Resting of sorghum-soya biscuit dough: effect on dough and biscuit physicochemical properties and consumer acceptability of the baked biscuits

By

Josephine Thandiwe Baloyi

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Pretoria, South Africa

DECLARATION

I, Josephine Thandiwe Baloyi declare that the dissertation, which I hereby submit for the degree of MSc Food Science at the University of Pretoria, is my own work and has not been previously submitted by me for a degree at any other tertiary institution

Signature: ____

Date: ___________________________

DEDICATION

This research is dedicated to my parents, Joseph Jimu and Juliet Baloyi and siblings Hungalani, Tafadzwa and Julia.

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This dissertation reflects the work of many people who deserve recognition. I am truly humbled and would like to thank and acknowledge the following:

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ABSTRACT

Resting of sorghum-soya biscuit dough: effect on dough and biscuit physicochemical properties and consumer acceptability of the baked biscuits

Student: Josephine Thandiwe Baloyi
Supervisor: Prof Henrietta. L de Kock
Co- supervisors: Prof J. R. N Taylor and Prof M. N. Emmambux
Degree: MSc Food Science

Previously researchers (also from the University of Pretoria) reported low acceptability ratings for sorghum and sorghum-soya composite biscuits because consumers perceived the biscuits as hard and gritty. Grittiness in sorghum biscuits may be attributed to limited hydration of starch during dough preparation due to the presence of hydrophobic sorghum protein bodies that surround starch granules. Hence, there is need for further research to improve the understanding of the sensory properties and also physicochemical properties of sorghum-soya dough and/or its biscuits.

In the current research, it was hypothesized that increasing the resting time of sorghum-soya composite biscuit dough would reduce the grittiness of sorghum-soya biscuits by promoting water absorption, starch granule swelling and starch gelatinisation. The objective of this study was to evaluate the effect of dough resting time (15 min and 24 h) on the physical properties, proximate composition, texture, thermal properties, starch and protein digestibility and consumer acceptability of sorghum-soya biscuits. A sorghum (70%) and full-fat enzyme active soya (30%) composite flour was mixed with other ingredients (water, sugar, baking powder, vanilla essence, margarine) to make a dough, followed by resting for 15 min or 24 h respectively, before baking.

Increasing dough resting time from 15 min to 24 h produced biscuits that were thicker (7.6 mm), with moisture content that increased by 30%, had lower stress and required less force to break (34.8 N). Biscuits made from dough rested for 15 min were 6.5 mm thick, had higher stress and required 51.1 N to break ($p < 0.05$). The higher moisture content of sorghum-soya biscuits made with dough rested for 24 h suggests that water acted as a plasticizer and reduced the rigidity of the biscuit matrix. Increase in biscuit thickness and diameter with dough resting time may have been influenced by aerating effect of baking powder that produced leavening gases during dough mixing and baking. Enzymatic action of proteinase and amylases on proteins and starch in sorghum-soya dough, respectively, may have been more pronounced during the longer dough resting time, resulting in more browning reactions to occur in biscuits made from dough rested for 24 h.

Fifty consumers assessed the acceptability using a 9-point hedonic scale and preference testing of the biscuits from dough rested for 15 min and 24 h. The consumer sensory evaluation test revealed that the sorghum-soya biscuits were equally liked for appearance, colour and texture, despite significant differences in instrumental texture and colour of biscuits with dough resting time ($p < 0.05$). Biscuits made from dough rested for 24 h received lower liking scores for taste and overall flavour, and were less preferred to biscuits made from dough rested for 15 min. The perceived bitter aftertaste in these biscuits may have been caused by formation of bitter peptides during enzymatic hydrolysis of proteins with dough resting time. Comments suggested that biscuits made from both resting times were grainy.

Increasing the dough resting time did not affect starch and protein digestibility of the biscuits. As expected, *in vitro* starch digestibility confirmed that the estimated glycaemic index (EGI) of sorghum-soya biscuits was lower (approx. 47%) than that of commercial digestive whole-wheat biscuits (64%) and white bread (94%). There was no significant difference ($p > 0.05$) in thermal properties of sorghum-soya (70:30) dough and/or biscuits with increasing dough resting time. Increasing sorghum-soya dough resting time to 24 h may have promoted hydration of some starch granules but not starch gelatinization. Longer dough resting treatment did not have a positive impact on the flavour, as consumers preferred the flavour of the biscuits from dough rested for 15 min. For this reason, it is recommended to not rest the sorghum-soya biscuit dough for 24 h.

Keywords: dough-resting, biscuit, sorghum, soya, consumer acceptance

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1 INTRODUCTION

Sorghum flour can be used to make gluten-free ready-to-eat baked biscuits that have similar texture to wheat biscuits (Adedara, 2016). Composited sorghum-legume biscuits have been proposed as a strategy to address protein energy malnutrition in Africa. Complementing sorghum flour with soya or cowpea flours greatly improves the protein quality of the composite biscuits (Serrem, 2010; Dovi, 2014). Past research has shown that composite biscuits made from sorghum-legume flours received low sensory scores for texture, as biscuits were described as gritty, hard and dense (Akubor and Ukwuru, 2003, Serrem, 2010, Omoba *et al*., 2015).

Sorghum (*Sorghum bicolor* L. Moench) is an indigenous, drought-resistant cereal crop that grows in Africa and can thrive in unfavourable conditions such as alkaline soils and high temperatures (Directorate of Plant Production and ARC, 2010). It is expected that the southern parts of Africa will become drier and hotter over the next coming years because of climate change. Apart from its climate-friendly advantages, sorghum is also a gluten-free cereal which can be beneficial to people with coeliac disease (Gallagher, 2009). Sorghum also has a low starch digestibility, which may benefit those suffering from type 2 diabetes (Srichuwong *et al*., 2017). Substitution of imported wheat flour in the production of snacks, with locally grown and available cereals such as sorghum, could potentially reduce production costs of snacks and/or open new opportunities for local farmers and snack processors.

A survey by Health (2016) showed that South African consumers enjoy eating baked snacks such as biscuits on different occasions. Serrem (2010) formulated sorghum-soya biscuits with inclusion of defatted soya flour at 28.6%, 50% and 71.4% to sorghum flour, respectively. Although there was an increase in protein content of sorghum-soya biscuits as soya flour increased, biscuits with 28.6% soya flour had the highest energy contribution of 1943 kJ per 100 g compared to biscuits with 50% soya flour (1924 kJ/100 g) or 71.5% soya flour (1873 kJ/100 g) (Serrem, 2010). Sorghum-soya biscuits have a high protein content and could be produced at an affordable cost (Serrem, 2010).

Although it was possible to produce sorghum-legume composite biscuits with sufficient nutritional content, the sensory properties such as consumer acceptability of sorghum-based products remains a challenge (Serrem, 2010). The unique structure of protein bodies surrounding starch granules in sorghum flour after milling may have been perceived as grittiness in sorghum-based products by consumers (Serrem, 2010). This could be caused by strong adhesion between starch granules and protein bodies that makes them difficult to separate during milling (Delcour and Hoseney, 2010). Disulphide bonding between cysteine molecules of kafirins makes sorghum protein bodies hydrophobic (Preedy *et al*., 2011), and could be a limitation for water absorption into the starch granules of sorghum flour during dough preparation (Duodu *et al*., 2002).

Increasing the dough resting time could potentially improve biscuit texture by allowing hydration of water into starch granules during dough resting. This is expected to enable gelatinisation of starch granules during baking and potentially reduce grittiness, and thus improve the texture of sorghum-soya composite biscuits. Attributes such as colour, flavour, taste and texture are important in determining the overall characteristics of food products (Hui and Corke, 2006). The aim of the study was to improve the texture and consumer acceptability of sorghum-soya composite biscuits.

2 LITERATURE REVIEW

Sorghum is a cereal crop that can be used as an alternative to wheat during baking. However, it is a challenge for scientists to produce sorghum products that have a similar texture to wheat products. Production of biscuits using gluten-free flours such as sorghum flour has been a challenge in dough handling properties and sensory quality of biscuits. Adedara (2016) reported that it is possible to produce sorghum biscuits with a similar texture to wheat biscuits since gluten is of low importance in producing short dough biscuits. In this literature review, the focus was on biscuit formulations, dough rheology, consumer acceptance and sensory challenges related to sorghum-soya biscuits. Fundamental analyses commonly used to measure biscuit texture was reviewed alongside the thermal properties of dough and biscuits, as well as *in vitro* starch and protein digestibility of baked biscuits.

2.1 Biscuit classification

Biscuits (made from wheat flour) can be classified into hard dough or short dough biscuits, based on the dough formulation, ratios of ingredients used in a formulation or method of biscuit manufacture (Delcour and Hoseney, 2010). During dough mixing, hard dough biscuits requires more water to allow for a developed gluten network (Delcour and Hoseney, 2010). On the other hand, short dough wheat biscuits require low water (8% - 35%), high sugar (30% - 70%) and high fat (12% - 50%) quantities in the formulation, that minimise gluten development and results in dough that lacks extensibility (Delcour and Hoseney, 2010).

100% sorghum biscuits made by Adedara (2016) were classified as short dough biscuits based on their formulation and ingredients. The author found that it was possible to produce sorghum biscuits with a similar texture to wheat biscuits since wheat biscuits produced from short dough require minimal or no gluten network development. This finding may suggest that gluten is of low importance in short dough formulations.

Classification of biscuits based on the method of dough placement on the baking tray includes biscuits made from rotatory-moulded, wire-cut, and cutting machine shaped biscuits (Delcour and Hoseney, 2010). Figure 2.1 shows rotatory mould biscuits dough that is forced into moulds on rotating rolls, extracted on the cavity and placed on the baking tray when the rolls are half way during rotation (Indiamart, 1996). The dough contains low water levels approximately

15% by flour basis, as well as a high content of sugar (30% - 70%) and fat (12% - 50%) (Delcour and Hoseney, 2010).

Figure 2.1 Industrial method of cutting dough using the rotatory cutter (Indiamart, 1996)

Wire-cut machine biscuits are made from soft doughs that are forced through an orifice and cut with wires into various shapes (Delcour and Hoseney 2010). Similar to the rotatory-mould biscuit formulation, the dough contains high levels of sugar and shortening and must have cohesive properties that will allow the dough to stick together during handling (Hui and Corke 2006).

Cut machine biscuit formulations require more water and less sugar to allow for gluten development, compared to rotatory moulded biscuits formula. After kneading, the dough is sheeted to a continuous sheet. A rotating cutter is then used to cut the dough into the desired shape and excess dough is scraped off, as shown in Figure 2.2 (Delcour and Hoseney, 2010; ReadingBakery, 2019).

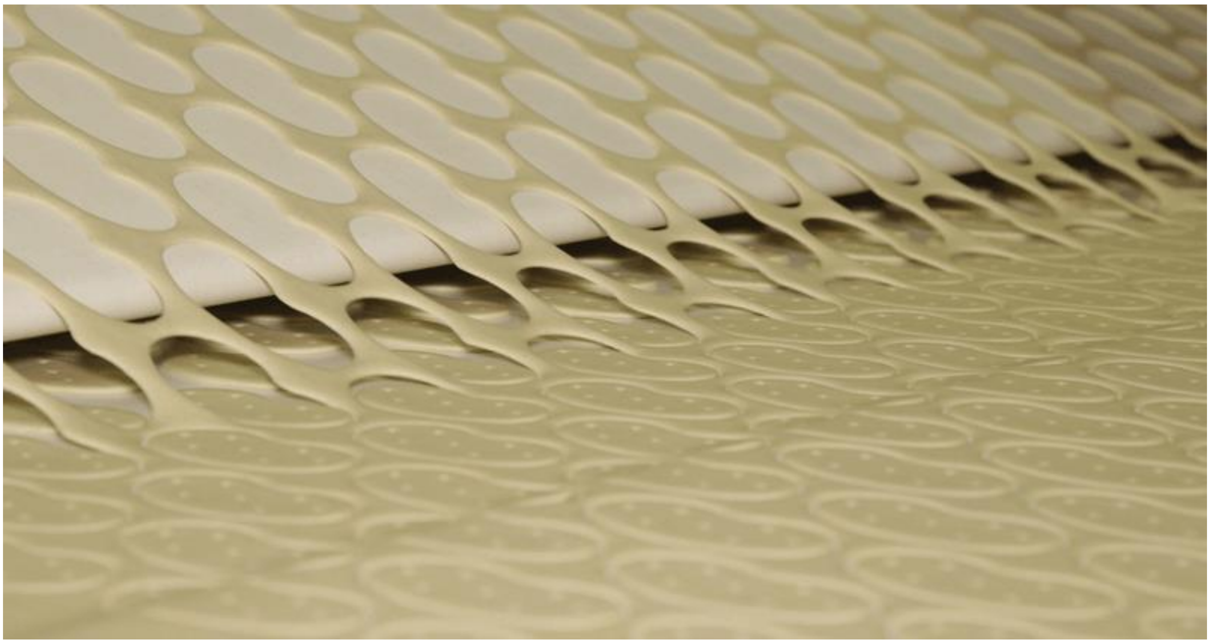

Figure 2.2 Industrial method of cutting dough for cut machine biscuits (ReadingBakery, 2019)

2.2 Complementation of sorghum-legume flours for biscuit manufacture

Cereals grains such as sorghum are limiting in the essential amino acids: lysine and tryptophan that are abundant in most leguminous pods (Snyder and Kwon, 1987). On the other hand, legumes are deficient in sulphur containing amino acids, which are abundant in sorghum (Snyder and Kwon, 1987). Soya is rich in protein (approximately 35%) and can be used as a relative alternative protein source when baking products such as bread (Taghdir *et al*., 2017). Hence, complimenting of sorghum flour with soya flours during baking can be done to boost the protein quality of biscuits produced. Most researchers have reported an increase in protein, fat, ash and fibre content as legume flours such as soya and cowpea were incorporated in the dough for baking biscuits and bread (Dovi, 2014, Taghdir *et al*., 2017). Addition of up to 40% of soya flour to malted sorghum increases the fat, crude fibre and protein content of the composite flour by 271%, 20.3% and 163%, respectively (Bolarinwa *et al*., 2015).

Also, incorporation of 40% soya flour to malted sorghum flour resulted in a decrease of anti-nutritional content of phytate and tannins in the composite flour by 27% (Bolarinwa *et al*., 2015). This was caused by the dilution effect as soya flour was incorporated because 100% malted sorghum flour had a higher phytate and anti-nutritional factor content in comparison to malted sorghum-soya composite flour (Bolarinwa *et al*., 2015). Tannins form complexes with food macro-molecules such as proteins and reduce their availability in the body (Preedy *et al*., 2011).

2.3 Formulation of sorghum and sorghum-legume biscuits

Table 2.1 and Figure 2.3 shows a biscuit formulation and flow diagram respectively for baking sorghum and sorghum-soya biscuits that was previously used by Nomsa Chimuti and Khuthadzo Mukheli (Masters students at the University of Pretoria).

Table 2.1 Formulations of sorghum, wheat and sorghum-soya composite biscuits expressed as percentage of sorghum and soya flour

Ingredients	100% wheat	10 % sorghum	70% wheat: 30% soya	70% sorghum: 30% soya	70% sorghum: 30% wheat
Toasted enzyme inactive defatted soya flour	0	0	30	30	0
Cake wheat flour	100	0	70	0	30
Red non-tannin sorghum flour	0	100	0	70	70
Vanilla essence	2.48	2.48	2.48	2.48	6.16
Sunflower oil	26.52	26.52	26.52	26.52	6.56
Sugar	24.88	24.88	24.88	24.88	6.22
Baking Powder	0.66	0.66	0.66	0.66	0.17
Water	35.56	18.13	43.20	42.66	6.66

Bakers % indicates the proportion of an ingredient relative to flour, expressed as a percentage (Manley, 2000).

Sorghum or, wheat or composite flour with defatted soya flour

↓

Mixing of dry ingredients which include flour, baking powder and sugar for 2 minutes in an electric mixer

↓

Gradually add vanilla essence, oil and water. Mix the dough for 7 minutes till firm

↓

Rest the dough 5 minutes

↓

Sheet the dough to a height of 5 mm using a pastry sheeter

↓

Cut the dough into circular shapes (5 mm) with a biscuit cutter

↓

Transfer the cut dough pieces to a baking tray lined with silicone paper

↓

Bake at 200 °C for 20 ± 5 min

↓

Cool overnight at ambient temperature

↓

Pack biscuits in polyethylene bags and store at -20 °C

Figure 2.3 Flow diagram for the preparation of sorghum- soya and/or cake wheat biscuits made by Chimuti and Mukheli (2017) – MSc Food Science students.

Tables 2.1, 2.2 and 2.3 shows the amount of water added to sorghum and sorghum-composite doughs by different researchers. The amount of water used in sorghum-cowpea and sorghum-soya biscuit formulation by Dovi (2014) and Serrem (2010) respectively were based on preliminary trial results. In biscuit dough formulations by Serrem (2010), low water (40.8 g flour) levels were required by for formulations of 100% sorghum biscuits in comparison to sorghum-soya (70 g - 90 g) or wheat-soya composite (90 g - 100 g) biscuits, (per 225 g flour) as shown in Table 2.2. This is because soya flour has higher water and oil hydration properties than sorghum flour, which could be contributed by the higher soya protein content (approximately 35%) (Akubor and Ukwuru, 2003, Sciarini *et al*., 2012). The high-water absorption capacity of soya could be due to the hydrophilic nature of soya globulin proteins, that can form hydrogen bonds with water molecules (Nehete *et al*., 2013, Taghdir *et al*., 2017).

Table 2.2 Formulations for sorghum, wheat and soya composite biscuits (Serrem, 2010)

Ingredients (g)		Sorghum-soya		Soya		Wheat-soya	
	100:0	71.4:28.6	50:50	100:0	100:0	71.4:28.6	50:50
Defatted soya flour	0	64	112.5	225	0	64	112.5
Sorghum flour	225	161	112.5	0	0	0	0
Wheat flour	0	0	0	0	225	161	112.5
Sugar	56	56	56	56	56	56	56
Sunflower oil	66	66	66	66	66	66	66
Baking powder	1.5	1.5	1.5	1.5	0.25	0.5	1
Vanilla essence	13.5	13.5	13.5	13.5	13.5	13.5	13.5
Water	40.8	70	90	160	60	90	100
Total dough weight	402	432	452	522	421	451	462

Serrem (2010) used toasted enzyme inactive defatted soya flour and red non-tannin commercial sorghum flour, while Dovi (2014) used wholegrain milled sorghum flour and cowpea flour for the production of biscuits (Table 2.3).

Table 2.3 Formulation of sorghum and cowpea composite biscuits (Dovi, 2014)

Ingredients	Sorghum 100:0	Sorghum-cowpea 60:40
Whole grain sorghum flour (g)	450	270
Whole grain cowpea flour (g)	0	180
Sugar	112	112
Sunflower oil	132	132
Baking powder	3	3
Vanilla essence	27	27
Water	157.5	103.5
Total dough weight	881.5	827.5

2.4 Science and technology of sorghum-soya biscuit manufacture

Biscuits are baked using different ingredients in various quantities. The role of some ingredients, as shall be discussed below, is most applicable to biscuits made from dough containing gluten, such as wheat.

Gluten-free biscuits made using sorghum can be produced using a short dough formulation because gluten is of low importance (Adedara, 2016). Gluten-free doughs rely on high fat (12% - 50%) and sugar (30% - 70%) quantities of short dough formulation to create a plastic and cohesive environment that provides structure in baked biscuits (Delcour and Hoseney, 2010). Water is a medium for dissolving ingredients during mixing in order to form a dough (Figoni, 2011). To minimise gluten formation in short wheat doughs, very little or no water is added because fat coats flour particles during mixing and limits the hydration of proteins in the flour (Manley, 2000).

2.4.1 Sorghum grain and its flour

Sorghum (*Sorghum bicolor* L. Moench) is a drought-resistant cereal crop that grows in semi-arid areas. A sorghum grain constitutes of pericarp, endosperm and germ (Kent, 1975, Delcour and Hoseney, 2010). The outer layer of the sorghum grain, the pericarp, is rich in dietary fibre and is composed of the epicarp, mesocarp and endocarp layer (Preedy *et al*., 2011). In some sorghum cultivars, a pigmented layer called the testa can be found between the pericarp and endosperm. The testa contains condensed tannins and phenolic acids (Hoseney *et al*., 1974). The latter is believed to have health-promoting effects, and they contribute to the colour of the grain or flours (Awika, 2017).

The endosperm contains starch granules and protein bodies. The endosperm is composed of the aleurone layer, corneous and floury endosperm (Preedy *et al*., 2011). Glutelins and prolamins are the major storage proteins found in sorghum grains (Hoseney *et al*., 1974). Kafirin, the main prolamin proteins of sorghum are further classified into α-, β-, γ- and δ-subclasses (Belton and Taylor, 2002, Preedy *et al*., 2011). In general, sorghum kafirins are hydrophobic because they contain high levels of prolamin and glutamine amino acids (Preedy *et al*., 2011).

The corneous endosperm cells consist of tightly packed proteins bodies and starch granules that are difficult to separate during milling, while the floury endosperm contains loosely packed starch granules and protein bodies (Hoseney *et al*., 1974). The germ contains mostly lipids, triglycerides rich in unsaturated fatty acids such as linoleic and oleic fatty acids (Preedy *et al*., 2011). Sorghum proteins contain abundant non-polar proline and glutamine residues but are limiting in the essential amino acid lysine (Delcour and Hoseney, 2010).

2.4.2 Soya bean and its flour

Soya (*Glycine max*) is a legume mostly grown in tropical and subtropical areas. Commercially, soya beans are processed into edible oils due to the high oil content of approximately 18% to 22% (Hamm *et al*., 2013). Soya is rich in unsaturated fatty acids, linoleic and linolenic acids (Manley, 2011). Soya bean is high in protein (approx 33% to 39%), plus it is a good source of essential amino acids (Wrigley *et al*., 2004). Soya flour can be used to boost the protein content of sorghum biscuits; a strategy to improve protein-energy malnutrition (PEM) (Serrem, 2010). Starch is a storage polysaccharide, found in quantities less than 1% (db) of soya bean flour (Karr-Lilienthal *et al*., 2005). Soya bean products include flours and grits, soya protein concentrates and soya protein isolates (Lapedes, 1977). In addition to high protein content, soya contributed improved functional properties in soybean-cassava composite dough such as dough handling and extensibility properties (Akubor and Ukwuru, 2003).

2.4.3 Sugar

Sugar contributes to the sweetness, colour and texture of biscuits (Manley, 2011). The hardness of biscuits, after cooling, is contributed by recrystallization of sugar after baking, causing the formation of an amorphous glass layer that changes from a rubbery to a glassy state (Manley, 2011). The rate of sugar dissolution during mixing depends on the quantity of sugar added and the amount of water available. During baking, undissolved sugar melts, increasing the liquid phase of the dough that affects cookie spread properties and diameter (Doescher *et al.*, 1987, Manohar and Rao, 1997).

The size of sugar particles affects the spread of the dough during baking. Sugar with fine particles dissolves faster than coarse sugar, resulting in baked biscuits with a more uniform appearance (Hui and Corke, 2006). Coarse sugar partially dissolves during mixing if there is limited water present in the formulation. Uneven distribution of undissolved sugar in the dough can cause the formation and appearance of black or white spots on the baked product's surface (Hui and Corke, 2006).

2.4.4 Fats and oils

Fat is used as a shortening agent and a lubricant in baking, as it imparts a texture to biscuits. During the mixing stage, fat molecules surround flour particles, and this limits the interaction of water molecules with gluten proteins (in the case of wheat) (Figoni, 2011). In gluten-containing flours, the heat generated during dough mixing and water hydration of flour proteins may strengthen the dough through gluten development, by forming a viscoelastic and extensible network (Manley, 2000). Thus, less water (approx. 5% to 15% by weight of flour) and minimal mixing is required in short dough formulations to reduce gluten formation (Delcour and Hoseney, 2010).

2.4.5 Other ingredients

Other ingredients such as baking powders are used in minute quantities, singular or in combination with other leavening agents (Manley, 2000). Most baking powders contain leavening agent(s) that form gases such as carbon dioxide, through dissociation of bicarbonates or chemical reactions of bicarbonate with salts in the presence of heat (Delcour and Hoseney, 2010). Leavening gases form pockets that allow for expansion of dough during preparation and baking, thus increasing the volume of baked products (Damodaran *et al*., 2008). In the presence of moisture and acid in the dough, sodium bicarbonate decomposes to carbon dioxide gas, water and salt (Figoni, 2011).

2.5 Rheological changes in dough during dough resting and fermentation

In gluten-containing flours, water is initially absorbed by starch and proteins during dough mixing (Manley, 2000). After mixing, it is recommended to rest wheat biscuit dough for approximately 30 minutes to allow for water hydration of proteins and starch in flour (Manley, 2000). During dough resting, the hydrated proteins form a gluten network causing the tightening of dough, as the dough becomes less extensible (Manley, 2000). Piazza and Schiraldi (1997) reported that wheat biscuits made from 120 min dough resting time had a higher moisture content in comparison to biscuits made from dough rested for shorter resting times, resulting in loss of crispiness and reduced strength of biscuits.

Manohar and Rao (2002) measured the rheology of wheat dough to predict the quality of biscuits using elastic recovery. Elastic recovery was determined by measuring the change in dough height after compression was applied and removed on the dough. Manohar and Rao (2002) reported that biscuit thickness was influenced by dough elastic recovery and cohesiveness. Elastic recovery was higher in the wheat dough with a protein content of 10.8% gluten, in comparison to the wheat dough with lower protein (8.8% gluten) (Manohar and Rao, 1999). In the same study, increasing water levels also increased biscuit hardness and thickness. Results from these studies show that high flour protein and water contents in dough promote gluten development, thus affecting the quality of biscuits produced.

Annan *et al*. (2005) determined the effect of soybean fortification on maize dough with 50% moisture content, fermented for 72 h at 30 °C. Although there were no significant differences between dough pH with and without 20% full-fat soya fortification, the authors attributed the increase in total acidity (expressed as lactic acid), to the buffering effect of soya soluble proteins. Other researchers have also reported a decrease in pH with natural fermentation, from 5.7 to 3.7 for sorghum flour (Yousif and El Tinay, 2001) and from 6 to 4.3 for sorghum flour supplemented with whey, at 37 °C for 36 h (Ibrahim *et al*., 2005).

There was a decrease in the pH of sorghum flour with fermentation from 6.2 to 3.4 (Hugo *et al*., 2003). Addition of sourdough to sorghum-wheat dough produced bread with higher moisture content, increased weight and volume in comparison to sorghum-wheat bread made with fermented and dried sorghum, flour (Hugo *et al*., 2003). The same authors attributed the increase in bread volume with an improved gas holding capacity of the composite dough. Water holding capacity of sorghum flour has also been reported to increase with sorghum flour fermentation, ranging from 98% to 110% for three different white sorghum varieties (Abd El-Moneim *et al*., 2015). Previous research shows that change in pH and rheological changes during dough resting or fermentation can affect the quality of baked products.

2.6 Physicochemical properties and consumer acceptance of sorghum and sorghum-legume biscuits

Whole grain sorghum biscuits were described as gritty, crumbly, fragile, coarse and with an unappealing appearance of white specks on the surface (Hoseney and Badi, 1975). The perceived grittiness and coarse texture in biscuits may be due to the formation of hard particles as a result of strong adhesion between proteins bodies and starch granules. It is difficult to separate the protein bodies and starch granules even with milling (Hoseney *et al*., 1974). Hoseney and Badi (1975) found out that increasing dough pH by replacement of sodium carbonate with sodium bicarbonate was effective for reducing grittiness in sorghum biscuits but not fragility. Sodium bicarbonate dissolves in water to form carbon dioxide gas and sodium carbonate salt, increasing the pH of the dough (Manley, 2000).

Reducing the particle size of sorghum flour through re-milling to 500 µm has been used in an attempt to reduce coarseness and grittiness in sorghum-soya and sorghum-cowpea biscuits by authors Serrem (2010) and Dovi (2014), respectively. The formulation for sorghum-soya (50:50) biscuits dough made with re-milled sorghum flour required double the amount of water compared to the amount that was required before re-milling flour, from 20% to 40% (on flour basis) in order to make a workable dough (Serrem, 2010). The amount of water added by these authors was based on preliminary trials. Despite the reduction in sorghum flour particle size, sorghum-soya and sorghum-cowpea biscuits produced were reported as gritty (Serrem, 2010, Dovi, 2014).

Dayakar Rao *et al*. (2016) determined the effect of different particle size (251 µm, 178 µm, 152 µm, 104 µm and 75 µm) on hydration properties of sorghum flour as well as the quality of sorghum biscuits. In their studies, the authors showed that there was an increase in water absorption and swelling capacity of sorghum flour as particle size was decreased. Furthermore, the hardness of sorghum biscuits was highest in biscuits made with 75 µm and 104 µm flour particle sizes, compared to 152 µm, 178 µm and 251 µm flour particle sizes (Dayakar Rao *et al*., 2016). Reduction in flour particle size did not reduce grittiness in sorghum biscuits by Serrem (2010) and Dovi (2014). An increase in the water-binding capacity of flour with a decrease of flour particle size may be associated with the large surface area of flour particles as the proportion of damaged starch increases, allowing more water uptake and hydration by flour particles (Gallagher, 2009).

Adedara (2016) determined the effect of different water levels (30%, 35%, 40% and 50%) by weight of flour, on the appearance and texture of 100% sorghum dough and biscuits. The author reported that the 100% sorghum doughs softened as the water levels in the formulation increased. Increasing water in the sorghum dough formulation could be sufficient to dissolve water-soluble ingredients and provide moisture for starch gelatinisation, which would reduce grittiness in sorghum biscuits. However, excess water in sorghum dough formulations is unfavourable for biscuits because it produces a batter-like system that is more favourable for the production of gluten-free bread (Taylor *et al*., 2006).

Sorghum biscuits became darker, and biscuit hardness increased as the water level increased from 35% to 40%, as measured instrumentally with a texture analyser (Adedara, 2016). There were no significant differences in brittleness and texture properties between sorghum biscuits and commercial wheat 'Marie' biscuits. Instrumental texture results showed that it is possible to produce 100% sorghum biscuits with a similar texture to wheat biscuits (Adedara, 2016).

Dovi (2014) reported that higher hardness (17.3 N) in red sorghum biscuits than white sorghum biscuits (7.2 N) might be attributed to the more corneous texture of red sorghum grain than white sorghum grain. White sorghum-cowpea (60:40) biscuits made with baking margarine had a more open structure and required less force to break compared to biscuits made with sunflower oil (Dovi, 2014). Dovi (2014) related the difference in biscuit crumb structure to the entrapment of more air bubbles during the creaming method with margarine, providing a stable framework for gas expansion in the dough during baking (Hui and Corke, 2006).

Cookies made from red and white sorghum flours were optimised for flavour, fragility and crispiness by addition of guar gum and baking powder (De Petre *et al*., 2016). De Petre *et al*. (2016) found that increasing guar gum levels from 0.5 g to 1.5 g caused a reduction in flavour, fragility and crispiness of biscuits. Increasing baking powder from 1 g to 3 g baking powder increased the flavour intensity, fragility and crispness of the biscuits. Adedara (2016) found that the presence of gelatinised starch in sorghum dough affected biscuit texture. Increasing the ratio of pre-cooked:raw sorghum flour (20%, 40% and 100%) reduced the hardness of sorghum biscuit by 23%, 45% and 80% in comparison to biscuits made from 100% uncooked sorghum flour. Polarised light microscopy showed an increase in swollen starch granules with partial birefringence, as the pre-cooked flour ratio increased. Findings from De Petre *et al*. (2016) and Adedara (2016) could suggest that starch granule swelling, increase in dough viscosity and irreversible breakage of intermolecular bonds caused by starch gelatinisation may be responsible for the reduction in biscuit hardness.

Chiremba et al. (2009) used a consumer panel to determine the acceptability of sorghum and wheat flour biscuits, made using condensed tannin and tannin-free sorghum. Biscuits made from condensed tannin sorghum were less acceptable due to their darker colour, hard texture and were more gritty in comparison to biscuits made from condensed tannin-free sorghums (Chiremba et al., 2009). Enrichment of sorghum flour with soya in biscuit making increased scores for biscuit liking by 5% - 6% in comparison to 100% sorghum biscuits, in a consumer test by children (Serrem, 2010). Descriptive panel results of sorghum-soya biscuits showed that biscuits were less hard, dense, chewy and more crispy than 100% sorghum biscuits (Serrem, 2010).

Consumers perceived a nutty flavour in sorghum-cowpea biscuits, which could be due to the flavour compounds from cowpea (Dovi, 2014). Sorghum and sorghum-cowpea biscuits received lower sensory acceptability scores than commercial wheat biscuits, and this could be because of less familiarity with sorghum products amongst consumers and less desirable texture properties (Dovi, 2014). 100% sorghum biscuits received the lowest intent to buy scores in comparison to sorghum-cowpea and commercial wheat biscuits, as these scores may have been affected by sensory attributes such as texture and flavour attributes of the biscuits.

Omoba *et al.* (2015) determined the effect of sourdough fermentation on sensory characteristics of sorghum and pearl millet biscuits. There were no significant differences in the texture of sorghum biscuits made with and without sorghum sourdough addition (Omoba *et al*., 2015). Whole grain sorghum-soya biscuits with sourdough addition were reported as more bitter, sour and had intense fermented aroma compared to biscuits where sourdough was not added (Omoba *et al*., 2015). Addition of sourdough also reduced sweetness and sorghum aroma in biscuits made from red sorghum composited with soya flour biscuits. Omoba *et al*. (2015) attributed the increase in sourness and fermented aroma in sorghum-soya biscuits with added sourdough, to the action of lactic acid bacteria and yeasts that may have liberated amino acids and other compounds during fermentation.

Although instrumental techniques can provide information on some texture attributes, it is not sufficient to rely only on such texture measurement. Sensory results obtained from human panellists, combined with data from instrumental techniques, are required to fully understand product texture (Serrem, 2010, Dovi, 2014).

2.7 Thermal properties of biscuit dough and baked biscuits

There was no literature available on the measurement of thermal properties of sorghum dough and biscuits. As previously mentioned, sorghum biscuits are mainly reported as gritty (Serrem, 2010, Dovi, 2014). Grittiness in baked products may be caused by the presence of hydrophobic protein bodies that surround starch granules and limit hydration of water into starch during dough mixing (Preedy *et al*., 2011).

By allowing the dough to rest for a longer time, water molecules may hydrate starch granules present in the dough. Differential scanning calorimetry can be used to measure the starch gelatinisation and or the amount of energy required to melt crystalline material in a biscuit dough system or baked products (Fennema *et al*., 2008). Differential scanning calorimetry (DSC) is a thermo-analytical tool that has been used by various researchers to determine changes in phase behaviour of materials during heating or cooling and measures the amount of heat that flows through a product/sample as a function of time (Delcour and Hoseney, 2010).

As water becomes limited due to starch hydration, water will no longer be available to act as a plasticizer and gelatinise the starch. Higher temperatures will be required to initiate starch gelatinisation or melting of crystalline/semi-crystalline areas (Baks *et al*., 2007). Increasing sugar in a wheat dough increased the gelatinisation temperature of starch (Chevallier *et al*., 2000). DSC thermographs of wheat dough had three endotherms, relating to melting of starch crystallites at 60 °C, melting of remaining crystallites around 90 °C and melting of the amylose-lipid complex at a higher temperature around 122 °C (Chevallier *et al*., 2000).

The presence of oil/fat does not affect the gelatinisation of starch, although an endotherm peak for the melting of fat/oil may appear. Biscuit dough containing fat, specifically coconut oil and palm oil had two endotherm curves, for fat melting between 21 °C and 52 °C due to the different melting temperatures of the oils used, as well as temperatures between 71 °C - 123 °C attributing to melting of starch molecules (Chevallier *et al*., 2000).

Baking does not gelatinise all starch granules due to limited water in the formulation used in short dough biscuits. Results showed that there was very little starch gelatinisation, approximately 4%, in wheat cookies after baking (Lineback and Wongsrikasem, 1980, Doescher *et al*., 1987, Baltsavias *et al*., 1999). Scanning electron microscopy (SEM) further showed that most starch granules were still intact, and some were slightly swollen after baking cookies (Lineback and Wongsrikasem, 1980). When measured, starch gelatinisation in the baked product was in the range of 2% - 9% (Wootton and Chaudhry, 1980, Baltsavias *et al*., 1999, Lineback and Wongsrikasem, 1980). Little starch gelatinisation is expected in baked products such as biscuits, where there are low quantities of water in the formulation.

2.8 *In vitro* methods to determine the starch and protein digestibility of biscuits

2.8.1 *In vitro* starch digestibility and estimated glycaemic index

Sorghum grain and flour has been reported as gluten-free and having lower starch digestibility than maize (Duodu *et al*., 2003), which may be beneficial for people who have coeliac disease or non-communicable diseases such as diabetes (Srichuwong *et al*., 2017). Different factors, such as grain morphology or food processing conditions, can affect starch and protein digestibility of sorghum flour and products (Duodu *et al*., 2003). Processing conditions such as cooking have been reported to reduce starch digestibility in sorghum porridge, but there is very little information on the effect of other processing conditions such as baking on starch and protein digestibility of sorghum containing products, such as biscuits. Duodu *et al*. (2003) proposed that cooking reduces starch and protein digestibility due to the formation of disulphide crosslinks between cysteine molecules, that are resistant to degradation by digestive enzymes in the gut.

In an experiment to determine the effect of phenolic compounds on starch digestibility, Lemlioglu-Austin *et al*. (2012) found that there was a reduction in starch digestibility of sorghum porridges that had phenolic compounds and tannin bran added to them. Condensed tannins form complexes with proteins, restricting access by enzymes, thus reducing starch and protein digestibility (Awika, 2017). Sorghum bread had a high glycaemic index of 72 (Wolter *et al*., 2013) while sorghum biscuits had a glycaemic index of 54 (Prasad *et al*., 2015).

In vitro starch digestibility is used to provide an estimated glycaemic index (EGI) of food products. Sorghum chips had an EGI of 79.9 and enrichment with soya flour produced chips with an EGI of 70.6. Soybean chips composed of sorghum flour, soya protein isolate (SPI) and soya flour had an EGI of chips to 59.8 (Jiang *et al*., 2018). The authors concluded that the decrease in EGI with enrichment of soya flour and soy protein isolate might have been caused by possible interactions between protein and starch molecules, that restrict digestion of starch.

2.8.2 *In vitro* protein digestibility

Sorghum has a lower *in vitro* protein digestibility (IVPD) compared to wheat and maize (Duodu *et al*., 2002). Duodu *et al*. (2002) proposed that the protein digestibility of sorghum decreased with wet heat cooking due to the formation of heat induced disulphide cross-linkages during cooking. Low protein digestibility was further reviewed by Belton *et al*. (2006). The authors proposed that application of heat during food processing causes amino acid conformational changes, thus promoting protein-protein interaction through disulphide links.

Red and white sorghum biscuits had protein digestibility of 34% and 28%, respectively (Dovi, 2014). Serrem (2010) reported 30% protein digestibility for sorghum biscuits made from red non-tannin sorghum flour. Protein digestibility of sorghum-soya biscuits increased by 148%, 170% and 191% on complementation of sorghum flour with 28.6%, 50%, and 71.4% of defatted soya flours, respectively (Serrem, 2010).

Complementation of white or red sorghum flours with 40% of cowpea flour also increased the protein digestibility of white sorghum-cowpea (60:40) and red sorghum-cowpea (60:40) biscuits by 71% and 77%, respectively (Dovi*,* 2014). The increase in *in vitro* protein digestion of sorghum-soya and sorghum-cowpea composite biscuits, with an increase in soya and cowpea flour inclusion, respectively, was attributed to the increase in more soluble globulin proteins found in legume flours that can be more easily digested by digestive enzymes (Duodu and Apea-Bah, 2017).

Proteins are digested in the stomach, and pepsin secreted by stomach walls hydrolyses proteins in food to polypeptides and amino acids (Caballero, 2005). Pancreatic juices contain trypsin and chymotrypsin enzymes that hydrolyse protein polypeptides into oligopeptides (Caballero, 2005). To simulate the human gastrointestinal (GI) tract, multi-enzyme and pepsin digestibility techniques are used to determine *in vitro* protein digestibility. Dovi (2014) reported higher results for multi-enzyme digestion than pepsin only digestion method on sorghum-cowpea composite biscuits. (Hsu *et al*., 1977) concluded that lower digestibility results for pepsin only digestion in comparison to the multi-enzyme technique could be because of the differences in methodologies used. A multi-enzyme digestibility method contains three enzymes namely protease, trypsin and chymotrypsin, while the pepsin digestibility method only uses pepsin enzyme. Trypsin preferentially hydrolyses protein polypeptide bonds with amino acids that contain positively charged groups such as lysine and chymotrypsin cleave polypeptide bonds of aromatic amino acids (Campbell, 2007). Protease enzymes selectively hydrolyse peptide bonds based on specificity for amino acid residues (Whitaker *et al*., 2003).

2.9 Conclusions

Progress has also been made in using locally available cereals such as sorghum, to produce gluten-free products such as biscuits that have textural properties similar to wheat biscuits. However, the texture of these biscuits produced has often received lower sensory acceptance scores, partly because consumers are not familiar with gluten-free products and their sensory properties. With the rise in utilisation of indigenous crops and production of gluten-free foods generally, there is a need to do more research on the consumer acceptability of these products. By understanding the rheological changes that take place during dough resting, this may lead to the knowledge of how processing techniques such as baking, affect a product’s sensory profile.

3 HYPOTHESES AND OBJECTIVES

3.1 Hypothesis

Allowing sorghum-soya (70:30) biscuit dough to rest for 24 h will reduce grittiness of the biscuits and improve consumer acceptability of sorghum-soya biscuits. Perceived grittiness in sorghum biscuits is caused by the limitation of water to reach the starch granules for gelatinisation and thereby softening the particles. In sorghum, the corneous endosperm starch granules are surrounded by hydrophobic protein bodies that limit water hydration (Delcour and Hoseney, 2010). A longer dough-resting period will allow more time for starch granules to absorb the water available in the dough. During dough mixing, water forms a thin film around starch granules and is absorbed through amorphous channels on the surface of the granule (Gallant *et al*., 1997). Heat application during biscuit baking will enable the hydrated starch granules to swell and gelatinise with the leaching of amylose molecules (Kent, 1975; Fennema *et al*., 2008). Optimum starch gelatinisation during biscuit baking will reduce biscuit grittiness and ensure optimum consumer acceptability.

3.2 Objectives

1. To determine the effects of dough resting time (15 min; 24 h) on physical and texture properties as well as consumer acceptance of sorghum-soya (70:30) biscuits.

2. To determine the effect of dough resting time (15 min; 24 h) on thermal properties and *in vitro* digestibility of sorghum-soya (70:30) dough and or biscuits.

3.3 Experimental Design

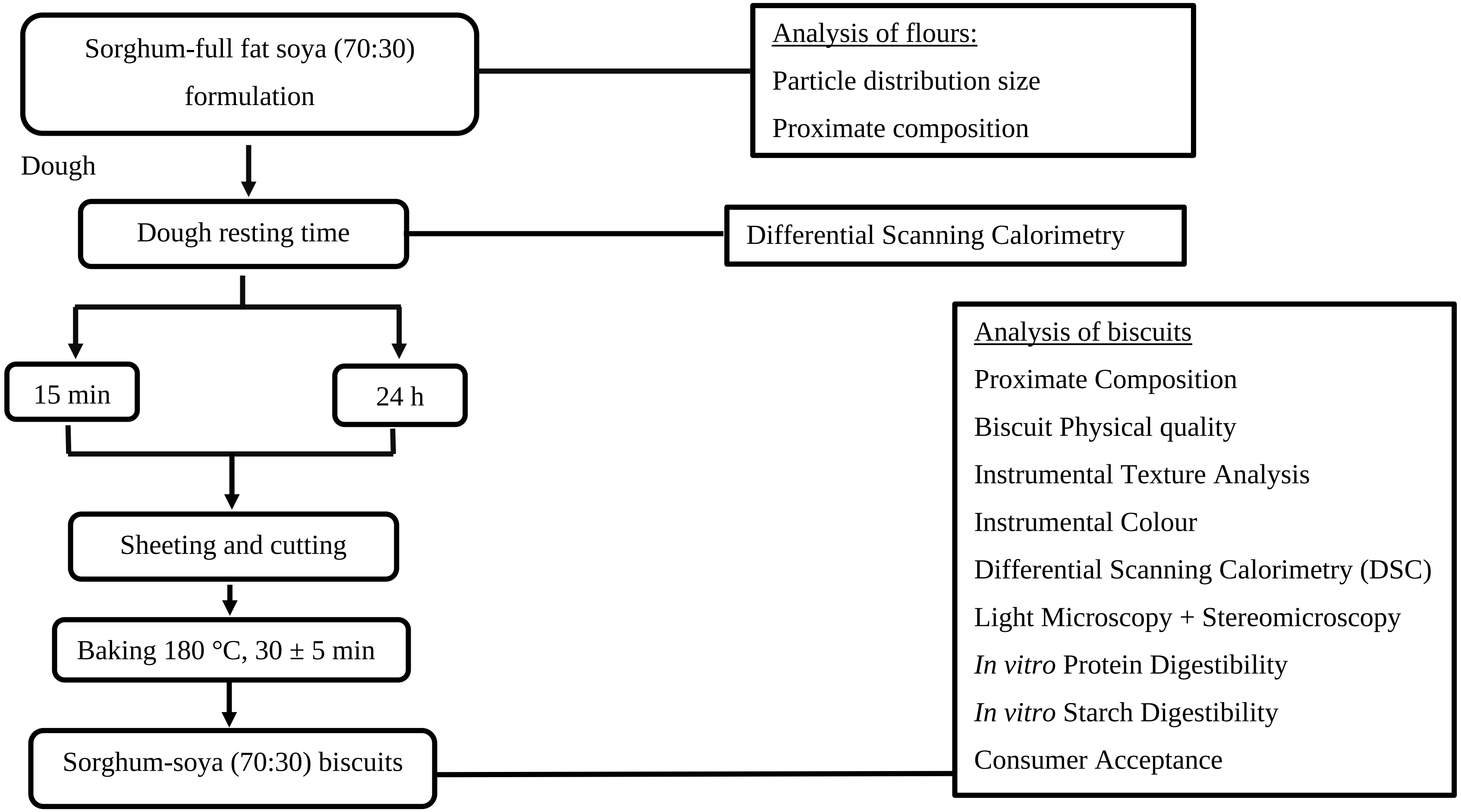

Figure 3.1 Experimental design for sorghum-soya (70:30) biscuits

4 RESEARCH CHAPTER

Abstract

Previously sorghum-soya composite biscuits developed by researchers at the University of Pretoria received relatively low sensory scores and acceptability ratings, as consumers perceived the biscuits as gritty and hard. Grittiness may be attributed to limited hydration of starch granules during dough preparation. A hypothesis identified was that increasing dough resting time of sorghum-soya dough could increase water absorption of flour, enabling starch gelatinisation to take place during baking, thus reducing grittiness in the biscuits. The objective of the current study was to evaluate the effect of biscuit dough resting time (15 min and 24 h) on proximate composition, physical and textural properties as well as consumer acceptability of sorghum-soya (70:30) biscuits. Sorghum-soya dough with 37% moisture (on flour basis) was sheeted, cut and used to bake biscuits. Fifty consumers assessed the acceptability and preference for biscuits made from the two treatments. Dough rested for 24 h produced biscuits with that were visually darker with thickness 7.6 mm, requiring 34.8 N to break while biscuits from 15 min dough resting were 6.7 mm thick and needed 51.1 N to break. It is possible that when the dough was rested for 24 h, some starch granules were hydrated and gelatinised during baking, contributing to the overall thickness of the biscuits. Increasing dough resting time to 24 h increased sorghum-soya biscuit moisture content by 30% and may have contributed to differences in biscuit texture properties, as water may have acted as a plasticiser that reduced biscuit matrix rigidity. Thermal properties showed that there were no significant differences ($p > 0.05$) between endotherm peaks of the sorghum-soya doughs and biscuits with dough resting time. Despite instrumental colour and texture differences, biscuits were equally liked for appearance, colour and texture irrespective of dough resting time. Biscuits made from dough rested for 15 min were less acceptable for the liking of taste and overall flavour, but were preferred over biscuits made from dough rested for 24 h. Using *in vitro* starch digestibility, there was no significant difference ($p > 0.05$) in *in vitro* protein digestibility of sorghum-soya biscuits with dough resting time. The estimated glycaemic index of sorghum-soya biscuits was lower (47%), compared to commercial digestive whole-wheat biscuits and white bread (64% and 94.6% respectively). A longer dough resting time did not necessarily improve the texture of sorghum-soya biscuits because biscuits made from both treatments were commented as grainy.

Keywords: sorghum, soya, biscuits, nutrition, texture, consumer acceptability

4.1 Introduction

Sorghum and sorghum-soya biscuits have previously been described as gritty and hard (Serrem, 2010, Dovi, 2014). Grittiness perceived in sorghum biscuits may be attributed to limited water absorption in starch granules during dough preparation, thereby reducing starch gelatinisation and softening of some flour particles. The unique arrangement of protein bodies surrounding starch granules in the corneous endosperm of sorghum may be a contributing factor for limited water absorption of starch granules in flour (Belton *et al*., 2006). A number of studies have been done in an attempt to improve the textural properties of sorghum-based biscuits. However, work that focuses on sensory acceptability is still lacking.

Increasing dough resting time could potentially improve sorghum-soya (70:30) biscuit texture and consumer acceptance, through absorption of water into amorphous regions of starch granules causing them to swell, enabling gelatinisation of hydrated starch granules during baking, thus reducing grittiness. Unlike wheat, sorghum does not form gluten during dough mixing; therefore, various ingredients are added in an attempt to improve the textural properties of the final baked product (Delcour and Hoseney 2010). Using wheat, Manley (2000) recommended that allowing the dough to rest for approximately 30 min after ingredient mixing promotes gluten formation as it allows for hydration of proteins and starch.

The texture of sorghum biscuits can be influenced by the particle size of the flour used during mixing. Reducing the particle size of sorghum flour to 500 µm through re-milling has been proposed as a way to reduce grittiness in sorghum-soya and sorghum-soya and sorgjum-cowpea biscuits by Serrem (2010) and Dovi (2014), respectively, but has been reported to cause an increase in damaged starch and water binding capacity of sorghum flour. Similar to Serrem (2010) and Dovi (2014), Dayakar Rao *et al*., (2016) reported an increase in sorghum biscuits hardness with a decrease in sorghum flour particle sizes, from 251 µm to 75 µm.

Soya flour is added to boost the protein content and quality of baked products. Also, it can be used to improve the functional properties in dough such as dough handling and extensibility properties (Akubor and Ukwuru, 2003). In a consumer test with children, enrichment of sorghum flour with soya in biscuit making increased biscuit liking scores by 5% to 6% in comparison to 100% sorghum biscuits (Serrem, 2010). Descriptive sensory panel results by Serrem (2010) showed that sorghum-soya biscuits were less hard, less dense, less chewy and more crispy than 100% sorghum biscuits. Whole grain sorghum-soya biscuits with sourdough addition were reported as bitter, sour and had a more intense fermented aroma compared to biscuits prepared without sourdough addition (Omoba *et al*., 2015). The perceived bitterness in sorghum biscuits made with added sourdough may have been caused by the conversion of phenolic acid esters to phenolic acids during lactic acid fermentation (Omoba *et al*., 2015).

Thermo-analytical tools such as differential scanning calorimetry (DSC) can be used to determine starch gelatinisation in dough and biscuits. However, there is limited literature available on thermal properties of sorghum biscuit dough and or biscuits. DSC analysis conducted for wheat dough showed three endotherm peaks of fat that melted at temperatures around 25 °C - 30 °C, undissolved sugar melted as the temperature is increased and starch gelatinisation occurred at temperatures above 100 °C (Abbound and Hoseney, 1983). The starch gelatinisation of sorghum is proposed to be between 68 °C - 78 °C (Kulamarva *et al*., 2009). Normal sorghum starch had the lowest onset and peak temperatures of 68 °C, while those of waxy and heterowaxy starches ranged from 68 °C - 73 °C (Sang et al., 2008).

In a dough system, the presence of crystalline material such as sucrose competes with flour for the available water before baking and increasing the fructose or glucose content from 0.26 g to 0.60 g per 100 g dry starch delays starch gelatinisation (Sopade *et al*., 2004). Water hydrates into amorphous regions of starch granules and causes them to swell. On the application of heat during baking, granule swelling and gelatinisation coincide while moisture present in the dough is lost in the form of water vapour (Baks *et al*., 2007). In systems with limited moisture content, starch gelatinisation is initiated at higher temperatures. The higher kinetic energy and mobility of molecules generated by the heat are sufficient to cause breakage of hydrogen bonds and melting of crystalline or semi-crystalline regions of starch granules (Moreira *et al*., 2015).

Sorghum is a gluten-free cereal that has lower starch and protein digestibility than maize (Duodu *et al*., 2003). Products made from sorghum are beneficial to people who have celiac disease or non-communicable diseases such as diabetes because of the low starch digestibility (Srichuwong *et al*., 2017). Enriching sorghum with soya flour or soya protein isolate has not only been reported to have improved the protein quality but also resulted in a reduction in the estimated glycaemic index and glycaemic load of sorghum chips (Jiang *et al*., 2018).

Complementation of red and white sorghum flours with 40% cowpea flour increased the protein digestibility of biscuits by 80.9% and 71.1%, respectively (Dovi, 2014). There was a gradual increase in protein digestibility with complementation of 28.6%, 50% and 71.4% soya flour by 147%, 172% and 191%, respectively (Serrem, 2010). Using the pepsin method, 100% sorghum biscuits made from red and white sorghum flour had protein digestibility in the range 27.8% and 33.9% respectively (Dovi, 2014), while 100% red sorghum biscuits by Serrem (2010) had protein digestibility of 30. Increase in protein digestibility could be because soya and cowpea flours contain high protein contents of 23.5% and 36.4% respectively, and gut enzymes quickly digest their proteins than sorghum proteins (Duodu and Apea-Bah, 2017).

Although it is possible to produce sorghum-legume composite biscuits with high nutritional content, the sensory properties of sorghum-based products remain a challenge. Attributes such as colour, flavour and texture are essential in determining the overall characteristics of food products (Hui and Corke, 2006). The aim of this study was to determine the effect of dough resting time (15 min and 24 h) on consumer acceptance and quality aspects of sorghum-soya biscuits.

4.2 Materials and Methods

4.2.1 Biscuit ingredients

The ingredients for the biscuits were commercial sorghum super fine meal (approx. 90% extraction rate) from red non-tannin sorghum (King Corn Fine Mabele meal, Tiger Consumer Brands Ltd, Bryanston, South Africa), vanilla essence (Premier Foods Ltd, Bryanston, South Africa), full-fat soya flour (RCL Foods Ltd, South Africa), Marvello margarine (Unilever South Africa (Pty) Ltd), baking powder (Robertson's Unilever, Durban, South Africa), white castor sugar (Illovo sugar group, Durban, South Africa) and tap water.

4.2.2 Biscuit preparation

A batch of sorghum-soya biscuits was made with 2 000 g sorghum fine meal flour, 920 g soya flour, 720 g white castor sugar, 840 g baking margarine, 780 g water, 80 g vanilla essence and 18g baking powder. Sugar and baking margarine were creamed together using a Diosna electric spiral mixer (Dierks and Sohne GmbH, Osnabruck, Germany) at slow speed. Flours and baking powder were sifted, added to the creamed mixture and mixed until homogeneous. Water and vanilla essence was gradually added while mixing until a firm and homogenous dough was formed. The dough was divided into two batches for resting for 15 min and 24 h respectively, before sheeting with a Rollfix 300 pastry sheeter (Fritsch, Bahnhofstrasse, Germany). Dough rested for 15 min was stored in the refrigerator at 4 °C, and the dough rested for 24 h was stored in a closed plastic container, at room temperature overnight (minimum temperature ± 17 °C). A temperature of 30 °C was recorded while activities continued in the bakery during the day, but unfortunately, the temperature drop during the night time was not recorded. Dough sheeting was done in three reduction steps, decreasing the gap between the rolls from 12 mm, 9 mm and 7 mm until a thickness of 4 mm was achieved. The excess dough was removed and added to the next batch of dough to be sheeted. The dough was cut into circular shapes using a 50 mm biscuit cutter. Cut pieces were transferred onto a baking tray lined with silicone baking paper (Bidvest Bakery solutions (Pty) Ltd. Modderfontein, South Africa). Biscuits were baked in a preheated deck oven (Miwe-Condo 4E, Germany) at 180 °C, for 30 ± 5 min. Biscuits were cooled at ambient temperature for 30 min before packaging and storage at 4 °C. For comparison between biscuits, commercial 'digestive' whole-wheat biscuits (Snackworks, National Brands) were used as a standard for some analyses.

4.2.3 Sorghum, soya and sorghum-soya flour particle size distribution

Commercial red non-tannin sorghum flour and full-fat soya flour were purchased from supermarkets in Pretoria, South Africa. The distribution of particles according to size in the flour was determined by using a vibratory sieve shaker (Fritsch Pulverisette, Analysette, Laborette, Idar-Oberstein, Germany). The procedure involved sifting 50 g of flour through screen tests sieves of 710 µm, 500 µm, 250 µm, 180 µm and 106 µm. The vibratory shaker was set at an amplitude of 4 mm for 5 min. Flour from each sieve was gently brushed off after shaking, weighed and expressed as a percentage of the total 50 g. Determination of the distribution of particle size in the flour was done in triplicate.

4.2.4 Proximate analysis of flours and biscuits

Proximate analysis of sorghum-soya (70:30) biscuits was carried out by the South African Grain Laboratory (SAGL). An internal SAGL control sample was included in each set of analyses. Replicate analyses required for the calculation of standard deviations were not performed.

4.2.4.1 Moisture content

The moisture content of flour and biscuits was determined using method 44-15A (AACC, 2000). Biscuits were ground into a fine powder using a pestle and mortar. A sample of 2 g was dried at 105 °C for 3 h. Moisture content was determined by calculating loss in moisture as a percentage of the original wet weight of the sample.

4.2.4.2 Ash content

Ash content of flours and biscuits was determined using method 08-02.01 (AACC, 2000). Approximately 3 g sample of ground biscuits and 5 ml of alcohol magnesium acetate solution was mixed in a crucible. Samples were ignited and heated in a silica crucible at 700 ± 10 °C for 1 h using a muffle furnace oven. Ash content was obtained by calculating the weight of the residue as a percentage of the original sample weight.

4.2.4.3 Fat content

Fat content of flours and biscuits was determined by a hot solvent extraction method 30-25.01 (AACC, 2000). A ground biscuit sample of 5 g was weighed into an extraction thimble and fat was extracted using petroleum ether (40 °C - 60 °C). The petroleum ether extract was dried in an oven at 95 °C - 100 °C for 3 h. Total fat content was obtained by calculating the weight of the extract as a percentage of the original sample weight.

4.2.4.4 Dietary fibre content

Total dietary fibre of biscuits was determined using the enzymatic and gravimetric method 985.29 (AOAC, 2000). Ground biscuit samples were first enzymatically digested with amylase, protease and amyloglucosidase for protein and starch removal and washed with ethanol for precipitation of soluble fibre. Samples were dried overnight in an air oven at 105 °C.

4.2.4.5 Protein content

Protein content for biscuits and flour was determined by the use of the Dumas method 46-30.01 as specified in AACC (2000), using N x 6.25 as the conversion factor.

4.2.4.6 Carbohydrate content

Percentage of carbohydrate in biscuits was calculated by difference, by summing up the percentage weight of determined values of protein, fat, ash, dietary fibre and moisture and subtracting from the total percentage weight of the food (Standard operating procedure MC 23 of SAGL).

4.2.5 Determination of physical and texture properties of sorghum-soya biscuits

4.2.5.1 Instrumental colour analysis

Colour of sorghum-soya (70:30) biscuits was measured using a CR 210 Minolta chromameter model CR-400 (Osaka, Japan). The chromameter was calibrated using a standard white plate. Readings were recorded as L*, a* and b* for nine biscuits from each treatment. L* represents lightness, a* for redness (+) and greenness (-) as well as b* for blueness (+) and yellowness (-) respectively. A total of nine biscuits from each treatment were analysed.

4.2.5.2 Thickness, diameter and density of biscuits

The diameter and thickness of sorghum-soya and digestive wheat biscuits were measured by a vernier calliper on sorghum-soya (70:30) and biscuits were taken randomly in each treatment. A biscuit was placed edge to edge on the vernier calliper and diameter was measured. Each biscuit was rotated at a 90 °C angle and diameter was re-measured. Biscuit thickness was measured by placing each biscuit on the jaws of the vernier calliper. A total of 15 biscuits from each treatment were measured.

The density of biscuits was measured by the solid displacement technique using small millet grains, according to the method described by Baltsavias *et al*. (1997). A container of known volume and mass was filled with pearl millet grains, tapped and excess grains were removed carefully. The bottom of the cylinder was first filled with some grains, and a biscuit of known mass was placed in the container. The container was filled with the remaining grains, tapped and the weight of displaced grains was measured. The density of grains and the weight of displaced grains were used to calculate the volume of biscuit. For each treatment, a mean of six biscuits was used to calculate biscuits density.

4.2.5.3 Instrumental texture analysis of biscuits

The texture analysis of sorghum-soya (70:30) and digestive whole wheat biscuits were done using the EZ-Test analyser (Model EZ-L, Shimadzu Tokyo, Japan). Biscuit hardness was determined by measuring the force required to break the biscuits. Maximum peak force during the first compression was measured using a three-point bend rig (HDP/3PB) attachment comprising of an upper blade and rig base with two adjacent supports. A vertical force was applied using an upper blade on the biscuit placed horizontally like a bridge over the two supports at a crosshead. The test speed was set at 3.0 mm/min, and the grip at a 3-point bend rig. The distance between the two steel bars supporting a biscuit was set at 30 mm. Fracture properties of biscuits were analysed using the following formula.

$$\text{Stress } (\sigma) = 3FL/\ 2bh^2 \qquad \text{Strain } (\varepsilon) = 6hY^2 / L^2$$

Where σ is the Stress midpoint (kPa),

ε is the Strain,

F is the force at the beam centre (N),

L is the distance between the supports or span length (mm),

b is the biscuit diameter (mm),

h is the biscuit thickness (mm),

Y is the deformation or deflection at the beam centre under the load (mm).

4.2.6 Microscopy of biscuits

4.2.6.1 Polarising light microscopy

A polarizing light microscope (Zeiss Axio Image. Alm, Jena, Germany) was used to analyse starch in sorghum biscuits. 50 mg of milled sorghum biscuit samples were weighed into Eppendorf tubes, and 0.5 ml of 30% glycerol solution was added. Tubes were vortexed at high speed for approximately 30 seconds to disperse and suspend the biscuit particles. Glass slides containing samples were placed on the microscope stage and analysed with visual magnification.

4.2.6.2 Stereomicroscopy of biscuits

Pictures of the microstructure of sorghum-soya (70:30) biscuit crumbs were analysed and taken using a stereomicroscope (Zeiss Discover V20, Jena, Germany). Biscuit samples were fractured and viewed through the cross-section with field view of 3.5 mm, 1.8 µm resolution and 64 µm depth of field.

4.2.7 Consumer evaluation of biscuits

Consumers used for this study were recruited from the University of Pretoria, Hatfield Campus and screened using a screening questionnaire (see Appendix 1). The study was conducted in the sensory laboratory, Department of Consumer and Food Sciences, University of Pretoria. Ethics approval for the use of human subjects in this study was approved by the Faculty of Natural and Agriculture Science (180000140). A consumer test was used to compare consumer liking as well as preference for sorghum-soya (70:30) biscuits prepared from dough rested for 15 min or 24 h. Fifty (50) consumers (34% males and 66% females) with 94% in the age group (18 – 25 years) and 6% from 26 – 34 years participated in the study. Each tasting session was approximately 20-30 minutes and accommodated a maximum of sixteen panellists at a time due to the facility chosen. Before the evaluation, participants were asked to sign a consent form (see Appendix 2).

Participants were presented with two biscuits, one from each treatment (15 min dough resting and 24 h dough resting), each in a zip-lock bag of 100 mm x 80 mm, labelled with a randomly selected three-digit code. The temperature in the room was approximately 25 °C. Filtered water was used as a palate cleanser. The order of presenting the biscuits was balanced over the group of consumers. Participants were asked to taste the biscuits, rate the liking of appearance, colour, taste, texture and overall flavour attributes of each biscuit using the 9-point hedonic scale where 1 = dislike extremely and 9 = like extremely (Peryam and Pilgrim, 1957). They were also asked to indicate which one of the two biscuits they preferred. Each attribute and preference question had a comment section available (see Appendix 3).

4.2.8 Thermal properties of the dough and biscuits

Thermal properties of sorghum-soya (70:30) dough and biscuits were determined using a Differential Scanning Calorimeter DSC (HP DSC827e, Mettler Toledo, Greifensee, Switzerland). Sorghum-soya (70:30) dough was prepared in the same way as in method 4.2.3. Dough with 37% moisture (by flour weight) was rested in the refrigerator at 4 °C before taking samples for dough rested for 15 min. Sorghum, soya and sorghum-soya (70:30) flours were analysed under conditions of one-part solids and three-parts water (1:3). To determine the effect of moisture content; sorghum-soya (70:30) slurries, sorghum flour, soya flour and sorghum-soya (70:30) flour were prepared under conditions of one-part solids and three-parts water. After dough preparation, doughs and slurries were rested for 24 h, hermetically sealed and stored at room temperature overnight. Biscuits were baked from dough that was rested for 15 min and 24 h. Biscuits for analysis were randomly selected from three batches and ground to flour using an analytical mill (IKA® A11 Basic analytical mill, Merck).

The DSC was calibrated with indium, and an empty aluminium pan was used as a reference. 50 µg of biscuit dough was weighed into a 100 µl aluminium pan and hermetically sealed. Aluminium pans containing 10 µg of flour or milled biscuit flour were prepared with the ratio of one-part solid and three parts water, and pans were hermetically sealed. Hermetically sealed pans containing flour or milled biscuits were left to equilibrate at room temperature for 24 h before analysis. Samples were scanned from 30 °C - 200 °C under 40 bar pressure, at a heating rate of 10 °C/minute. Dough, flours and biscuit samples were analysed in triplicate.

4.2.9 *In vitro* digestibility

4.2.9.1 *In vitro* starch digestibility and estimated glycaemic index

The method according to Goñi *et al*. (1997) was used to determine the *in vitro* starch digestibility of sorghum-soya (70:30) biscuits. Boiling water (1 mL) was added to 50 mg of milled biscuit for easy dispersion of sample, before the addition of 10 mL of HCl-KCl buffer (pH 1.5) and 0.2 mL solution containing 1 mg of pepsin (Sigma-Aldrich P7000-100G) was added. After incubation of the solution at 40 °C for 60 min in a shaking water bath, 10 mL of tris-maleate buffer (pH 6.9) was added and pH was adjusted with 1 M NaOH. The volume was made up to 25 ml with tris-maleate buffer, an aliquot of 0.1 mL was taken out for 0 min before addition of 5 mL tris-maleate buffer (pH 6.9) containing 2.16 U of pancreatic α-amylase with an activity of 19.6 units/mg (Sigma-Aldrich A-3176) and incubation at 37 °C with constant shaking. Aliquots of 0.1 mL were taken at 5, 30, 60, 90, 120 and 180 min. Tubes containing aliquots were placed in a boiling water bath for 15 min to inactivate the α-amylase enzyme. One mL 0.4 M sodium-acetate buffer (pH 4.75) and 90 µL of the amyloglucosidase with the activity of 64.7 U/mg (Megazyme E-AMGDF) was added to aliquots and incubated at 60 ° C for 45 min. The glucose concentration per sample was measured using the glucose oxidase-peroxide kit (Megazyme D-glucose Assay Kit), and the rate of *in vitro* starch gelatinisation was expressed as the percentage of the total starch digested at time intervals of 0, 5, 30, 60, 90, 120 and 180 min.

Estimated Glycaemic Index

The first order equation proposed by Goñi *et al*. (1997) was used to describe the kinetics of starch hydrolysis.

$$C = C_{\infty}(1 - e^{-kt})$$

where C is the concentration at time t,

C_{∞} is the percentage of starch hydrolysed after 180 min,

k is the kinetic constant (min^{-1}) and t is the time (min).

Parameters k and C_∞ were estimated for each treatment based on data obtained from the *in vitro* hydrolysis procedure. The equation by Jaisut *et al*. (2008) was used to calculate the area under curve (AUC).

$$AUC = C_\infty ((t_f - t_0) - C_\infty/K)(1\text{-}\exp(-K(t_f\text{-}t_0)))$$

Where t_f is the final time (180 min),

t_0 is the initial time (time 0).

The hydrolysis index (HI) was defined as the area under the hydrolysis curve of a sample divided by the corresponding area of white bread. The estimated glycaemic index was estimated using the equation by Goñi *et al*. (1997).

$$EGI = 39.71 + 0.549\ HI$$

4.2.9.2 *In vitro* assays for protein digestibility

4.2.9.2.1 Multi-enzyme protein digestibility

A multi-enzyme protein digestibility method was used to determine protein digestibility of biscuits made from dough rested for 15 min and 24 h, based on Hsu *et al*. (1977). A multi-enzyme solution was prepared containing, bovine trypsin (T0303-IG), bovine chymotrypsin (C4129-1G) and protease (P5747-5G) from *Streptomyces griseus* (Sigma-Aldrich). The multi-enzyme solution was added to biscuit sample suspensions that were adjusted to pH 8.0 with 0.1 M HCL and/or NaOH and incubated at 37 °C. The drop in pH of the suspension was recorded over 10 min, at 1 min intervals. The % *in vitro* protein digestibility was calculated by using the equation Y=210.46 – 18.10x, where x is the pH of the product after 10 min digestion and Y is the percentage *in vitro* digestibility (Hsu *et al*., 1977). The multi-enzyme solution was freshly prepared for each analysis.

4.2.9.2.2 Pepsin protein digestibility

The pepsin protein digestibility assay used was based on Hamaker et al. (1986). Biscuit samples were digested with porcine pepsin P7000-100g (Sigma-Aldrich, St Louis, MO) for two hours at 37 °C. The products of digestion were pipetted off using a Pasteur pipette. The remaining contents were washed with distilled water, centrifuged and the clear supernatant was pipetted off. The residues were dried overnight at 100 °C in a forced draft oven and protein content of the dried residues was determined using Dumas method 46-30.01 as specified in AACC (2000) using N x 6.25 as the conversion factor. The *in vitro* protein digestibility was expressed as a percentage by calculating the difference between the initial total weight of the protein and residual weight of protein after pepsin digestion.

4.2.10 Statistical Analysis

Descriptive statistics were used to analyse sorghum flour particle size distributions. *In vitro* digestibility, instrumental colour and thermal properties data was analysed with a t-test to determine the effect of treatment using SPSS (IBM, Armonk. New York, USA) and expressed as means ± standard deviations. The Tukey Honestly Significant Difference (HSD) test was used to compare means at $p < 0.05$.

Hedonic consumer data were analysed using two-way ANOVA (treatment and consumer) to determine the effects of dough resting treatment and consumers on the responses for the liking of appearance, colour, taste, texture and overall flavour of sorghum-soya (70:30) biscuits. MS Excel's binomial distribution function was used for the analysis of the paired preference results at $p < 0.05$.

Word clouds (https://worditout.com) were used to summarise key terms and phrases visually in comments written by consumers for sorghum-soya biscuit attributes. In the resulting figures, font size represented the frequency of mention of the same terms. All comments were analysed for each section. The larger the word/phrase appears in a word cloud image, the higher the frequency of mention of the same terms. Keywords and phrases were selected from sentences written by consumers. For instance, if a comment on biscuit liking for colour was written as: 'The colour is dark which makes me think it is rich with ingredients but also makes me think it was about to be burnt'. Keywords selected were 'dark' and 'burnt', which appeared as separate terms. Short key phrases were also selected as expressed by consumers and connected with a hyphen to appear as one term.

To capture the different words/expressions used by consumers, scores of the biscuit were also used to assign the comments into a positive or negative category. Some examples of comments treated using this strategy were: ‘Quite dark for a biscuit’, ‘Colour is a bit dark’ and ‘the darker colour looks more appealing’. Keywords and phrases selected then were ‘dark’, ‘dark’ and ‘dark-appealing’ respectively. In the above examples, biscuits had received high and positive ratings (above 5). Comments such as ‘It has a good taste, but more sugar would've been added’ or ‘It’s not too sweet’ were categorised as negative because they received a lower rating (below 5).

4.3 Results

4.3.1 Sorghum, soya and sorghum soya composite flour particle size

In the current study, approximately 80% of the commercial red sorghum flour, soya flour and sorghum-soya (70:30) composite flour passed through the 500 µm sieve (Figure 4.1).

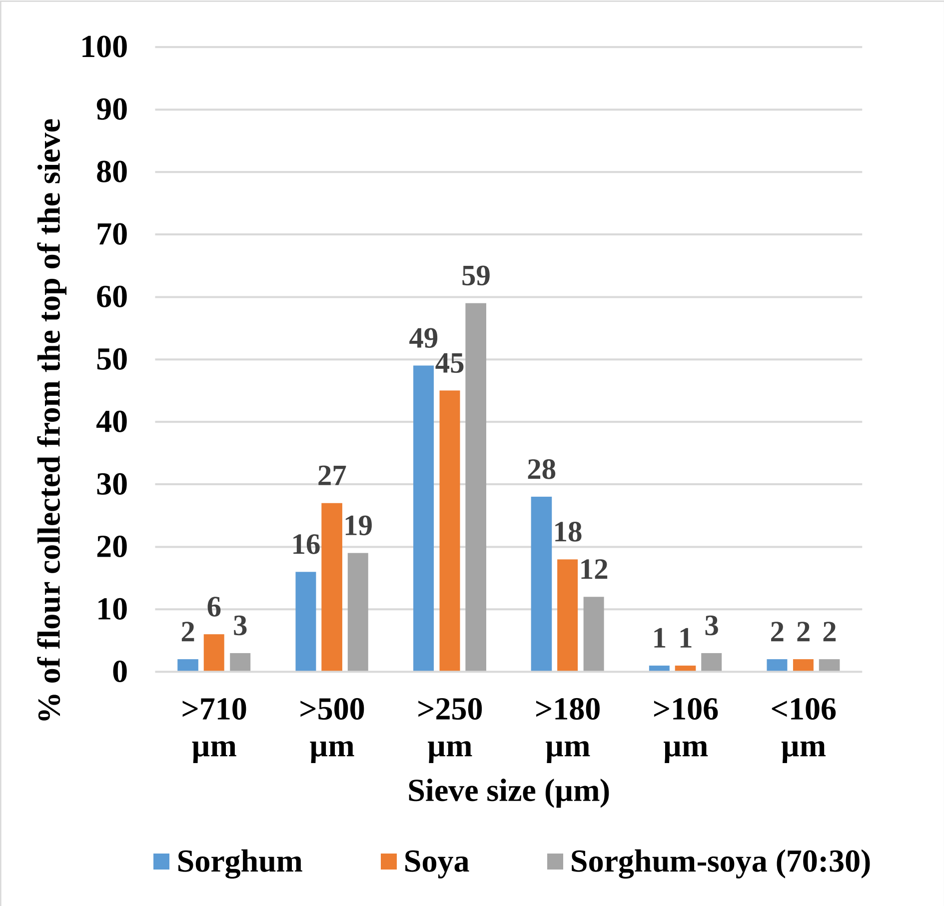

Figure 4.1 Distribution of size particles in the sorghum, soya and sorghum-soya composite flours

4.3.2 Proximate composition of flours and biscuits

After mixing sorghum and full-fat soya flour, the protein, ash and fat content of the sorghum-soya (70:30) composite flour increased by approximately 90%, 130% and 263%, respectively ($p < 0.05$) (Table 4.1).

Table 4.1 Proximate composition of sorghum, soya and composite sorghum-soya (70:30) flours (g/100 g)

Flour type	Moisture	Protein	Fat	Ash
Sorghum	10.00 ^{c} ± 0.01	9.43 ^{a} ± 0.06	2.16 ^{a} ± 0.09	0.85 ^{a} ± 0.01
Full-fat soya	7.68 ^{a} ± 0.18	36.70 ^{c} ± 0.17	21.04 ^{c} ± 2.51	4.64 ^{c} ± 0.04
Sorghum-soya (70:30)	9.13 ^{b} ± 0.36	18.10 ^{b} ± 0.18	7.85 ^{b} ± 0.30	2.03 ^{b} ± 0.08

Means ± standard deviation of two analyses. Values with different letters in the same column differ significantly ($p \leq 0.05$).

Irrespective of dough resting time, the protein, fat and ash content of sorghum-soya biscuits were similar and higher than the wheat control biscuit (Table 4.2). Of interest, the moisture content of sorghum-soya biscuits made from dough rested for 24 h was 30% higher compared to biscuits made from dough rested for 15 min. The fat content of the sorghum-soya biscuits and that of digestive whole-wheat biscuits (control) were similar, and within the range (10 – 25 g oil per 100 g) as recommended by FAO/WHO (1994).

Table 4.2 Proximate composition of sorghum-soya and digestive whole-wheat biscuits (g/100 g)

Biscuits	Dough Resting time	Moisture	Protein	Fat	Ash	Dietary fibre	Carbohydrates
Sorghum-soya (70:30)	15 min	5.00	11.94	19.90	1.47	4.70	57.00
	24 h	6.50	11.83	19.30	1.51	ND	ND
Digestive whole-wheat	-	4.21	7.60	17.50	ND	2.90	66.00

ND-Not determined. Proximate composition of digestive whole-wheat biscuits was obtained from information provided on the product's packaging.

4.3.3 Instrumental colour of biscuits

Biscuits made from dough rested for 24 h had lower L* values (darker) and higher a* values (redness) ($p \leq 0.01$), than biscuits made from dough rested for 15 min (Table 4.3). This implies that there were colour differences between biscuits made from the two treatments, as shown in Figure 4.2 by definite visual colour differences between biscuits. There were no significant differences in b* values of biscuits, with an increase in dough resting time ($p > 0.05$).

Table 4.3 Effect of dough resting time (15 min versus 24 h) on colour of sorghum-soya biscuits.

Dough Resting time	L*	a*	b*
15 min	57.40 ^{b} ± 1.20	15.70 ^{a} ± 0.45	30.90 ^{a} ± 0.31
24 h	50.00 ^{a} ± 1.02	13.00 ^{b} ± 0.72	31.20 ^{a} ± 1.50

Means ± standard deviation. Means in the same column with different letters differ significantly at $p \leq 0.05$

Figure 4.2 Visual presentation of sorghum-soya biscuits made from dough rested for 15 min (left) and 24 h (right)

4.3.4 Physical and textural properties of biscuits

The cross-section of the biscuits showed that the sorghum-soya biscuits made from dough rested for 15 min had an open crumb structure with visible air pockets, indicating that the dough might have trapped air produced by leavening agents from baking powder (Figure 4.3). Biscuits made using dough rested for 24 h had few air pockets and a compact structure that seemed to have swollen granules embedded within the matrix after baking (Figure 4.3).

There were significant differences ($p \leq 0.05$) between mass, diameter and density properties of sorghum-soya biscuit, with the increase in dough resting time from 15 min to 24 h (Table 4.4).

Biscuits made from dough rested for 24 h were thicker and required less force to break (N), resulting in lower stress and higher strain compared to biscuits made from dough rested for 15 min.

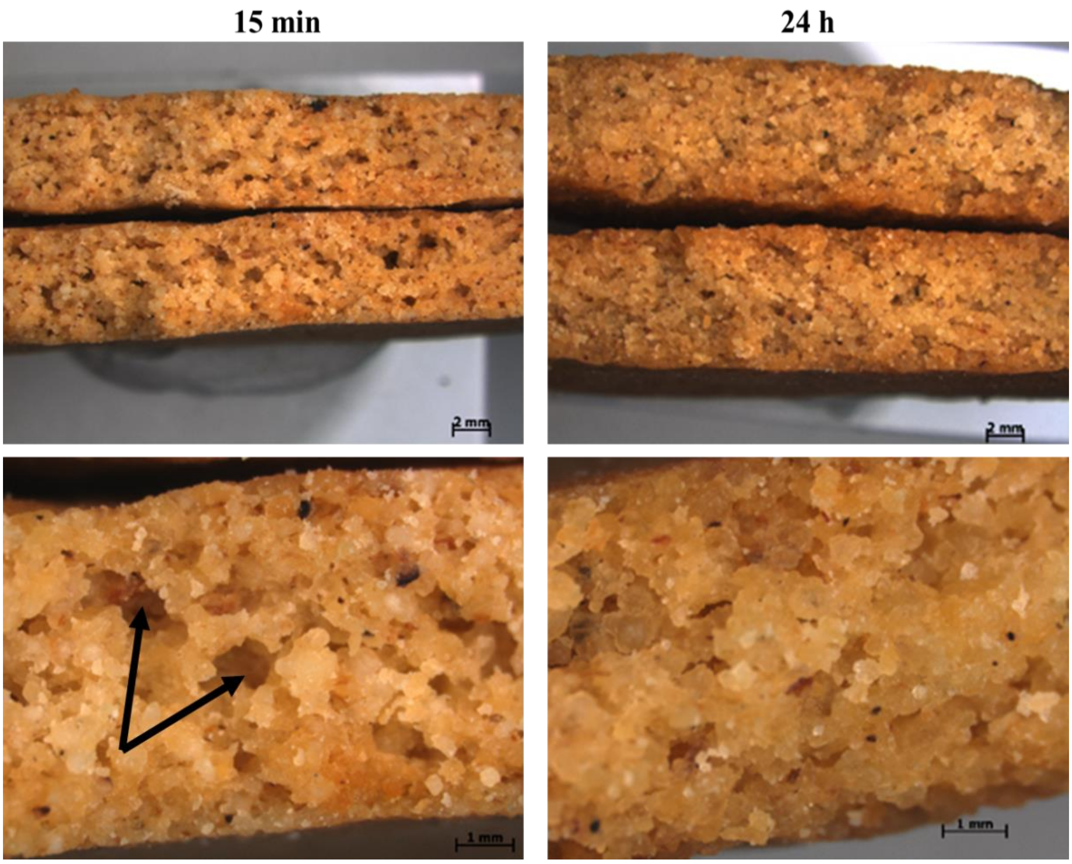

Figure 4.3 Effect of dough resting time (15 min; 24 h) on the biscuit matrix and the structure of sorghum-soya biscuits. Arrows indicate open spaces in crumb structure

Table 4.4 Effect of dough resting time on textural and physical properties of sorghum-soya and digestive whole-wheat biscuits

Biscuits	Dough resting time	Mass (g)	Diameter (mm)	Thickness (mm)	Density (g/cm^3)	Force to break (N)	σ Stress (kPa)	ε Strain (%)
Sorghum-soya	15 min	$11.5^a \pm 0.8$	$53.0^a \pm 0.3$	$6.5^a \pm 0.8$	$0.81^a \pm 0.0$	$51.1^c \pm 12.0$	$1057.6^c \pm 311$	$1.0^a \pm 0.0$
	24 h	$14.5^b \pm 0.9$	$54.1^b \pm 1.0$	$7.6^b \pm 0.6$	$0.84^b \pm 0.0$	$34.8^b \pm 2.9$	$506.2^b \pm 86.0$	$1.1^b \pm 0.0$
Digestive whole-wheat	-	$11.8^a \pm 0.1$	$63.2^c \pm 0.9$	$6.5^a \pm 0.1$	$0.82^a \pm 0.0$	$12.7^a \pm 1.5$	$213.2^a \pm 30.6$	$1.3^c \pm 0.0$

Means ± standard deviation. Means in the same column with different letters differ significantly at $p \leq 0.05$

4.3.5 Microscopy of sorghum-soya biscuits.

Biscuits made from dough rested for 15 min had more intact starch granules that appeared in clusters showing that the proportion of partial and non-birefringent starch granules in sorghum-soya biscuits was higher than for dough rested for 24 h (Figure 4.4). Biscuits made from dough rested for 24 h had a lower proportion of Maltese crosses, showing that there was more hydration in starch granules with 24 h dough resting compared to 15 min dough resting time.

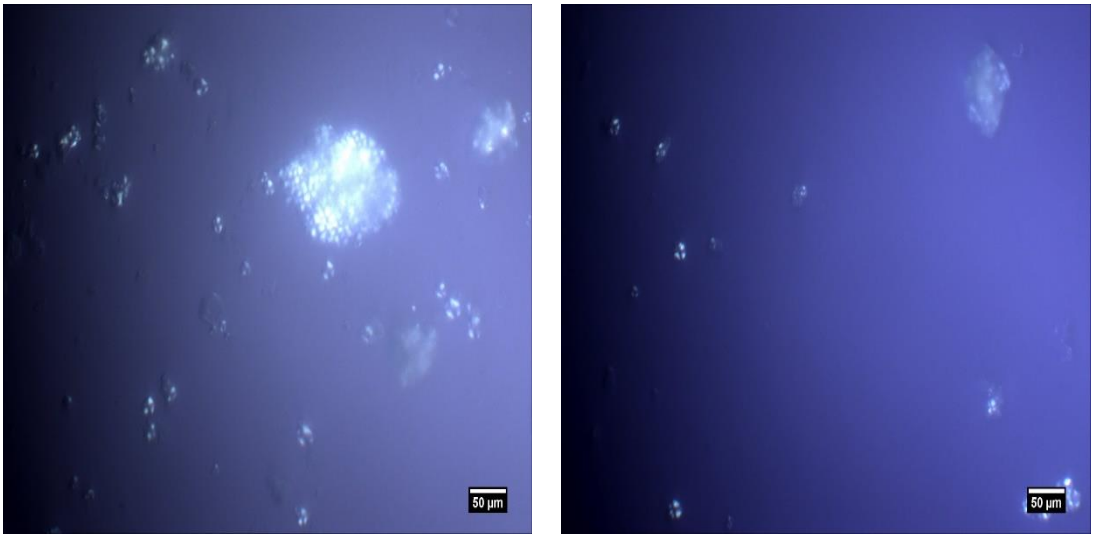

Figure 4.4 Polarised light microscopy showing starch granule birefringence, for sorghum-soya biscuits made from dough rested for 15 min (left) and 24 h (right)

4.3.6 Consumer evaluation

Consumer evaluation test results showed that resting time did not have a significant effect ($p > 0.05$) on liking scores for appearance, colour and texture of sorghum-soya (70:30) biscuits. Biscuits made from dough rested for 15 min received higher scores for the liking of taste and overall flavour (Table 4.5). In addition, these biscuits were preferred over biscuits made from dough rested for 24 h dough (Figure 4.10). Figure 4.5 - 4.9 shows the distribution of consumer responses over the 9-point hedonic scale and liking means for attributes of appearance, colour, taste, texture and overall flavour, where 1=Dislike extremely and 9 = Like extremely. Figure 4.11 – 4.16 shows consumers' comments as word clouds, where font size represented the frequency of mention. The larger the word, the higher the frequency of mention of a specific comment.

Sorghum-soya biscuits made from 15 min and 24 h dough resting times had an appealing shape to consumers. Sorghum specks on the surface of biscuits were found to be unappealing more in biscuits made from 24 h dough resting time than 15 min dough resting time (Figure 4.11). Figure 4.12 shows that sorghum-soya biscuits were appealing. Biscuits made from 15 min dough resting time were mainly described as healthy and pale while biscuits from 24 h dough resting time were dark. Biscuits from 24 h dough resting time had an after taste as well as a bitter aftertaste, and biscuits made from 15 dough resting were described as bland and lacked flavour (Figure 4.13).

Sorghum-soya biscuits made from different resting times (15 min and 24 h) were commented as grainy, hard and had some particles after-feel. In addition to comments, biscuits from 24 h resting time were softer than biscuit made from 15 min dough resting time (Figure 4.14). Comments indicated that the biscuits from 24 h dough resting time were healthy and bland, while biscuits from both treatments were sweet (Figure 4.15).

In terms of preferences, consumers commented that biscuits made from both resting times were tasty, had appealing colours (Figure 4.16). Biscuits from 24 h dough resting time were perceived as not too sweet and healthy, while biscuits from 15 min dough resting time were grainy.

Table 4.5 Effect of dough resting time on consumer liking of sensory characteristics of sorghum-soya biscuits (n=50)

Biscuits from dough resting time	Appearance	Colour	Taste	Texture	Overall flavour
15 min	7.14 ^{a} ± 1.20	6.90 ^{a} ± 1.50	6.90 ^{a} ± 1.60	6.30 ^{a} ± 1.70	7.10 ^{a} ± 1.40
24 h	7.30 ^{a} ± 1.12	7.20 ^{a} ± 1.40	6.20 ^{b} ± 2.30	6.20 ^{a} ± 1.90	6.10 ^{b} ± 2.20

Means ± standard deviation. Means with the same superscript in the same column are not significantly different, at $p \leq 0.05$.

Biscuits were evaluated using a 9-point hedonic scale (1 = Dislike extremely and 9 = Like extremely)

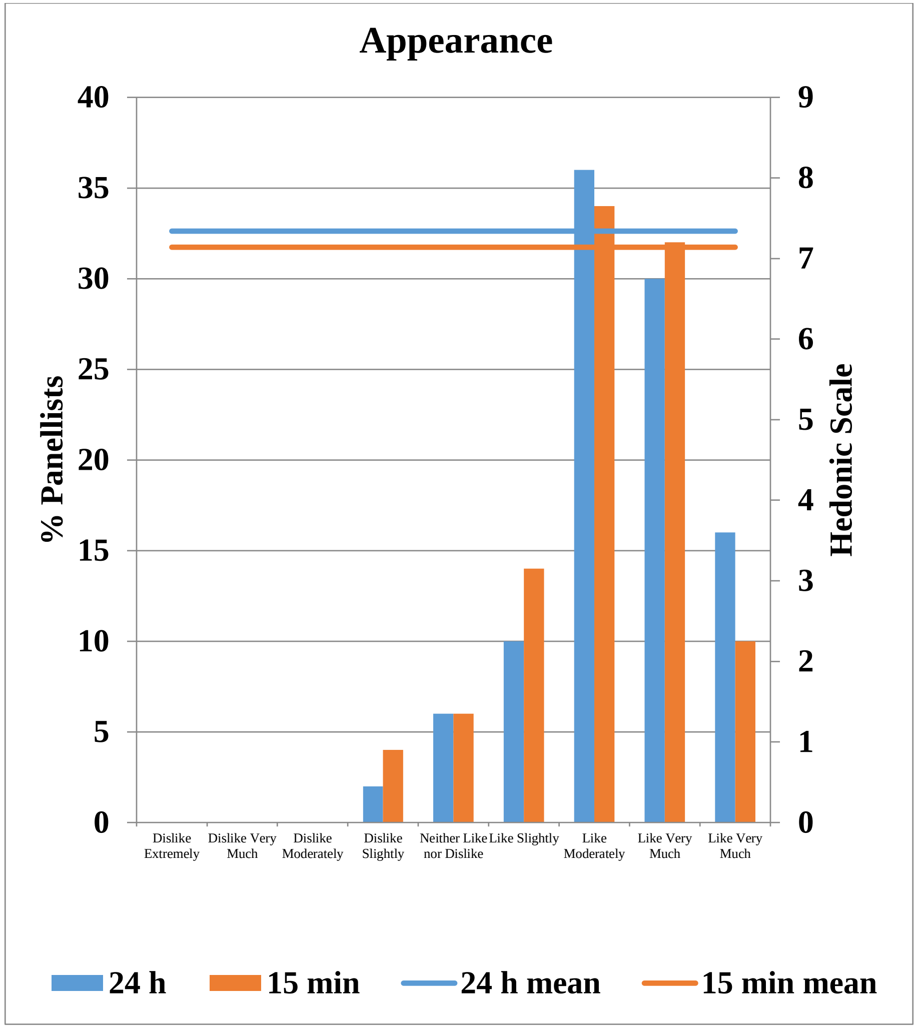

Figure 4.5 Consumer liking for the appearance of sorghum-soya biscuits baked using dough rested for 15 min or 24 h

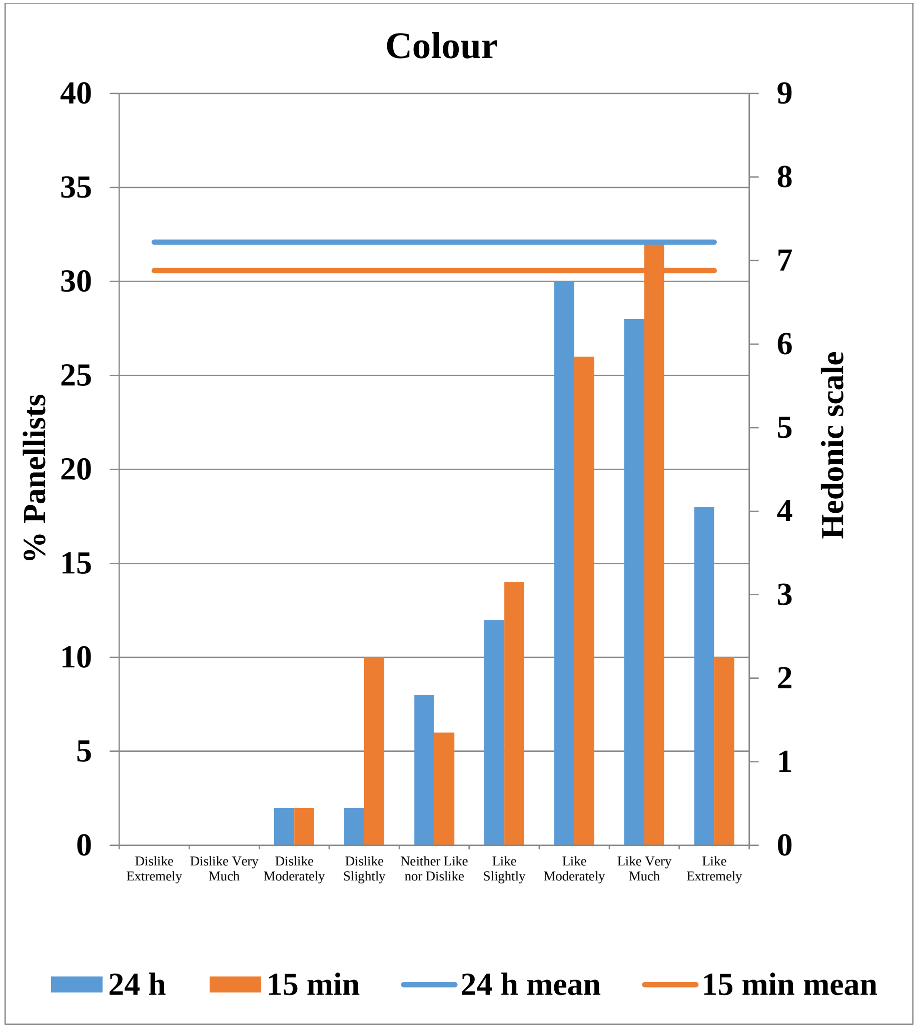

Figure 4.6 Consumer liking for the colour of sorghum-soya biscuits baked using dough rested for 15 min or 24 h

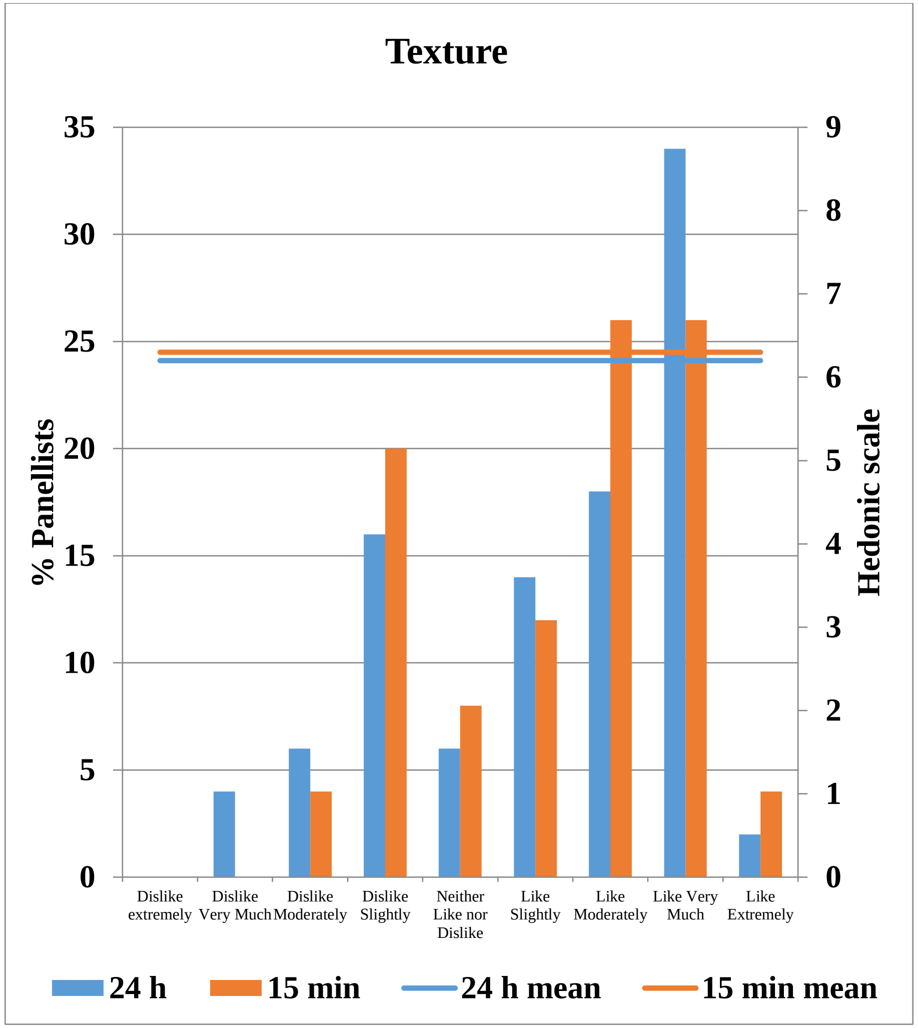

Figure 4.7 Consumer liking for the texture of sorghum-soya biscuits baked using dough rested for 15 min or 24 h

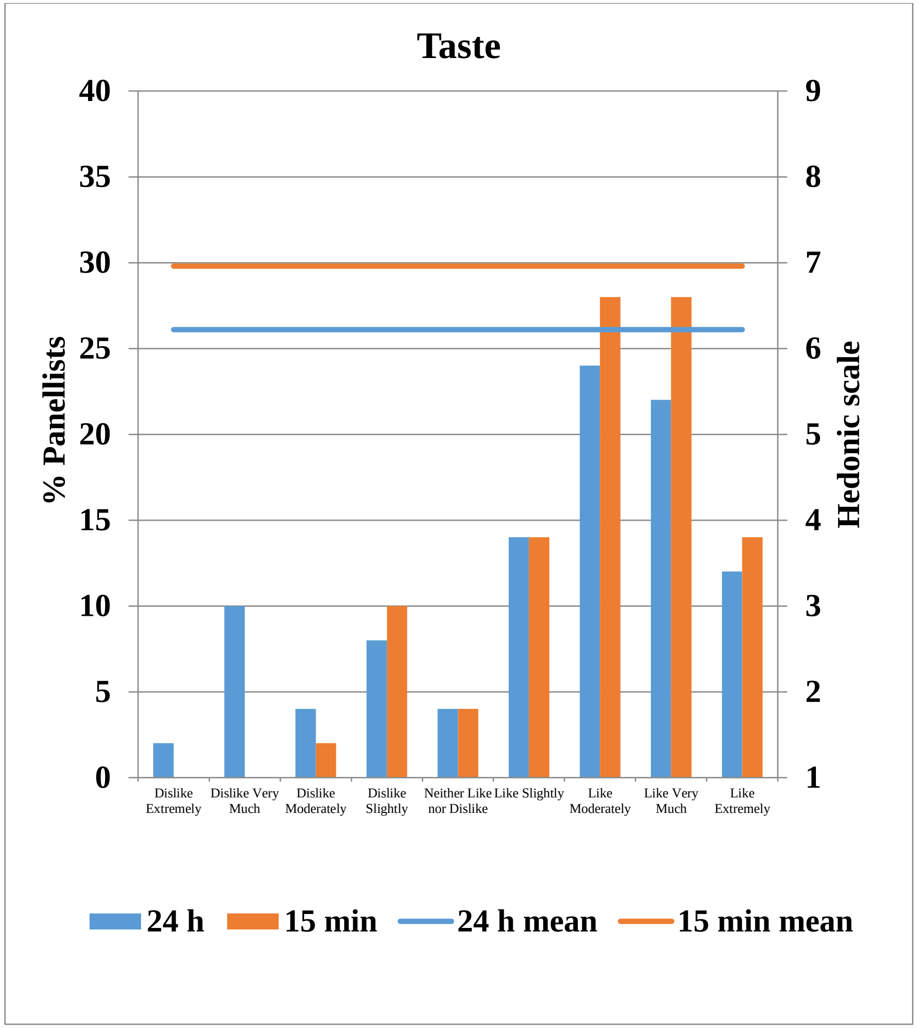

Figure 4.8 Consumer liking for the taste of sorghum-soya biscuits baked using dough rested for 15 min or 24 h

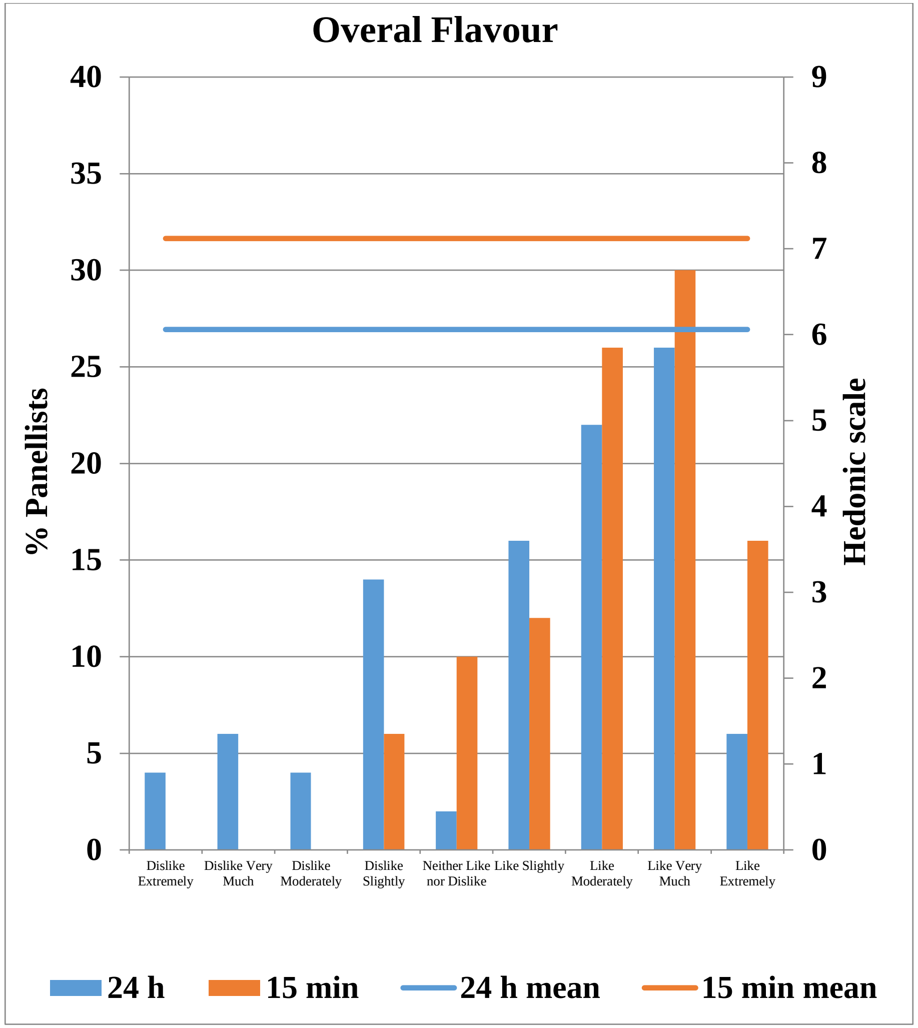

Figure 4.9 Consumer liking for the overall flavour of sorghum-soya biscuits baked using dough rested for 15 min or 24 h

Figure 4.10 shows that there were significant differences $p < 0.05$ in biscuit preference, with biscuits made from dough rested for 15 min preferred to biscuits made from dough rested for 24 h.

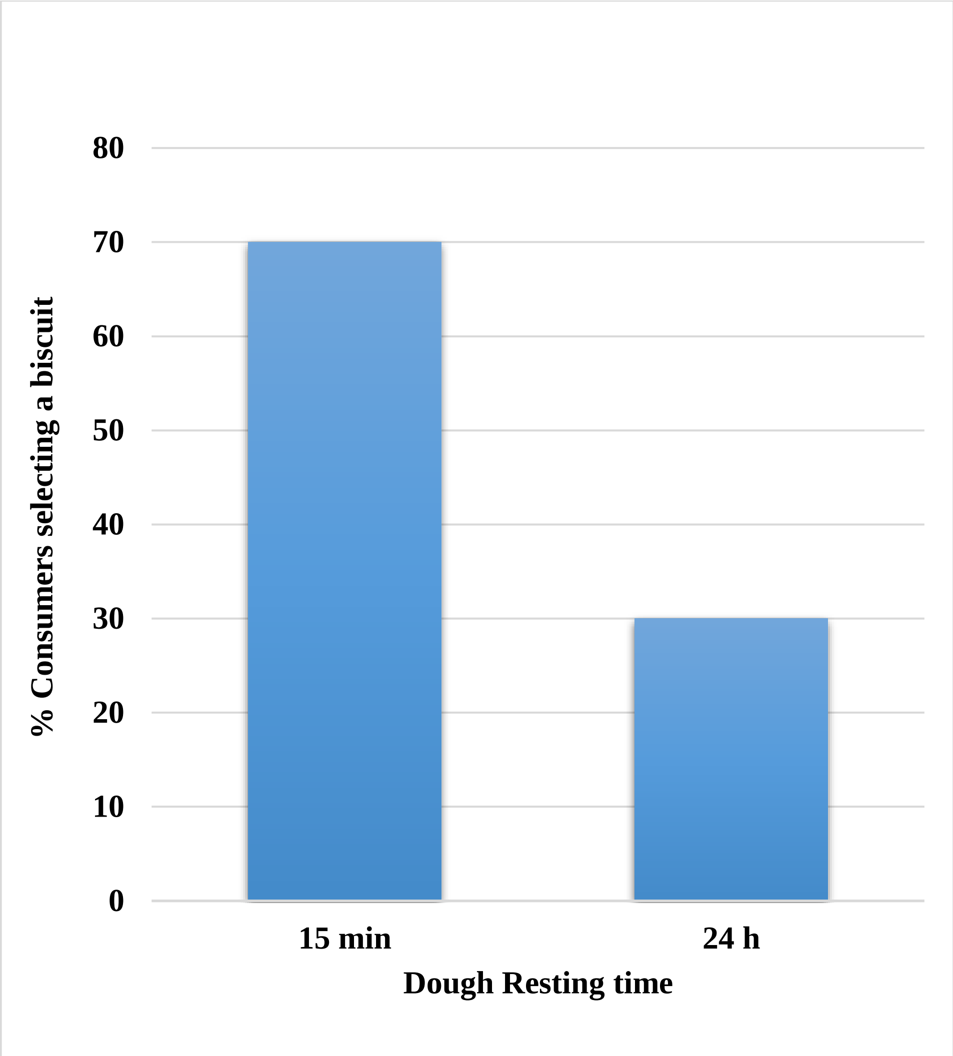

Figure 4.10 Comparison of % consumers preferring sorghum-soya biscuits from dough rested for 15 min or 24 h (n = 50).

Appearance Comments

Figure 4.11 Comments for the liking of the appearance of sorghum-soya (70:30) biscuits made from dough rested for 15 min (left) and 24 h (right)

Colour Comments

Figure 4.12 Comments for the liking of the colour of sorghum-soya (70:30) biscuits made from dough rested for 15 min (left) and 24 h (right)

Taste Comments

Figure 4.13 Comments for the liking of the taste of sorghum-soya (70:30) biscuits made from dough rested for 15 min (left) and 24 h (right).

Texture Comments

Figure 4.14 Comments for the liking of the texture of sorghum-soya (70:30) biscuits made from dough rested for 15 min (left) and 24 h (right)

Overall Flavour Comments

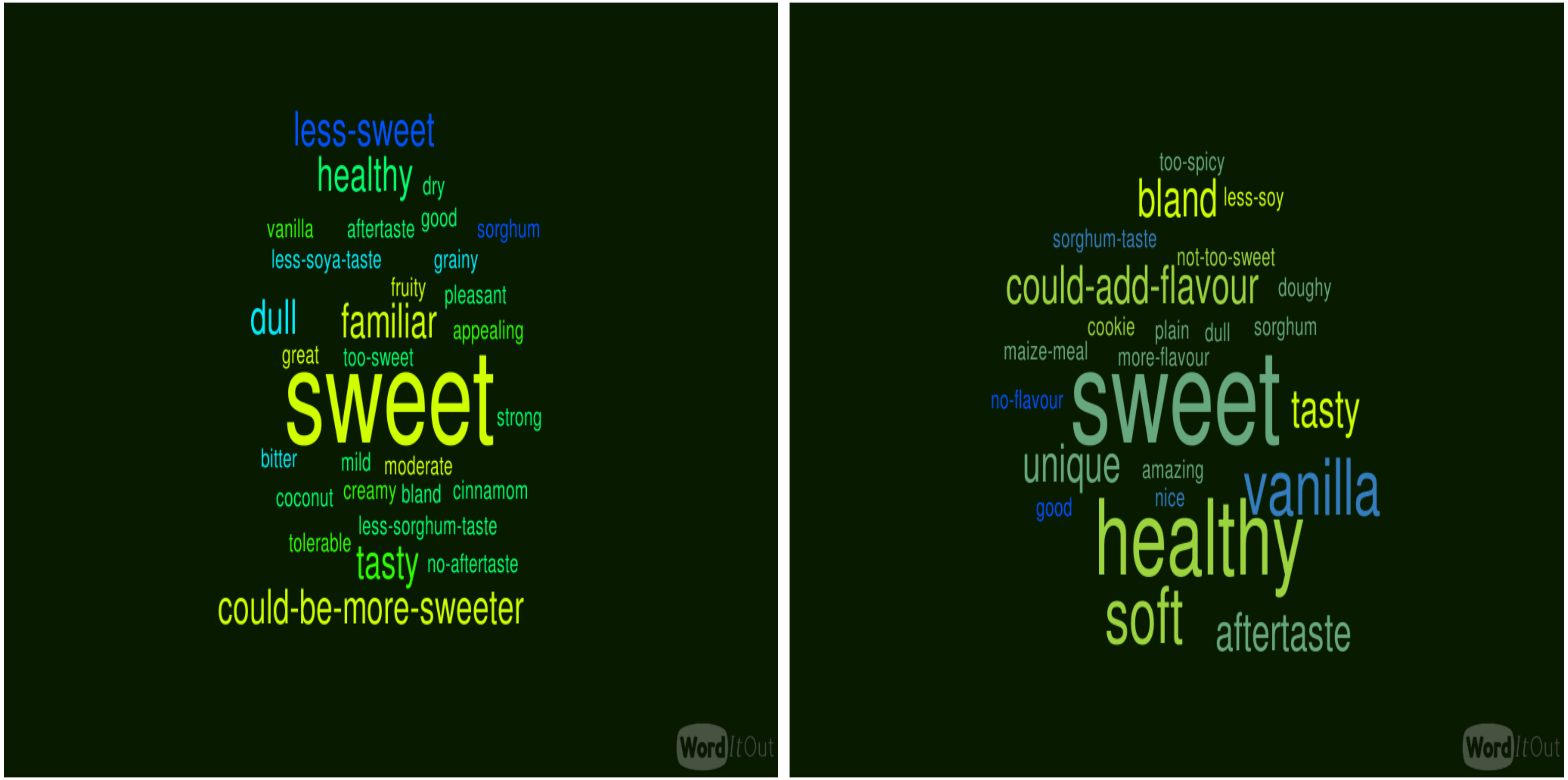

Figure 4.15 Comments for the liking of the overall flavour of sorghum-soya (70:30) biscuits made from dough rested for 15 min (left) and 24 h (right)

Preference

Figure 4.16 Comments for the preference of sorghum-soya (70:30) biscuits made from dough rested for 15 min (left) and 24 h (right)

4.3.7 Thermal properties of sorghum-soya dough and biscuits

4.3.7.1 Thermal properties of sorghum-soya dough, slurries and flours

Figure 4.17 shows the thermal properties of sorghum-soya dough rested for 15 min and 24 h respectively. There was no significant difference ($p > 0.05$) in thermal properties of sorghum-soya dough with the increase in dough resting time. There were two distinct endothermic peaks Peak 1 (a, c) and Peak 2 (b, d) for each endotherm of sorghum-soya dough. Peak 1 had mean endotherm temperature of 106.8 °C for both dough resting times. Peak 2 had mean endotherm temperatures of 131.1 °C and 132 °C, for sorghum-soya dough with increasing dough resting time. (Table 4.6). The enthalpy (ΔH) of the second peak (d) for dough rested for 24 h was higher (0.8 J/g) than for peak (b) in dough rested for 15 min (0.5 J/g).

The thermal temperatures obtained for sorghum-soya dough appeared at higher temperatures than expected. This could be due to low moisture content present in the dough, that shifted endotherms to higher temperatures. To investigate the effect of moisture content on thermal properties of sorghum-soya dough, slurries in the ratio one part solids and three parts water were prepared and analysed using a DSC.

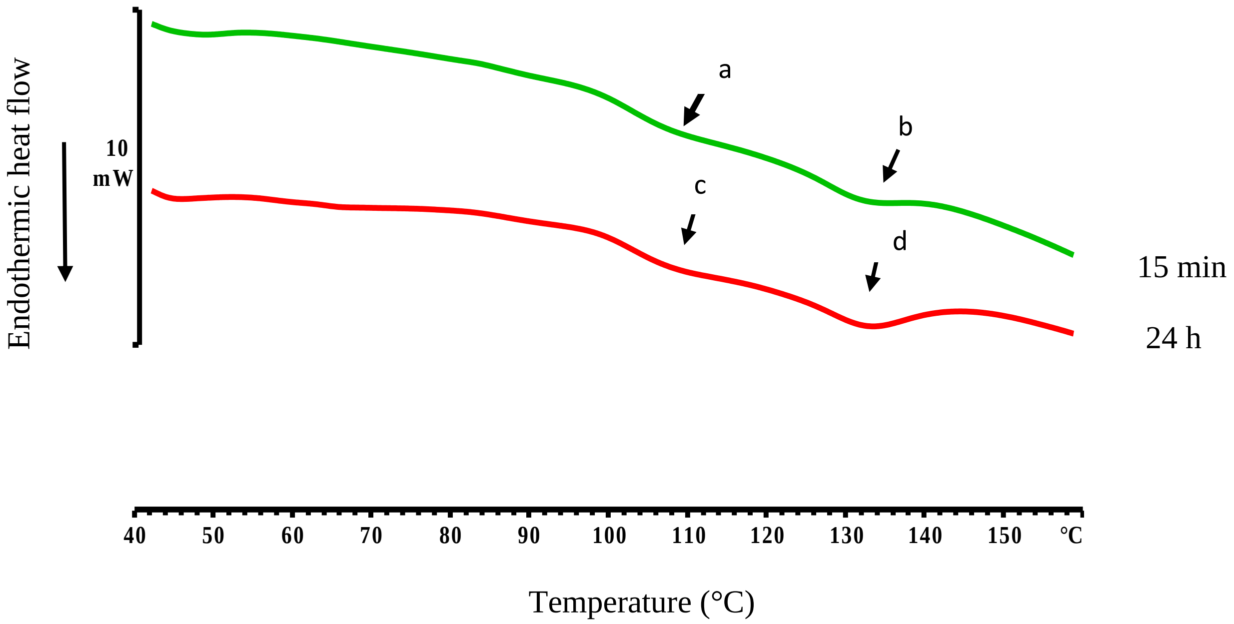

Figure 4.17 DSC thermogram of sorghum-soya dough rested for 15 min and 24 h

Table 4.6 Effect of dough resting time (15 min and 24 h) on thermal properties of sorghum-soya (70:30) dough.

Dough resting time	Peak 1				Peak 2			
	Onset (T_o)	Peak (T_p)	Conclus-ion (T_c)	Enthalpy (J/g)	Onset (T_o)	Peak (T_p)	Conclus-ion (T_c)	Enthalpy (J/g)
15 min	99.5^a ±0.7	106.8^a ± 0.0	116.8^a± 0.2	0.3^a±0.0	124.7^a ± 0.9	131.1^a± 0.0	138.7^a± 0.3	0.4^a± 0.0
24 h	98.2^a ±0.8	106.8^a ± 1.2	115.7^a± 0.3	0.3^a±0.0	123.4^a ± 0.2	132.0^a ± 0.3	140.6^a± 1.3	0.7^b± 0.0

Mean±Standard. Means with the same superscript per column are not significantly different at $p > 0.05$.

T_o- gelatinisation onset temperature; Tp – gelatinisation peak temperature; Tc- gelatinisation conclusion temperatures.

Figure 4.18 shows thermograms of thermal properties of sorghum-soya slurries rested for 15 min and 24 h, each with two endothermic peaks. The endothermic peaks could relate to starch gelatinisation (a, c) and denaturation of soya proteins (b, d). The endothermic peaks obtained had shifted to lower temperatures, compared to endotherm peaks obtained for sorghum-soya dough (Table 4.16).

Results showed that there was no significant difference ($p > 0.05$) in thermal denaturation properties of sorghum-soya slurries (Peak 2) with an increase in dough resting time. The mean peak, conclusion and enthalpy of the sorghum-soya (70:30) slurries increased with increase in dough resting time, suggesting that more energy was required to melt starch crystallites (Table 4.7).

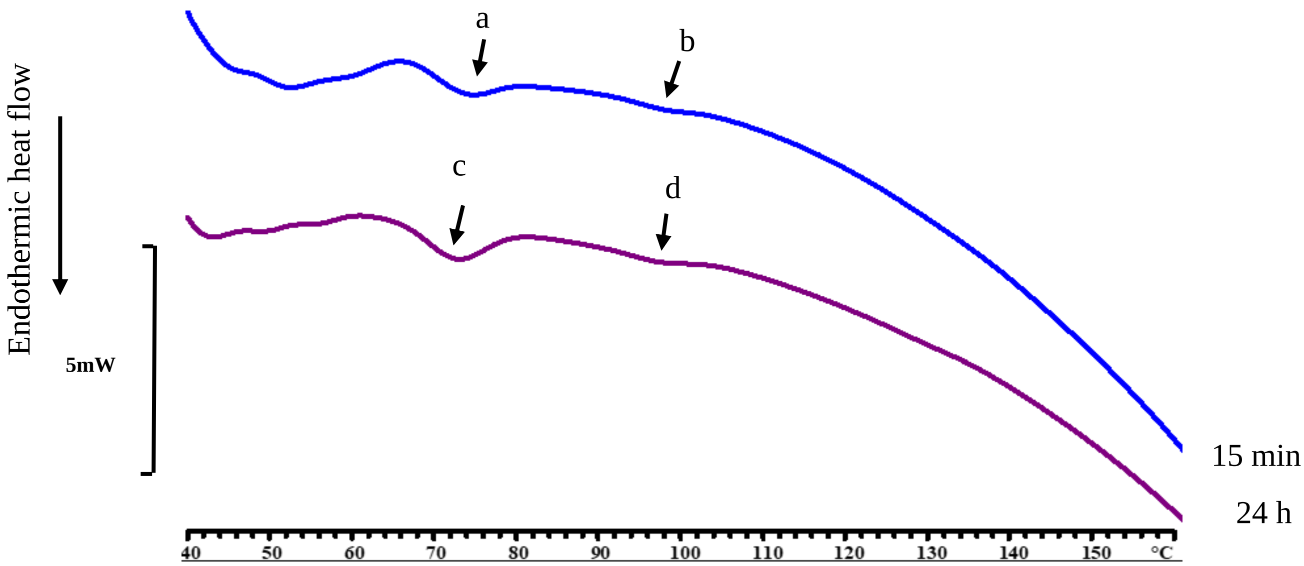

Figure 4.18 DSC thermogram of sorghum-soya slurries (70:30), rested for 15 min and 24 h.

Table 4.7 Effect of dough resting time (15 min and 24 h) on sorghum-soya slurries (70:30)

	Peak 1				Peak 2			
Dough resting time	Onset (T_o)	Peak (T_p)	Conclusion (T_c)	Enthalpy (J/g)	Onset (T_o)	Peak (T_p)	Conclusion (T_c)	Enthalpy (J/g)
15 min	65.8[a]± 2.0	70.8[a]± 1.5	76.9[a]± 0.1	0.4 [a]±0.0	93.0[a]± 1.1	96.2[a]± 0.2	101.9[a]±1.3	0.1[a]±0.0
24 h	64.2[a]± 0.2	72.2[b]± 0.0	80.4[b]± 0.1	0.8 [b]±0.1	90.5[a]± 1.6	96.8[a]± 0.9	104.0[a]±1.7	0.1[a]±0.0

Mean±standard deviation. Means with the same superscript per column are not significantly different at $p > 0.05$.

T_o- gelatinisation onset temperature; Tp – gelatinisation peak temperature; Tc- gelatinisation conclusion temperature

Figure 4.19 shows thermograms for sorghum, soya and sorghum-soya composite flour. Sorghum, soya and sorghum-soya (70:30) flours were mixed with water in the ratio one-part flour: three parts water and analysed using a DSC, in order to identify thermal properties of soya proteins. Sorghum flour only had one endothermic peak (a) for starch gelatinisation with temperature 70.7 °C and enthalpy ΔH 4.9 J/g.

Soya and sorghum-soya (70:30) flours had three endothermic peaks on each endotherm. The first peak was in the range of 58.4 °C - 65.8 °C (ΔH 0.3 J/g) for soya flour and 54.6 °C to 62.7 °C (ΔH 0.3 J/g) for the sorghum-soya composite flour. Soya flour had two other endothermic peaks with mean peak temperatures at 73.3 °C, ΔH 0.4 J/g (b) and 95.5 °C, ΔH 2.2 J/g (c), for denaturation of soya proteins. The other two extra endothermic peaks, for sorghum-soya (70:30) flour had mean temperatures at 72.2 °C, ΔH 2.6 J/g (d) for starch gelatinisation and 93.7 °C, ΔH 0.3 J/g (e) for soya protein denaturation. Endotherm peak (b) for sorghum-soya composited flour may be an indication of a merged endotherm for starch gelatinisation and denaturation of soya proteins that occurred concurrently.

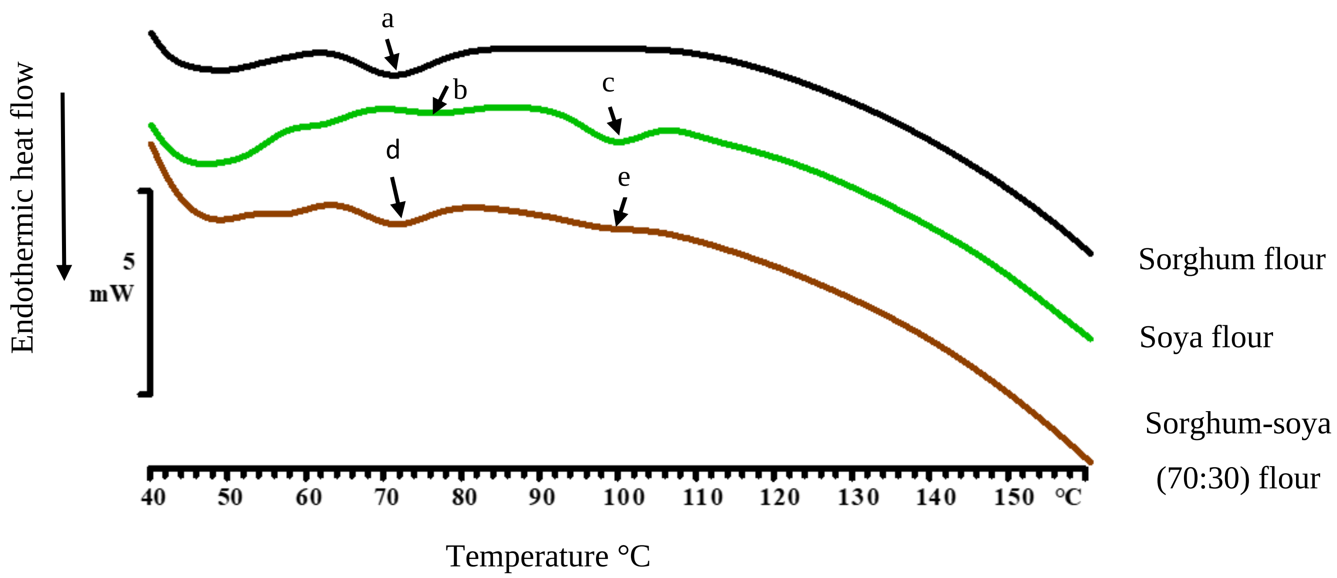

Figure 4.19 DSC thermograms of sorghum, soya and sorghum-soya (70:30) composite flours in ratios of one part flour and three parts water

4.3.7.2 Thermal properties of sorghum-soya biscuits

Figure 4.20 shows two thermograms of sorghum-soya biscuits made from dough rested for 15 min and 24 h, respectively, each with two endothermic peaks. Peak 1 (a, c) could be for melting of soya lipids, and peak 2 (b, d) indicates starch gelatinisation. Dough resting time did not have a significant effect on starch gelatinisation of sorghum-soya biscuits ($p > 0.05$), but there were differences in melting of lipids (peak 1).

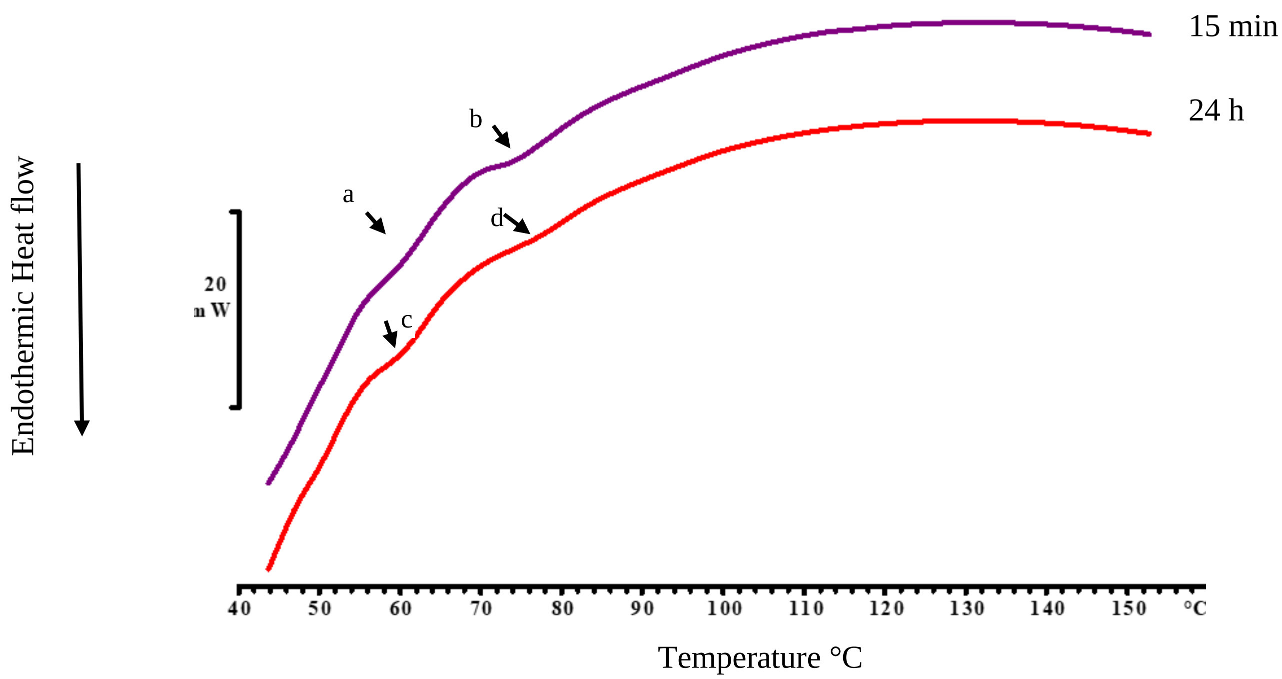

Figure 4.20 DSC thermogram of sorghum-soya biscuits, made from dough rested for 15 min and 24 h.

Table 4.8 Effect of dough resting time (15 min and 24 h) on thermal properties of sorghum-soya biscuits in the ration one part solids and three parts water

	Peak 1				Peak 2			
Dough resting time	Onset (T_o)	Peak (T_p)	Conclusion (Tc)	Enthalpy (J/g)	Onset (T_o)	Peak (T_p)	Conclusion (Tc)	Enthalpy (J/g)
15 min	$48.5^a \pm 0.4$	$55.9^a \pm 0.2$	$61.9^a \pm 0.7$	$1.1^a \pm 0.2$	$69.9^a \pm 1.1$	$74.7^a \pm 1.2$	$81.4^a \pm 0.2$	$3.6^a \pm 0.5$
24 h	$56.0^b \pm 0.0$	$60.2^b \pm 0.2$	$65.9^b \pm 0.5$	$2.7^b \pm 0.1$	$70.5^a \pm 0.4$	$76.6^a \pm 0.3$	$82.8^a \pm 0.8$	$1.3^b \pm 0.3$

Mean±Standard deviation. Means with the same superscript per column are not significantly different at $p > 0.05$

T_o- gelatinisation onset temperature; Tp – gelatinisation peak temperature; Tc- gelatinisation conclusion temperature.

4.3.8 *In vitro* starch digestibility of sorghum-soya biscuits .

Figure 4.21 shows the extent of sorghum-soya and digestive whole-wheat biscuits starch hydrolysis, in comparison to a white wheat bread standard over a 3 h period. The starch hydrolysis of wheat biscuits (44.2%) was lower than white bread (100%) but higher than values for sorghum-soya biscuits (13.5% -14.2%). There was immediate starch hydrolysis for sorghum-soya biscuits during the first 5 min, and the rate continued to increase until 90 min. After 90 min, sorghum-soya biscuits were slowly digested until maximum hydrolysis was achieved. Hydrolysis in white bread was rapid during the first 30 min, where there was a constant rate of hydrolysis between 60-180 min. Starch hydrolysis in digestive whole-wheat biscuit was also rapid during the first 5 min, reaching its maximum after 30 min and became constant.

There was no significant difference in starch hydrolysis and estimated glycaemic index (EGI) for sorghum-soya biscuits with an increase in dough resting time. Sorghum-soya biscuits had the lowest EGI in comparison to white bread and digestive whole-wheat biscuits. Sorghum-soya biscuits had the highest resistant starch ranging from 84.1% - 85.5%, followed by digestive whole-wheat biscuits (37.7%) and white bread (11.1%) (Table 4.9).

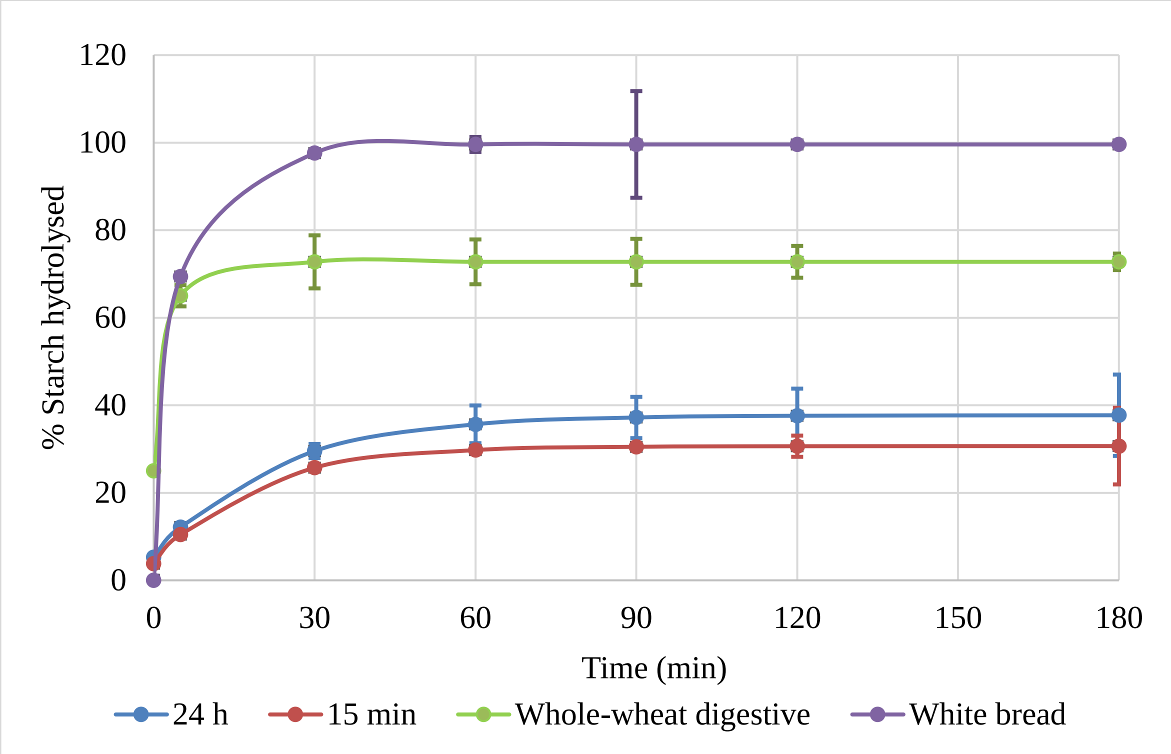

Figure 4.21 Effect of dough resting time (15 min, 24 h) on starch hydrolysis of sorghum-soya biscuits. White bread was used as a reference sample.

Table 4.9 Effect of dough resting time on the percentage of starch hydrolysed after 180 min and estimated glycaemic index of sorghum-soya biscuits

Sample	Sorghum-soya (70:30) biscuit		Digestive whole-wheat biscuit	White bread
Dough resting time	15 min	24 h	-	-
Area under curve	$2337^{a} \pm 172$	$3051^{a} \pm 800$	$7176.0^{b} \pm 1.2$	$17371.0^{c} \pm 1.3$
Hydrolysis Index (%)	$13.5^{a} \pm 0.1$	$14.2^{a} \pm 0.1$	$44.2^{b} \pm 6.9$	$100.0^{c} \pm 0.8$
Estimated Glycemic Index	$47.1^{a} \pm 0.5$	$47.5^{a} \pm 0.0$	$64.0^{b} \pm 3.8$	$94.6^{c} \pm 0.4$
C_{∞}	$24.5^{a} \pm 8.7$	$31.3^{a} \pm 9.3$	$71.4^{b} \pm 1.9$	$48.5^{c} \pm 1.0$
K	$0.05^{a} \pm 0.0$	$0.05^{a} \pm 0.0$	$0.08^{ab} \pm 0.0$	$0.1^{b} \pm 0.0$
Rapidly Digestible Starch (%)	$6.4^{a} \pm 0.4$	$9.6^{a} \pm 1.4$	$34.2^{b} \pm 5.4$	$86.5^{c} \pm 0.6$
Slowly Digestible Starch (%)	$8.0^{a} \pm 2.8$	$6.3^{a} \pm 7.8$	$28.0^{b} \pm 2.4$	$2.3^{a} \pm 0.6$
Resistant Starch (%)	$85.5^{c} \pm 2.5$	$84.1^{c} \pm 6.4$	$37.7^{b} \pm 3.0$	$11.1^{a} \pm 0.0$

Mean ± Standard deviation of two replicate analyses. Means with the same superscript per row are not significantly different at $p > 0.05$

$C\infty$ is the percentage of starch hydrolysed after 180 min, k is the kinetic constant (min^{-1}) and t is the time (min).

EGI was calculated usng the equation (39.71-0.549HI) according to Goñi et al. (1997)

White bread was used as a reference

4.3.9 *In vitro* protein digestibility (IVPD)

Table 4.10 shows the amount of protein digestible, using the multi-enzyme method and pepsin method. There was no significant effect ($p > 0.05$) on the *in vitro* protein digestibility using both the multi-enzyme and pepsin methods, with increase in dough resting time.

Table 4.10 Multi-enzyme and pepsin protein digestibility of sorghum-soya biscuits made from dough rested for 15 min or 24 h and digestive whole-wheat biscuits

Biscuit Type	Dough resting time	Multi-enzyme IVPD (%)	Pepsin IVPD (%)
Sorghum-soya (70:30)	15 min	88.8 [b] ± 1.0	85.7 [a] ± 0.9
	24 h	88.9 [b] ± 0.4	84.0 [a] ± 0.8
Digestive whole-wheat	Not applicable	82.4 [a] ± 0.7	89.7 [b] ± 0.7

Means ± standard deviation. Mean values with different letters in the same column differ significantly ($p \leq 0.05$)

4.4 Discussion

To produce the sorghum-soya biscuits, commercial sorghum super fine meal (approx. 90% extraction rate) from red non-tannin sorghum was used. The relative proportion of particles of different sizes in sorghum flour is affected by sorghum variety (Kebakile *et al*., 2008). In this study, more than 82% of the particles passed through a 500 µm sieve (Figure 4.1). This was likened to milled whole grain red sorghum flour that was used for sorghum bread making by Nkhabutlane *et al*. (2014), who reported that 77% of the particles passed through the 500 µm sieve. For 100% sorghum and 50:50 ratio sorghum-soya composite biscuits, Serrem (2010) re-milled commercial sorghum meal to achieve a maximum particle size of 500 µm and had to double the water added in formulation from 8.8% to 17.7% and 20% to 40% (by recipe basis), respectively. The size of the flour particles is important when making biscuits because it affects the water absorption, as fine particles have a larger surface area and absorb more water than coarse particles (Kebakile *et al*., 2008).

Biscuits made from dough rested for 24 h had a higher moisture content of 6.5 g/100 g, compared to 5.0 g/100 g of biscuits made from 15 min dough resting time (Table 4.2). High moisture content is undesirable in biscuits as it increases water activity and provides a medium that supports the growth of moulds, consequently reducing biscuits shelf life (Manley, 2000). For shelf-stable baked products such as biscuits, water activity (a_w) of less than 0.65 is recommended because it does not support microbial growth (Manley, 2000). The moisture content for sorghum-soya biscuits made from dough rested for 15 min in this study is 5.0%, similar to 4.9% moisture content for sorghum-soya (71.4 : 28.6) biscuits made using defatted soya flour by Serrem (2010). As expected, the commercial digestive whole-wheat biscuits used in this study had a relatively low moisture content of 4.2%, that is within the recommended range (2.5 - 4.5%) of moisture content for wheat short dough biscuits (Manley, 2000).

It is possible that moisture in biscuits made from dough rested for 24 h was bound to the sugar during resting. Hence, it would be less easily removed during baking (Manley, 2000). According to Manley (2011), dissolving sugar in water increases the liquid phase of the solution by at least 60%. In support of this hypothesis, Adedara (2016) observed an increase in softness of sorghum dough containing sugar pre-dissolved in water than undissolved sugar, but no significant differences in colour of sorghum biscuits made from the two treatments. This suggests that the sugar dissolving during the 24 h dough resting period could have increased the liquid volume of the solution, which affected biscuit moisture content.

The moisture content of biscuits made from dough rested for 24 h may also be attributed to the increase in water absorption and stability of the dough, as more water interacted with the soya protein during resting (Yasumatsu *et al*., 1972). As dough resting time increased from 15 min to 24 h, the mass (g), diameter, thickness and density of sorghum-soya biscuits also increased (Table 4.4). The increase in biscuit thickness with an increase in dough resting time may be attributed to the hydration of starch granules that occurred during dough resting, and the swelling of the hydrated granules during baking.

Polarised light microscopy confirmed that sorghum-soya biscuits made from dough rested for 24 h had a higher proportion of partial birefringence of starch granules (Figure 4.4), indicating partial gelatinisation of starch granules. These results could provide more evidence for the absorption of water into the amorphous rings of the starch granules during longer resting time of 24 h, through channel pores located on the surface of the granules (Gallant *et al*., 1997). Sorghum-soya biscuits made from dough rested for 15 min showed more non-birefringent starch granules, indicating that most starch granules remained intact and were not gelatinised during baking. Intact starch granules with Maltese crosses are birefringent (Kent, 1975). Similar observations on the crumb structure of sorghum biscuits have been reported by Adedara (2016) who reported a decrease in biscuit hardness with increase in the proportion of pre-cooked sorghum flour in the formulation. The author attributed this to an increase in starch gels and swollen starch granules, which made the biscuit matrix less cohesive.

Furthermore, biscuits made from dough rested for 24 h required nearly 32% less force to break (N), resulting in lower stress and higher strain compared to biscuits made from dough rested for 15 min (Table 4.4). This is probably due to the water acting as a plasticizer (Fennema *et al*., 2008), that reduced the rigidity of the biscuit matrix. The differences in biscuit thickness, of biscuits made from dough rested for 15 min and 24 h made it difficult for comparisons of biscuit texture properties such as stress and strain to be made. Results on biscuit moisture content with increase in dough resting time are similar to those of Piazza and Schiraldi (1997). The authors reported that wheat biscuits made from 120 min dough resting time had a higher moisture content in comparison to biscuits made from shorter resting times, resulting in loss of crispiness and reduced strength of biscuits. However, there was no difference in consumer liking for the texture of the biscuits with increase in dough resting time, as consumers reported that biscuits made with both treatments were grainy and hard.

Biscuits made from dough rested for 24 h were heavier and more dense than biscuits made from dough rested for 15 min, possibly because the biscuits were thicker (Table 4.4). In addition to the presence of swollen starch granules, the action of proteinase enzymes during sorghum-soya dough resting may have weakened the dough structure, resulting in a dense matrix that was unable to support the expansion of gas during baking. Carbon dioxide formed may have either escaped from dough during the longer resting time or during the initial stages of baking. In wheat doughs, proteinase enzymes weaken gluten as it becomes more extensible (Manley, 2000). Biscuits made from dough rested for 15 min had more air spaces which may have been caused by carbon dioxide production from baking powder during baking (Damodaran *et al*., 2008). Baking powder dissociates to produce leavening gases such as carbon dioxide that form air pockets and allow for dough expansion during dough preparation, thus contribute to an increase in the thickness of the product during baking (Damodaran *et al*., 2008).

Baking powder →carbon dioxide(g), sodium hydrogen carbonate(salt) and an acid (salt).

Wheat dough stiffness increased with dough resting time, suggesting that there may be structural changes such as cross-linking of polymer chains that took place during 120 min dough resting time (Piazza and Schiraldi, 1997).

Figure 4.3 showed that biscuits made from dough rested for 24 h were darker, with lower L* values (Table 4.3) compared to biscuits from dough rested for 15 min which were pale in colour. The colour/browning of baked products and biscuits is usually related to enzymatic or non-enzymatic Maillard reactions that also increase during baking at high temperatures (Leiva-Vanzuela *et al*., 2018). In addition to Maillard reactions, it is possible that the colour of biscuits made from dough rested for 24 h was also contributed by enzymatic reactions.

The enzyme active full-fat soya flour (used in this study) contains enzymes (amylases, proteases and lipoxygenase) that were possibly active in dough during the long dough resting (Figoni, 2011). During baking, these enzymes are thermally deactivated and denatured by the high oven temperatures (Fennema *et al*., 2008). It is likely that as dough resting time increased, the enzymatic activity in the dough also increased. Amylase enzymes hydrolyze starch into sugars such as maltose and glucose (Figoni, 2011). The proteinase enzymes present in soya flour may have hydrolyzed proteins into peptides and amino acids such as lysine (Figoni, 2011). However, baking reduces the nutritive value of foods containing soya (Snyder and

Kwon, 1987). Lysine is an essential amino acid abundant in soya, that reacts with sugars in a series of biochemical and chemical reactions to form brown pigments that contribute to colour changes, such as caramelisation and Maillard browning reactions (Coultate, 2002, Fennema *et al*., 2008). The small amounts of sugars and amino acids produced by starch and protein hydrolysis, respectively (Fennema *et al*., 2008) during 24 h dough resting may have been enough to contribute to more browning reactions.

Despite the instrumental colour differences between the biscuits, the colour of sorghum-soya biscuits made from dough rested for 15 min and 24 h were liked equally by consumers. Of interest, the colour of biscuits made from dough rested for 24 h were likened by the consumers to traditional cookies such as ginger biscuits that are commercially available in the market. Liking of the flavour and taste attributes were significantly different between the two treatments (Table 4.5). Biscuits made from dough rested for 24 h received lower liking scores for taste and overall flavour than biscuits made from dough rested for 15 min. The former was described as having a bitter aftertaste. Perceived bitterness may have been caused by the formation of bitter peptides during protein hydrolysis, or hydrolysis of phenolic compound dough during the long dough resting time. Bitterness has been reported in soya and sorghum products by other researchers. Bitterness in soya protein has been reported in soya protein isolate and hydrolysates by Meinlschmidt *et al*. (2016). Saha and Hayashi (2001) and Sun (2011) proposed that bitterness in soya is caused by low molecular, hydrophobic peptides that are formed after enzymatic hydrolysis.

Concerning sorghum, Omoba *et al.* (2015) found that addition of sourdough to red and white sorghum flour resulted in sorghum biscuits with a bitter taste and fermented aroma and flavour, caused by lactic acid action during fermentation. Kobue-Lekalake *et al*. (2007) reported that bitterness and astringency in sorghum bran infusion and sorghum rice were directly proportional to the total phenolic content present in sorghum. Increase in phenolic compounds during fermentation is attributed to either the release of bound phenolic compounds or hydrolysis of phenolic acid glycerol esters present in sorghum sourdough (Svensson *et al*., 2010).

Thermal analysis revealed two endotherm peaks for the sorghum-soya doughs rested for 15 min and 24 h (Figure 4.17). The endotherm peaks indicated starch gelatinisation (peak 1) and possibly denaturation of soya proteins (peak 2), at 98.9 °C - 117.5 °C and 123.9 °C - 139.9 °C, respectively. The thermal temperatures for sorghum-soya dough thermograms were not expected because endotherm peaks occurred at temperatures that had initially suggested the presence of type IIa or IIb amylose-lipid complexes. Amylose lipid type IIa and IIb complexes melt at temperatures in the range of 115 °C -125 °C (Tufvesson *et al*., 2003; Wang and Copeland, 2013). Thermal temperatures obtained for sorghum-soya dough could have been influenced by limited water available the in the dough, which shifted endotherms of starch gelatinisation and soya protein denaturation to higher temperatures (Fennema *et al*., 2008).

To investigate the effect of moisture content on dough properties, sorghum-soya (70:30) slurries were prepared under conditions of one-part solids and three parts moisture and rested for 15 min and 24 h. Sorghum-soya slurries prepared from both resting times had two endothermic peaks (Table 4.7 or Figure 4.18). Starch gelatinisation (peak 1) had endotherm temperature in the range of 64.3 °C - 80.4 °C and soya protein denaturation (peak 2) had endotherm temperature in the range 90.4 °C - 104 °C. Both endotherm peak 1 and peak 2 for slurries appeared at lower temperatures compared to sorghum-soya doughs, with the increase in dough resting time.

Under low or limiting moisture conditions, starch gelatinisation is initiated at higher temperatures because more energy is required for molecular mobility and melting of starch crystallites (Sopade *et al*., 2004, Baks *et al*., 2007). Concerning the effects on the soya proteins, of relevance is the findings by Kitabatake *et al*. (1990). The authors reported that reducing the moisture content of isolated 11S and 7S soya proteins from 94% to 47% resulted in an increase of endotherm temperatures from 93.9 °C to 149 °C and from 76.5 °C to 118.7 °C, respectively for their thermal denaturation. These results show that the low moisture conditions present in sorghum-soya dough were responsible for the shift in endothermic peaks of starch gelatinisation and possibly thermal denaturation of the soya proteins, to higher temperatures.

To investigate the thermal denaturation of the soya proteins; sorghum, soya and sorghum-soya (70:30) flours were mixed in the ratio of one-part flour to three-parts water. Sorghum flour only had one endotherm for starch gelatinisation with peak temperature at 70.7 °C (Figure 4.19), which was within the gelatinisation temperature range 68 °C - 78 °C for sorghum starch (Kulamarva *et al.*, 2009). Soya flour had two endotherm peaks in the ranges of 68.3 °C - 79.2 °C and 90.7 °C - 102.6 °C, which could be for denaturation of 7S and 11S soya proteins, respectively. The two endothermic peaks obtained for soya flour in this study are in agreement with finding of Sciarini *et al.* (2012) who reported two endotherms in enzyme active soya flour for denaturation of β-conglycinin (7S) and glycinin (11S) soya proteins, with temperature ranges of 85 °C – 93 °C and 106.5 °C - 116.7 °C, respectively. Similar to Sciarini *et al*. (2012), Li *et al*. (2014) also reported two endotherm peaks in soybean protein isolate, which they attributed to thermal denaturation of 7S and 11S proteins at 89.9 °C and 108.8 °C, respectively. There were no endotherm peaks for the soya proteins in the biscuit thermograms (Figure 4.20). This was presumably because the 7S and 11S proteins were denatured during baking, which took place at a temperature of 180 °C.

Sorghum-soya (70:30) flour also had two endotherm peaks, with the first peak in the range of 65.7 °C - 79.6 °C (Figure 4.19). This peak could be an overlapped endothermic peak of starch gelatinisation and denaturation of 7S soya proteins that occurred concurrently because sorghum flour had an endotherm with a peak temperature of 70.7 °C. The second peak was in the range 87.5 °C - 100.4 °C, for denaturation of 11S soya proteins (Sciarini *et al*., (2012).

Soya proteins denaturation in dough rested for 24 h had a higher enthalpy than dough rested for 15 min, suggesting that more energy was required to denaturing 11S proteins in the dough (Table 4.6). During longer dough resting, water may have been absorbed in the amorphous regions of the starch granules, enabling granule swelling or leaching out of amylose in some granules (Sopade *et al*., 2004). There may also have been some interaction or complex formation during the DSC scan between the free amylose molecules, with either short-chain fatty acids or proteins present in the dough. Melting of complexes requires more energy in order to break covalent bonds between the complexes (Delcour and Hoseney, 2010).

There were no significant differences in the thermal properties of sorghum-soya biscuits with increase in dough resting time, and each thermogram for biscuits had two endotherm peaks (Figure 4.20). The first endotherm peak was over the range of 52.5 °C - 63.5 °C, which could be due to lipids or fats present in soya flour. Soya oil contains saturated fatty acids such as myristic (C14:0), palmitic (C16:0) and stearic acid (C18:0) at levels of approximately 0.3%, 11.4% and 3.8%, respectively (Duodu and Apea-Bah, 2017). Myristic and palmitic acid have melting points of 53.2 °C and 63.1 °C respectively (Tufvesson et al., 2003), temperatures which coincided with endotherm peak temperatures of 58.3 °C - 65.7 °C and 54.4 °C - 62.6 °C, for soya flour and sorghum-soya flour thermogram, respectively (Figure 4.19). This suggests that endotherm peaks appearing below 65 °C were for melting of soya lipids. Abboud and Hoseney (1984) attributed endotherms between 25 °C and 55 °C to the melting of fats in DSC thermograms of wheat cookies.

Concerning starch digestibility, there were no significant differences in starch digestibility of the biscuits with an increase in dough resting time (Table 4.9). The estimated glycaemic index (EGI) for sorghum-soya biscuits was in the range of 47.1% - 47.5%, significantly lower than that of digestive whole-wheat and white bread. Similarly, Jiang *et al.* (2018) found that sorghum-soya chips had an estimated glycaemic index of 70.6%, 100% sorghum chips had a glycaemic index of 79.9% and sorghum-soya chips enriched with soya protein isolate had a glycaemic index of 59.8%. The authors attributed the decrease in the glycaemic index of chips with enrichment of soya flour and soy protein isolate to possible protein and starch interactions that reduced starch digestion.

The results for IVPD of sorghum-soya and digestive whole-wheat biscuits using multi-enzyme and pepsin methods gave contradicting results. Multi-enzyme protein digestion results of sorghum-soya biscuits were higher than those obtained using the pepsin digestion method. Similarly, Dovi (2014) reported higher results for *in vitro* protein digestibility using the multi-enzyme method than pepsin *in vitro* digestibility methods on sorghum-cowpea composite biscuits and flours. This could be because the multi-enzyme technique uses three enzymes (peptidase, trypsin and chymotrypsin) while pepsin method only uses one type of enzyme that is pepsin (Hsu *et al*., 1977). Trypsin and chymotrypsin preferentially hydrolyse protein polypeptide bonds with amino acids that contain positively charged groups and aromatic amino acids, respectively (Campbell, 2007). Peptidase hydrolyses proteins into protein polypeptides and amino acids (Caballero, 2005).

Sorghum-soya biscuits had a higher protein digestibility than digestive whole-wheat biscuits, using multi-enzyme method. This was expected because soya has a high protein content and its proteins are more digestible by enzymes than wheat proteins (Duodu and Apea-Bah, 2017). In contrast, the sorghum-soya biscuits had a lower pepsin digestibility than the wheat biscuits. Serrem (2010) used the pepsin *in vitro* digestibility method and reported protein digestibility of 74.3%, 81.1%, and 87.3% in sorghum-soya biscuits, complemented with 28.6%, 50.0% and 71.4% of defatted soya flour, respectively. Generally, sorghum has a lower *in vitro* protein digestibility (IVPD) in comparison to wheat and maize (Duodu *et al*., 2002). Duodu *et al.* (2002) had proposed that cooked sorghum protein is indigestible due to the formation of heat-induced disulphide cross-links that are not easily digested by enzymes. Regardless of the method used, there were no significant differences in protein digestibility of sorghum-soya biscuits with increase in dough resting time.

4.5 Conclusions

Biscuits made from 24 h dough resting time had higher moisture content, which could reduce the shelf life of biscuits. The outcomes of this study suggest that enzymatic action in sorghum-soya dough was more pronounced during 24 h dough resting time, contributing to differences in biscuit colour, taste and flavor between the two treatments. Biscuits made from both resting times were equally liked for biscuit texture, colour and appearance. Biscuits made from dough rested for 24 h received lower liking scores for taste and overall flavour and were commented as bitter and with an aftertaste. Increasing dough resting time to 24 h may have promoted hydration of some starch granules and partial gelatinisation during baking, resulting in thicker biscuits. A longer dough resting time did not necessarily improve the biscuits thermal properties and consumer acceptance, because consumers commented that biscuits made from both treatments were grainy. Consumers preferred biscuits made from dough rested for 15 min than 24 h dough. This is the first report to report a low EGI of the sorghum-soya biscuits, compared to wheat biscuits. The biscuits have potential to enhance satiety feelings and slower digestion in comparison to commercial wheat alternatives.

5 GENERAL DISCUSSION

The formulation for sorghum-soya biscuits used in this study was adapted from a formulation for sorghum-soya biscuits developed by Serrem (2010). Sunflower oil was partially replaced with baking margarine containing 18% moisture. Defatted soya flour was replaced with enzyme-active full-fat soya flour which also provided some of the oil. Table sugar was replaced with castor sugar. Dovi (2014) compared the effect of sunflower oil and creamed baking margarine on the textural properties of sorghum-cowpea (60:40) composite biscuits. Sorghum-cowpea biscuits made with baking margarine had a more open crumb structure and required less force to break compared to biscuits made with sunflower oil. Creaming enables entrapment of air bubbles in baking margarine and provides a framework for gas expansion during baking (Hui and Corke, 2006). Full-fat soya flour was used in this study because it made the sorghum-soya dough easier to roll into a continuous sheet without crumbling, unlike sorghum-defatted soya dough (personal observation). Substitution of defatted soya flour with full-fat soya flour led to the addition of less fat and water in the sorghum-soya dough formulation, on account of their presence in full-fat soya flour and baking margarine, respectively. In this study, full-fat soya flour contained 21 g / 100g of fat and baking margarine contained 18% of moisture.

Sugar is one of the main ingredients that contribute to sweetness, colour, and it also affects the texture of sorghum biscuits. The same amount of white sugar used by (Serrem, 2010) was replaced with castor sugar (by weight). Castor sugar was used as an ingredient because of its reduced granular size in comparison to white table sugar. Sugar with fine particles dissolves faster than coarse sugar and results in baked biscuits with a more uniform appearance (Hui and Corke, 2006).

After formulation optimisation for sorghum-soya biscuits, 100% sorghum biscuits could not be used as a control using the same formulation as sorghum-soya biscuits because the dough was batter-like, sticky and impossible to either roll or form into a dough (personal observation). Serrem (2010) reported adding less water in 100% sorghum dough compared to sorghum-soya and wheat-soya dough. This meant that the formulation would have to be adjusted and optimised specifically for 100% sorghum biscuits. Instead, sorghum-soya biscuits were compared to a commercially available digestive whole-wheat biscuits that were used as a control for some analyses. This is because digestive whole-wheat biscuits are classified under short dough biscuits, similar to biscuits made in this study.

A limitation of the study was that there were only two dough resting times (15 min and 24 h) were used. Biscuit treatments made were compared with each other or with commercial digestive whole-wheat biscuits in some analyses. It would have been beneficial to have more dough resting time intervals and measure the rheological changes in the dough that took place over the resting period.

Sorghum-soya biscuits made from dough rested for 24 h were thicker, with higher moisture content, mass and density than biscuits made from dough rested for 15 min. Dough functional properties such as water holding capacity during the 24 h dough resting time may have been influenced by changes in dough pH. There is a decrease in pH with natural fermentation of sorghum flour due primarily to the production of lactic acid (Yousif and El Tinay, 2001). For example, pH reductions of pH 5.7 to 3.7 (Yousif and El Tinay, 2001) and from pH 6.0 to 4.3 for sorghum flour supplemented with whey (Ibrahim *et al*., 2005) have been reported. Hugo *et al*. (2003) investigated the use of naturally fermented sorghum in composite wheat-sorghum bread making. They observed a decrease in pH of sorghum flour with fermentation from pH 6.2 to 3.4. Inclusion of the fermented sorghum resulted in an increase in dough viscosity and water-holding with the consequent increase in bread loaf volume and weight. Abd El-Moneim *et al*. (2015) also reported an increase in the water-holding capacity of sorghum flour fermentation, ranging from 98% to 110% for 3 different white sorghum varieties. Parameters such as water-holding capacity and pH of sorghum-soya dough after 15 min and 24 h intervals were unfortunately not measured in this study. It is possible that dough may have become softer and more extensible with resting due to the action of the hydrolytic enzymes.

Dough rested for 24 h was stored in a closed plastic container, at room temperature overnight. A room temperature of 30 °C was recorded while activities continued in the bakery during the day of baking, but the temperature fluctuations during the night time were not recorded. It was estimated to be minimum ± 17 ºC using data for the past forecast from AccuWeather (2019). In addition to the temperature conditions in the facility, heat energy may have been generated during dough mixing and increased the dough temperature (Hui and Corke, 2006), causing the sorghum-soya dough to become soft, sticky and difficult to roll, sheet or cut without sticking on the machinery (personal observation). For this reason, the dough was rested in the refrigerator at 4 °C for 15 min to make it more stiff, easier to roll and cut into dough pieces, without the dough sticking onto the rollers or cookie-cutter surfaces (personal observation). Dough rested for 24 h was stored in a closed plastic container, at room temperature overnight but temperature fluctuations in the dough for the duration of the long resting time were not recorded. After 24 h resting time, the dough was neither sticky nor difficult to roll, when compared to sorghum-soya dough immediately after dough mixing.

Enzyme active full-fat soya flour used in this study was not characterised for enzyme activity before it was used for baking. This was a disadvantage the rheological changes in dough during longer resting time are suspected to have been caused by enzymatic action in the dough. Ribotta *et al*. (2005) reported a nitrogen solubility index in the range of 82% - 95% for enzyme-active soya flours and 11.6% for heat-treated enzyme inactive soya flour. Heat treatment causes unfolding and denaturation of proteins in flour, thus reducing the solubility of proteins in soya (Hsu *et al*., 1977).

The available information on dough resting is mostly applicable to hard sweet biscuit types, where gluten development is desirable and causes some structural modification to take place in dough (Manley, 2000). In these formulations, dough is recommended to rest for approximately 30 minutes to allow for hydration of proteins and starch in flour, forming a gluten network (Manley, 2000). In short dough biscuits (as used in this study), gluten development is undesirable in order to produce biscuits that are not hard. Gluten development in short dough is minimised by the low water (8% - 35%) and high fat levels (12% - 50%) in a formulation, because fat coats flour particles during mixing and limits the hydration of proteins in the flour (Delcour and Hoseney, 2010). Dough temperature in the production of hard sweet biscuits is critical because it influences the behaviour of dough during handling or product quality (Manley, 2000). Warm wheat dough (approximately 27 °C) promotes hydration of flour, gluten development or yeast fermentation (Figoni, 2011).

The reduction in thickness of sorghum-soya dough was fast and achieved by using a pastry sheeter. Dough sheeting was done in three reduction steps by decreasing the gaps between the rolls from 12 mm, 9 mm, 7 mm to 4 mm, to produce even dough with uniform thickness before cutting. The conveyor belts on the pastry reversible sheeter were flexible and moved at different speeds, although in the same direction (personal observation). As a result of the high fat content in the sample, molten dough fat often accumulated on the space between the rolls and conveyer belt of the pastry causing slipping rather than roller grip. The sheeter had to be constantly cleaned to remove the fat build-up that accumulated. It is possible that the biscuit dough pieces from the two treatments were not the same thickness after sheeting (this was however not measured) due to the flexibility of the sheeter used during sheeting which may have caused variation in thickness of the sheeted dough. Pastry sheeters are preferably used for flattening or laminating pastry doughs containing solid fat, to produce pastry that is flaky (Tufvesson *et al*., 2003, Figoni, 2011). More efficient sheeting equipment for this specific dough type is required.

For oven selection, sorghum-soya biscuits were baked in a convection oven as well as a bread oven for trials. Biscuits baked in a Miwe-Condo convection oven required approximately 14 ± 2 min to bake, while biscuits baked in a bread deck oven required approximately 30 ± 5 min. Baking biscuits in the convection oven required less time compared to the bread oven because it had a steam outlet that allowed the escape of moisture evaporated from the surfaces of the biscuit during baking (Manley, 2000). The convection oven accommodated only one tray, whereas the bread oven could fit three trays placed next to each other on one oven deck. Each tray could fit approximately 80 cut dough pieces. Therefore, to reduce variability between biscuits made in different batches, the bread deck oven was selected because more trays could be fitted in the oven and biscuits could be baked at the same time.

Possible experimental errors during baking were inconsistent baking times between biscuit trays placed in the same deck oven, as well as between biscuits made from 15 min and 24 h dough resting times. For the trays placed next to the sides of the deck oven, unequal convection heat movement within the oven caused biscuits placed on the edge of the trays to develop colour quicker than biscuits that were on other positions within the same tray (personal observation). The tray in the middle of the oven was least affected with heat movements, as biscuits took longer to be baked (personal observation). To avoid inconsistencies of baking times between biscuits in trays placed in the same oven, trays positions were sometimes shuffled. After the estimated baking time (30 min) had elapsed, sufficient baking of biscuits was estimated by observing surface colouration, with an additional baking time of 5 minutes.

A limitation with the proximate analysis performed on the sorghum-soya biscuits in this study was that replicate analyses required for the calculation of standard deviations was not performed. This is because an internal control sample was included in each set of analyses. Information on the proximate composition for the commercial digestive whole-wheat biscuits was obtained from the packaging container. The recommended moisture content for short dough wheat biscuits is between 2% and 4% (Manley, 2000). Other researchers have reported higher moisture contents for sorghum and sorghum-composite biscuits. Red and white sorghum cookies had moisture content of 5.54% and 5.39%, respectively (De Petre *et al*., 2016). Serrem

(2010) reported moisture content of 3.2% and 4.9% for 100% sorghum and sorghum-soya (71.4: 28.6) biscuits, respectively. The average moisture content of sorghum and or sorghum-soya biscuits needs to be studied further. It would be recommended to include free water activity as analysis to measure the amount of water that is available in a product (Fennema *et al*., 2008).

The texture of biscuits was measured using a texture analyser. The calculation equations for stress and strain properties for biscuits were similar to those used by (Baltsavias *et al*., 1997). The equations were based on the assumptions for square or rectangular biscuits, as for uniform force distribution in the sample. However, the same equations were used for round sorghum-soya biscuits because no literature was found with equations that are applicable to round biscuits.

Dough rested for 15 min showed starch granules with visible Maltese crosses that appeared in clusters, showing that most starch granules were birefringent and remained intact after baking (Kent, 1975). Biscuits made from dough rested for 24 h had a lower proportion of starch granules with visible Maltese crosses, suggesting hydration of some starch granules during longer dough resting time. On the other hand, microscopy results for dough rested for 24 h microscopy were not in agreement with results obtained for thermal properties of sorghum dough and or biscuits with increase in resting time. There were no significant differences in starch gelatinisation temperatures for sorghum-soya biscuits. This suggests that although there was some starch hydration during longer dough resting, the low water present in the formulation, which was not sufficient to gelatinise most starch granules.

In vitro starch digestibility was used to estimate the glycaemic index of sorghum-soya biscuits. In this study, sorghum-soya biscuits had low EGI than for digestive whole-wheat and white bread. A potential target market for biscuits with a low GI are individuals who have diabetes. Most research has been done on sorghum and or wheat flours and porridges, products that are not comparable to the sorghum-soya biscuits produced in this study.

A consumer panel (n=50) was used to assess the consumer acceptability of sorghum-soya biscuits made from dough rested for two different times. Consumers commented that biscuits made from dough rested for 24 h had a bitter taste and an aftertaste. It is recommended that in the next stage of the project a descriptive panel is used to provide descriptive profiles and to rate the intensity of each attribute, while a consumer panel would be used to determine acceptability of the biscuits. 100% sorghum biscuits and 100% soya biscuits (made with enzyme active soya flour) should be added to the study as controls, in order to determine the cause of bitter after taste in biscuits with longer dough resting time. Saha and Hayashi (2001) and Sun (2011) proposed that bitterness in soya is caused by the formation of bitter peptides after enzymatic hydrolysis of proteins. Bitterness has also been reported in whole grain sorghum-soya biscuits with sourdough addition by Omoba *et al*., (2015). It was difficult to the precise the cause of bitter taste in sorghum-soya biscuits with a longer dough resting time, as it may have been either from the sorghum and soya flours used, or caused by other reactions that took place in dough during the longer resting time.

The paired preference test that was applied, was a forced-choice test with two samples, where consumers only had to select the sample that they preferred (Lawless and Heymann, 2013). Unlike the 9-point hedonic scale, there was no scale provided for the preference question, which did not give any further information on the size of the preference by consumers. There was a comment section provided after the attribute and preferences question, with the aim of consumers providing more information on the motivation of consumer's selection of responses on the attribute. The heading before each attribute and preference comment was phrased as 'Any comment on biscuit sample ABC?' and 'Any comment?', which suggested non-directional comments expected from consumers.

Table 5.1 has a summary of results, showing the effect of dough resting time (15 min and 24 h) on the physicochemical properties of dough and/or biscuits, as well as the consumer acceptance of sorghum-soya biscuits.

Table 5.1 Summary of results showing the effect of dough resting time (15 min; 24 h) on physicochemical properties or sorghum-soya (70:30) dough and/or biscuits, and consumer acceptability.

	Dough resting time		
	15 min	24 h	Possible reasons
Proximate analysis	Lower biscuit moisture content (5%)	Moisture content of biscuit (6.5%) increased by 30%	- Differences in sheeting thickness due to the dough handling properties of the biscuits. - Inconsistent baking times between biscuits made from 15 min and 24 h dough resting times.
Instrumental colour of biscuits L*- lightness a* - redness b* - yellowness	Higher L* value Higher a* value no significant differences in b* value	Lower L* (darker) Lower a* value no significant differences in b* value	- With longer resting time, more enzymatic action of amylase and proteinase enzymes on starch and proteins in dough respectively, producing reducing sugars and free amino acids respectively, that took part in browning reactions such as caramelisation or Maillard browning.
Instrumental texture	Higher force to break (N) Higher Stress (kPa) Lower strain (%)	Lower force to break (N) Lower stress (kPa) Higher strain (%)	- Biscuits made from dough rested for 24 h had higher moisture content than biscuits made from dough rested for 15 min. The water acted as a plasticizer, reducing rigidity of biscuit matrix.
Biscuit physical quality	Lower thickness Lower mass	Higher thickness Higher mass	- Hydration of some starch granules during 24 h dough resting time. - Partial starch gelatinization of hydrated starch granules during baking. - Increase in biscuit thickness with increase in dough resting time.
Biscuit physical quality	Lower biscuit density	Higher biscuit density	- Presence of swollen starch granules and starch gels in dough rested for 24 h. - Proteinase enzymes may have weakened dough structure during longer resting time, resulting in a matrix that was not able to support gas expansion during baking. - Baking powder may have acted as aerating agent during 15 h dough resting.

Table 5.2 . (continued)

	Dough resting time		Possible reasons
	15 min	24 h	
Consumer acceptance	Equal liking for biscuit colour, texture and appearance	Equal liking for biscuit colour, texture and appearance	- Consumers related the colour of sorghum-soya biscuits to commercially available ginger and digestive whole-wheat biscuits, available in the South African markets. - Biscuits from both resting times were grainy.
	Higher biscuit liking scores for taste and overall flavour Biscuits were preferred more.	Lower biscuit liking scores for taste and overall flavour	- Perceived bitter taste and aftertaste in biscuits made from 24 h resting time. Bitterness may have been caused by the formation of hydrophobic bitter peptides during enzymatic hydrolysis of proteins in the dough, during longer dough resting time. - Enzymatic action on proteins may have influenced flavour profiles of biscuits.
Thermal properties Dough	No significant difference		- No significant difference with increase in dough resting time.
Biscuits	No significant difference		- No significant difference with increase in dough resting time
Extent of starch gelatinisation	More intact starch granules shown by a higher presence of Maltese crosses, indicating limited starch gelatinisation on dough rested for 15 min	Lower proportion of Maltese crosses, showing more starch gelatinisation	- Low water content used in biscuit formulation was insufficient to gelatinise all starch granules - Water hydration of some starch granules during longer dough resting. - Increase in water holding capacity with longer dough resting time, influenced by rheological changes such as decrease in pH.
***In vitro* protein digestibility**	No significant difference		- No significant difference with dough resting

6 CONCLUSIONS AND RECOMMENDATIONS

Previously sorghum and sorghum-legume composite biscuits have been described by researchers as being gritty and hard. These aspects were considered as reasons for the products receiving relatively low scores for consumer acceptability. Perceived grittiness is attributed to be as a result of limited hydration of sorghum starch granules during dough mixing due to hydrophobic protein bodies that surround the starch granules. In this study, it was hypothesized that a longer dough resting time (24 h) will promote hydration and swelling of starch granules in the sorghum flour. Application of heat during baking will enable further granule swelling and starch gelatinization to occur more optimally in the 24 h rested dough compared to 15 min rested dough, thereby reducing the perceived grittiness of the biscuits and improving consumer acceptability scores of the biscuits.

Increasing dough resting time to 24 h produced thicker biscuits with 30% higher moisture content, lower stress and required less force to break, compared to biscuits made with dough rested for 15 min. This suggests that the high biscuit moisture content may have acted as a plasticizer that reduced the rigidity in the biscuit matrix. Thickness may be explained by the aerating effect of baking powder producing leavening gases during dough mixing and baking, given the longer time to act in the dough during dough resting.

Enzymatic action of proteinase and amylases on proteins and starch, respectively, in sorghum-soya dough could have been more pronounced during 24 h dough resting time, resulting in more browning reactions to occur in biscuits. Protein hydrolysis during longer dough resting may be responsible for production of bitter peptides, that were perceived by consumers as a bitter taste in biscuits. Despite significant differences in instrumental texture and colour results, sorghum-soya biscuits made from dough rested for 15 min and 24 h were equally liked for colour, texture and appearance by consumers. Biscuits made from dough rested for 15 min received higher liking scores for taste and overall flavour,and were more preferred than biscuits made from dough rested for 24 h.

Sorghum-soya biscuits have lower starch digestibility compared to commercial digestive whole-wheat biscuits and white bread. There were no significant differences between thermal properties of sorghum-soya dough and/or biscuits as well as *in vitro* protein and *in vitro* starch digestibility of the biscuits with an increase in dough resting time. This is the first report on thermal properties of sorghum-soya dough and EGI of sorghum-soya biscuit. Increasing the dough resting time of the sorghum-soya biscuit formulation seems to may have promoted hydration and gelatinization in some starch granules. This made the biscuits less hard, and the treatment did not have a positive impact on the flavour of the biscuits. Consumers preferred the flavour of the biscuits from dough rested for 15 min. A longer dough resting did not contribute to starch gelatinization of sorghum-soya dough between treatments. For this reason, it is recommended to not rest the sorghum-soya biscuit dough for 24 h. The behaviour of gluten-free composite dough such as sorghum-soya dough, using enzyme active or enzyme inactive soya flours, at constant dough resting temperatures need to be studied further.

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8 APPENDICES

Appendix 1 Screening questionnaire

Dear Sir/Madam

The Consumer and Food Sciences Department of the University of Pretoria is conducting a sensory tasting session on sorghum-soya biscuits. As a consumer, you play a vital role in product development. We are interested in your opinion about the taste of biscuits. Our objective is to determine consumer liking and preference of biscuits.

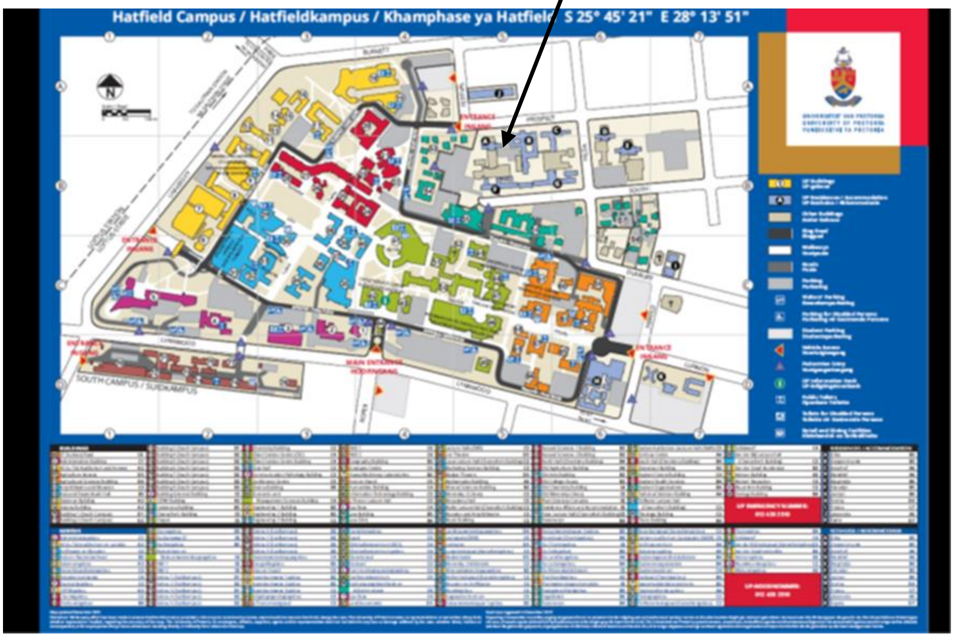
The tasting session will take approximately 20-30 minutes. Participants will taste a total of 2 biscuit samples and answer a few questions. As an incentive, each participant will receive a gift voucher worth R25 on completion of the test.

Date: Thursday 17 May 2018

Venue: Sensory Lab, Room 2-6 Old Agricultural Building.

Time: 10:30am, 11:30am, 12:30pm

Building 20: Sensory Evaluation on laboratory. Department of Consumer and Food Sciences



Kind Regards

Josephine Baloyi (MSc Food Science)

Old Agriculture Building Room 2-

University of Pretoria
PRETORIA 0002
South Africa

Tel: Number +27 (0)12 420 3238

Fax: Number +27 (0)12 420 2839

Email address: riette.dekock@up.ac.za

www.up.ac.za

To determine if you qualify for the tasting, please answer the questions below.

1. On average, how many times per week do you consume biscuits? *Listen to the answer*

At least once a week	
2-4 times a week	
Never	Terminate

2. Do you have any food allergies?

No	
Yes	Terminate interview

3. The product that you will be asked to taste contains an allergen soya, as one of the ingredients. Are you willing to taste the products?

Yes	
No	Terminate interview

4. Please indicate (tick) your preferred timeslot.

10:30 am	11:30 am	12:30 pm

Thank you, qualify for the tasting session. Kindly may you provide your name, surname and contact details. This information will be treated confidentially by the researchers.

Name	
Email	
Phone No.	

Interviewer section: I ………………………………………declare that I have screened the respondent and that the above information is correct and valid to the best of my knowledge.

Signature: ………………………. **Date: ……………………………**

Appendix 2 Consent Form

Student Researcher: Josephine Baloyi Contact number: +27 797121743 Email: u12134695@tuks.co.za	Supervisor: H.L de Kock Associate Professor E-mail: riette.dekock@up.ac.za
Department of Consumer and Food Science, University of Pretoria Private Bag X20 Hatfield, Pretoria 0028, South Africa Tel:+27 12 420 3238 Fax:+27 12 420 2839	

This is a consent form for research participation. It contains important information about this study and what to expect if you decide to participate. Please consider the information carefully. Feel free to ask questions before making your decision on whether or not to participate. If you decide to participate, you will be asked to sign this form. Participation in this study is voluntary.

Purpose: The purpose of this project is to get information on consumer preference and liking for sorghum biscuits.

Procedures/Tasks: You will be presented with two biscuits in total, asked to taste and answer a few questions.

Duration: This study will take approximately 20-30 minutes to complete.

DISCLAIMER:

The University of Pretoria, nor any of its representatives, cannot be held responsible in the unlikely event of any injury or illness as a direct or indirect result of your participation in this project.

CONTACT INFORMATION:

You can contact the Principal Investigator at the number listed on the front page if you have any questions about this study.

Name of participant (print)	Signature of participant	Date
Signature of Supervisor	Signature of Student Researcher	

Appendix 3 Sensory Evaluation questionnaire

Welcome to the sensory evaluation of sorghum biscuits and thank you for your willingness to participate.

By clicking the NEXT button to begin, you acknowledge and agree that you fully understand the conditions of participation and consent to participate. If you have any questions, please do not hesitate to ask any of the assistants or contact the project supervisor Prof Riette de Kock - Tel: 012 420 3238.

Tray number

Please enter your tray number

Tray Number confirmation

Please confirm if you have received the tray number shown below:

123

Instructions:

1. You are provided with two coded biscuit samples. Taste the biscuits in the order given, from left to right.
2. Remember to rinse your mouth before and in-between tasting of the different samples.

- Please ensure that you have received the correct samples and their codes matches the ones shown on the screen.
- For the purpose of concentration, it is recommended that you REFRAIN from using your mobile devices and / chatting during food evaluation session Please ask for assistance if you do not
- understand anything.

How much do you like or dislike the colour of biscuit BC111?

- [ ] Like Extremely
- [ ] Like Very Much
- [ ] Like Moderately
- [ ] Like Slightly
- [ ] Neither Like nor Dislike
- [ ] Dislike Slightly
- [ ] Dislike Moderately Dislike
- [ ] Very Much
- [ ] Dislike Extremely

Any comments on biscuit sample BC111
Sample: BC111

How much do you like or dislike the appearance of biscuit BC111?

- [ ] Like Extremely
- [ ] Like Very Much
- [ ] Like Moderately
- [ ] Like Slightly
- [ ] Neither Like nor Dislike
- [ ] Dislike Slightly
- [ ] Dislike Moderately
- [ ] Dislike Very Much
- [ ] Dislike Extremely

Any comments on biscuit sample BC111

Sample: BC111

How much do you like or dislike the texture of biscuit BC111?

- Like Extremely
- Like Very Much
- Like Moderately
- Like Slightly
- Neither Like nor Dislike
- Dislike Slightly
- Dislike Moderately
- Dislike Very Much
- Dislike Extremely

Any comments on biscuit sample BC111
Sample: BC111

How much do you like or dislike the taste of biscuit BC111?

- Like Extremely
- Like Very Much
- Like Moderately
- Like Slightly
- Neither Like nor Dislike
- Dislike Slightly
- Dislike Moderately
- Dislike Very Much
- Dislike Extremely

Any comments on biscuit sample BC111

Sample: BC111

How much do you like or dislike the overall flavour of biscuit BC111?

- [ ] Like Extremely
- [ ] Like Very Much
- [ ] Like Moderately
- [ ] Like Slightly
- [ ] Neither Like nor Dislike
- [ ] Dislike Slightly
- [ ] Dislike Moderately
- [ ] Dislike Very Much
- [ ] Dislike Extremely

Any comments on the biscuit sample BC111?

Which sample do you prefer?

BC111	BC222

Any comment?

Sample: BC111

Please indicate your gender

- ○ Male
- ○ Female

Please indicate your age

- ○ 18-25
- ○ 26-34
- ○ 35-44
- ○ above 44

Thank you for completing this test!

Finished | Back

Appendix 4 Conference Presentations:

Baloyi, J. T., Emmambux M.N., Taylor, J. R. N., and de Kock H.L. 2019. Effect of dough resting time on physical properties and consumer acceptance of sorghum-soya biscuits. Poster presentation at SAAFOST Congress 2019, Food Science and Technology for the 21st Century, Birchwood Hotel and Conference Centre, Johannesburg, South Africa, 1 – 4 September 2019

Baloyi, J. T., Emmambux M.N., Taylor, J. R. N., and de Kock H.L. 2018. The effect of dough resting time on thermal properties and texture or sorghum-soya (70:30) dough and biscuits. Poster presentation at The International Sorghum Conference, Sorghum in the 21st Century. Century City Conference Centre, Cape Town, South Africa, 9 - 12 April 2018