EFFECT OF SORGHUM FLOUR SUBSTITUTION ON PASTING BEHAVIOR OF WHEAT FLOUR AND APPLICATION OF COMPOSITE FLOUR IN BREAD

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ผลของการแทนที่ด้วยแป้งข้าวฟ่างต่อพฤติกรรมการเกิดเพสต์ของแป้งสาลีและการประยุกต์แป้งเชิงประกอบในขนมปัง

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ยูนิส มุน มูมา : ผลของการแทนที่ด้วยแป้งข้าวฟ่างต่อพฤติกรรมการเกิดเพสต์ของแป้งสาลีและการประยุกต์แป้งเชิงประกอบในขนมปัง. (EFFECT OF SORGHUM FLOUR SUBSTITUTION ON PASTING BEHAVIOR OF WHEAT FLOUR AND APPLICATION OF COMPOSITE FLOUR IN BREAD) อ.ที่ปรึกษาหลัก : ธนจันทร์ มหาวนิช, อ.ที่ปรึกษาร่วม : ศิริมา พ่วงประพันธ์

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งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของการแทนที่แป้งสาลีด้วยแป้งข้าวฟ่างต่อสมบัติการเกิดเพสต์และสมบัติทางความร้อนของแป้งเชิงประกอบและความแน่นเนื้อของเจล รวมทั้งเพื่อศึกษาการประยุกต์แป้งเชิงประกอบในขนมปัง อัตราส่วนของแป้งสาลีต่อแป้งข้าวฟ่างที่ใช้ในงานวิจัยนี้ได้แก่ 100:0 (wheat control), 0:100 (sorghum control), 80:20, 60:40, 40:60 และ 20:80 โดยใช้ตัวอย่าง wheat control เป็นเกณฑ์มาตรฐาน แป้งสาลีและแป้งข้าวฟ่างมีปริมาณความชื้นประมาณ 10% แป้งข้าวฟ่างมีปริมาณโปรตีนหยาบเท่ากับ 14.90% ซึ่งต่ำกว่าแป้งสาลี (16.91%) แป้งข้าวฟ่างมีปริมาณสตาร์ชเท่ากับ 60.19% (โดยมีปริมาณแอมิโลสเท่ากับ 14.88%) ในขณะที่แป้งสาลีมีปริมาณสตาร์ชเท่ากับ 73.03% (โดยมีปริมาณแอมิโลสเท่ากับ 23.87%) แป้งข้าวฟ่างมีปริมาณไขมันหยาบและเส้นใยหยาบเท่ากับ 1.76% และ 2.27% ตามลำดับ ซึ่งมีปริมาณที่สูงกว่าแป้งสาลี สมบัติการเกิดเพสต์ที่ศึกษาโดยใช้ Rapid Visco Analyzer แสดงให้เห็นว่า peak viscosity มีค่าลดลงอย่างมีนัยสำคัญ ($p \leq 0.05$) เมื่ออัตราส่วนของแป้งข้าวฟ่างเพิ่มขึ้น จาก 1538.33 cP ใน wheat control เป็น 1265.33 cP ในแป้งผสมที่มีอัตราส่วนของแป้งสาลีต่อแป้งข้าวฟ่างเท่ากับ 20:80 ตัวอย่าง sorghum control มีค่า breakdown viscosity (400.33 cP) ต่ำกว่า wheat control (716.33 cP) อย่างมีนัยสำคัญ มีผลให้ตัวอย่างแป้งเชิงประกอบมี breakdown viscosity ลดลงเมื่ออัตราส่วนของแป้งข้าวฟ่างเพิ่มสูงขึ้น ในแง่แป้งเชิงประกอบพบว่าตัวอย่างที่มีอัตราส่วนของแป้งสาลีต่อแป้งข้าวฟ่างเท่ากับ 20:80 มี final viscosity สูงที่สุด และ final viscosity มีค่าลดลงเมื่ออัตราส่วนของแป้งสาลีเพิ่มขึ้น สำหรับ setback viscosity ตัวอย่าง sorghum control มีค่า setback viscosity (1478.67 cP) สูงกว่า wheat control (1108.00 cP) ค่า setback viscosity ของแป้งเชิงประกอบจึงมีค่าเพิ่มขึ้นเมื่ออัตราส่วนของแป้งข้าวฟ่างเพิ่มขึ้น สำหรับการเกิดเจลาทิไนเซชันของสตาร์ช พบว่าแป้งเชิงประกอบทุกตัวอย่างมีอุณหภูมิเจลาทิไนเซชันและเอนทาลปีของการเกิดเจลาทิไนเซชัน (DH_G) ใกล้เคียงกับ wheat control ในแง่การเกิดรีโทรเกรเดชันพบการเพิ่มขึ้นของเอนทาลปีของการหลอมเหลวผลึกแอมิโลเพกทิน (DH_R) ในทุกตัวอย่างโดยพบในปริมาณที่ต่างกัน โดยตัวอย่างที่มีอัตราส่วนของแป้งสาลีต่อแป้งข้าวฟ่างเท่ากับ 20:80 มีการเพิ่มขึ้นของ DH_R ต่ำที่สุดในระหว่างการเก็บรักษา สำหรับความแน่นเนื้อของเจล เจลแป้งข้าวฟ่างที่เตรียมใหม่ๆ มีความแน่นเนื้อของเจลสูงกว่าเจลแป้งสาลี ความแน่นเนื้อของเจลแป้งเชิงประกอบจึงมีค่าเพิ่มขึ้นเมื่ออัตราส่วนของแป้งข้าวฟ่างเพิ่มขึ้น ในด้านสมบัติของขนมปัง พบว่าตัวอย่างขนมปังมีปริมาณความชื้นและวอเตอร์แอกทิวิตีลดลงเมื่อระยะเวลาการเก็บรักษาเพิ่มขึ้น ปริมาตรจำเพาะของก้อนขนมปังมีค่าลดลงเมื่ออัตราส่วนของแป้งข้าวฟ่างเพิ่มขึ้น ตัวอย่างขนมปังที่ทดแทนด้วยแป้งข้าวฟ่างพบว่ามีเซลล์อากาศที่มีรูปร่างและขนาดที่ไม่สม่ำเสมอ สำหรับสมบัติด้านเนื้อสัมผัส พบว่าขนมปังมีค่า hardness, gumminess และ chewiness เพิ่มขึ้นเมื่ออัตราส่วนของแป้งข้าวฟ่างและระยะเวลาการเก็บรักษาเพิ่มขึ้น ปริมาณสตาร์ชที่ละลายน้ำได้มีค่าลดลงจาก 6.14% ในขนมปังจากแป้งสาลีเป็น 1.38% ในขนมปังจากแป้งเชิงประกอบที่มีอัตราส่วน 20:80 สำหรับสมบัติด้านสีพบว่าเปลือกนอกและเนื้อในของขนมปังมีสีเข้มขึ้นเมื่ออัตราส่วนของแป้งข้าวฟ่างเพิ่มขึ้น จากงานวิจัยนี้พบว่าแป้งเชิงประกอบจากแป้งสาลีและแป้งข้าวฟ่างสามารถใช้เพื่อทดแทนแป้งสาลี 100% โดยสามารถใช้ได้จนถึงระดับการแทนที่ 40% ในพื้นที่ที่ข้าวฟ่างสามารถปลูกได้และมีราคาที่ต่ำ การใช้แป้งข้าวฟ่างทดแทนแป้งสาลีบางส่วนจึงช่วยลดปริมาณการนำเข้าของข้าวสาลีได้

สาขาวิชา	วิทยาศาสตร์และเทคโนโลยีทางอาหาร	ลายมือชื่อนิสิต ....
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Eunice Muute Muema : EFFECT OF SORGHUM FLOUR SUBSTITUTION ON PASTING BEHAVIOR OF WHEAT FLOUR AND APPLICATION OF COMPOSITE FLOUR IN BREAD. Advisor: Asst. Prof. THANACHAN MAHAWANICH Co-advisor: Asst. Prof. SIRIMA PUANGPRAPHANT

The objective of this study was to investigate the effect of sorghum flour substitution to wheat flour on pasting and thermal properties of the composite flours as well as firmness of the flour gels and application of composite flour in pan bread. Wheat-to-sorghum flour ratio used were 100:0 (wheat control), 0:100 (sorghum control), 80:20, 60:40, 40:60 and 20:80, with the wheat control serving as a benchmark. Both flours had approximately 10% moisture content. Crude protein content of sorghum flour was 14.90%, which was lower than that of wheat flour (16.91%). Sorghum flour contained 60.19% starch (with 14.88% amylose) as compared to wheat flour which contained 73.03% starch (with 23.87% amylose). Crude fat and crude fiber contents of sorghum flour were 1.76% and 2.27%, respectively, which were higher than those of wheat flour. Pasting properties as monitored using a Rapid Visco Analyzer showed a significant decrease ($p \leq 0.05$) in peak viscosity with increasing level of sorghum substitution from 1538.33 cP in wheat control to 1265.33 cP in the 20:80 composite. Sorghum control demonstrated significantly lower breakdown (400.33 cP) than wheat control (716.33 cP) and this led to a progressive decrease in breakdown of the wheat-sorghum composites. Among the composite flours, the 20:80 blend exhibited the greatest final viscosity of which the value decreased with increasing ratio of wheat flour. In terms of setback, sorghum control was higher in setback (1478.67 cP) as compared to wheat control (1108.00 cP). Setback of the wheat-sorghum composites, therefore, increased with increasing sorghum proportion. Regarding starch gelatinization, all wheat-sorghum composites had similar temperature and enthalpy of gelatinization (DH_G) to those of wheat control. As to retrogradation, an increase in enthalpy for melting of amylopectin crystallites (DH_R) was observed in all samples but at a different degree, with the 20:80 composite revealed the smallest increase in DH_R with increasing storage time. In the matter of flour gel firmness, freshly prepared sorghum gel was of higher firmness than wheat gel. Gel firmness of the wheat-sorghum composites correspondingly increased with increasing sorghum ratio. For the bread properties, moisture content and water activity of the bread samples were found to decrease with increasing storage time. Specific loaf volume decreased with increasing sorghum flour substitution. Pertaining to the air cells, it was found that there was increasing irregularity in cell size and shape upon substitution with sorghum flour. For crumb texture, hardness, gumminess, chewiness increased with increasing sorghum ratio and storage time. Water-soluble starch content decreased from 6.14% in wheat bread to 1.38% in the 20:80 composite bread. Color of crust and crumb progressively became darker as the level of sorghum flour substitution increased. Wheat-sorghum composite is a viable alternative to 100% wheat flour at levels up to 40% substitution. In areas that sorghum is locally grown and of lower price, this could reduce the volume of wheat importation.

Field of Study:	Food Science and Technology	Student's Signature ....
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		Co-advisor's Signature ....

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CHAPTER 1
INTRODUCTION

The absolute number of people in the world affected by undernourishment, or chronic food deprivation, is estimated to nearly 690 million or 8.9% of the world population in 2019 (FAO, 2020). The number of people affected by severe food insecurity, which is another measure that approximates hunger, shows a similar upward trend. In 2019, close to 750 million or nearly one in ten people in the world were exposed to severe levels of food insecurity. The situation is worsening in South America and most regions of Africa. The prevalence of undernourishment in Africa was 19.1% of the population in 2019. This prevalence is more than twice the world average (8.9%) and is the highest among all regions. Without increased efforts, there is a risk of falling far short of achieving the sustainable development goals (SDG) of hunger eradication by 2030. The world is not on track to achieve Zero Hunger by 2030. If recent trends continue, the number of people affected by hunger would surpass 840 million by 2030 (FAO, 2020).

About 234.7 million people in Sub-Saharan Africa are suffering from chronic undernutrition (FAO, 2020). Kenya suffers from a level of hunger which is classified as ‘serious’ with about 3.4 million Kenyans being severely food insecure. One in every three Kenyans is grappling with severe food insecurity and poor nutrition. In Kenya, food consumption is outpacing food production (Welborn, 2018). Food security in Kenya thus remains a distant goal with country heavily dependent on food imports to bridge the deficits.

Wheat is the second most important cereal in Kenya after maize and contributes substantially to food security, poverty reduction and employment creation. The national demand for wheat and wheat products is on the increase, this is due to high population growth, increased urbanization, and related changing trends in food consumption patterns. However, the local wheat production has not been able to meet this demand leading to importation of large quantities to plug the gap between supply and demand (KALRO, 2016).

Demand for wheat-based products, particularly in developing countries, has been on the rise. In Kenya, wheat provides 50.4 % of dietary needs and this is likely to even increase as the demand for wheat bread escalates due to growing urbanization, population growth, rising incomes and changing consumer preferences and tastes (Mariera et al., 2017). Despite this, the supply of wheat has and is likely to remain on the decline creating a deficit which must be bridged through importation. This has made bread and other baked products from wheat to be expensive.

Due to the increased costs associated with imported wheat, the use of other flours from locally grown crops which are cheaper is being investigated for their appropriateness in substitution to wheat. Such crops include maize, sweet potato, cassava, millet, and sorghum. Out of these, sorghum has potential to be used as a substitute to wheat in terms of agronomic aspects and flour properties (Wambua et al., 2016). (Adebowale et al., 2012) reported that sorghum flour has a neutral smell and blends well with wheat. However, characteristics of bread is inevitably affected by substitution of wheat with other flours (Abdelghafor et al., 2011) .

The objective of this study was therefore to assess pasting and thermal properties as related to gelatinization and retrogradation of wheat, sorghum flour and their composites as well as baking and keeping quality of bread prepared from the composite flours.

CHAPTER 2
LITERATURE REVIEW

2.1 Sorghum

Sorghum is a monocotyledonous plant that belongs to the Gramineae (Poaceae) family, Panicoideae subfamily and Andropogoneae tribe (de Morais Cardoso et al., 2017). Two of the best-known species are *Sorghum vulgare* and *Sorghum bicolor* (L.) Moench. Sorghum is called great millet and guinea corn in West Africa, kafir corn in South Africa, *dura* in Sudan, *mtama* in East Africa, *jowar* in India, *kaoliang* in China, milo or milo-maize in the USA, and *khaofang* in Thailand. Sorghum kernels are typically round varying in weight from 3 to 80 mg.

Sorghum is highly tolerant to drought and able to withstand periods of water-logging. The crop is characterized by an extensive root system, waxy bloom on leaves that reduces water loss, ability to stop growth during drought and resume it when the stress is relieved, and C4 photosynthesis. As a result, sorghum crop can survive the harsh climatic conditions of the arid environments. It has ability to maintain relatively high levels of stomatal conductance, maintenance of internal tissue water potential through osmotic adjustment and phenological plasticity. The crop grows well in areas between 500 and 1700 m above sea level, with seasonal rainfall of 300 mm and above (Abdelhalim et al., 2019; Hadebe et al., 2017; Muui et al., 2013).

2.1.1 Grain structure

2.1.1.1 Kernel

The kernel is considered a naked caryopsis, typically 2-5 mm in length and 2-3 mm thick at the widest point. Due to genetic diversity, the grains vary widely in color, shape and size. Kernel color varies from white or yellow to red, whereas the endosperm color can be yellow or white (Mutahi, 2012). The grain is made up of a pericarp, endosperm or storage tissue and germ or embryo.

2.1.1.2 Pericarp

The pericarp region comprises a pericarp, testa and aleurone layer. In most sorghum varieties, the pericarp is the thick outer layer consisting of three layers, which are epicarp, mesocarp and endocarp. Pericarp thickness ranges from 8 to 160 μm and varies within an individual kernel. The sections below the style and hilum are the thickest and the sides of the kernel are the thinnest. The outermost layer or epicarp is usually covered with a thin protective layer of wax. The epicarp is two to three layers thick and is made of rectangular cells often containing pigmented material. Sorghum is unique in that it is the only cereal grain that has starch granules in the pericarp. Sorghum with thick pericarp, contains three to four mesocarp cell layers filled with small starch granules. The inner pericarp tissue or endocarp, is composed of cross and tube cells (Bianchi, 2013).

2.1.1.3 Testa

The pigmented inner integument, also known as testa layer or seed coat, may or may not be present in mature sorghum caryopsis based on genetic factors. The testa separates the pericarp from the aleurone layer. The pigmentation is due to the presence of condensed tannins in the testa layer. These molecules affect agronomic profile of the crop as well as end-use of the grain. For instance, high concentration of tannins results in greater pest resistance, especially against birds, but this lowers protein digestibility. The testa is thin in low tannin sorghum varieties but thicker and highly pigmented in high tannin sorghums (Belton & Taylor, 2002).

2.1.1.4 Endosperm

Like most cereal grains, sorghum endosperm is essentially a storage tissue. This storage organ is an assembly of the aleurone layer, peripheral, floury, and corneous regions. The aleurone cells are the outer cover of the endosperm consisting of a single layer of rectangular cells adjacent to the testa or tube cells. The outer edge of the endosperm is composed of the aleurone layer containing lipids, enzymes and protein bodies. Under the aleurone layer is the outer corneous endosperm fraction which is a hard, homy, vitreous layer surrounding an inner floury and soft core. The outer corneous endosperm is tightly packed with starch bodies covered with a continuous protein matrix, whereas the floury endosperm, in the centre of the kernel, is loosely packed with a discontinuous protein matrix and round starch granules (Mutahi, 2012).

The peripheral area is composed of many layers of dense cells containing more protein and smaller starch granules than the corneous area. Both the peripheral and corneous areas appear translucent, or vitreous, and they affect processing and nutrient digestibility. Waxy sorghums contain larger starch granules and less protein in the peripheral endosperm than regular sorghums (Bianchi, 2013).

The corneous and floury endosperm cells are composed of starch granules, protein matrix, protein bodies, and cell walls rich in cellulose, β-glucans, and hemicellulose. Starch granules and protein bodies are embedded in the continuous protein matrix in the peripheral and corneous areas. The starch granules are polygonal and often contain dents from the protein bodies. Their size varies from 4 to 25 μm, with the average being 15 μm. Granules present in the corneous endosperm are smaller and angular whereas those in the floury endosperm are larger and spherical. The opaque, floury endosperm is located near the center of the caryopsis. It has a discontinuous protein phase, air voids, and loosely packed, round and lenticular shaped starch granules (Bianchi, 2013). The presence of air voids diffracts incoming light, giving the floury endosperm an opaque or chalky appearance.

2.1.1.5 Germ

The germ consists of two major parts that is the embryonic axis and scutellum. The embryonic axis contains the new plant and is divided into a radicle and plumulae. Upon germination and development, the radicle forms primary roots whereas the plumulae forms leaves and stems. The scutellum is the single cotyledon and contains reserved nutrients including, moderate amounts of oil, protein, enzymes, and minerals, and serves as the bridge between the endosperm and germ (Bianchi, 2013).

2.1.2 Grain composition

Sorghum proximate composition varies significantly due to genetic and environment factors. Sorghum protein content is usually the most variable and can range from 7 to 16% of the whole kernel. Sorghum is similar in composition to corn (*Zea mays*) but sorghum usually contains slightly more protein and has less oil than yellow dent corn (Bianchi, 2013). The pericarp is rich in fiber, whereas the germ has a high crude protein, fat and ash content. Regarding the endosperm, it contains essentially starch and protein with little amounts of fat and fiber.

2.1.2.1 Starch

The major component of sorghum is starch which accounts for approximately 50 to 75% of the caryopsis total weight. Starch is located in the endosperm and pericarp of the grain. Starch granules of sorghum range from 2 to 30 μm in diameter. Starch molecules are arranged in highly organized granules, with the linear amylose and branched amylopectin molecules binding together via hydrogen bonding. This arrangement makes starches pseudo crystals with a crystalline and an amorphous area (Bianchi, 2013; Jambunathan & Subramanian, 1988).

Sorghum endosperm is similar to that of maize. The starch granules and protein bodies are in very close association with each other. The polygonal and tightly packed starch granules are surrounded with numerous spherical protein bodies embedded in a protein matrix. The granules are often misshaped due to the compressive effects of contact with the protein bodies and as a result assuming many complex shapes (Mutahi, 2012). While starch granules present in the corneous endosperm are smaller and angular, those in the floury endosperm are larger and round. Regular endosperm sorghum types contain 20-30% amylose and 70-80% amylopectin (Mariera et al., 2017). The gelatinization temperature normally ranges from 70-80°C. Water binding capacity of sorghum starch is lower than that of regular yellow corn. Soluble sugars represent a significant part of sorghum carbohydrates, particularly in sugary cultivars. In such varieties, sucrose is the major soluble sugar in the dry grain (Bianchi, 2013).

Starch is a dominant component that plays a crucial role in food products and is often used as a thickening and gelling agent (Chanapamokkhot & Thongngam, 2007). Starch is important in bread making due to its water absorption property, gelatinization, pasting behavior, crystallization, and retrogradation behavior during dough development and baking (Onyango, 2016). Apart from starch, sorghum also contains arabinoxylans which play a role on mechanical properties of dough, as well as texture and other quality characteristics of the final products (Izydorczyk & Biliaderis, 1995).

Sorghum flour has comparable amounts of starch as to wheat flour, but with significantly lower amylose content when compared to wheat flour (Kulamarva et al., 2009). Sorghum starch is characterized by high gelatinization temperature (70-80°C), but there are considerable differences among the sorghum cultivars. Starch isolated from the corneous endosperm has higher gelatinization temperature and intrinsic viscosity and lower iodine binding activity than starch from the floury endosperm (Mutahi, 2012; Taylor & Emmambux, 2010).

Normally, sorghum has lower starch digestibility than maize. Therefore, it has been suggested that sorghum may be a particularly suitable food for diabetic and obese people (Taylor & Emmambux, 2010). Lower starch digestibility of sorghum-containing foods is not an intrinsic property of the sorghum starch granules themselves but appears to be a consequence of the interactions of the starch with the endosperm proteins, as well as with cell wall materials and polyphenolic compounds, as condensed tannins and flavonoids. These interactions retard the action of carbohydrate-hydrolyzing enzymes, such as α-glucosidase and α-amylase. Presence of the protein matrix has also been associated with reduced starch gelatinization during cooking resulting in partially-gelatinized starch granules (Stefoska-Needham et al., 2015) that may resist *in vivo* enzymatic degradation.

2.1.2.2 Proteins

Protein, the second major component of sorghum grain, is approximately 6-18% of the grain (De Mesa-Stonestreet et al., 2010). Protein content and composition can vary greatly due to different factors such as sorghum genotype, water availability, soil fertility, temperatures, and environmental conditions during grain development.

Approximately 81, 15 and 4% of the sorghum protein are located in the endosperm, germ and pericarp, respectively. Protein from different part of sorghum grain normally contains different protein fractions. The germ tends to be rich in albumins and globulins while the endosperm contains prolamins, which are known as kafirins, together with glutelins.

Kafirins are the major storage protein of sorghum that serve as nitrogen reserve for the next generation of plant. Kafirins represent 72-82% of the total protein (Abdelghafor et al., 2011; Mutahi, 2012). The proteins are mainly located within the spherical protein bodies. They are protease-resistant, and thus responsible for the poor nutritional quality of sorghum (Kulamarva et al., 2009). They are classified based on molecular weight into three fractions, namely α-, β- and γ-kafirins (Bianchi, 2013).

Depending on whether it is floury or vitreous, sorghum endosperm contains about 66-84% α-kafirin, 8-13% β-kafirin, and 9-21% γ-kafirin plus low levels of the poorly characterized δ-kafirin. α-Kafirin is composed of two groups of polypeptides with molecular weights of 23,000 and 25,000 daltons. These proteins are rich in non-polar amino acids and are found primarily as monomers and oligomers. These proteins do not crosslink extensively and form mainly intra-molecular disulfide bonds. β-Kafirin, with a molecular weight of approximately 18,000 daltons, is rich in sulfur-containing amino acids methionine and cysteine, and is found in monomeric and polymeric forms. γ-Kafirin weighs approximately 20,000 daltons and is rich in proline, cysteine, and histidine. Both β- and γ-kafirins form inter- and intra-molecular disulfide bonds and are highly crosslinked (De Mesa-Stonestreet et al., 2010).

Normal sorghum protein bodies are presumably made rigid by the disulfide-linked polymeric nature of the β- and γ-kafirins found at the body periphery. In food uses, to maximize sorghum protein functional quality, the protein bodies must be disrupted or modify which can be done using shear force or high pressure-high temperature such as in extrusion processing (Chanapamokkhot & Thongngam, 2007). The kafirins differ in structure from wheat gliadin and glutenin (Stefoska-Needham et al., 2015). They are poorly digestible, especially when cooked in water, as occurs during most food preparation processes (Dahir et al., 2015). Sorghum contains very little lysine which is essential for growth, bone health and for fat conversion into energy but has high levels of proline, glutamic acid and leucine. Digestibility of the protein *in vitro* and *in vivo* ranges between 49.5-70% as compared to 81% for wheat, 73% for maize and 66% for rice (Mariera et al., 2017).

The presence of protein and lipid in flour can inhibit the swelling of starch granules (Chanapamokkhot & Thongngam, 2007). Proteins exert a suppressive effect on the swelling of starch granules consequently causing reduction in viscosity of the starch paste (Pu et al., 2017). Protein and liberation of free fatty acids during storage were reported to be responsible for different pasting properties of sorghum flour (Winger et al., 2014).

2.1.2.3 Lipids

Lipids are relatively minor constituent in sorghum grain. The crude fat content of sorghum grain is 3%, which is higher than that of wheat (2.7%) and rice (2.3%) but lower than maize (4.1%) (Bianchi, 2013). The majority of the lipids present in sorghum are neutral triglycerides. The germ and aleurone layers are the main contributors to the lipid fraction. The lipids are mainly located in the germ, although there are smaller amounts in the endosperm (Mutahi, 2012). The germ itself provides about 80% of the total fat. The triglycerides of sorghum are rich in unsaturated fatty acids (Arendt & Zannini, 2013). The main fatty acids are linoleic (38-49%), oleic (31-38%) and palmitic (14%) acids (Serna-Saldivar, 1995).

2.1.2.4 Fibers

Fiber is an endogenous component of the plant material which is resistant to digestion by human enzymes. Sorghum kernel consists of 2.3-2.9% fiber and a single serving of sorghum provides 48% of the Recommended Dietary Intake (Mariera et al., 2017). Bran of sorghum is excellent source of fiber (Mehmood et al., 2015). The major sorghum grain fibers are hemicellulose, cellulose, lignin, and pectin which are normally located in the pericarp and endosperm cell walls. These dietary fibers are in general classified according to their solubility in water. Insoluble fiber components are present primarily in the pericarp where they have essential structural and protective functions. In sorghum, most of the dietary fiber is insoluble, representing about 86% of the total fiber (Bianchi, 2013). Insoluble fiber fraction consists of pectin, arabinoxylan and β-glucans. These fibers have the role in lowering serum cholesterol through bulking and binding of cholesterol and preventing its digestion. Fibers also help in elimination of carcinogenic and harmful substances hence exhibiting a protection role against heart diseases, atherosclerosis, obesity, diabetes, and cancers, as well as a role in maintenance of gastrointestinal health (Mariera et al., 2017).

2.1.2.5 Vitamins and minerals

Sorghum is rich in minerals whose bioavailability ranges from low to less than 1% for some forms of Fe to higher than 90% for Na and K. when compared with barley and rye, sorghum grain contains low amount of P, K, Mg, Ca, Na, Zn, Fe, Mn and Cu. There is a similarity in mineral content between sorghum grain and finger millet grain. K and P were reported to be dominant minerals in sorghum grain (Pontieri et al., 2014). Minerals in sorghum grain are located in the pericarp, aleurone layer and germ. Therefore, refined sorghum products possess reduced amount of these important nutrients, as in all other refined cereal products.

Sorghum grain is an important source of fat-soluble and B vitamins, except vitamin B12. Among the B vitamins, concentrations of thiamine, riboflavin and niacin in sorghum were comparable to those in maize grain. Some yellow-endosperm sorghum varieties contain β-carotene which can be converted to vitamin A by the human body. Detectable amounts of other fat-soluble vitamins, namely D, E and K are also present in sorghum germ (Mohammed et al., 2015).

2.1.2.6 Phytochemicals

Sorghum grain tannins are almost exclusively of the condensed type, i.e., proanthocyanidins and procyanidins, and are only present in pigmented cultivars, which are also known as brown sorghum. Previous studies revealed that tannin levels vary among different genotypes and range from 10.0-68.0 mg/g dry weight. They are primarily located in the pigmented testa, comprising approximately 5-6% dry weight of the kernel. The role of tannins is to protect the plant from environmental effects and against attack by pests, as birds, insects, molds and bacteria. The agronomic protective effects of tannins are conversely accompanied by nutritional inconvenience and reduction in food quality. Condensed tannins are known to bind to proteins, carbohydrates and minerals, thus reducing their digestibility and bioavailability by 5-15%. In order to reduce these negative effects, various processing mechanisms, such as decortication, fermentation, germination or malting and chemical treatments such as the use of chloric acid, formaldehyde and alkali are applied. Among them, malting effectively lowers up to 43% of the assayable levels of sorghum tannins. However, during malting, tannins were found to pose an effect on malt amylase activity. Alkaline and formaldehyde treatments effectively counteract this phenomenon (Mohammed et al., 2015). In term of food uses, tannins are potent antioxidant which is mainly attributed to their chemical structure containing many aromatic rings and hydroxyl groups.

All sorghum varieties contain phenolic acids, located in the pericarp, testa, aleurone layer and endosperm. Previous studies showed that total phenolic content and antioxidant activity vary depending on the genotype. Total phenolic and flavonoid contents are higher in whole grain than in flour, with the same effect being observed for antioxidant activity. The phenolic acids of sorghum are derivatives of benzoic or cinnamic acid. As in other cereals grains, phenolic acids in sorghum grain are mainly concentrated in the pericarp and occur mostly in a bound form by being esterified to cell wall polymers. A positive correlation was reported between total phenolic content and antioxidant activity. Sorghum whole grain is a rich source of antioxidants and phenolic compounds and hence recommended for using as a functional ingredient in food (Cristina et al., 2018). The most abundant phenolic acids identified in sorghum grain include gallic, vanillic, protocatechuic, cinnamic, *p*-coumaric, *p*-hydroxybenzoic, syringic, ferulic, caffeic and sinapic acids (Xiong et al., 2019). The phenolic acids are thought to play a role in plant defence against pests and pathogens. (Mohammed et al., 2015) reported that phenolic acid content in sorghum highly correlated with *in vitro* antioxidant activity and health benefits may be associated with consumption of whole-grain sorghum (Figure 2.1)

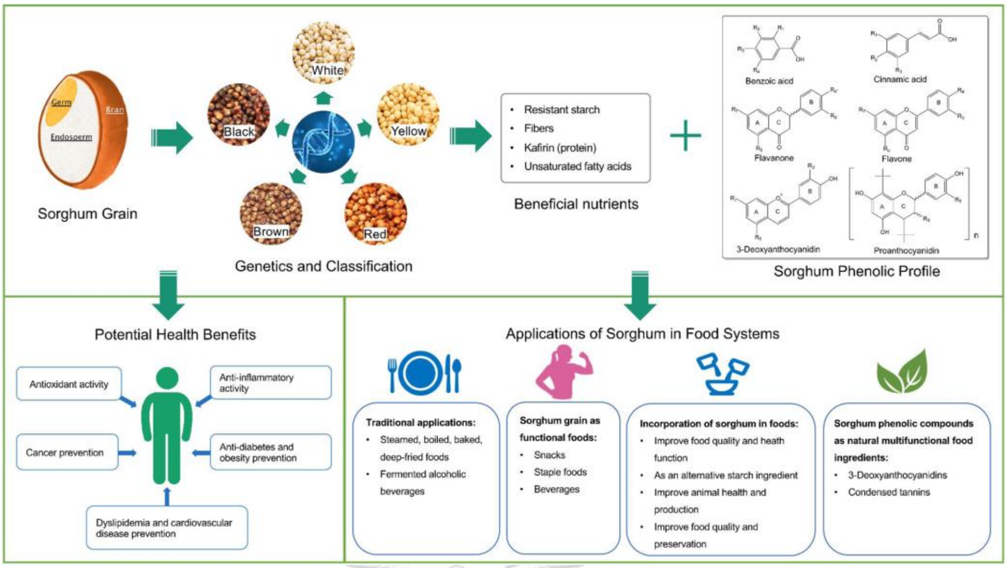


Figure 2. 1 Schematic diagram of sorghum grain genotypes, nutritional components, potential health benefits and food applications
(Xiong et al., 2019)

2.1.3 Processing and utilization

In terms of production, sorghum is the fifth most important cereal in the world after wheat, rice, maize and barley. However, in respect of human consumption, it is considered an under-utilized crop. It is used as food in selected cultures as in the arid and semi-arid regions of sub-Saharan Africa because it is drought-tolerant and can grow well where other crops fail (Abdelhalim et al., 2019). In Kenya, sorghum ranks third after maize and wheat regarding production volume. It has been noted to thrive well on a wide range of soils including those with very low fertility. Sorghum is an indigenous Kenyan crop and is grown in the often drought-prone marginal agricultural areas (Muui et al., 2013). The potential of sorghum to catalyze regional development and improve food security is considerably high (Mwadalu & Mwangi, 2013). In Kenya, sorghum has been utilized as food, feed and for manufacturing of industrial products, with more than 35% produced directly for human consumption.

Traditionally, sorghum flour has been used to prepare a variety of foods as pancakes, porridges, beer and flat breads throughout different cultures, such as Indian *jowar* roti, African sorghum *ugali* (thick porridge) and Chinese *maotai* and *kaoliang* liquor. In the United States, it is becoming more common to use sorghum flour in baked products, such as Trader Joe's® gluten-free whole grain bread. As to industrial products, the grain is used to manufacture wax, starch, syrup, alcohol, dextrose, edible oils and feed. For Kenya, the food security programs target sorghum and other legumes for reduction of food insecurity to 52% in food-insecure population (Mariera et al., 2017).

While some gluten-free flours, such as rice flour, can cause gritty texture to baked products, sorghum flour was reported to possess smoother texture that many consumers prefer. Due to its very mild taste, sorghum flour is a great choice to incorporate into sweet breads, biscuits, cookies and other baked products. The use of sorghum flour has been investigated in different baking systems such as cookies, muffins, breads and tortillas. Also, the flour has been used to fortify crackers, crisps and other confectionaries so as to improve the nutritional content, dietary fiber and sensory properties (Adeyeye, 2016; Pezzali et al., 2020). (Ratnavathi & Patil, 2013) produced cake and noodles from non-pigmented sorghum composite flour.

Sorghum flour is also used as an antioxidant supplement, natural food preservative and anti-caloric agents. Sorghum composite flour has been used for production of ready-to-use therapeutic foods (RUTF) such as Plumpy'Nut® and also to increase energy, protein and minerals in biscuits (Mariera et al., 2017). According to experiments carried out in Sudan and Senegal, sorghum composite breads were found to be organoleptically acceptable.

2.2 Principal changes of starch during food processing and storage

Starch is the main carbohydrate in most cereals and grains and has many useful properties for food as well as non-food applications, including thickening, coating, gelling, binding and encapsulation (Chanapamokkhot & Thongngam, 2007). During processing and storage, the starch in food undergoes various changes which can be monitored using various advanced analytical techniques (Dupuis & Liu, 2019; Yang, 2020).

2.2.1 Gelatinization

Starch is insoluble in cold water. Cold water can penetrate the amorphous regions of the granules but not for the hydrogen-bonded highly-ordered crystalline regions. Heating of the starch dispersion breaks the hydrogen bonds. Water penetrates the interior of the starch granule, hydrating the free hydroxyl groups of amylose and amylopectin. This process leads to irreversible swelling of the starch granules known as gelatinization (Alcázar-Alay & Meireles, 2015). The point of initial gelatinization and the range over which it occurs are governed by starch concentration, method of observation, granule type, and heterogeneity among the granules under observation (Wang et al., 2015).

Pasting is a phenomenon following gelatinization. Friction among the swollen granules leads to an increase in paste viscosity. Pasting involves a myriad of changes occurring to starch, including granular swelling, exudation of molecular components from the granule and eventual total disruption of the starch granule upon prolonged heating. Pasting property is an important index in determining the cooking and baking qualities of starches and flours and is often used as index of predicting the ability of starch-based food to form a viscous paste or gel when subjected to heat application and shearing. It is also used to depict starch or flour behavior during and after cooking. These properties affect the texture, stability and digestibility as well as the end use of starch-based food commodities (Inyang & Elijah, 2020). Pasting is influenced by the amylose-to-amylopectin ratio, and the properties of amylose and amylopectin in terms of molecular weight, distribution, degree and length of branching, and conformation (Yang, 2020).

Pasting profiles of flour-water or starch-water mixtures can be studied using a Rapid Visco Analyzer (RVA). RVA is basically a viscometer with heating and cooling cycles which monitors the viscosity of a sample under controlled heating and shearing. Amylopectin contributes to the swelling of starch granules, while amylose and lipids retard granular swelling. Amylopectin chain length and the molecular size of amylose also have a great effect on viscosity of the starch paste (Yang, 2020). In the case of flour sample, pasting profile may also be affected by protein and lipid contents.

2.2.2 Retrogradation

Retrogradation is an ongoing process, which initially involves rapid reassociation or recrystallization of amylose molecules, followed by a slower recrystallization of amylopectin. In a system with limited water, retrograded amylose was reported to form an A-type helix with 9 molecules of water trapped inside. In the case of a system with excess water, both retrograded amylose and amylopectin assume a conformation of B-type helix with 36 molecules of water trapped inside. The changes that starch undergoes during gelatinization and retrogradation are major determinants of its functional properties for food processing, during digestion, and in industrial applications. Starch retrogradation is usually accompanied by a series of physical changes such as increased viscosity and turbidity of pastes, gel formation, exudation of water and increased degree of crystallinity. These properties determine the quality, acceptability, nutritional value and shelf-life of the end products (Wang & Copeland, 2013).

Amylose retrogradation determines the initial hardness of a starch gel and the stickiness and digestibility of processed foods. The long-term development of crystallinity of processed starch, which is involved in the staling of breads and cakes, is largely due to retrogradation of amylopectin. Starch retrogradation is often considered to have unappealing effects because it results in reduced shelf-life and consumer acceptance leading to significant waste, and thereby poses significant challenges to food processors (Mtelisi et al., 2020). However, a certain degree of starch retrogradation is desirable in some applications like in the production of breakfast cereals, parboiled rice, dehydrated mashed potato, and Chinese rice vermicelli, due to the modification effect on the structural, mechanical and sensory properties of the products. Starch retrogradation is also desirable in terms of nutritional significance, due to the slower enzymatic digestion of retrograded starch and moderated release of glucose into the blood stream (Wang et al., 2015).

Differential scanning calorimetry (DSC) is one of the proven as an extremely valuable and sensitive tool to characterize flour/starch retrogradation as well as gelatinization. In the case of starch retrogradation, DSC endothermic changes provides quantitative measurements of enthalpy and transition temperature for the melting of the recrystallized amylopectin (Abd Karim et al., 2000). Starch retrogradation has been shown to be affected by many factors, such as moisture content, starch type and storage condition. The presence of other components, such as lipids, carbohydrates, salts, proteins and peptides, has also appeared to play an important role on the rate of starch retrogradation (Wang et al., 2015).

2.3 Breads

Bread is a baked product that is produced mainly from wheat flour, yeast, water and salt, by a process that involves a series of activities including mixing, kneading, proofing, shaping and baking. Wheat flour is the main bread ingredient due to the functional protein gluten. There are many types of bread which are differentiated by either flavour constituents, textural properties, size, shape and baking conditions. Demand for wheat bread is increasing in the world especially in many parts of developing countries following the growing urbanization, population growth, rising incomes and changing consumer preferences and taste (Mariera et al., 2017). However, in certain areas like the tropical regions where wheat does not grow well, bread and other baked products are becoming less affordable because they normally lean on imported wheat flour (Wambua et al., 2016).

Worldwide, billions of people rely on wheat for a large part of their diets (Mariera et al., 2017). In Africa, although wheat is not historically part of the staple diet, its consumption has been increasing in recent years. The rise in consumption trend has been a result of several factors that include increased urbanization, changes in lifestyles and growing population. With estimations that about 60% of the African population is projected to live in urban areas by 2050, the demand for wheat-based foods such as bread is expected to surge (Sibanda et al., 2015).

At current rates, Africa produces only about 44% (27 million tons) of the wheat that it needs with the balance being provided by imports. This dependence on imports has a huge impact on African economies, a situation made worse by the rising prices of wheat on the international market. Wheat consumption in Sub-Saharan Africa is increasing rapidly, faster than any other major food grain. It is projected that consumption will be approximately 1.28 million tons by the year 2030.

According to Mariera et al. (2017) decline in wheat production has been caused by high production costs, biotic stress, pests, lack of credit to growers, low level of technology adaptation, reduced arable land and unpredictable weather. Yield losses have led to increased food insecurity and undernutrition. The high cost of wheat products in many developing countries has led to the use alternative flours from locally grown crops to mitigate against doubling of international food prices especially wheat, maize and hoarding of cereals (Sasson, 2012). Also, creating awareness on consumption of different foods is necessary for dietary diversity. The increased use of locally grown cereals like sorghum is intended to improve the nutritional quality and diet diversity especially for poor populations in developing countries.

2.4 Composite flour technology

Africa is not a major wheat growing region, but it produces large quantities of other legumes and cereals such as sorghum. The use of composite flour has been identified by researchers as possible avenue of producing high-quality nutritious food products, utilizing indigenously grown cereals, reducing end product costs, bringing in varieties with different texture and flavor and being a means of reducing the huge amount of foreign exchange spent in the importation of wheat flour (Abdelghafor et al., 2011; Ohizua et al., 2017).

Composite flour technology refers to the process of mixing wheat flour with cereal and legume flours for making bread. However, the term can also be used with regards to mixing of non-wheat flours, roots and tubers, legumes and other raw materials. For composite flour, proper blending to get the desired end properties requires understanding of the interactions of the different components at the physicochemical level. The use of composite flour has been reported to affect dough properties and quality of the end products. As to this study, only sorghum-containing composite flour will be focused here.

The relative quantity of water absorbed by key components of flour is hypothesized as principal parameters adding the rheological properties of dough and flour quality traits as far as baking is concerned. Lower water absorption is not desirable because it is associated with quicker staling of the bread (Torres et al., 1993). (Akajiaku et al., 2017) investigated sorghum flour substitution in wheat noodles. It was found that water absorption of composite flour was found to increase with increasing sorghum flour substitution. The authors suggested that this was attributed to the higher protein content of sorghum flour (14.2%) as compared to that of wheat flour used in the study (11.2%). Torres et al. (1993) reported that particle size of sorghum flour also affected water absorption of dough. It was found that dough containing up to 30% of finely ground sorghum flour had higher water absorption than those containing the same concentration of coarsely ground flour. Water absorption in gluten-free sorghum noodles was affected by the flour particle size, starch particle size and extent of starch granule damage (Winger et al., 2014).

Dough development time and dough stability were found to decrease with an increase in sorghum substitution level of the sorghum-wheat composite flour. The increase in the dough development time combined with the decrease of dough stability implied the weakening of gluten network structure (Sibanda et al., 2015). Another researcher established that, addition of extruded sorghum flour increased water absorption and dough development time but decreased the stability of wheat-sorghum composite dough. Starch gelatinization is thought to be responsible for the increase of the water absorption index of the sorghum flour through extrusion cooking. Increasing water absorption may, in turn, decrease water-gluten interactions, resulting in an increase in dough development time (Mtelisi et al., 2020).

Dough with extensibility is particularly important for gas retention which results in a loaf with the required volume (Bugusu et al., 2001). Sibanda et al. (2015) reported a reduction in volume of sorghum-containing breads. These changes possibly resulted from an increase in sorghum kafirins which are not functional as far as giving the necessary viscoelastic qualities to the dough.

Sorghum proteins, similar to those of other cereal grains with exception of wheat, and to a lot lesser degree rye and triticale, are not able to form a gas-holding, viscoelastic dough on account of the poor viscoelastic properties of kafirin proteins contrasted with wheat gluten (Mtelisi et al., 2020). (Khating et al., 2014) reported a reduction in protein content of wheat-sorghum composite flour with increasing sorghum ratio. This was due to the lower protein content of sorghum flour as compared to the wheat flour used in the study. Hussein et al. (1977) reported similar trend for wheat bread with 40% sorghum substitution. The inferior gas-holding capacity of the sorghum containing dough is responsible for the lower loaf volume of the resulted bread. This is attributable to the gluten dilution effect caused by sorghum substitution. In addition, the high hydrophobicity of kafirins may also contribute to poor gas retention ability of the dough. It was proposed that kafirins are encapsulated in protein bodies in the sorghum endosperm which makes them inaccessible for participation in dough fibril formations (Bugusu et al., 2001). Consequently, it can be concluded that the reduction in peak viscosity, dough extensibility, water absorption capacity, and dough stability upon adding sorghum is due to the reduction of the protein quality as well as quantity (Mtelisi et al., 2020).

Baking characteristics are affected when sorghum flour is increased to higher levels in bread making. According to Mariera et al. (2017), a lower loaf volume was observed in sorghum-containing bread as compared to the wheat control. To obtain a product with satisfactory quality, modification of the ingredients in bread recipe as well as dough preparation and baking techniques may be needed. (Ratnavathi & Patil, 2013) reported that wheat bread needed 50 min-proofing time and 250°C baking for 8-10 min while sorghum composite bread needed 45 min-proofing time and 212°C baking for 18 min.

In a study to characterize four sorghum hybrids both as a kernel and as a flour and to evaluate their physicochemical and sensory properties in gluten-free tortillas (Winger et al., 2014). It was noted that understanding quality characteristics of each sorghum variety is very important in translating to end-product use. The study showed that sorghum hybrids differed in kernel and flour properties. Sorghum flour with smaller particle size and greater starch damage yielded tortillas with better qualities. Through control of sorghum flour quality characteristics, gluten-free tortilla could be prepared with acceptable quality attributes.

Apart from breads, attempts have also been made for using sorghum in other starch-based products. For example, Adeyeye (2016) prepared cookies from sorghum-wheat composite flours. It was revealed that cookies of acceptable and desirable physical properties and chemical composition comparable to that made from wheat flour could be prepared from sorghum-wheat composite flour.

Akajiaku et al. (2017) prepared noodles from sorghum-wheat composite flours. It was found that up to 60% of sorghum flour can be used to partly substitute wheat flour in the noodle recipe. The authors also reported that the noodles made from composite flour were comparable in terms of nutritional quality to the wheat noodle.

CHAPTER 3
MATERIALS AND METHODS
3.1 Materials

Sorghum flour (Unsurpassed Product, Zhejiang, China)

Wheat flour (unbleached bread flour), Shuttle^{TM} Brand

(Thai Flour Mill Industry, Samut Prakan, Thailand)

Instant dried yeast, Bruggeman^{TM} (Algist Bruggeman, Ghent, Belgium)

Iodised salt, Tipp^{TM} (Saha Pathanapibul, Bangkok, Thailand)

Refined sugar, Mitr Phol^{TM} (Mitr Phol Sugar, Bangkok, Thailand)

Shortening, Cream Topp$^{®}$ (Three Top Chemical & Foods, Bangkok, Thailand)

Calcium propionate (Chemipan, Bangkok, Thailand)

3.2 Equipment

Spectrophotometer, model CM-600d (Minolta, Tokyo, Japan)

Food mixer, model CHEF XL elite (Kenwood, Thailand)

Differential scanning calorimeter (DSC), Diamond DSC$^{®}$

(Perkin Elmer, Waltham, MA, USA)

Laboratory hot air oven, model 600 (Memmert, Schwabach, Germany)

Rapid visco analyzer (RVA), model RVA-4 (Newport Scientific,

Warriewood, NSW, Australia)

Shaking water bath, model SW23 (Julabo labortechnik, Seelbach, Germany)

Texture analyzer, model TA-XT2i (Stable Micro Systems, Surrey, UK)

Water activity meter, AquaLab^{TM}, series 3TE (Decagon Devices, Pullman, WA, USA)

Spectrophotometer, GENESYS 20 (Thermo Scientific, Waltham, MA, USA)

Infrared food oven, model PL-6 (Kluaynumthaitowop, Bangkok, Thailand)

3.3 Methods

3.3.1 Wheat and sorghum flour composition and property

3.3.1.1 Moisture content

Moisture content of wheat and sorghum flours was determined using air-oven method according to AOAC (1990).

3.3.1.2 Crude protein content

Nitrogen content of wheat and sorghum flours was determined using Kjeldahl method as described by AOAC (1990). The nitrogen content obtained was multiplied by a factor of 5.70 and 6.25 to obtain the crude protein content of wheat and sorghum flours, respectively.

3.3.1.3 Crude fat content

Crude fat content of wheat and sorghum flours was analyzed using Soxhlet extraction as described by AOAC (1990).

3.3.1.4 Crude fiber content

Crude fiber content of wheat and sorghum flours was determined using the procedure as described by AOAC (1990)

3.3.1.5 Ash content

Ash content of wheat and sorghum flours was determined according to AOAC (1990).

3.3.1.6 Starch content

Starch content of wheat and sorghum flours was determined using the amyloglucosidase/α-amylase method according to AOAC (2007).

3.3.1.7 Amylose content

Amylose content of wheat and sorghum flours was analyzed using amperometric method (Gibson et al., 1997; Takeda et al., 1987).

3.3.1.8 Water absorption capacity

Water absorption capacity of wheat and sorghum flours was determined using the method 56-11 (AACC, 2000). Flour sample (5 g) was transferred to a centrifuge tube and then added with excess amount of water (25 mL). The mixture was vigorously shaken for 5 s and kept for 20 min with intermittent shaking every 5 min. The mixture was then centrifuged at 1000×*g* for 15 min. The supernatant was decanted and the sediment was weighed. The absorbed water was calculated by subtracting the flour weight from the sediment weight. Water holding capacity was calculated using Equation (1):

Water holding capacity (%) = (weight of absorbed water/weight of flour sample) ×100 … (1)

3.3.2 Effect of sorghum flour substitution on pasting behavior, gelatinization and retrogradation of wheat flour

Wheat-to-sorghum flour ratio used in this study were 100:0 (wheat control), 0:100 (sorghum control), 80:20, 60:40, 40:60 and 20:80. The flour samples were determined for the following properties:

3.3.2.1 Pasting behavior

Pasting behavior of the flour samples was monitored using an RVA (model RVA-4, Newport Scientific, Warriewood, NSW, Australia). The analysis was carried out according to the method described earlier (Bao, 2008). Three g of flour sample (10% moisture, wet basis) were mixed with 25 g of distilled water in an RVA sample canister. The idle temperature was set at 50°C, and the 13-min test profile was run according to Table 3.1. Pasting properties were determined using

Thermocline for Windows software (Newport Scientific, Warriewood, NSW, Australia).

3.3.2.2 Gelatinization

A DSC (Diamond DSC®, Perkin Elmer, Waltham, MA, USA) was used to monitor temperature and enthalpy of gelatinization of the flour samples according to (Karim et al., 2008). Flour and distilled water at a ratio of 1:2 (w/w) were hermetically sealed in a 60 μL stainless-steel DSC pan (Perkin Elmer, Waltham, MA, USA) and heated from 30 to 100°C at a rate of 10°C/min. Onset (T_o), peak (T_p) and conclusion (T_c) temperatures together with enthalpy of gelatinization (ΔH_G) were obtained from the DSC endotherms using Pyris software, version 3.01 (Perkin Elmer, Waltham, MA, USA).

Table 3. 1 Testing profile for RVA experiments

Stage	Temperature/speed	Time
1	50°C	0 min, 0 s
2	960 rpm	0 min, 0 s
3	160 rpm	0 min, 0 s
4	50°C	1 min, 0 s
5	95°C	4 min, 42 s
6	95°C	7 min, 12 s
7	50°C	11 min, 0 s
End of test		13 min, 0 s
Time between readings		4 s

3.3.2.3 Retrogradation

Long-term retrogradation was investigated by monitoring amylopectin recrystallization. The sample pans obtained from gelatinization study as described in 3.3.2.2 were stored at 4°C for 10, 12 and 14 days. Thermal properties related to the melting of amylopectin crystallites were determined by heating the samples from 30 to 100°C at a rate of 10°C/min (Karim et al., 2008). T_o, T_p, T_c and enthalpy for melting of amylopectin crystallites (ΔH_R) were extracted from the DSC curve using Pyris software, version 3.01 (Perkin Elmer, Waltham, MA, USA).

3.3.2.4 Gel firmness

Starch retrogradation was also monitored through changes in flour gel firmness. Flour gel samples were prepared according to the method of (Muadklay & Charoenrein, 2008) with some modifications. Flour dispersion was prepared using 10 g of flour and 40 g of distilled water. The dispersion was stirred for 30 min and then heated at 95°C for 60 min, after which it was transferred to a mold and cooled at 25°C for 60 min. Gel firmness of the freshly prepared gel (Day 0) as well as those stored at 4°C for 10, 12 and 14 days was determined using a Texture Analyzer (model TA-XT2i, Stable Micro Systems, Surrey, UK) equipped with a spherical stainless-steel probe (P/0.25S). A 25×25×11 mm piece of gel was used for the test. The test was conducted using pre-test speed of 1 mm/s, test speed of 10 mm/s and 50% strain deformation. From each force-time curve, maximum force required to accomplish the designated deformation during the compression was taken as gel firmness.

3.3.3 Effect of sorghum flour substitution on baking and keeping quality of pan bread

Wheat-to-sorghum flour ratio used in this study were 100:0 (control bread), 80:20, 60:40, 40:60 and 20:80. Since all-sorghum bread could not successfully be made, the 0:100 bread was thus omitted in this experiment. Pan bread was prepared using a straight dough method according to that described by Choondee (2003). The bread ingredients included 500.0 g of flour, 5.0 g of dried yeast, 7.5 g of salt, 25.0 g of sugar, 25.0 g of shortening, 300.0 g of water and 1.5 g of calcium propionate (as mold inhibitor).

To prepare the dough, dry ingredients including flour, yeast and calcium propionate were blended and sifted into a Kenwood mixing bowl. The bowl was placed in the receptacle of a food mixer (model CHEF XL elite, brand - Kenwood, Kittisit Enterprise Co., Ltd) attached with a dough hook. In a separate mixing bowl, sugar and salt were dissolved into 300 g of water and then added to the dry ingredients. The mixture was mixed at Speed 3 for 3 min. Shortening was then added and mixed at Speed 6 for 10 min.

The dough was then taken out from the machine, hand kneaded and fermented at 32°C for 1 h. After that, the dough was divided into 250 g pieces, kneaded and rested in a tray at 32°C for 30 min. After resting, the dough was hand kneaded again, rolled into a log shape, placed in a baking pan and proofed at 32°C for 1 h. Once the time had elapsed, the pan was placed in an oven pre-heated to 200°C and baked for 30 min. The bread was then removed from the pan and cooled at room temperature for 1 h before being packed in a polyethylene bag. Freshly baked bread (Day 0) as well as those stored at room temperature for 10, 12 and 14 days were analyzed for the following properties:

3.3.3.1 Moisture content and water activity

Moisture content of the bread samples was determined using air-oven method according to AACC (2000). Water activity was measured using an AquaLab^{TM} water activity meter (series 3TE, Decagon Devices, Pullman, WA, USA) at 25°C.

3.3.3.2 Specific loaf volume

To determine specific loaf volume, bread loaf was weighed and the loaf volume was measured using rapeseed displacement method according to (Bárcenas & Rosell, 2007). Container of the size that could accommodate the bread loaf was first determined for its volume by over-filling the container with rapeseeds. A straight-edged spatula was used to level the seeds until they were even with the top of the container. The seeds in the container were then transferred to a measuring cylinder and the volume was recorded as V_1. With the same container, bread loaf was placed inside and the container was over-filled with rapeseeds. A straight-edged spatula was used to level the seeds. The seeds were then transferred to a measuring cylinder while the bread loaf was removed. The volume of the seeds was recorded as V_2. The difference between V_1 and V_2 was designated as loaf volume. Specific loaf volume was calculated by dividing loaf volume with loaf weight.

3.3.3.3 Texture

Texture profile analysis (TPA) of bread crumb was determined using texture analyzer (model TA-XT2i, Stable Microsystems, Surrey, UK) equipped with P/100 aluminium compression platen (100 mm diameter). Bread crumb sample (30×30×30 mm) was compressed at a test speed of 1 mm/s with 70% strain deformation and waiting time of 5 s before starting the second compression (Itthivadhanapong & Sangnark, 2016). Hardness, adhesiveness, cohesiveness, springiness, gumminess and chewiness were obtained from the TPA curve.

3.3.3.4 Water-soluble starch content

Water-soluble starch content was determined according to the method described by (Shaikh et al., 2007). Two hundred mg of bread crumb was added to 15 ml of distilled water. The mixture was shaken at 25°C for 20 min. The slurry obtained was then centrifuged at 1000×*g* for 10 min and the supernatant was subjected to starch content determination using iodine titration technique.

3.3.3.5 Crumb structure

Photographs of crumb structure were taken using a digital camera.

3.3.3.6 Color

Color of the bread crumb and crust was measured in CIELAB system using a spectrophotometer (model CM-600d, Minolta, Tokyo, Japan). The measurements were done under D65 illuminant with a 10° observer.

3.3.4 Statistical analysis

All experiments were done in three replicates using a completely randomized design (CRD). Data were analyzed using Analysis of Variance (ANOVA) at p=0.05. Difference between means was determined by the Duncan's new multiple range test (Cochran & Cox, 1957) using SPSS Statistics 22.0 (IBM, Armonk, NY, USA).

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Wheat and sorghum flour composition and property

The composition and property of the wheat and sorghum flour used in the present study are given in Table 4.1. Both flours had approximately 10% moisture content, typical of cereal flours of which specifications usually limit the moisture content to 14% or less. Crude protein content of the sorghum flour was 14.90%, which was lower than that of wheat flour (16.91%). Protein content is an important factor affecting flour properties and creating food structure (Ragaee & Abdel-Aal, 2006). Sorghum flour was higher in crude fat content (2.17%) than wheat flour (0.87%). The role of flour lipid, particularly lipid that was complexed with amylose, could affect the swelling and pasting properties of the flour (Chanapamokkhot & Thongngam, 2007). Sorghum flour also had higher contents of ash and crude fiber which was in agreement with previous report (Sibanda et al., 2015). Starch content of wheat and sorghum flour were 73.03 and 60.19%, respectively. The starch content of the sorghum flour fell within the range reported for different sorghum varieties (Mutahi, 2012). The amylose content of wheat flour (23.87%) was greater than that of sorghum flour (14.88%). The sorghum flour used in this study could be classified as heterowaxy sorghum which contains about 14% amylose (Sang et al. (2008). The amylose content could play a major role in granular swelling, pasting properties and gel firmness (Chanapamokkhot & Thongngam, 2007).

Water absorption capacity represents the water absorbed by the flour in the presence of excess water. Our results show that sorghum flour had slightly higher water absorption capacity (387.2%) than wheat flour (354.7%).

Table 4. 1 Chemical composition and water absorption capacity of wheat and sorghum flour

Composition/property	Wheat flour	Sorghum flour
Proximate composition		
Moisture (% wet basis)	11.28±0.03	9.97±0.20
Crude protein (% wet basis)	16.91±0.03	14.90±0.11
Crude fat (% wet basis)	0.87±0.08	2.17±0.14
Ash (% wet basis)	0.59±0.00	1.76±0.01
Crude fiber (% wet basis)	0.21±0.00	2.27±0.02
Other property		
Starch (% wet basis)	73.03±0.86	60.19±0.84
Amylose (% of starch)	23.87±0.38	14.88±0.44
Water absorption capacity (% dry basis)	354.7±1.26	387.2±0.21

Mean±SD of three replicates

4.2 Effect of sorghum flour substitution on pasting behavior, gelatinization and retrogradation of wheat flour

4.2.1 Pasting behavior

The RVA curves and the corresponding pasting properties of wheat flour substituted with different levels of sorghum flour are presented in Figure 4.1 and Table 4.2, respectively. The result showed significant differences ($p \leq 0.05$) in pasting properties among the flour samples because of the differences in composition between wheat and sorghum flours.

In terms of pasting temperature, even though that of wheat and sorghum flours were significantly different ($p \leq 0.05$), the temperature of the two controls, as well as the composites, still varied within a narrow range, around 87-88°C. According to Sandhu et al. (2007), pasting temperature provides an indication of the minimum temperature required to cook the flour. A higher pasting temperature indicates more difficult gelatinization tendency and lower swelling properties (Panghal et al., 2019).

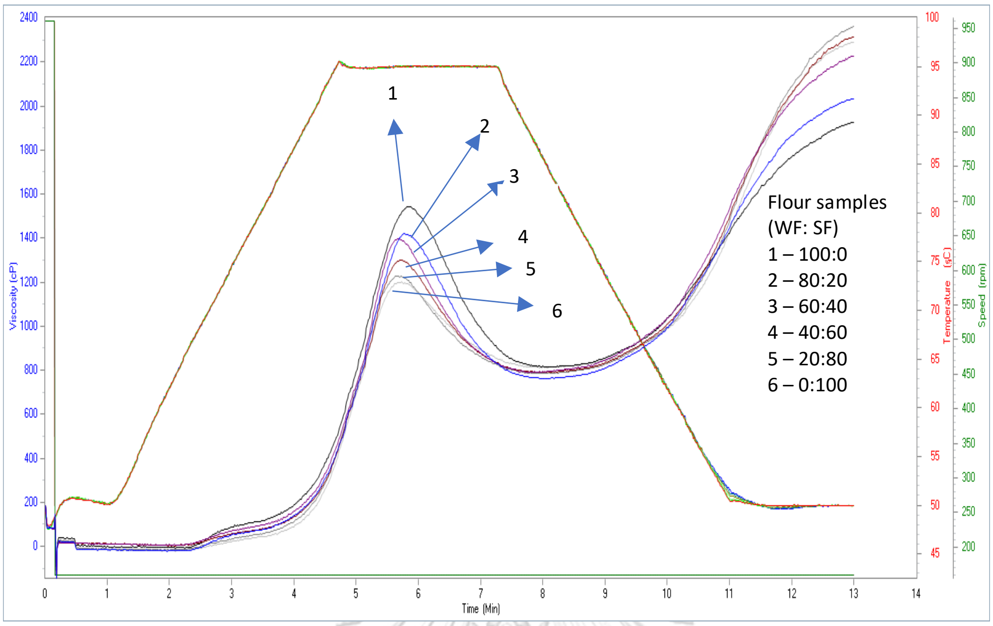


Figure 4. 1 RVA profiles of wheat and sorghum flour and their composites. WF: wheat flour, SF: sorghum flour.

Peak time is the time to reach peak viscosity and it ranged between 5.70 to 5.87 min with wheat flour exhibiting significantly higher peak time than sorghum flour ($p \leq 0.05$). However, that of the wheat-sorghum composites was not different from the wheat and sorghum controls ($p > 0.05$).

Peak viscosity which is the maximum viscosity developed during or soon after the heating cycle of the test (Ohizua et al., 2017). It is indicative of the viscous load likely to be encountered during heating and mixing (Maziya-Dixon et al. (2007). It is often correlated with final product quality and it occurs at the equilibrium point between swelling that causes an increase in viscosity and granule rupture and starch chain alignment that cause its decrease (Adebowale et al., 2011). In this study, peak viscosity of wheat and sorghum flours were different significantly ($p \leq 0.05$), with wheat being of higher peak viscosity than sorghum flour. High peak viscosity is correlated with high starch content (Adebowale et al., 2012; Babajide & Olowe, 2013) and this could explain the higher peak viscosity of wheat flour compared to sorghum flour and the composites. Peak viscosity of the composites then demonstrated a decreasing trend with increasing sorghum flour substitution. These results are in agreement with Dahir Mohammed et al. (2015) who reported that, the rise of the viscosity will discontinue when granules reach adequate internal pressure followed by drop in pasting viscosity due to granule rupture. Similar results were reported by (Panghal et al., 2019), who studied the effects of finger millet on pasting profile of wheat flour. The authors suggested that the presence of lipid, ash, and fiber in finger millet can hinder water uptake and swelling of starch granules, hence causing a reduction in peak viscosity.

Trough viscosity is the lowest viscosity after peak viscosity. It indicates shear-thinning behavior of the flour. Similar trough viscosity was demonstrated by both wheat and sorghum flour. Therefore, all the composites were similar in trough viscosity to one another and to the controls, except the 80:20 blend.

Breakdown is related to the stability of starch granule under a high shear condition. From this study, it was found that wheat flour demonstrated greater breakdown (716.33 cP) as compared to sorghum flour (400.33 cP). Lower breakdown conferred the paste ability to withstand heat and shear (Tiga et al., 2021). This implies that sorghum substitution was proved to help increase the paste stability under high heat and shear. Sang et al. (2008) suggested that amylose-lipid complexation may be responsible for rigidity of starch granules by limiting swelling, which might explain the decrease in breakdown viscosity of sorghum flour.

As the starch paste cools, there is a decrease in kinetic energy, which allows the starch molecules to reassociate and form network. The reassociation, which usually occurs in a matter of hours after cooling, is mainly due to amylose retrogradation (Majzoobi et al. (2015). This short-term retrogradation results in an increase in final viscosity as well as textural changes of the cooked paste (Chanapamokkhot & Thongngam, 2007).

Final viscosity reflects the ability of a starch sample to form a viscous paste or gel upon cooling (Ajatta et al., 2016). In this study, it was found that sorghum flour exhibited significantly higher final viscosity than wheat flour ($p \leq 0.05$). The composite flours therefore demonstrated an increase in final viscosity with increasing sorghum flour concentration. Final viscosity has been reported to depend on starch, amylose and amylopectin contents, as well as amylose-to-amylopectin ratio (Inyang &Elijah,2020).

Table 4. 2 Pasting characteristics of wheat, sorghum flours and their composites

Flour samples (WF: SF)	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown viscosity (cP)	Final viscosity (cP)	Setback viscosity (cP)	Peak time (min)	Pasting temperature (°C)
100:0 (Wheat control)	1538.33 ± 8.14^{a}	822.00 ± 8.19^{a}	716.33 ± 9.45^{a}	1930.00 ± 8.66^{e}	1108.00 ± 2.65^{e}	5.87 ± 0.04^{a}	87.18 ± 0.33^{c}
0:100 (Sorghum control)	1209.33 ± 32.32^{e}	809.00 ± 30.51^{a}	400.33 ± 8.14^{f}	2287.00 ± 63.50^{bc}	1478.67 ± 33.01^{b}	5.70 ± 0.11^{b}	88.42 ± 0.46^{ab}
80:20	1393.67 ± 27.15^{b}	753.00 ± 22.11^{b}	640.67 ± 15.82^{b}	2006.33 ± 46.23^{d}	1253.33 ± 24.66^{d}	5.74 ± 0.03^{ab}	87.62 ± 0.38^{bc}
60:40	1390.33 ± 29.28^{b}	803.00 ± 27.78^{ab}	587.33 ± 16.65^{c}	2229.33 ± 30.57^{c}	1426.33 ± 11.50^{c}	5.76 ± 0.09^{ab}	87.78 ± 0.88^{ab}
40:60	1318.67 ± 20.74^{c}	824.67 ± 30.89^{a}	494.00 ± 19.31^{d}	2342.67 ± 34.27^{ab}	1518.00 ± 24.98^{ab}	5.83 ± 0.09^{ab}	88.72 ± 0.53^{a}
20:80	1265.33 ± 41.49^{d}	815.33 ± 41.20^{a}	450.00 ± 7.21^{e}	2359.67 ± 21.01^{a}	1544.33 ± 28.38^{a}	5.73 ± 0.05^{ab}	87.90 ± 0.44^{ab}

Mean±SD of three replicates

Means in the same column with different superscript letters differ significantly at $p=0.05$.

WF: wheat flour, SF: sorghum flour

Setback value is the tendency of starch to reassociate and retrograde upon cooling. According to Gujral et al. (2019), when hot starch paste cools, reordering and recrystallization of the starch molecules occur, also known as retrogradation. This results in an increase in torque, also referred to as setback. According to Dube et al. (2021), higher setback viscosity indicates a higher tendency for retrogradation. High setback may also be associated with syneresis (Ajatta et al., 2016; Ragaee & Abdel-Aal, 2006). Paste with higher setback tends to produce a harder gel.

From this study, sorghum flour was shown to possess significantly greater setback viscosity than wheat flour ($p \leq 0.05$). The composite flours displayed accordingly increasing setback with increasing level of sorghum flour substitution. According to Lu et al. (2020), the setback of starch is affected by the fine structure of amylopectin, amylose-to-amylopectin ratio, and moisture content.

4.2.2 Gelatinization

Thermal properties as related to starch gelatinization of wheat and sorghum flours, along with their composites are summarized in Table 4.3. Starch gelatinization is a process that breaks down the intermolecular bonds of starch molecules in the presence of water and heat, allowing the hydrogen bonding sites, the hydroxyl hydrogen and oxygen to engage more water. Gelatinization temperature is regarded as the temperature at which the phase transition of starch granules from an ordered state to a disordered state occurs.

In this study, sorghum flour exhibited slightly wider range of gelatinization temperature than wheat flour. However, T_p of the two controls were of similar values ($p>0.05$). Gelatinization of the composites thereby changed accordingly to the ratio of sorghum flour. With respect to gelatinization enthalpy (ΔH_G), sorghum flour unveiled a lower value than wheat flour ($p \leq 0.05$). Therefore, that of the wheat-sorghum composites decreased with increasing ratio of sorghum flour. Gelatinization temperature is believed to be an indicator of crystallinity, which is related to double helix length, whereas gelatinization enthalpy is a measure of the loss of molecular order (Sang et al., 2008).

Table 4. 3 Temperature and enthalpy of gelatinization of wheat, sorghum flours and their composites

Flour samples (WF: SF)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_G (J/g)
100:0 (Wheat control)	61.31±0.30^{ab}	66.85±0.11^{c}	71.95±0.68^{d}	12.36±0.30^{a}
0:100 (Sorghum control)	60.63±0.41^{c}	67.01±0.09^{bc}	73.74±0.30^{bc}	8.80±1.15^{b}
80:20	61.59±0.23^{a}	67.29±0.35^{abc}	73.24±0.74^{c}	11.21±0.83^{ab}
60:40	60.95±0.59^{bc}	67.52±0.36^{a}	73.93±0.79^{bc}	10.15±1.00^{ab}
40:60	60.85±0.16^{bc}	67.41±0.16^{ab}	74.66±0.67^{ab}	10.44±2.52^{ab}
20:80	60.54±0.07^{c}	67.25±0.28^{abc}	75.48±0.58^{a}	10.64±1.61^{ab}

Mean±SD of three replicates

Means in the same column with different superscript letters differ significantly at p=0.05.

WF: wheat flour, SF: sorghum flour

4.2.3 Retrogradation

Amylose gelation involves a rapid network development, typically less than 1 day, via chain entanglement; while amylopectin is responsible for slow development of the crystallinity in the polymer-rich regions, which may continue for weeks (León et al., 2006). The degree of retrogradation varies by type of starch (Ploypetchara et al., 2015). Thermal properties as related to amylopectin crystallite melting in wheat and sorghum flours, along with their composites stored at 4°C for 10, 12 and 14 days are presented in Table 4.4.

From this study, it was found that all samples demonstrated similar temperature for melting of amylopectin crystallites, with T_P around 56°C. For the degree of retrogradation, as implied by ΔH_R, at 10 days of storage, sorghum flour had significantly lower ΔH_R than wheat flour ($p \leq 0.05$) and, in general, the composite flours with greater ratio of sorghum flour exhibited lower degree of retrogradation.

However, with prolonged storage (12 and 14 days), all samples seemed to have similar degree of retrogradation. Each sample displayed an increase in ΔH_R with increasing storage time.

Table 4. 4 Temperature and enthalpy of amylopectin crystallite melting of wheat, sorghum flours and their composites stored at 4°C up to 14 days

Flour samples (WF: SF)	T_o (°C)			T_p (°C)			T_c (°C)			ΔH_R (J/g)		
	Day 10^{ns}	Day 12 ^{ns}	Day 14	Day 10	Day 12	Day 14	Day 10	Day 12 ^{ns}	Day 14	Day 10	Day 12 ^{ns}	Day 14
100:0	45.19	47.40	44.55	57.00	55.57	55.90	60.40	64.02	64.03	0.83	0.84	1.28
	±4.53	±1.04	±1.00^{c}	±0.54^{ab}	±0.34^{c}	±0.66^{b}	±0.73^{c}	±0.64	±0.57^{ab}	±0.08^{a}	±0.22	±0.13^{a}
0:100	48.61	47.81	48.14	57.13	57.24	57.20	77.10	65.00	64.63	0.60	0.97	1.23
	±1.41	±0.69	±1.21^{ab}	±0.96^{ab}	±0.25^{a}	±0.29^{a}	±3.78^{a}	±1.08	±0.60^{a}	±0.04^{bc}	±0.16	±0.11^{a}
80:20	47.40	46.69	47.33	56.05	56.02	56.06	62.32	65.12	64.09	0.76	1.19	1.25
	±3.34	±1.61	±1.33^{ab}	±0.29^{bc}	±0.29^{bc}	±0.48^{b}	±2.15^{bc}	±0.15	±0.64^{ab}	±0.13^{a}	±0.22	±0.09^{a}
60:40	47.66	47.70	46.15	56.05	56.63	56.52	60.97	64.43	63.93	0.54	0.81	1.33
	±1.78	±0.74	±1.42^{c}	±0.60^{bc}	±0.51^{abc}	±0.82^{ab}	±1.04^{c}	±0.41	±0.29^{ab}	±0.01^{c}	±0.14	±0.39^{a}
40:60	49.37	47.62	47.47	55.49	56.69	56.48	62.99	65.23	63.55	0.49	0.87	1.04
	±1.31	±1.65	±0.29^{ab}	±0.59^{c}	±1.02^{ab}	±0.42^{ab}	±2.45^{bc}	±1.19	±0.18^{b}	±0.11^{c}	±0.09	±0.02^{ab}
20:80	46.39	47.04	48.93	57.49	56.52	56.03	66.65	65.82	63.33	0.71	0.80	0.84
	±0.68	±1.10	±0.86^{a}	±0.10^{a}	±0.60^{abc}	±0.60^{b}	±2.98^{b}	±1.79	±0.80^{b}	±0.09^{ab}	±0.29	±0.01^{b}

Mean±SD of three replicates

Means in the same column with different superscript letters differ significantly at $p=0.05$.

^{ns} Means in the same column were not significantly different at $p=0.05$.

WF: wheat flour, SF: sorghum flour

4.2.4 Gel firmness

Increase in firmness during storage of starch/flour gel has been reported to be related to starch retrogradation. Gel firmness of the flour samples are shown in Table 4.5. Freshly prepared (Day 0) wheat control gel (100:0) demonstrated significantly lower firmness than sorghum control gel (0:100) and composite flour gels ($p \leq 0.05$). Similar results were reported by (Abebe & Ronda, 2014) who indicated that lower initial firmness of wheat flour gel could be attributed to gluten-starch interactions that hinder starch molecules to reorganize and retrograde.

Table 4. 5 Gel firmness of wheat, sorghum flours and their composites stored at 4°C up to 14 days

Flour samples (WF: SF)	Gel firmness (N)			
	Day 0	Day 10	Day 12	Day 14
100:0 (Wheat control)	0.08±0.00^{c}	0.12±0.00^{e}	0.12±0.01^{d}	0.16±0.01^{c}
0:100 (Sorghum control)	0.13±0.02^{ab}	0.17±0.01^{d}	0.15±0.02^{c}	0.17±0.02^{c}
80:20	0.11±0.01^{b}	0.24±0.02^{a}	0.24±0.01^{a}	0.21±0.01^{ab}
60:40	0.13±0.01^{ab}	0.22±0.02^{bc}	0.21±0.02^{ab}	0.23±0.01^{a}
40:60	0.12±0.01^{b}	0.20±0.01^{c}	0.19±0.02^{bc}	0.19±0.02^{bc}
20:80	0.15±0.01^{a}	0.22±0.02^{ab}	0.21±0.03^{ab}	0.24±0.02^{a}

Mean±SD of three replicates

Means in the same column with different superscript letters differ significantly at $p=0.05$.

WF: wheat flour, SF: sorghum flour

In addition, wheat control gel had the least firmness for the whole storage period. However, it is worth noting that at the end of storage (Day 14), wheat control gel showed the highest increase in firmness from the value at Day 0 (2-time increase) as compared to sorghum control gel and composite flour gels which demonstrated an increase in firmness around 1.3-1.9 times from the values at Day 0. According to Charoenkul et al. (2011), the increase in firmness of flour gels might also be affected by other flour components such as nonstarch polysaccharides, lipid and protein. These materials may possibly reduce starch reassociation by acting as physical barriers to hydrogen bond formation between the starch molecules, or by interacting with the starch molecules, thus reducing the interaction between them. The chemical nature of these materials such as hydrophilicity or hydrophobicity, and the ability to create complex formations, may also influence the firmness of gel. From this study, it appeared that sorghum flour replacement may result in a product with greater initial firmness but it could retard the increase in firmness over prolonged storage.

4.3 Effect of sorghum flour substitution on baking and keeping quality of pan bread

4.3.1 Moisture content and water activity

Moisture content is an important factor in food stability and quality (Twinomuhwezi et al., 2020). Moisture content of the bread samples are shown in Table 4.6. It was found that there was no significant difference in moisture content among freshly prepared (Day 0) bread samples. Moisture content of all bread samples decreased as the storage time increased. This could be attributed to moisture migration from the crumb to crust and also from the crust to the air surrounding the loaf. However, at Day 10 and beyond, moisture content of the bread samples stayed relatively constant at about 33%. Therefore, substitution of sorghum flour to wheat flour in a bread recipe did not seem to pose an effect on the bread moisture content.

Our results are in agreement with (He & Hoseney, 1990) who reported that bread moisture content decreased sharply with time. The researchers indicated that, the crumb of freshly baked bread contained about 47% moisture, during 2 h of cooling the moisture dropped to 41%. With additional storage time, the moisture continued to decrease, but at a slower rate. The researchers also added that after bread was stored for 30 days, the moisture of the bread crumb was essentially constant, about 31.5%.

In terms of water activity (Table 4.7), it was found that at Day 0, there was no significant difference among the samples. At Day 10, water activity of the breads slightly decreased as compared to those at Day 0. However, after Day 10, water activity was almost constant in all samples.

Table 4. 6 Moisture content of bread substituted with different levels of sorghum flour

Bread samples (WF:SF)	Moisture content (%)			
	Day 0 ^{ns}	Day 10 ^{ns}	Day 12	Day 14
100:0 (Wheat control)	40.78±0.24	34.60±0.84	33.82±0.94^{ab}	32.28±0.11^{c}
80:20	40.82±0.46	33.83±0.96	33.77±0.96^{b}	33.07±0.74^{bc}
60:40	41.32±0.31	33.29±0.79	33.56±0.80^{ab}	32.03±0.76^{c}
40:60	40.80±0.34	33.63±1.26	33.78±1.06^{ab}	33.64±0.81^{b}
20:80	41.33±0.55	34.76±0.15	34.74±0.68^{a}	34.41±0.81^{a}

Mean±SD of three replicates

Means in the same column with different superscript letters differ significantly at p=0.05.

^{ns} Means in the same column were not significantly different at p=0.05.

WF: wheat flour, SF: sorghum flour

Table 4. 7 Water activity of bread substituted with different levels of sorghum flour

Bread samples (WF:SF)	Water activity			
	Day 0 ^{ns}	Day 10	Day 12	Day 14
100:0 (Wheat control)	0.97±0.00	0.96±0.00^{a}	0.96±0.00^{a}	0.96±0.00^{a}
80:20	0.97±0.00	0.95±0.01^{ab}	0.96±0.00^{a}	0.96±0.01^{a}
60:40	0.97±0.01	0.95±0.01^{ab}	0.95±0.01^{b}	0.95±0.01^{b}
40:60	0.97±0.01	0.95±0.00^{b}	0.95±0.00^{b}	0.95±0.00^{b}
20:80	0.97±0.00	0.96±0.00^{a}	0.96±0.00^{a}	0.96±0.00^{a}

Mean±SD of three replicates

Means in the same column with different superscript letters differ significantly at p=0.05.

^{ns} Means in the same column were not significantly different at p=0.05.

WF: wheat flour, SF: sorghum flour

4.3.2 Specific loaf volume

Specific loaf volume is an important technological characteristic of the bread because it reflects appearance and sensory acceptance and provides quantitative measurement of baking performance. Loaf volume is used as a criterion to measure the quality of bread in quality control in industry and by consumers. The effect of sorghum flour substitution on specific loaf volume is summarized in Table 4.8. It was found that sorghum substitution resulted in a significant decrease in specific loaf volume ($p \leq 0.05$). The volume ranged from highest in wheat (5.31 cm^3/g) to lowest in 20:80 composite (2.34 cm^3/g) for freshly baked bread (Day 0). Gluten assumes a key role in the remarkable volume of wheat bread. Proteins present in non-wheat flours do not have the capacity to form viscoelastic networks responsible for holding the gas produced during fermentation (Torbica et al., 2019).

Table 4. 8 Specific loaf volume of bread substituted with different levels of sorghum flour

Bread samples	Specific loaf volume (cm^3/g)			
(WF:SF)	Day 0	Day 10	Day 12	Day 14
100:0 (Wheat control)	5.31 ± 0.06^{a}	4.91 ± 0.01^{a}	4.91 ± 0.06^{a}	4.87 ± 0.03^{a}
80:20	5.12 ± 0.05^{ab}	4.85 ± 0.01^{a}	4.65 ± 0.01^{b}	4.59 ± 0.07^{b}
60:40	4.88 ± 0.01^{bc}	4.79 ± 0.12^{a}	4.52 ± 0.05^{c}	4.51 ± 0.05^{bc}
40:60	4.60 ± 0.03^{c}	4.56 ± 0.10^{b}	4.42 ± 0.03^{c}	4.40 ± 0.02^{c}
20:80	2.34 ± 0.46^{d}	2.27 ± 0.13^{c}	2.26 ± 0.13^{d}	2.23 ± 0.13^{d}

Mean±SD of three replicates

Means in the same column with different superscript letters differ significantly at $p=0.05$.

WF: wheat flour, SF: sorghum flour

Sorghum substitution resulted in gluten dilution and consequently weakened the gluten network in the dough. The higher level of sorghum flour further reduced specific volume of the composite bread. In addition, the presence of sorghum flour might have increased solid-like behavior that can inhibit the expansion of dough during fermentation and in turn decreased specific volume of the bread.

Relationship between gluten content and loaf volume was reported earlier. For example, Adeyeye et al. (2019) reported a decrease in the volume of bread made from wheat-rice composite flour. According to Chisenga et al. (2020) breads with higher volume were associated with higher gluten content, which promotes appreciable pore formation and better gas retention during proofing. In the current study, the highest loaf volume among the composite breads belonged to the 80:20 sample, which was not significantly different (p>0.05) from that of the wheat control. The decrease in loaf volume of the sorghum-substituted bread was so obvious, particularly at higher ratio of sorghum flour substitution (Figure 4.2). This decrease in loaf volume may be the major limitation of sorghum substitution to leavened bread.

Figure 4. 2 Relative loaf size of bread substituted with different levels of sorghum flour.

(From left to right), WF: SF of 100:0 (wheat control), 80:20, 60:40, 40:60 and 20:80.

Even though specific loaf volume is mainly governed by gluten content of the bread, other constituents such as starch and fiber might also pose an effect on the loaf volume (Zaidel et al. (2010). The higher fiber content of sorghum flour may be another factor contributing to the observed decrease in specific volume in this study. The low starch content of sorghum flour may also be responsible the low specific loaf volume of sorghum-containing bread. Schober et al. (2005) suggested that gluten-free breads produced from whole-grain flours that are lower in starch and higher in fiber had lower loaf volume than that made from wheat flour.

Apart from gluten content, property of wheat protein, specifically the glutenin, may also play a role on dough elasticity. (Greene & Bovell-Benjamin, 2004) investigated the difference of breads made from soft and hard wheat flour and reported that soft wheat flour yielded bread with lower loaf volume. The authors explained that this was due to the difference in the molecular mass distribution of their proteins. Hard wheat flour contained glutenin with higher chain length which made the protein phase, and consequently the dough, more extensible.

4.3.3 Texture

TPA parameters of bread crumb substituted with different levels of sorghum flour during storage were shown in Tables 4.9-4.12.

Hardness is the peak force required to deform the sample. From this study, a marked increase in crumb hardness of freshly baked bread (Day 0) was demonstrated with increasing sorghum substitution level, from 128.08 g_f in wheat control (100:0) to 1037.32 g_f in the bread with WF:SF ratio of 20:80. Greater hardness of sorghum-substituted breads correlated well with their lower specific loaf volume (Section 4.3.2). Our results are in agreement with Banu & Aprodu (2020) who reported an increase in crumb firmness with increasing sorghum flour in bread formulation. Increasing crumb hardness and crumb firmness has been well recognized in breads substituted with or made with non-wheat flour. For instance, (Trappey et al., 2015) reported that gluten-free rice bread was 4-times greater in crumb firmness than wheat bread. In the present study, the high fiber content of sorghum flour may be another factor contributing to crumb hardness of sorghum-substituted bread.

Cohesiveness is internal cohesion of the bread material. Cohesiveness is obtained by dividing the energy consumed during second compression by the energy consumed during first compression of the TPA analysis (Gupta et al., 2009). There was no major difference observed in cohesiveness with increasing sorghum flour as well as increasing storage time. Wheat bread and the 80:20 composite bread were not significantly different in crumb cohesiveness (p>0.05) while the 60:40, 40:60 and 20:80 composite breads were similar in their crumb cohesiveness. Results from this study are in agreement with Patil et al. (2016) who reported that there was no significant difference in cohesiveness of composite bread substituted with different level of extruded finger millet.

Springiness indicates the degree of recovery of the sample during the time that elapses between the end of first bite and the start of the second bite. Meanwhile, adhesiveness represents the work required to overbear the attractive forces between two contacted surfaces. From this study, it was found that sorghum substitution posed a minimal effect on both springiness and adhesiveness of the bread crumb.

Gumminess and chewiness are secondary parameters. Gumminess is a product of hardness and cohesiveness. There was a significant difference ($p \leq 0.05$) in gumminess as the level of sorghum flour increased. The results showed that gumminess increased with increasing amount of sorghum flour. For the freshly baked bread (Day 0), gumminess was found to increase from 101.23 g_f in wheat control bread (100:0) to 550.52 g_f for the 20:80 composite bread. Chewiness is the energy required to chew the bread crumb to a state ready for swallowing and is a product of hardness, cohesiveness and springiness. The results showed that chewiness was highly dependent on hardness. With increasing hardness, chewiness also increased from 37.46 g_f in wheat control (100:0) to 224.19 g_f in the 20:80 composite bread. Our results are in agreement with (Abdelghafor et al., 2011) and Patil et al. (2016) who investigated the substitution of sorghum and finger millet flour, respectively.

Physico-chemical changes during bread storage leads to crumb firming, flavor changes and loss of crispy crust, all of which constitute a process known as staling. Loss of moisture, as well as starch retrogradation, are two of the basic mechanisms operating in the firming of the crumb. Bread firming during storage is mainly caused by recrystallization of amylopectin fraction (Aguirre et al., 2011).

As the storage time increased, hardness, chewiness and gumminess were found to increase. A previous study by (He & Hoseney, 1990) indicated that the increase in firmness is related to the decrease in moisture. Moisture content has been shown to be inversely proportional to the rate of bread firming. In bread, water acts as a plasticizer. When moisture content decreases, it accelerates the interactions among starch molecules. This, in turn, speeds up the crumb firming. Results from this study are in agreement with (Baik & Chinachoti, 2000) who reported that extended storage beyond seven days led to an adverse staling or firming in bread with crust intact, possibly due to moisture migration from crumb to crust.

Table 4. 9 TPA parameters of bread substituted with different levels of sorghum flour at Day 0

Bread samples (WF:SF)	Hardness (g$_f$)	Adhesiveness	Cohesiveness	Springiness^{ns}	Gumminess (g$_f$)	Chewiness (g$_f$)
100:0 (Wheat control)	128.08±3.47^{e}	-0.28±0.48^{a}	0.79±0.10^{a}	0.37±0.03	101.23±3.76^{e}	37.46±3.81^{c}
80:20	166.40±2.52^{d}	-0.05±0.09^{a}	0.78±0.02^{a}	0.37±0.04	129.84±4.63^{d}	48.25±6.87b^{c}
60:40	192.66±3.99^{c}	-2.99±2.63^{b}	0.72±0.01^{b}	0.41±0.04	138.86±4.19^{c}	56.87±3.89^{b}
40:60	260.52±7.57^{b}	-0.82±0.29^{ab}	0.71±0.01^{b}	0.34±0.04	185.21±4.89^{b}	63.10±7.05^{b}
20:80	1037.32±9.02^{a}	-0.88±1.36^{ab}	0.53±0.00^{c}	0.41±0.03	550.52±5.13^{a}	224.19±17.67^{a}

Mean±SD of three replicates

Means in the same column with different superscript letters differ significantly at $p=0.05$.

^{ns} Means in the same column were not significantly different at $p=0.05$.

WF: wheat flour, SF: sorghum flour

Table 4. 10 TPA parameters of bread substituted with different levels of sorghum flour at Day 10

Bread samples (WF: SF)	Hardness (g_f)	Adhesive-ness ^{ns}	Cohesiveness	Springiness	Gumminess (g_f)	Chewiness (g_f)
100:0 (Wheat control)	650.23±4.12^{c}	-1.92±2.13	0.46±0.01^{b}	0.39±0.02^{b}	296.33±1.47^{d}	116.08±5.68^{c}
80:20	683.51±2.73^{b}	-2.81±2.60	0.46±0.00^{b}	0.39±0.01^{b}	314.60±1.78^{c}	123.41±2.74^{c}
60:40	689.81±1.18^{b}	-1.48±1.97	0.51±0.00^{a}	0.38±0.01^{b}	350.15±1.09^{b}	133.81±3.96^{c}
40:60	694.00±3.17^{b}	-0.78±1.35	0.50±0.01^{a}	0.38±0.03^{a}	349.10±2.41^{b}	175.12±9.27^{b}
20:80	1662.68±22.48^{a}	-0.65±0.27	0.39±0.01^{c}	0.38±0.06^{b}	640.30±6.80^{a}	240.43±37.22^{a}

Mean±SD of three replicates

Means in the same column with different superscript letters differ significantly at $p=0.05$.

^{ns} Means in the same column were not significantly different at $p=0.05$.

WF: wheat flour, SF: sorghum flour

Table 4. 11 TPA parameters of bread substituted with different levels of sorghum flour at Day 12

Bread samples (WF:SF)	Hardness (g_f)	Adhesiveness^{ns}	Cohesiveness^{ns}	Springiness	Gumminess (g_f)	Chewiness (g_f)
100:0 (Wheat control)	762.32±15.41^{d}	-0.01±0.02	0.47±0.00	0.44±0.01^{b}	357.87±6.77^{d}	156.86±1.14^{d}
80:20	807.46±4.03^{c}	-1.49±1.55	0.43±0.00	0.46±0.00^{a}	348.04±2.36^{e}	160.89±1.33^{cd}
60:40	813.45±13.44^{c}	-0.03±0.05	0.46±0.00	0.45±0.00^{ab}	373.34±3.63^{c}	167.37±1.69^{c}
40:60	975.93±4.84^{b}	-0.55±0.96	0.41±0.00	0.45±0.01^{ab}	396.73±2.31^{b}	180.55±0.83^{b}
20:80	1902.82±9.56^{a}	-0.25±0.43	0.43±0.00	0.38±0.02^{c}	817.13±3.27^{a}	310.29±10.36^{a}

Mean±SD of three replicates

Means in the same column with different superscript letters differ significantly at $p=0.05$.

^{ns} Means in the same column were not significantly different at $p=0.05$.

WF: wheat flour, SF: sorghum flour

Table 4. 12 TPA parameters of bread substituted with different levels of sorghum flour at Day 14

Bread samples (WF: SF)	Hardness (g_f)	Adhesiveness[ns]	Cohesiveness	Springiness	Gumminess (g_f)	Chewiness (g_f)
100:0 (Wheat control)	815.08±4.73[e]	-1.42±1.31	0.45±0.00[c]	0.41±0.00[b]	370.39±2.17[e]	151.49±2.06[e]
80:20	837.59±10.56[d]	-2.39±4.15	0.48±0.01[b]	0.42±0.01[b]	402.92±8.60[d]	169.64±2.25[c]
60:40	867.74±3.78[c]	-3.22±3.79	0.49±0.01[a]	0.43±0.02[ab]	430.76±5.46[c]	185.64±2.25[c]
40:60	945.08±5.19[b]	-2.54±2.50	0.49±0.00[ab]	0.46±0.03[a]	464.80±2.87[b]	213.84±17.44[b]
20:80	1945.90±12.61[a]	-1.69±1.58	0.40±0.01[d]	0.41±0.01[b]	788.51±9.04[a]	321.08±9.53[a]

Mean±SD of three replicates

Means in the same column with different superscript letters differ significantly at p=0.05.

[ns] Means in the same column were not significantly different at p=0.05.

WF: wheat flour, SF: sorghum flour

4.3.4 Water-soluble starch content

Water-soluble starch content of the bread samples was shown in Table 4.13. Upon retrogradation, starch chains re-align and re-associate via hydrogen bonds, resulting in an increase in ordered crystalline structure, which has lower water solubility as compared to the amorphous structure. Water-soluble starch content thus could be used as an index of starch retrogradation. In this study, it was found that water-soluble starch content was significantly different ($p \leq 0.05$) among the freshly baked breads (Day 0). As the level of sorghum substitution increased, water-soluble starch content decreased from 6.14% in wheat control (100:0) to 1.38% in the 20:80 composite bread. This is in agreement with the increase in RVA setback with increasing sorghum ratio (Section 4.2.1). The water-soluble starch content became decreasing as the storage time increased in all bread samples, with the greatest decrease demonstrated by the wheat control. Upon prolonged storage (Days 12 and 14), water-soluble starch content displayed only a slight difference among the samples. This is also in accord with the changes in ΔH_R and gel firmness as described earlier in Sections 4.2.3 and 4.2.4. Results from this study are in agreement with

Ghiasi et al. (1979) and (Morad & Rubenthaler, 1983) who reported a decrease in soluble starch content as the bread aged.

Table 4. 13 Water-soluble starch content of bread substituted with different levels of sorghum flour

Bread samples	Water-soluble starch content (%)			
(WF: SF)	Day 0	Day 10	Day 12	Day 14
100:0 (Wheat control)	6.14±0.16^{a}	3.19±0.11^{a}	1.60±0.71^{a}	1.49±0.10^{a}
80:20	3.75±0.31^{b}	2.75±0.03^{b}	1.40±0.21^{ab}	1.42±0.07^{a}
60:40	2.72±0.18^{c}	2.35±0.09^{c}	1.19±0.07^{abc}	1.36±0.14^{a}
40:60	2.04±0.03^{d}	2.03±0.11^{d}	0.78±0.05^{c}	0.91±0.06^{b}
20:80	1.38±0.17^{e}	1.91±0.11^{d}	1.02±0.53^{bc}	0.86±0.35^{b}

Mean±SD of three replicates

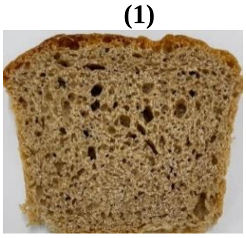
Means in the same column with different superscript letters differ significantly at p=0.05.

WF: wheat flour, SF: sorghum flour

4.3.5 Crumb structure

The crumb structure is a very important feature which affect consumers' perception and it directly relates to the loaf volume (Jafari et al., 2018). It is a common agreement that good quality bread should have high porosity with fine and regular-shaped air cells. Air cell size and number are usually determined by the gluten quality and quantity. Cell size and structure greatly influence how the crumb feels by touch or in the mouth. Thin walled, uniformly sized cells yield a soft and elastic bread texture, the properties that are usually preferred by consumers (Angioloni & Collar, 2013). Crumb structure of the bread samples are depicted in Figure 4.3.

(1) **(2)** **(3)** **(4)**



(5)

Figure 4. 3 Crumb structure of bread substituted with different levels of sorghum flour (1) 100:0 (wheat control), (2) 80:20, (3) 60:40, (4) 40:60 and (5) 20:80

On examination of the crumb structure, it revealed that the wheat control bread had numerous small air cells with regularly round shape and evenly distributed throughout the crumb. For the 80:20 and 60:40 composite breads, the air cells appeared to be of irregular shape and uneven size, with a number of larger air cells present. This was due to the reduction in gluten which decreased the ability of the dough to retain the gas. For the 40:60 and 20:80 composite breads, the crumb was very dense with fewer air cells and this resulted in the bread with very low loaf volume. Results from this study are in agreement with Chisenga et al. (2020). de la Hera et al. (2013) concluded that denser crumb and irregular air cells result in lower loaf volume. During wheat bread production, the extent to which cells are formed is a function of the protein-starch interactions more specifically from gluten that provide viscoelastic properties to the dough. As gluten-free bread lacks the means necessary to produce such a network (Trappey et al., 2015).

4.3.6 Color

Color is very important parameter in judging the properly baked bread that not only reflects the suitable raw material used for the preparation but also provides information about the formulation and quality of the product (Chavan et al. (2014). Also, color of bread is an important quality factor responsible for the consumer acceptance and influences the way a consumer evaluates a product when making a purchase (Zhang et al., 2010). The CIELAB color parameters of the bread crumb and crust are presented in Tables 4.14-4.17.

Addition of sorghum flour at different ratios affected the L*, a* and b* values of both crumb and crust. The L* of crumb and crust were significantly reduced as the levels of sorghum flour substitution increased ($p \leq 0.05$). This could be due to the inherent light brown color of the sorghum flour. Our results are in agreement with Chavan et al. (2014) who concluded that the incorporation of up to 20% of sorghum flour in bread formulation darkened both the crumb and crust. Other flours which are darker in color than wheat flour was also reported to cause a bread with darker color. For example, Mohammed et al. (2014) reported that color of crust and crumb became progressively darker as the level of chickpea substitution increased. Similarly, Zhang et al. (2010) studied the effect of sweet potato flour on the color characteristics of noodles and reported that the addition of sweet potato flour into noodles decreased the L* value.

As the amount of sorghum flour increased, there was significant increase in redness (+a*) of the crumb. The positive a* value suggests that the red tone is dominant which is most probably due to the presence of high levels of anthocyanins in sorghum (Yousif et al. (2012). For crust, there was a decrease in +a* values as the level of sorghum flour increased. The yellowness value (+b*) of the bread crumb increased as the level sorghum flour substitution increased. The positive b* value indicates a strong predominance of yellow over blue coloration. On the other hand, b* for crust became decreasing as the level of substitution increased.

Hue angle of the crumb changed from yellowness (90°) towards redness (70°) with substitution of sorghum flour. Meanwhile, chroma of bread crumb significantly increased as the sorghum substitution increased ($p \leq 0.05$), indicating the increasing color saturation. Both hue angle and chroma of the crust slightly decreased as the level of sorghum flour substitution increased. Moreover, the increasing of sorghum flour substitution caused an increase in color difference (ΔE^*) of the bread sample from the wheat control. All color parameters were consistent with the visual perception of the crumb and crust. The color parameters did not demonstrate a major change during storage.

Table 4. 14 CIELAB color parameters of bread substituted with different levels of sorghum flour at Day 0

Bread samples	Crumb						Crust					
(WF: SF)	L*	a*	b*	Hue angle	Chroma	ΔE*	L*	a^{*ns}	b*	Hue angle	Chroma	ΔE*
100:0	74.77	0.11	13.94	89.55	13.94	-	51.67	15.12	29.84	63.15	33.46	-
(Wheat control)	$\pm1.56^{a}$	$\pm0.11^{e}$	$\pm0.56^{d}$	$\pm0.44^{a}$	$\pm0.56^{e}$		$\pm1.12^{a}$	±1.08	$\pm0.24^{a}$	$\pm1.48^{a}$	$\pm0.69^{a}$	
80:20	69.49	2.04	14.61	82.04	14.75	5.69	50.80	14.59	28.04	62.52	31.60	2.08
	$\pm1.50^{b}$	$\pm0.06^{d}$	$\pm0.22^{c}$	$\pm0.16^{b}$	$\pm0.23^{d}$	$\pm1.35^{d}$	$\pm0.31^{a}$	±0.31	$\pm0.39^{a}$	$\pm0.36^{a}$	$\pm0.46^{b}$	$\pm0.52^{b}$
60:40	65.06	3.61	15.62	76.97	16.03	10.46	41.88	14.57	21.15	55.39	25.69	13.10
	$\pm1.72^{c}$	$\pm0.10^{c}$	$\pm0.22^{b}$	$\pm0.26^{c}$	$\pm0.23^{c}$	$\pm1.65^{c}$	$\pm1.08^{b}$	±0.06	$\pm1.36^{c}$	$\pm1.58^{b}$	$\pm1.15^{d}$	$\pm1.71^{a}$
40:60	62.86	4.72	15.99	73.54	16.67	12.94	41.99	14.79	21.60	55.58	26.17	12.72
	$\pm0.20^{c}$	$\pm0.47^{b}$	$\pm0.47^{b}$	$\pm0.89^{d}$	$\pm0.41^{b}$	$\pm0.21^{b}$	$\pm0.73^{b}$	±0.37	$\pm1.08^{c}$	$\pm1.04^{b}$	$\pm1.04^{d}$	$\pm1.25^{a}$
20:80	54.59	6.22	17.23	70.16	18.32	21.34	41.81	14.66	23.58	58.06	27.80	11.76
	$\pm1.35^{d}$	$\pm0.23^{a}$	$\pm0.41^{a}$	$\pm0.61^{e}$	$\pm0.18^{a}$	$\pm1.33^{a}$	$\pm1.33^{b}$	±0.98	$\pm1.62^{b}$	$\pm3.39^{b}$	$\pm0.94^{c}$	$\pm1.65^{a}$

Mean±SD of three replicates

Means in the same column with different superscript letters differ significantly at $p=0.05$.

^{ns} Means in the same column were not significantly different at $p=0.05$.

WF: wheat flour, SF: sorghum flour

Table 4. 15 CIELAB color parameters of bread substituted with different levels of sorghum flour at Day 10

Bread samples (WF: SF)	Crumb						Crust					
	L*	a*	b*	Hue angle	Chroma	ΔE*	L*	a*	b*	Hue angle	Chroma	ΔE*
100:0	72.23	0.24	13.37	88.95	13.34	-	53.14	13.56	28.73	64.73	31.77	-
(Wheat control)	$\pm0.20^{a}$	$\pm0.04^{e}$	$\pm0.33^{d}$	$\pm0.15^{a}$	$\pm0.33^{d}$		$\pm0.69^{a}$	$\pm0.20^{ab}$	$\pm0.14^{a}$	$\pm0.37^{a}$	$\pm0.14^{a}$	
80:20	67.17	2.24	14.78	81.37	14.95	5.76	43.83	13.77	22.90	58.98	26.73	11.02
	$\pm3.27^{b}$	$\pm0.11^{d}$	$\pm0.33^{c}$	$\pm0.27^{b}$	$\pm0.35^{c}$	$\pm2.93^{d}$	$\pm1.25^{b}$	$\pm0.82^{a}$	$\pm0.40^{b}$	$\pm1.66^{b}$	$\pm0.47^{b}$	$\pm1.20^{b}$
60:40	63.18	3.61	15.77	77.10	16.18	10.06	41.29	12.80	20.58	58.10	24.24	14.40
	$\pm3.15^{c}$	$\pm0.12^{c}$	$\pm0.40^{b}$	$\pm0.11^{c}$	$\pm0.42^{b}$	$\pm2.66^{c}$	$\pm0.90^{c}$	$\pm0.27^{c}$	$\pm0.91^{c}$	$\pm1.30^{b}$	$\pm0.78^{c}$	$\pm1.26^{a}$
40:60	56.48	4.62	16.14	74.01	16.76	16.60	41.31	13.20	20.95	57.79	24.77	14.17
	$\pm1.33^{d}$	$\pm0.21^{b}$	$\pm0.53^{b}$	$\pm0.66^{d}$	$\pm0.54^{b}$	$\pm1.22^{b}$	$\pm0.29^{c}$	$\pm0.48^{ab}$	$\pm0.35^{c}$	$\pm0.52^{b}$	$\pm0.56^{c}$	$\pm0.32^{a}$
20:80	52.90	6.19	17.76	70.78	18.81	20.71	40.05	14.05	20.28	55.27	24.68	15.59
	$\pm0.33^{d}$	$\pm0.18^{a}$	$\pm0.45^{a}$	$\pm0.35^{e}$	$\pm0.44^{a}$	$\pm0.36^{a}$	$\pm0.86^{c}$	$\pm0.25^{a}$	$\pm0.92^{c}$	$\pm0.77^{c}$	$\pm0.90^{c}$	$\pm1.21^{a}$

Mean±SD of three replicates

Means in the same column with different superscript letters differ significantly at $p=0.05$.

WF: wheat flour, SF: sorghum flour

Table 4. 16 CIELAB color parameters of bread substituted with different levels of sorghum flour at Day 12

Bread samples (WF: SF)	Crumb						Crust					
	L*	a*	b*	Hue angle	Chroma	ΔE*	L*	a*	b*	Hue angle	Chroma	ΔE*
100:0 (Wheat control)	72.60 ±2.60^{a}	0.29 ±0.03^{e}	12.27 ±0.43^{d}	88.65 ±0.09^{a}	12.27 ±0.43^{d}	-	50.25 ±2.19^{a}	15.39 ±0.53^{a}	29.41 ±0.43^{a}	62.38 ±0.85^{a}	31.19 ±0.49^{a}	-
80:20	67.35 ±2.64^{b}	1.61 ±0.26^{d}	11.91 ±0.50^{d}	82.32 ±0.96^{b}	12.02 ±0.53^{d}	5.48 ±2.53^{d}	48.66 ±0.77^{a}	12.40 ±0.20^{d}	19.29 ±0.48^{b}	57.27 ±0.67^{b}	22.93 ±0.45^{b}	10.69 ±0.51^{b}
60:40	63.47 ±1.32^{c}	3.12 ±0.12^{c}	13.16 ±0.19^{c}	76.68 ±0.55^{c}	13.52 ±0.19^{c}	9.60 ±1.29^{c}	42.37 ±0.83^{b}	13.81 ±0.10^{bc}	19.91 ±0.55^{b}	55.25 ±0.74^{c}	24.23 ±0.47^{b}	12.45 ±0.93^{b}
40:60	59.27 ±1.31^{d}	4.30 ±0.09^{b}	14.56 ±0.27^{b}	73.53 ±0.18^{d}	15.18 ±0.28^{b}	14.12 ±1.16^{b}	39.87 ±0.61^{c}	13.54 ±0.38^{c}	19.12 ±1.19^{b}	54.66 ±1.33^{cd}	23.43 ±1.13^{b}	14.75 ±1.22^{a}
20:80	53.19 ±0.58^{e}	6.03 ±0.14^{a}	16.03 ±0.16^{a}	69.37 ±0.41^{e}	17.12 ±0.17^{a}	20.59 ±0.59^{a}	38.92 ±0.81^{c}	14.36 ±0.16^{b}	18.92 ±0.88^{b}	52.78 ±1.56^{d}	23.75 ±0.62^{b}	15.48 ±1.18^{a}

Mean±SD of three replicates

Means in the same column with different superscript letters differ significantly at p=0.05.

WF: wheat flour, SF: sorghum flour

Table 4. 17 CIELAB color parameters of bread substituted with different levels of sorghum flour at Day 14

Bread samples (WF: SF)	Crumb						Crust					
	L*	a*	b*	Hue angle	Chroma	ΔE*	L*	a*	b*	Hue angle	Chroma	ΔE*
100:0 (Wheat control)	67.87 $\pm 2.77^{a}$	0.24 $\pm 0.24^{e}$	14.07 $\pm 1.38^{b}$	89.10 $\pm 0.83^{a}$	14.08 $\pm 1.39^{b}$	-	54.17 $\pm 5.00^{a}$	13.44 $\pm 1.02^{a}$	33.18 $\pm 1.58^{a}$	67.90 $\pm 2.37^{a}$	31.02 $\pm 1.16^{a}$	-
80:20	67.81 $\pm 1.83^{a}$	1.54 $\pm 0.13^{d}$	11.86 $\pm 0.27^{c}$	82.60 $\pm 0.46^{b}$	11.96 $\pm 0.29^{c}$	2.98 $\pm 0.21^{d}$	46.02 $\pm 1.62^{b}$	13.53 $\pm 0.22^{a}$	20.58 $\pm 0.90^{b}$	56.66 $\pm 1.41^{b}$	24.63 $\pm 0.70^{c}$	15.08 $\pm 0.54^{c}$
60:40	62.80 $\pm 1.57^{b}$	3.28 $\pm 0.33^{c}$	13.84 $\pm 0.46^{b}$	76.71 $\pm 0.86^{c}$	14.23 $\pm 0.52^{b}$	5.96 $\pm 1.42^{c}$	43.15 $\pm 1.47^{bc}$	10.42 $\pm 0.65^{b}$	15.83 $\pm 0.74^{c}$	56.65 $\pm 1.69^{b}$	18.96 $\pm 0.81^{d}$	20.81 $\pm 1.04^{a}$
40:60	59.20 $\pm 0.91^{c}$	4.33 $\pm 0.07^{b}$	14.69 $\pm 0.29^{ab}$	73.58 $\pm 0.24^{d}$	15.31 $\pm 0.29^{b}$	9.62 $\pm 0.84^{b}$	40.78 $\pm 1.44^{d}$	13.69 $\pm 0.39^{a}$	20.00 $\pm 1.20^{b}$	55.51 $\pm 2.02^{b}$	24.24 $\pm 1.81^{c}$	18.81 $\pm 2.27^{ab}$
20:80	52.27 $\pm 0.75^{d}$	6.02 $\pm 0.24^{a}$	15.78 $\pm 0.51^{a}$	69.12 $\pm 0.16^{e}$	16.89 $\pm 0.56^{a}$	16.74 $\pm 0.73^{a}$	42.09 $\pm 1.18^{bc}$	14.50 $\pm 0.29^{a}$	22.41 $\pm 1.13^{b}$	57.07 $\pm 1.84^{b}$	26.69 $\pm 0.79^{b}$	16.22 $\pm 1.65^{bc}$

Mean±SD of three replicates

Means in the same column with different superscript letters differ significantly at p=0.05.

WF: wheat flour, SF: sorghum flour

CHAPTER 5
CONCLUSION

When compared to wheat flour, sorghum flour had higher contents of crude fat, ash and crude fiber but it was lower in crude protein, starch and amylose. Sorghum flour had slightly higher water absorption capacity than wheat flour.

Addition of sorghum flour to wheat flour significantly affected the pasting properties of composite flour systems by increasing pasting temperature, final and setback viscosity but decreasing peak, trough, and breakdown viscosity as well as peak time. On gelatinization temperature and enthalpy, sorghum and wheat-sorghum composite flours exhibited similar gelatinization temperature to wheat flour. Sorghum flour demonstrated lower gelatinization enthalpy than wheat flour. However, in the case of the composite flours, gelatinization enthalpy was not different from that of wheat flour. This implied that replacement of wheat flour by wheat-sorghum composites may not require significant change in the heating process.

As of retrogradation, sorghum substitution seemed to help retard amylopectin recrystallization, particularly up to 10 days of storage. In spite of that, sorghum flour substitution was found to result in an increase in initial firmness of the flour gel, but it could decelerate the increase in firmness during storage.

With the application of wheat-sorghum composite flours in pan bread, there was no significant difference in moisture content as well as water activity of all freshly prepared bread samples. At Day 10 of storage, water activity slightly decreased as compared to Day 0. However, after Day 10, water activity was almost constant in all samples.

One major impact of sorghum substitution was on specific loaf volume. Specific loaf volume significantly decreased with increasing level of sorghum flour substitution. The decrease could be mainly attributed to gluten dilution effect. Texture was another attribute substantially affected by sorghum flour substitution. Crumb hardness increased considerably as the level of sorghum flour increased. Since chewiness and gumminess are highly dependent on hardness, they were also found to increase upon sorghum substitution. Low number of air cells along with their irregular shape and size were responsible for the inferior texture and loaf volume of the sorghum-containing bread.

For water-soluble starch, as the level of sorghum substitution increased, water-soluble starch content became decreasing, implying a lower amount of crystalline structure, or, in other words, a lower degree of starch retrogradation.

Color of crumb and crust was another quality that was greatly affected by sorghum substitution. The L* of crumb and crust significantly reduced as the levels of sorghum flour substitution increased. Upon addition of sorghum flour, redness (+a*) of the crumb increased due to the anthocyanins naturally present in sorghum. Yellowness (+b*) of the crumb also increased as the level sorghum flour substitution increased. The changes in hue angle indicate a change in crumb color from yellow to red upon adding sorghum flour. Chroma also increased as the sorghum flour substitution increased, indicating the increase in color saturation. In contrary, chroma of the crust became slightly decreasing as the level of sorghum flour substitution increased, indicating a decrease in color saturation.

In summary, sorghum flour, to a certain extent, can be used as a substitute to wheat flour in pan bread recipe. Wheat-sorghum composite is a viable alternative to 100% wheat flour at levels up to 40% substitution. The bread made from 80:20 and 60:40 wheat-sorghum composites exhibited a 30 and 50% increase in hardness from wheat bread, respectively. In areas that sorghum is locally grown and of lower price, this could reduce the volume of wheat importation.

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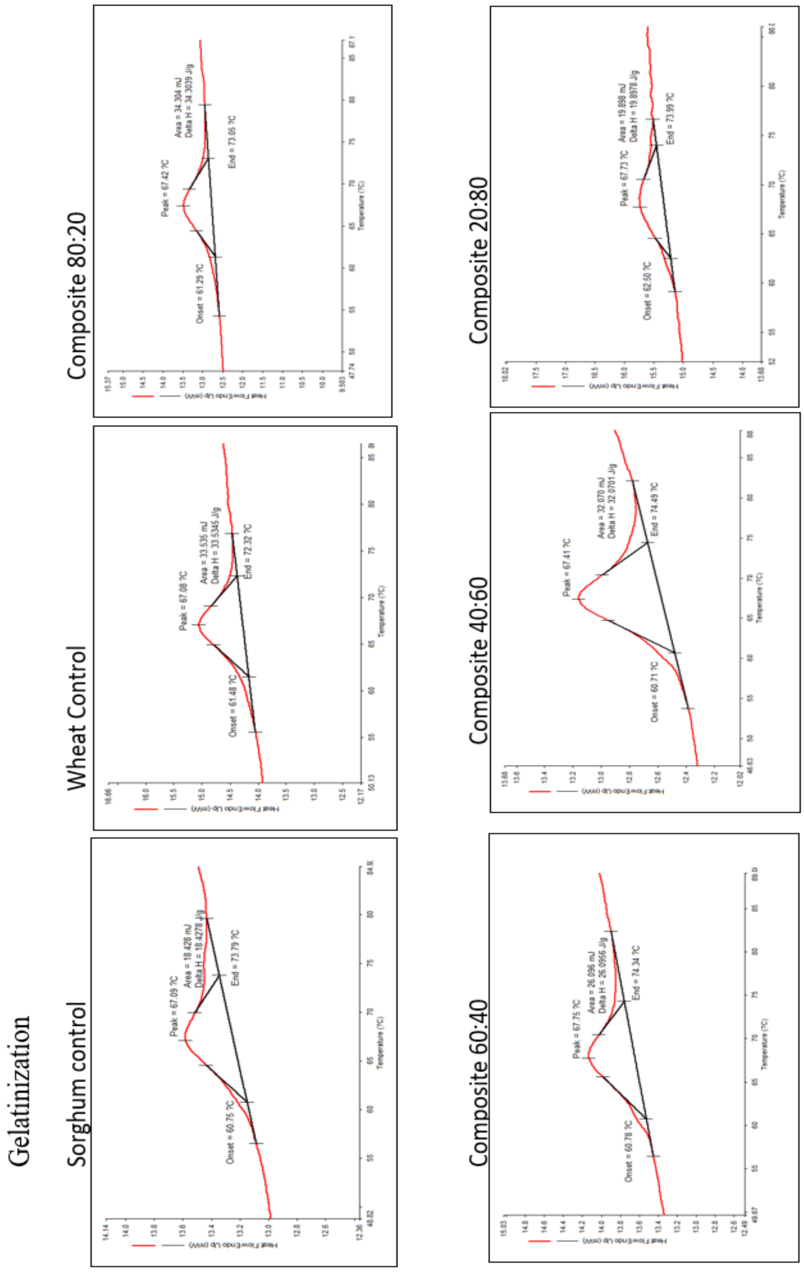
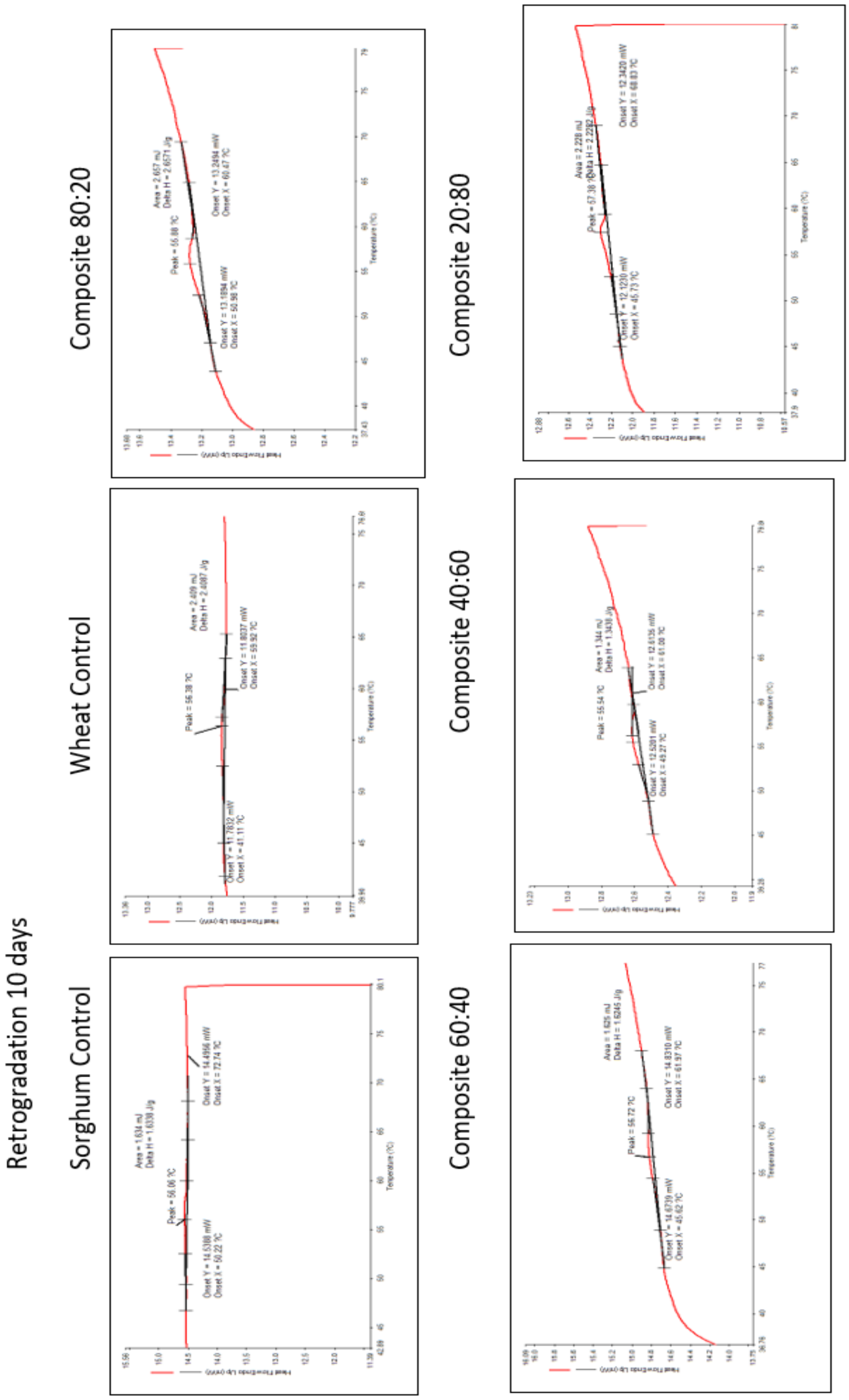
APPENDICES

Thermograms

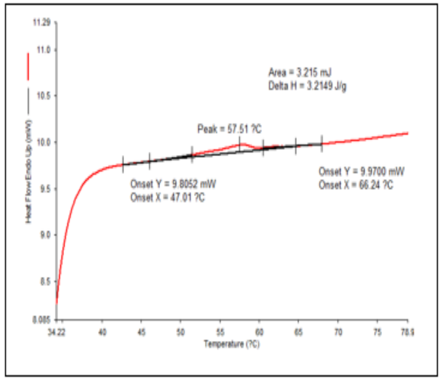


Retrogradation

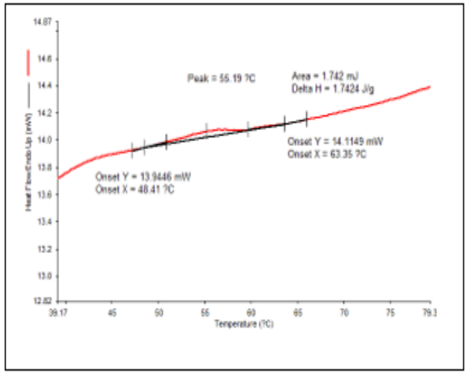


Retrogradation 12 days

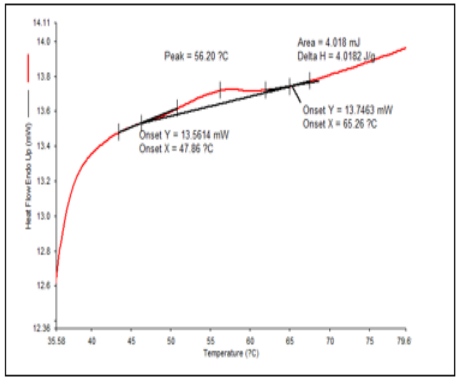
Sorghum Control



Wheat Control



Composite 80:20



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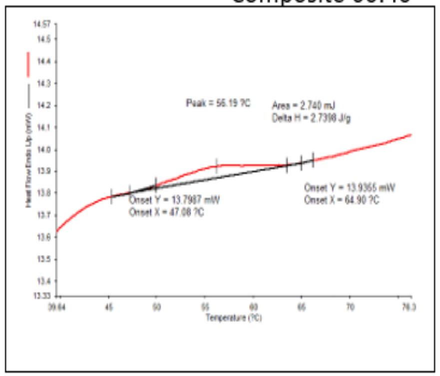

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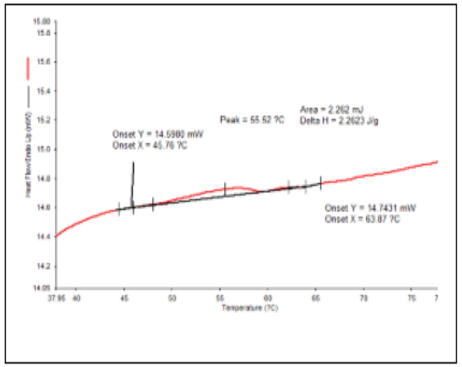
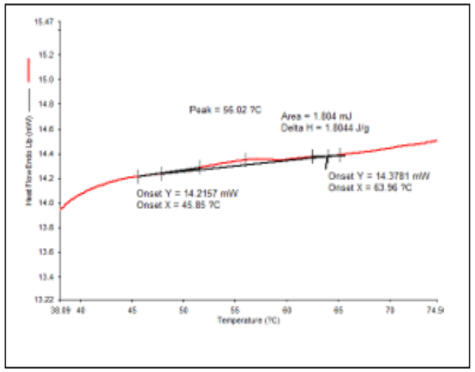
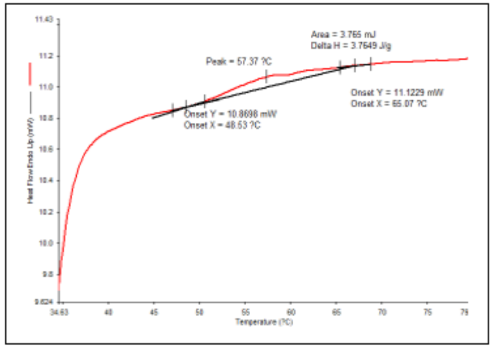


Composite 20:80



Retrogradation 14 days

Sorghum Control



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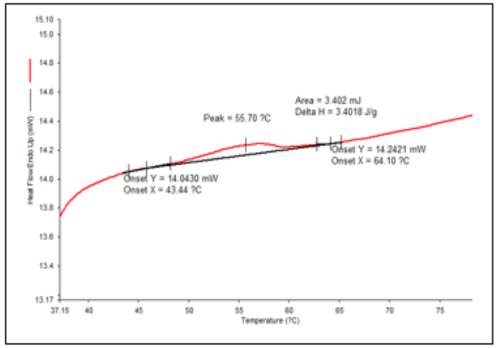

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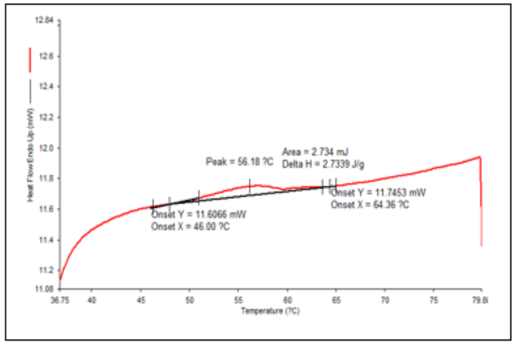

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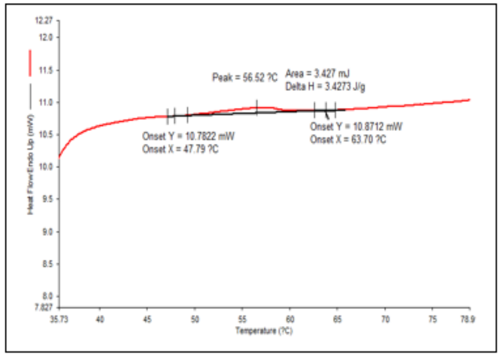

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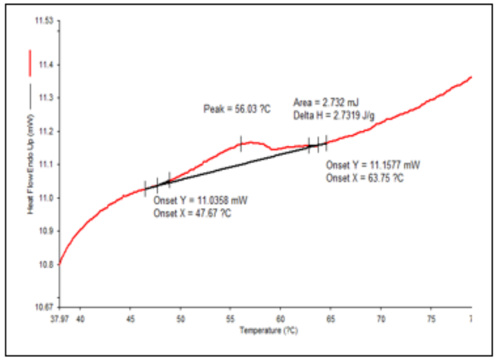

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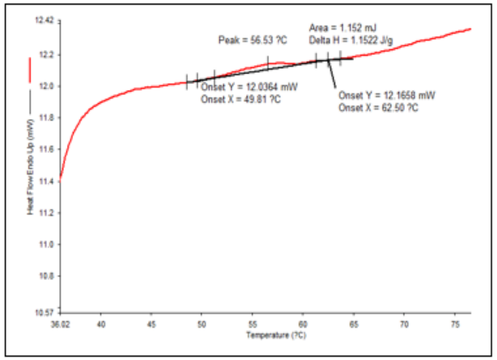

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