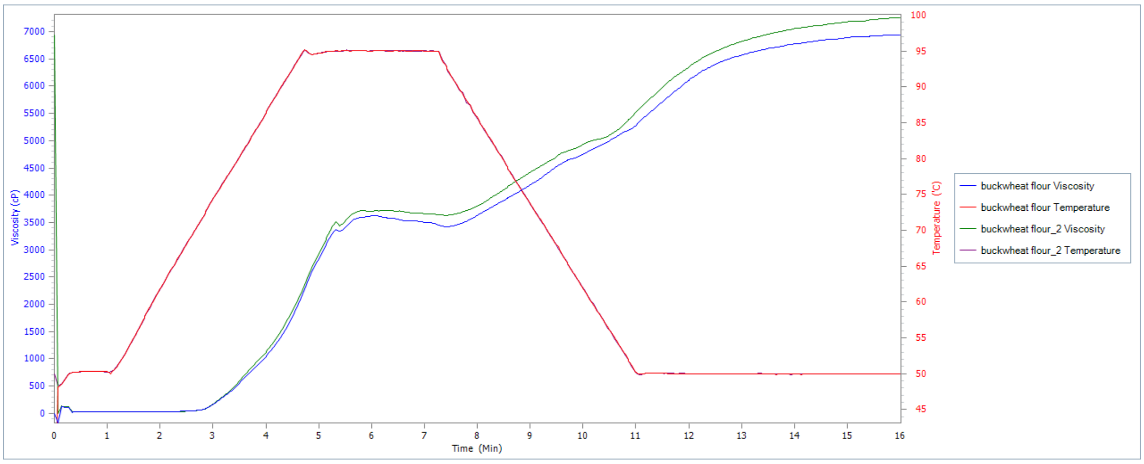


Figure 3 RVA pasting curves of pseudocereal (buckwheat flour).

Buckwheat flour protein has high globulins and albumins, low amount of prolamins and glutenins content and does not contain any gluten structure so it cannot be developed into the dough with good elasticity and plasticity (Krishnaswamy Gomathi & Parameshwari, 2022). The viscosity curve of buckwheat flour is shown in **Figure 3**. Based on the graph, it is possible to conclude that buckwheat flour, during gelatinization, has a good water absorption ability, its viscosity is higher than that of wheat flour and the viscosity increases quickly with the temperature decrease during the cooling stage. The same results were observed by Wet et al. (1995).

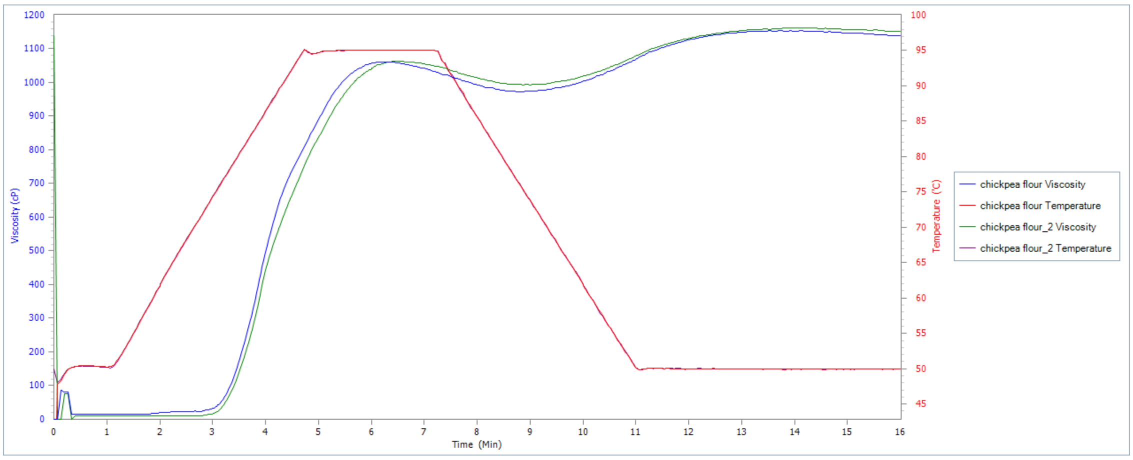


Figure 4 RVA pasting curves of legume flour (chickpea flour).

In this graph (**Figure 4**) are demonstrate the behaviour of legume flour – chickpea. According to **Table 3**, chickpea flour has the lowest peak viscosity, breakdown, and final viscosity. Chickpea flour also has the lowest value of setback 161.5±0.2 cp which indicates a lower tendency of the starch granules to retrograde during staling.

Another important aspect of this method is that the shape of the pasting flour curves helps to predict the final product behaviour and its interaction with the environment. According to the **Figure 1**, buckwheat flour has the highest value of retrogradation, and chickpea flour the lowest value. Based on the literature, the product shelf-life is directly influenced by the retrogradation of the starch. The product shelf-life decreases when the retrogradation increases. Thus, is expect that biscuits with buckwheat flour in their composition (higher SB value) will have lower shelf-life and biscuits with chickpea flour higher shelf-life.

Results discussion of sensory acceptance

The water absorption of each pure flour and their mixture, in different proportions, with other flours was previously determined using a farinograph according to AACCI Method 54-21.02. Thus, according to the water absorption value, was possible to establish a correlation with the water that was needed to add to each recipe of biscuits.

For the biscuits recipe with wholegrain in wheat flour were added different amounts of water, **Annexe 1**, depending on the type of wholegrain wheat flour percentage. Based on the results, as is possible to see in **Figure 5** and **Figure 6**, the biscuit with pure wholegrain flour is darker than the others. The colour of the biscuits is lightening proportionally to the decrease in the percentage of wholegrain wheat flour.

According to the sensory analyses performed by the consumer panel, **Annexe 3** and **Annexe 4**, the BWF+WWF (50:50) was the biscuit with the best acceptance with 81%, followed by BWF+WWF (75:25) and BWF+WWF (25:75) with the same overall acceptability (79%). The BWF+WWF (0:100) had low acceptance but the BWF+WWF (100:0) had the worst score, 75%. Overall, all the biscuits were easy to masticate and non-sticky. Their colour was well accepted and regarding the hardness and crispness, all were soft. Their sweetness intensity, flavour, odour and taste, the score was around 3 and 4 which means that the panel liked but it was just satisfactory.

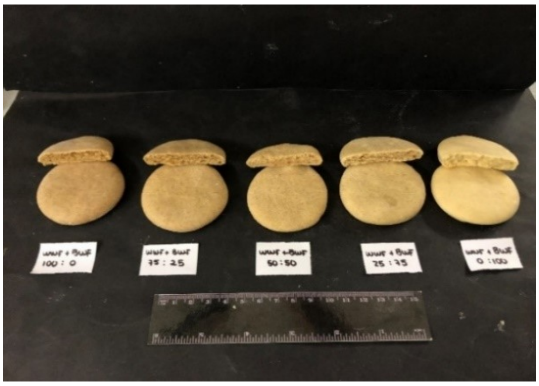


Figure 5 and *Figure 6* Biscuits with different ratios of biscuit wheat flour and wholegrain wheat flour from different perspectives.

For the biscuits recipe with buckwheat flour were added different amounts of water, **Annexe 1**, depending on the type of buckwheat flour percentage. According to the overall acceptability, **Annexe 3** and **Annexe 4**, the BWF+BF (75:25), with 75%, was the most liked biscuit, followed by BWF+BF (50:50), BWF+BF (25:75), and then BWF+BF (0:100) with overall acceptability of 53%. This fact implies that the acceptability of the biscuits by the consumer panel is inversely proportional to the increase in the buckwheat flour content in the recipe.

Based on the results, as is possible to see in **Figure 7** and **Figure 8**, the biscuit BWF+BF (100:0) had the lightest colour and the BWF+BF (0:100) had a greyish tone. Regarding the smoothness of the surface, all of them had some bubbles and the colour of BWF+BF (50:50) and BWF+BF (25:75) are not homogeneous, except the BWF+BF (0:100). Their hardness and texture were soft and a bit harder and all of them had a low sweetness and indistinct taste. However, the flavour and the taste of BWF+BF (0:100) had the lowest score since the panel dislike it extremely.

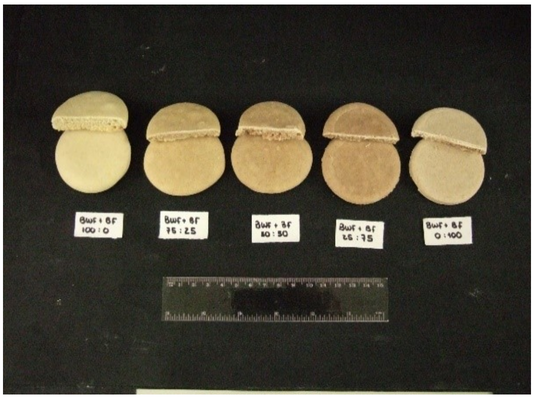


Figure 7 and *Figure 8* Biscuits with different ratios of biscuit wheat flour and buckwheat flour from different perspectives.

For the biscuits recipe with wholegrain rye flour were added different amounts of water, **Annexe 1**, depending on the type of wholegrain rye flour percentage. According to the overall acceptability, all the combinations with different ratios of biscuit wheat flour and wholegrain rye flour showed higher acceptance compared to the other type of biscuits. The BWF+WRF (50:50) had the highest score, 84%, and the BWF+WRF (100:0) was the lowest (75%).

The colour of the biscuits was well accepted, and it was darker with a bigger ratio of wholegrain rye flour. The BWF+WRF (75:25) had shown some bubbles and the hardness and the texture of all of them were soft and granular. The flavour, odour and taste were highly scored, although they were not so sweet, with moderate intensity. All of them were easy to masticate and were not very sticky in the mouth (**Figure 9** and **Figure 10**).

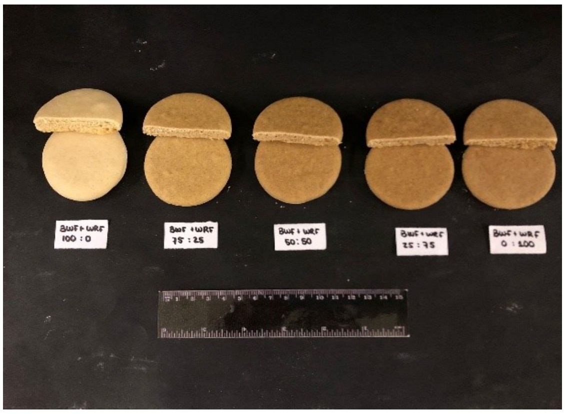
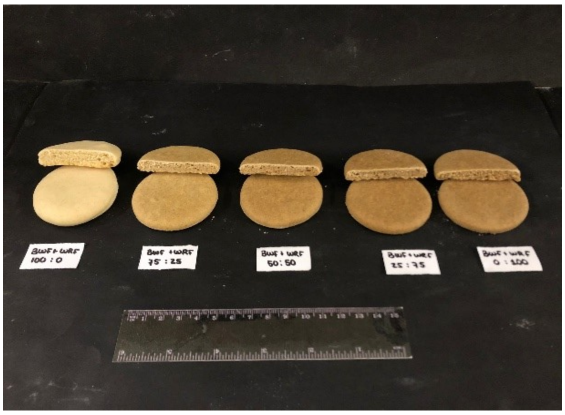
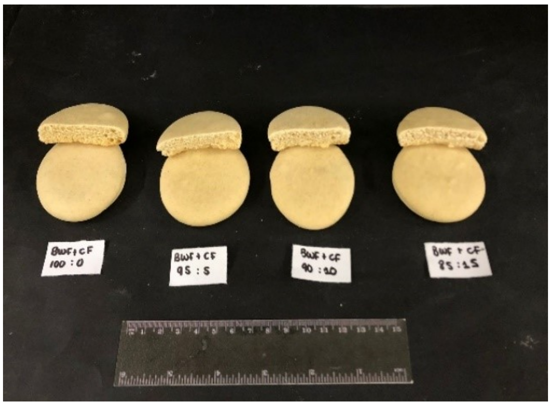



Figure 9 and *Figure 10* Biscuits with different ratios of biscuit wheat flour and wholegrain rye flour from different perspectives.

For the biscuits recipe with chickpea flour (**Figure 11** and **Figure 12**) were added different amounts of water, **Annexe 1**, depending on the type of chickpea flour percentage. Chickpea flour has a strong flavour and yellow colour. Thus, the formulation of the biscuits, was used in the blends with ratios of 95:5, 90:10, and 85:15.

Based on the overall acceptability, the increase of the ratio in chickpea was associated with a lower acceptance. The BWF+CF (95:5), with 68%, were the most liked biscuit, followed by BWF+CF (90:10), and the BWF+CF (85:15) had the lowest value, 60%. The biscuit's colour was noticeably light and the BWF+CF (85:15) was slightly darker than the others. All of them have some bubbles on their surface and were soft and non-crispy.

Regarding sweetness, all biscuits were evaluated with 2 (on a scale of 5), which means that they were not sweet, and the consumer panel did not like them. The flavour, odour, flavour, and taste were indistinct and score with a 2 or 3. Also, they were easy to masticate and not sticky in the mouth.



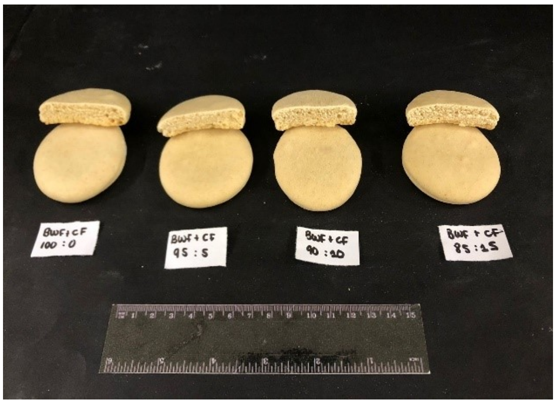
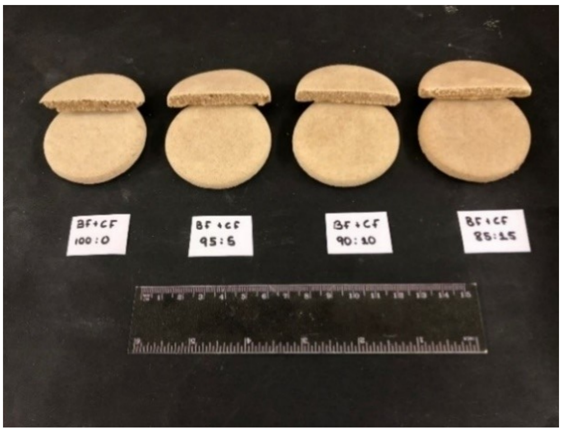


Figure 11 and *Figure 12* Biscuits with different ratios of biscuit wheat flour and chickpea flour.

For the biscuits recipe with buckwheat and chickpea flours (**Figure 13** and **Figure 14**) were added different amounts of water, **Annexe 1**, depending on the blends. For the biscuits recipe with buckwheat flour and wholegrain rye flour, the ratio of each type of biscuit was 100:0, 95:5, 90:10, and 85:15 since both flours have a strong flavour.

The BF+CF (90:10) had the highest value, 60%, followed by BF+CF (95:5), 50%, and BF+CF (85:15) with 49%. The colour of biscuits was not homogenized and showed some bubbles. All of them were soft and broke easily. Their overall acceptance was scoured with low values, which means that the recipes for gluten free biscuits still need to be improved.



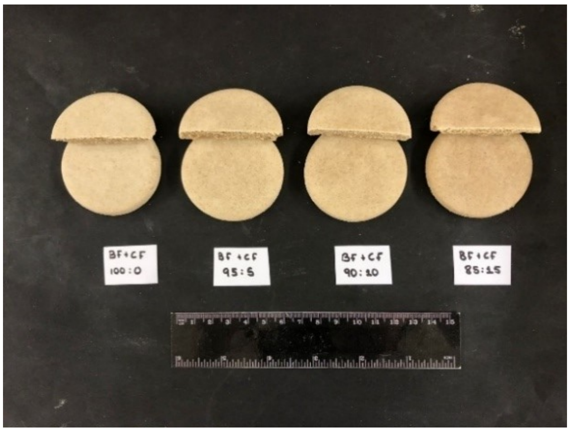


Figure 13 and *Figure 14* Biscuits with different ratios of buckwheat flour and chickpea flour.

Texture analyses

Food texture is an important attribute in consumer acceptance. It has been defined as "the sensory and functional manifestation of the structural, mechanical, and surface properties of foods detected through the senses of vision, hearing, touch, and kinesthetics" (Szczesniak, 2002; Tunick, 2011).

Based on texture analyses, there are some mechanical parameters that can be measured, such as hardness, cohesiveness, adhesiveness, fracturability, and chewiness. Hardness can be defined as the force required to achieve a given deformation and cohesiveness as extent to which a product can be deformed before breaking. Fracturability is the strength with which a product fractures (with high degree of hardness and low degree of cohesiveness). Adhesiveness is the work required to overcome the attractive forces between the surface of the product and the surface of the other materials with which the product comes into contact and chewiness is the energy required to chew a solid food to a state ready to be swallowed (Szczesniak, 2002).

Therefore, in **Table 11** (**Annex 4**) are demonstrated the texture analyses results of each sample of biscuit and in **Figure 15** and **Figure 16** are two examples of the graphical result of the texture analyses. The force, expressed in newton (N), simulates the force needed to break the biscuit with the teeth and the distance, expressed in mm, simulates the time it takes to break the biscuit (time 0 to the peak of the curve). The area, expressed in N.sec, is the force needed per second during process of carving the biscuit and breaking it.

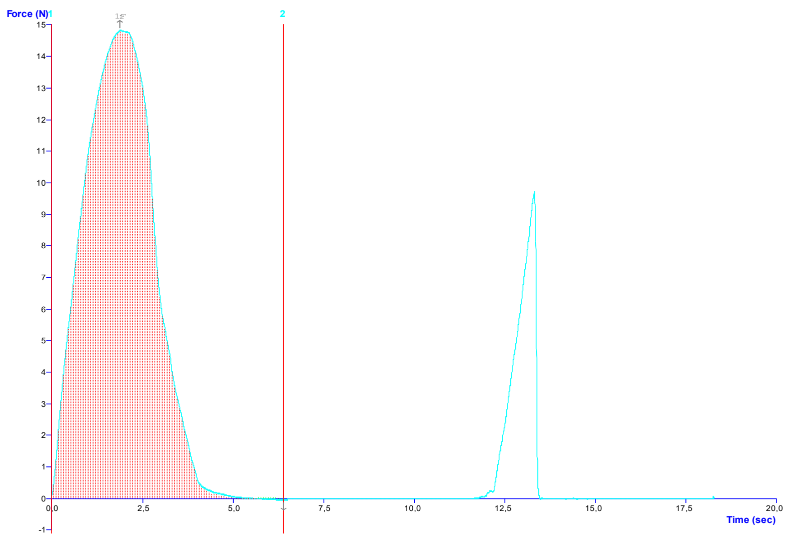


Figure 15 Graphical result of the texture analyses of the biscuit with biscuit wheat flour (BWF) and wholegrain wheat flour (WWF) in ratio 50:50.

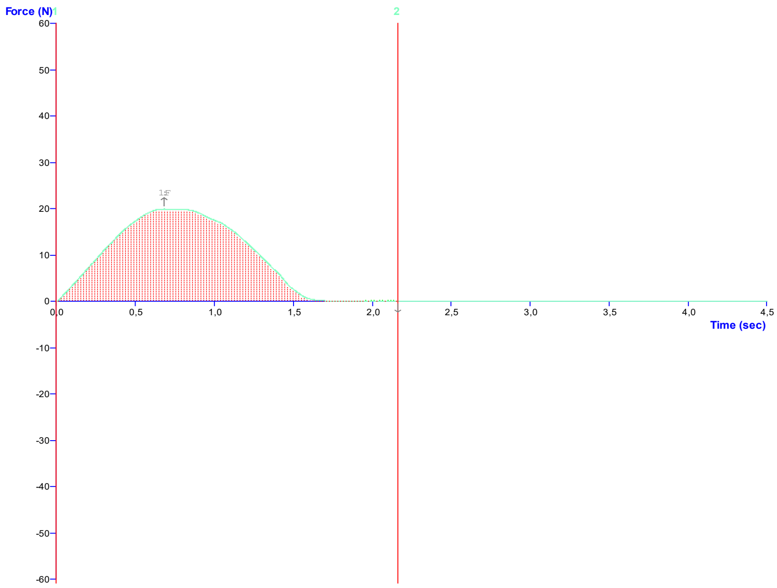


Figure 16 Graphical result of the texture analyses of the biscuit with buckwheat flour (BF) and chickpea flour (CF) in ratio 90:10.

The shape of the curve gives information regarding the hardness. It means that when the curve is sharper, it needs more force to break the biscuit. In turn, the smoother or softer the curve, the less force is needed. In these two different cases, BWF+WWF (50:50) is harder than BF+CF (90:10) and it can be confirmed by the area values, in **Table 11** (**Annex 4**), 33.56 N·sec and 22.12 N·sec, respectively.

Also, it is possible to do a correlation between these results and the results of sensory evaluation (**Annex 2**). Comparing theses values it can be concluded that almost all of samples were evaluated as soft and non-crispy except the biscuit BWF+BF (0:100) which was evaluated as a bit hard. The BWF+WRF (75:25), BWF+WRF (50:50), and BF+CF (90:10) were the most positively scored samples regarding texture.

Statistical analysis

The resulting data were processed using correlation analysis and principal component analysis (PCA). Statistical methods were used to describe the relationships between the various wheat flour quality traits that could be used to predict the quality of flour and bakery products.

The correlation analyses were performed from the data set of 5 pure flour samples described by qualitative traits (**Table 11** and **Table 12**, **Annex 5**). Statistically significant strong negative correlations between ash content and total starch content, total starch content and total fibre content ($r = -0.81$, $p<0.05$; $r = -0.92$, $p<0.05$, respectively) were determined and confirmed by the literature (Hemdane et al., 2015). Minerals (determined as content of ash) and fiber are found in the coating seed layers and aleurone layer, while starch is exclusively in the endosperm.

Interestingly, a negative correlation was found between insoluble fiber content and viscosity parameters (PeakVisc and SetBack) ($r = -0.79$, $p<0.05$; $r= -0.80$, $p<0.05$). Higher proportions of fibre (especially water insoluble fibre) affect the behaviour of the suspension during heating. The relationships between total fibre content and viscosity RVA parameters (PeakVisc, Final Visc and SetBack) showed a similar trend.

Statistically and logically related correlations were found for SRC tests: the SRC sucrose correlated with Pasting Temp ($r = -0.88$, $p<0.05$) and Breakdown ($r = -0.81$, $p<0.05$). Higher proportions of arabinoxylans in the flour may influence the viscosity behaviour of the suspension during RVA measurements. SRC of carbonate showed a negative correlation with Peak Time ($r = -0.85$, $p<0.05$). Higher proportion of damaged starch was related to lower gelatinization temperature of the suspension. SRC of sucrose was correlated with specific volume of the biscuit ($r = -0.94$, $p<0.05$). Higher proportion of fiber and its components may cause reduced volume and slight deterioration in texture of the biscuit.

The parameters describing the composition of flours and flour blends, and biscuits quality were subjected to correlation analyses (**Table 13**, **Annex 5**). Statistically significant positive correlations were confirmed between ash content and insoluble fibre content ($r = 0.85$, $p<0.05$), strong negative correlations between ash content and total starch content ($r = -0.96$, $p<0.05$), and negative correlations between total starch content and total fibre content ($r = -0.83$, $p<0.05$). Also, it is possible to conclude (confirmed by texture analyses – **Table 10**, **Annex 4**) that there is a significant positive correlation between total starch and distance ($r = 0.82$, $p<0.05$), total dietary fibre and area ($r = 0.90$, $p<0.05$), and between biscuit weight and distance ($r = 0.85$, $p<0.05$).

The method of principal component analysis (PCA) was chosen to describe the relationships between the measured features and also between the studied samples without significant loss of information. The original multidimensional data are transposed into a new coordinate system using linear combinations, preserving the distances and angles between the objects - the studied samples. Principal components form the axes of this new system and are calculated to explain all the variability of the original features (**Table 14**, **Annex 5**). In this study, these were the most significant components PC1 and PC2. In total, 74% and 91% of the variance in the data was explained in this way.

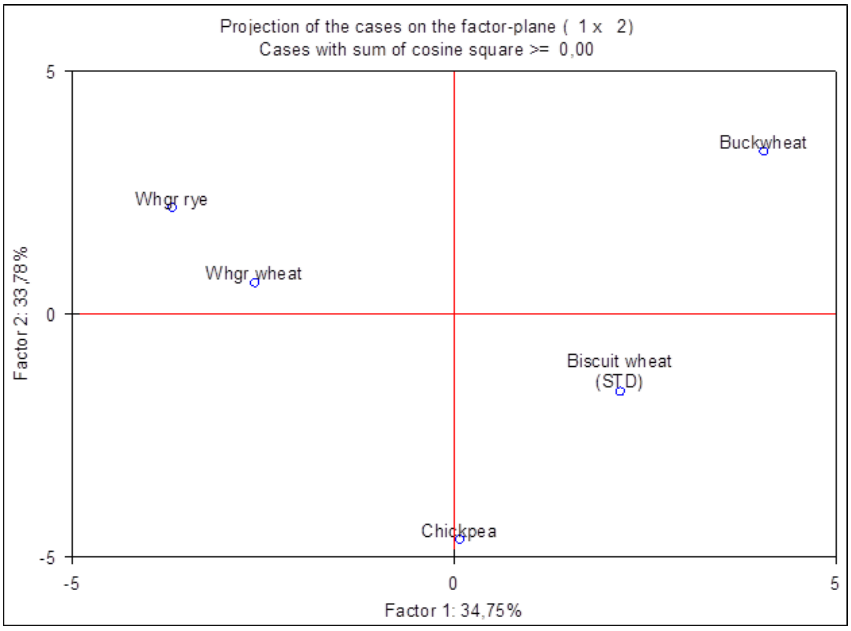


Figure 17 PCA Projection of the all-pure flours (buckwheat, chickpea, biscuit wheat wholegrain rye and wheat flours) on the factor-plane (1×2).

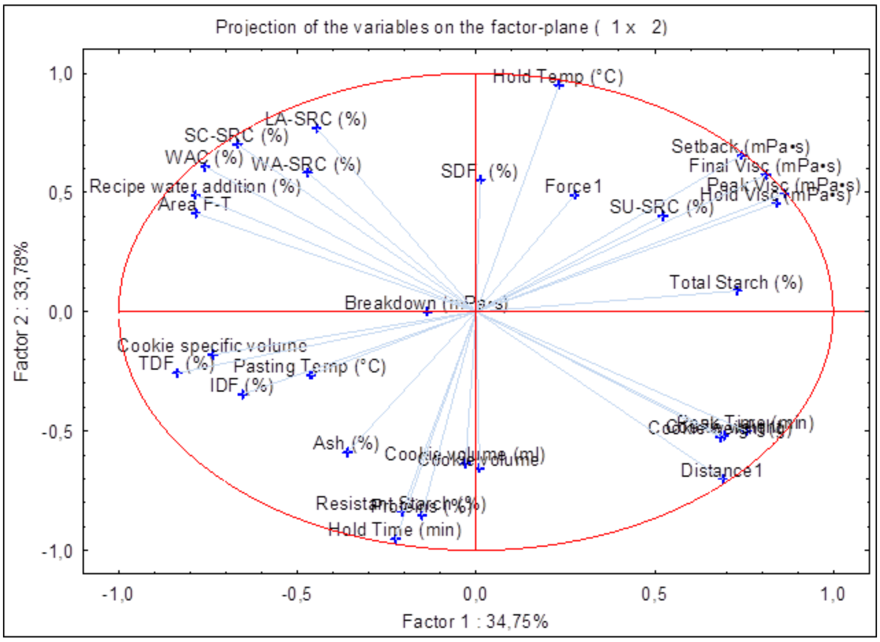


Figure 18 PCA Projection of the variables on the factor-plane (1×2).

WAC – water absorption, *DW* – demineralized water, *LA-SRC* - lactic acid, *SC-SCR* – sodium carbonate, *SU-SCR* – sucrose, *IDF* – insoluble dietary fibre, *SDF* – soluble dietary fibre, *TDF*- total dietary fibre, *RS* – resistant starch

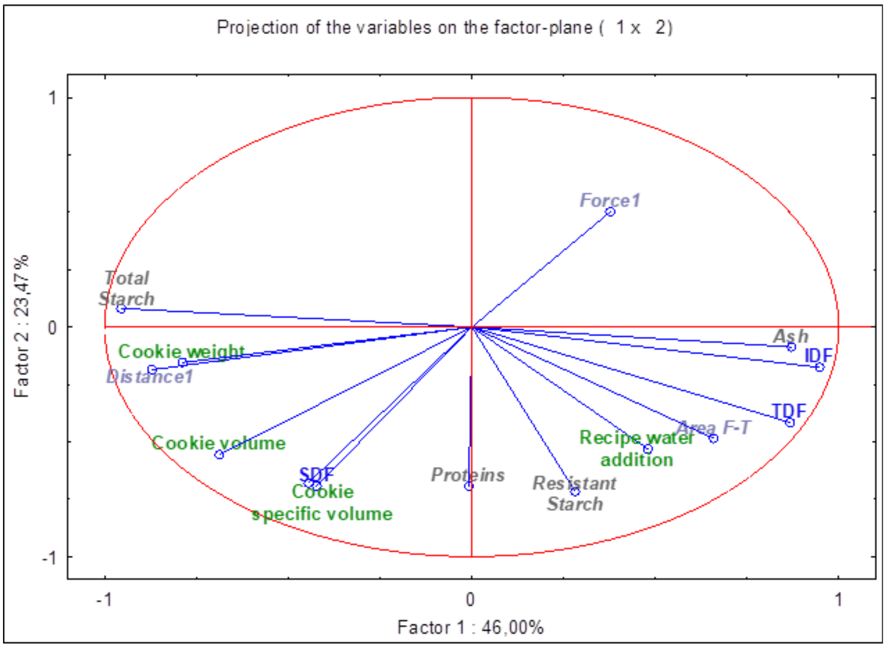


Figure 19 PCA Projection of the variables on the factor-plane (1×2).

IDF – insoluble dietary fibre, *SDF* – soluble dietary fibre, *TDF*- total dietary fibre, *RS* – resistant starch

Based on the different physicochemical properties, the different flours in the PCA were divided into sub-areas, with buckwheat flour (right in **Figure 17**; gluten-free flour from pseudocereals) and wholegrain wheat and wholegrain rye flours (close left in **Figure**

17) in the PC1 area; cereal flours showed similar chemical composition and technological purpose), in PC2 chickpea flour (legume flour; gluten-free) and biscuit wheat flour (soft wheat flour for standard biscuit production). In projection PC1, buckwheat flour (top right in **Figure 17**) differed from the other flours in the FinalVisc and PeakVisc parameters (top right in **Figure 18**) and the Force parameter (**Figure 19**) for final biscuit parameter. Wholegrain wheat and rye flours (top left, projection **Figure 18**) differed from the others in the LA-SRC characteristic and recipe water addition and Area F-T. In projection PC2, chickpea flour showed differences from the other flours in the parameter resistant starch content and protein, and the parameters Hold Time and Hold Temp (graphs of **Figure 18** and **Figure 19**).

Nutritional value of the biscuits

As mentioned in literature and verified experimentally, the wholemeal flours (wheat and rye) and chickpeas are rich in fibre. This fact leads biscuits baked with high percentage of these flours also have higher fibre content.

According to regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutritional and health claims made on foods, when the product contains at least 3g of fibre per 100g or at least 1.5g of fibre per 100 kcal can be claim that is a source of fibre. Additionally, foods can be labelled as high in fiber if they contain at least 6 g of fiber per 100 g or 3 g of fiber per 100 kcal. Thus, based on that and on the nutritional labels in **Annex 6** it can be concluded that all of the biscuits are source of fibre and some of them are high in fibre such as the BWF+WWF (50:50), and BWF+WRF (25:75). Depending on consumers' desire and nutritional need, it is possible, in this case, to opt for a product claimed by "source of fibre" or "high in fibre."

Regarding protein, the chickpea flour has the highest content, so biscuits with a higher proportion of chickpea flour should be nutritionally preferable since lower glycemic index is expected.

Shelf-life of the biscuits

The quality of food decreases with holding time or storage. The shelf-life can be defined as the time during which all primary characteristics of the food remain acceptable for consumption (Robertson, 2011). The storage conditions (temperature, relative humidity, and light), the product properties (moisture content and physicochemical properties), the processing parameters (microorganisms, water activity, pH, enzymes, concentration of reactive compounds), and the packaging material impact on the product shelf-life (Galić et al., 2009).

Thus, to determine the biscuits' shelf-life is necessary to put them to certain conditions of time, temperature, and packaging (Gebreselassie & Clifford, 2016). In this study, due to the limited time, was not possible to experimentally determine it.

Therefore, based on literature and scientific articles, it is possible to determine which conditions and determinations are necessary to estimate an approximate shelf-life of the biscuits. According to Kumari et al. (2021), to experimentally test the shelf-life of biscuits, after baking and chill time, the biscuits should be stored at room temperature and drawn at interval of 15 days of storage for 75 days. During this period of time, should be evaluated various sensory parameters such as appearance, colour, aroma, texture, taste, and overall acceptability. According to Nagi et al. (2012), besides the sensory evaluation, it also should be estimated the water activity, moisture content, microbiological quality, and free fatty acids at regular intervals of 1 month over the period of 3 months. So, in order to experimentally evaluate the maintenance of the acceptable quality of the biscuits, it should be combined the analyses of these two studies and the result should be approximately stored at 25ºC with a relative humidity of 65-70%.

CONCLUSIONS

Based on the nutritional value of the biscuits it can be concluded that biscuits with higher proportion of chickpea flour in their composition should be nutritionally preferable, from the point of view of protein and starch content, since their higher content of protein, lower content of starch and, as mentioned on the literature, lower glycemic index. Also, with the incorporation of wholegrain wheat and rye flours and chickpea flour, the biscuits are fortified with fibre. All of them can be label with "source of fibre" and some of them with "high in fibre."

Thus, combined these results with the flours' chemical properties, and texture analyses of the biscuits concludes that biscuits with buckwheat flour and chickpea flour in a ratio of 90:10 are a viable choice for people with gluten-sensitive or who have celiac disease. Biscuits with biscuit wheat flour and wholegrain rye flour in a ratio of 25:75, biscuits with only wholegrain rye flour or with only wholegrain wheat flour in their composition are also a desirable choice for consumers who desires products with high content of fibre. However, these recipes, based on panel's overall acceptance, still need to be improved. Although on a nutritional level, the biscuits meet consumers' expectations, on an organoleptic level they still need to be improved. To achieve the success of a given product, it has to fulfill all the requirements, and consumer acceptability is one of them. Furthermore, in order to continue and enhance this study, in a next step, acrylamide, lipid oxidation during staling and shelf-life should be tested experimentally. Also, it will be necessary to conduct a hedonic test with a representative panel of adequate dimension in order to obtain reliable results.

Sumi up, this study allowed me to go deepen my knowledge of innovation and development of new products and gain more interest for ongoing research in this area of science.

FORTHCOMING WORK

Given the relevance of this study, it would be interesting to continue the work done so far.

The nutritional value of the biscuit, its sensory value, acceptability to the average consumer (price, availability, interest, and re-purchase of the biscuit) are important. Thus, since the acceptability of the biscuit does not meet their potential nutritional value, it would be necessary to improve the recipes.

The amount of water added in the recipes has a significant impact on the final performance of the biscuit dough. Thus, it would be necessary to perform more tests in order to correct the values. The water absorption was analysed by farinograph which is a rheological instrument simulating the dough's behaviour during its formation and kneading. However, since it since it mixes distinct types of flours, there may be an associated error since the properties of the flours are different and behave differently when mixed. Another point of view is that rheological instruments are only suitable for wheat flour/dough under given standard conditions, so it could have an impact on the result.

Besides that, the amount and type of oil also has influenced the final result. Thus, different combinations of amount and types of oils should be tested. One of the reasons to replacing fats with other ingredients is to reduce the energy value of the final product while maintaining its functional and sensory properties.

Another factor that could be developed would be decrease the fat content of the biscuits so they could have the "low fat" claim. This can be achieved by carbohydrate-based compounds (e.g., hydrocolloids such as various fibres, maltodextrins, modified starches, resistant starch, microcrystalline cellulose, or polydextrose), protein-based compounds such as modified whey protein concentrate, fat-based compounds (esters of sucrose with fatty acids) or combined compounds (mixtures of carbohydrates and proteins or carbohydrates and fats).

Thus, based on that it should be done a search for suitable ingredients, prepare recipes, and test healthy biscuits. Also, substitution of fat/oil, or even sugar substitution with different ingredients such us mimetics, fat substituents, and fibre preparations could be interesting for the study.

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ANNEXES

Annex 1 Amount of water used in different biscuit recipes

Table 4 Amount of water, in millilitres, used in different biscuit recipes with biscuit wheat flour (BWF) and wholegrain wheat flour (WWF).

Flour blends	Amount of water (ml)
BWF + WWF 100:0	48
BWF + WWF 75:25	59.5
BWF + WWF 50:50	63
BWF + WWF 25:75	60
BWF + WWF 0:100	60

Table 5 Amount of water, in millilitres, used in different biscuit recipes with biscuit wheat flour (BWF) and buckwheat flour (BF).

Flour blends	Amount of water (ml)
BWF + BF 100:0	48
BWF + BF 75:25	53
BWF + BF 50:50	55
BWF + BF 25:75	58
BWF + BF 0:100	51

Table 6 Amount of water, in millilitres, used in different biscuit recipes with biscuit wheat flour (BWF) and wholegrain rye flour (WRF).

Flour blends	Amount of water (ml)
BWF + WRF 100:0	48
BWF + WRF 75:25	51
BWF + WRF 50:50	52
BWF + WRF 25:75	57
BWF + WRF 0:100	60

Table 7 Amount of water, in millilitres, used in different biscuit recipes with biscuit wheat flour (BWF) and chickpea flour (CF).

Flour blends	Amount of water (ml)
BWF + CF 100:0	48
BWF + CF 95:05	55
BWF + CF 90:10	47
BWF + CF 85:15	46

Table 8 Amount of water, in millilitres, used in different biscuit recipes with buckwheat flour (BF) and chickpea flour (CF).

Flour blends	Amount of water (ml)
BF + CF 100:0	51
BF + CF 95:05	48
BF + CF 90:10	48
BF + CF 85:15	46

Annex 2 Farinograph curve of mixture of flours

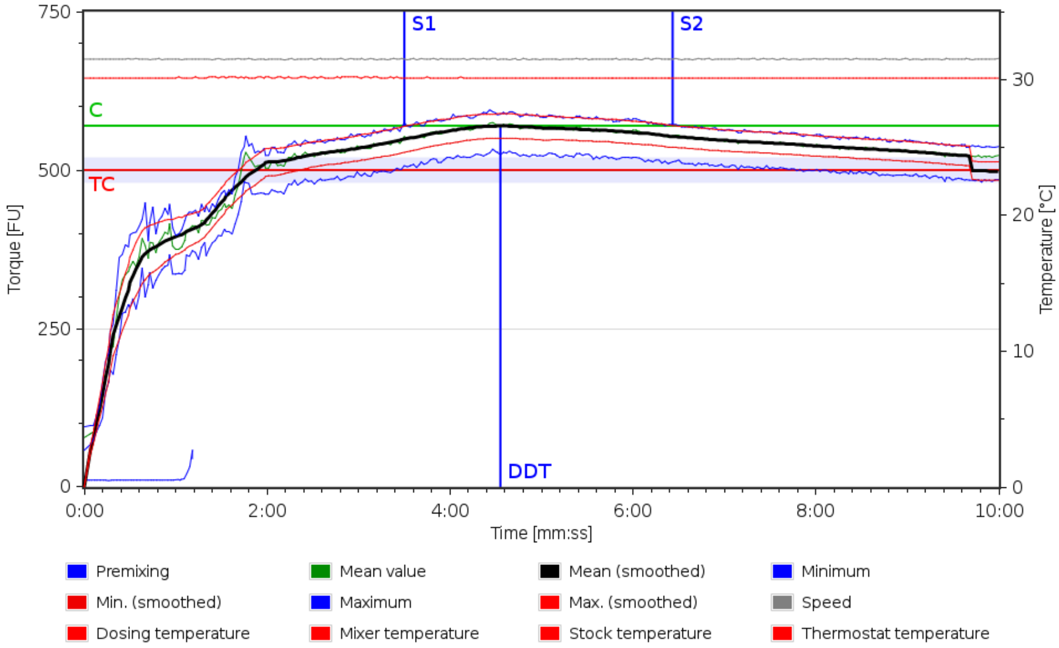

Figure 20 Farinograph curve when testing the consistency (570 FU) of the mixture of flours with biscuit what flour and wholegrain rye flour in a ratio of 25:75.

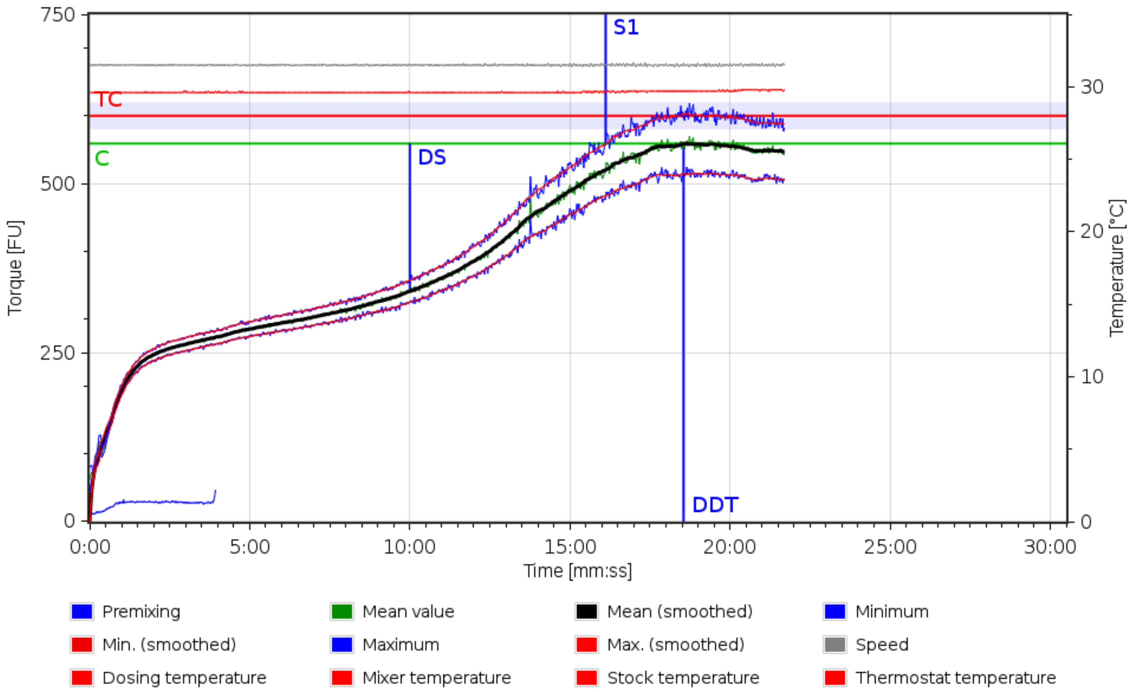

Figure 21 Farinograph curve when testing the consistency (559 FU) of the mixture of flours with biscuit wheat flour and wholegrain wheat flour in a ratio of 75:25.

Annex 3 Key to sensory acceptance of biscuits

Table 9 Key used to sensory acceptance of laboratory baked biscuits.

	Biscuit quality attribute		Point scale	*Comment*
1	**Colour and surface character**	5	like extremely	*just-about-right colour, optimal*
		4	like slightly	
		3	neither like nor dislike	*a bit lighter or darker*
		2	dislike slightly	
		1	dislike extremely	*unevenly coloured, too light, or dark*
2	**Smoothness (surface appearance)**	5	like extremely	*properly flat, smooth surface*
		4	like slightly	
		3	neither like nor dislike	*with some bubbles*
		2	dislike slightly	
		1	dislike extremely	*vaulted, uneven, rough*
3	**Total hardness / crispness**	5	like extremely	*proper thick, crispy*
		4	like slightly	
		3	neither like nor dislike	*soft, non-crispy*
		2	dislike slightly	
		1	dislike extremely	*extremely hard, or gummy*
4	**Texture**	5	like extremely	*friable, crispy*
		4	like slightly	
		3	neither like nor dislike	*granular, a bit harder or softer*
		2	dislike slightly	
		1	dislike extremely	*extremely hard or soft, crumbly, dry, sticky, oily*
5	**Sweetness intensity**	5	like extremely	*very well, typical, pleasant*
		4	like slightly	
		3	neither like nor dislike	*less or more sweet, moderate intensity*
		2	dislike slightly	
		1	dislike extremely	*extremely high or low sweetness, not noticeable*
6	**Flavour, odour & taste**	5	like extremely	*pleasant, balanced, well baked, tasty*
		4	like slightly	
		3	neither like nor dislike	*indistinct but satisfactory*
		2	dislike slightly	
		1	dislike extremely	*too strong or weak, salty, burnt flavour*
7	**Chewing sensation**	5	like extremely	*easy to masticate, to form a mouthful*
		4	like slightly	
		3	neither like nor dislike	*yet forming a mouthful*
		2	dislike slightly	
		1	dislike extremely	*hardly to mix with the saliva and soften*

	Biscuit quality attribute	Point scale		*Comment*
8	**Stickiness to palate**	5	like extremely	*non-sticky, easily falls off the soft palate*
		4	like slightly	
		3	neither like nor dislike	*moderately sticky (also to teeth)*
		2	dislike slightly	
		1	dislike extremely	*very wet and sticky, or dry and crumbly*

Annex 4 Sensory profile

Table 10 Average of Sensory Profile of the samples of biscuits.

Sample	At1	At2	At3	At4	At5	At6	At7	At8	*points*	*Overall acceptability*
BWF+WWF (100:0)	3.67	4.00	3.67	3.67	4.00	3.67	3.67	3.67	30.00	75%
BWF+WWF (75:25)	4.00	3.67	3.33	4.00	3.33	4.33	4.33	4.67	31.67	79%
BWF+WWF (50:50)	4.33	3.67	3.67	3.67	3.67	4.33	4.33	4.67	32.33	81%
BWF+WWF (25:75)	5.00	3.50	3.00	3.00	4.00	4.50	4.00	4.50	31.50	79%
BWF+WWF (0:100)	5.00	3.50	3.00	3.50	3.50	4.00	4.00	4.00	30.50	76%
BWF+BF (75:25)	4.00	3.33	3.33	3.00	3.67	4.33	4.67	3.67	30.00	75%
BWF+BF (50:50)	4.00	3.33	3.00	3.00	2.67	3.67	4.33	4.00	28.00	70%
BWF+BF (25:75)	3.67	3.33	3.00	3.00	3.00	3.33	3.33	2.00	24.67	62%
BWF+BF (0:100)	3.50	4.50	2.00	2.00	2.00	2.00	2.50	2.50	21.00	53%
BWF+WRF (75:25)	4.33	4.00	4.00	4.00	3.00	3.00	4.67	4.00	31.00	78%
BWF+WRF (50:50)	5.00	4.33	4.00	4.00	2.67	4.33	4.33	5.00	33.67	84%
BWF+WRF (25:75)	4.67	4.00	3.67	3.67	3.00	3.33	4.00	4.00	30.33	76%
BWF+WRF (0:100)	4.67	4.00	3.67	3.33	2.00	3.67	4.33	5.00	30.67	77%
BWF+CF (95:5)	3.33	3.33	3.67	3.33	2.67	3.33	4.00	3.67	27.33	68%
BWF+CF (90:10)	3.33	3.00	3.33	3.00	2.33	2.67	4.00	4.33	26.00	65%
BWF+CF (85:15)	3.33	3.00	2.67	2.67	2.67	2.67	3.67	3.33	24.00	60%
BF + CF (95:5)	2.00	3.50	3.00	2.50	2.00	2.00	2.50	2.50	20.00	50%
BF + CF (90:10)	2.50	3.50	4.00	3.00	2.00	2.50	3.50	3.00	24.00	60%
BF + CF (85:15)	1.50	3.00	3.50	2.50	1.00	1.50	3.00	3.50	19.50	49%

Annex 5 Texture analyses results

Table 11 Texture analyses results of force (N), distance (mm) and area (N·sec) of each sample of biscuit.

Sample	Force (N)	Distance (mm)	Area (N·sec)
BWF+WWF (100:0)	17.83	7.32	26.32
BWF+WWF (50:50)	14.66	5.02	33.56
BWF+WWF (75:25)	17.17	6.29	31.94
BWF+WWF (25:75)	16.60	5.77	31.18
BWF+WWF (0:100)	13.77	4.82	31.85
BWF+BF (50:50)	14.88	6.57	18.88
BWF+BF (75:25)	15.11	7.33	21.64
BWF+BF (25:75)	12.60	5.45	13.87

Sample	Force (N)	Distance (mm)	Area (N·sec)
BWF+BF (0:100)	22.00	5.98	22.63
BWF+CF (95:05)	16.68	7.92	24.50
BWF+CF (90:10)	16.77	7.87	28.95
BWF+CF (85:15)	16.09	8.93	30.41
BWF + WRF (50:50)	20.73	5.35	38.29
BWF + WRF (75:25)	25.74	6.57	30.57
BWF + WRF (25:75)	20.02	4.09	49.37
BWF + WRF (0:100)	21.88	4.38	54.17
BF+CF (95:05)	20.21	5.66	23.45
BF+CF (90:10)	22.01	6.00	22.12
BF+CF (85:15)	20.71	5.65	19.99

Annex 6 PCA test results

Table 12 Correlation for 5 pure flours – biscuit wheat flour, wholegrain wheat and rye flours, buckwheat, and chickpea flour. Marked correlations are significant at $p<0.05$.

Variable (N = 5)	Water absorption corrected	Recipe water addition	Cookie weight	Cookie volume	Cookie specific volume	Force1	Distance1	Area F-T
Proteins	-0.40	-0.22	0.25	0.18	-0.05	-0.44	0.41	-0.35
Ash	-0.14	0.01	0.05	-0.05	-0.10	-0.15	0.03	-0.01
Total Starch	-0.45	-0.55	0.47	0.33	-0.11	0.03	0.57	-0.49
Resistant Starch	-0.42	-0.30	0.37	0.33	0.00	-0.19	0.36	-0.14
IDF	0.18	0.26	-0.15	-0.01	0.15	0.05	-0.32	0.44
SDF	0.46	0.36	-0.42	-0.03	0.39	-0.21	-0.24	0.13
TDF	0.39	0.45	-0.32	0.05	0.40	-0.04	-0.47	0.63
WA-SRC	0.56	0.45	-0.35	-0.29	0.04	0.70	**-0.79**	**0.85**
SU-SRC	-0.22	-0.21	0.15	-0.70	**-0.94**	0.62	-0.06	-0.27
SC-SRC	**0.88**	**0.81**	-0.74	-0.44	0.26	0.35	**-0.98**	**0.88**
LA-SRC	0.72	0.56	-0.49	-0.17	0.32	0.52	**-0.81**	**0.87**
Pasting Temp	0.36	0.41	-0.42	0.34	**0.81**	**-0.86**	-0.02	0.06
Peak Time	**-0.84**	-0.73	0.64	-0.03	-0.69	-0.14	**0.81**	**-0.95**
Peak Visc	-0.33	-0.41	0.29	-0.35	-0.70	0.40	0.26	-0.51
Hold Time	-0.37	-0.24	0.29	0.65	0.44	-0.68	0.54	-0.26
Hold Temp	0.36	0.24	-0.28	-0.64	-0.44	0.68	-0.53	0.26
Hold Visc	-0.38	-0.43	0.32	-0.50	**-0.89**	0.54	0.21	-0.50
Breakdown	0.22	0.15	-0.17	0.50	0.73	-0.49	0.08	0.09
Final Visc	-0.26	-0.34	0.23	-0.46	**-0.76**	0.50	0.14	-0.42
Setback	-0.14	-0.24	0.13	-0.41	-0.60	0.44	0.07	-0.34
Water absorption corrected	1.00	**0.98**	**-0.95**	-0.42	0.49	-0.11	**-0.94**	0.75
Recipe water addition	**0.98**	1.00	**-0.99**	-0.48	0.46	-0.26	**-0.89**	0.64
Cookie weight	**-0.95**	**-0.99**	1.00	0.57	-0.38	0.31	**0.85**	-0.52
Cookie volume	-0.42	-0.48	0.57	1.00	0.55	-0.14	0.54	0.02
Cookie specific volume	0.49	0.46	-0.38	0.55	1.00	-0.47	-0.27	0.57
Force1	-0.11	-0.26	0.31	-0.14	-0.47	1.00	-0.20	0.31
Distance1	**-0.94**	**-0.89**	**0.85**	0.54	-0.27	-0.20	1.00	**-0.81**
Area F-T	0.75	0.64	-0.52	0.02	0.57	0.31	**-0.81**	1.00

Table 13 Correlations matrix among analytic features of flour or flour blends and biscuit quality characteristics. Marked correlations are significant at p<0.05.

Variable (N = 5)	Proteins	Ash	Total Starch	Resistant	IDF	SDF	TDF	WA-SRC	SU-SRC	SC-SRC	LA-SRC	Pasting	Peak Time	Peak Visc	Hold Time	Hold Temp	Hold Visc	Breakdown	Final Visc	Setback
Proteins	1.00	**0.89**	-0.50	**0.94**	0.64	**-0.78**	0.50	-0.37	-0.08	-0.45	-0.74	0.07	0.51	-0.56	**0.79**	**-0.79**	-0.38	-0.40	-0.57	-0.71
Ash	**0.89**	1.00	**-0.81**	**0.91**	**0.89**	**-0.89**	**0.76**	0.08	0.12	-0.05	-0.41	-0.17	0.25	-0.64	0.52	-0.52	-0.36	-0.66	-0.57	-0.73
Total Starch	-0.50	**-0.81**	1.00	-0.55	**-0.93**	0.58	**-0.92**	-0.52	-0.09	-0.54	-0.14	0.09	0.29	0.71	-0.14	0.14	0.46	0.56	0.58	0.67
Resistant Starch	**0.94**	**0.91**	-0.55	1.00	**0.76**	**-0.88**	0.63	-0.14	-0.08	-0.34	-0.56	-0.08	0.37	-0.63	0.75	-0.75	-0.42	-0.47	-0.62	**-0.77**
IDF	0.64	**0.89**	**-0.93**	**0.76**	1.00	**-0.76**	**0.96**	0.46	0.00	0.35	0.03	-0.16	-0.19	**-0.79**	0.34	-0.34	-0.53	-0.56	-0.69	**-0.80**
SDF	**-0.78**	**-0.89**	0.58	**-0.88**	**-0.76**	1.00	-0.55	-0.12	-0.37	0.19	0.42	0.53	-0.43	0.35	-0.36	0.36	0.04	**0.81**	0.27	0.48
TDF	0.50	**0.76**	**-0.92**	0.63	**0.96**	-0.55	1.00	0.53	-0.22	0.49	0.21	0.05	-0.44	**-0.89**	0.33	-0.34	-0.70	-0.34	**-0.80**	**-0.85**
WA-SRC	-0.37	0.08	-0.52	-0.14	0.46	-0.12	0.53	1.00	0.27	**0.88**	**0.84**	-0.46	-0.70	-0.18	-0.58	0.58	-0.04	-0.37	-0.04	-0.04
SU-SRC	-0.08	0.12	-0.09	-0.08	0.00	-0.37	-0.22	0.27	1.00	0.07	-0.03	**-0.88**	0.42	0.61	-0.63	0.63	**0.84**	**-0.81**	0.72	0.57
SC-SRC	-0.45	-0.05	-0.54	-0.34	0.35	0.19	0.49	**0.88**	0.07	1.00	**0.89**	-0.08	**-0.85**	-0.25	-0.57	0.56	-0.19	-0.11	-0.13	-0.07
LA-SRC	-0.74	-0.41	-0.14	-0.56	0.03	0.42	0.21	**0.84**	-0.03	**0.89**	1.00	-0.10	**-0.89**	-0.03	-0.66	0.66	-0.08	0.15	0.05	0.16
Pasting Temp	0.07	-0.17	0.09	-0.08	-0.16	0.53	0.05	-0.46	**-0.88**	-0.08	-0.10	1.00	-0.29	-0.46	0.53	-0.54	-0.70	**0.81**	-0.56	-0.41
Peak Time	0.51	0.25	0.29	0.37	-0.19	-0.43	-0.44	-0.70	0.42	**-0.85**	**-0.89**	-0.29	1.00	0.41	0.29	-0.28	0.50	-0.37	0.37	0.22
Peak Visc	-0.56	-0.64	0.71	-0.63	**-0.79**	0.35	**-0.89**	-0.18	0.61	-0.25	-0.03	-0.46	0.41	1.00	-0.65	0.65	**0.94**	-0.06	**0.99**	**0.98**
Hold Time	**0.79**	0.52	-0.14	0.75	0.34	-0.36	0.33	-0.58	-0.63	-0.57	-0.66	0.53	0.29	-0.65	1.00	-1.00	-0.67	0.21	-0.74	**-0.77**
Hold Temp	**-0.79**	-0.52	0.14	-0.75	-0.34	0.36	-0.34	0.58	0.63	0.56	0.66	-0.54	-0.28	0.65	-1.00	1.00	0.67	-0.21	0.74	**0.77**
Hold Visc	-0.38	-0.36	0.46	-0.42	-0.53	0.04	-0.70	-0.04	**0.84**	-0.19	-0.08	-0.70	0.50	**0.94**	-0.67	0.67	1.00	-0.40	**0.97**	**0.89**
Breakdown	-0.40	-0.66	0.56	-0.47	-0.56	**0.81**	-0.34	-0.37	**-0.81**	-0.11	0.15	**0.81**	-0.37	-0.06	0.21	-0.21	-0.40	1.00	-0.19	0.02
Final Visc	-0.57	-0.57	0.58	-0.62	-0.69	0.27	**-0.80**	-0.04	0.72	-0.13	0.05	-0.56	0.37	**0.99**	-0.74	0.74	**0.97**	-0.19	1.00	**0.97**
Setback	-0.71	-0.73	0.67	**-0.77**	**-0.80**	0.48	**-0.85**	-0.04	0.57	-0.07	0.16	-0.41	0.22	**0.98**	**-0.77**	**0.77**	**0.89**	0.02	**0.97**	1.00
Water absorption corrected	-0.40	-0.14	-0.45	-0.42	0.18	0.46	0.39	0.56	-0.22	**0.88**	0.72	0.36	**-0.84**	-0.33	-0.37	0.36	-0.38	0.22	-0.26	-0.14
Recipe water addition	-0.22	0.01	-0.55	-0.30	0.26	0.36	0.45	0.45	-0.21	**0.81**	0.56	0.41	-0.73	-0.41	-0.24	0.24	-0.43	0.15	-0.34	-0.24
Cookie weight	0.25	0.05	0.47	0.37	-0.15	-0.42	-0.32	-0.35	0.15	-0.74	-0.49	-0.42	0.64	0.29	0.29	-0.28	0.32	-0.17	0.23	0.13
Cookie volume	0.18	-0.05	0.33	0.33	-0.01	-0.03	0.05	-0.29	-0.70	-0.44	-0.17	0.34	-0.03	-0.35	0.65	-0.64	-0.50	0.50	-0.46	-0.41
Cookie specific volume	-0.05	-0.10	-0.11	0.00	0.15	0.39	0.40	0.04	**-0.94**	0.26	0.32	**0.81**	-0.69	-0.70	0.44	-0.44	**-0.89**	0.73	**-0.76**	-0.60
Force1	-0.44	-0.15	0.03	-0.19	0.05	-0.21	-0.04	0.70	0.62	0.35	0.52	**-0.86**	-0.14	0.40	-0.68	0.68	0.54	-0.49	0.50	0.44
Distance1	0.41	0.03	0.57	0.36	-0.32	-0.24	-0.47	**-0.79**	-0.06	**-0.98**	**-0.81**	-0.02	**0.81**	0.26	0.54	-0.53	0.21	0.08	0.14	0.07
Area F-T	-0.35	-0.01	-0.49	-0.14	0.44	0.13	0.63	**0.85**	-0.27	**0.88**	**0.87**	0.06	**-0.95**	-0.51	-0.26	0.26	-0.50	0.09	-0.42	-0.34

Table 14 Table with correlations matrix among analytic features of flour and flour blends and biscuit quality characteristics.

Variable (N = 19)	***Proteins***	***Ash***	***Total Starch***	***Resistant Starch***	**IDF**	**SDF**	**TDF**	**Recipe water addition**	**Cookie weight**	**Cookie volume**	**Cookie specific volume**	***Force1***	***Distance1***	***Area F-T***
Proteins	1.00	0.28	-0.10	0.55	0.01	0.52	0.14	0.33	0.02	0.18	0.24	-0.46	0.25	0.05
Ash	0.28	1.00	-0.96	0.27	0.85	-0.48	0.72	0.41	0.60	-0.52	-0.34	0.15	-0.69	0.44
Total Starch	-0.10	-0.96	1.00	-0.25	-0.94	0.46	-0.83	-0.51	0.69	0.55	0.31	-0.23	0.82	-0,59
Resistant Starch	0.55	0.27	-0.25	1.00	0.46	0.32	0.64	0.16	0.08	0.16	0.16	-0.01	0.10	0.70
IDF	0.01	0.85	-0.94	0.46	1.00	-0.39	0.95	0.43	-0.59	-0.46	-0.26	0.33	-0,75	0.79
SDF	0.52	-0.48	0.46	0.32	-0.39	1.00	-0.09	0.33	0.18	0.48	0.55	-0.44	0.44	0.06
TDF	0.14	0.72	-0.83	0.64	0.95	-0.09	1.00	0.53	-0.53	-0.32	-0,09	0.24	-0.64	0.90
Recipe water addition	0.33	0.41	-0.51	0.16	0.43	0.33	0.53	1.00	-0.53	-0.11	0.21	-0.38	-0.54	0.39
Cookie weight	0.02	-0.60	0.69	0.08	-0.59	0.18	-0.53	-0.53	1.00	0.77	0.42	-0.30	0.85	-0.31
Cookie volume	0.18	-0.52	0.55	0.16	-0.46	0.48	-0.32	-0.11	0.77	1.00	0.90	-0.47	0.69	-0.08
Cookie specific volume	0.24	-0.34	0.31	0.16	-0.26	0.55	-0.09	0.21	0.42	0.90	1.00	-0.47	0.39	0.09
Force1	-0.46	0.15	-0.23	-0.01	0.33	-0.44	0.24	-0.38	-0.30	-0,47	-0.47	1.00	-0.22	0.33
Distance1	0.25	-0.69	0.82	0.10	-0.75	0.44	-0.64	-0.54	0.85	0.69	0.39	-0.22	1.00	-0.42
Area F-T	0.05	0.44	-0.59	0.70	0.79	0.06	0.90	0.39	-0.31	-0.08	0.09	0.33	-0.42	1.00

Table 15 Communalities, portion (%) of explained variability by four principal components (PC1, PC2, PC3 and PC4).

		Variable	**PC1**	**PC2**	**PC3**	**PC4**	***Sum***
Analytics	***Basic***	Proteins	2	73	17	8	***100***
		Ash	13	35	50	3	***100***
		Total Starch	53	1	43	3	***100***
		Resistant Starch	4	**70**	25	0	***100***
	Fibre	IDF	43	12	44	1	***100***
		SDF	0	31	**68**	1	***100***
		TDF	**70**	7	21	2	***100***
	Retention capacity	WA-SRC	22	34	21	22	***100***
		SU-SRC	27	16	55	2	***100***
		SC-SRC	45	49	4	2	***100***
		LA-SRC	20	**59**	0	20	***100***
Rheology	***Rapid Visco Analyser***	Pasting Temp	21	7	**60**	12	***100***
		Peak Time	**57**	25	6	12	***100***
		Peak Visc	**74**	25	0	0	***100***
		Hold Time	5	**91**	4	1	***100***
		Hold Temp	5	**90**	4	1	***100***
		Hold Visc	**71**	21	8	1	***100***
		Breakdown	2	0	**98**	0	***100***
		Final Visc	**66**	33	1	0	***100***
		Setback	55	43	1	0	***100***
	FAR	WAC	**58**	37	2	4	***100***
Cookies	***Baking***	Recipe water addition	**62**	24	0	14	***100***
		Cookie weight	47	28	1	25	***100***
		Cookie volume	0	40	23	37	***100***
		Cookie weight	48	27	2	23	***100***
		Cookie volume	0	43	25	32	***100***
		Cookie specific volume	54	3	39	4	***100***
	Texturo-meter	Force1	8	24	24	45	***100***
		Distance1	48	49	3	0	***100***
		Area F-T	**62**	17	0	21	***100***

Annex 7 Nutritional label of the biscuits

Table 16 Nutritional label of biscuits with 100% biscuit wheat flour in their composition.

	Per 100g	**Per 33g**
Energy	1796 kJ/ 429 kcal	593 kJ/ 142 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	62.23 g	20.54 g
Sugar	20.00 g	6.60 g
Starch	37.2 g	12.28 g
Fibre	5.03 g	1.66 g

Protein	9.54 g	3.15 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 17 Nutritional label of biscuits with 100% wholegrain wheat flour in their composition.

	Per 100g	**Per 33g**
Energy	1684 kJ/ 402 kcal	556 kJ/ 133 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	59.99 g	19.80 g
Sugar	20.00 g	6.60 g
Starch	29.76 g	9.82 g
Fibre	10.23 g	3.38 g
Protein	12.56 g	4.14 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 18 Nutritional label of biscuits with 100% wholegrain rye flour in their composition.

	Per 100g	**Per 33g**
Energy	1735 kJ/ 415 kcal	573 kJ/ 137 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	65.22 g	21.52 g
Sugar	20.00 g	6.60 g
Starch	31.77 g	10.48 g
Fibre	13.45 g	4.44 g
Protein	8.43 g	2.78 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 19 Nutritional label of biscuits with 100% buckwheat flour in their composition.

	Per 100g	**Per 33g**
Energy	1783 kJ/ 426 kcal	588 kJ/ 141 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	63.15 g	20.84 g
Sugar	20.00 g	6.60 g
Starch	37.97 g	12.53 g
Fibre	5.18 g	1.71 g
Protein	8.01 g	2.64 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 20 Nutritional label of biscuits with 75% biscuit wheat flour and 25% wholegrain wheat flour in their composition.

	Per 100g	**Per 33g**
Energy	1782 kJ/ 426 kcal	588 kJ/ 141 kcal
Fat	16.67 g	5.50 g

Available carbohydrates	61.66 g	20.35 g
Sugar	20.00 g	6.60 g
Starch	35.33 g	11.66 g
Fibre	6.33 g	2.09 g
Protein	10.3 g	3.40 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 21 Nutritional label of biscuits with 50% biscuit wheat flour and 50% wholegrain wheat flour in their composition.

	Per 100g	**Per 33g**
Energy	1758 kJ/ 420 kcal	580 kJ/ 139 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	61.11 g	20.17 g
Sugar	20.00 g	6.60 g
Starch	33.48 g	11.05 g
Fibre	7.63 g	2.52 g
Protein	11.05 g	3.65 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 22 Nutritional label of biscuits with 25% biscuit wheat flour and 75% wholegrain wheat flour in their composition.

	Per 100g	**Per 33g**
Energy	1739 kJ/ 416 kcal	574 kJ/ 137 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	60.54 g	19.98 g
Sugar	20.00 g	6.60 g
Starch	31.61 g	10.43 g
Fibre	8.93 g	2.95 g
Protein	11.81 g	3.90 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 23 Nutritional label of biscuits with 75% biscuit wheat flour and 25% wholegrain rye flour in their composition.

	Per 100g	**Per 33g**
Energy	1768 kJ/ 423 kcal	583 kJ/ 140 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	62.98 g	20.78 g
Sugar	20.00 g	6.60 g
Starch	35.84 g	11.83 g
Fibre	7.14 g	2.36 g
Protein	9.26 g	3.06 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 24 Nutritional label of biscuits with 50% biscuit wheat flour and 50% wholegrain rye flour in their composition.

	Per 100g	**Per 33g**
Energy	1743 kJ/ 417 kcal	575 kJ/ 138 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	63.72 g	21.03 g
Sugar	20.00 g	6.60 g
Starch	34.48 g	11.34 g
Fibre	9.24 g	3.05 g
Protein	8.99 g	2.97 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 25 Nutritional label of biscuits with 25% biscuit wheat flour and 75% wholegrain rye flour in their composition.

	Per 100g	**Per 33g**
Energy	1712 kJ/ 409 kcal	565 kJ/ 135 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	64.47 g	21.27 g
Sugar	20.00 g	6.60 g
Starch	33.12 g	10.93 g
Fibre	11.35 g	3.75 g
Protein	8.71 g	2.87 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 26 Nutritional label of biscuits with 95% biscuit wheat flour and 5% chickpea flour in their composition.

	Per 100g	**Per 33g**
Energy	1788 kJ/ 427 kcal	590 kJ/ 141 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	61.74 g	20.37 g
Sugar	20.00 g	6.60 g
Starch	36.33 g	11.99 g
Fibre	5.41 g	1.79 g
Protein	10.03 g	3.31 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 27 Nutritional label of biscuits with 90% biscuit wheat flour and 10% chickpea flour in their composition.

	Per 100g	**Per 33g**
Energy	1784 kJ/ 426 kcal	589 kJ/ 141 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	61.24 g	20.21 g
Sugar	20.00 g	6.60 g
Starch	35.44 g	11.69 g
Fibre	5.80 g	1.91 g
Protein	10.53 g	3.47 g

Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 28 Nutritional label of biscuits with 85% biscuit wheat flour and 15% chickpea flour in their composition.

	Per 100g	**Per 33g**
Energy	1778 kJ/ 425 kcal	587 kJ/ 140 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	60.75 g	20.05 g
Sugar	20.00 g	6.60 g
Starch	34.57 g	11.41 g
Fibre	6.18 g	2.04 g
Protein	11.02 g	3.64 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 29 Nutritional label of biscuits with 75% biscuit wheat flour and 25% buckwheat flour in their composition.

	Per 100g	**Per 33g**
Energy	1793 kJ/ 429 kcal	592 kJ/ 142 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	62.46 g	20.61 g
Sugar	20.00 g	6.60 g
Starch	37.39 g	12.34 g
Fibre	5.07 g	1.67 g
Protein	9.16 g	3.02 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 30 Nutritional label of biscuits with 50% biscuit wheat flour and 50% buckwheat flour in their composition.

	Per 100g	**Per 33g**
Energy	1790 kJ/ 428 kcal	591 kJ/ 141 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	62.69 g	20.69 g
Sugar	20.00 g	6.60 g
Starch	37.58 g	12.40 g
Fibre	5.11 g	1.69 g
Protein	8.78 g	2.90 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 31 Nutritional label of biscuits with 25% biscuit wheat flour and 75% buckwheat flour in their composition.

	Per 100g	**Per 33g**
Energy	1787 kJ/ 427 kcal	590 kJ/ 141 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	62.92 g	20.76 g
Sugar	20.00 g	6.60 g
Starch	37.78 g	12.47 g

Fibre	5.14 g	1.70 g
Protein	8.39 g	2.77 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 32 Nutritional label of biscuits with 95% buckwheat flour and 5% chickpea flour in their composition.

	Per 100g	**Per 33g**
Energy	1777 kJ/ 429 kcal	592 kJ/ 142 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	62.61 g	20.66 g
Sugar	20.00 g	6.60 g
Starch	37.05 g	12.23 g
Fibre	5.56 g	1.83 g
Protein	8.58 g	2.83 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 33 Nutritional label of biscuits with 90% buckwheat flour and 10% chickpea flour in their composition.

	Per 100g	**Per 33g**
Energy	1771 kJ/ 423 kcal	584 kJ/ 140 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	62.07 g	20.48 g
Sugar	20.00 g	6.60 g
Starch	36.14 g	11.93 g
Fibre	5.93 g	1.96 g
Protein	9.15 g	3.02 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g

Table 34 Nutritional label of biscuits with 85% buckwheat flour and 15% chickpea flour in their composition.

	Per 100g	**Per 33g**
Energy	1765 kJ/ 422 kcal	582 kJ/ 139 kcal
Fat	16.67 g	5.50 g
Available carbohydrates	61.53 g	20.30 g
Sugar	20.00 g	6.60 g
Starch	35.22 g	11.62 g
Fibre	6.31 g	2.08 g
Protein	9.72 g	3.21 g
Salt	1.00 g	0.33 g
Minerals	0.54 g	0.18 g