JOY CHINENYE MBA

EVALUATION OF THE PHYSICAL, CHEMICAL, TECHNOLOGICAL AND SENSORIAL PROPERTIES OF EXTRUDATES AND COOKIES FROM COMPOSITE SORGHUM AND COWPEA FLOURS

Dissertation submitted to the Food Science and Technology Graduate Program of the Universidade Federal de Viçosa, in partial fulfillment of the requirements for the degree of *Magister Scientiae.*

Advisor: Frederico Augusto Ribeiro de Barros

Co-advisors: Maria Herminia Ferrari Felisberto
Carlos Wanderlei Piler Carvalho

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Dedicated to my daughters, Cherish and Olivia Mba, as a motivation to pursue greater heights, no matter the obstacle on their way.

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ABSTRACT

MBA, Joy Chinenye, M.Sc., Universidade Federal de Viçosa, May, 2023. **Evaluation of the physical, chemical, technological, and sensorial properties of extrudates and cookies from composite sorghum and cowpea flours.** Advisor: Frederico Augusto Ribeiro de Barros. Co-advisors: Maria Herminia Ferrari Felisberto and Carlos Wanderlei Piler Carvalho.

In recent years, there has been a growing demand for gluten-free and functional products, driven by consumer preferences for healthier and more diverse food choices. Then, there is a need to explore new ingredients that can be used as alternatives to traditional gluten-containing grains like wheat. Sorghum and cowpea are two such ingredients that have great potentials. This work evaluated the physical, chemical, technological, and sensorial properties of extrudates and cookies from composite sorghum and cowpea flour. Extrudates and cookies were produced from composite flour of sorghum and cowpea, at ratios (sorghum: cowpea) of 70:30, 50:50 and 30:70. The control samples were 100% sorghum and cowpea extrudates and cookies. Raw flour, extrudates and cookies were characterized (dietary fiber, resistant starch, proteins, antioxidant capacity, pasting properties, etc). Results obtained for particle size distribution and bulk density indicated that the addition of cowpea flour decreased the particle sizes of flour but increased the bulk densities. Also, the addition of cowpea flour increased the expansion ratio of cookies, but did not affect the weight loss. Color parameters (L*, a*, b*, H, and C) for extrudates were increased with the addition of cowpea flour, but that of cookies were not affected. Furthermore, the proximate composition results obtained for flour, extrudates and cookies differed significantly ($p<0.05$). The protein, ash, and dietary fiber contents were observed to increase with an increase in the percentage substitution of cowpea flour. In addition, the values obtained for free phenolics ranged between 0.62-45.34 mg GAE/g, values for tannin ranged between 0.38-47.25 mg Catechin equiv./g, antioxidant capacity ranged from 7.40-211.20 Micromole TE/g sample and that of resistant starch ranged from 0.16-36.29 g/100g. Free phenolics, tannin, and antioxidant capacity decreased with increase in percentage addition of cowpea flour. The addition of cowpea flour increased the crispiness of extrudates, but was not observed to play a role in the hardness of cookies. On the other hand, pasting properties of flour, extrudates and cookies decreased with addition of cowpea flour. Finally, the sensory evaluation scores for extrudates and cookies indicated that the products had good acceptability, with all evaluated products having scores above the cutoff score of 5. In conclusion, gluten-free extrudates and cookies of good acceptability can be produced from sorghum flour with up to 50% substitution of cowpea flour.

Keywords: Gluten-free cookie. Phenolics. Tannins. Antioxidant capacity. Resistant starch. Extrusion. Baking.

RESUMO

MBA, Joy Chinenye, M.Sc., Universidade Federal de Viçosa, maio de 2023. **Avaliação das propriedades físicas, químicas, tecnológicas e sensoriais de extrusados e biscoitos tipo "cookies" de farinhas compostas de sorgo e feijão-caupi.** Orientador: Frederico Augusto Ribeiro de Barros. Coorientadores: Maria Herminia Ferrari Felisberto e Carlos Wanderlei Piler Carvalho.

Nos últimos anos, tem havido uma demanda crescente por produtos sem glúten e funcionais, impulsionada pelas preferências dos consumidores por escolhas alimentares mais saudáveis e diversificadas. Então, há uma necessidade de explorar novos ingredientes que possam ser usados como alternativas aos grãos tradicionais que contêm glúten, como o trigo. O sorgo e o feijão-caupi são dois desses ingredientes com grande potencial. Este trabalho avaliou as propriedades físicas, químicas, tecnológicas e sensoriais de extrusados e biscoitos de farinha composta de sorgo e feijão-caupi. Extrudados e biscoitos foram produzidos a partir de farinha composta de sorgo e feijão caupi, nas proporções (sorgo: feijão caupi) de 70:30, 50:50 e 30:70. As amostras controle foram 100% sorgo e extrusados e biscoitos de feijão-caupi. Farinha crua, extrusados e biscoitos foram caracterizados (fibra alimentar, amido resistente, proteínas, capacidade antioxidante, propriedades de pasta, etc). Os resultados obtidos para distribuição granulométrica e densidade aparente indicaram que a adição de farinha de feijão-caupi diminuiu a granulometria da farinha, mas aumentou a densidade aparente. Além disso, a adição de farinha de feijão-caupi aumentou a taxa de expansão dos biscoitos, mas não afetou a perda de peso. Os parâmetros de cor (L*, a*, b*, H e C) dos extrusados aumentaram com a adição de farinha de feijão-caupi, mas os biscoitos não foram afetados. Além disso, os resultados de composição centesimal obtidos para farinha, extrusados e biscoitos diferiram significativamente ($p<0,05$). Observou-se que os teores de proteína, cinzas e fibra alimentar aumentaram com o aumento da porcentagem de substituição da farinha de feijão-caupi. Além disso, os valores obtidos para fenólicos livres variaram entre 0,62-45,34 mg GAE/g, os valores para taninos variaram entre 0,38-47,25 mg Catechin equiv./g, a capacidade antioxidante variou de 7,40-211,20 Micromole TE/g de amostra e a de resistente amido variou de 0,16-36,29 g/100 g. Fenólicos livres, taninos e capacidade antioxidante diminuíram com o aumento da porcentagem de adição de farinha de feijão-caupi. A adição de farinha de feijão-caupi aumentou a crocância dos extrusados, mas não influenciou na dureza dos biscoitos. Por outro lado, as propriedades de pasta das farinhas, extrusados e biscoitos diminuíram com a adição de farinha de feijão-caupi. Por fim, as notas da avaliação sensorial para extrusados e biscoitos indicaram que os produtos tiveram boa aceitabilidade, com todos os produtos avaliados tendo notas acima da nota de corte de 5. Em conclusão, extrusados sem glúten e biscoitos de boa aceitabilidade podem ser produzidos a partir de farinha de sorgo com até 50% de substituição da farinha de feijão-caupi.

Palavras-chave: Biscoito sem glúten. Fenólicos. Taninos. Capacidade antioxidante. Amido resistente. Extrusão. Cozimento.

SUMMARY

1 INTRODUCTION

In recent years, there has been a growing demand for gluten-free and functional products, driven by consumer preferences for healthier and more diverse food choices (Lavriša *et al*., 2020). According to a report by Grand View Research, the global gluten-free products market size was valued at USD 5.9 billion in 2021 and is expected to grow at a compound annual growth rate (CAGR) of 9.8% from 2022 to 2030. This growth can be attributed to an increasing prevalence of celiac disease, rising consumer awareness regarding gluten intolerance, and the availability of a wide range of gluten-free products in the market (Grand view Research, 2021). As the demand for gluten-free products and functional foods continues to grow, there is a need to explore new ingredients that can be used as alternatives to traditional gluten-containing grains like wheat, barley, and rye (Šmídová, & Rysová, 2022).

Sorghum (*Sorghum bicolor)* is a versatile, drought-tolerant cereal commonly grown in semi-arid regions of Africa, Asia, Australia, North and South America (Hossain *et al*., 2022). Sorghum is known for its amazing agronomic performance due to its ability to adapt to a variety of environments (Hossain *et al*., 2022). In addition to its agronomic benefits, sorghum grain is gluten-free, high in resistant starch, nutrient dense and, most importantly, it contains a variety of phenolic compounds such as phenolic acids, flavonoids, and condensed tannins (Awika & Rooney, 2004; Dykes & Rooney, 2007; Girard & Awika, 2018). These bioactive compounds have been linked to several health benefits such as improvements in glucose and lipid metabolism, insulin sensitivity and decreased gastric emptying time, in addition to reducing fat accumulation, and markers of oxidative stress and inflammation (Shen *et al.,* 2018; Girard & Awika, 2018).

Cowpea (*Vigna unguiculata*) is a nutritious crop that thrives in locations unsuitable for growing most other edible legumes as it is heat and drought tolerant, making it an environmentally and climate friendly crop (Mekonnen *et al*., 2022). It is an important source of high-quality dietary protein for millions of people living in semi-arid regions and is the most produced legume after the common dry bean (*Phaseolus vulgaris*) and chickpea (*Cicer arietinum*) (FAO, 2014; Abebe & Alamayehu, 2022). Along with the nutritional benefits, cowpea is also rich in dietary fiber, flavonols, flavan-3-ols, and bioactive peptides that contribute to the prevention of diseases such as cancer and diabetes (Abebe & Alamayehu, 2022; Ojwang *et al*., 2013; Quansah *et al*., 2013; Segura-Campos *et al*., 2011).

In addition to the numerous health benefits associated with the consumption of the bioactive compounds present in sorghum and cowpea separately, researchers have also reported that the synergistic interaction of bioactive compounds from these grains can enhance their bioavailability, leading to improved health benefits (Awika *et al.,* 2018; Chen *et al.,* 2022). For instance, in a study conducted by Agah *et al.* (2017), they demonstrated that sorghum-cowpea flavones showed strong anti-inflammation synergy, when they combined flavonoids extracted from sorghum and cowpea. Awika et al. (2018) noted that the key bioactive components (phenolic compounds and dietary fiber) of sorghum and cowpea are structurally different and have been reported to provide complementary health benefits to consumers beyond their complementary amino acid nutrition. Hence, combining sorghum and cowpea would most likely result in better balanced and enhanced bioactive compounds that would potentially address most inflammation-related health issues (Apea-Bah *et al.*, 2014; Awika *et al.,* 2018).

Despite possessing many bioactive compounds and almost similar nutritional content compared to other cereals and legumes, products from sorghum and cowpea on both small and large scales are far below their potentials (Apea-Bah *et al.*, 2014). Moreover, sorghum is underappreciated by many communities and viewed as an inferior grain that should only be eaten by poor and vulnerable people, as it has been reported to have reduced protein digestibility and high contents of phytic acids and tannins (Rooney, 2003), whereas cowpea is mostly used in traditional dishes as a result of its beany flavor (Okaka & Potter, 1979). This is one of the challenges preventing these crops from reaching their full market potentials. Interestingly, these challenges can be overcome by adequate processing of sorghum and cowpea, through their use in various value-added products, with improved nutritional and functional properties. For instance, cowpea has been used to improve technological and nutritional profiles of baked and fried products, and comminuted meat products like chicken nuggets and meat balls (Phebean *et al.*, 2017; Ngoma, *et al.*, 2018; Marchini *et al.*, 2021), and sorghum bran can improve antioxidant capacity and dietary fiber of cookies (Queiroz *et al.*, 2022). Thus, this research is aimed at evaluating, for the first time, the physical, chemical, technological, and sensorial properties of extrudates and cookies from mixed tannin sorghum and white cowpea flours.

2. LITERATURE REVIEW

2.1 Sorghum

Sorghum (*Sorghum bicolor* L. Moench) is a versatile, drought-tolerant crop commonly grown in semi-arid regions of Africa, Asia, Australia, and North and South America (Hossain *et al*., 2022). It is one of the leading cereal crops in the world, ranking fifth among the highest-producing cereal crops after corn, wheat, rice, and barley, with annual global production of 57.6 million tonnes in 2017 (FAO, 2017). Sorghum has been widely grown in tropical and subtropical regions. In Asian and African countries like India and Nigeria, sorghum is used to make foods like bread and porridge. In some underdeveloped and semi-arid regions, it serves as the main source of energy and food for humans and livestock (Rooney & Waniska, 2000). In western countries like the United States, Mexico and Australia, sorghum is grown primarily as animal feed. However, due to the natural compounds found in sorghum that are beneficial for the development of healthy and functional foods, there is increasing interest in cultivating sorghum for biofuel production as well as food for human consumption (Taleon *et al.,* 2012).

Sorghum is known for its amazing agronomic performance due to its ability to adapt to a variety of environments. It is drought tolerant, heat tolerant, and can grow at high altitudes and on saline-alkaline and barren soils (Hossain *et al*., 2022). This is because sorghum has a well-developed root system with a high root-to-leaf ratio and the leaves are protected by wax and can also self-roll in response to external threats/stimuli (Rooney & Waniska, 2000). In addition to its agronomic benefits, sorghum grain is gluten-free, high in resistant starches, nutritious, and most importantly, contains a variety of bioactive phenolic compounds (Awika & Rooney, 2004; Dykes & Rooney, 2007). Sorghum contains more abundant and diverse phenolic compounds compared to other major grains; it contains almost all classes of phenolic compounds, with simple phenolic acids, flavonoids and tannins being the dominant groups (Dykes & Rooney, 2007; Shen *et al.,* 2018).

2.2 Sorghum grain Genotypes

The bran layer (pericarp and testa), the endosperm, and the germ make up the sorghum grain (naked caryopsis), although some sorghum varieties may have a pigmented testa situated between the pericarp and endosperm (Earp *et al*., 2004; Waniska & Rooney, 2000). The pericarp and testa of the sorghum bran layer include non-starch polysaccharides, a wide range of phenolic substances such phenolic acids, flavonoids, and condensed tannins, as well as certain vitamins like carotenoids. Starch, proteins, and a few vitamins and minerals (including the vitamin B complex) make up most of the endosperm. According to Earp *et al*. (2004), the germ fraction is mostly made up of lipids and proteins and is also rich in vitamins and minerals, particularly the vitamin B complex and fat-soluble vitamins.

The phenolic profile of sorghum varies significantly between varieties and is influenced by sorghum genotypes as well as growth and environmental conditions (Wu *et al*. 2016). With more than 235,000 sorghum germplasm accessions having been gathered globally, sorghum has a very diversified genetic makeup and a vast range of cultivars that come in different colors, sizes, structures, and shapes (Awika & Rooney, 2004; Bean *et al.*, 2016). The genes that affect the pericarp color (R and Y gene), pericarp thickness (Z gene), and the existence of pigmented testa all play a role in determining the color, which is strongly tied to the phenolic profile of the grain, especially the bran layer of the grain (Rooney & Waniska, 2000). Sorghum can be roughly grouped into five categories based on color, phenolic profile, and genotypes: white, yellow, red, brown, and black sorghums.

When the Y gene is recessive, regardless of the R gene, white sorghum has a white or colorless pericarp (rryy or R yy). White sorghum has very low or no quantities of tannin and 3-deoxyanthocyanidin, as well as low levels of total phenolic content (Awika & Rooney, 2004). When the R gene is recessive and the Y gene is dominant (rrY), yellow sorghum develops a yellow pericarp, is high in flavanones, and has a little greater total phenolic content than white sorghum (Dykes *et al.*, 2011). The genes R and Y are both dominant in sorghum varieties with pericarps that are red or black (R Y). Red sorghum lacks tannins but contains moderately high quantities of phenolic chemicals. Black sorghum is a unique variety of red sorghum that is genetically red. As it matures, the red pericarp turns black due to exposure to sunlight. In the pericarp of black sorghum, there are large concentrations of phenolic compounds, especially 3-deoxyanthocyanidins (Dykes *et al.,* 2013; Dykes, *et al.,* 2005; Dykes *et al.*, 2009). Tannin sorghum, commonly referred to as brown sorghum, has colored testa and a lot of condensed tannins (Awika & Rooney, 2004).

Fig. 1: Black, white, and red sorghum grain phenotypes (Source: Awika, 2017).

2.3 Nutritional Profile of Sorghum grain

The nutrient composition of sorghum grains varies between varieties. In general, carbohydrates (starch and non-starch polysaccharides), proteins, and lipids are the main components of grain (Hill, *et al.,* 2012; Leder, 2004). On average, 100 g of grain contains about 72.1 g of carbohydrates, 12.4 g of water, 10.6 g of protein, 6.7 g of fiber and 3.5 g of lipids, providing about 1,377kJ of energy (USDA, 2019).

Starch is the dominant carbohydrate in sorghum and is stored as granules in the endosperm. The starch content varies greatly between varieties from 32.1 to 72.5 g per 100 g grain (Udachan *et al.,* 2012). Sorghum starch consists mainly of amylose and amylopectin, but some waxy sorghums may have little or no amylose (Dicko *et al.,* 2006). The high content of resistant starch and slow-digesting starch, as well as strong interactions between starch granules, endosperm proteins and condensed tannins reduce the starch digestibility of sorghum compared to other cereal crops (Barros *et al*., 2012). Sorghum is a rich source of fiber because the non-starch carbohydrate in sorghum consists primarily of insoluble fiber (75% to 90%) and soluble fiber (10% to 25%) found in the cell walls of the pericarp and endosperm about 6 to 15 g per 100 g grain (Taylor & Emmambux, 2010). Sorghum non-starch carbohydrate consists primarily of arabinoxylans and β-glucans. The arabinoxylans are essentially glucuronoarabinoxylans and contain bound p-coumaric acid and ferulic acid, important phenolic acids in sorghum (Verbruggen *et al.,* 1998).

The protein in sorghum can be broadly divided into prolamin proteins (like kafirins) and non-prolamin proteins (like globulins, glutelins, and albumins). Kafirins are the main form of protein storage in sorghum grain, accounting for 70% of the total protein in whole sorghum grain, with the remaining albumins, glutelins and globulins making up the remainder (Belton, *et al*., 2006). There are four types of kafirin based on molecular weight namely α-, β-, γ, and δ -kafirin and these proteins are hydrophobic proteins and are stored in tightly coated protein bodies in the endosperm (Belton *et al.,* 2006). Sorghum grain is rich in glutamic acid, proline, and leucine. However, like other cereal grains, lysine deficiency can occur, although this problem can be improved by sorghum breeding or food fortification (Belton & Taylor, 2004; Galili & Amir, 2013).

Sorghum proteins are not easily digested. Sorghum kafirins have a high degree of polymerization and extensive disulfide bonds that are resistant to enzymatic digestion in the digestive tract; their strong interaction with tannins and starch also hinders protein digestion (Belton et al., 2006; Da Silva et al., 2011). Despite these properties, sorghum's low starch and protein digestibility makes it a promising food source for people with obesity and diabetes.

The lipid in the sorghum grain consists mainly of unsaturated fatty acids, with polyunsaturated fatty acids being the most common. The primary fatty acids in sorghum are oleic, linoleic, palmitic, linolenic, and stearic acids; the lipid profile is like that of corn but more unsaturated (Adeyeye & Ajewole, 1992; USDA, 2019). Sorghum is also a good source of vitamins and minerals. The vitamin B complex (pyridoxine, riboflavin, and thiamine) and some fat-soluble vitamins (vitamins A, D, E, and K) are the major vitamins in sorghum, with potassium, phosphorus, magnesium, and zinc being the major minerals (Leder, 2004; Martino et al., 2012). The high content of resistant starch, fiber, relatively difficult to digest proteins (kafirins) and unsaturated fatty acids gives sorghum unique nutritional properties that are beneficial to health. The nutritional composition of sorghum grain as reported by Makokha et al. (2002) is shown on Table 1.

Table 1. Nutritional composition of sorghum

Constituents (%)	**Range**
Protein	4.40-21.10
Water soluble protein	0.30-0.90
Lysine	1.06-3.64
Starch	55.60-75.20
Amylose	21.20-30.20
Soluble sugar	0.70-4.20
Reducing sugar	0.05-0.53
Crude fiber	1.00-3.40
Fat	2.10-7.60
Ash	1.30-3.30
Minerals (mg/100g)	
Calcium	11.00-586
Phosphorus	167-751
Iron	0.90-20
Vitamins (mg/100g)	
Thiamine	0.24-0.54
Niacin	2.90-6.40
Riboflavin	0.10-0.20
Antinutritional factors	
Tannin (%)	0.1-7.22
Phytic acid (mg/100g) as Phytin Phosphate	875-2211.90

Source: Makokha *et al.* [2002]

2.4 Bioactive Compounds in Sorghum Grain

Most bioactive compounds in cereal grains are secondary metabolites made by plants as defense or signaling molecules or as structural molecules; most of these compounds are structurally categorized as polyphenols or lipids. The phenolic compounds in sorghum mainly include phenolic acids, flavonoid derivatives, condensed tannins, stilbenes and lignins produced by the phenylpropanoid pathway, while lipids mainly include esters of phytosterols/stanols and policosanols (Figure 2.2). Occasionally, the lipids are found esterified with phenolic compounds, particularly phenolic acids (Awika and Rooney, 2004).

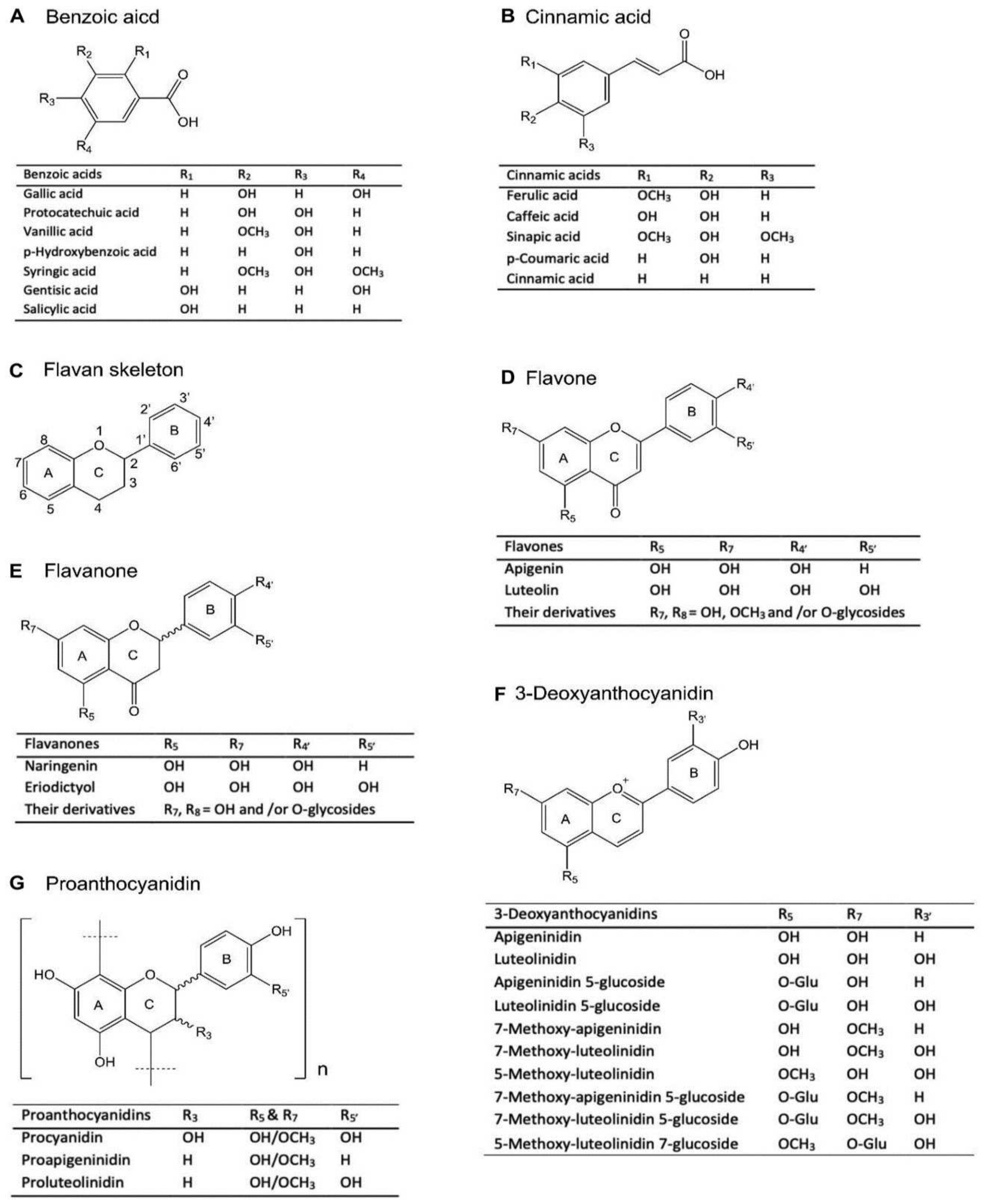
In sorghum, as in other cereal grains, the bioactive compounds are mainly found in the bran fraction (pericarp, testa and aleurone tissues). The compounds coexist with the abundant cell wall polysaccharides in cereal bran and contribute significantly to the health benefits attributed to whole grain ingestion (Awika *et al.*, 2018; Vitaglione *et al.*, 2015). Consequently, food processing methods that remove the bran will severely impact the health benefits of cereal grains. Sorghum has a more diverse and/or higher content of polyphenols and bioactive lipids than most other cereal grains.

2.4.1 Phenolic acids

Phenolic acids are the simplest but most abundant phenolic compounds present in all sorghum grains, with a total concentration of 445 to 2,850 g/g (Girard & Awika, 2018). The phenolic acids can be divided into two categories according to their structure: benzoic and cinnamic acid. The major phenolic acids reported in sorghum grain are gallic, vanillic, protocatechuic, cinnamic, p-coumaric, p-hydroxybenzoic, syringic, ferulic, caffeic, and sinapic acid (Althwab, *et al.*, 2015; Vanamala, *et al.*, 2018).

The phenolic acids are present in the endosperm, pericarp and testa of grain and exist in both free and bound forms. The free phenolic acids, which are extractable by organic solvents, are not bound to the cell wall and are mainly found in the pericarp and testa. They are often conjugated with monomeric carbohydrate and glycerol and exist in the free form as esters (conjugated) or aldehydes (non-conjugated). The ester conjugates are the primary extractable phenolic acids in sorghum (Dykes & Rooney, 2006; Svensson, *et al.*, 2010; Yang, *et al.*, 2012).

The bound phenolic acids are bound to the cell wall (lignin) via covalent bonds and are also part of the cell wall structure, and the extraction requires acidic or alkaline conditions and high temperatures to break the covalent bonds (Wu *et al.*, 2017). Most of the phenolic acids (about 70% to 95%) in sorghum are present in a bound form. Among them, ferulic acid (100 to 500 g/g in grain) is the most common and can account for up to 90% of the total bound phenolic acids (Chiremba, *et al.*, 2012; Yang *et al.*, 2012). The bound phenolic acids have low bioavailability due to the extensive covalent bonds that are resistant to enzymatic digestion (Saura-Calixto, 2010). Furthermore, since the bound phenolic acids are part of the cell wall constituents, its concentration is also directly related to grain hardness, with higher concentrations being associated with harder grain (Chiremba *et al*., 2012).



Benzoic acids	R_1	R_2	R_3	R_4
Gallic acid	H	OH	H	OH
Protocatechuic acid	H	OH	OH	H
Vanillic acid	H	OCH_3	OH	H
p-Hydroxybenzoic acid	H	H	OH	H
Syringic acid	H	OCH_3	OH	OCH_3
Gentisic acid	OH	H	H	OH
Salicylic acid	OH	H	H	H

Cinnamic acids	R_1	R_2	R_3
Ferulic acid	OCH_3	OH	H
Caffeic acid	OH	OH	H
Sinapic acid	OCH_3	OH	OCH_3
p-Coumaric acid	H	OH	H
Cinnamic acid	H	H	H

Flavones	R_5	R_7	$R_{4'}$	$R_{5'}$
Apigenin	OH	OH	OH	H
Luteolin	OH	OH	OH	OH
Their derivatives	R_7, R_8 = OH, OCH_3 and /or O-glycosides			

Flavanones	R_5	R_7	$R_{4'}$	$R_{5'}$
Naringenin	OH	OH	OH	H
Eriodictyol	OH	OH	OH	OH
Their derivatives	R_7, R_8 = OH and /or O-glycosides			

3-Deoxyanthocyanidins	R_5	R_7	$R_{3'}$
Apigeninidin	OH	OH	H
Luteolinidin	OH	OH	OH
Apigeninidin 5-glucoside	O-Glu	OH	H
Luteolinidin 5-glucoside	O-Glu	OH	OH
7-Methoxy-apigeninidin	OH	OCH_3	H
7-Methoxy-luteolinidin	OH	OCH_3	OH
5-Methoxy-luteolinidin	OCH_3	OH	OH
7-Methoxy-apigeninidin 5-glucoside	O-Glu	OCH_3	H
7-Methoxy-luteolinidin 5-glucoside	O-Glu	OCH_3	OH
5-Methoxy-luteolinidin 7-glucoside	OCH_3	O-Glu	OH

Proanthocyanidins	R_3	R_5 & R_7	$R_{5'}$
Procyanidin	OH	OH/OCH_3	OH
Proapigeninidin	H	OH/OCH_3	H
Proluteolinidin	H	OH/OCH_3	OH

Figure 2: Structure of major sorghum phenolic compounds (Source: Xiong, *et al.,* 2019).

2.4.2 Flavonoids

Flavonoids are mainly found in sorghum bran, and the types and concentrations are related to the color and thickness of the pericarp and the presence of pigmented testa (Awika *et* *al.*, 2005; Dykes *et al.*, 2005). They are the largest class of phenolic compounds in plants and represent the most abundant and diverse phenolic compounds in sorghum. Flavonoids share the basic flavan skeleton and their classification is based on the presence of C2-C3 double bonds and substituent groups on the C-ring (Buer, *et al.*, 2010). A broad class of flavonoids have been found in sorghum, including anthocyanins (3-deoxyanthocyanidins), flavones, flavanones, flavan-3-ols, flavan-4-ols, flavonols and dihydroflavonols (Awika, 2017; de Morais Cardoso *et al.*, 2017). Among the flavonoids, 3-deoxyanthocyanidins, flavones and flavanones are the dominant compounds in sorghum.

2.4.2.1 Flavones

Flavones are yellow-colored flavonoids that are commonly found in fruits, vegetables, legumes, and in grains. Although cereal grains generally contain modest amounts of flavones, they represent one of the main dietary sources of flavones and therefore play an important role in human nutrition (Zamora-Ros *et al.,* 2016). The flavone content of sorghum grain is around 20 to 390 g/g, relatively low compared to other flavonoids (Girard & Awika, 2018). Some flavonoids occur naturally in the form of glycosides such as luteolin, but others such as apigenin exist primarily as aglycones (Yang *et al.,* 2012; Yang, *et al.,* 2015). The glycosides in sorghum are predominantly O-glycosides and are very unstable in acidic environments because the glycosidic bonds are easily hydrolyzed, forming the aglycones; the aglycones of luteolin and apigenin are the dominant flavones in sorghum (Dykes *et al.*, 2011; Yang *et al.,* 2015).

In general, both luteolin and apigenin are the dominant flavones in sorghum; However, in other cereal grains, the flavones are mainly present as apigenin and in the form of C-glycosides (Dykes & Rooney, 2007; Dykes *et al.,* 2011; Dykes *et al.,* 2009). The O-glycosides are more biologically accessible than C-glycosides in the acidic gastric environment due to the easily hydrolyzable property and therefore have high bioactivity at low concentrations (Yang et al., 2015; Zamora-Ros *et al.,* 2016). Therefore, sorghum flavones are more abundant than other grains with higher bioavailability. Sorghum varieties with red and yellow pericarp are often said to be high in flavones (Dykes *et al.,* 2011; Dykes *et al.,* 2009).

2.4.2.2 Flavanones

Flavanones are widespread in food plants, with naringenin and its derivatives being the dominant ones. They are the main intermediates in the biosynthesis of flavonoids, but are rarely found in cereals (Awika, 2017). Sorghum seems to be an exception and some genotypes have been reported to have the highest levels of flavanones among food crops (Awika, 2017). The flavanone content of sorghum ranges from 0 to 2,000 µg/g. The lowest level is reported in white sorghum and the highest level is found in sorghum with a yellow pericarp (Bhagwat *et al.,* 2014; Dykes *et al*., 2011; Yang *et al*., 2015). Naringenin and eriodyctiol glycosides are the main flavanones in sorghum, while their aglycones and O-methylated derivatives are relatively limited (Yang *et al*., 2012; Yang *et al.,* 2015). Like the flavones, the flavanone glycosides are mainly the O-glycosides, sensitive to low pH, easily hydrolyzed and highly bioavailable (Yang *et al*., 2015).

2.4.2.3 3-Deoxyanthocyanidins

The unique characteristic of sorghum flavonoids is their anthocyanin content. Anthocyanins are a source of natural water-soluble pigments and antioxidants (Riaz, *et al.,* 2016). Most of the natural anthocyanins in plants are C-3-hydroxylated anthocyanins; However, the anthocyanins found in sorghum are almost exclusively the C-3 deoxylated analogs- 3-deoxyanthocyanidins, which represent a rare subclass of anthocyanins (Awika, *et al.,* 2004; Xiong, *et al.,* 2019). Both 3-deoxyanthocyanidins and anthocyanins originate from the flavanone biosynthetic pathway but are distinct from flavanone intermediates (Kawahigashi *et al*., 2016; Liu *et al*., 2010). The main difference between 3-deoxyanthocyanidins and anthocyanins is the lack of an -OH group in position C-3, and this structural difference gives 3-deoxyanthocyanidins unique chemical properties (Awika *et al.,* 2004).

The main 3-deoxyanthocyanidins in sorghum are apigeninidin and luteolinidin aglycones. Their derivatives including the methoxylated form (7-methoxy-apigeninidine, 7-methoxy-luteolinidine and 5-methoxy-luteolinidine), glycosides (apigeninidine-5-glucoside and luteolinidine-5-glucoside) and methoxylated glycosides (7-methoxy-apigeninidine 5 - glucoside, 7-methoxy-luteolinidin-5-glucoside, and 5-methoxy-luteolinidin-7-glucoside) have also been reported and are present in relatively moderate or small amounts (Dykes *et al.,* 2013; Petti *et al*., 2014; Wu & Prior, 2005). In addition, other new derivatives such as dimeric 3-deoxyanthocyanidins (e.g., apigeninidin flaven dimer) and pyran-3-deoxyanthocyanidins (an extra ring attached to the C-4 and C-5 position) have also been reported and demonstrated promising color stability (Bai *et al.,* 2014; Geera, *et al.,* 2012; Khalil, *et al.,* 2010).

3-Deoxyanthocyanidins are one of the most common flavonoids in sorghum, with a total concentration of 200 to 4,500 µg /g. In some sorghum varieties, 3-deoxyanthocyanidin content can account for up to 80% of the total flavonoids in the grain (de Morais Cardoso *et al*., 2017; Girard & Awika, 2018). 3-deoxyanthocyanidins are up to four to five times more concentrated in the bran layer of the grain than in the whole grain, and sorghum with a red pericarp genotype

(RY) is particularly rich in 3-deoxyanthocyanidins (Awika *et al.,* 2005). Among red pericarp sorghum genotypes, black sorghum bran (genotypically red but phenotypically black) has the highest levels of 3-deoxyanthocyanidins (1,790 to 6,120 µg/g), at least two times higher than red (both genotypically and phenotypically red), and brown sorghum bran (red genotype with pigmented testa) (Awika *et al.,* 2005; Dykes *et al*., 2005). In addition, 3-deoxyanthocyanidins are also distributed in other plant tissues of sorghum such as the husk and leaves at a concentration of up to 90,000 µg/g (Geera *et al.,* 2012; Petti *et al*., 2014).

Apart from providing attractive orange/red to blue/purple colors for plants, 3-deoxyanthocyanidins are powerful antioxidants with antimicrobial activity (Xiong *et al.,* 2019). Sorghum is considered the main food source of 3-deoxyanthocyanidins for humans.

2.4.3 Condensed tannins

Tannins are one of the most studied polyphenols in sorghum. Of course, they are widely distributed in plants such as grapes, tea and legumes as monomers or low molecular weight oligomers (Ojwang, *et al*., 2013; Savolainen, 1992). However, sorghum tannins exist in a condensed form with high molecular weight and a high degree of polymerization (DP), which are not common in major cereals (Wu *et al*., 2012). Sorghum-condensed tannins are composed of oligomers or polymers of primarily flavan-3-ol and flavan-3,4-diol and are linked primarily by B-type bonds, with an average DP of about 20 compared to other tannin-containing cereal grains of 3 to 10 DP (Dykes & Rooney, 2006; Girard & Awika, 2018). The low molecular weight forms such as monomers (mainly catechin) and dimers (mainly procyanidin B1) are present in small amounts in sorghum grains (Awika & Rooney, 2004).

In general, sorghums with pigmented testa have high levels of condensed tannin on grain (Dykes *et al.,* 2013; Girard & Awika, 2018). High tannin sorghums have agronomic benefits by protecting the plant from pathogens and birds and have been commonly grown in some underdeveloped regions with food security issues (Kil *et al*., 2009; Taylor, 2003).

2.5 Health Benefits of Sorghum Grain’s Bioactive Compounds

Due to its nutritional value and the availability of phytochemicals that help prevent non-communicable diseases, sorghum grain consumption has demonstrated health advantages (Stefoska-Needham *et al.,* 2015). Several research have demonstrated the health advantages of sorghum, which can vary from antioxidant and anti-inflammatory potential to metabolic effects on obesity and glycemic indices (Martinez *et al*., 2021; Silva *et al.,* 2020; Arbex *et al.,* 2018; Moraes *et al.,* 2015).

2.5.1 Antioxidant activity

The main cause of various chronic diseases is oxidative stress, which is an imbalance of free radicals and antioxidants (Lee *et al*., 2011). The antioxidant activity of sorghum phenolic compounds appears to play a key role in health promotion and disease prevention associated with sorghum consumption. Several methods have been used to measure the antioxidant activity of natural compounds and these methods are based almost exclusively on colorimetric methods using *in vitro* assays. Oxygen Radical Absorption Capacity (ORAC), ferric iron reducing antioxidant power, 2,2-azino-bis- (3- ethylbenzothiazoline -6-sulfonic acid) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) to scavenge free radicals are some of the methods currently used widely for estimating the antioxidant activity of sorghum *in vitro*.

The phenolic compounds extracted from sorghum grain have the highest antioxidant activity among cereals when compared with wheat, rice and corn and are also comparable to common fruits and vegetables (Adom & Liu, 2002). Awika *et al*. (2003b), noted that antioxidant activity is closely related to total phenolic content, mainly condensed tannin content, in sorghum. Sorghums with condensed tannins (black and brown sorghums) have consistently shown high antioxidant activity *in vitro*, particularly in the bran where phenols are concentrated (Awika *et al*., 2005; Dykes *et al.,* 2005).

Aside from the direct antioxidant effects, the phenolic compounds from sorghum have been shown to induce endogenous detoxifying enzymes (phase II enzymes) which are responsible for converting the harmful reactive oxygen or nitrogen species into non-toxic compounds and thus indirectly enhancing the body's anti-oxidative defense mechanism thereby improving stress (Awika, *et al.,* 2009; Yang *et al.,* 2009).

Among the phenolic compounds present in sorghum, 3-deoxyanthocyanidins have shown the strongest influence on the phase II enzyme activity, particularly, the activity of the enzyme NADH:quinonoxyreductase (NQO). 3-deoxyanthocyanidins are strong NQO inducers; Both 3-deoxyanthocyanidin standards and 3-deoxyanthocyanidin-rich sorghum extract have been reported to significantly increase NQO activity in some cancer cells (Gonzalez-Montilla *et al*., 2012), and such an effect has not been reported in their anthocyanin analogues. The inducing ability of 3-deoxyanthocyanidins on the phase II enzyme varies widely with their structure and substitution. Methoxylated substitution at positions C-5 and C-7, such as 7-methoxyapigeninidine and 5,7-dimethoxyapigeninidine, can considerably enhance the inducing effect on NQO activity (Awika *et al.,* 2009; Yang *et al.,* 2009).

2.5.2 Anti-inflammatory activity

Prolonged oxidative stress can lead to chronic inflammation and thus to various chronic diseases. Through inflammation, several pro-inflammatory compounds such as interleukin (IL), cyclooxygenase (COX)-2, tumor necrosis factor (TNF) and prostaglandin E2 (PG-E2) are formed (Shim*, et al.,* 2013). Many phenolic compounds from sorghum grain have been shown to inhibit the production of these pro-inflammatory compounds (Burdette, 2007; Funakoshi-Tago, *et al*., 2011; Makanjuola, *et al*., 2018). Phenolic acids found in sorghum, such as gallic acid and ferulic acid, have been reported to suppress the COX-2 enzyme, and ferulic acid has been shown to inhibit the production of TNF- (Burdette, 2007). Flavone apigenin and luteolin have been reported to inhibit the production of COX-2 and inhibit the transcription factor (nuclear factor kappa B) that activates the production of these pro-inflammatory compounds (Agah, *et al.* 2017; Funakoshi-Tago *et al.*, 2011). 3-Deoxyanthocyanidins have also been shown to subdue the production of COX-2 and PG-E2 (Makanjuola *et al*., 2018).

The inhibitory effects against the pro-inflammatory compounds are believed to be important for disease prevention. Recent studies have also shown that the combination of flavone-apigenin and flavonol-quercetin, as well as the apigenin-rich extract of sorghum and the quercetin-rich extract of cowpea, has a powerful synergistic anti-inflammatory effect by stimulating their bioavailability through the suppression of the phase II metabolism and ATP binding cassette membrane transporter function in cellular models (Agah *et al*., 2017; Ravisankar *et al*., 2019). It has been proposed that the C2-C3 conjugation structure of apigenin and quercetin may play an important role in enhancing anti-inflammatory effects (Ravisankar *et al*., 2019).

2.5.3 Cancer prevention

The phenolic compounds from sorghum have shown anticancer activity, and consumption of whole sorghum grain may reduce the risk of developing certain cancers (Chen, *et al.,* 1993; Isaacson, 2005). The anticancer activity of sorghum can be attributed to the potent antioxidant activity and phase II enzyme induction of its phenolic compounds (Awika *et al.,* 2009).

Among the sorghum phenolic compounds, 3-deoxyanthocyanidins have been the most widely studied. Both 3-deoxyanthocyanidins and 3-deoxyanthocyanidin-rich sorghum extract have been shown to be effective against the growth of various cancer cells, including colon, hepatoma, esophageal, intestinal epithelial, leukemia, breast, and gastric cancer cells. These compounds act directly against cancer by inducing cell apoptosis and inhibiting cancer cell proliferation and metastasis (Devi, *et al.,* 2011; Woo *et al*., 2012; Suganyadevi, *et al.,* 2013; Massey, *et al.,* 2014).

Sorghum condensed tannin may also play a significant role in cancer prevention. Sorghum tannins have been shown to inhibit aromatase (an enzyme implicated in breast cancer), thereby preventing the formation of unwanted cancer growth stimuli (Hargrove, *et al.,* 2011; Huang, *et al*., 2009). In addition, other phenolic sorghum compounds such as flavones and flavanones have also shown anticancer activity, particularly the flavone apigenin, which has been reported to activate estrogenic activity and induce apoptosis of colon cancer cells (Yang *et al.,* 2015).

2.5.4 Antidiabetics and obesity prevention

Obesity is an increasing problem in the western world and is linked to several disease states including cardiovascular disease and diabetes. Dietary intake (high calorie content) and lifestyle (lack of physical activity) are responsible for most obesity cases. Whole grain sorghum is an excellent food for people with obesity and diabetes because it has relatively low starch digestibility. This is because sorghum endosperm contains high levels of resistant and slow-digesting starch (Taylor & Emmambux, 2010; Barros *et al.,* 2012).

During hydrothermal food processing, extensive cross-links form between sorghum protein (kafirin) and starch, and the cross-linking consists mainly of the strong disulfide bonds that are resistant to digestion (Duodu, *et al.,* 2003; Ezeogu, *et al.,* 2008). Additionally, condensed tannins from sorghum can react with starch and proteins to form bulk complexes in the gastrointestinal tract, making them even less digestible or indigestible (Barros *et al.,* 2012; Amoako & Awika, 2019). These complexes can provide satiety and reduce caloric intake, and induce a low glycemic response, which is desirable for those with obesity and diabetes (Zhang & Hamaker, 2009).

Sorghum also has possible anti-diabetic activities. Sorghum grain phenolic extract has been shown to have inhibitory activity against digestive enzymes such as *Bacillus stearothermophilus* α-glucosidase, porcine pancreatic α-amylase, and human salivary α-amylase, thereby lowering blood sugar levels. Some sorghum cultivars have shown even greater inhibitory effects on α-glucosidase than the common antidiabetic drug acarbose (Kim, *et al.,* 2011). Sorghum phenols may play a role in insulin regulation and act as an adjuvant in the management of diabetes (Chung *et al*., 2011). Additionally, eating muffins with the addition of sorghum has been shown to affect blood sugar and insulin levels and improve glycemic response in healthy people (Poquette *et al.,* 2014). Sorghum's antidiabetic activity can be partially attributed to the condensed tannins. Including sorghum in the general diet could help prevent obesity and diabetes and improve human health.

2.5.5 Dyslipidemia and cardiovascular disease prevention

Sorghum grain contains various bioactive phenolic compounds, and these compounds may also offer protection against the risk of dyslipidemia and cardiovascular disease. Sorghum lipids from phytosterols and polycosanols have been shown to promote cardiovascular health by regulating the absorption, excretion, and synthesis of cholesterol (Martnez *et al.*, 2009). Sorghum phenolic compounds may also function in cholesterol metabolism. Researchers have reported that administration of sorghum phenol extract significantly reduced plasma cholesterol and triacylglycerol levels in hyperlipidemic or diabetic rats (Kim, *et al.,* 2011; Chung, *et al.,* 2011).

Whole sorghum grains/ingredients and extracted sorghum phenolic compounds have been shown to have beneficial health effects. These benefits are derived primarily from the antioxidant effects of phenolic compounds, which can prevent several negative oxidative chain reactions, as well as reducing food digestion through their slow-digesting starches and proteins and their complexation with the phenolic compounds.

2.6 Food applications of sorghum

2.6.1 Traditional application of sorghum in the food system

For centuries, sorghum has been a staple feeding millions of people worldwide, and is particularly important in some underdeveloped regions of Asia and Africa (Hossain *et al.*, 2022). Sorghum has traditionally been used to make various foods. The traditional food uses of sorghum can be roughly divided into five groups: steamed products, boiled products, baked foods, fried products and fermented alcoholic beverages.

Steamed products, such as sorghum grain granules (often mixed with other grains), like those made from wheat and millet, are the most important staple food in North Africa (Dicko, *et al.,* 2006). Cooked products such as sorghum porridge and soup are commonly consumed in Africa. Sorghum porridge is made from whole sorghum grains (often mixed with other grains such as corn and millet) with or without fermentation. For example, ogi, a thin and fermented porridge, is an important weaning food for babies in Africa (Anglani, 1998). Baked goods such as flatbreads (tortillas) and pancakes (roti) are made from sorghum flour (often mixed with corn) and baked as flat and unleavened bread or pancakes. In some regions of Asia and Africa, they are the leading staple food. Since the sorghum protein lacks gluten, the dough cannot form a network to hold gas, and therefore the baked sorghum products often have low volume, lack elasticity and dark color. Despite these drawbacks, these products are still widely accepted (Anglani, 1998; Le´der, 2004).

Fried sorghum products such as tortilla chips and jowar crunch, made from whole sorghum grain or sorghum batter, are popular in Asia and Africa (Vivas-Rodrguez, *et al*., 1990). Sorghum is also traditionally used in homemade alcoholic beverages. In East Asia, red sorghum was an important ingredient in the production of high-alcohol spirits such as Chinese Gaoliang and Maotai spirits. In Africa, white and red sorghum are traditionally used to make opaque, low-alcohol beers; particularly in Nigeria, sorghum has been used on a large industrial scale to produce beers such as lager and stout. These beers are gluten free and have a pleasant fruity flavor and have achieved tremendous commercial success (Kayode *et al*., 2007).

2.6.2 Sorghum grain as functional foods and beverages

The development of new foods and beverages with functional and health benefits has been one of the recent core innovations of the food industry, and various sorghum foods have been produced and studied. Unlike other important gluten-containing grains like wheat and barley, sorghum is considered gluten-free and is a promising and safe alternative food source for people with celiac disease. No symptoms of gastrointestinal distress were observed in celiac disease patients after consumption of sorghum foods (Ciacci *et al*., 2007; Koehler & Wieser, 2013). Due to the ever-increasing demand for gluten-free foods in western and developing countries, sorghum represents an excellent opportunity to be used as a gluten-free food ingredient and offers a rich source of nutrients and bioactive phenolic compounds that other foods may not offer.

Sorghum can be used to make gluten free healthy snacks like cookies and biscuits. Sorghum grain biscuits have been shown to have high phenolic content and antioxidant activity, particularly those made from tannin sorghum, which has up to 20 times more antioxidant activity than wheat biscuits (Chiremba, *et al.,* 2009). However, biscuits made from tannin sorghum have low sensory acceptability despite their high antioxidant activity and major health benefits (Chiremba *et al*., 2009). Non-tannin sorghum biscuits have shown similar sensory acceptability to wheat biscuits, with slightly lower phenolic content and antioxidant activity than tannin sorghum biscuits (Chiremba *et al*., 2009). Tannin-free sorghum biscuits have the potential for commercialization and large-scale production. As discussed above, sorghum biscuits have been shown to reduce oxidative stress and inflammation and improve glycemic response in humans, and are an ideal alternative snack for those with obesity and diabetes

(Stefoska-Needham *et al*., 2016, 2017).

Sorghum can also be used to make functional staple foods. Not only can sorghum staple foods benefit human health, but they also provide a perfect option for people with gluten intolerance whose diets are heavily dependent on gluten-based (or wheat-based) foods such as pasta, bread, and grains. Attempts have been made to use sorghum to make Chinese egg noodles (Liu *et al*., 2012). The noodles made from hulled non-tannin sorghum had a completely different and probably poor physical quality compared to wheat noodles. Despite these results, by controlling flour quality (i.e., particle size and starch damage), it was possible to produce sorghum noodles with good physical properties (Liu *et al.,* 2012).

Sorghum can also be used to make low glycemic index (GI) bread. Bread made from sorghum flour has been reported to have a lower predicted GI than other gluten-free breads (Wolter *et al.,* 2014). Low GI foods are often associated with high levels of resistant starch and therefore poor starch digestibility; sorghum bread can be consumed by humans to improve glycemic response (Wolter *et al*., 2014). Sorghum porridge has also been shown to improve glycemic responses. Decorticated sorghum grain porridge has been reported to have a significant slower gastric emptying rate, more than two times longer than other staple foods such as rice, potatoes, and pasta (Cisse *et al*., 2018).

There has been a trend to use grains such as Tartary buckwheat and barley to make functional grain tea beverages due to the pleasant aroma and flavor and the health benefits associated with their consumption (Guo *et al.,* 2017). Several attempts have been made to use sorghum to make grain tea beverages (Wu *et al*., 2013; Xiong *et al.,* 2019). Wu et al. (2018) developed a sorghum grain tea using the whole grain of a red sorghum variety and traditional processing techniques (steeping, steaming, and roasting). The tea showed antioxidant activity and glucosidase and amylase inhibitory activities.

Xiong et al. (2019) also investigated this and prepared a sorghum grain tea from white sorghum. Various volatile compounds were detected in the tea, contributing to an overall floral, sweet, waxy, and nutty taste, although the phenolic content and antioxidant activity were low and further optimization work was needed. Anunciacao *et al*. (2018) developed a low-calorie and nutritious sorghum powder drink using the extrusion technique. Whole grain sorghum was simply extruded to make powders ready to be infused with water or milk to make a drink. The drink was low in fat but high in fiber and protein and high in phenolic compounds, and showed high sensory acceptability and purchase intent (Anunciacao *et al*., 2018). The presence of tannin has not been reported to have a negative impact on sensory acceptability; instead, it enhanced the health and functional properties of the drink (Queiroz *et al.,* 2018).

Sorghum offers the possibility of making gluten-free beers for people with celiac disease. A study showed that white sorghum beer had more than two times higher phenolic content than barley beer, contributing to its high antioxidant activity (Garzon *et al.*, 2019). The beer also contained significant amounts of ɤ-aminobutyric acids with potential antihypertensive effects and had α-glucosidase inhibitory effects (Garzon *et al.,* 2019). This beer was low in ethanol content and consumption of this beer could benefit human health if consumed in moderation.

2.7 Cowpea

Cowpea (*Vigna unguiculata* (L.) Walp.) is a nutritious crop that, due to its heat and drought tolerance, thrives well in locations unsuitable for the growth of most other edible legumes, making it an environmentally and climate friendly crop (Mekonnen *et al*., 2022). It is an important source of high-quality dietary protein for millions of people living in semi-arid regions and is the most produced pulse after the common dry bean (*Phaseolus vulgaris*) and chickpea (*Cicer arietinum*) (Abebe & Alamayehu, 2022). The world's largest production and consumption of cowpeas occurs in West Africa, which accounts for more than 87% of global production and use (FAO, 2014). Cowpea remains more popular than common dry beans in production and use in Africa. With growing interest in diet diversification and alternative food ingredients in developed regions, cowpea is likely to play a more prominent role globally (Abebe & Alamayehu, 2022).

Apart from the nutritional benefits, cowpea is also rich in important bioactive compounds such as dietary fiber, antioxidants, polyunsaturated fatty acids, and polyphenols, that can benefit human health in a variety of ways (Carneiro da Silva *et al*., 2021). The most important group of bioactive compounds in cowpea are the polyphenols, which are mainly concentrated in the seed coat (Sosulski & Dabrowski, 1984). The compounds are relevant not only for their health-promoting properties, but also because their composition directly influences the selection and use of cowpeas in different crops, mainly due to their effect on seed coat color. The phenolic compounds are responsible for most of the coloration observed in various cowpea seeds, ranging from white, red, cream, bronze, purple to black (Sombié *et al*., 2018). The most important phenolic compounds in cowpeas include the phenolic acids and flavonoids (Dueas *et al.,* 2005; Ojwang *et al.,* 2013). Results have shown that cowpeas have a unique profile of some polyphenols, particularly the flavonols and flavan-3-ols (Ojwang *et al*., 2013), implying that they may offer unique bioactive properties that complement other foods. Other important bioactive compounds in cowpeas are specific peptides that are increasingly being studied, particularly for their beneficial effects on cardiovascular health (Quansah *et al.,* 2013; Segura-Campos *et al.,* 2011).

2.8 Cowpea genotypes

The full mechanism of seed coat coloration is complex and not fully known (Fery, 1985). According to research done by Padi (2003) on the genetic variation of cowpea seeds, the presence of pigment predominates over the absence of pigment, and black seed eyes outnumber brown seeds eyes (Figure 2.3). His research looked at the inheritance of leaf node pigmentation, flower (petal) color, immature pod color, seed coat color, and seed eye color pattern. He also claimed that the Holstein eye type partially dominated the very small eye pattern.

Fig. 3: Cowpea genotypes (Source: Weng *et al.,* 2018).

Other researchers have reported that the pod pigmentation is digenic and follows two patterns of inheritance-monogenic and digenic (Harland, 1920; Mustapha and Singh, 2008). In West Africa, the color of the cowpea seed coat is a crucial agricultural characteristic for the market. The inheritance of several features in cowpea was also explored by Lachyan *et al.* (2016). All four qualitative traits—growth habit, flower color, seed coat color, and seed coat pattern—were found to have monogenic inheritance. Seed coat color and seed coat pattern were found to segregate together. Lopes *et al*. (2003), also reported that cowpea has five genes that regulate its 100-seed weight, and its high narrow sense heritability values suggest that seed size can be selected for in the earliest generations.

2.9 Chemical characterization of Cowpea

The protein content of cowpea seeds has been reported to range from 203 to 394 g/kg, with globulin being the predominant protein in cowpeas, followed by albumin, glutelin and prolamin (Carvalho *et al.,* 2012). The actual amount of each protein depends on the cowpea variety studied (Kachare *et al.,* 1988). When estimating essential amino acids, various reports on the subject have shown that the limiting amino acids in cowpea are methionine and cysteine, followed by tryptophan and threonine (Vasconcelos *et al.,* 2010), while cowpea is rich in lysine (Iqbal *et al.,* 2006). The total amino acid content in cowpea seeds, the ratio of essential amino acids to total amino acids and the ratio of essential amino acids to non-essential amino acids are on average 23.3, 35.6 and 55.2%, respectively, which suggests cowpea flours have the potential to meet human nutritional needs (Xiong *et al.,* 2013).

It is important to note that 5.0-37.0% of the total protein in cowpeas (mainly globulins) has been reported to be nutritionally unavailable (Kachare *et al*., 1988). However, bioactive peptides with antioxidant activity have been obtained from the enzymatic proteolysis of cowpea proteins, indicating their potential as functional food ingredients (Xiong *et al.,* 2013). Also, Cowpeas have been reported to have a low-fat content (31-304 g/kg) compared to other legumes (chickpeas, split peas, lentils, green chickpeas, and lupins) (Kalogeropoulos, 2010). This is an indication that cowpeas could be used in weight-loss diets (Aremu *et al.,* 2015). In addition, the lipid profile of cowpea shows a dominance of triglycerides, followed by phospholipids, monoglycerides, free fatty acids, diglycerides, sterols, hydrocarbons, and sterol esters (Antova *et al.,* 2014).

In terms of fatty acids, palmitic acid is predominant, followed by linoleic acid, linolenic acid, and stearic acid. Polyunsaturated fatty acids make up most fatty acids, ranging from 40.1 to 78.3% of the total (Kalogeropoulos *et al.,* 2010). Furthermore, cowpea contains a high proportion of carbohydrates (504-658 g/kg), which are a potential source of dietary fiber and resistant starches (Kalogeropoulos *et al*., 2010). Fiber content in cowpeas ranges from 160 to 209 g/kg (Granito *et al.,* 2005). Researchers have reported that pigmented varieties contain more than twice the fiber than unpigmented varieties (Madod *et al.,* 2012). Cowpea also has the highest levels of starch content and *in vitro* starch hydrolysis (223-665 g/kg) compared to other legumes (Torres *et al*., 2013). In addition, cowpea have been reported to contain eight sugars, namely; Stachyose, sucrose, verbascose, raffinose, glucose, fructose, galactose, and maltose (Sreerama *et al.,* 2012).

Cowpea is also a good source of essential macro and micronutrients (Luthria *et al.,* 2014). Vitamin B complex are the main vitamins in cowpea, they have been reported to be found in the following descending order: niacin > panthothenic acid > thiamine > pyridoxine > folic acid > riboflavin > biotin > cobalamin (traces) (Tresina and Mohan, 2011). Also, the brown variety has been reported to contain more vitamins than the blue-eyed and black varieties (Gonçalves *et al.,* 2016). Cowpea is also a good source of vitamin C (Tresina and Mohan, 2011) and carotenoids, which are precursors to vitamin A (Gonçalves *et al.,* 2016). This vitamin contributes to the antioxidant properties of this legume. Lutein accounts for over 70.0% of the carotenoids found in cowpea seeds. β-Carotene, ɤ-carotene and cryptoxanthin are other carotenoids found in cowpeas (Hashim and Pongjata, 2000). Among the various vitamin E vitamers present in cowpeas, δ-tocopherol has been reported to have the highest concentration, followed by ɤ-tocopherol, and ɤ-tocotrienol (Antova *et al.,* 2014).

In addition, studies have shown that the mineral composition of cowpea differs significantly between the varieties evaluated. However, it has been reported that potassium is the most abundant mineral found in cowpea seeds and leaves. Calcium, zinc, and iron are also present in large quantities (Carvalho *et al.,* 2012).

2.10 Food applications of cowpea

2.10.1 Composite flour and baking applications

Due to their low natural fat content (1-2%), matured, dry cowpeas can be processed relatively easily into flour-like products, whereby defatting before grinding is not necessary (McWatters *et al*., 1995). Blending cowpea with cereals improves the protein quality of the blend and allows cowpea to be expanded into cereal-based baked goods and snack chips (Philips *et al*., 2003). Bread containing 15% extruded cowpea flour had a similar moisture content (35%) as the control (34.5%), but was heavier, had lower loaf volume, and contained more oil and protein (Ahmed and Campbell, 2002). Using 30% extruded cowpea flour or 15 or 30% unheated cowpea flour gave breads that were not as acceptable.

Cake-type buttermilk doughnuts prepared with 10, 20, or 30% cowpea flour from non-decorticated cream-type cowpeas (Dixie Cream variety) performed well for mechanical cutting, dispensing, and frying, and finished products compared favorably to 100% wheat flour control in sensory quality attributes. However, their open-grain structure contributed to excess fat absorption during frying, 31.7-34.6% for 10 and 30% wheat flour substitutes, respectively, compared to 27.7% fat for the 100% wheat flour control (McWatters, 1982a).

Doughnut quality was significantly improved when cowpeas in the form of finely ground flours were used instead of grist (McWatters, 1982b). The sensory quality of doughnuts made with only 10% cowpea flour or with 3% soy flour (a fat absorption control agent) was like controls made with 100% wheat flour and contained about the same amount of fat as the control (30 % fat).

Tortillas are unleavened bread made from corn or wheat flour. They are a staple in many countries and are growing in popularity in the United States. The usual ingredients are flour, salt, shortening and water with the flattened pieces of dough cooked in a pan or on a griddle. Modeling, optimization, and verification methods were used to evaluate the performance of cowpea (White Acre variety), peanut and wheat in preparing tortillas (Holt *et al.,* 1992b). Replacing wheat flour with 0-24% cowpea flour, 0-46% peanut flour, and combinations thereof resulted in tortillas with quality characteristics like 100% wheat (Shamki *et al.,* 2019). These insights and the mixture design approach are useful in developing baked goods using flours from indigenous sources in areas where wheat flour is not widely available.

2.10.2 Traditional processing of cowpea seeds

Whole cowpea seeds, flours or meals, and pastes were all used in traditional processing. The Hausa, Yoruba, and Fulani peoples of West Africa, as well as other ethnic groups in Ghana, make more than 20 home-cooked dishes using cowpea seeds (Dovlo *et al*., 1976). To make soups, stews, steamed or fried cakes, and sauces, traditional recipes employ a variety of techniques. There are also additional components or staples included, such as meats, seafood, eggs, certain vegetables, and herbs. A popular dish in West Africa is *akara*, a fried cowpea paste. Cowpeas are used to make *idhli, dhosal/dosa*, and *papad*, a traditional snack meal in India (Bhagirathi *et al*., 1992). They are also used to make sev, an extruded and fried dough product (Enwere & Ngoddy, 1986). *Acaraje*, a fried fritter like *akara*, is sold by street vendors in Brazil. Cowpeas are primarily eaten as cooked whole seeds in the United States of America, where they can be purchased in raw/dry, canned, or frozen forms. Cowpeas are typically consumed as part of meals that also include various vegetables and/or fried corn cakes or baked corn bread.

2.10.3 Extruded products

Nutritious weaning foods made from cowpeas have been prepared via extrusion processing. Cowpea flour/meal, alone or in combination with other components such as sorghum, can be extruded to generate snack products and weaning foods (Affrifah *et al.,* 2021). In a single-barrel extruder, Phillips *et al.* (1984) extruded cowpea meal at temperatures ranging from 150°C to 200°C and measured the extrudates' physical and rheological characteristics. At

20% and 40% moisture content respectively, the highest and lowest expansion ratios were noted. The maximum expansion and lowest bulk density, both deemed ideal for a snack food, were found in the 20% MC/175°C extrudate. According to a texture examination, greater temperatures and lower MC created crisper product, whereas lower temperatures and higher MC produced softer product. Using a pilot-scale single-screw extruder, Falcone, and Phillips (1988) extruded sorghum and cowpea (67:33) at 20.5-25% MC and 175°C-205°C. The effect of the process factors (MC and temperature) on the expansion ratio, density, and rheological properties were obtained. Temperature was most significant, although MC was more essential as cowpea ratio increased. Cowpea blends with maize and rice have also been reported to be extruded (Sefa-Dedeh and Saalia, 1997; Marengo *et al.,* 2017).

2.11 Benefits of complimenting Cereals with Legumes

Cereals and legumes are primary staples often consumed together, and contribute a major portion of daily human calorie and protein intake globally. They thus impact human nutrition and health in important ways (Awika *et al*., 2018). Protective effects of consuming whole grain cereals and legumes against various inflammation-related chronic diseases, including cardiovascular disease, type-2 diabetes, and cancer, among others, are well documented (Moraes *et al.,* 2015; Stefoska-Needham *et al.,* 2015; Awika *et al*., 2016; Arbex *et al.,* 2018; Silva *et al.,* 2020; Martinez *et al*., 2021). Also, potential benefits of combined intake of whole cereals and legumes beyond their complementary amino acid nutrition has been reported (Awika *et al.,* 2018). There is ample evidence that key bioactive components (polyphenols, dietary fiber, and flavonoids) of whole grain cereals and legumes are structurally different and have been reported to also provide complementary health benefits to consumers (Awika *et al*., 2018). Hence, combining cereals and legumes would most likely result in better balanced and enhanced bioactive compounds that would potentially address most inflammation-related health issues (Apea-Bah *et al*., 2014; Awika *et al.,* 2018).

Cereals are known to be high in carbohydrates and dietary fiber. They are also protein sources that could be expanded in the future by combining them with legumes (Lafiandra *et al.,* 2014; Poutanen *et al.,* 2014). Because cereals have a low protein content, they must be supplemented with protein-rich supplies from other sources to achieve a nutritional balanced diet (Onwulata & Konstance, 2006). This thus entails the partial substitution of cereals with other nutrient sources such as legumes to diversify as well as upgrade the nutritive value of indigenous agricultural food products.

Below are some examples of researchers combining grains and legumes and obtaining positive outcomes in terms of improved nutritious content in the product. Sorghum is a staple grain in Africa. For weaning infants, Asma *et al*. (2006) created low-cost, high-protein food supplements from sorghum, cowpea/pigeon pea, and groundnut/sesame seed mixes. Researchers have also investigated the effects of mixing fermented grains with legumes. Mbata *et al.* (2009) found that combining maize flour with 30% Bambara (*Vigna subterranean*) groundnut increased the mineral and amino acid profile of the composite mix significantly. Sensory testing revealed that the Bambara-maize 'ogi' was generally well accepted. Ojokoh & Bello (2014) also found that supplementing millet with soybean improved the nutritious characteristics of the grain. According to Jackson *et al.* (2013), combining traditional sorghum porridge with sugar beans increased the food's nutritious value. In terms of protein content and essential amino acid profile, Kayitesi *et al*. (2012) found that combining sorghum with marama bean (*Tylosema esculentum*) (an underutilized legume) improved its nutritional quality when compared to sorghum porridge. The energy values of the sorghum marama bean porridges improved significantly when compared to sorghum porridge

Furthermore, findings from reviewed literatures show that mixing grains and legumes boosted the food's nutritious content. Full fat or defatted soy flour (Junqueira *et al*., 2008), defatted wheat germ (Arshad *et al.,* 2007), flaxseed (Koca & Anil, 2007), sunflower seed (Skrbic & Filipcev, 2008), and lupin flour have all been used as flour alternatives in bread items (Hall & Johnson, 2004). Because soy protein is a high source of lysine, it helps to increase the essential amino acid content of peanut-cereal blends or any other legume-cereal mix (Lang *et al.,* 1999). Several more research on mixing grains and legumes to improve the nutritional profile of food products have also been conducted. The majority, if not all, of the participants reported improved nutritional, physical, chemical, and sensory characteristics (Gonzalez-Agramon & Serna-Saldivar, 2006; Chinma & Gernah, 2007; Singh & Mohamed, 2007; Okoye *et al.,* 2010; Chinma *et al*., 2012).

Recently, there has been a rise in the commercial manufacturing of unique cereal-based food products that are intended to supplement the daily diet with additional proteins (Vitali *et al.,* 2008). These are snacks that have been nutritionally enhanced to lessen the risk of acquiring nutrient deficient disorders (Serrem *et al*., 2011). When compared to other foods, their inexpensive cost of manufacture, varied taste, convenience of availability, and extended shelf life are some of the reasons for their increased appeal (Sudha *et al*., 2007). Generally, combining cereals and legumes will likely result in more balanced and increased bioactive chemicals (Apea-Bah *et al*., 2014), which might potentially solve most inflammation-related health issues.

3 OBJECTIVES

3.1 General Objective

To evaluate the physical, chemical, technological, and sensorial properties of extrudates and cookies from composite tannin sorghum and white cowpea flours.

3.2 Specific Objectives

The specific objectives of the research were to:

- Elaborate ready-to-eat expanded extrudates and cookies using composite tannin sorghum and white cowpea flours.
- Characterize the flours, extrudates and cookies (proximate composition, free phenolic compounds, antioxidant capacity, resistant starch, and tannin content).
- Determine the pasting properties of raw flours, extrudates and cookies.
- Determine the texture properties of extrudates and cookies.
- Evaluate the sensory properties of the extrudates and cookies

4. METHODOLOGY

Proximate composition of samples was done at Food analysis and Chemistry Laboratories; cookies was formulated and baked at Bakery and Pasta laboratory; the technological analysis was also carried out in the same lab; Phenolic content, antioxidant capacity, tannin content, and resistant starch analyses were carried out at BIOCARB laboratory; Sensory evaluation of samples was done at Sensory evaluation lab, all in the Department of Food Science and Technology, UFV. Dietary fiber was analyzed at the Laboratory of Experimental Nutrition, Nutrition department, UFV. Production of extrudates, paste viscosity (RVA), Texture of extrudates, Particle size distribution of flours, and Bulk density, were done at Embrapa Food Technology, Rio de Janeiro, Brazil.

4.1 Materials

A sorghum genotype with high tannin and resistant starch content (BRS 305) (Fig. 4) was obtained from Embrapa Maize and Sorghum (Sete Lagoas, Brazil). Commercial white cowpea (“Feijão Fradinho”) (Fig. 5) was kindly donated by Granfino Alimentos (Nova Iguaçú, Brazil). Ingredients used to make the cookies (Qualy cremosa margarine, Alvinho crystal sugar, Cisne tradicional salt, Viçosa mixed spice (cinnamon, nutmeg), egg, cocoa powder, and Royal baking powder) were purchased from Escola supermarket at UFV (Viçosa, Brazil). Hydrochloric acid and Sulfuric acid were purchased from Synth, sodium carbonate from Alphatec, Petroleum ether from Quimica, Folin-Ciocalteau, gallic acid, sodium carbonate, Trolox, ABTS, Vanillin, Maleic acid, Gallic acid, and Ethanolamine were purchased from Sigma Aldrich. Dietary fiber assay kit and Resistant starch assay kit, were purchased from Megazyme Ltd, Wicklow, Ireland.

Fig 4: BRS 305 sorghum grain

Fig. 5: Commercial cowpea grain (called "Feijão Fradinho" in Brazil)

4.2. Obtention and characterization of sorghum, cowpea, and composite flours

Matured dried seeds of sorghum and cowpea were sorted, to remove damaged seeds, foreign materials, dirt, and stones. The grains were ground in a laboratory hammer mill model 3100 (Perten, Huddinge, Sweden) with a 0.8 mm opening. In order to produce the composite whole grain flours, sorghum and cowpea flours were mixed at ratios (sorghum:cowpea) of 70:30, 50:50, 30:70 (w/w) in a homogenizer for 15 min to homogenize the flour particles. 100% sorghum and cowpea flours served as controls, totaling 5 samples: 100S, 70S:30C, 50S:50C, 30S:70C and 100C. Flour particle size distribution was measured in water using a Mastersizer S3500 particle size analyzer (Microtrac, Montgomery, USA). Values of D (10) (83 µm), D (50) (330 µm), and D (90) (1600 µm), which represent the maximum particle diameter below which 10%, 50%, and 90% of the sample fall, respectively, were obtained. All the measurements were carried out in duplicate. The produced composite flours were dried in an oven with air circulation at 35°C for 15 hours, cooled, and stored in polyethylene packages at a temperature of -22 °C until needed for analysis.

4.3 Preparation of extrudates

The samples were extruded at Embrapa Food Technology, Rio de Janeiro, Brazil, according to the method described by Galdeano et al. (2018). The extrusion process was done using an Evolum HT25 co-rotating, intermeshing twin-screw extruder (Clextral Inc., Firminy, France) with the aim to produce expanded or puffed extrudates. The screw diameter was 25 mm, with a diameter ratio of 40:1, ten heating zones (25, 25, 50, 70, 100, 100, 100, 100, 120 and 120 °C) were used, and the screw and cutter speeds were set at 600 rpm and 80 rpm respectively. The flours were mixed with 7% sugar and 0.7% salt in a homogenizer, before being fed through a twin-screw gravimetric feeder model GRMD15 (Schenck Process, Darmstadt, Germany) at a constant rate of 10 kg/h, and the process was monitored by Schenck Process Easy Serve software (Schenck Process, Darmstadt, Germany). Deionized water was injected between the first and second modular zones through a port with a 5.25 mm internal diameter using a plunger metering pump model Super K PP 6.35 (Clextral DKM Pumps, Firminy, France) set to compensate moisture differences in the samples and provide a final moisture of 12% content. The collected extrudates were dried in a forced air oven at 60 °C for 1 hour, packaged in plastic bags and stored at 25 °C until needed for analysis.

4.4 Formulation of cookies

After several adjustments and testing of formulations, the formulation of Soares *et al.* (2013) was used as a base, with slight modification in the amount of ingredients used as follows: 17.97% sugar, 21.71% margarine, 1.03% egg yolk, 0.26% mixed spice (nutmeg, clove powder, and cinnamon powder), 1.54% ammonium bicarbonate, and 2.57% cocoa powder. Two mixing stages were used for the cookie's formulation. First, sugar, fat, and egg yolk were creamed in a high-speed planetary mixer (Kitchen aid, St Joseph, USA) for 10 min. The other ingredients were then added and homogenized for approximately 5 min at a low speed. The optimum development of the dough is the point at which all ingredients have been properly incorporated, and the dough is homogeneous and at an optimum point, which allows the cookies to be molded. The dough was then rolled out on 6 mm thick sheets, cut into cycles 41 mm in diameter, and baked for about 15 min in an oven, model HF4B (Haas, Curitiba, Brazil), at surface and ceiling temperatures of ~150 °C and 200 °C, respectively. Afterwards, the cookies were cooled for 30 min, packed, and stored at room temperature, protected from light, until analysis. The amount of ingredients used is shown in Table 2.

Table 2: Cookie formulation (%)

Sample	100S	70S:30C	50S:50C	30S:70C	100C
Sorghum flour	51.34	35.94	25.65	15.40	-
Cowpea flour	-	15.40	25.65	35.94	51.34
White Sugar	17.97	17.97	17.97	17.97	17.97
Margarine	25.30	25.30	25.30	25.30	25.30
Baking powder	1.54	1.54	1.54	1.54	1.54
Cocoa powder	2.57	2.57	2.57	2.57	2.57
Egg yolk	1.03	1.03	1.03	1.03	1.03
Spice	0.26	0.26	0.26	0.26	0.26

*S= sorghum flour; C= cowpea flour

4.5 Physical and chemical characterization of flour, extrudates and cookies

4.5.1 Bulk density (BD) of the flour

The method described by Oladele and Aina (2007) was used for the determination of bulk density of flour. Fifty grams (50 g) of flour was put into 100 mL measuring cylinder. The measuring cylinder was then tapped continuously on a laboratory table until a constant volume was obtained. Bulk density was calculated using following the formula:

BD (g/cm^3) = weight of the sample/volume of sample

4.5.2 Cookies expansion ratio and weight loss

The diameter and thickness of cookies were measured using a Vernier caliper and the expansion ratio was obtained by dividing the diameter by the thickness values, according to method 10-50.05 (AACC, 2010). The weight loss was calculated as a ratio between raw dough and baked cookies' weight. All analyses were performed on 6 cookie samples.

4.5.3 Color measurements

The color parameters (L*, a*, b*, C and H) for extrudates and cookies were evaluated according to the CIELAB system, in a colorimeter CR-400 (Konica Minolta, Japan), using illuminant D65 and observer angle 2º, and the readings were performed using six replicates per extrudate and cookie sample.

4.5.4 Water activity (a_w)

Water activity (aw) of cookies was determined by the water activity meter using a dew point sensor (Aqualab, 4TEV, Decagon, Pullman, USA), and the readings were performed in triplicate at room temperature (25 °C).

4.5.5 Proximate composition analysis

The chemical composition of raw flour, extrudates and cookies was performed according to the AACC (2000) official analytical methods: moisture (method 44-15A), fat was extracted with petroleum ether (method 30-10.1), total protein (method 46-12.01, conversion factor of 14, protein equivalent of 5.27), ash (method 08-01.01). The total dietary fiber (soluble and insoluble) was determined by the enzymatic method (AOAC, 2012), while the carbohydrate was determined by the difference (% Carbohydrate = % Moisture + % fat + % Protein + % ash).

4.5.6 Free phenolic compounds

The extraction of free phenolic compounds from flour, extrudates and cookies were carried out using ethanol as solvent. The samples were mixed with ethanol in water (80% v/v) (1:10 w/v) by stirring for 1 hour at room temperature (25 º C) on a magnetic stirrer. The suspension was centrifuged at 3100 g for 10 minutes. After this time, the supernatant was removed, and used for the determination of free phenolics. The method used was the Folin–Ciocalteau method as described by Blainski et al. (2013). The phenolic concentration was expressed in milligrams of gallic acid equivalent per gram of sample (mg GAE/g).

4.5.7 Total condensed tannins

Total condensed tannin of flour, extrudates and cookies was quantified using the vanillin acidified method described by Broadhurst and Jones (1978). Results were expressed in mg of catechin equivalent (mg CE/g) by using a calibration curve of catechin.

4.5.8 Antioxidant capacity

Antioxidant capacity (µmoles Trolox equivalent/g sample) of the flour, extrudates and cookies were determined according to the ABTS (Re *et al.,* 1999) method. Absorbance was read at 517 nm after 30 minutes and results were expressed in µmoles Trolox equivalent/g sample.

4.5.9 Resistant starch content

Resistant starch (RS) content of the flour, extrudates and cookies were measured according to the resistant starch assay kit from Megazyme International (Wicklow, Ireland) (AACC method 32-40). Total starch (TS) was measured in the Total starch assay kit from Megazyme (AACC method 76-13).

4.6 Technological properties of flour, extrudates and cookies

4.6.1 Pasting properties

A Rapid Visco Analyzer series 4 RVA (Newport Scientific Pty Ltd., Warriewood, Australia) was used to measure the paste viscosities of the raw flour, extrudates and cookies according to the methodology reported by Carvalho et al. (2010). Three grams of each gluten-free whole grain flour adjusted to 14% of moisture (wet basis) were placed along with 25 mL of distilled water in the sample holder (aluminum cup) of the equipment. The test conditions were: mixing at 160 rpm at 25 °C for 2 min, heating up to 95 °C at a constant rate of 14 °C/min and kept for 3 min and then cooled to 25 °C in 5 min at the same rate, with a total time of 23 min. The results were analyzed by the software Thermoclines for Windows. The pasting properties measured were Trough viscosity (25 °C), Peak viscosity at 95 °C, Final viscosity (cold paste viscosity), Breakdown viscosity (BDV= Peak Viscosity-Trough), Setback viscosity (SBV=FV-PV). Measurements were performed in duplicates.

4.6.2 Texture analysis of extrudates and cookies

The texture of extrudates were measured using the method described by Alzuwaid et al. (2021) with slight modifications. The instrument used was a Texture Analyzer TA-XT Plus (Stable Micro Systems, Surrey, England) running on the Exponent software 6.1.11.0 (Stable Micro Systems, Surrey, England) fitted with a load cell of 30 kg. The test was adjusted in the compression mode, pre-test speed at 2 mm/s, test speed at 1 mm/s and post-test speed of 10 mm/s. Compression occurred until reaching 50% of the sample height (strain) and trigger contact force of 0.5 N. Hardness was defined as the maximum force (N) required to puncture the extrudates. Crispiness was evaluated using the equations of Bouvier et al. (1997) as described by da Silva et al. (2004).

Texture of the cookies were determined using the TA-XT Plus texture analyzer (Stable Micro Systems, Surrey, England) using a 50 kg load cell, equipped with a three-point bend rig (HDP/3PB) and heavy-duty platform. Maximum force was recorded as the hardness value and test conditions were: pre-test speed of 1 mm/s, test speed of 3 mm/s, post-test speed of 10 mm/s, and penetration distance of 5 mm. The readings were performed on 10 cookie samples.

4.7 Sensory evaluation of extrudates and cookies

Approval was sort before recruiting participants. The approval number was CAAE: 63482522.3.0000.5268. Sensory evaluation of extrudates and cookies was done separately, using 60 panelists for each product. Participants who identified as frequent consumer of breakfast cereals and cookies were recruited from staff and students at Universidade Federal De Viçosa, via posters and WhatsApp messages. Participants were provided with an information sheet and consent forms. People with any food allergies were excluded from the evaluation.

Three samples of extrudates (100S, 70S:30C and 50S:50C) and five cookies' samples (100S, 70S:30C, 50S:50C, 30S:70C and 100C) were subjected to evaluation by the participants using a 9-point Hedonic scale as described by Larmond (1991) with scores of 1 representing "dislike extremely" and 9 "like extremely" respectively. The samples of extrudates 30S:70C and 100C were not evaluated because they presented a very strong beany flavor. Participants evaluated the products for acceptability seated in individual booths under cool, natural, fluorescent lights. The samples were marked with random 3 digits code and presented in a randomized order. Participants marked their perception of the acceptability of color, appearance, texture (on eating), flavor and overall acceptance on the questionnaire. Participants rinsed their mouth with water between each sample. The cut-off score was 5.

4.9 Statistical analysis

All data were reported as mean ± standard deviation. One-way ANOVA with Tukey post-hoc test was used to identify significant differences between samples for all measured parameters. $P < 0.05$ was considered as significant. SPSS Statistics v.22 (IBM, New York, USA) was used for all analyses.

5 RESULTS AND DISCUSSION

5.1 Physical properties of flour, extrudates and cookies

5.1.1 Particle Size distribution and bulk density of flour

The average particle size of the flour used for production of the samples (extrudates and cookies) is shown on Table 3. The flour presented an average within the range of particle sizes of the fractions that comprised it. The average particle size of flour decreased as the percentage of cowpea flour increased up to 50%, but increased as the cowpea flour reached 70%. The increase followed the same pattern for all blends with D (10) having the least values and D (90) the highest. Also, the results show that 100% sorghum flour had the largest particles with D10 = 15.63 µm, D50 = 136 µm, D90 = 391.90 µm, and 50% sorghum:50% cowpea flour had the finest particle size with D10 = 9.64 µm, D50 = 63.14 µm and D90 = 307.80 µm.

In general, the average size distributions of the flour used in this research showed a bimodal distribution and were like those applied in other studies on gluten-free cookie formulations (Mancebo *et al.,* 2015; Rao *et al.,* 2016).

Table 3: Particle size distribution (µm) and bulk density (g/cm^3) of flours

Sample	**100S**	**70S:30C**	**50S:50C**	**30S:70C**	**100C**
D10	15.63±0.30^{a}	11.92±0.55^{c}	9.64±0.61^{e}	10.81±0.35^{d}	12.43±0.15^{b}
D50	136.00±7.20^{a}	80.31±12.09^{c}	63.14±9.80^{e}	68.31±7.66^{d}	84.66±6.55^{b}
D90	391.90±45.70^{a}	290.00±39.20^{e}	307.80±30.60^{d}	333.30±30.00^{c}	371.10±38.20^{b}
Bulk density	0.09±0.15^{d}	0.17±0.07^{a}	0.15±0.51^{b}	0.17±0.27^{a}	0.09±0.27^{c}

Values are mean ± Standard deviation of duplicate readings. Data in the same row bearing different superscript differ significantly ($p < 0.05$).

100 S= 100% sorghum flour; 70S:30C: flour from 70% sorghum and 30% cowpea, 50S:50C= flour from 50% sorghum and 50% cowpea, 30S:70C= flour from 30% sorghum and 70% cowpea, 100C= 100% cowpea flour.

The bulk density obtained for the flour in this study increased with the addition of cowpea flour and ranged between 0.09-0.17 g/cm^3. The composite flour had higher bulk densities than their individual flour. This increase in bulk density of the composite flour could be attributed to the higher fiber content of the cowpea flour, as researchers have reported that high fiber increases bulk density (Onwulata *et al.*, 2001). The images of the flour used are shown in the Figure 6.

Fig. 6A: 100S

Fig 6B: 100C

Fig 6C: 70S:30C

Fig 6D: 50S:50C

Fig 6E: 30S:70C

Figure 6: Flour used in the study. 100S=100% sorghum flour (Fig. 6A), 100C=100% cowpea flour (Fig. 6B), 70S:30C=70% sorghum flour:30% cowpea flour (Fig. 6C), 50S:50C=50% sorghum flour:50% cowpea flour (Fig. 6D), 30S:70C=30% sorghum flour:70% cowpea flour (Fig. 6E).

5.1.2 Weight loss, expansion rate, water activity and color

The physical properties of cookies (weight loss, expansion ratio, water activity and color) and extrudates (color) are shown on Table 4. Weight loss obtained in the cookies were not significantly different ($p>0.05$) and ranged from 7.48-9.71 %. Cookies produced from 50% sorghum and 50% cowpea flour had the least value (7.48) for weight loss, while cookies from 30% sorghum and 70% cowpea flour had the highest value (9.71), despite not being statistically different. Weight loss results from the evaporation of water in dough during baking. Addition of cowpea flour to cookies did not play a significant role in weight loss.

The expansion ratio of cookies differed significantly ($p<0.05$) and ranged from 5.18-5.45, with sample 30S:70S having the highest value and sample 100S the least value. The addition of cowpea flour increased the expansion slightly. This increase could be attributed to the fine particle size of the cowpea flour. Cowpea produced a large amount of fine dust during milling, and had particle size less than 500 µm. This theory is backed by the findings of Moraru and Kokini (2003), who reported that fine particle size increase expansion because they show greater elasticity with water compared to coarse particles. Expansion is also caused by loss of moisture and gases, and has been reported to improve the sensory properties of foods. Moraes et al. (2010) added that sugar and fat also play a role in expansion of cookies during baking. This was however not obvious, since the amounts used were same for all formulations.

Also, the values obtained for water activity of the samples were low (ranged between

0.35-0.46), and were within the acceptable range of water activity given for cookies (<0.6). It was observed from the result that sample 50S:50C and sample 100C had the highest value for water activity. The low values obtained in this study could be as a result of the dry heat treatment used in baking the cookie samples. Water activity gives an indication of the keeping quality of a food product.

Table 4: Some physical properties of cookies and extrudates

Sample	**100S**	**70S:30C**	**50S:50C**	**30S:70C**	**100C**
Cookies					
Weight loss (%)	8.50±0.23^{a}	9.06±0.20^{a}	7.48±0.58^{a}	9.71±0.20^{a}	8.80±0.45^{a}
Expansion ratio	5.18±0.00^{d}	5.32±0.00^{c}	5.38±0.00^{b}	5.45±0.00^{a}	5.26±0.00^{c}
Water activity	0.39±0.00^{bc}	0.43±0.03^{ab}	0.46±0.00^{a}	0.35±0.02^{c}	0.44±0.01^{a}
Color parameters					
Luminosity (L*)	33.75±0.44^{a}	32.73±0.61^{b}	34.22±0.50^{a}	34.13±0.39^{a}	34.42±0.88^{a}
Redness (a*)	7.38±0.25^{bc}	7.13±0.29^{c}	7.90±0.30^{ab}	7.80±0.28^{ab}	8.15±0.63^{a}
Yellowness (b*)	9.08±0.34^{c}	9.10±0.44^{c}	10.02±0.44^{b}	10.10±0.46^{b}	10.97±0.70^{a}
Chroma (C)	11.70±0.43^{b}	11.57±0.50^{b}	12.78±0.52^{a}	12.77±0.49^{a}	13.65±0.52^{a}
Hue (h)	50.83±0.20^{c}	51.95±0.31^{b}	51.68±0.59^{b}	52.33±0.42^{b}	53.33±0.46^{a}
Extrudates					
Color parameters					
Luminosity (L*)	42.18±1.62^{c}	42.15±1.07^{c}	45.83±0.87^{c}	49.97±1.57^{b}	54.75±2.68^{a}
Redness (a*)	10.35±2.93^{a}	10.62±0.88^{a}	11.22±1.18^{a}	10.95±0.45^{a}	10.20±0.68^{a}
Yellowness (b*)	12.32±2.26^{c}	12.28±1.60^{c}	13.62±0.88^{bc}	15.43±1.22^{b}	19.02±1.05^{a}
Chroma (C)	16.18±3.09^{e}	16.25±1.75^{d}	17.68±1.27^{c}	18.93±1.23^{b}	21.58±0.72^{a}
Hue (H)	50.52±7.21^{b}	48.98±1.64^{b}	50.60±2.26^{b}	54.62±1.42^{b}	61.70±2.75^{a}

Values are mean scores± Standard deviation of duplicate readings. Data in the same row bearing different superscript for extrudates and cookies respectively differ significantly ($p < 0.05$). 100S= samples from 100% sorghum flour; 70S:30C= samples from 70% sorghum and 30% cowpea flour, 50S:50C= samples from 50% sorghum and 50% cowpea flour, 30S:70C= samples from 30% sorghum and 70% cowpea flour, 100C=samples from 100% cowpea flour.

Furthermore, the values obtained for Luminosity (L*), Redness (a*), Yellowness (b*), Chroma (C), and Hue (H) of extrudates and cookies are shown on Table 4. The values ranged from 32.73-34.42, 7.13-8.15, 9.08-10.97, 11.37-13.65, 50.83-53.33 for cookies and 42.15-54.75, 10.20-11.22, 12.28-19.02, 16.18-21.58, and 48.98-61.70 for extrudates respectively. The predominant chromatic coordinate in all samples was luminosity, related to lightness, with values greater than 30 in all the samples. The main color differences promoted by formulation changes were manifested in an increase in all the color parameters. The addition of cowpea flour to sorghum flour increased the luminosity, yellowness, chroma and hue of the extrudates, but did not affect the redness, as the value obtained for redness of extrudates were not significantly different from the 100S formulation. On the other hand, cowpea flour increased the yellowness, chroma and hue of the cookies, but did not affect so much the luminosity and redness of the samples. This could be as a result of the addition of cocoa powder to cookies, causing a color change of all the samples to a dark brown.

All the observed color modifications were related to the natural color of the added ingredients, but could also have been from the brown pigments generated due to the Maillard reaction (Ačkar *et al.,* 2018). Also, Tamanna and Mahmood (2015) reported that processes driven by mechanical energy, such as extrusion, develop mild Maillard reaction products compared to those driven by time-temperature factors like baking/toasting.

5.2 Chemical properties of flour, extrudates and cookies

5.2.1 Proximate composition of flour, extrudates and cookies

The proximate composition of whole grain flour, extrudates and cookies is shown on Table 5. Moisture content ranged from 11.73-12.97, 4.96-8.00, and 3.71-5.80 % for flour, extrudates and cookies respectively. Cookie sample 50S:50C, which was observed to loss the least weight had the highest moisture content (5.80), which indicates a correlation between weight loss and moisture content. Also, extrusion cooking and baking significantly decreased the moisture content of extrudates and cookies compared to their respective flour. The water reduction is likely due to moisture loss that occurred during the expansion, extrusion and oven drying procedures. In addition, the moisture content of the extrudates and cookies was below 10%, which according to Zambrano (2019) is the optimal moisture content for preventing the proliferation of microorganisms in foods.

The fat content ranged between 0.84-2.95, 0.34-0.44, and 22.11-24.20 % for flour, extrudates and cookies respectively. The inclusion of cowpea flour slightly increased the fat content of the extrudates produced from the composite flour. The fat contents were 0.44, 0.39, and 0.35% respectively compared to 0.34% obtained for extrudates from 100% sorghum and cowpea flour. This finding suggests that cowpea flour can be added to cereal extrudates without changing the foods' nutritional fat content. When compared to raw flour, extrusion reduced the fat content of the extrudates (Table 5). De Pilli et al. (2012) provided evidence in favor of this claim, finding that starch-lipid complexes formed under a variety of extrusion circumstances and that the only factor significantly affecting the development of the complex was barrel temperature. The consensus is that during the extrusion process, fat can combine with starch and protein to create complexes, which can prevent the oxidation phenomenon of extruded products during storage and thereby increase the product shelf life (Wang *et al*., 2022). Also, the fat content of the cookies ranged from 22.11-24.20% and was not significantly different (p>0.05). This could be because the fat and ingredients used in the cookie's formulation were standardized for all samples, and their interaction could have increased the stability of the fats. However, the fat contents obtained in this study were closer to the lowest range of fat content given for commercial cookies (20-70%), indicating a healthier cookie.

Furthermore, the protein values ranged between 9.19 to 18.69, 8.60 to 18.01 and 5.98 to 11.28 % for flour, extrudates and cookies respectively. The addition of cowpea flour to the extruded samples and cookies increased the protein content of all the products. All combined samples showed significantly ($p < 0.05$) higher protein content than the sorghum control sample (100% Sorghum) (Table 5). The increase in percent protein of the extrudates and cookies were proportional to the amount of cowpea flour added. The 70S:30C, 50S:50C and 30S:70C sorghum-cowpea ratios showed a 31.05%, 56.16% and 77.44% increase respectively for extrudates, and a 24.92%, 38.63% and 61.71% increase for cookies respectively. This could be because of additive effect of cowpea inclusion, as cowpea flour has been shown to have higher protein content (18.69%) than sorghum (9.19%). The obtained results indicated that adding different amount of cowpea to sorghum based extrudates and cookies can significantly increase the protein content of the products. Similar results were obtained by Gularte et al. (2011), with the addition of 50% of different legumes (Chickpea, lentils, bean, and pea) to the rice-based gluten free layer cakes increasing the protein content. Also, Pastor-Cavada et al. (2011) observed an increase in corn and rice based extrudates after adding legumes. Similarly, Zucco (2011) reported that adding wild legumes to wheat-based cookies increases the protein levels of the cookies.

For the ash content, the values ranged from 1.46 to 2.80, 1.73 to 3.40 and 1.92 to 2.81 % for flour, extrudates and cookies respectively. The inclusion of cowpea flour to sorghum flour significantly increased ($p<0.05$) the ash content of the samples (Table 5). The increase was observed to be proportional with an increase in the quantity of cowpea flour for both the extrudates and cookies. This could be because cowpea flour had higher ash content (2.80 %) compared to the sorghum flour (1.46) as seen on Table 5.

Table 5: Proximate composition of flours, breakfast cereals and cookies (%)

Sample	Moisture	Fat	Protein	Ash	Carbohydrate	Total dietary fiber	Soluble fiber	Insoluble fiber
Flours								
Sorghum	12.97±0.16^{a}	2.95±0.17^{a}	9.19±0.05^{b}	1.46±0.09^{b}	73.43±0.00^{a}	14.77±2.78^{b}	0.87±2.73^{a}	13.90±0.05^{b}
Cowpea	11.73±0.08^{b}	0.84±0.30^{b}	18.69±0.10^{a}	2.80±0.00^{a}	65.94±0.00^{b}	20.11±0.55^{a}	0.49±0.55^{b}	19.63±0.00^{a}
Extrudates								
100S	6.14±0.04^{c}	0.34±0.03^{c}	8.60±0.00^{e}	1.73±0.09^{e}	83.19±0.00^{a}	10.63±0.02^{b}	0.39±0.39^{e}	10.24±0.37^{a}
70S:30C	8.00±0.05^{a}	0.44±0.07^{a}	11.27±0.00^{d}	2.13±0.09^{d}	78.16±0.00^{b}	11.47±1.22^{a}	2.38±0.71^{a}	9.09±0.51^{c}
50S:50C	5.74±0.76^{d}	0.39±0.08^{b}	13.43±0.00^{c}	2.40±0.00^{c}	78.04±0.00^{c}	09.09±0.19^{d}	0.61±0.04^{d}	9.29±0.22^{b}
30S:70C	6.40±0.12^{b}	0.35±0.02^{bc}	15.26±0.20^{b}	2.60±0.00^{b}	75.39±0.00^{d}	10.15±0.64^{c}	1.08±0.20^{c}	9.07±0.84^{c}
100C	4.96±0.02^{e}	0.34±0.00^{c}	18.01±0.05^{a}	3.40±0.00^{a}	73.29±0.00^{e}	11.43±1.15^{ab}	1.19±0.75^{b}	10.24±1.90^{a}
Cookies								
100S	5.10±0.12^{b}	24.20±0.35^{a}	5.98±0.05^{e}	1.92±0.04^{c}	62.82±0.00^{a}	11.06±2.59^{b}	0.21±0.30^{d}	10.92±2.19^{b}
70S:30C	4.95±0.22^{b}	23.22±1.39^{a}	7.47±0.05^{d}	2.12±0.01^{bc}	61.20±0.00^{c}	12.94±1.02^{a}	0.52±0.00^{b}	12.42±0.86^{a}
50S:50C	5.80±0.07^{a}	22.11±0.08^{a}	8.29±0.05^{c}	2.26±0.04^{b}	61.57±0.00^{b}	09.47±0.66^{d}	0.27±0.07^{c}	9.31±0.89^{c}
30S:70C	3.71±0.12^{c}	22.86±0.16^{a}	9.67±0.05^{b}	2.64±0.11^{a}	61.23±0.00^{c}	09.15±0.27^{e}	0.13±0.18^{e}	9.06±0.02^{d}
100C	5.01±0.25^{b}	23.66±0.01^{a}	11.28±0.05^{a}	2.81±0.01^{a}	57.27±0.00^{d}	09.55±0.21^{c}	1.89±0.72^{a}	7.65±0.51^{e}

Values are mean scores± Standard deviation of duplicate readings. Data in the same column bearing different superscript for flours, extrudates and cookies respectively differ significantly ($p < 0.05$). 100S= samples from 100% sorghum flour; 70S:30C= samples from 70% sorghum and 30% cowpea flour, 50S:50C= samples from 50% sorghum and 50% cowpea flour, 30S:70C= samples from 30% sorghum and 70% cowpea flour, 100C=samples from 100% cowpea flour.

In addition, the values obtained for total, soluble and insoluble dietary fiber ranged from 14.77 to 20.11, 09.09 to 11.47, 9.15 to 12.94; 0.49 to 0.87, 0.39 to 2.38, 0.13 to 1.89; and 13.90 to 19.63, 9.07 to 10.24, 7.65 to 12.42 for flour, extrudates and cookies respectively. The effect of the addition of cowpea flour did not follow a trend as the dietary fiber of some samples increased, whereas others decreased. For instance, the dietary fiber content of the 70S:30C extrudate was increased, but that of the 50S:50C and 30S:70C extrudates were decreased. Dietary fiber is the part of a plant's edible portion that are resistant to digestion and absorption in the small intestine of humans and ferment completely or partially in the large intestine of humans (Gómez et al., 2003). Dietary fiber has been reported to increase appetite, improve metabolic health, and prevent cardiovascular diseases (Barber *et al*., 2020).

5.2.2 Free phenolics, tannin, antioxidant capacity, and resistant starch contents of samples

The results for the free phenolics, tannin, antioxidant capacity, and resistant starch contents of the flour, extrudates, and cookies are shown on Table 6. The values obtained for free phenolics were significantly different ($p<0.05$) and ranged between 0.90 to 45.34, 0.62 to 2.56, and 1.48 to 7.16 mg GAE/g for flour, extrudates and cookies respectively. The free phenolic compounds present in sorghum flour was higher than that in cowpea flour. Generally, the same trend was observed in the extrudates and cookies- the phenolics decreased with an increase in the percentage substitution of cowpea flour. The data obtained for the phenolic content of extrudates agreed with that of de Morais et al. (2015), who assessed how the extrusion procedure affected the free total phenolic content of various sorghum genotypes and found that it decreased by between 11.8 and 20.0% as a result of the phenolic compounds' thermal breakdown or interaction with the nutrients released from the food matrix. Brennan *et al.* (2011), noted that the effect of extrusion on bioactive compounds was cultivar dependent and that decrease may also be due to decarboxylation of phenolic acids during extrusion.

Furthermore, the results obtained for cookies agreed with that of Chiremba et al. (2009), who reported that the phenolic content of whole grain tannin sorghum cookies was 6.5 mg GAE/g, but were lower than 8.5 to 11.1 mg GAE/g reported by Queiroz et al. (2022) for black tannin sorghum bran cookies. The higher values reported by the latter researchers could be because they used sorghum bran, and sorghum's phenolic content has been reported to be concentrated in the bran of genotypes with deeper colors, particularly those with condensed tannins (Moraes 2015).

Table 6: Free phenolics, Tannin and Antioxidant properties of samples

Sample	Free phenolics (mg GAE/g)	Tannin (mg catechin equiv./g)	Antioxidant capacity (µMol TE/g sample)	Resistant starch (g/100g)
Flours				
Sorghum	45.34±1.44^{a}	47.25±0.00^{a}	211.20±13.50^{a}	36.29±3.13^{a}
Cowpea	0.90±0.00^{b}	0.65±0.01^{b}	20.10±1.90^{b}	2.18±0.19^{b}
Extrudates				
100S	2.56±0.01^{a}	5.53±0.01^{a}	38.10±4.10^{a}	0.52±0.10^{a}
70S:30C	2.22±0.02^{b}	4.58±0.02^{b}	27.70±3.50^{b}	0.16±0.01^{d}
50S:50C	1.95±0.01^{c}	4.05±0.01^{c}	19.70±1.10^{c}	0.28±0.02^{c}
30S:70C	1.38±0.00^{d}	2.78±0.00^{d}	12.90±0.90^{d}	0.31±0.04^{c}
100C	0.62±0.00^{e}	0.38±0.00^{e}	7.40±0.70^{e}	0.48±0.04^{b}
Cookies				
100S	7.16±0.01^{a}	30.51±0.02^{a}	60.20±5.30^{a}	4.67±0.42^{a}
70S:30C	5.28±0.01^{b}	15.84±0.01^{b}	49.30±3.60^{b}	3.68±0.42^{b}
50S:50C	3.90±0.03^{c}	9.22±0.00^{c}	36.40±2.90^{c}	3.03±0.08^{c}
30S:70C	2.53±0.02^{d}	5.12±0.01^{d}	21.10±2.70^{d}	2.79±0.23^{d}
100C	1.48±0.01^{e}	2.30±0.02^{e}	12.60±0.90^{e}	2.06±0.24^{e}

Values are mean ± standard deviation of triplicates. Data in the same column bearing different superscript for flours, extrudates and cookies respectively differ significantly ($p < 0.05$). 100S= samples from 100% sorghum flour; 70S:30C= samples from 70% sorghum and 30% cowpea flour, 50S:50C= samples from 50% sorghum and 50% cowpea flour, 30S:70C= samples from 30% sorghum and 70% cowpea flour, 100C=samples from 100% cowpea flour.

The result for tannin content of flour, extrudates and cookies as shown on Table 6 differed significantly ($p<0.05$). The values obtained ranged between 0.65 to 47.25, 0.38 to 5.53 and 2.30 to 30.51 mg Catechin equiv./g. The decrease followed the same pattern for the extrudates and cookies, as it was proportional to percentage increase in substitution of cowpea flour. However, the cookies retained more of their tannins compared to the extrudates. This could be because the cookies contained more flour than the extrudates. It could also be related to the different heat treatments applied in processing the products, as the high temperature and pressure used during extrusion has been reported to lead to more degradation of tannins compared to baking (Anton *et al.*, 2009). The reduction in tannin content during extrusion cooking may also be attributed to the binding of tannins with protein (Emmambux and Taylor, 2003) and other cell wall macromolecules thereby reducing their extractability (Taylor and Duodu, 2015). Dlamini et al. (2007) added that extrusion cooking at high temperatures can denature protein, causing it to assume a more open structure with exposed regions that encourages tannin-protein interaction. Similar results have also been reported by several researchers for the tannin content of processed foods. For instance, Awika et al., (2003) and Dlamini et al. (2007) reported a reduction in tannin content upon extrusion of sorghum. Alonso et al. (2000) reported same result as above for faba bean and kidney bean.

About the antioxidant capacity, the values obtained for the flour, extrudates and cookies differed significantly ($p<0.05$) and ranged from 20.10 to 211.20, 7.40 to 38.10 and 12.60 to 60.20 µMol TE/g sample for flours, extrudates and cookies respectively. The sorghum flour had the highest value which could be linked to the high content of phenolics and tannins present in the grain as seen in Table 6. Phenolic acids have been reported to scavenge peroxyl radicals (Abdel-Aal *et al*., 2022). The antioxidant capacity was also observed to decrease with increase in the substitution of sorghum flour with cowpea flour in both the extrudates and cookies, which could be related to decreased phenolic content of samples. The obtained results for extrudates were like that of Korus et al. (2007), who investigated the effect of extrusion on polyphenol content and antioxidant activity of common bean, and reported that a significant decrease in antioxidant activity occurred after extrusion.

Similarly, Delgado-Licon et al. (2009) observed a significant decrease in the antioxidant activity during extrusion of bean/corn mixture. Awika et al., (2003) and Dlamini et al. (2007) also reported a reduction in antioxidant activity upon extrusion of sorghum. The reduction in radical scavenging activity during extrusion cooking may be attributed to binding of tannins with protein (Emmambux and Taylor, 2003) and other cell wall macromolecules thereby reducing their extractability (Taylor and Duodu, 2015). For the cookies, the obtained results agreed with that of Chiremba et al. (2009), who studied the phenolic content, antioxidant capacity and consumer acceptability of sorghum cookies, and reported that the sorghum flour had slightly higher phenolic content and antioxidant activity values than their corresponding cookies. Contrarily to this, Shafi et al. (2016) noted that baking resulted in an increase in the antioxidant capacity of cookies in comparison to flour in his study of the effect of baking on antioxidant properties of wheat-water chestnut cookies.

On the other hand, the resistant starch content of the flour, extrudates and cookies as presented in Table 6 differed significantly and ranged between 2.18-36.29, 0.16-0.52, and 2.06-4.67 g/100g for flour, extrudates and cookies respectively. The sorghum flour had the highest content of resistant starch in this study (36.29 g/100g), which was expected since the sorghum BRS 305 has been reported to be high in resistant starches (de Teixeira *et al*., 2016). This result indicated that products containing BRS 305 sorghum flour can contribute to improved health of consumers as researches have shown that consumption of foods rich in resistant starches have physiological effects, which is due to their fermentation in the large intestine by the gut microbiota. This is possible because the digestion process produces methane, hydrogen, and short-chain fatty acids (Deehan *et al*., 2020), which has been reported to reduce cholesterol and triglyceride levels in the blood (Bojarczuk *et al.,* 2022).

Also, the cookies were observed to retain more resistant starch than the extrudates. This could be related to the heat treatments applied to them, as food products that undergo dry heat treatment (e.g., cookies) tend to retain higher resistant starch content from the original flour used, compared to wet heat processing (extrudates) (Englyst *et al.* 2003). In addition, a reduction in the resistant starch content of the cookies and extrudates was observed to occur with an increase in the addition of cowpea flour. This could be related to the low resistant starch content of cowpea flour (2.18 g/100g) as shown on Table 6. Generally, the resistant starch content of a food substance is affected by factors like tannin-starch interaction, the formation of indigestible complexes, intensification of already-existing interactions and type of processing techniques used (Barros *et al*., 2012; Soares *et al.,* 2019).

5.3 Technological properties of extrudates and cookies

5.3.1. Texture properties of extrudates

The values for textural properties of extrudates obtained via the puncture test is summarized on Table 7. The frequency of structural ruptures (N$_{sr}$), which is related to the specific mechanical energy applied to extrudates during processing ranged from 0.49-0.76 mm^{-1}. Extrudates from 100S had the highest value, while that from 70S:30C had the least value. The result obtained in this study were higher than 0.12-0.25 mm $^{-1}$ reported by da Silva et al. (2014) for extrudates from corn flour and defatted carioca bean flour blend. The lower Nsr values could be related to lower specific mechanical energy spent during the extrusion process (Bouvier *et al.* 1997; da Silva *et al*., 2014).

Table 7: Texture properties of cookies and extrudates

Sample	100S	70S:30C	50S:50C	30S:70C	100C
Cookies					
Hardness (N)	12.75±1.07^{bc}	20.98±4.61^{a}	9.41±2.32^{c}	19.24±5.14^{ab}	16.03±1.92^{abc}
Extrudates					
Frequency of structural ruptures (mm^{-1}).	0.76±0.15^{a}	0.49±0.12^{b}	0.59±0.23^{ab}	0.68±0.17^{ab}	0.52±0.17^{b}
Av. Spec. force of structural ruptures (N)	0.02±0.01^{a}	0.03±0.03^{a}	0.04±0.04^{a}	0.03±0.02^{a}	0.02±0.01^{a}
Average of compression force (N)	0.23±0.12^{b}	0.53±0.16^{ab}	0.36±0.37^{b}	0.44±0.36^{b}	0.86±0.31^{a}
Crispness work (N mm)	0.31±0.17^{b}	1.21±0.58^{ab}	0.83±0.94^{b}	0.75±0.72^{b}	1.98±1.37^{a}

Values are mean scores± Standard deviation of duplicate readings. Data in the same row bearing different superscript for extrudates and cookies respectively differ significantly ($p < 0.05$). 100% S= samples from 100% sorghum flour; 70S:30C= samples from 70% sorghum and 30% cowpea flour, 50S:50C= samples from 50% sorghum and 50% cowpea flour, 30S:70C= samples from 30% sorghum and 70% cowpea flour, 100% C=samples from 100% cowpea flour.

The average specific force of structural ruptures (F_{sr}) was also low, with values ranging from 0.02-0.04 N. The values were lower than 0.30-1.09 N reported by da Silva et al. (2014) for extrudates from corn flour and defatted carioca bean flour blend. The average compression force (F), which reflects the force required to penetrate, with a probe, the cell walls of the extrudates ranged from 0.23-0.86 N. The values were within the range of 0.09-0.35 N reported by da Silva et al. (2014). Addition of cowpea flour to extrudates increased the compression force across the samples. This could be related to increased protein content of the extrudates as Chanvrier et al. (2013) noted that increase in protein content increased hardness. Similar result was reported by Azzollini et al. (2018) when they studied the effects of formulation and process conditions on microstructure, texture, and digestibility of extruded insect-rich snacks, and reported that formulations with increased protein showed high compactness, which they associated with modification of the pore wall composition and their morphology.

In addition, the results for crispness work (Wc) ranged between 0.31 and 1.98 N mm (Table 7). This property combines information about Nsr, Fsr and F values, and it is directly related to crispness of the extrudate material. It can be interpreted as the sensory parameter of fracturability and describes the work required to fracture one pore or a group of pores. Extrudates from 100C had the highest value for crispiness work, and was significantly different from other samples, while the 100S cookies had the least value. The difference could be related to the protein content of both flours used in the cookie's formulation. Saeleaw et al. (2012) noted that an increase in moisture content might cause reduction of expansion by consequently reducing the formation of air bubbles and number of internal cells in the extrudates, which could lead to a decrease in crispiness of products. Pezalli et al. (2020) added that crispiness could also be related to expansion and cell structure development within the starch matrix during extrusion (Pezalli *et al*., 2020).

5.3.2. Texture properties of cookies

The results of the texture of extrudates are presented in Table 7. The values obtained for hardness of cookies differed significantly ($p<0.05$) and ranged between 9.06-21.02 N. The cookies from 70S:30C blend had the highest value for hardness. This could be as a result of the higher bulk density recorded for this blend and the lower moisture content of the sample. Interestingly, the results obtained in this study were like that of Garźon et al. (2020), who reported that cookies made with native white sorghum flour had a hardness of 11.38 N at 20 °C.

Similarly, Ibrahim (2017) reported that cookies made with 80% sorghum flour and containing approximately 16% sugar had a hardness of 21.78 N.

Generally, the results did not follow a trend, which could be because of the large variability in the particle size of the flours. Belorio at al. (2019) noted that an increase in average particle size can increase diameter and spread factor of the cookies but decreases their hardness. Hardness of cookies is instigated by the moisture content, and the starch-protein interactions, through hydrogen bonds (Brites *et al.*, 2019), and is proportional to the force applied to cause a deformation, the greater the force needed to penetrate the food, the greater its hardness (Celia *et al.,* 2022). It is an important parameter for the acceptance of cookies, since it is desirable as they represent the crunchiness of the food.

5.3.2. Pasting properties of flours, extrudates and cookies

The results of pasting properties of flour, extrudates and cookies are shown on Table 8. The values obtained differed significantly ($p<0.05$). A remarkable decrease was observed in the pasting properties of the cookies and extrudates when compared to that of the raw flours. Generally, the pasting properties were seen to decrease with substitution of cowpea flour.

The trough viscosities obtained in this study differed significantly ($p<0.05$) and ranged between 26.50 to 49, 52 to 87 and 33.50 to 48.00 cP for flour, extrudates and cookies respectively. Trough viscosity measures the ability of paste to resist breakdown during cooling. The results obtained for trough in this study indicates that raw sorghum and cowpea starch granule had better resistance against breakage compared to their processed counterparts. The result agreed with the findings of Hashimoto et al. (2020) who studied the pasting properties of raw (BRS Guariba) and extruded (BRS Novaera) cowpea cotyledons flour and reported that BRS Guariba starch granules resisted better ($p < 0.05$) the breakage than BRS Novaera. Similar result was also obtained by Wang et al. (2020) for sorghum flour (893.7 RVU) and sorghum-chickpea extrudates (93.3 to 120.3 cP).

Table 8: Pasting properties (cP) of flour, extrudates and cookies

Sample	Trough viscosity	Peak viscosity	Final viscosity	Breakdown Viscosity	Set back viscosity
Flours					
100S	26.50 ± 1.00^{d}	1069.00 ± 1.00^{a}	2708.00 ± 1.00^{a}	1042.50 ± 1.00^{a}	1639.00 ± 1.00^{a}
70S:30C	49.00 ± 2.00^{a}	988.00 ± 2.00^{b}	1743.00 ± 2.00^{d}	939.00 ± 2.00^{b}	755.00 ± 2.00^{d}
50S:50C	27.00 ± 3.00^{d}	805.50 ± 3.00^{d}	1541.50 ± 3.00^{e}	778.50 ± 3.00^{d}	736.00 ± 3.00^{e}
30S:70C	36.00 ± 3.50^{c}	766.50 ± 3.50^{e}	1907.00 ± 3.50^{c}	730.50 ± 3.50^{e}	1140.50 ± 3.50^{b}
100C	47.50 ± 1.42^{b}	852.50 ± 1.42^{c}	1963.50 ± 1.42^{b}	805.00 ± 1.42^{c}	1111.00 ± 1.42^{c}
Extrudates					
100S	81.00 ± 2.00^{c}	123.00 ± 2.00^{a}	134.00 ± 2.00^{b}	82.00 ± 2.00^{a}	11.00 ± 2.00^{d}
70S:30C	87.50 ± 7.00^{a}	118.50 ± 7.00^{b}	146.50 ± 7.00^{a}	71.00 ± 7.00^{b}	28.00 ± 7.00^{a}
50S:50C	85.00 ± 0.50^{b}	115.00 ± 0.50^{c}	127.00 ± 0.50^{c}	70.00 ± 0.50^{c}	12.00 ± 0.50^{c}
30S:70C	67.50 ± 2.00^{d}	93.00 ± 2.00^{d}	94.00 ± 2.00^{d}	65.50 ± 2.00^{d}	1.00 ± 2.00^{e}
100C	52.00 ± 1.00^{e}	53.00 ± 1.00^{e}	68.50 ± 1.00^{e}	41.00 ± 1.00^{e}	15.50 ± 1.00^{b}
Cookies					
100S	48.00 ± 0.00^{a}	95.50 ± 0.00^{a}	165.50 ± 0.00^{a}	47.50 ± 0.00^{a}	70.00 ± 0.00^{a}
70S:30C	35.00 ± 1.50^{d}	75.00 ± 1.50^{d}	97.00 ± 1.50^{e}	40.00 ± 1.50^{c}	22.00 ± 1.50^{e}
50S:50C	33.50 ± 1.00^{e}	91.00 ± 1.00^{b}	137.00 ± 1.00^{b}	57.50 ± 1.00^{b}	46.00 ± 1.00^{b}
30S:70C	36.50 ± 0.50^{c}	75.00 ± 0.50^{d}	98.00 ± 0.50^{d}	38.50 ± 0.50^{d}	23.00 ± 0.50^{d}
100C	43.00 ± 1.50^{b}	83.50 ± 1.50^{c}	111.50 ± 1.50^{c}	40.50 ± 1.50^{c}	28.00 ± 1.50^{c}

Values are mean ± standard deviation of triplicates. Data in the same column bearing different superscript for flours, extrudates and cookies respectively differ significantly ($p < 0.05$). 100S= samples from 100% sorghum flour; 70S:30C= samples from 70% sorghum and 30% cowpea flour, 50S:50C= samples from 50% sorghum and 50% cowpea flour, 30S:70C= samples from 30% sorghum and 70% cowpea flour, 100C=samples from 100% cowpea flour.

Also, the peak viscosities of samples ranged from 766.50 to 1069, 53 to 123 and 75 to 95.50 cP for flour, extrudates and cookies respectively. The 100% sorghum flour and 100% sorghum cookies exhibited the highest peak viscosities and were significantly different ($P < 0.05$) from the rest of the flour and cookie samples, while the extrudate made with 70S:30C extrudate had the highest value for peak viscosity. The peak viscosities of the flour and extrudates decreased with an increase in the substitution of cowpea flour, that of the cookies did not follow any trend. The low peak viscosities exhibited by the extrudates and cookies could be an indication of various levels of starch depolymerization and gelatinization that occurred during extrusion and baking (Adegunwa *et al*., 2012). The peak viscosities obtained in this study were within the range of 158 to 2121 RVU reported by Kesselly et al. (2023) for cowpea flours and extrudates and 107.3 to 1162.3 cP reported by Wang et al. (2019) for sorghum-chickpea flour and extrudates.

About the final viscosities, the values obtained for flour, extrudates and cookies ranged from 1541.50 to 2708.50, 68.50 to 146.50, and 97 to 165.50 cP respectively. The final viscosities of the flour, extrudates and cookies in this study decreased with substitution of cowpea flour, but did not follow a trend. Extrusion and baking also played a role in decreasing the final viscosities. A similar trend was reported by Hashimoto et al. (2020) for extruded cowpeas. They reported that the final viscosity of extrudates were 18 to 30 times lower than that of the raw samples. Kesselly et al. (2023) also reported a final viscosity range of 86-2737 RVU for cowpea extrudates. All final viscosity values were higher than trough viscosities, this increase in viscosity value at the end of the cooling cycle could be due to the alignment of amylose chains and other interactions between proteins, lipids, and complex carbohydrates (Kaur *et al.,* 2007).

Furthermore, the values obtained for breakdown viscosities ranged from 730.50 to 1042.50, 41.00 to 82.00 and 38.50 to 57.50 cP for flour, extrudates and cookies respectively. The 100% sorghum flour had the highest breakdown and was significantly different ($P < 0.05$) from the rest of the samples. The breakdown viscosity is an index of the stability of the starch and a measure of the ease with which the swollen granules can be disintegrated (Kaur *et al*., 2007). It gives an indication of the ability of flour to withstand heating and shear stress during cooking (Adebowale *et al*., 2005). The values obtained in this research were lower than 1169 to 3171 mPa s reported by Palavecino et al. (2016) for sorghum flour but within the range of 8.70 to 30.70 RVU reported by Wang et al. (2019) for sorghum-chickpea extrudates.

In addition, the setback viscosities ranged from 736.00 to 1639.00, 1.00 to 28.00 and 22.00 to 70.00 cP for flour, extrudates and cookies respectively. The 100 % sorghum flour had the highest values for setback and was significantly different ($P < 0.05$) from other flour samples, while extrudate from 30S:70C had the least value among the extrudates. The low setback values observed in the extrudates and cookies after addition of cowpea flour also indicated decreased starch retrogradation, which could be related to the depolymerization of the starch and its subsequent complexation with other components in sorghum and cowpea flours (Naiker *et al.,* 2019). The values obtained in this study were within the range of 28-1115 RVU reported by Kesselley et al. (2023) for cowpea extrudates and 18.7 to 15.3 cP reported by Wang et al. (2020).

5.5 Sensory evaluation of extrudates and cookies

The sensory attributes of extrudates and cookies from composite flours of sorghum and cowpea are shown on Table 9. A total of 120 consumers participated in the present study, 60 different panelists for each product. Majority of the panelists (75%) were graduate students and university staff of UFV. The scores of sensory attributes of all extrudates analyzed were higher than the cutoff score (5), suggesting the acceptance of all samples by panelists. Generally, there were significant differences ($p<0.05$) in the sensory attributes among the extrudates samples, except for aroma. The sensory attributes were seen to decrease with percentage increase in cowpea flour addition. The extrudate made from 100% sorghum flour had the highest value for all sensory parameters evaluated, and had an intention to purchase score of 68.33%, indicating an acceptance of the product. According to Awika and Rooney (2004), the sensory characteristics of sorghum-based food products may be influenced by color of the sorghum pericarp, which changes with sorghum genotype. The BRS 305 sorghum had a brown-colored pericarp, which gave the 100S extrudate the color of chocolate, which most panelists considered acceptable. The higher scores obtained for 100S extrudate could have also been as a result of the beany flavor of the cowpea flour that was noticeable in the extrudate samples that had up to 50% cowpea flour substitution. This was also the reason for the exclusion of samples 30S:70C and 100C from the sensory evaluation. The low scores obtained for the extrudates containing cowpea flour, probably had a considerable influence on purchase intent of consumers, which led to an acceptance index below 50% for the 70S:30C and 50S:50C extrudates.

Table 9: Sensory evaluation scores for extrudates and cookies

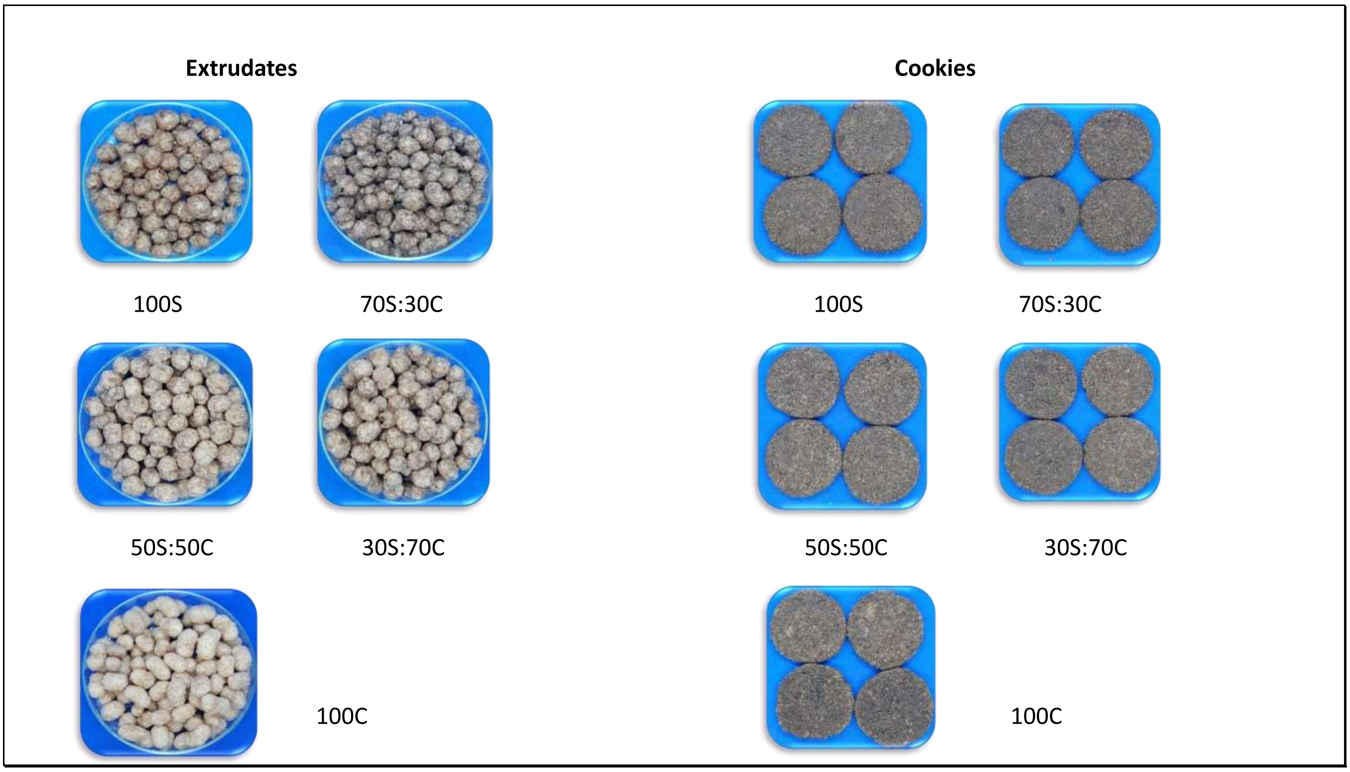
Sample	Appearance	Aroma	Flavour	Texture	Overall acceptance	Intention to purchase (%)	
Extrudates						**Yes**	**No**
100S	7.35±1.31^{a}	6.77±1.48^{a}	6.20±1.77^{a}	7.16±1.71^{a}	6.52±1.60^{a}	68.33	31.67
70S:30C	6.43±1.41^{b}	6.45±1.48^{a}	5.43±2.10^{ab}	5.68±2.35^{b}	5.58±5.58^{b}	43.33	56.67
50S:50S	7.33±1.87^{a}	6.22±1.81^{a}	4.98±2.47^{b}	6.43±2.13^{ab}	5.31±5.31^{b}	38.33	61.67
Cookies							
100S	7.00±1.56^{a}	6.65±1.78^{a}	6.48±1.85^{ab}	6.23±2.01^{a}	6.45±1.67^{ab}	58.33	41.67
70S:30C	6.97±1.59^{a}	6.47±1.96^{a}	6.73±1.51^{ab}	6.43±1.95^{a}	6.50±1.64^{ab}	76.67	23.33
50S:50C	6.60±1.68^{a}	6.58±1.53^{a}	5.98±1.90^{b}	6.13±1.82^{a}	6.07±1.61^{b}	46.67	53.33
30S:70C	6.75±1.73^{a}	6.70±1.66^{a}	7.00±1.56^{a}	6.97±1.78^{a}	7.03±1.52^{a}	76.67	23.33
100C	6.58±1.65^{a}	6.48±1.63^{a}	5.88±2.02^{b}	6.12±2.06^{a}	5.80±1.77^{b}	55.00	45.00

Values are mean scores± standard deviation. Data in the same column bearing different superscript for extrudates and cookies respectively differ significantly ($p < 0.05$). 100% S= samples from 100% sorghum flour; 70S:30C= samples from 70% sorghum and 30% cowpea flour, 50S:50C= samples from 50% sorghum and 50% cowpea flour, 30S:70C= samples from 30% sorghum and 70% cowpea flour, 100% C=samples from 100% cowpea flour.

On the other hand, the sensory scores of the cookies did not differ significantly ($p>0.05$) amongst the samples. Sample 30S:70C had the highest score for all sensory parameters measured, except for appearance. Sample 100S had the highest value for appearance. Samples 30S:70C and 70S:30C had the same value for intention to purchase (76.67 %). The high acceptance of the sample could be as a result of the nutty flavor developed in cookies substituted with cowpea flour during baking, which most of the panelist considered acceptable. Like this, earlier research found that cookies manufactured with gluten-free flours had sensory scores that were higher than the 5 for all sensory qualities (Giuberti *et al*., 2018; Hussain *et al*., 2020;

Aljobair, 2022). Another study (Paesani *et al.*, 2020) found no appreciable variations in the sensory characteristics of cookies baked with gluten-free flours. The qualitative attributes of cookies made with several gluten-free flour blends were also tested by Rai et al. (2014), who found that sorghum flour samples had the highest overall acceptability scores.

The samples are shown in the figures below:



6 CONCLUSIONS

This study has evaluated the physical, chemical, technological, and sensorial properties of extrudates and cookies from composite sorghum and cowpea flour. The results obtained indicated that cowpea flour improved the physical, nutritional, technological, and sensory properties of extrudates and cookies. The high protein and dietary fiber content of cowpea flour and the rich phenolic and tannin content of sorghum flour have enhanced the nutritional profile of the products. The extrusion and baking processes have also contributed to the development of desirable texture and sensory attributes. The sensory evaluation of the extrudates and cookies showed that they were well-liked by consumers, with no significant difference in preference between the two. Extrudates and cookies from sample 70S:30C were the best samples, as they were observed to retain more of the chemical properties of their raw flour, and had good acceptability. Sorghum flour can be substituted with cowpea flour up to 50% for extrudates and 70% for cookies without affecting the sensorial properties of the final products. These findings suggest that composite sorghum and cowpea flours can be used to develop nutritious gluten-free and functional foods that are acceptable to consumers. Further research is needed to optimize the formulations and processing conditions to enhance the properties of the final products.

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Appendix

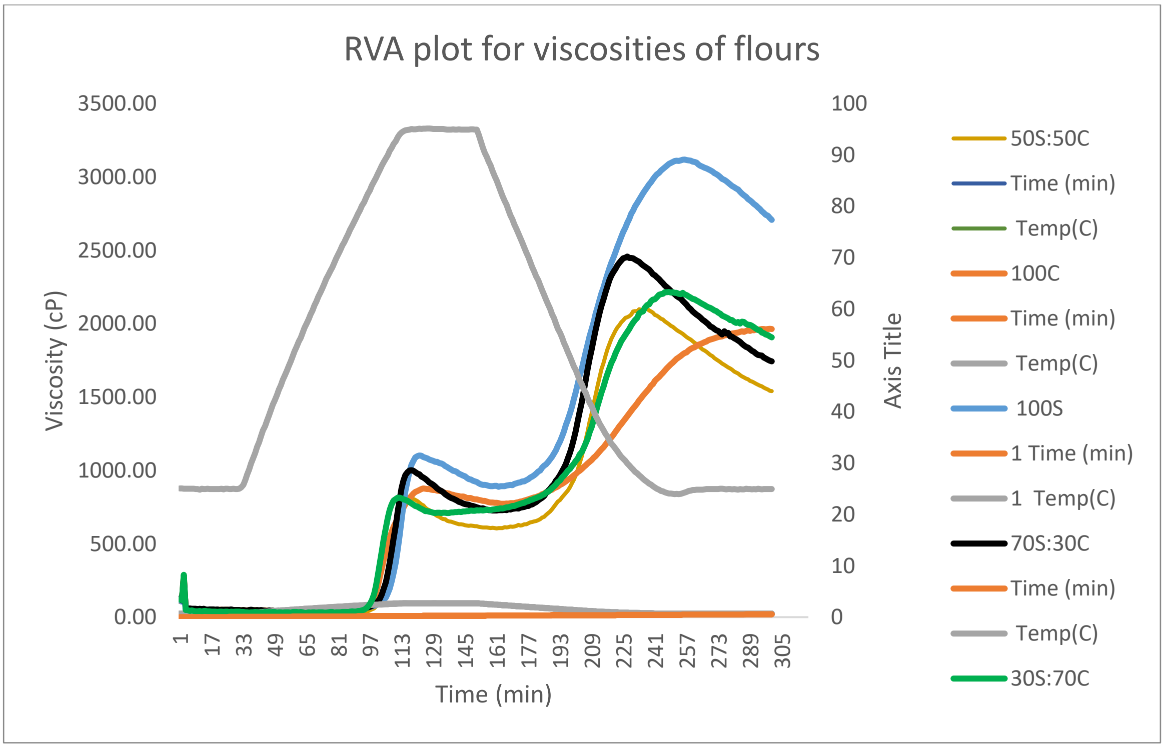

Figure 8a: RVA plots of flours

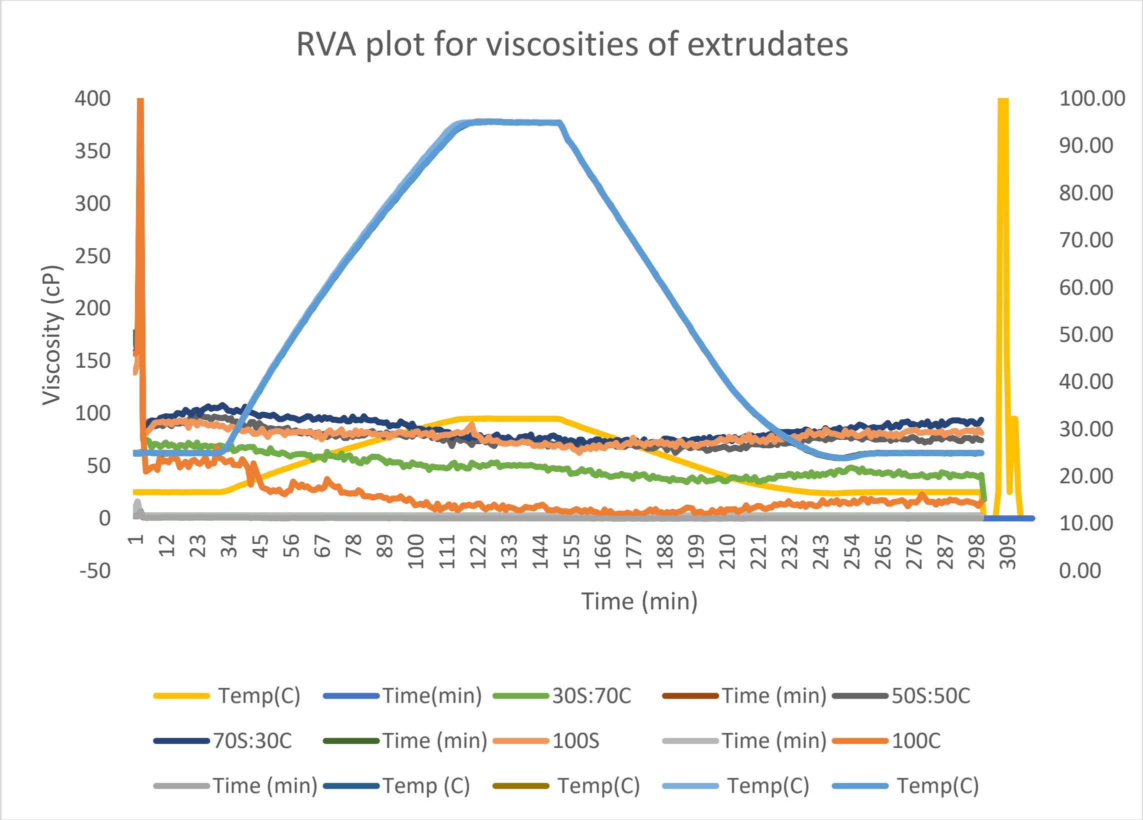

Figure 8b: RVA plot of extrudates

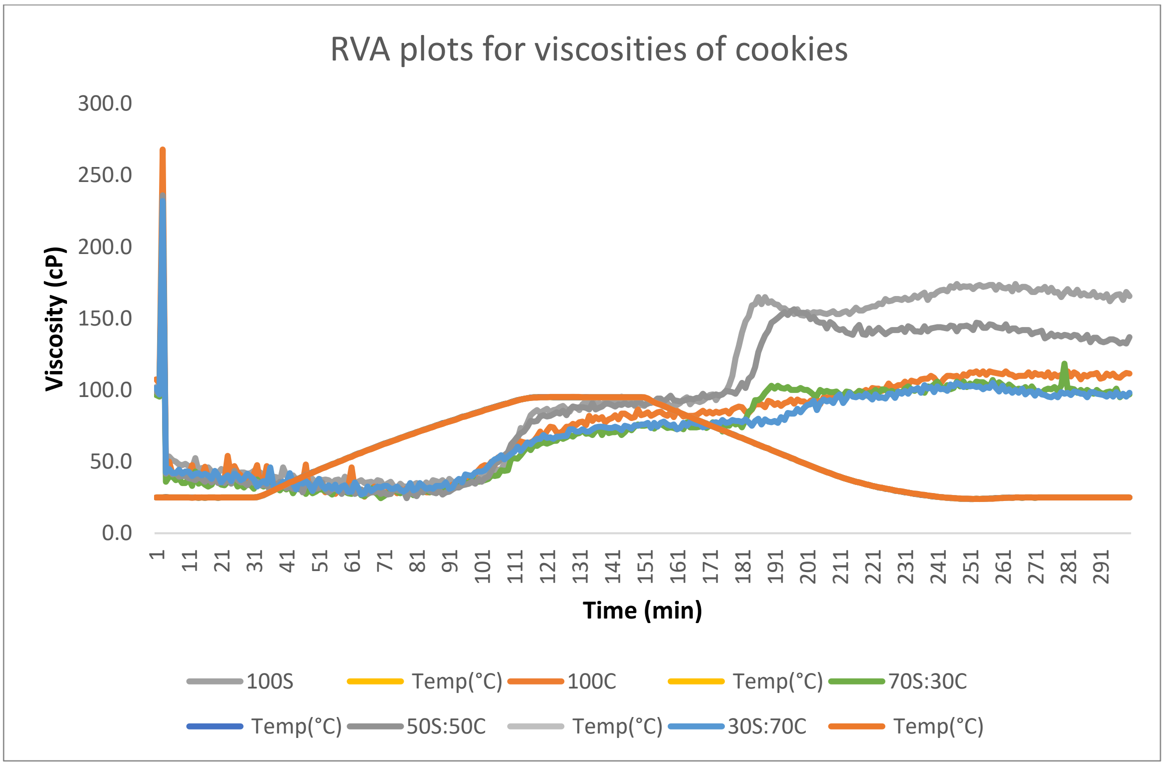

Figure 8c: RVA plots of cookies

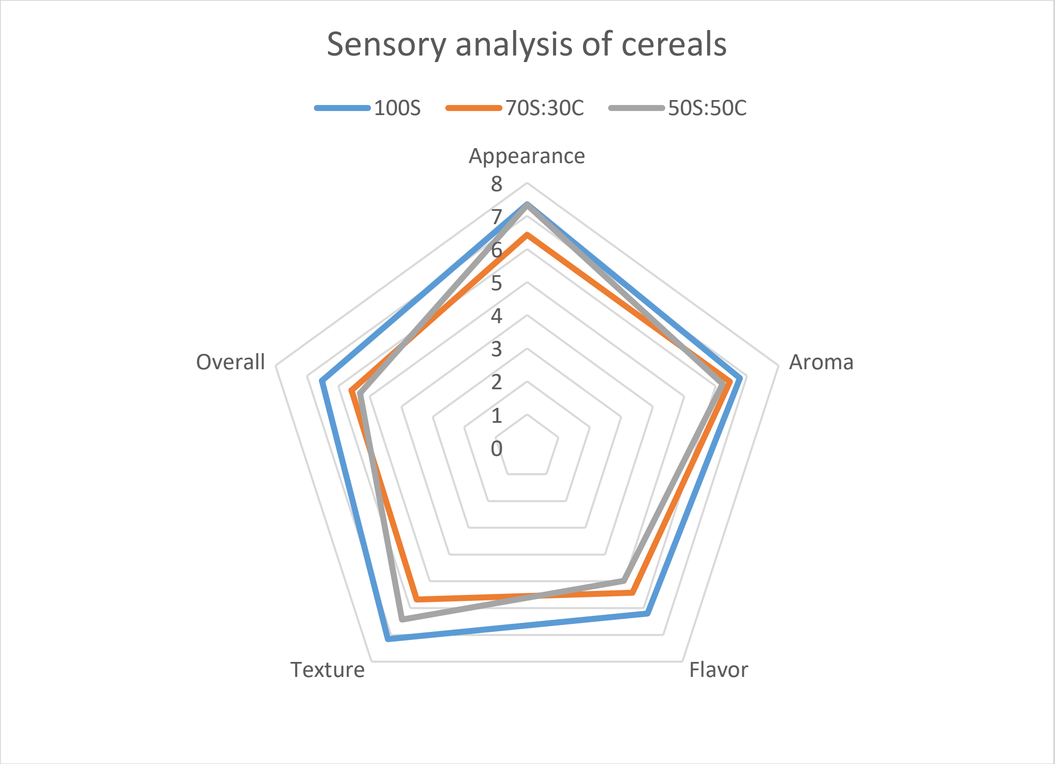

Figure 9a: Sensory profile of cereals

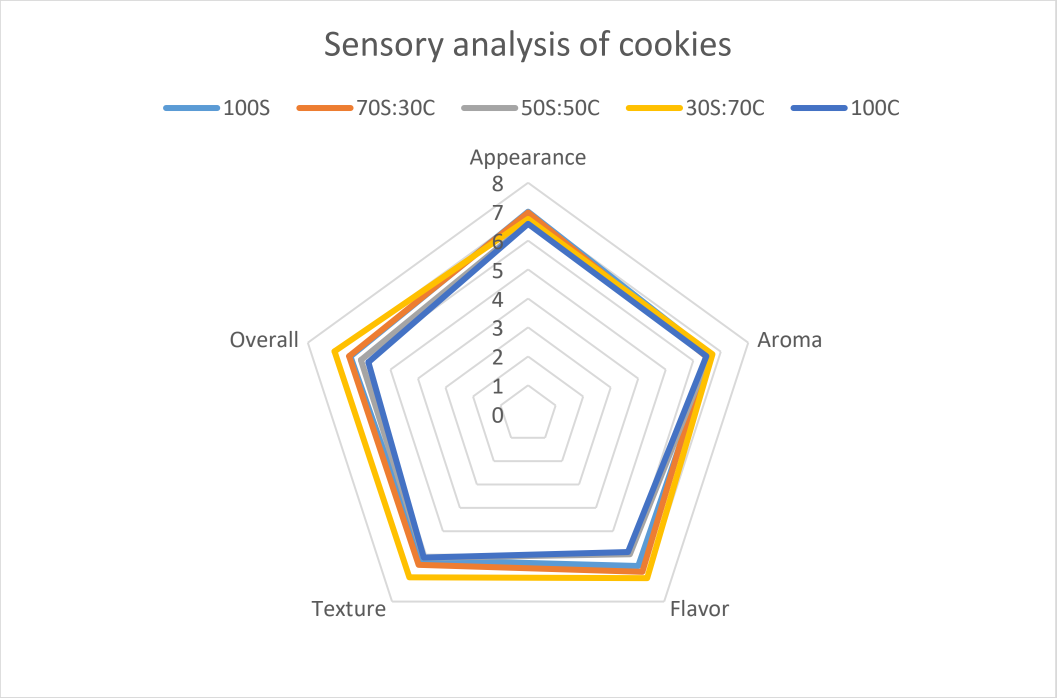

Figure 9b: Sensory profile of cookies