EFFECT OF COMBINING HIGHLY DIGESTIBLE PROTEIN AND WAXY STARCH TRAITS ON SORGHUM ENDOSPERM FUNCTIONALITY AND PROTEIN DIGESTIBILITY

A Dissertation

by

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ABSTRACT

Sorghum is known for its resilience to climate change and suitability to arid areas, having several potential health benefits such as controlling blood glucose and inflammation. However, functionality of sorghum as a food ingredient is limited due to the hydrophobic proteins. Sorghum mutant with a highly digestible (HD) protein (irregularly shaped) has potential for food application. The goal of this research was to establish the relationship between combined HD - waxy starch in hard endosperm sorghum on its functionality and protein digestibility.

First, the suitability of the standard *in vitro* pepsin assay to identify the HD protein traits in hard endosperm sorghum was checked. The pepsin assay was not reliable in distinguishing the HD and regular low digestible (LD) protein as the digestibility was not consistent across locations and year. For the first time, field emission scanning electron microscope (FE-SEM) was effective to qualitatively identifying the HD protein body.

The physicochemical properties of improved HD sorghum lines in waxy versus normal starch background were compared to the LD counterparts. HD-waxy combinations had better functionality in terms of water solubility (%) (7.10 versus 4.68 for normal LD), pasting properties (higher peak (more starch swelling) and lower final (slower retrogradation) viscosities), that indicated better interaction with water. The HD sorghum also had higher lysine content (2.15%) compared to the wild LD control (1.47% of total protein).

Finally, the improved sorghum lines with the HD protein traits were tested in model foods in comparison with the LD ones and wheat controls. Pancake, cookie and bread were

selected as model foods. The HD-normal gave pancakes with higher diameter (mm, 89.6 versus 87.5 for the LD normal) as well as higher moisture (% db, 45.2 versus 41.2 for the LD-normal) at 100% sorghum, most likely due to higher level of water absorption by the HD-protein. The same treatment also resulted in cookies of lower hardness (g, 1715 versus 4705 for the LD-normal). The HD sorghum resulted in denser bread crumb with faster firming, likely due to retained moisture migrating during storage (staling). The implication is that the improved HD-normal sorghum gave pancakes and cookies with desirable attributes making it an appealing ingredient in batter-based products as a partial substitute for wheat, or as gluten-free healthy grain with improved processing and nutritional qualities.

DEDICATION

This dissertation is dedicated to my mother and teacher, Yigebashal Gebrehana and her husband Eng. Wogderes Azene, whose unreserved support and care rescued me from dropping out of high school and maintained me on the track of success and whose advice and direction made me the person I am today. The generous support and care of Yigebashal Gebrehanna has been nurturing me from first year of high school all the way to graduate studies that led me to earning a terminal degree in one of the best universities in the world. I want to emphasize that, without the support of this kind-hearted Ethiopian Mom, I wouldn't have gotten to the stage I am at today.

This dissertation is also dedicated to my wife Tseganesh Yitbarek Haile for nursing our children, managing family and supporting me with love and affection to my success in graduate studies at Texas A&M University. Tseganesh has been a dependable and supportive partner in my life by nursing our handsome boys, Natnael and Keareyam and supporting me and the entire family not only during my PhD study but also during my masters. Tseganesh has been a "single Mom" for the last 4 years sending 3 boys including the grown up, myself. She proved to be not only the strongest and kindest mother, but also one of the committee members of my PhD dissertation research, giving me all the necessary psychological supports I needed to endure the vigorous challenges of graduate school. I truly appreciate the supports all my family: my Dad Abbu (Fikre Teferra), My Mom Abayo (Tiringo Hailu) and my siblings who have encouraged me during this time. The encouragements I have gotten from Tsegi's side of the family: Tsehay Yitbarek, Tewodros and Dagem Cherinet, Getnet, Befikadu, Sinidu, Tigist and Workiye Yitbarek as well as Adane Kefele are unforgettable.

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This work was supervised by a dissertation committee consisting of the following Lead Scientists: Professor Joseph M. Awika [Chair], who runs the Cereal Quality Lab of the Department of Soil and Crop Sciences and Professor of Food Science and Technology in the Department of Nutrition and Food Science, at Texas A&M University. Professor William L. Rooney [Member] runs Sorghum Improvement Program and is a Professor of Plant Breeding and Genetics in the Department of Soil and Crop Sciences at Texas A&M University. Professor Rhonda K. Miller [Member] is Professor of Meat Science in the Department of Animal Science at Texas A&M University. Professor Miller leads the Meat Sensory Science Lab and is also a faculty of the department of Nutrition and Food Science. Dr. Carmen Gomes [Member] was an associate professor of Nano Technology in the Department of Biological and Agricultural Engineering at Texas A&M University and is currently a professor at the Department of Mechanical Engineering, Iowa State University.

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NOMENCLATURE

AACC	American Association of Cereal Chemists [International]
ANOVA	analysis of variance [statistics]
EC	enzyme commission
EPC	extractable phenolic content
E-SEM	environmental scanning electron microscope
FC	Folin-Ciocalteu [method]
FE-SEM	field emission scanning electron microscope
FMOC	fluoromethyl-chloroformate
GAE	gallic acid equivalent
HD	highly digestible [proteins]
HG	high gluten [flour]
HI	[kernel] hardness index
HMT	heat-moisture treatment
HPLC	high pressure liquid chromatography
HSD	honestly significant difference
LD	low digestible [proteins]
LM	low magnification [microscope]
OPA	*o*-phthalaldehyde

PA	phenolic acid
PF	pastry flour
PTFE	polytetrafluoroethylene [membrane]
RS	resistant starch
RTE	ready-to-eat
RVA	rapid visco analyzer
SDS	slowly digestible starch
SE	standard error [of means]
SEM	scanning electron microscope
SKCS	single kernel characterization system
SOD	superoxide dismutase
TEM	transmission electron microscope
TKW	thousand kernel weight
UGWW	ultra-ground whole wheat [flour]
USA	United States of America
WAI	water absorption index
WSI	water solubility index

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CHAPTER I

INTRODUCTION

Grain Sorghum (*Sorghum bicolor* L. Moench) is an important food crop in arid and semi-arid areas of Africa, Asia and Latin America. As food grain, sorghum use is increasing in the western diets because of its gluten-free nature and a higher proportion of slowly digestible starch that contributes to a reduced glycemic effect as well as resistant starch (Lehmann & Robin, 2007; Lemlioglu-Austin, Turner, McDonough, & Rooney, 2012). Sorghum is also known for its beneficial health components, including dietary fibers and classes of bioactive compounds that provide cardio-protective effect and antioxidant/anti-inflammation properties (Awika & Rooney, 2004; Stefoska-Needham, Beck, Johnson, & Tapsell, 2015).

However, sorghum food applications are limited by the poor functionality of its endosperm. The reduced functionality is due to the hydrophobic proteins (kafirins) (Belton, Delgadillo, Halford, & Shewry, 2006), which limit hydration of sorghum starch, requiring higher pasting temperature and resulting in dry and sandy product texture (Wong, Lau, Cai, Singh, Pedersen, Vensel, et al., 2009). Sorghum also has a lower protein digestibility compared to other cereal grains (Duodu, Taylor, Belton, & Hamaker, 2003), which further decreases during cooking due to formation of disulfide cross-linkage of the kafirin proteins (Aboubacar, Axtell, Huang, & Hamaker, 2001). Kafirin protein bodies are organized in such a way that the γ- and β- kafirins (poorly digestible), enclose the more digestible α-kafirin (80%) (Winn, Mason, Robbins, Rooney, & Hays, 2009).

Sorghum mutants with irregularly shaped protein bodies have increased protein digestibility and may have enhanced functionality in applications for food and bioethanol production (Da Silva, Taylor, & Taylor, 2011; Oria, Hamaker, Axtell, & Huang, 2000; Weaver, Hamaker, & Axtell, 1998). Thus, the irregular protein body sorghum mutants are commonly referred to as highly digestible (HD) protein sorghum (Oria, Hamaker, Axtell, & Huang, 2000) due to their elevated *in vitro* pepsin digestibility. The HD mutation in sorghum is also associated with enhanced protein quality (increased lysine content – the most limiting amino acid in cereals) and could benefit protein malnutrition in populations that consume sorghum as a staple. The enhanced protein quality also makes the mutant sorghum appealing to the feed industry.

However, the sorghum mutant with the HD protein trait has soft endosperm, which is undesirable as it is negatively associated with agronomic production and grain milling. Soft endosperm increases susceptibility to grain pests and causes difficulty in postharvest handling and processing. Thus, breeding efforts to introduce the HD trait in agronomically competitive hard endosperm sorghum are underway. However, methods to easily identify the HD traits are not available. Furthermore, how endosperm properties (protein and starch nature) impact functionality of HD sorghum is unknown. It is believed that improvement in the major endosperm components, protein and starch is important for better functionality of sorghum in foods.

Starch is the single most abundant storage polysaccharide on earth and supplies the majority of calorie to humans. Starch for food and industrial applications are mainly obtained from cereal grains and comprises about 60 – 80% of cereal grain dry matter (Ai &

Jane, 2016). Starch has two types of polymers, amylose and amylopectin. Amylose is mainly a linear chain of glucose monomers linked by α (1-4) glycosidic bonds while amylopectin has additional branched chains with α (1-6) linkages. The proportion of amylose and amylopectin polymers in a starch granule is a major determinant of functionality in foods. Sorghum starch is classified as normal (20-30% amylose and 70-80% amylopectin) and waxy (<1% amylose and >99% amylopectin) (Dicko, Gruppen, Traoré, Voragen, & Van Berkel, 2006). Normal starch gelatinizes on cooking and rapidly re-associates on cooling forming a strong gel, which may be associated with rapid staling in baked foods. Waxy starch on the other hand, gelatinizes faster on cooking and re-associates slowly forming thin batter that does not gel. These properties are desirable in some applications, as faster swelling is related to lower energy requirement and slower re-association means delayed staling. However, lack of gelling affects the structures of baked goods like bread and cakes, ending up with a sticky mass (Sang, Bean, Seib, Pedersen, & Shi, 2008; Yan, Wu, Bean, Pedersen, Tesso, Chen, et al., 2011) that collapses easily. Starch composition is, therefore, a crucial determinant of grain functionality as ingredient in different foods and other industrial products. In an attempt to improve the endosperm functionality, the effect of combining waxy starch with HD protein traits needs to be investigated. Enhancement in the sorghum endosperm functionality has the potential to increase sorghum use in healthy foods.

Due to the importance of sorghum as a healthy food and its acceptable flavor, there has been an increasing interest in its application as a gluten-free food ingredient. There is therefore a need for improving sorghum endosperm functionality for modern food processing. The HD trait presents an important opportunity to enhance the functionality and protein digestibility of sorghum. We hypothesize that combining HD and waxy traits in hard endosperm sorghum will enhance functionality of sorghum in food applications, while also improving the nutritional quality of sorghum proteins. The goal of this study is therefore, to establish the effect of combining HD protein and waxy starch traits on sorghum endosperm functionality and protein digestibility. The specific objectives of this research are:

- To establish the suitability of *in vitro* pepsin assay to identify the HD protein trait in hard endosperm sorghum.
- To determine the physicochemical properties of hard endosperm HD sorghum lines.
- To establish the effect of combining HD and waxy traits on endosperm functionality in dough and batter-based model food systems

CHAPTER II

IDENTIFYING HD PROTEIN TRAIT IN HARD ENDOSPERM SORGHUM USING *IN VITRO* PEPSIN ASSAY *

Background

Advantages of sorghum as a food ingredient

Sorghum is known for its resilience to harsh environment and grows better than many crops in arid and semi-arid areas of Africa, Asia and Central America (Afify, El-Beltagi, El-Salam, & Omran, 2012; Morris, Ramu, Deshpande, Hash, Shah, Upadhyaya, et al., 2013; Wong, et al., 2009). Sorghum is a staple crop for half a billion people in Sub-Saharan Africa and Asia (Rhodes, Hoffmann Jr, Rooney, Ramu, Morris, & Kresovich, 2014). Some of the popular staple foods and beverages of importance include injera (leavened pancake like large traditional bread) from Ethiopia and Movite (an instant porridge) from south Africa (Taylor, 2003). There are also beverages (alcoholic and non-alcoholic) consumed in different parts of Africa that includes Obushera of Uganda (Mukisa, Byaruhanga, Muyanja, Langsrud, & Narvhus, 2017) and Gowe from Benin (Vieira-Dalodé, Akissoé, Hounhouigan, Jakobsen, & Mestres, 2015). The Ethiopian traditional "*borde*" (Abegaz, 2007) and Nigerian Milo and Malta (commercial) as well as Obiolor (traditional) (Ajiboye, Iliasu, Adeleye, Ojewuyi, Kolawole, Bello, et al., 2016) are

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also commonly made of sorghum. Sorghum is widely used for making many other traditional foods and beverages in other African countries and other regions of the World.

Sorghum use is limited to mainly animal feed and biofuel in developed world (Stefoska-Needham, Beck, Johnson, & Tapsell, 2015; Vu, Bean, Hsieh, & Shi, 2017). There is an increasing interest in the use of sorghum as food ingredient as it has many advantages. Sorghum is a gluten-free whole grain that appeals to the baked and snack foods industries. It is known to have high levels of dietary fiber, mineral nutrients and health beneficial bioactive compounds (Stefoska-Needham, Beck, Johnson, & Tapsell, 2015). Sorghum is also known for its higher proportions of resistant starch (RS) and slowly digestible starch (SDS) compared to other grains (Lehmann & Robin, 2007). The higher resistant and slowly digestible starches help the consumer experience satiety for longer time, reducing the frequency of snacking and hence cuts caloric intakes. Overall, about 50% of sorghum production goes to direct human consumption (Awika, 2011), where the proportion is much higher in Africa and India and almost negligible in the United States and other developed countries. There is an expanding use of grain sorghum in gluten-free formulations, syrup production and many other novelty products.

Limitations of sorghum use as food ingredient

The food use of sorghum is limited due to two main reasons. One of the reasons that limits sorghum use in foods is low starch functionality and it is related to the hydrophobicity of the proteins that surround the starch and limits its hydration, swelling and pasting properties. The low starch functionality results in products of dry, sandy or gritty texture that is not appreciated by the consumers (Wong, et al., 2009). This is also associated with higher pasting temperature (Taylor & Emmambux, 2010), which requires higher processing energy, potentially demanding different processing conditions from those used for other grains. The second limitation of sorghum application in foods is its extremely low protein digestibility (Duodu, Tang, Grant, Wellner, Belton, & Taylor, 2001; Duodu, Taylor, Belton, & Hamaker, 2003; Mertz, Hassen, Cairns-Whittern, Kirleis, Tu, & Axtell, 1984; Oria, Hamaker, & Shull, 1995), which further drops after wet cooking. This has limited the nutritional qualities of the foods available to large proportion of the population who live in the sorghum belt of Africa and Asia.

Sorghum storage proteins are generally known for their surface hydrophobicity and belong to prolamins called kafirins. Kafirins in sorghum exist as smooth spherical bodies (approximately 1 μm in diameter) that are distributed in the endosperm distinctly or sometimes fused to each other, starch and cell wall materials. Kafirins are classified into α, β and γ sub classes based on solubility, molecular weight and structure (Aboubacar, Axtell, Nduulu, & Hamaker, 2003). The protein bodies are arranged in a manner where the more digestible α-kafirins (80% of kafirins or 60-70% of total proteins) are surrounded by layers of the less digestible β and γ-kafirins (Hamaker, Mohamed, Habben, Huang, & Larkins, 1995). The peripheral arrangement of the β and γ-kafirins in the protein bodies, affects digestibility and functionality of both proteins and starch in sorghum and has limited its use in foods. The need to improve the protein and the starches in the sorghum endosperm to make it more functional in foods as ingredient is well recognized.

Highly digestible (HD) sorghum mutant proteins

The HD sorghum line with unusually high raw and cooked protein digestibility was discovered from the high-lysine mutant population crossed with normal line (Oria, Hamaker, Axtell, & Huang, 2000). In the HD mutant sorghum, the kafirins are organized into an irregularly shaped protein matrix with numerous pores and foldings (Hamaker, Mohamed, Habben, Huang, & Larkins, 1995; Oria, Hamaker, Axtell, & Huang, 2000). The better digestible α-kafirins are more exposed with the pores extending deep into the protein body matrices (Massafaro, Thompson, Tuinstra, & Weil, 2016) and this increases exposure surface area of the proteins for the protease enzymes to act on.

The major constituent of sorghum prolamin proteins is kafirin that makes >70% of all proteins (Stefoska-Needham, Beck, Johnson, & Tapsell, 2015). Kafirins are similar in composition to the maize zein but have higher hydrophobicity with the uncharged amino acids comprising about 61% (Xiao, Li, Li, Gonzalez, Xia, & Huang, 2014). The sorghum kafirin also has lower digestibility than maize zein. Looking at the amino acid contents, sorghum proteins are generally rich in glutamic acid, proline, leucine, alanine, valine and aspartic acid and is poor in cysteine, lysine, methionine, tyrosine and threonine with leucine and lysine being among the most abundant and limiting ones, respectively (Ahmed, Eltayeb, & Babiker, 2015). Lysine is one of the most limiting amino acids and hence its level is used as an indication of protein quality in sorghum. The HD mutant line was reported to have significantly higher lysine content than regular (LD) sorghum lines (Tesso, Ejeta, Chandrashekar, Huang, Tandjung, Lewamy, et al., 2006). In fact, Massafaro, Thompson, Tuinstra, and Weil (2016) reported that the same mutation is associated to both increased protein digestibility (HD) and lysine content. The combination of the HD protein and waxy starch trait is expected to have, not only better protein digestibility, but also better lysine levels implying improved overall protein quality. The HD proteins are also reported to have better overall functionality than the regular ones in dough and batter systems (Taylor, Belton, Beta, & Duodu, 2014; Taylor, Taylor, Campanella, & Hamaker, 2016). Goodall, Campanella, Ejeta, and Hamaker (2012) reported that the HD sorghum-wheat composite formed a viscoelastic dough mass and bread crumb texture while the normal sorghum-wheat composite did not, showing that the HD lines having better functionality compared to the LD ones.

Materials and Methods

Materials description

A total of 86 experimental lines under development to transfer the HD trait into commercial hard endosperm sorghum samples were obtained from Texas A&M University Sorghum Improvement Center. The lines were grown in 2014 and 2015 crop years in College Station and Halfway, Texas. Both normal and waxy starch traits with and without HD protein traits were included in the trial. Pepsin enzyme powder (EC 3.4.23.1, lot number: SLBP2152V, Activity ≥ 250 units/mg) was obtained from Sigma Aldrich.

HD protein identification

Identification of sorghum lines with the HD protein trait was important for screening those hybrids with improved protein quality and overall endosperm functionality. Suitability of the traditional pepsin *in vitro* digestibility assay for the HD proteins in hard endosperms were investigated first. Microscopic methods with simpler sample preparation procedures were also studied.

In vitro pepsin digestibility assay

In vitro protein digestibility was investigated using rapid pepsin assay as described by Mertz, Hassen, Cairns-Whittern, Kirleis, Tu, and Axtell (1984) and modified by Aboubacar, Axtell, Huang, and Hamaker (2001). Ground samples (200 mg, ≤1 mm particle size) for raw digestibility and wet cooked flour samples (1 mL water added, cooked at 95ºC for 25 min, then cooled to room temperature) for cooked digestibility were suspended in 35 mL pepsin solution(Mertz, Hassen, Cairns-Whittern, Kirleis, Tu, & Axtell, 1984) (1.5 mg/mL of phosphate buffer at pH = 2) and the suspension was incubated in a gently shaking water bath at 37ºC for 2 h. Immediately after incubation, the enzyme activity was stopped by changing the pH of the system with addition of 2 mL 2 N NaOH. The suspension was then centrifuged at 4900 g/4ºC for 20 min. The residue was washed twice in phosphate buffer (pH = 7) and then dried in a forced air oven at 40ºC for 24 h. Nitrogen contents of raw flours and the digestion left-over residue was determined using LECO combustion method as described by Sweeney and Rexroad (1987) and Sweeney (1989). A factor of 6.25 was used to estimate protein content. Protein digestibility was calculated as:

$Digestibility\ (\%) = \left(\frac{P_{Samples} - P_{Residue}}{P_{Samples}}\right) \times 100$, where P = protein content

Microscopy technique

Environmental scanning electron microscope (E-SEM; Model: Tescan Vega 3, Brno-Kohoutovice, Czech Republic), was used for the characterization of the structures and morphologies of protein body isolates first from known HD and LD control lines and then the new HD ones. The E-SEM was not able to clearly identify the differences between the HD and normal (LD) protein bodies. A more advanced electron one, field emission scanning electron microscope (FE-SEM) was used to check if the HD protein body structure could be distinguished.

Isolation of the protein bodies was achieved as per the method reported by Buffo, Weller, and Parkhurst (1998) with some modifications. The isolated protein body powder was used for imaging after sputter-coating (5 nm thickness) with a mixture of platinum (80%) and palladium (20%) to improve the conductivity of the sample surface.

For whole grain, dried seeds were freeze-fractured to obtain clean cleavage of the endosperm morphology and to avoid damages to the starch and protein structures. This was achieved by keeping the seed in liquid nitrogen for 3 min and gently cracking it using a small mortar and pestle. One-half of the cracked seed was then mounted on a sample holder using double-sticky carbon tape and sputter-coated similar to that for the protein isolates.

The samples were then loaded into the vacuum chamber of the FE-SEM, (Model JEOL JSM-7500F, Japan). The pressure in the specimen chamber was maintained below 3.6×10^{-4} Pa. The region of interest of the endosperm were selected under low magnification (LM) and the desired scanning/imaging of the morphology was done using the SEM mode in the range of ≤ 1 μm. The protein bodies and protein matrices were imaged and compared for different samples. In instances of excessive static charging, scanning was done in freeze-mode to obtain good quality images.

Statistical analysis

The *in vitro* pepsin digestibility of the samples was analyzed using factorial ANOVA to assess effects of genotype, growing environment and waxy starch endosperm. The mean protein digestibility of the new experimental lines was compared to know HD and LD controls using Dunnett's mean comparison procedures. Data were presented in partition distribution and box plots to show the segregation of the digestibility for the different genotype categories and environments respectively.

Results and Discussions

Classifying sorghum protein digestibility based on the in vitro pepsin assay

Consistent data that is minimally influenced by the environment and other factors is important for precise tracking of heritability of the HD protein body mutation in sorghum breeding programs. To determine if the pepsin assay could readily distinguish the HD trait in hard endosperm sorghum, the protein digestibility of 86 experimental lines known to segregate for the HD mutation from two locations (Halfway and College Station, Texas) and two cropping years (2014 and 2015), were compared to control soft endosperm HD sorghum and commercial normal protein body (LD) lines. The *in vitro* protein digestibility of the experimental lines was greatly influenced by environment (location and year) (Figure 1), with significant genetics by environment interactions (Table 1). The *in vitro* digestibility data for the raw set of experimental samples averaged 62 – 72% (range 44 –

79%) across three environments, compared to soft endosperm HD control (average 80%), and LD controls (67%). As expected, the digestibility values for the cooked samples were lower than raw samples, and averaged 50 – 56% (range 37 – 69%). Cooked HD and LD controls had digestibility averages of 65% and 49%, respectively.

Based on the *in vitro* pepsin digestibility data for raw and cooked samples, it was not possible to readily identify which of the experimental lines had the HD mutation. For one, the data among the sample set at each environment formed a continuum without any clear segregation among presumed HD vs LD lines as was previously reported (Aboubacar, Axtell, Huang, & Hamaker, 2001). Furthermore, due to significant genetics X environment interactions, several samples that would be classified as highly digestible in one environment would be low digestible in another. Another variable further confounding the pepsin digestibility data was the relative change in digestibility for cooked samples compared to the raw ones. For example, even though the average drop in protein digestibility upon cooking was 20% for the experimental lines, the individual sample variability was significant, from a 50% drop to slight increase in digestibility for some lines (see Fig 1). By comparison, the HD control dropped in digestibility an average of 17%, whereas control LD dropped by 28%.

Another variable further confounding the pepsin digestibility data was the relative change in digestibility for cooked samples compared to the raw ones. For example, even though the average drop in protein digestibility upon cooking was 20% for the experimental lines, the individual sample variability was significant, from a 50% drop to

slight increase in digestibility for some lines (see Fig 1). By comparison, the HD control dropped in digestibility an average of 17%, whereas control LD dropped by 28%.

The data clearly indicates that *in vitro* protein digestibility of sorghum is influenced by other factors in major ways that cannot be readily overcome by the presence of HD protein mutation. Non-protein related factors like polyphenol content (specifically presence and level of condensed tannins) (Awika & Rooney, 2004) and grain hardness, among others (Duodu, Taylor, Belton, & Hamaker, 2003), are known to have significant effect on protein digestibility. Because tannins can especially be a major confounder of protein digestibility, we screened for the presence of tannins in all the samples. None of the samples contained detectable tannins based on the Vanillin-HCl test (data not shown). Furthermore, the crude Folin phenol content among the samples were comparable (not shown).

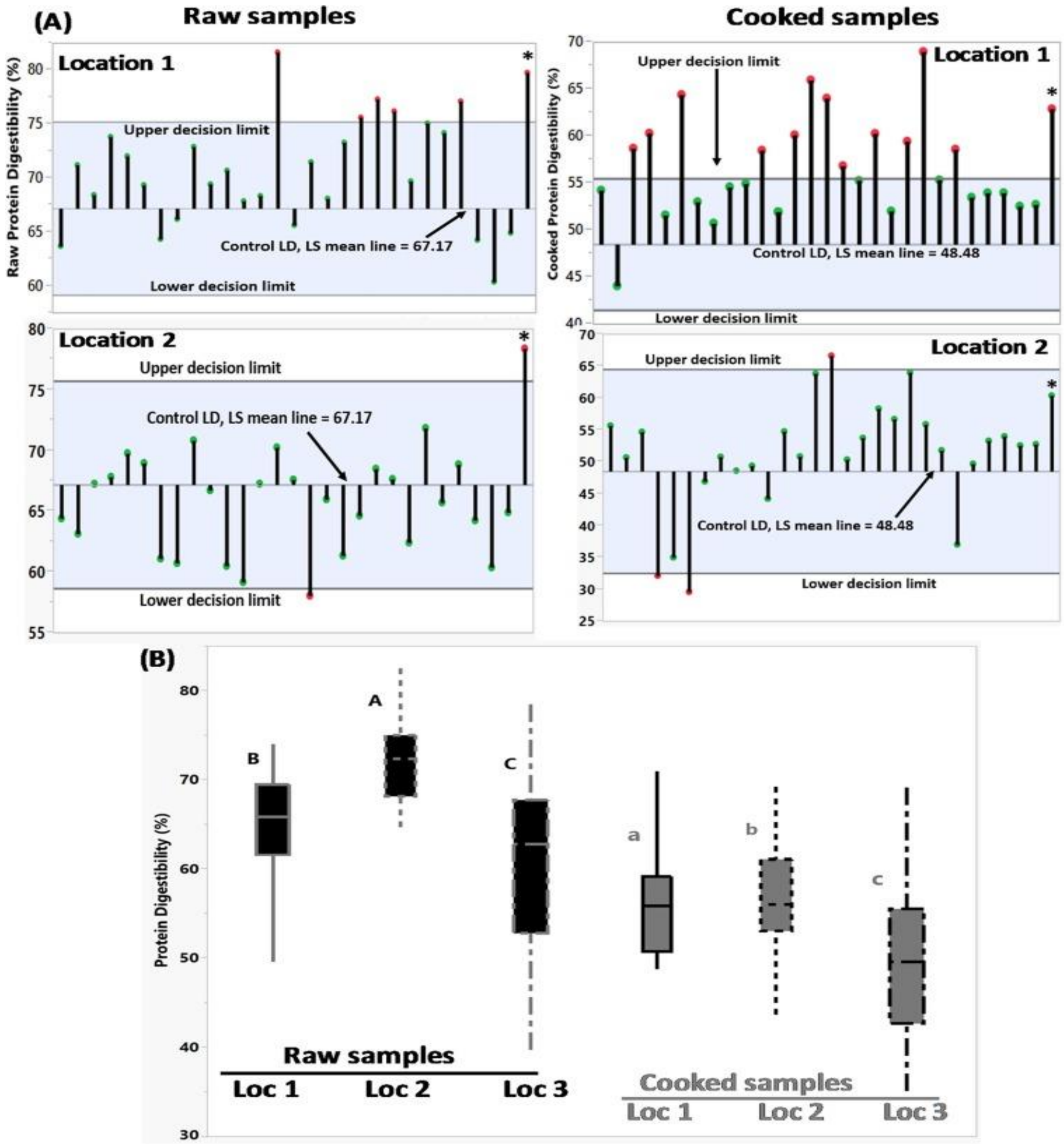

Figure 1: Needle plot of individual samples (A) and aggregate box plot (B) showing variability in protein digestibility of raw and cooked experimental sorghum samples grown in different environments. (A) Samples sequentially arranged by genotype for both locations; Control HD sample marked with *; upper and lower decision limits based on comparison to control LD (Dunnett's test, $p < 0.05$). (B) Boxes with different letters of same style are significantly different ($p < 0.05$); Loc = location. Samples were grown in College Station and Halfway, Texas in 2014, and in College Station in 2015.

Table 1: Analysis of variance table showing significant main and interaction effects environment and genetics on experimental sorghum protein digestibility.

Responses	Source	DF	Sum of Squares	F Ratio	Prob > F
Raw Protein Digestibility	Genotypes	12	1648.5602	23.8423	<.0001*
	Environment	2	1152.7531	100.0301	<.0001*
	Genotypes*Environment	24	1722.0093	12.4523	<.0001*
Cooked Protein Digestibility	Genotypes	12	1855.4666	8.2205	<.0001*
	Environment	2	516.9229	13.7412	<.0001*
	Genotypes*Environment	24	1013.5617	2.2453	0.0120*

Thus, the phenolic profile of the samples was likely not a factor in the varied protein digestibility. Obviously, specific factors that account for such wide variations in sorghum protein digestibility and cooking-induced changes in the digestibility need investigating. In all, the evidence indicates that *in vitro* pepsin digestibility may not be appropriate for screening the HD mutation trait in hard endosperm sorghum lines.

Qualitative identification of HD protein mutation using field emission scanning electron microscopy (FE-SEM)

Scanning electron microscopy is a common tool used to characterize cereal grain endosperm and starch morphology. However, the method, to our knowledge, has not been successfully used to investigate cereal protein body structure. As a potential screening tool, SEM is far simpler and quicker than the current gold standard, TEM, or even the pepsin digestibility assay. We thus initially isolated sorghum proteins to determine whether the method was viable as a tool to identify the HD protein body morphology. As previously mentioned, environmental scanning electron microscopy was unable to resolve the protein body structure. However, using the ultra-high-resolution FE-SEM, the protein body morphologies of the control HD and LD samples were easily distinguishable (Figs 2 a – c). The protein bodies of the LD sorghum, as expected, were discrete and spherical in shape, averaging ≈0.8-1 μm in diameter, whereas the HD mutant had what appeared to be a fused matrix of irregular-shaped protein bodies. The distinguishing feature of the HD sorghum mutants is the protein body with invaginated surface morphology (Oria, Hamaker, Axtell, & Huang, 2000).

With the initial success using isolated protein bodies, we used the method to characterize the sorghum grain protein body structure in situ. Again, clear differences were apparent among control and experimental HD and LD protein body structure in the endosperm (Figs 2 d – l). Our data, for the first time, provides insight on the 3-dimensional morphology and arrangement of the HD mutant protein bodies in mature sorghum endosperm. When viewed up close, the HD mutant proteins appear to form a continuous protein matrix (Fig 2 k&l), instead of the discrete spherical protein bodies in the wild type (Figs 2 j).

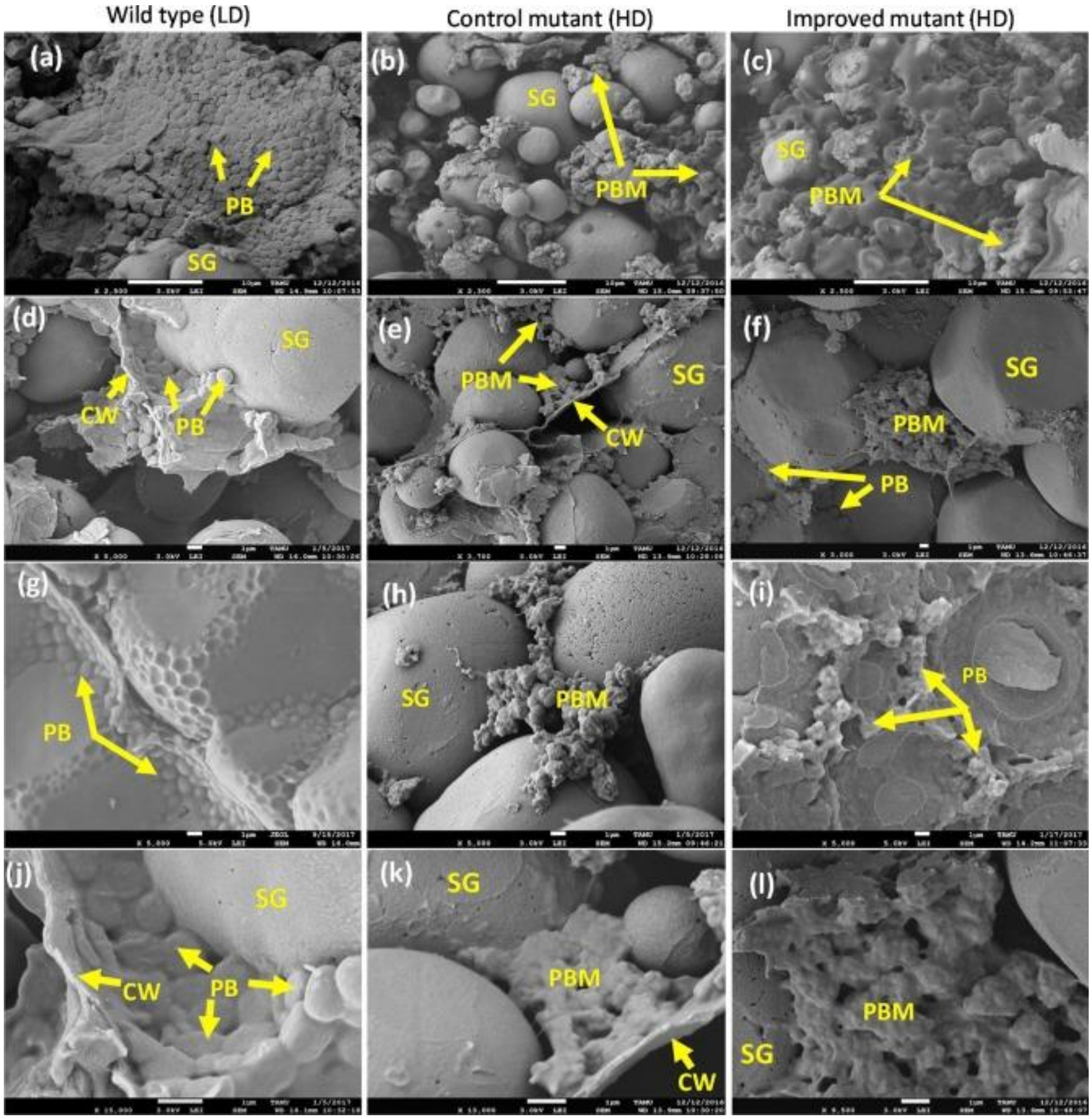

Figure 2: FE-SEM images of sorghum protein bodies from wild type (left column), soft endosperm 'high digestible' mutant control, P850029 (middle column), and experimental hard endosperm sorghum with the 'high digestible' protein body mutation (right column). First row (a-c) represent isolated protein bodies, whereas 2^{nd} - 4^{th} rows are in situ images of endosperm at medium (d-i) and high magnification (j-l). PB = protein bodies, PBM = protein body matrix; SG = starch granule; CW = cell wall; bars represent 10 µm (a-c) and 1 µm (d-l).

Thirty-six samples from the 86 experimental lines that represented wide variations in protein digestibility (Table 2), were imaged to establish the relationship between in vitro digestibility and the presence of the HD trait. The classification of the lines based on *in vitro* digestibility using Dunnett's mean separation ($p < 0.05$, against HD and LD controls) did not agree with the qualitative FE-SEM imaging results for a significant number of samples. For the raw digestibility, 82.6% of the experimental lines that had the HD mutation based on FE-SEM (Table 2, (+)) were misclassified (significantly lower in digestibility than the control HD and/or not significantly higher than control LD). Looking at the experimental LD lines, only 15.4% were misclassified as having high *in vitro* digestibility while lacking the HD protein mutation based on FE-SEM (Table 2, (-)). The large misclassification of experimental HD samples suggests that the hard endosperm trait plays an important role in influencing sorghum protein digestibility, and thus masks the ability of the pepsin assay to identify the HD trait.

Table 2: Comparison of *in vitro* protein digestibility and qualitative FE-SEM method for identification of HD protein mutation in hard endosperm sorghum.

Lines	Means PD (%)		Imaging identification (+/-)	Classification^		Lysine, (%) Total Protein
	Raw	Cooked		Raw	Cooked	
Control HD	80.1*	66.0*	+	✓	✓	2.54[a]
HD1	67.2	54.7*	+	×	✓	NA
HD2	69.8	61.0*	+	×	✓	NA
HD3	66.7	59.6*	+	×	✓	NA
HD4	69.8	35.0	+	×	×	NA
HD5	72.1	50.1	+	×	×	NA
HD6	61.4	40.2	+	×	×	1.89[f]
HD7	64.0	62.0*	+	×	✓	NA
HD8	77. 0*	62.4*	+	✓	✓	2.38[ab]
HD9	70.2	63.8*	+	×	✓	NA
HD10	82.5*	67.0*	+	✓	✓	NA
HD11	67.6	66.6*	+	×	✓	NA
HD12	66.0	66.4*	+	×	✓	NA
HD13	62.8	52.7	+	×	×	2.25[bc]
HD14	61.3	58.3	+	×	✓	NA
HD15	73.4	59.4	+	×	✓	NA
HD16	65.4	52.3	+	×	×	2.04[def]
HD17	68.5	64.0*	+	×	✓	NA
HD18	80.8*	60.4	+	×	✓	NA
SE	3.52	1.66				0.04

PD = protein digestibility; SE = standard error [of means]

Table 2 Continued

Lines	Means PD (%)		Imaging identification (+/-)	Classification^		Lysine, (%) Total Protein
	Raw	Cooked		Raw	Cooked	
HD19	74.7*	55.8	+	✓	×	NA
HD20	67.7	55.8	+	×	×	NA
HD21	74.7*	68.8*	+	✓	✓	NA
HD22	64.8	54.3	+	×	×	2.57[a]
HD23	72.5	57.6*	+	×	✓	2.20[bcd]
Control LD	67.2	48.5**	+	✓	✓	1.47[g]
LD1	56.0**	40.4**	-	✓	✓	1.98[ef]
LD2	59.5**	41.2**	-	✓	✓	2.04[def]
LD3	62.6**	36.5**	-	✓	✓	1.92[f]
LD4	46.2**	38.2**	-	✓	✓	2.14[cde]
LD5	64.4**	55.6	-	✓	×	NA
LD6	65.4**	52.4	-	✓	✓	NA
LD7	63.1**	50.6	-	✓	✓	NA
LD8	71.5	44.5**	-	×	✓	NA
LD9	49.9**	37.6**	-	✓	✓	NA
LD10	60.0**	61.7	-	✓	×	NA
SE	3.52	1.66				0.04

PD = protein digestibility; SE = standard error [of means]

Table 2 Continued

Lines	Means PD (%)		Imaging identification (+/-)	Classification^		Lysine, (%) Total Protein
	Raw	Cooked		Raw	Cooked	
LD11	65.7	49.6	+	✓	✓	2.02^{cde}
LD12	74.4	52.3	+	×	×	NA
LD13	63.4	36.9	+	✓	✓	1.71^{f}
SE	3.52	1.66				0.04
Summary				% miss-classification		
Range, HD	61.3 – 77.0	35.0 – 67.0	+	82.6	34.8	1.89-2.57
Mean, HD (n=46)	69.3	57.7	+			2.31^{A}
Range, LD	46.2 – 74.4	36.5 – 61.7	-	15.4	23.08	1.71-2.14
Mean, LD (n=26)	62.4	46	-			2.09B

PD = protein digestibility; SE = standard error [of means]

For the cooked digestibility, 34.8% of the experimental HD lines were misclassified (significantly lower than the HD control and/or not significantly higher than the LD control). For instance, three waxy lines (HD4, HD5 and HD6) had low *in vitro* digestibility on cooking (Table 2) and were statistically categorized as LD lines. The FE-SEM technique, however, showed that all these waxy lines had the HD protein trait. On the other hand, four other lines (LD5, LD6, LD10 and LD12) had high *in vitro* digestibility for cooked samples comparable (p>0.05, Dunnett's) to the control HD, but were found to lack the HD protein mutation and had the regular spherical LD protein bodies. The HD mutation is assumed to limit extensive disulfide cross-linkages believed responsible for the reduced digestibility of sorghum proteins upon wet cooking (Hamaker, Kirleis, Butler, Axtell, & Mertz, 1987). However, the fact that the drop in protein digestibility on cooking were significant for both the true HD and true LD lines (Table 2), suggests that this assumption may not be entirely accurate. There is a possibility that a yet unidentified factor(s) leads to the reduced digestibility of proteins in hard endosperm sorghum, independent of the HD mutation. The data definitely brings to question the true magnitude of impact of the protein body mutation on sorghum protein digestibility, given the 'highly digestible protein' classification was originally mostly based on sorghums that had softer endosperm than wild types used for comparison (Weaver, Hamaker, & Axtell, 1998). Thus, in hard endosperm sorghum, the protein body mutation should perhaps not be referred to as 'highly digestible' as is standard in literature, until more reliable *in vivo* or human clinical data on the true protein digestibility becomes available.

The data distribution (Figure 3) as well as the range and means of the experimental HD (henceforth referring to the protein body mutation, not pepsin digestibility) and LD lines that were imaged (Table 2) showed significant overlap in protein digestibility for the two categories. This confirms that the *in vitro* enzyme assay is not an effective way of screening the HD mutation in hard endosperm sorghum. The qualitative FE-SEM, where available, should be a valuable tool for definitively identifying the HD mutation in sorghum grain.

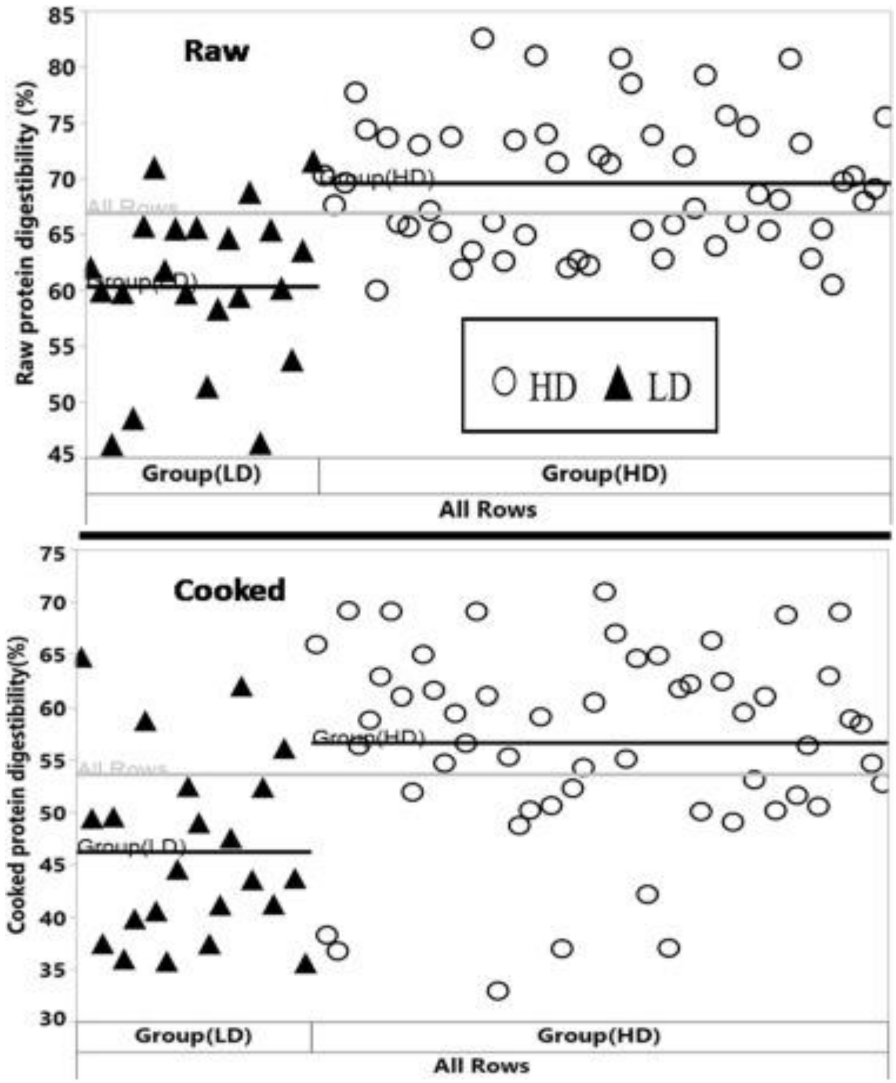

Figure 3: Scatter plot of *in vitro* protein digestibility values for raw and cooked samples of the experimental sorghum lines. Without (LD; ▲) and with the 'high digestible' protein body mutation (HD; ○); after samples qualitatively classified as LD or HD based on FE-SEM imaging.

Among samples that were imaged, the mean *in vitro* digestibility (%) of the experimental lines having the HD mutation ranged between 61.3 and 77.0, and between 35.0 and 67.0 for the raw and cooked samples, respectively. By comparison, the soft endosperm control HD had protein digestibility of 80.1 for raw and 66.0% for cooked samples, (Table 2). The mean digestibility values (%) for the LD samples (no HD protein mutation trait), ranged between 46.2 and 74.4 for the raw and between 36.5 and 61.7 for the cooked samples compared to the LD control mean values of 67.2 for the raw and 48.5 for the cooked samples. Despite the relatively poor predictive power of the pepsin assay, it was encouraging to note that the average protein digestibility for the experimental HD lines was significantly higher than the LD lines (Table 2; Fig 3). The experimental HD lines average digestibility was 69.3% for the raw and 57.7% for the cooked samples; whereas the values were 62.4% and 46.0% for the raw and cooked experimental LD samples, respectively. Furthermore, the average drop in cooked digestibility was higher for the LD lines (23%) compared to HD lines (17%). These differences are practically relevant and imply obviously superior nutritional quality of the HD sorghum lines. However, this should be confirmed in *in vivo* models. On average, introducing the HD mutation in hard endosperm sorghum improves protein nutrition quality of sorghum, regardless of starch composition.

Chapter Summary

Protein digestibility analysis using *in vitro* pepsin assay was inconsistent in establishing presence of 'HD' protein mutation in hard endosperm sorghum, and is thus not recommended for definitive trait identification. The assay appears to be strongly influenced by other factors unrelated to the protein body mutation, including growth environment and grain hardness, among others. We showed, for the first time, that the relatively rapid high-resolution field emission scanning electron microscopy (FE-SEM) can readily distinguish the protein body mutation from normal sorghum protein body structure in sorghum, regardless of endosperm hardness. The method is thus useful for tracking the heritability of the mutated protein body trait. However, capital cost of the FE-SEM equipment is a limitation. Because the pepsin digestibility assay did not accurately predict presence of the trait in hard endosperm sorghum, it is debatable as to whether the protein body mutants should be referred to as HD (highly digestible protein) sorghums.

CHAPTER III

PHYSICOCHEMICAL CHARACTERIZATION OF IMPROVED HD LINES†

Background

Characterization of the new HD-waxy experimental lines in comparison with the wild types is important to see if there is any change in the functionality of the endosperms due to the combinations of the HD proteins and waxy starches into hard endosperm hybrids. Looking at the well-established quality parameters as indicators of overall performance of the new lines is an easy strategy. The starch functionality using the pasting characteristics, water absorption and solubility indices were selected to show the interactions of the flours with water. Protein composition in terms of essential amino acid (lysine) contents was chosen to see of the improved HD lines have better protein quality compared to the wild types. Phenolics levels, phenolic acid and flavonoid profiles as indicators of bioactive compounds were chosen. The objective of this component of the research was to establish the effect of HD trait on physicochemical properties of sorghum in waxy and normal starch backgrounds and correlate the properties to grain functionality.

Starch functionality in normal and waxy sorghum

Starch functionality is important physicochemical properties of flours from different sources in order to predict their performances in different food applications. Starch is a polymer organized into a defined semi-crystalline shape. Starch granule has two

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types of polymers, namely amylose and amylopectin. Amylose is a linear chain of glucoses attached via α (1-4) linkages whereas amylopectin is a branched chain with additional α (1-6) linkages (5%) at the branching points. The proportion of the amylose and amylopectin in the granules determine the functionality of the starch and this in turn dictates the desirability in different forms of foods. Starch is classified into two types (normal and waxy) based on the proportion of amylose and amylopectin. In sorghum, normal starch has amylose content of 20-30% and the amylopectin is 70-80%, whereas waxy starch has mostly (>99%) amylopectin (Dicko, Gruppen, Traoré, Voragen, & Van Berkel, 2006)

Native starch (non-processed, raw) is water insoluble and forms a slurry regardless of its type. When heated in excess water, however, normal and waxy starches behave differently. Starch absorbs water and starts to swell, which results in increased viscosity. With continued heating, the starch granules bursts and result in a slight drop in viscosity. Normal starch (amylose) swells and bursts slowly, requiring higher processing energy.

Waxy starch (amylopectin) on the other hand, absorbs water and swells more rapidly due to the branched nature of the polymers. Waxy starch is very slow to retrograde due to steric hindrance and is associated to reduced rate of staling. It was reported that waxy sorghum is highly suitable for low moisture ready-to-eat (RTE) snack products such as flakes and granolas that are made by micronization and popping processes (Celis, Rooney, McDonough, & Production, 1996). However, waxy starch was reported to have poor performance in high moisture baked products as it resulted in sticky and dense crumbs (Taylor, Schober, & Bean, 2006). Waxy sorghum starch was reported to perform well in brewing (Pozo-Insfran, Urias-Lugo, Hernandez-Brenes, & Saldivar, 2004).

Figueroa, Martinez, and Rios (1995) demonstrated that waxy sorghum adjunct required lower gelatinization temperature and shorter conversion time. It was also reported that waxy sorghum wort had a high filtration rate (Osorio-Morales, Saldivar, Contreras, Almeida-Dominguez, & Rooney, 2000) and the fermentation process (Moguel, Gante, & Saldivar, 2001). The obtained beer (Moguel, Gante, & Saldivar, 2001) were also similar to those of other grain adjuncts. We hypothesize that having waxy starch and HD protein mutation combined in a hard endosperm will enhance sorghum endosperm interaction with water and results in less gritty food products due to the branched amylopectin starch and the irregularly shaped protein body mutants.

Water absorption and solubility indices

Water absorption index (WAI (g/g)) and water solubility index (WSI (%)) are important quality parameters for grains flours and other particulate food ingredients used in high moisture intermediate food formulations such as batter and dough in baked foods and extrudate processing operations. WAI and WSI indicate how readily the ingredients interact with water in the mixing processes. WAI shows the level of moisture absorbed where as WSI indicates the amount of soluble components of flour preparations that goes into the water (Mahasukhonthachat, Sopade, & Gidley, 2010). The WAI and WSI parameters can also be used to characterize the composition in terms of starch and protein types that are available in the ingredients (Pelembe, Erasmus, & Taylor, 2002). The improved sorghum lines, with HD protein and waxy starch, are expected to have better interaction with water due to ready hydration of both the waxy starch and HD protein components.

Amino acid profile of sorghum

The kafirin protein of grain sorghum (Xiao, Li, Li, Gonzalez, Xia, & Huang, 2014) is closely related to the zein of maize (Gianazza, Viglienghi, Righetti, Salamini, & Soave, 1977) in terms of amino acid composition. Both kafirin and zein are characterized as having higher proportion of glutamic acid, leucine, alanine and proline as abundant amino acids. Leucine is the most abundant essential amino acids with lysine being the most limiting one (Ahmed, Eltayeb, & Babiker, 2015).

The HD mutant sorghum was reported to have higher lysine content than the regular sorghum cultivars (Winn, Mason, Robbins, Rooney, & Hays, 2009). It was recently reported that the same mutation is responsible for both improved digestibility and increased lysine contents in the HD mutant sorghum line (Massafaro, Thompson, Tuinstra, & Weil, 2016). Characterization of the new experimental HD lines in terms of their lysine contents is important.

Phytochemicals in sorghum and their health benefits

Sorghum has appreciable concentrations of health beneficial phytochemicals including phenolic compounds, phytosterols and policosanols (Awika & Rooney, 2004; Girard & Awika, 2018). The dominant phytochemicals in sorghum are the phenolic compounds, including the phenolic acids and flavonoids. The phenolic acids are mainly benzoic and cinnamic acid derivatives, while flavonoids are classified into several subclasses including 3-deoxyanthocyanins, flavones, flavanones and condensed tannins (proanthocyanidins) (Awika & Rooney, 2004; Dykes, Seitz, Rooney, & Rooney, 2009; Girard & Awika, 2018). Phytochemicals have gained increasing interest as functional components of human diets because they play important roles as antioxidant, anti-inflammation, cholesterol lowering agents and other potential health promoting effects. A recent study involving healthy human subjects showed that pasta containing red whole grain sorghum significantly improved antioxidant status by increasing plasma polyphenols, antioxidant capacity and superoxide dismutase (SOD) activity, while decreasing a marker of protein oxidation compared to a control pasta from durum wheat semolina (Khan, Yousif, Johnson, & Gamlath, 2015). Apart from the antioxidant activities, polymeric tannins from sorghum were reported to reduce *in vitro* digestibility of partially gelatinized starch, with the potential of reducing caloric intake (Amoako & Awika, 2016). Girard and Awika (2018) recently reviewed evidences of health beneficial effects of sorghum polyphenols from *in vitro, in vivo* and human trials.

Sorghum polyphenols have also been shown to be important natural functional ingredients as food colorant and dough and batter improvers. Condensed tannins (proanthocyanidins) from sorghum were reported to improve dough rheology by interacting with gliadin and glutenin fractions of wheat glutens, which increased dough strength and resistance to overmixing (Girard, Bean, Tilley, Adrianos, & Awika, 2018; Girard, Castell-Perez, Bean, Adrianos, & Awika, 2016). It was shown in the same research that proanthocyanidins from sorghum performed better than oligomeric tannins from grapes, showing the potential of sorghum use as ingredient for both nutritional and functional advantages. Characterization of the new experimental hybrids with combined HD proteins and waxy starch in comparison with their wild counterparts is important as phytochemical composition is one of the crucial health beneficial and functional properties that make sorghum appealing to the food industry and the consumers.

Materials and Methods

Physical properties of sorghum

A total of 86 sorghum lines were characterized. These included 25 lines from 2014 grown at College Station and Halfway and the remaining from 2015 crop year grown in College Station, Texas. Rapid iodine staining technique described by Pedersen, Bean, Funnell, and Graybosch (2004) was used to identify waxy phenotypes. Single seeds were randomly selected and crushed in 6 replications. The samples were transferred into a small test tube with 1 mL of water and cooked in a water bath at 95℃ for 25 min. The cooked seeds (gelatinized starch) were cooled to room temperature for 25 min and 50 µL of iodine solution (2.5 g potassium iodate (KI), 250 mg I_2, 125 mL of distilled water). The color score was made in less 60 min where those developing dark blue were considered normal and those forming various shades of magenta were considered waxy. The approximate percentage of waxy seeds was calculated by counting the number of seeds with the magenta color out of the total replications. The kernel hardness and weight were assessed using a single kernel characterization system (SKCS 4100, Perten Instruments, Springfield, Illinois). The system records kernel hardness, seed diameter and weight for 300 individual kernels and reports the average values. Grain density was analyzed using a gas displacement multi pycnometer (MVP-1, Quanta Chrome).

The samples were cleaned and milled (UDY Cyclone sample mill model: 3010-014, Fort Collins, USA) to pass through a 1 mm screen size. The samples were then packed in plastic bags and stored in a refrigerator until used for different analysis.

Water absorption and solubility indices

Water absorption index (WAI) and water solubility index (WSI) are important properties of flours and other dry food ingredients. These properties indicate how readily an ingredient hydrate and incorporate well with others in dough and batter-based food systems. The WAI and WSI of the ground sorghum samples were analyzed using the methods explained by Mahasukhonthachat, Sopade, and Gidley (2010) with a slight modification. Flour sample of 1 g (W_1) was mixed with 15 mL distilled water in a previously weighed (W_2) centrifuge tubes and then incubated at room temperature (25ºC) with orbital shaking (VWR orbital shaker advanced 3500, VWR, USA) at 184 cycles/min for 30 min. The mixture was then centrifuged (Heraeus, Thermo Scientific, USA) at 2795 g for 20 min. The residue (with the absorbed water and tube) was weighed and the weight of the absorbed water (W_3) is determined after deducting the weights of the tube (W_1) and the flour samples (W_2). The supernatant was dried in an oven at 105ºC for 24 h and weighed (W_4). The tube with the residue (gel) was weighed (W_5). The WAI and WSI were computed as:

$$WAI\,(g/g) = \frac{W_5 - W_2}{W_1}\,;\; WSI\,(\%) = \left(\frac{W_4 - W_3}{W_1}\right) \times 100$$

Starch pasting properties

Pasting properties of the sorghum lines were determined using a Rapid Visco Analyzer (RVA) (RVA-4, Newport Scientific, Maryland, USA) based on the method used by Yan, et al. (2011). For sample preparation, 3 g of sorghum flour (12% moisture basis) and distilled water (25 mL) are used. The standard 23 min analysis profile (AACC International Approved Method 76-21.01, 2010) was followed. The slurry was heated to

90°C and held at this temperature for 6 min and then cooled with continuous stirring and measurement of viscosity.

Chemical Characterization

Amino acid content: lysine

Lysine is the most limiting amino acids in sorghum (Virupaksha & Sastry, 1968) and the importance of increasing lysine content was recognized in the 1970s. The HD sorghum mutant originally discovered was naturally high in lysine content (Mohan, 1975). In the current study, lysine content was compared for selected improved HD and LD sorghum lines as well as the controls. Massafaro, Thompson, Tuinstra, and Weil (2016) recently reported that the same mutation is responsible for both improved digestibility and increased lysine contents in the HD mutant sorghum lines. The amino acid profile of selected samples were analyzed using the method described by Dai, Wu, Jia, and Wu (2014) with some modifications. Finely milled samples (100 mg) were acid hydrolyzed using 300 µM of 6 N HCl at 100ºC for 22 h. Hydrolyzed proteins samples and free amino acids were derivatized pre-column with *o*-phthalaldehyde (OPA) and 9-fluoromethyl-chloroformate (FMOC) prior to separation and quantitation by reverse phase HPLC (Agilent 1260) equipped with an auto-sampler and fluorescence detector. Quantification of each of the amino acids was done using respective commercial standards.

Extractable phenolic compounds (EPC)

The levels of total phenolic compounds in sorghum and its correlations with protein digestibility is important to investigate. The extractable phenolic content of selected lines were determined using the Folin-Ciocalteu (FC) method described by Kaluza, McGrath, Roberts, and Schroeder (1980). Extraction was done using acidified (1%) methanol where 100 mg of flour samples are mixed with 10 mL of the solvent and shaken for 2 h. The extracts were separated by centrifugation at 671 g for 5 min. The extracts (0.1 mL) were added into 1.1 mL distilled water and then reacted with 0.4 mL FC reagent and 0.9 mL 0.5 M ethanolamine for 20 min at room temperature. The absorbance was measured using a UV-visible spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan) at 600 nm against a reagent blank. The EPC values were calculated as per the following equation and expressed as micrograms of gallic acid equivalent per gram of sorghum samples (μg GAE/g) on dry weight basis using the calibration curve of gallic acid as a standard.

$$Total\ phenols\ \left(\frac{\mu g}{g}\right) = \left(\frac{A - b}{m}\right) \times (V_{ES} \div V_S \div W_S)$$

where A = absorbance; b = intercept and m = slope of the standard curve; V_{ES} = volume (mL) of extraction solvent and V_S = volume of sample extract used; W_S = weight (g) of the sample used for extraction.

Phenolic acid and flavonoid profiles using HPLC

The phenolic acid and flavonoid profiles of the sorghum is crucial for potential impact on protein digestibility, sensory properties, and also for beneficial health effects of the compounds in the improved hybrids as ingredient. Extraction of soluble phenolics were performed according to Ravisankar, Abegaz, and Awika (2018), with slight modifications. Ground sample of 1 g was extracted in 80% methanol (1:5 flour:solvent, w/v) for 2 h continuously shaking at 200 cycles/min at room temperature (Standard Analog Shaker, VWR, Radnor, PA). The extract was centrifuged (10,000 g for 10 min) using a Heraeus

Megafuge 11R Centrifuge (Thermo Fisher Scientific, Asheville, NC) at 20°C and the supernatant was transferred to a new set of tubes. The residue was further extracted twice (1:2.5 residue:solvent (same solvent), w/v) each time for 30 min and centrifuged. The supernatants from the different batches were combined and stored at −20°C until further use.

For the HPLC profiling of the extracts from the new HD lines, aliquots of the purified extract (400 μL) was each separately mixed with 400 μL of methanol acidified with 0.05% formic acid and filtered through a syringe with a 0.2 μm polytetrafluoroethylene (PTFE) membrane filter prior to injection into the HPLC. The HPLC analysis was conducted using an Agilent 1200 series LC system, equipped with a G1322A vacuum degasser, a G1311A quaternary pump, G1364C analytical auto-sampler, G1316B thermostated column compartment, and a G1315C Diode Array Detector (DAD, Agilent Technologies, Palo Alto, CA, USA) at Multiple Wavelength (280, 325, 340, 360, 480 and 520 nm. Chromatographic separation was performed on a Phenomenex Luna-C18(2) column (4.6 × 150 mm, 5 μm, 100A; Phenomenex Companies, CA, USA). The mobile phase consisted of aqueous 2% v/v formic acid solution (A) and acetonitrile acidified with 2% formic acid (B). Using an injection volume of 20 μL in each analysis, the elution gradient was carried out at a flow rate of 1.0 mL/min with the column temperature thermostated at 40°C. The gradient profile was programmed at 0 – 10% B from 0 – 4 min, 0 – 20% B from 5 – 10 min, 0– 32% B from 11 – 25 min, 32 – 50% B from 26 – 32 min, 50 – 10% B from 33 – 36 min.

Statistical Analysis

Data on in vitro protein digestibility, lysine content, starch pasting properties and other quantitative physical parameters were obtained in duplicates and processed in a factorial analysis of variance (ANOVA), where genotype, growing environment and other variables were considered separately and in combination. Dunnett's mean separation procedure was employed to compare *in vitro* protein digestibility of the experimental sorghum lines with positive (HD) and negative (LD) control lines. Tukey's multiple comparison was used for comparing the means of other physical parameters.

Results and Discussions

Effect of HD mutation on sorghum kernel physical properties

The experimental HD lines exhibited kernel hardness index values (68.3 – 77.6) comparable to the experimental LD lines (77.1 -82.2), and significantly ($p<0.05$) higher than control HD (29.7) (Table 3). The improved hardness in the experimental HD lines corresponded to increased proportion of corneous to floury endosperm based on visual and microscopy observations (not shown). The kernel hardness did not significantly differ for the HD and LD experimental lines when further categorized into waxy and normal starch traits. A weak but significant ($p<0.05$) negative correlation was observed between kernel hardness and protein digestibility ($r = –0.32$ for the raw and –0.37 for the cooked samples). This suggests that, kernel hardness, though important to grain quality, may to some extent negatively contribute to sorghum protein digestibility even when HD trait is present. This factor should be taken into account in breeding programs.

Thousand kernel weights, TKW, (21.9 – 31.2 g), grain density 1.34 – 1.39 g/cc), and seed size (2.41 – 2.80 mm) were within expected ranges (Table 3). The experimental LD lines seemed to have higher TKW values compared to their HD counterparts, and this may have been due to their slightly larger seed size; however, these differences were not practically relevant. As expected, grain density had significant positive correlation ($r = 0.70$) with the kernel hardness values, because more dense and compacted kernels (high density) generally are harder to crush or break.

Effect of HD trait on sorghum flour water absorption index (WAI) and water solubility index (WSI)

We did not detect significant differences in WAI of the HD and LD lines, although overall trends showed higher WAI for the HD lines (Table 3). Similar to a previous report (Elhassan, Naushad Emmambux, Hays, Peterson, & Taylor, 2015), the soft endosperm HD control had higher ($p < 0.05$) WSI than the control LD sorghum (Table 3). This could be partly attributed to the less compact (soft) endosperm structure of the HD sorghum, which produces finer flour particles upon grinding, and thus increased surface area for hydration. However, this trend was maintained in the hard endosperm experimental lines, where the HD samples generally tended to have higher WSI than LD lines of similar starch composition (Table 3). This suggests the HD protein body structure likely contributes to the improved WSI of sorghum flour. The fact that the hydrophilic α-kafirin proteins are more exposed in the HD protein bodies (Oria, Hamaker, Axtell, & Huang, 2000) likely makes the HD proteins absorb water more readily and solubilize. This may confer an advantage to the HD sorghums in dough and batter-based systems (Elhassan, Naushad

Emmambux, Hays, Peterson, & Taylor, 2015). Higher WSI indicates readiness of flour components to hydrate better and mix with other food ingredients.

As expected, waxy trait significantly enhanced WSI of the experimental sorghum lines (Table 3). This effect was more pronounced than the effect of the HD trait. For example, among the experimental lines, the waxy lines had mean WSI of 8.0% compared to 4.9% for non-waxy lines. By comparison, the experimental HD lines averaged WSI of 6.7% vs 5.9% for experimental LD lines. These values are within ranges previously reported for waxy and non-waxy sorghums (Elhassan, Naushad Emmambux, Hays, Peterson, & Taylor, 2015). The higher WSI of the waxy sorghum starch is expected because of the weaker structure of waxy starch granule, and thus easier ability to release amylopectin into solution from starch damaged during the grinding process. The HD protein trait further enhanced the WSI of the waxy and heterowaxy sorghums (Table 3), suggesting that combining HD and waxy traits can enhance functionality of sorghum endosperm in dough and batter systems.

Starch pasting properties

The pasting profile of the experimental lines varied widely by trait and environment, which was expected due to the major role of endosperm hardness and environmental conditions during maturation on starch pasting properties. However, a few relevant trends were discernible, especially for peak and final viscosities (Table 3). The control HD had a slightly (non-significant) higher peak viscosity than the control LD; however, this may have been partly due to its softer endosperm, which allowed for easier hydration and less restricted swelling of starch. Among the experimental lines, the non-waxy HD lines had peak viscosities similar to the LD lines. On the other hand, among the waxy and heterowaxy lines, the HD trait resulted in significantly increased peak viscosity (Table 3), which suggests interactive effect of the HD trait with amylopectin.

The final viscosity (gel strength) of the non-waxy HD lines (both control and experimental) were generally lower than their LD counterparts. On the other hand, the opposite effect was observed for the waxy and heterowaxy lines, where the HD lines tended to have higher final viscosities than LD lines. The lower final viscosity of non-waxy HD lines might be due to increased surface area of protein bodies and their better hydration as they have more exposed hydrophilic α-kafirins (Massafaro, Thompson, Tuinstra, & Weil, 2016). The hydrated α-kafirins (bulk of the protein body proteins) may interact with each other to form a continuous matrix during the cooking process; this matrix may interact with the amylose starch polymers and limit/delay amylose-amylose re-association during cooling. This is a desirable functionality since it may lead to reduced staling in products.

On the other hand, the ability of the α-kafirins to interact with starch may have produced the opposite effect in the waxy and heterowaxy lines. Waxy starch normally does not gel due to steric effect of the highly branched polymers, thus generally produce lower final viscosities (Sang, Bean, Seib, Pedersen, & Shi, 2008). The higher final viscosities of HD waxy/heterowaxy lines compared to their LD counterparts suggests that the α-kafirins likely interacted with amylopectin to form a gel-like network, with strengths closer to those induced by presence of amylose. Thus, the HD trait may have important functional impact on sorghum starch relevant to food product quality.

Table 3: Mean values of physical and starch pasting properties of experimental HD and LD sorghum lines of various starch composition

Experimental Lines	**N**	**Hardness (HI)**	**TKW (g)**	**Seed Density (g/cc)**	**Seed Size (mm)**	**WAI (g/g)**	**WSI (%)**	**Pasting properties**	
								Peak V (cP)	**Final V (cP)**
Control HD	2	29.7±18.6^{b}	29.7±5.2^{a}	1.35±0.00^{bc}	2.43±0.20^{b}	2.17±0.13^{a}	5.92±0.09^{bc}	2129±8^{ab}	2863±70^{b}
Control LD	2	82.5±18.5^{a}	25.1±5.4^{b}	1.38±0.00^{a}	2.50±0.30^{ab}	2.52±0.13^{ab}	4.48±0.03^{e}	1850±50^{b}	3757±127^{a}
Ex. HD, Nml	34	68.3±11.8^{a}	25.7±3.0^{b}	1.34±0.02^{c}	2.51±0.08^{b}	2.51±0.05^{ab}	5.28±0.66^{cd}	1930±145^{b}	3032±343^{ab}
Ex. LD, Nml	8	80.7±7.5^{a}	29.9±1.4^{a}	1.39±0.01^{a}	2.80±0.14^{a}	2.40±0.05^{ab}	4.68±0.48^{de}	1928±176^{b}	3460±747^{a}
Ex. HD, HWX	6	77.1±12.3^{a}	21.9±5.1^{c}	1.37±0.00^{abc}	2.41±0.27^{b}	2.63±0.18^{a}	6.63±0.22^{ab}	2440±54^{a}	3034±10^{ab}
Ex. LD, HWX	2	82.2±11.5^{a}	30.7±5.5^{a}	1.38±0.00^{a}	2.60±0.30^{ab}	2.32±0.18^{ab}	5.72±0.03^{bc}	1433±64^{c}	2551±40^{bc}
Ex. HD, WX	10	77.6±3.1^{a}	25.7±3.9^{b}	1.37±0.01^{ab}	2.62±0.12^{ab}	2.51±0.09^{ab}	7.10±0.44^{a}	1936±314^{b}	2921±714^{b}
Ex. LD, WX	2	77.1±3.0^{a}	31.2±0.1^{a}	1.36±0.01^{bc}	2.51±0.05^{b}	2.42±0.09^{ab}	7.46±0.68^{a}	2445±102^{a}	2272±39^{c}

HD = highly digestible, LD = low digestible [proteins], HI = hardness index, TKW = thousand kernel weight, WAI = water absorption index, WSI = water solubility index,

Physicochemical properties

Effect of the HD protein mutation on lysine content

Selected lines of the experimental HD and LD samples (as classified by FE-SEM) were analyzed for amino acid profile to establish whether inheritance of the HD trait influenced lysine content. Lysine is the leading of the limiting amino acids in cereal grains, and its level is associated with protein quality and biological value. As expected, the soft endosperm control HD had significantly higher lysine content (2.54 g/100 g proteins) than the commercial control LD sorghum (1.47 g/100 g protein) and the experimental (2.02 g/100 g protein) LD ones (Figure 4A&B). Wide variation in lysine content was apparent among the experimental lines in both the HD (range: 1.71 – 2.57 g/100 g protein) and LD (1.93 – 2.25 g/100 g protein) categories (Figure 4A). There was no significant difference between the experimental HD (2.15 g/100 g protein) and experimental LD (2.02 g/100 g protein) categories (Figure 4B) which may indicate that the protein composition is independent of the protein structure (HD mutant versus regular globular protein bodies). The finding in this work for the experimental LD lines seem to be in contrary to the report of Massafaro, Thompson, Tuinstra, and Weil (2016), which concluded that the same mutation is responsible for both HD protein and high lysine content in sorghum. The experimental LD lines had significantly higher lysine content than the control LD one, which may be of interest to breeders for improving the lysine levels of established LD hybrids for animal feed or other uses where protein functionality is not of great interest. The overall observation was that the improved HD lines have better protein quality than the wild types and hence can improve the nutrition of consumers if used as food.

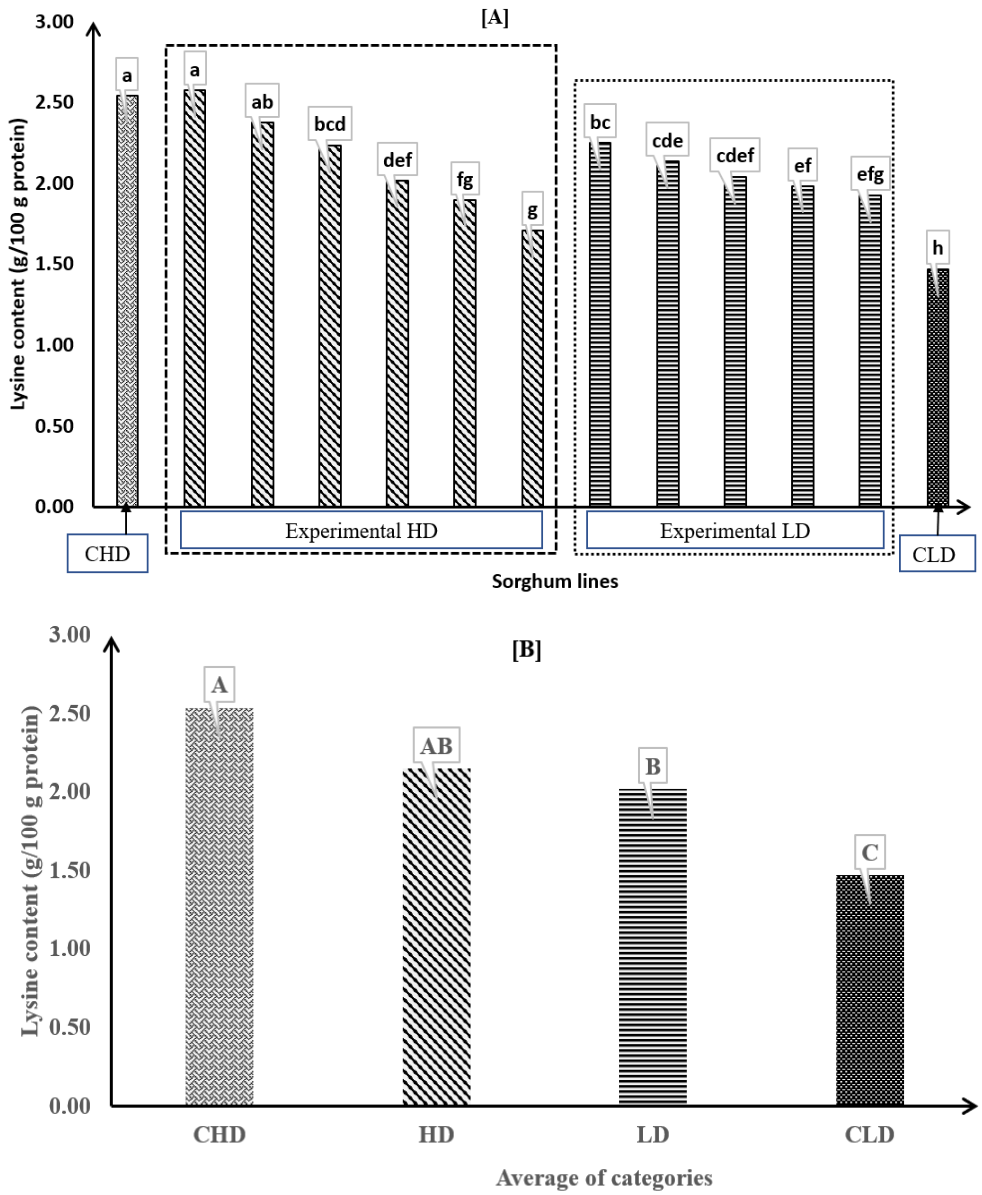

Figure 4: Lysine content of individual lines from the HD and LD categories [A] and their category averages [B] compared to the HD and LD controls.

The lysine data were consistently (about 40%) lower than those previously reported for HD and LD sorghums (Tesso, et al., 2006). This may be partly due to the differences in the analysis methods used. However, the trends were consistent; in our study the experimental hard endosperm HD sorghums had 85% of the lysine content of the soft endosperm control HD, comparable to Tesso, et al. (2006) who reported that a set of hard endosperm HD sorghum mutants had on average 92% of the lysine content of a soft endosperm HD line. The observed variability in the lysine levels both in the experimental HD and LD groups is expected as the lines are the F2 generation. The normal control sorghum in our study had only 58% of the lysine content of control HD, also comparable to Tesso, et al. (2006) report (64% of lysine content in HD) for their normal sorghum. An important finding in our data is that even in experimental lines that did not express the HD trait, use of a HD mutant parent may boost overall sorghum lysine content (experimental LD vs control LD) (Figure 4).

The lysine content and *in vitro* digestibility of cooked samples weakly, but significantly correlated ($p<0.05$, $r = 0.47$ and 0.44, for the raw and cooked samples, respectively). The result of current work seems to agree with previous reports (Winn, Mason, Robbins, Rooney, & Hays, 2009), that the HD mutation is associated with increased synthesis of lysine-rich proteins in sorghum seeds, with suppressed synthesis of kafirins. This was also emphasized by Massafaro, Thompson, Tuinstra, and Weil (2016) where the same mutation was responsible for both increased protein digestibility and lysine content, although the values for the experimental LD were not in compliance.

Extractable phenolic contents

The extractable phenolic content (EPC) was analyzed as part of the physicochemical properties of the experimental lines. There was no significant difference in the extractable phenolic contents of the HD and LD proteins as well as the waxy and normal starch types over the different growing environments. The levels of TPC (mg GAE/g) ranged from 1.14 to 3.89, with the average of 2.53, which was similar to those reported by Dykes, Rooney, Waniska, and Rooney (2005) for sorghum cultivars of thin pericarps of different colors.

The correlation between the EPC and protein digestibility was also analyzed and significant ($p<0.05$) but weak negative correlations ($r = -0.30$ for the raw and $r = -0.48$ for the cooked samples) were observed only for the samples from the 2014 crop year. The general observation was that the EPC did not significantly contribute to the limited protein digestibility. It was previously reported that tannin may bind proteins and make them undigestible, but the lines investigated in this work are known non-tannin cultivars. In non-tannin sorghum lines, although flavonoids and phenolic acids may interact with proteins as they have hydroxyl groups, there is no conclusive evidence that they bind proteins to an extent that significantly affects protein digestibility (Duodu, Taylor, Belton, & Hamaker, 2003). This means that the effect of EPC is not of concern to protein digestibility regardless of the protein nature (HD and LD). The other important point is that the EPC of the new HD-waxy hybrids are comparable to those reported for wild types (Dykes, Rooney, Waniska, & Rooney, 2005), making the improved lines viable sources of functional bioactive components.

Phenolic acid and flavonoid profiles

The general flavonoid and phenolic acid profiles of selected lines were determined and there was no noticeable difference among the different lines of varying protein and starch types (Figure 4). All categories of experimental lines had high and a greater number of peaks at wavelength of 325 and 340 nm, showing that phenolic acids and flavones are the dominant phytochemicals. The high peaks were observed at retention times of 7, 9, 18 and 23.12 min at the DAD wavelength of 325 nm, indicating the abundance of phenolic acid derivatives. Several peaks were observed at different retention times for all the lines analyzed and this shows the presence of many phenolic acid and flavonoid derivatives. Luteolin and apigenin were flavonoids observed to be present.

The analysis was important to identify potential experimental lines that has additional health benefits from the phytochemical composition stand point. As expected, there existed no differences in the flavonoids and phenolic acid profiles of the HD versus LD protein or the waxy versus normal starch experimental lines. The research confirmed that the improvement in the protein and starch functionality for food application was achieved without alterations in other heath beneficial traits in sorghum.

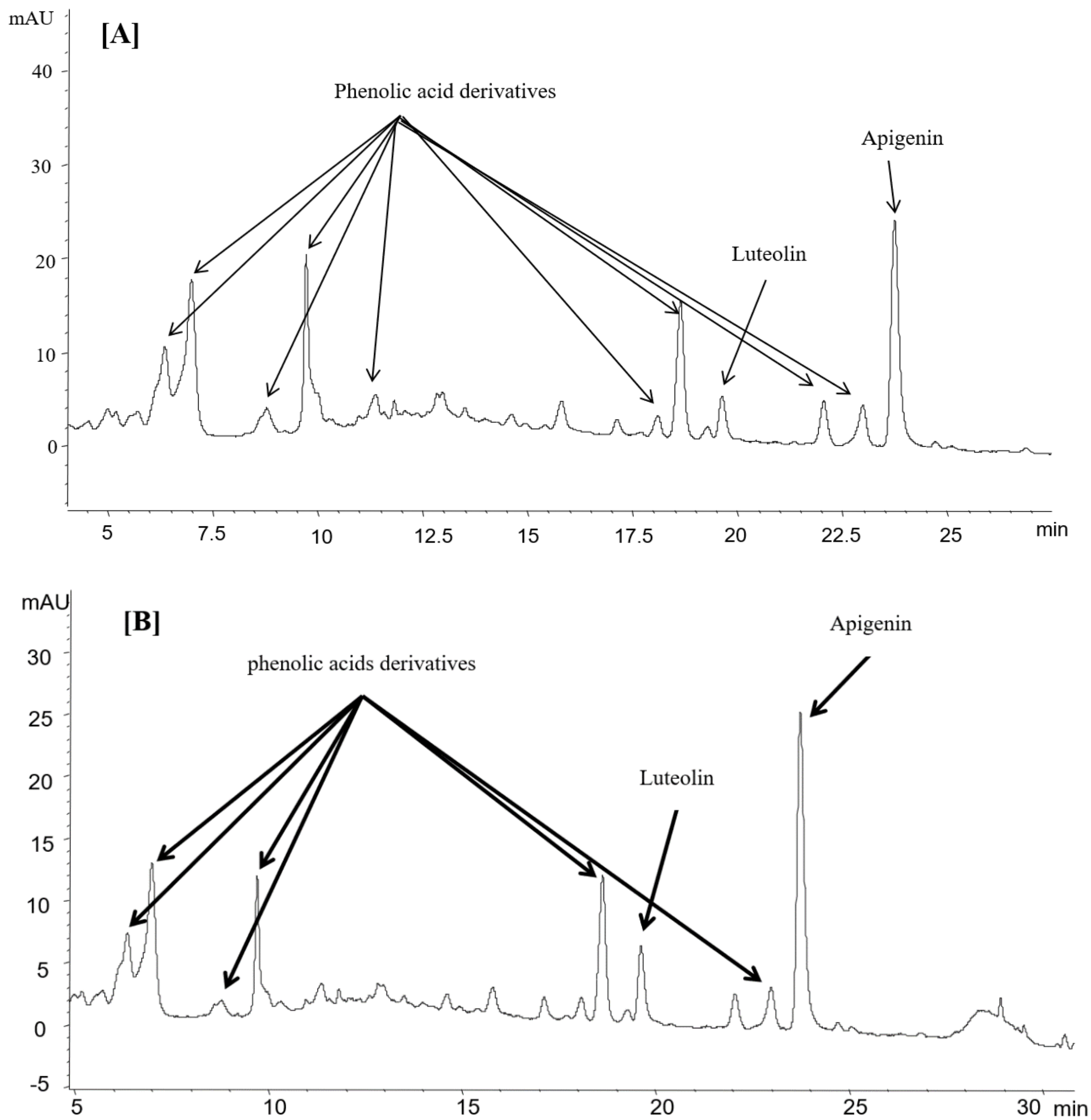

Figure 5: A representative HPLC chromatogram of phenolic extracts for experimental sorghum lines at a wavelength of 325 [A] and 340 [B] nm.

Chapter Summary

The overall evidence suggests that the protein body mutation trait, regardless of endosperm hardness, is associated with improved sorghum quality, including significantly higher protein digestibility and lysine content, as well as improved pasting and other functional properties relevant to food processing. Waxy and heterowaxy traits further enhance the functional properties of the mutated protein body of sorghum. Thus, the protein body mutation trait has the potential to significantly expand sorghum food use and add value to the commodity without changes in other important physicochemical and health beneficial properties, including the TPC, flavonoid and phenolic acid profiles.

CHAPTER IV

FUNCTIONALITY OF HD TRAIT IN MODEL FOOD SYSTEMS

Background

Sorghum is used as a staple food in the arid and semi-arid regions of Africa, Asia and Latin and Central America for its better adaptability to harsh environment compared to other crops (Dicko, Gruppen, Traoré, Voragen, & Van Berkel, 2006; Taylor, Schober, & Bean, 2006). Sorghum is commonly used in the preparation of traditional fermented foods and beverages and nonfermented products in African and Asian cultures (Dahlberg, Berenji, Sikora, & Latković, 2012). Nonfermented breads such as flat-breads in different parts of Africa, Roti of India and tortilla of Latin and Central America; fermented breads such as injera of Ethiopia and kisra of Sudan; porridges and fermented alcoholic and nonalcoholic beverages are made of sorghum and consumed on a daily bases (Asante, 1995; Rooney & Murty, 1982).

There are, however, limitations in sorghum food use that are mainly associated with the hydrophobic nature of the kafirin proteins that limits hydration of the starch and affect its functionality, such as starch swelling. Bacteria and their enzymes during sourdough fermentation are reported to break down the hydrophobic kafirin proteins, making them soluble. These results in strong starch gel formation for a uniform crumb without the interference of the protein chinks (Schober, Messerschmidt, Bean, Park, & Arendt, 2005). The metabolites of phenolic acids and flavonoids produced during sourdough fermentation also provide additional nutraceutical benefits (Omoba, Taylor, & de Kock, 2015; Svensson, Sekwati-Monang, Lutz, Schieber, & Ganzle, 2010), particularly with products containing rich phytochemical grain sources like sorghum. The use of sour dough culture in fermented products from sorghum may therefore enhance its functionality and nutritional desirability.

Sorghum is also used in commercially processed staples and snacks in the western diets. The emergence of products based on sorghum and other "ancient grains" in all natural innovative products such as sorghum snacks in the western food market is encouraged by a growing demand (John & Awika, 2017). The application of sorghum in commercial products is, however, still limited by poor endosperm functionality due to the hydrophobic proteins that limits the starch swelling during processing. The potential of highly digestible protein (HD) mutant sorghum in enhancing sorghum flour performance and nutritional quality was reported (Wu, Jampala, Robbins, Hays, Yan, Xu, et al., 2010). The HD protein mutants, having irregularly shaped, porous and invaginated protein bodies (Figure 2 under section 2.3.2.), exposing the better functional α kafirins enhances its interaction with water and other ingredients. The more hydrophobic and resistant kafirin sub-classes, β and γ enclose the α in the wild-type sorghum protein bodies.

The amylopectin components in the waxy starch absorbs water in to its branched structures and is expected to enhance the functionality of the flour, which was indicated by better water absorption (WAI) and solubility (WSI) indices as well as pasting properties (Table 3, section 3.3.3.). The combination of the HD proteins and waxy starch is therefore, expected to further improve sorghum grain functionality for food application. The objective of this research is to investigate if the improved HD trait in waxy/normal lines perform any better than their LD counterparts in selected food systems.

Materials and Methods

Bakery ingredients including whole and refined wheat flour (Ardent Mills, CO, USA), shortening (Cargill Inc, MN, USA), instant dry yeast (Red Star instant dry yeast, WI, USA) and baking Powder (H-E-B, TX, USA) were obtained from their respective suppliers. Other basic ingredients including sugar, table salt and milk powder were purchased from local stores. Sourdough culture was obtained from King Arthur flour and bakery (Skagit Valley, WA, USA). Five separately milled experimental sorghum lines (HD and LD sorghum in normal and waxy backgrounds, as well as the control HD) were prepared. The milling was done using Quadrumat® Senior roller milling machine (Model: 880220.002, Brabender GmbH & Co. KG, Duisburg, Germany). The machine has two units with 4 rollers each the first for breaking (crushing) and the second for grinding. After the bran was separated, the refined and shorts components of the flour were mixed to have sorghum flour (86.8% average flour yield), which was stored in thick plastic bags at room temperature until used for the experiment.

Effect of HD trait in waxy vs normal starch backgrounds was tested in batter and dough-based food models in comparison to wheat-based controls. Model products were selected from a wide range of categories to check the relative performance of improved HD lines in different food matrices. The food models were selected from low moisture (rich products) and high moisture (lean products). The model products chosen were also different based on processing techniques, including; pancakes for chemically leavened batter-based products, bread for biologically leavened dough-based products, as well as cookies for minimally mixed dough products were used. Parameters of the batter systems (pancakes) and texture attributes were measured and compared for the different categories of sorghum lines.

Pancakes

The influence of the HD protein traits on the baked products was assessed in terms of batter properties and product characteristics. Two levels of sorghum (50 and 100%) were used for pancakes and compared with controls made from pastry flour based on the recipe indicated in Table 4. The dry ingredients were sieved into a bowl and mixed well before they were whisked first with milk and then with a molten shortening. Batter viscosity was measured using Brookfield viscometer using the methods explained by Kim and Walker (1992), with some modification. Batter sample was filled into a 100 mL beaker with continuous stirring to avoid sedimentations. The viscosity measurement was done in triplicates at room temperature (23±2°C) using spindle number 4 at the speed of 10 rpm for a minute. The pancakes were then baked on a greased hot griddle heated to a 350°F for about a minute and half on each side. The pancakes were cooled for 10 min before the different physical and textural measurements took place. Physical parameters of the pancakes (height/thickness and diameter/spread) were measured using a digital caliper. Pancake textural quality indicators such as the height, diameter, hardness, chewiness, cohesiveness, springiness, resilience and adhesiveness were determined using a Texture Profile Analyzer (TA.XT Plus, Model: PLUS-UPGRADE, Texture Technologies, MA, USA), as described by Finnie, Bettge, and Morris (2006).

The texture analyzer was equipped with a 50 kg load cell and a flat round 75 mm diameter compression probe. The texture analyzer had a computer interphase with Exponent TPA software (version 32). Three pancakes of each treatment were stacked up with the first baked sides facing down. The pancake stalk was compressed first to 50% of its original height at a constant rate of 1 mm/s. The probe was retracted and rested for 5 s before the second compression to 50% of the original height. The pancake compression test was carried out in triplicates with the three pancakes in a given stack being randomly selected and pressed in a random order. Different texture parameters were derived from the TPA curve and compared for the HD and LD lines as well as waxy and normal starch types at the different formulations. The instrument was configured to the same setting until all the pancake experiment sessions were complete to avoid errors.

Table 4: Basic pancake recipe and treatment formulations

Ingredient	Control, (g)	Baker's %	Treatment 1 (g)	Baker's %	Treatment 2 (g)	Baker's %	Treatment 3 (g)	Baker's %
Pastry flour	112.5	100	_	_	56.75	50	56.75	50
Sorghum	_	_	112.5	100	56.75	50	_	_
Whole wheat, UG	_	_	_	_	_	_	56.75	50
Sugar	15	12.5	15	12.5	15	12.5	15	12.5
Salt	1.25	1	1.25	1	1.25	1	1.25	1
Baking powder	7.5	6	7.5	6	7.5	6	7.5	6
Milk	225	200	225	200	225	200	225	200
Melted shortening	27.5	25	27.5	25	27.5	25	27.5	25

Control is 100% pastry flour, UG = ultra-ground

Table 5: Basic cookies recipe and treatment formulations

Ingredient	Control (g)	Baker's %	Treatment 1 (g)	Baker's %	Treatment 2 (g)	Baker's %	Treatment 3 (g)	Baker's %	Treatment 4 (g)	Baker's %
Shortening	125	40	125	40	125	40	125	40	125	40
Sugar	155	50	155	50	155	50	155	50	155	50
Salt	2.5	0.8	2.5	0.8	2.5	0.8	2.5	0.8	2.5	0.8
Milk	30	10	30	10	30	10	30	10	30	10
Flour (cookie)	312.5	100	_	_	156.25	50	156.25	50	_	_
Sorghum	_	_	312.5	100	156.25	50	_	_	_	_
Whole wheat (UG)	_	_	_	_	_	_	156.25	50	312.5	100
Baking powder	9	3	9	3	9	3	9	3	9	3

Control is 100% cookies flour, UG = ultra-ground

Table 6: Descriptive texture attributes and references

Products	Attributes	Definitions	References
Pancakes	Bitter	The fundamental taste factor associated with a caffeine solution	0.01% caffeine solution = 2.0 (flavor) 0.02 caffeine = 3.5 (flavor)
	Denseness	The compactness of the sample cross section	Cool whip = 0.5; Marshmallow fluff = 2.5; Nugget = 4.0; Melted milk balls = 6.0; Frankfurter = 9.5; Fruits jellies = 15.0
	Grittiness	The fundamental texture of associated with grit or sand	Miracle whip = 0.0; Instant cream of wheat and sour cream = 5.0 (f); Hellman's mayo and corn meal = 10.0 (f)
	Cohesiveness of mass	The degree to which chewed samples hold together in a mass	Carrot = 2.0; Mushrooms = 4.0; Frankfurter = 7.5; American processed cheese = 9.0; Soft brownie = 13.0; Pillsbury/country biscuit dough = 15.0
Cookies	Hardness	The force to attain a given deformation, such as force to compress with the molars, as above; force to compress between tongue and palate; force to bite through with incisors	Cream cheese = 1.0; Egg white = 2.5; Yellow American cheese = 4.5; Olives = 6.0; Hebrew National Frankfurter = 7.0; Planters peanut = 9.5; Carrots = 11.0; Life severs = 14.5
	Fracturability	The force with which the sample breaks	Corn muffin = 1.0; Graham crackers = 4.2; Melba toast = 6.7; Pita chips = 11.5; Life saver = 14.5
	Surface roughness		Gelatin dessert = 0.0; Orange peel = 5.0; Potato chips = 8.0; Hard granola bar = 12.0
	Crispiness	The force (noise) with the which the product breaks or fractures, characterized by the many small breaks	Granola bar = 2.0; Club crackers = 5.0; Graham crackers = 6.5; Oat cereal = 7.0; Corn flakes = 14.0

Reference scores are based on universal scale ranging from 0 to 15 where zero means none and 15 means extremely intense.

Cookies

Cookies were also made from the same proportions as indicated for the pancakes and compared with controls made from cookies and ultra-ground whole wheat flours based on the recipe presented in Table 5. The sugar and shortening were creamed together for 3 min and 1 min after the milk was added. The flour and the remining dry ingredients were folded in and mixed until all are fully incorporated. The dough was sheeted and cut into round shapes, put on parchment paper lined trays and baked at 375°F for 12 min. The cookies were cooled for 60 min before the different measurements were taken. Cookies quality parameters such as spread, spread ratio, thickness, hardness, breaking strength and colors were measured and compared. The texture of the cookies was analyzed using the three point break (also called the triple beam snap) method as described by Sindhuja, Sudha, and Rahim (2005) with some modifications. The cookie was placed on two parallel beams fixed at 35 mm apart. The cutting fixture attached to the head of the texture analyzer (5 kg load cell), was brought down to the sample at a speed of 3 mm/s. The peak force (g) required to break the sample was taken as the breaking strength and compared for the HD versus LD as well as the waxy versus normal sorghum types and selected controls.

The color of the cookie was measured using the L*a*b* color system, where "L*" is darkness to lightness (0 to 100), "a*" as greenness to redness (-50 to +50), and "b*" blueness to yellowness (-50 to +50). The measurement was done using the Chroma Meter (model: CR – 300), of Konica Minolta, (Japan) in triplicates after calibration of the machine as described by Gouveia, Batista, Miranda, Empis, and Raymundo (2007).

Sensory analysis of pancakes and cookies

The pancakes and cookies were analyzed using descriptive sensory analysis with 5 trained panelists. Institutional review for the compliance of the research with protection of human research participants was done by and exemption was obtained from Texas A&M University Human Research protection program (IRB number IRB2018-1515). The sensory test focused on the textural difference between the HD and LD protein sorghums in different starch backgrounds (Table 4 for pancakes and Table 5 for cookies). The panelists were trained with appropriate references (Table 6) and taste products until they were familiarized with the attributes of importance for each of the products assessed. Hardness, fracturability, surface roughness and crispiness for cookies as well as bitterness, denseness, grittiness and cohesiveness of mass for the pancakes were used.

The cookies were baked a day before the taste session and the pancakes were baked on the same day of tasting (on average 1 hour before the session). The samples were coded with a random three-digit number and were served to the panelists in a random order on each day.

Bread

Sourdough breads with substitution of wheat up to 20% using the HD and waxy sorghum lines was made based on the recipe presented in Table 7. The sourdough model was used to partially hydrolyze the pericarp and protein components of whole grain sorghum with enzymes from the cocktail of different bacteria and yeast strains to improve the properties of the bread (Schober, Bean, & Boyle, 2007). Control bread samples were also made using yeast starter. A straight-dough method was used where all the ingredients were mixed to optimal gluten development (20 ± 5 min) and bulk fermented for 40 min in a proofer at 80°F and 85% RH. The yeast dough was then punched and divided, fermented for an hour and baked at 400°F for 30 min. For the sour dough bread the culture was made to adopt to the formula by feeding on it twice before it was used for the actual baking. Mixing was done for the same duration as the yeast bread and the sour dough was fermented in bulk for 60 min before it was divided into small loaves of equal weight (188 ± 5 g), followed by proofing for 5 ± 0.5 h. The sour dough bread was baked at the same condition as for the yeast bread. The loaves were cooled at room temperature for 2 h before further analysis took place.

The bread samples were then characterized in terms of loaf volume, loaf density, specific volume, moisture content, crust and crumb color as well as instrumental texture parameters. The dough volume for the specific volume was determined using rapeseed displacement method (AACC method 10-05). For the crumb texture analysis a compression technique described by Galle, Schwab, Dal Bello, Coffey, Gänzle, and Arendt (2012), with some modification. The texture analyzer was equipped with 50 kg cell load and a cylindrical aluminum probe (35 mm diameter). Two slices (15 mm each) were stacked on the on the sample stage and compressed to 60% of the original height at 1 mm/sec with a trigger force of 1 g. The peak force (g) required for the compression was taken as the hardness (firmness) of the crumb and was compared for the different variables. The performance of the HD lines was compared with LD and ones. Both yeast and sourdough bread made of ultra-ground whole and high gluten wheat flours were used as positive checks (controls).

The culture was made to sustain by feeding it initially with high gluten flour for two rounds. The culture was then made to grow on the experimental flour blend (wheat and sorghum) (two rounds of feeding) before it was used for baking. The sourdough breads from the different formulations were compared to their yeast controls for all the parameters assessed. The formulas with the sorghum substitution were also compared with those substituted with the same level of ultra-ground whole wheat flour. Bread samples made of 100% high gluten bread flour and ultra-ground whole wheat were also used as controls.

The color of the crust of the fresh loaves and the crumb of the fresh and stored (24 h) were measured using the “L*”, “a*”, and “b*” systems, where “L*” is darkness to lightness (0 to 100), “a*” as greenness to redness (-50 to +50), and “b*” blueness to yellowness (-50 to +50). Measurement was done using the Chroma Meter (model: CR – 300), of Konica Minolta, Foster City, CA, USA). Each measurement was done in triplicates after calibration of the colorimeter as described by Bize, Smith, Aramouni, and Bean (2017).

Many of the texture parameters are good indicators of product quality and stability. More specifically, shelf stability and the rates of product staling were also estimated based on changes in the texture profile and moisture content parameters.

Table 7: Basic and treatment bread formulations

Ingredients	Control (g)	Baker's %	Treatment 1 (g).	Baker's %	Treatment 2 (g)	Baker's %
Bread flour	500	100	450	100	400	100
Sorghum/ UGWW	–	_	50	10*	100	20*
Sugar	30	6	30	6	30	6
Shortening	15	3	15	3	15	3
Sourdough/ Yeast**	11	2.2	11	2.2	11	2.2
Salt	9	1.8	9	1.8	9	1.8
Water (cold)	300	60	302.5	60	3005	60

*Baker's % based on total flour weight, **values are for yeast only and 1 cup of sourdough for the sour bread, Sorg. = sorghum, CF = cookies flour, UGWW = ultra-ground whole wheat flour, UG = ultra-ground

Statistical analysis

The product model trials were organized in 2x2x2 factorial design (2 level of formulation, 2 types of protein and 2 types of starch) for the pancakes and cookies where as a 2x2x2x2 factorial design was used for the breads where an additional fermentation type (sourdough versus yeast) was considered (Table 8). The data were analyzed using ANOVA in full factorial settings for the factorial design and additionally a one-way

ANOVA was used in comparing the different combinations of the factors with the controls. Means with their standard errors were reported and the mean comparisons were done using Tukey's HSD procedure.

Table 8: Summary of variables for the different food product models

Fermentation	Formulations	Proteins	Starch	Products
	50% and 100% sorghum	HD versus LD	Waxy versus normal	Pancakes and cookies
Sourdough versus yeast	10% and 20% sorghum	HD versus LD	Waxy versus normal	Bread

Results and Discussions

Effect of HD trait in waxy and normal starch sorghum on batter and pancake properties

Batter viscosity

The levels of sorghum protein and starch types as well as sorghum flour incorporation, significantly ($p<0.05$) influenced the pancake batter consistency (Table 9). Comparing the HD and LD protein as well as the waxy versus normal starch sorghum traits, both the HD and waxy traits resulted in higher viscosities than their LD and normal counterparts, respectively. Pancake batter viscosity is important for the formation of pancakes of desirable thicknesses. The higher batter viscosity produced by the HD and waxy traits might be due to their better interactions with water (better water holding) and other components. The HD trait has irregular protein bodies with numerous foldings where the better soluble and better digestible α kafirins are more exposed for better water holding compared to the globular shaped protein bodies of the LD lines. In the globular LD proteins, the more resistant β and γ kafirins engulf the α kafirins making it unavailable for interaction. For the waxy starch trait, the branching in the amylopectin increased the water holding capacity, resulting in a thicker and more viscous batter (Witczak, Korus, Ziobro, & Juszczak, 2019).

The interaction effects of the different variables were also significant ($p<0.05$). The two-way (Table 10) and three-way (Table 11) combination of the variables follow the same trend as in the separate effects (Table 8). The most important observation was that HD-waxy trait had significantly higher batter viscosity than the LD-waxy, indicating that the combination of the HD protein and waxy starch traits synergistically resulted in better interaction with water and contributed to better functionality compared to the HD-normal as well as LD-waxy/normal counterparts.

The better water interaction of the HD-waxy traits is expected to enhance hydration of the flour (starch) and swelling that may result in products of desirable texture by overcoming the dryness and grittiness often reported for wild type sorghum-based foods (Rooney, Miller, & Mertin, 1981; Wong, et al., 2009). This implies that the new sorghum lines with combined HD and waxy traits in hard endosperm have enhanced interaction with water that may result in pancakes with desirable textural properties.

The HD-waxy combination had higher batter viscosity values (4613 cP for 100% and 2767 cP for the 50% substitutions) which were also closest to those for the 50% whole wheat flour-based controls (4133 cP), while all the other combinations ranged from 1273 cP for the 50% HD control to 2380 cP for the 100% LD-waxy (Tables 11&12). This implies that the HD-waxy combinations gave batter viscosities comparable to the whole wheat controls and hence may provide improved functionality in gluten-free whole grain batter-based applications.

Pancake physical properties

The physical properties of the pancakes were also significantly different for the different types of proteins and starches at the different formulation levels, although the means for some of the parameters seem to be very close to one another (Table 9). The thickness (height) of the pancakes was significantly lower for the HD protein compare to the LD ones. The pancake thickness was also lower for samples containing waxy starch traits compared to their normal counterparts. The lower thickness of the pancakes made from HD or waxy sorghum lines was due to greater spread (diameter) which were significantly higher than those for the LD protein and normal starch traits, respectively. Although the batter viscosity was higher (lower flowability) for the HD or waxy samples (section 4.3.1.1), the lower pancake thickness might be due to higher degree of starch solubilization (section 3.3.3.) that most likely resulted in a denser mass. This relationship was also supported by a significant and strong negative correlation between the thickness and diameter (r = -0.88, Appendix A- 1). The higher diameter (better flowability) and lower thickness of the HD protein or waxy starch traits is likely due to greater degree of solubilization and cooking leading reduced gas holding resulted from the better interaction of the flour with the moisture.

Table 9: Effects of sorghum level, protein and starch types on batter viscosity and pancake quality

Variables	Batter viscosity (cP)	Height (mm)	Diameter (mm)	MC (%)	Hardness (g)	Adhesiveness (g/mm)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)	Resilience
Protein type											
HD	2632^{a}	8.43^{b}	94.87^{a}	45.17^{a}	5.25^{a}	-4.86^{b}	0.59^{b}	0.60^{a}	3.00^{a}	1.76^{a}	0.26^{b}
LD	2170^{b}	8.82^{a}	93.62^{b}	41.82^{b}	6.40^{a}	-2.43^{a}	0.62^{a}	0.63^{a}	3.87^{a}	2.32^{a}	0.27^{a}
Starch type											
Normal	1827^{b}	9.02^{a}	94.53^{a}	42.22^{b}	5.64^{a}	-3.19^{a}	0.62^{a}	0.63^{a}	3.48^{a}	2.12^{a}	0.27^{a}
Waxy	2975^{a}	8.23^{b}	93.96^{a}	44.77^{a}	6.01^{a}	-4.10^{a}	0.59^{b}	0.60^{b}	3.40^{a}	1.97^{a}	0.25^{b}
Formula											
100% Sorg.	2712^{a}	10.26^{a}	87.43^{b}	43.38^{a}	7.23^{a}	-2.13^{a}	0.50^{b}	0.54^{b}	3.85^{a}	1.93^{a}	0.23^{b}
50% Sorg.	2090^{b}	6.99^{b}	101.06^{a}	43.61^{a}	4.42^{a}	-5.16^{b}	0.71^{a}	0.69^{a}	3.02^{a}	2.15^{a}	0.30^{a}
Std Err	49.74	0.1	0.4	0.17	1.1	0.42	0.01	0.01	0.58	0.29	0.004

MC = moisture content (% db), HD = highly digestible [protein], LD = low digestible [protein], values are means of triplicate experiments and those with different superscript letters in the same column are significantly different ($p<0.05$); parameters with no unit (springiness, cohesiveness and resilience) are ratios of dimensions.

Table 10: Batter viscosity and pancake characteristics as influenced by two-way interaction of the different variables

Interaction (2-way)	**Batter viscosity (cP)**	**Height (mm)**	**Diameter (mm)**	**MC (%)**	**Hardness (g)**	**Adhesiveness (g/mm)**	**Springiness**	**Cohesiveness**	**Gumminess (g)**	**Chewiness (g)**	**Resilience**
Protein by starch											
HD, Nml	1573^{c}	9.11^{a}	95.28^{a}	45.14^{a}	4.19^{a}	-3.94^{a}	0.62^{a}	0.64a	2.67^{a}	1.70^{a}	0.28^{a}
wx	3690^{a}	7.74^{b}	94.46^{ab}	45.20^{a}	6.30^{a}	-5.78^{b}	0.56^{b}	0.57b	3.34^{a}	1.82^{a}	0.23^{b}
LD, Nml	2080^{b}	8.93^{a}	93.78^{ab}	39.30^{c}	7.10^{a}	-2.45^{a}	0.62^{a}	0.63a	4.29^{a}	2.52^{a}	0.27^{a}
wx	2260^{b}	8.71^{a}	93.47^{b}	44.34^{b}	5.71^{a}	-2.41^{a}	0.63^{a}	0.62a	3.46^{a}	2.11^{a}	0.27^{a}
Protein type by formulations											
HD 100%	3047^{a}	9.58^{b}	88.16^{b}	44.66^{b}	6.13^{a}	-3.28^{b}	0.49^{d}	0.53b	3.04^{a}	1.46^{a}	0.22^{c}
50%	2217^{b}	7.28^{c}	101.57^{a}	45.68^{a}	4.37^{a}	-6.44^{c}	0.70^{b}	0.68a	2.96^{a}	2.06^{a}	0.29^{b}
LD 100%	2377^{b}	10.94^{a}	86.69^{b}	42.09^{c}	8.34^{a}	-0.97^{a}	0.52^{c}	0.56b	4.66^{a}	2.40^{a}	0.23^{c}
50%	1963^{c}	6.70^{d}	100.56^{a}	41.54^{c}	4.47^{a}	-3.89^{b}	0.73^{a}	0.7a	3.09^{a}	2.23^{a}	0.31^{a}
Std Err	70.34	0.14	0.58	0.23	1.52	0.59	0.01	0.01	0.82	0.4	0.005

MC = moisture content (% db), HD = highly digestible [protein], LD = low digestible [protein], nml = normal starch, wx = waxy starch, values are means of triplicate experiments and those with different superscript letters in the same column are significantly different ($p<0.05$); parameters with no unit (springiness, cohesiveness and resilience) are ratios of dimensions.

Table 10 Continued

Interaction (2-way)	**Batter viscosity (cP)**	**Height (mm)**	**Diameter (mm)**	**MC (%)**	**Hardness (g)**	**Adhesiveness (g/mm)**	**Springiness**	**Cohesiveness**	**Gumminess (g)**	**Chewiness (g)**	**Resilience**
Starch by formulations											
Nml 100%	1927[c]	10.62[a]	88.56[b]	43.22[b]	6.64[a]	-2.03[a]	0.52[c]	0.58b	3.76[a]	1.90[a]	0.25[b]
50%	1727[c]	7.42[c]	100.50[a]	41.22[c]	4.64[a]	-4.35[b]	0.73[a]	0.69a	3.20[a]	2.32[a]	0.30[a]
Waxy 100%	3497[a]	9.89[b]	86.30[c]	43.53[b]	7.83[a]	-2.22[a]	0.49[c]	0.51c	3.94[a]	1.96[a]	0.21[c]
50%	2453[b]	6.56[d]	101.63[a]	46.01[a]	4.19[a]	-5.97[b]	0.7[b]	0.69a	2.85[a]	1.97[a]	0.29[a]
Std Err	70.34	0.14	0.58	0.23	1.52	0.59	0.01	0.01	0.82	0.4	0.005

MC = moisture content (% db), HD = highly digestible [protein], LD = low digestible [protein], nml = normal starch, wx = waxy starch, values are means of triplicate experiments and those with different superscript letters in the same column are significantly different ($p<0.05$); parameters with no unit (springiness, cohesiveness and resilience) are ratios of dimensions.

Table 11: Batter viscosity and pancake characteristics as influenced by three-way interaction of the different variables

Interactions (3-ways)	Batter viscosity (cP)	Height (mm)	Diameter (mm)	MC (%)	Hardness (g)	Adhesiveness (g/mm)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)	Resilience
HD nml 100%	1480^{e}	10.49^{b}	89.62^{b}	45.23^{b}	3.91^{a}	-2.72^{abc}	0.52^{c}	0.58^{b}	2.26^{a}	1.17^{a}	0.25^{bc}
HD wx 100%	4613^{a}	8.66^{c}	86.71^{c}	44.09^{c}	8.34^{a}	-3.84^{bc}	0.46^{d}	0.47^{c}	3.83^{a}	1.75^{a}	0.20^{e}
LD nml 100%	2373^{c}	10.75^{ab}	87.49b^{c}	41.21^{e}	9.37^{a}	-1.35^{ab}	0.52^{c}	0.57^{b}	5.26^{a}	2.63^{a}	0.24^{cd}
LD wx 100%	2380^{c}	11.12^{a}	85.88^{c}	42.98^{d}	7.31^{a}	-0.60^{a}	0.52^{c}	0.54^{b}	4.06^{a}	2.17^{a}	0.23^{d}
HD nml 50%	1667de	7.73^{d}	100.94^{a}	45.05^{bc}	4.47^{a}	-5.15^{c}	0.72^{a}	0.69^{a}	3.07^{a}	2.22^{a}	0.30^{a}
HD wx 50%	2767^{b}	6.82^{ef}	102.20^{a}	46.32^{a}	4.27^{a}	-7.72^{d}	0.67^{b}	0.67^{a}	2.85^{a}	1.90^{a}	0.27^{b}
LD nml 50%	1787^{d}	7.10^{e}	100.06^{a}	37.38^{f}	4.82^{a}	-3.55^{ab}	0.73^{a}	0.69^{a}	3.32^{a}	2.41^{a}	0.30^{a}
LD wx 50%	2140^{c}	6.30^{f}	101.05^{a}	45.70^{ab}	4.11^{a}	-4.23^{c}	0.73^{a}	0.71^{a}	2.86^{a}	2.05^{a}	0.31^{a}
Std Err	99.47	0.2	0.82	0.33	2.15	0.84	0.01	0.02	1.17	0.58	0.008

MC = moisture content (% db), HD = highly digestible [protein], LD = low digestible [protein], nml = normal starch, wx = waxy starch, values are means of triplicate experiments and those with different superscript letters in the same column are significantly different ($p<0.05$); parameters with no unit (springiness, cohesiveness and resilience) are ratios of dimensions.

Table 12: Pancake comparing with different controls

Treatments versus control	**Batter viscosity (cP)**	**Height (mm)**	**Diameter (mm)**	**MC (%)**	**Hardness (g)**	**Adhesiveness (g/mm)**	**Springiness**	**Cohesiveness**	**Gumminess (g)**	**Chewiness (g)**	**Resilience**
100% Exptl HD nml	1480^{fg}	10.49^{a}	89.62^{c}	45.23^{bc}	3.91^{a}	-2.72^{a}	0.52^{cd}	0.58^{bc}	2.26^{a}	1.17^{a}	0.25^{cd}
100% Exptl HD wx	4613^{a}	8.66^{b}	86.71^{de}	44.09^{cde}	8.34^{a}	-3.84^{a}	0.46^{d}	0.47^{d}	3.83^{a}	1.75^{a}	0.20^{e}
100% Exptl LD nml	2373^{cd}	10.75^{a}	87.49^{de}	41.21^{g}	9.37^{a}	-1.35^{a}	$0.52c^{d}$	0.57^{c}	5.26^{a}	2.63^{a}	0.24^{cd}
100% Exptl LD wx	2380^{cd}	11.12^{a}	85.88^{e}	42.98^{ef}	7.31^{a}	-0.60^{a}	0.53^{c}	0.54^{cd}	4.06^{a}	2.17^{a}	0.22^{de}
50% Exptl HD nml	1667^{fg}	$7.73b^{cd}$	100.94^{ab}	45.05^{bcd}	4.47^{a}	-5.15^{a}	0.72^{ab}	0.69^{a}	3.07^{a}	2.22^{a}	0.30^{ab}
50% Exptl HD wx	2767^{c}	6.82^{de}	102.20^{a}	46.32^{ab}	4.27^{a}	-7.72^{a}	0.67^{b}	0.67^{ab}	2.85^{a}	1.90^{a}	0.27^{bc}
SE	82.41	0.19	0.7	0.31	2.18	6.95	0.01	0.02	1.29	0.8	0.007

MC = moisture content (% db), Exptl = experimental sorghum lines, HD = highly digestible [protein], LD = low digestible [protein], nml = normal starch, wx = waxy starch, UGWW = ultra-ground whole wheat, values are means of triplicate experiments and those with different superscript letters in the same column are significantly different ($p<0.05$) parameters with no unit (springiness, cohesiveness and resilience) are ratios of dimensions.

Table 12 Continued

Treatments versus control	Batter viscosity (cP)	Height (mm)	Diameter (mm)	MC (%)	Hardness (g)	Adhesiveness (g/mm)	Springiness	Cohesiveness	Gummines s (g)	Chewiness (g)	Resilience
50% Exptl LD nml	1787[ef]	7.10[cde]	100.06[ab]	37.38[h]	4.82[a]	-3.55[a]	0.73[a]	0.69[a]	3.32[a]	2.41[a]	0.30[ab]
50% Exptl LD wx	2140[de]	6.30[e]	101.05[ab]	45.70[b]	4.11[a]	-4.23[a]	0.73[a]	0.70[a]	2.86[a]	2.05[a]	0.31[a]
100% Control HD	1273[g]	11.33[a]	88.27[de]	47.708[a]	11.98[a]	-70.83[b]	0.52[cd]	0.54[cd]	6.42[a]	3.32[a]	0.23[d]
100% Pastry flour	2222[d]	6.41[e]	101.88[a]	42.42[fg]	4.40[a]	-23.96[a]	0.76[a]	0.72[a]	3.16[a]	2.40[a]	0.32[a]
50% Control HD	1747[ef]	8.06[bc]	98.01[bc]	45.15[bc]	8.66[a]	-2.63[a]	0.71[ab]	0.68[a]	5.89[a]	4.22[a]	0.30[ab]
50% UGWW flour	4133[b]	8.03[bc]	95.93[c]	43.49[def]	4.84[a]	-7.13[a]	0.72[ab]	0.68[a]	3.29[a]	2.40[a]	0.29[ab]
SE	82.41	0.19	0.7	0.31	2.18	6.95	0.01	0.02	1.29	0.8	0.007

MC = moisture content (% db), Exptl = experimental sorghum lines, HD = highly digestible [protein], LD = low digestible [protein], nml = normal starch, wx = waxy starch, UGWW = ultra-ground whole wheat, values are means of triplicate experiments and those with different superscript letters in the same column are significantly different ($p<0.05$) parameters with no unit (springiness, cohesiveness and resilience) are ratios of dimensions.

The two-way interactions of the variables were also significant for most of the parameters. The protein and starch type showed significant interaction (Table 10), with the HD protein and waxy starch showing faster setting and more cooking with enhanced water interaction that results in soft pancakes.

In general, pancake from sorghum lines with the HD protein traits had significantly higher ($p<0.05$) moisture content than those from the LD ones. Pancake made from waxy starch also had significantly higher moisture content than those from the normal type. The higher moisture levels might be due to better interaction of water with the flour of the HD protein or waxy starch that resulted in better water holding capacities with respect to the irregularly shaped protein matrices and the branched amylopectin containing starches, which also goes in line with the higher batter property. The low moisture content of the LD protein or normal starch on the other hand, was possibly due to the evaporation of the free water on baking as there is no much interaction with the protein and starch components, respectively.

The HD-waxy lines in the 100% formulations showed lower height (thickness) and diameter than HD and LD-normal ones at the same level of sorghum incorporation, which shows higher degree of cooking associated with better interaction of both the proteins and starch with water. This is also an indication that the lines with the combined HD-waxy traits absorb more water and hold it whereas the LD-normal starches interact less with moisture. The irregular shape of the HD proteins and the greater branching of the amylopectin in the waxy starch contribute to the better water holding characteristic of the lines with the HD-waxy combinations. Compared to the refined pastry flour control (6.41

mm), all pancakes with sorghum and whole wheat incorporations (Table 12) had higher thickness values ranging from 7.10 to 11.33 mm (with the exception of the waxy lines at 50%, 6.82 mm for HD and 6.30 mm for LD). This implies that pancakes with the waxy starch are fairly comparable in thickness to those made from refined pastry flour controls, although the instrumental sensory properties were less desirable (section 4.3.1.3.).

Pancake texture

Looking at the textural characteristics (Table 9), the HD line had significantly lower ($p<0.05$) adhesiveness (g/mm, -4.86 versus -2.43 for LD), springiness (0.59 versus 0.62 for LD) and resilience (0.26 versus 0.27 for LD), which might be due to better dissolution of the starches and protein bodies that gave products with tender textures. Adhesiveness is a negative force during retraction of the texture analyzer probe and it represents the stickiness of the product to the surface of the plate or the base (Finnie, Bettge, & Morris, 2006). Less adhesiveness therefore, means better holding-together of the dough/batter as well as the pancake mass due to better dissolution or water holding tendency of the flours from HD sorghum. The moisture content of the pancakes had a weak but significant negative correlation with adhesiveness ($r = -0.35$, Appendix A- 1). The samples with the waxy starch had lower springiness, cohesiveness and resilience compared to samples with the normal starch. The lower springiness, cohesiveness and resilience for the waxy trait might be due to a sticky mass formation after cooking/baking resulted from the higher moisture absorption tendency. The waxy starch results in less spongy and stickier pancake crumb and is not desirable.

Significantly lower adhesiveness, springiness, cohesiveness and resilience were observed for the HD lines at both 50 and 100% sorghum additions, with the LD lines behaving in an opposite manner. Similarly, lower values of the aforementioned parameters were reported for the waxy starches in both levels of formulation. The pancakes from the HD-waxy lines were observed to have lower adhesiveness (g/mm, -5.78 versus -3.94 for the HD-normal), lower springiness (0.56 versus 0.62 for the HD-normal), lower cohesiveness (0.57 versus 0.64 for the HD-normal) and lower resilience (0.23 versus 0.28 for the HD-normal). Similarly, The HD-waxy traits resulted in pancakes of least resilience compared to the HD-normal and LD (both normal and waxy). Comparing the sorghum incorporated pancakes with those with 50% wheat and wheat controls (Table 12), the general observation was that the 100% sorghum pancakes had significantly lower ($p<0.05$) springiness (0.46-0.53 versus 0.67 – 0.76 for the 50 – 100% wheat containing ones); lower cohesiveness (0.47 – 0.58 versus 0.67 – 0.72 for the 50 – 100% wheat containing ones), lower resilience (0.20 – 0.25 versus 0.27 – 0.32 for the wheat containing samples). The low springiness, cohesiveness and springiness of the pancakes from HD-waxy are indications of less spongy and more sticky mass showing poor structural integrity of the pancake crumb. The HD and LD-normal combinations at 50% substitution gave pancakes with more spongy and fluffy textural attributes that are comparable to that of pastry flour-based control. The HD-normal combination at 100% level is a potential ingredient in gluten-free and healthy whole grain-based applications with respect to the better processing functionality and nutritional quality discussed in Chapter 3. There was no significant difference in the hardness, gumminess and chewiness of the samples with respect to the protein, starch, formulation levels, separately or combined.

Effect of HD trait on physical and textural properties of cookies

Physical properties of cookies

There were significant differences ($p<0.05$) in the physical parameters including spread, spread ratio, thickness and moisture content due to the different sorghum traits (protein, starch) at different formulation levels (Table 13), although mean values for some of these were observed to be close to each other. Comparing the HD versus LD protein trait, cookies with the HD proteins had significantly lower spread as well as higher lightness and moisture content. Lower value of the spread for the HD (60.12 versus 61.52 mm for the LD) indicates that the HD flour has better water holding tendency, resulting into a thicker mass that does not readily flow on baking due to the fact that the proteins retain residual moisture. Cookies from the waxy starch sorghum, compared to the normal, were also observed to have lower spread (60.32 versus 61.40 mm for the LD), lighter color (69.15 versus 69.58) and lower moisture content (7.59 versus 7.75%, db) (Table 12). The lower spread is due to high water absorption of the branched amylopectin structure that resulted in thicker and less flowable dough.

The cookies made from sorghum having the HD-waxy traits had significantly lower spread (59.67 versus 60.75 mm), spread ratio (5.06 versus 5.39), brightness (69.01 versus 69.91) and moisture (7.52 versus 8.57% db) compared to the HD-normal. The spread (60.98 mm), brightness (69.30) and moisture content (7.66%, db) for the LD-waxy were higher than that of HD-waxy (Table 14).

Comparing the different sorghum-based cookie samples at different formulation levels with different controls (Tables 15 & 16) significant differences were observed. The

HD and waxy sorghum-based formulations (separately and combined), had lower spread, spread ration and higher thickness compared to the ultra-ground whole wheat (UGWW) and cookie flour (CF)-based controls. There was no clear segregation for the spread based on the protein and starch types of lines. The general observation was that the 100% sorghum containing cookies had lower spread (57.13 – 60.46 mm) compared to those having 50 – 100% wheat (61.19 – 66.46 mm). The 100% sorghum incorporated cookies were noted to have spread values (61.19 mm) comparable to those made of 100% whole wheat flour. The other more noticeable difference in the physical properties comparing the sorghum cookies with whole wheat and refined cookie flour-based ones was significantly higher moisture content of those made from control (9.44%) and experimental (8.82%) HD-normal traits at 100% formulations, which gave the cookies a softer texture (least hardness – sections 4.3.2.2. and 4.3.2.3.).

Spread and setting rate in cookies are reportedly influenced by moisture in the formula (Miller, Hoseney, & Morris, 1997). The lower spread of HD and waxy sorghum-based formulations (separately and combined), might be due to faster setting rate due to higher residual moisture by better interaction of the mutant protein and waxy starch. The higher moisture levels for the HD-normal traits might be due to higher moisture absorbed by the protein mutant and retained in the matrix due to the faster setting even further enhanced by faster reassociation of the amylose in normal starch compared to the waxy counter parts. The HD-normal combination seemed to produce cookies of desirable properties due to higher moisture that results into softer texture (least hardness – sections 4.3.2.2. and 4.3.2.3.) and better gelling of amylose of normal starch compared to amylopectin of the waxy type..

Table 13: Main Effect of variables on cookies parameters

Variables	Spread (mm)	Thickness (mm)	Spread ratio	Color L*	Color a*	Color b*	MC (%)	Hardness (g)		Fracturability (mm)	
								Week 0	Week1	Week 0	Week 1
Protein type											
HD	60.21[b]	11.50[a]	5.23[a]	69.46[a]	2.11[a]	18.90[a]	8.05[a]	2495[b]	3415[b]	-43.73[a]	-43.23[a]
LD	61.52[a]	11.60[a]	2.34[a]	69.27[b]	2.01[b]	18.34[b]	7.30[b]	3504[a]	4719[a]	-43.80[a]	-43.13[a]
Starch type											
Nml	61.40[a]	11.60[a]	5.32[a]	69.58[a]	2.59[a]	19.57[a]	7.75[a]	2840[b]	3724[b]	-43.56[b]	-43.00[a]
Wx	60.32[b]	11.50[a]	5.25[a]	69.15[b]	2.53[b]	17.67[b]	7.59[b]	3159[a]	4410[a]	-43.97[a]	-43.37[a]
Formulations											
50%	63.64[a]	12.10[a]	5.25[a]	71.19[a]	1.84[b]	18.88[a]	7.48[b]	2456[b]	3975[a]	-43.22[a]	-42.60[a]
100%	58.09[b]	11.00[b]	5.32[a]	67.55[b]	2.29[a]	18.36[b]	7.86[a]	3543[a]	4159[a]	-44.31[b]	-43.77[b]
SE	0.2	0.13	0.06	0.05	0.02	0.05	0.03	101	95.77	0.11	0.16

MC = moisture content (%db), HD = highly digestible [protein], LD = low digestible [protein], Nml = normal starch, Wx = waxy starch, SE = standard error [of means]. Values are means of triplicates and those with different superscript letters in the same column are significantly different ($p<0.05$).

Table 14: Interaction Effect (two-way) of variables on cookies parameters

Variables		Spread (mm)	Thickness (mm)	Spread ratio	Color L*	Color a*	Color b*	MC (%)	Hardness (g)		Fracturability (mm)	
									Week 0	Week 1	Week 0	Week 1
Protein by starch												
HD	Nml	60.75^{ab}	11.29^{a}	5.39^{a}	69.91^{a}	1.54^{d}	20.21^{a}	8.57^{a}	1693^{c}	2673^{c}	-43.55^{a}	-43.04^{a}
	Wx	59.67^{c}	11.80^{a}	5.06^{b}	69.01^{c}	2.68^{a}	17.60^{c}	7.52^{b}	3296^{b}	4157^{b}	-43.91^{a}	-43.43^{a}
LD	Nml	62.05^{a}	11.86^{a}	5.25^{ab}	69.24^{bc}	1.64^{c}	18.94^{b}	6.93^{c}	3987^{a}	4775^{a}	-43.57^{a}	-42.95^{a}
	Wx	60.98^{bc}	11.27^{a}	5.44^{a}	69.30^{b}	2.38^{b}	17.74^{c}	7.66^{b}	3021^{b}	4662^{ab}	-44.02^{a}	-43.31^{a}
Protein by formulation												
HD	50%	62.83^{b}	11.90^{ab}	5.28^{a}	71.19^{a}	1.88^{c}	19.14^{a}	7.81^{b}	2181^{c}	3577^{c}	-43.27^{a}	-42.87^{ab}
	100%	59.60^{c}	11.19^{bc}	5.17^{a}	67.73^{b}	2.34^{a}	18.66^{b}	8.29^{a}	2808^{b}	3253^{c}	-44.19^{b}	-43.60^{bc}
LD	50%	64.46^{a}	12.38^{a}	5.22^{a}	71.18^{a}	1.79^{c}	18.61^{b}	7.15^{d}	2732^{bc}	4372^{b}	-43.17^{a}	-42.33^{a}
	100%	58.58^{c}	10.76^{c}	5.47^{a}	67.36^{c}	2.24^{b}	18.07^{c}	7.44^{c}	4277^{a}	5065^{a}	-44.42^{b}	-43.94^{c}
	SE	0.28	0.19	0.08	0.69	0.03	0.07	0.04	142	135	0.16	0.22

MC = moisture content (%db), HD = highly digestible [protein], LD = low digestible [protein], Nml = normal starch, Wx = waxy starch, SE = standard error [of means]. Values are means of triplicates and those with different superscript letters in the same column are significantly different ($p<0.05$).

Table 14 Continued

Variables		Spread (mm)	Thickness (mm)	Spread ratio	Color L*	Color a*	Color b*	MC (%)	Hardness (g)		Fracturability (mm)	
									Week 0	Week 1	Week 0	Week 1
Starch by formulation												
Nml	50%	64.71[a]	12.20[a]	5.31[a]	71.63[a]	1.36[d]	19.52[a]	7.54[c]	2469[c]	3723[b]	-43.17[a]	-42.53[a]
	100%	58.09[c]	10.95[b]	5.32[a]	67.52[c]	1.83[c]	19.63[a]	7.96[a]	3210[b]	3725[b]	-43.95[b]	-43.46[bc]
Wx	50%	62.57[b]	12.07[a]	5.19[a]	70.74[b]	2.32[b]	18.24[b]	7.42[c]	2443[c]	4226[ab]	-43.27[a]	-42.66[ab]
	100%	58.08[c]	11.00[b]	5.32[a]	67.58[c]	2.75[a]	17.10[c]	7.77[b]	3875[a]	4593[a]	-44.66[c]	-44.08[c]
	SE	0.28	0.19	0.08	0.69	0.03	0.07	0.04	142	135	0.16	0.22

MC = moisture content (%db), HD = highly digestible [protein], LD = low digestible [protein], Nml = normal starch, Wx = waxy starch, SE = standard error [of means]. Values are means of triplicates and those with different superscript letters in the same column are significantly different ($p<0.05$).

Table 15: Interaction Effect (three-way) of variables on cookies parameters

Protein by starch by formulation	**Spread (mm)**	**Thickness (mm)**	**Spread ratio**	**Color L***	**Color a***	**Color b***	**MC (%)**	**Hardness (g)**		**Fracturability (mm)**	
								Week 0	**Week 1**	**Week 0**	**Week 1**
HD, Nml, 50%	64.38^{a}	11.99^{a}	5.37^{ab}	72.17^{a}	1.23^{f}	19.92^{b}	8.33^{b}	1670^{e}	2958^{c}	-43.25^{a}	-42.72^{ab}
HD, Nml, 100%	57.13^{d}	10.59^{bc}	5.40^{ab}	67.65^{de}	1.85^{d}	20.49^{a}	8.82^{a}	1715^{e}	2388^{c}	-43.86^{ab}	-43.35^{abc}
HD, Wx, 50%	61.28^{b}	11.81^{ab}	5.19^{ab}	70.21^{c}	2.53^{b}	18.37^{de}	7.28^{d}	2691^{cd}	4196^{ab}	-43.30^{a}	-43.01^{abc}
HD, Wx, 100%	58.07^{cd}	11.79^{ab}	4.94^{b}	67.82^{de}	2.84^{a}	16.83^{g}	7.76^{c}	3902^{ab}	4118^{b}	-44.53^{b}	-43.85^{bc}
LD, Nml, 50%	65.05^{a}	12.42^{a}	5.25^{ab}	71.10^{b}	1.48^{e}	19.12^{c}	6.74^{e}	3269^{bc}	4489^{ab}	-43.10^{a}	-42.34^{a}
LD, Nml, 100%	59.06^{c}	11.30^{abc}	5.24^{ab}	67.39^{de}	1.81^{d}	18.76^{cd}	7.11^{d}	4705^{a}	5061^{a}	-44.05^{ab}	-43.57^{abc}
LD, Wx, 50%	63.87^{a}	12.34^{a}	5.18^{ab}	71.27^{b}	2.10^{c}	18.11^{e}	7.56^{c}	2194^{de}	4256^{ab}	-43.24^{a}	-42.32^{a}
LD, Wx, 100%	58.09^{cd}	10.21^{c}	5.70^{a}	67.33^{e}	2.66^{b}	17.37^{f}	7.77^{c}	3849^{ab}	5069^{a}	-44.80^{b}	-44.31^{c}
SE	0.40	0.26	0.12	0.10	0.04	0.10	0.05	201	192	0.22	0.31

MC = moisture content (%db), HD = highly digestible [protein], LD = low digestible [protein], Nml = normal starch, wx = waxy starch, SE = standard error [of means], MC = moisture content. Values are means of triplicates and those with different superscript letters in the same column are significantly different ($p<0.05$).

Table 16: Comparison of HD versus LD sorghum-based cookies with wheat controls.

Variables	**Spread (mm)**	**Thickness (mm)**	**Spread ratio**	**Color L***	**Color a***	**Color b***	**MC (%)**	**Hardness (g)**		**Fracturability (mm)**	
								Week 0	**Week 1**	**Week 0**	**Week 1**
CHD 100%	60.46^{de}	11.07^{bcd}	5.47^{ab}	60.88^{h}	5.90^{a}	15.09^{l}	9.44^{a}	1446^{h}	2235^{f}	-43.82^{bc}	-43.37^{abcd}
HD Nml 100%	57.13^{g}	10.59^{cd}	5.40^{abc}	67.65^{ef}	1.85^{g}	20.49^{c}	8.82^{b}	1715^{gh}	2388^{f}	-43.86^{bc}	-43.35^{abcd}
HD wx 100%	58.07^{fg}	11.79^{ab}	4.94^{c}	67.82^{e}	2.84^{d}	16.83^{j}	7.76^{def}	3902^{bc}	4118^{bcd}	-44.53^{c}	-43.85^{cd}
LD Nml 100%	59.06^{ef}	11.30^{abcd}	5.24^{abc}	67.39^{ef}	1.81^{g}	18.76^{fg}	7.11^{h}	4705^{ab}	5061^{abc}	-44.05^{bc}	-43.57^{abcd}
LD wx 100%	58.09^{fg}	10.21^{d}	5.70^{a}	67.33^{ef}	2.66^{de}	17.37^{i}	7.77^{def}	3849^{bc}	5069^{ab}	-44.80^{c}	-44.31^{d}
CHD 50%	66.66^{a}	12.31^{a}	5.42^{abc}	67.42^{ef}	4.01^{c}	15.78^{k}	7.88^{de}	2375^{efgh}	4080^{cd}	-42.36^{a}	-42.31^{a}
HD Nml 50%	64.38^{bc}	11.99^{ab}	5.37^{abc}	72.17^{b}	1.23^{i}	19.92^{d}	8.33^{c}	1670^{gh}	2958^{ef}	-43.25^{ab}	-42.72^{abc}
HD wx 50%	61.28^{d}	11.81^{ab}	5.19^{bc}	70.21^{d}	2.53^{e}	18.37^{gh}	7.28^{gh}	2691^{def}	4196^{bcd}	-43.30^{ab}	-43.01^{abcd}
LD Nml 50%	65.05^{ab}	12.42^{a}	5.25^{abc}	71.10^{c}	1.48^{h}	19.12^{ef}	6.74^{i}	3269^{cde}	4489^{bcd}	-43.10^{ab}	-42.34^{ab}
LD wx 50%	63.87^{bc}	12.34^{a}	5.18^{bc}	71.27^{c}	2.10^{f}	18.11^{h}	7.56^{fg}	2194^{fgh}	4256^{bcd}	-43.24^{ab}	-42.32^{a}
UG WW 100%	61.19^{d}	11.47^{abc}	5.34^{abc}	63.14^{g}	5.25^{b}	24.52^{a}	7.50^{fg}	4942^{a}	5483^{a}	-43.96^{bc}	-43.66^{bcd}
UG WW 50%	63.22^{c}	11.45^{abc}	5.52^{ab}	67.10^{f}	4.14^{c}	23.81^{b}	7.98^{d}	3366^{cd}	5813^{a}	-43.99^{bc}	-43.43^{abcd}
CF 100%	66.46^{a}	11.64^{abc}	5.71^{a}	75.30^{a}	1.19^{i}	19.48^{de}	7.67^{ef}	2594^{defh}	3617de	-43.28^{ab}	-43.06^{abcd}
SE	0.37	0.23	0.10	0.14	0.04	0.10	0.06	187	197	0.21	0.27

CHD = control HD, HD = highly digestible [protein], LD = low digestible [protein], Nml = normal starch, wx = waxy starch, UG WW= ultra-ground whole wheat flour, CF = cookie flour, SE = standard error [of means], MC = moisture content. Values are means of triplicates and those with different superscript letters in the same column are significantly different ($p<0.05$).

Instrumental textural properties of cookies

There were significant differences ($p<0.05$) in the instrumental texture parameters, hardness and fracturability due to the different sorghum traits (protein and starch) at different formulation levels (Table 13). Sorghum lines with the HD protein trait were observed to have lower hardness (g) (2495 and 3415 for the fresh and 1-week stored samples, respectively), compared to the LD counterparts (3504 and 4719, for the fresh and 1w stored samples, respectively). The higher moisture absorption and retention by the HD mutant protein might have given the cookies a softer and more tender texture compared to the LD ones. Comparing the waxy and normal starch trait, the former had significantly higher hardness (g) (3159 for the fresh and 4410 for 1 w stored samples) than the normal starch types (2840 for the fresh and 3724 for 1 w stored). Looking at the percentage of sorghum addition, the 100% levels had higher hardness (3543 g) compared to the 50% ones (2456 g) for the fresh samples. The harder texture of the samples from sorghum with waxy starch might be due to higher absorption of moisture resulting in more cohesive mass compared to dry and crumbly mass for the normal starch counter parts.

Cookies from HD-normal lines had lower hardness (1693 g) than the HD-waxy ones (3296 g). In fact, the HD-normal combination had the least hardness values for both fresh and stored cookies compared to the ones from LD lines regardless of the starch type. The high level of moisture retained in the HD-waxy might resulted in the formation of more cohesive and harder mass due to the faster setting (Miller, Hoseney, & Morris, 1997). The higher hardness of the cookies with the LD proteins might be due to faster drying of the moisture due to limited interaction with the proteins that resulted in excessive crystallization of sugar.

In the 100% sorghum formulations (Table 15&16), HD-waxy had higher hardness values (3902 g) compared to the HD-normal (1715 g) on the day of baking. On the other hand, the samples from the LD-waxy had lower hardness values (3849 g) compared to the LD-normal ones (4705 g) at the 100% sorghum addition. Fresh cookies from the HD-normal had lower hardness (1715 g) than the LD-normal (4705 g) whereas the HD (3902 g) and the LD (3849 g) lines in the waxy starch background did not differ. The higher hardness values in the HD-waxy might be due to higher moisture retention by both the protein mutants and the amylopectin that led to a faster setting and the formation of a more cohesive mass. The soft texture of the HD-normal on the other hand might be due to the retention of moisture by the proteins but the absence cohesive mass formation due to limited level of the amylopectin, resulting into an easily snapping cookie. The highest hardness in the LD-normal is due to limited interaction of the flour (globular protein bodies and amylose rich starches) that led to higher level of evaporation of the water on baking, resulting in to cookies of drier texture, that can partly be due to higher degree of sugar crystallization.

The lower hardness values (1715 g) of the HD-normal based fresh samples was close to the values for the control HD (1446 g) and cookie flour (2594 g) controls. The least hardness in the HD-normal combination on the other hand might be due to better moisture (8.82% versus 7.11% for LD-normal) absorption and retention of the HD mutant proteins that resulted in soft texture. The normal starch (amylose) in the HD-normal combination might also contributed to the softer texture by limiting the extent of cooking, followed by faster retrogradation. It was apparent that HD-normal combination has textural properties fairly comparable to the cookie flour-based control, whereas those with waxy starch (both HD and LD) resulted in hard cookies. This indicates that the HD-normal at the 100% addition level can be a potential gluten-free, whole grain alternative for sugar snap cookies, which was also supported by desirable physical properties (section 4.3.2.1.). The relationship between some of the physical and texture properties was also shown by significant correlations. The moisture content and hardness were significantly and negatively correlated (r = -0.62 at W0 and r = -0.66 at W1, Appendix A- 2), showing that the higher the moisture, the softer the cookies in the absence of the waxy starch.

The important trend was that the cookies from the sorghum lines that had the least hardness were observed to have the highest fracturability (Table 13), which was also confirmed by a significant negative correlation (r = -0.44, Appendix A- 2) between the two parameters for the fresh samples. The physical parameter, spread was significantly and positively correlated with fracturability (r = 0.70 at W0 and r = 0.62 at W1, Appendix A- 2), indicating that the higher the spread the more fracturable the cookies are. The fructurability values of cookies made from the different sorghum types were not different from the whole and refined wheat as well as the HD sorghum controls (Table 15).

Changes in cookie textural properties over storage

As expected, the hardness of the cookies increased over a storage period of a week and the rate of hardening was significantly different with respect to the different protein and starch types (Figure 6A). A more rapid increase in hardness (steeper slope) (slope =

788) was observed for the LD lines than for the HD (slope = 445). Sorghum lines with the HD protein traits had the least rate of hardening whereas the LD ones had the highest. This suggests that the HD protein trait is associated with better shelf stability of the cookies compared to the LD trait. Comparing the different starch types, the waxy lines had higher rate of hardening (slope = 718) than the normal ones (slope = 515), which was most likely due to the formation of a more cohesive mass by the rapid cooking waxy starch.

Looking at the protein types in the different starch backgrounds (Tables 13 - 16, Figure 6B), HD-normal had lower magnitude of hardness and slower rate of hardening (slope = 672) compared to the HD-waxy with higher magnitude of hardness for fresh cookies (3902 g) but with the least rate of hardening (slope = 216). The results show that the HD-normal combination gave softer cookies compared to the HD-waxy. The cookies from HD-normal had half hardness values (2388 g) compared to the HD-waxy combinations (4118 g) after a week storage. LD-normal had the largest magnitude of hardness value (4705 g) and with a rate of hardening (slope = 356) compared to the LD waxy (3849 g with steepest slope = 1220). The slightly lower slope for the LD normal might be an indication that the cookie dough might become dry on baking due to the low interaction of the flour and water, that might have resulted into extensive crystallization of sugar. The steepest slope of the LD-waxy shows the slower retrogradation of the amylopectin over the storage days compared to the few hours for the amylose (Yu, Ma, & Sun, 2009).

The HD-normal combination was observed to have softer cookies even after a week storage compared to all other combinations and those having 50 – 100% wheat flour

(Tables 15 and 16). Cookies from 100% HD-normal were less chewy (2388 g after a week storage) compared to the those containing 50 – 100% wheat (2958 – 4489 g) or other combinations of sorghum protein and starch traits at the 100% addition levels (4118 – 5069 g) or the 50 – 100% whole wheat flour substitution for cookies flour (5483– 5813 g).

The hardness and the rate of hardening are associated to the capacity of the protein and starch to interact with water. The relationship between moisture content and hardness was also explained by a significant negative correlation (r = -0.66, Appendix A- 2) The hardness of the cookies (fresh) was observed to be significantly but weakly and negatively correlate with the fracturability (Appendix A- 2) in both week 0 (r = -0.44) and week 1 (r = -0.35).

In summary, in the cookies model the normal starch was more influenced by the HD trait than the waxy ones. The 100% HD-normal was the best combination of sorghum traits for making the softest (section 4.3.2.2.) and stable sugar snap cookie with desirable physical properties (section 4.3.2.1.) and can potentially be used as ingredient for a gluten-free, wheat free and whole grain options.

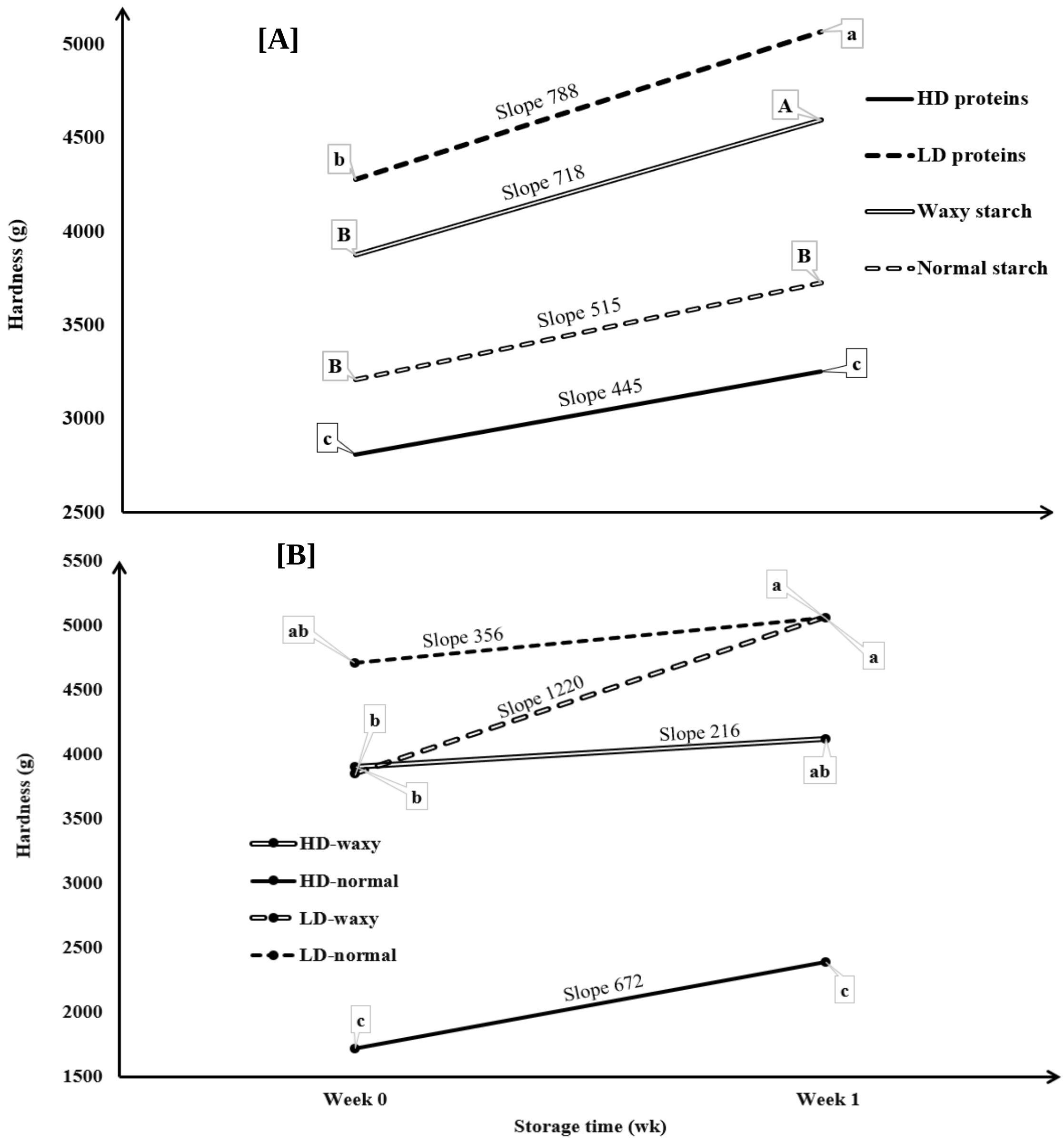

Figure 6: Change in the hardness of cookie over storage, for the [A] HD versus LD protein and waxy versus normal starch traits of sorghum; [B] HD-waxy and normal versus LD-waxy and normal. Slope indicates the rate of hardening; points with different letters of the same style are significantly different ($p<0.05$).

L*a*b* color parameters of the cookies

Looking at the color of the cookies, there were significant differences pertaining to the different variables. The other important color difference was that the cookies made from the whole wheat flour (ultra-ground) and the control HD sorghum were significantly higher in redness/yellowness (a* = 4.01-5.90) compared to all other formulations (a* = 1.19-2.84) (Table 15). The color of cookies from whole grain sorghum depends on the pericarp colors that may not directly be related to the protein or starch types. It is also plausible that the consistently slightly darker (Table 16) HD protein (L* = 67.65 – 67.82) and waxy starch-based cookies (L* = 67.33 – 67.82) at the 100% sorghum formulation might be due to more mass density caused by better moisture retention. The cookie formulas containing cookie flour (50 – 100%), were consistently lighter (L* = 70.21 – 75.30) compared to the 100% sorghum-based ones. The 100% sorghum formulas gave darker cookies with whole grain appearance that might appeal to health-conscious consumers.

Sensory analysis of pancakes and cookies

Descriptive sensory attributes of pancakes

The sensory attributes of the pancakes were not influenced by the HD protein trait (Table 17). However, the waxy starch resulted in pancakes of significantly higher cohesiveness of mass compared to the normal ones. This is most likely due to the formation of sticky mass on cooking and limited gelling on cooling, that may affect the texture of the pancakes. The effect of the waxy starch on the cohesiveness of mass was persistent regardless of the protein type and formulation levels. The improved sorghum lines with HD-normal traits were observed with more positive physical properties (section 4.3.1.2.) as well as instrumental texture (section 4.3.1.3.).

Sorghum incorporation level affected pancake bitterness, denseness and grittiness. Pancakes with 100% sorghum were more bitter (2.57 versus 1.95 for the 50%), dense (4.08 versus 3.30 for the 50%) and gritty (2.80 versus 1.80 for the 50%). The influence was consistent across the different starch and protein types. The 100% formulation had significantly higher bitterness but the score of 2-3 on the scale of 0 to 15 would most likely not impact the overall flavor in the product. The higher intensity of bitterness, denseness and grittiness was most likely due to the bran in the whole sorghum flour compared to those having 50% refined wheat (pastry) flour. The implication is that preparation of pancakes from 100% whole sorghum flour is feasible as far as sensory attributes are concerned and this makes the improved sorghum an appealing healthy, gluten and wheat-free ingredient for pancake making.

Descriptive texture analysis for cookies

The HD-protein trait influenced the crispiness of the cookies (Table 18), where cookies from sorghum flour with the HD-protein trait were crispier (4.19) than the LD counter parts. The higher crispiness of cookie samples from the HD-protein containing sorghum might be due to the higher moisture absorption and retention by the irregularly shaped protein mutants that resulted in a matrix that better holds together compared to the LD ones. The starch type (waxy versus normal) also significantly affected the hardness of the cookies where normal starch cookies were harder (6.18) than cookies containing the waxy counterpart (5.44). The harder normal starch cookies may be the result of limited interaction with moisture that might have resulted in extensive sugar crystallization as manifested in the instrumental texture properties (sections 4.3.2.2. and 4.3.2.3.).

Table 17: Descriptive sensory analysis of pancakes made from different sorghum lines and formulation levels

Variables		Bitterness	Denseness	Grittiness	Cohesiveness of mass
Protein	HD	2.30[a]	3.88[a]	2.20[a]	11.63[a]
	LD	2.23[a]	3.50[a]	2.42[a]	11.22[a]
	SE	0.14	0.18	0.17	0.22
Starch	Normal	2.30[a]	3.68[a]	2.35[a]	11.05[b]
	Waxy	2.23[a]	3.70[a]	2.28[a]	11.80[a]
	SE	0.14	0.18	0.17	0.22
Formulation	50	1.95[b]	3.30[b]	1.80[b]	11.38[a]
	100	2.57[a]	4.08[a]	2.80[a]	11.48[a]
	SE	0.14	0.18	0.17	0.22
Protein by starch	HD, Nml	2.30[a]	3.75[a]	2.35[a]	11.05[b]
	HD, Wx	2.30[a]	4.00[a]	2.05[a]	12.20[a]
	LD, Nml	2.30[a]	3.60[a]	2.35[a]	11.05[b]
	LD, Wx	2.15[a]	3.40[a]	2.50[a]	11.40[ab]
	SE	0.2	0.28	0.24	0.31

HD = highly digestible [protein], LD = low digestible [protein], Nml = normal starch, Wx = waxy starch, SE = standard error [of means], Values are means of duplicate scores and those with different superscript letters in the same column under the same variables and their combinations are significantly different ($p<0.05$).

Table 17 Continued

Variables		**Bitterness**	**Denseness**	**Grittiness**	**Cohesiveness of mass**
Protein by formulation	HD, 50%	1.90[b]	3.40[b]	1.65[c]	11.80[a]
	HD, 100%	2.70[a]	4.35[a]	2.75[ab]	11.45[a]
	LD, 50%	2.00[b]	3.20[b]	1.95[bc]	10.95[a]
	LD, 100%	2.45[ab]	3.80[ab]	2.90[a]	11.50[a]
	SE	0.2	0.25	0.24	0.31
Starch by formulation	Nml, 50%	1.95[b]	3.30[b]	1.55[c]	11.20[a]
	Nml, 100%	2.65[a]	4.05[a]	3.15[a]	10.90[a]
	Wx, 50%	1.95[b]	3.30[b]	2.05[bc]	11.55[a]
	Nml, 100%	2.50[ab]	4.10[a]	2.50[ab]	12.05[a]
	SE	0.2	0.25	0.24	0.31
Protein by starch by formulation	HD, Nml, 50%	1.80[b]	3.30[bc]	1.40[d]	11.70[ab]
	HD, Nml, 100%	2.80[a]	4.20[ab]	3.30[a]	10.40[c]
	HD, Wx, 50%	2.00[ab]	3.50[abc]	1.90[cd]	11.90[ab]
	HD, Wx, 100%	2.60[ab]	4.50[a]	2.20[bcd]	12.50[a]
	LD, Nml, 50%	2.10[ab]	3.30[bc]	1.70[d]	10.70[bc]
	LD, Nml, 100%	2.50[ab]	3.90[abc]	3.00[ab]	11.40[abc]
	LD, Wx, 50%	1.90[b]	3.10[c]	2.20[bcd]	11.20[bc]
	LD, Wx, 100%	2.40[ab]	3.70[abc]	2.80[abc]	11.60[abc]
	SE	0.28	0.36	0.39	0.44

HD = highly digestible [protein], LD = low digestible [protein], Nml = normal starch, Wx = waxy starch, SE = standard error [of means], Values are means of duplicate scores and those with different superscript letters in the same column under the same variables and their combinations are significantly different ($p<0.05$).

Sorghum incorporation level significantly influenced all the assessed attributes (Table 18). The 100% sorghum cookies had higher hardness (8.51 versus 3.11 for the 50%), fracturability (5.97 versus 2.25 for the 50%), surface roughness (5.34 versus 4.47 for the 50%) and crispiness (6.00 versus 1.28 for the 50% sorghum incorporated samples). The higher scores for the hardness, fracturability, surface roughness and crispiness of the 100% sorghum cookies might be due to the limited moisture retention compared to the those containing 50% wheat flour.

The two-way combination of protein and starch resulted in differences ($p<0.05$), although there were no clear trends pertaining to the HD-proteins in different starch backgrounds. However, all the combinations (both two and three-way) involving sorghum incorporation levels clearly showed higher scores for those having 100% sorghum regardless of the protein and starch types. This indicates that there is some room to improve the sensory texture attributes of sorghum cookies, although the instrumental texture analysis revealed clear differences among the different sorghum treatments. It was indicated that HD-normal sorghum combination was the best choice giving the softest cookies (sections 4.3.2.2. and 4.3.2.3.) and other physical characteristics (section 4.3.2.1).

Table 18: Descriptive sensory analysis of cookies made from different sorghum lines and formulation levels

Variables		**Hardness**	**Fracturability**	**Surface roughness**	**Crispiness**
	HD	5.87[a]	4.33[a]	5.01[a]	4.19[a]
Protein	LD	5.76[a]	3.89[a]	4.79[a]	3.08[b]
	SE	0.22	0.31	0.22	0.35
	Normal	6.18[a]	4.44[a]	4.73[a]	4.11[a]
Starch	Waxy	5.44[b]	3.78[a]	5.08[a]	3.17[a]
	SE	0.24	0.31	0.23	0.37
	50	3.11[b]	2.25[b]	4.47[b]	1.28[b]
Formulation	100	8.51[a]	5.97[a]	5.34[a]	6.00[a]
	SE	0.24	0.31	0.23	0.37
	HD, Nml	5.85[ab]	4.10[ab]	4.92[a]	3.88[a]
	HD, Wx	5.89[ab]	4.56[a]	5.11[a]	4.50[a]
Protein by starch	LD, Nml	6.51[a]	4.79[a]	4.53[a]	4.33[a]
	LD, Wx	5.00[b]	3.00[b]	5.06[a]	1.83[b]
	SE	0.29	0.44	0.32	0.53
	HD, 50%	2.89[b]	2.06[b]	4.44[b]	1.22[c]
	HD, 100%	8.85[a]	6.6[a]	5.85[a]	7.16[a]
Protein by formulation	LD, 50%	3.33[b]	2.44[b]	4.50[b]	1.33[c]
	LD, 100%	8.18[a]	5.34[a]	5.09[ab]	4.83[b]
	SE	0.32	0.44	0.32	0.53

HD = highly digestible [protein], LD = low digestible [protein], Nml = normal starch, Wx = waxy starch, SE = standard error [of means], Values are means of duplicate scores and those with different superscript letters in the same column under the same variables and their combinations are significantly different ($p<0.05$).

Table 18 Continued

Variables		Hardness	Fracturability	Surface roughness	Crispiness
Starch by formulation	Nml, 50%	3.11^{c}	2.33^{b}	4.44^{b}	1.33^{c}
	Nml, 100%	9.25^{a}	6.55^{a}	5.01^{ab}	6.88^{a}
	Wx, 50%	3.11^{c}	2.17^{b}	4.50^{b}	1.22^{c}
	Nml, 100%	7.78^{b}	5.39^{a}	5.67^{a}	5.11^{b}
	SE	0.32	0.44	0.32	0.53
Protein by starch by formulation	HD, Nml, 50%	3.00^{c}	1.89^{c}	4.22^{c}	1.00^{b}
	HD, Nml, 100%	8.69^{a}	6.31^{a}	5.62^{a}	6.77^{a}
	HD, Wx, 50%	2.78^{c}	2.22^{bc}	4.67^{abc}	1.44^{b}
	HD, Wx, 100%	9.00^{a}	6.89^{a}	5.56^{ab}	7.56^{a}
	LD, Nml, 50%	3.22^{c}	2.78^{bc}	4.67^{abc}	1.67^{b}
	LD, Nml, 100%	9.80^{a}	6.80^{a}	4.40^{abc}	7.00^{a}
	LD, Wx, 50%	3.44^{c}	2.11^{c}	4.33^{bc}	1.00^{b}
	LD, Wx, 100%	6.56^{b}	3.89^{b}	5.78^{a}	2.67^{b}
	SE	0.44	0.62	0.45	0.75

HD = highly digestible [protein], LD = low digestible [protein], Nml = normal starch, Wx = waxy starch, SE = standard error [of means], Values are means of duplicate scores and those with different superscript letters in the same column under the same variables and their combinations are significantly different ($p<0.05$).

Bread characteristic as influenced by the HD sorghum protein trait

Specific volume and moisture content

Comparing the sorghum with HD and LD protein types (Table 19), the bread samples with the HD lines were characterized by lower specific volume (4.37 versus 4.89

mL/g for the LD) (higher loaf density) and higher moisture content (33.80 versus 33.25% for LD). The lower specific volume (higher density) and higher moisture content for the samples with HD protein mutants are likely due to better water holding capacity of the proteins in the improved HD lines, that helps in better holding together of structure. The specific volume was also significantly and positively correlated with the lightness of the crumb (r = 0.73 for day 1 and 0.77 for day 2, Appendix A- 3). The sourdough bread samples were generally characterized by significantly lower specific volume and lower moisture content compared to their yeast counterparts. Considering the level of sorghum incorporation (Table 19), the specific volume decreased (4.77 to 4.48 mL/g) and the moisture content of the bread samples increased (33.39 to 33.66%, db) as the proportion of sorghum incorporation increased from 10 to 20%. The decrease in the specific volume is obviously due to the increased level of dilution of the gluten as the proportion of wheat flour decreases.

Sorghum lines with waxy starch types resulted in breads of significantly higher specific volume (4.75 mL/g) (lower loaf density) (Table 19) compared to those with normal starches (4.51 mL/g), which might be due to the limited gelling in the waxy sorghum flour component.

The improved HD-normal sorghum traits generally resulted in lower specific volume (4.15 mL/g) compared to the HD-waxy (4.59 mL/g). The LD-waxy/normal had higher specific volumes (4.88 – 4.90 mL/g) in both levels of formulations and the yeast versus sour dough cultures. Bread samples with the HD-normal also had highest moisture content (34.3%) compared to the HD-waxy (33.3%) and the LD waxy (33.3%) and normal

(33.2%) ones (Table 20). Similar tends were observed in the various levels of sorghum addition and the yeast versus sourdough cultures (Tables 21-22). The lower specific volume (higher loaf density) and the higher moisture content for the samples from the HD-normal is likely due to a combination of better interaction with and retention of moisture by the irregularly shaped protein bodies, as well as the stronger gel formed by the normal starches where the amylose partially re-associates. In the regular LD protein types, the kafirins might have limited the absorption of water into the matrix that might have limited swelling and gelatinization of the starches (both waxy and normal).

Comparing the sorghum treatments with controls (soft endosperm HD sorghum, 100% high gluten flour and ultra-ground whole wheat), there was significant differences in many of the assessed parameters (Table 23). The least specific volume (2.54 – 2.74 mL/g) (highest loaf density) and highest moisture content (34.90 – 37.0% db) were observed for ultra-ground whole wheat (UGWW) flour and LD sorghum treatments (34.9 – 36.9% db) compared to other combinations (3.50 – 6.33 mL/g for the specific volume and 30.4 – 34.2% db for the moisture). The specific volume (3.53 mL/g) and moisture level (32.9%) of the sourdough bread samples from 20% HD-normal sorghum were close to those made form 100% high gluten wheat control (4.24 mL/g and 30.4%, for the specific volume and moisture, respectively) than to the 100% ultra-ground whole wheat controls (2.54 mL/g and 37.0% for the specific volume and moisture, respectively). This implies that the 20% HD-normal may be a better alternative for whole grain incorporation than whole wheat.

Table 19: Bread characteristics as influenced by protein, starch and fermentation types and formulation levels

Variables	SV (ml/g)	MC (%)	Crust			Crumb						Firmness (g)	
			L*	a*	b*	Day 1			Day 2			Day 1	Day 2
						L*	a*	b*	L*	a*	b*		
Protein type													
HD	4.37^{b}	33.80^{a}	54.45^{a}	12.06^{a}	26.60^{a}	68.24^{a}	0.76^{a}	17.29^{a}	68.08^{a}	0.85^{a}	17.15^{a}	220^{a}	621^{a}
LD	4.89^{a}	33.25^{b}	49.95^{b}	12.28^{a}	25.19^{b}	67.88^{a}	0.75^{a}	17.14^{b}	68.13^{a}	0.72^{a}	16.87^{b}	183^{b}	404^{b}
Starch type													
Normal	4.51^{b}	33.75^{a}	52.34^{a}	12.30^{a}	26.31^{a}	67.68^{b}	0.65^{b}	17.39^{a}	67.63^{b}	0.61^{b}	17.09^{a}	227^{a}	504^{a}
Waxy	4.75^{a}	33.30^{b}	52.06^{a}	12.04^{a}	25.49^{b}	68.43^{a}	0.86^{a}	17.04^{b}	68.57^{a}	0.97^{a}	16.93^{a}	175^{b}	520^{a}
SE	0.03	0.06	0.25	0.09	0.1	0.18	0.03	0.04	0.14	0.1	0.06	5.71	18.11

SV = specific volume, MC = moisture content (%db), HD = highly digestible [protein], LD low digestible [protein], values are means of triplicate measurements and those with different superscript letters are significantly different ($p<0.05$), SE is standard error of means

Table 19 Continued

Variables	SV (ml/g)	MC (%)	Crust			Crumb						Firmness (g)	
			L*	a*	b*	Day 1			Day 2			Day 1	Day 2
						L*	a*	b*	L*	a*	b*		
Formulation													
10% Sorg.	4.77^{a}	33.39^{b}	51.36^{b}	12.71^{a}	26.03^{a}	68.96^{a}	0.45^{b}	17.50^{a}	68.59^{a}	0.53^{b}	17.03^{a}	184^{b}	448^{b}
20% Sorg.	4.48^{b}	33.66^{a}	53.04^{a}	11.63^{b}	25.77^{a}	67.16^{b}	1.06^{a}	16.92^{b}	67.61^{b}	1.05^{a}	17.00^{a}	218^{a}	577^{a}
Starter													
Sourdough	3.81^{b}	32.16^{b}	50.47^{b}	11.30^{b}	23.64^{b}	65.65^{b}	1.17^{a}	16.74^{b}	65.75^{b}	1.12^{a}	16.53^{b}	283^{a}	726^{a}
Yeast	5.45^{a}	34.89^{a}	53.93^{a}	13.04^{a}	28.16^{a}	70.76^{a}	0.34^{b}	17.68^{a}	70.45^{a}	0.45^{b}	17.49^{a}	119^{b}	298^{b}
SE	0.03	0.06	0.25	0.09	0.1	0.18	0.03	0.04	0.14	0.1	0.06	5.71	18.11

SV = specific volume, MC = moisture content (%db), HD = highly digestible [protein], LD low digestible [protein], values are means of triplicate measurements and those with different superscript letters are significantly different ($p<0.05$), SE is standard error of means

Table 20: Bread characteristics as influenced by two-way interaction of different variables

Interactions (2-way)	**SV (ml/g)**	**MC (%)**	**Crust**			**Crumb**						**Firmness (g)**	
						Day 1			**Day 2**				
			L*	**a***	**b***	**L***	**a***	**b***	**L***	**a***	**b***	**Day 1**	**Day 2**
Protein by starch													
HD Nml	4.15^{c}	34.3^{a}	54.2^{a}	12.5^{a}	27.0^{a}	68.1^{ab}	0.52^{c}	17.6^{a}	68.1^{b}	0.46^{b}	17.1^{a}	257^{a}	612^{a}
HD Wx	4.59^{b}	33.3^{b}	54.7^{a}	11.7^{b}	26.2^{b}	68.4^{a}	1.00^{a}	17.0^{b}	68.0^{b}	1.24^{a}	17.2^{a}	183^{b}	630^{a}
LD Nml	4.88^{a}	33.2^{b}	50.5^{b}	12.1^{ab}	25.7^{c}	67.3^{b}	0.78^{b}	17.2^{b}	67.2^{c}	0.75^{ab}	17.0^{ab}	198^{b}	397^{b}
LD Wx	4.90^{a}	33.3^{b}	49.4^{b}	12.4^{a}	24.7^{d}	68.4^{a}	0.72^{b}	17.1^{b}	69.1^{a}	0.70^{b}	16.7^{b}	168^{b}	411^{b}
Protein by formulation													
HD 10%	4.48^{c}	33.9^{a}	53.2^{b}	12.8^{a}	26.9^{a}	69.4^{a}	0.46^{b}	17.8^{a}	68.6^{a}	0.68^{ab}	17.2^{a}	218^{a}	568^{b}
HD 20%	4.26^{d}	33.7^{a}	55.7^{a}	11.3^{c}	26.3^{b}	67.0^{c}	1.07^{a}	16.8^{c}	67.6^{b}	1.25^{a}	17.1^{a}	221^{a}	674^{a}
LD 10%	5.07^{a}	32.9^{b}	49.5^{c}	12.6^{a}	25.2^{c}	68.5^{b}	0.45^{b}	17.2^{b}	68.6^{a}	0.37^{b}	16.9^{a}	150^{b}	328^{c}
LD 20%	4.71^{b}	33.6^{a}	50.4^{c}	12.0^{b}	25.2^{c}	67.3^{c}	1.05^{a}	17.1^{b}	67.6^{b}	1.08^{a}	16.9^{a}	215^{a}	479^{b}
SE	0.04	0.08	0.35	0.13	0.14	0.25	0.04	0.06	0.2	0.14	0.08	8.08	25.6

SV = specific volume, MC = moisture content (%db), HD = highly digestible [protein], LD = low digestibility [protein], Nml = normal starch, Wx = waxy starch, SD = sourdough starter, Yst = yeast, values are means of triplicate measurements and those with different superscript letters are significantly different ($p<0.05$), SE = Standard error of means.

Table 20 Continued

Interactions (2-way)	SV (ml/g)	MC (%)	Crust			Crumb						Firmness (g)	
						Day 1			Day 2				
			L*	a*	b*	L*	a*	b*	L*	a*	b*	Day 1	Day 2
Protein by starter													
HD SD	3.64^{d}	32.2^{c}	52.7^{b}	11.4^{c}	24.4^{c}	66.1^{b}	1.19^{a}	17.0^{b}	66.1^{c}	1.20^{a}	16.7^{b}	323^{a}	895^{a}
HD Yst	5.10^{b}	35.4^{a}	56.2^{a}	12.8^{b}	28.8^{a}	70.3^{a}	0.34^{b}	17.6^{a}	70.0^{b}	0.51^{b}	17.6^{a}	117^{c}	347^{c}
LD SD	3.97^{c}	32.1^{c}	48.2^{c}	11.2^{c}	22.9^{d}	65.2^{c}	1.15^{a}	16.5^{c}	65.4^{c}	1.05^{a}	16.4^{c}	244^{b}	558^{b}
LD Yst	5.80^{a}	34.4^{b}	51.7^{b}	13.3^{a}	27.5^{b}	70.6^{a}	0.35^{b}	17.8^{a}	70.9^{a}	0.40^{b}	17.4^{a}	121^{c}	250^{c}
Starch by formulation													
Nml 10%	4.66^{b}	33.6^{b}	51.8^{bc}	12.8^{a}	26.4^{a}	68.7^{a}	0.32^{c}	17.6^{a}	68.6^{a}	0.31^{b}	17.2^{a}	194^{b}	409^{c}
Nml 20%	4.37^{c}	33.9^{a}	52.9^{ab}	11.9^{b}	26.2^{a}	66.7^{c}	1.00^{a}	17.1^{c}	66.7^{b}	0.91^{a}	17.0^{ab}	260^{a}	599^{a}
Wx 10%	4.89^{a}	33.2^{c}	50.9^{c}	12.7^{a}	25.6^{b}	69.2^{a}	0.59^{b}	17.4^{b}	68.6^{a}	0.74^{ab}	16.9^{b}	174^{b}	487^{bc}
Wx 20%	4.60^{b}	33.4^{bc}	53.2^{a}	11.4^{b}	25.4^{b}	67.6^{b}	1.13^{a}	16.7^{d}	68.6^{a}	1.20^{a}	17.0^{ab}	176^{b}	554^{ab}
SE	0.04	0.08	0.35	0.13	0.14	0.25	0.04	0.06	0.2	0.14	0.08	8.08	25.6

SV = specific volume, MC = moisture content (%db), HD = highly digestible [protein], LD = low digestibility [protein], Nml = normal starch, Wx = waxy starch, SD = sourdough starter, Yst = yeast, values are means of triplicate measurements and those with different superscript letters are significantly different ($p<0.05$), SE = Standard error of means.

Table 20 Continued

Interactions (2-way)	SV (ml/g)	MC (%)	Crust			Crumb						Firmness (g)	
						Day 1			Day 2				
			L*	a*	b*	L*	a*	b*	L*	a*	b*	Day 1	Day 2
Starch type by starter													
Nml SD	3.73[c]	31.8[d]	50.3[b]	11.2[c]	23.7[c]	65.2[b]	1.03[b]	16.9[c]	65.5[c]	1.00[ab]	16.5[c]	336[a]	753[a]
Nml Yst	5.30[b]	35.7[a]	54.4[a]	13.4[a]	29.0[a]	70.1[a]	0.27[c]	17.9[a]	69.8[b]	0.22[c]	17.7[a]	118[c]	255[b]
Wx SD	3.88[c]	32.5[c]	50.6[b]	11.4[c]	23.6[c]	66.1[b]	1.31[a]	16.6[d]	66.1[c]	1.25[a]	16.5[c]	231[b]	699[a]
Wx Yst	5.61[a]	34.1[b]	53.5[a]	12.7[b]	27.4[b]	70.8[a]	0.41[c]	17.5[b]	71.1[a]	0.69[bc]	17.3[b]	119[c]	342[b]
Formulation by starter													
10% SD	3.86[c]	31.6[d]	50.3[c]	11.7[c]	23.9[b]	66.8[c]	0.85[b]	17.2[c]	66.2[c]	0.98[a]	16.5[b]	263[b]	647[b]
10% Yst	5.69[a]	35.2[a]	52.4[b]	13.7[a]	28.2[a]	71.1[a]	0.06[d]	17.9[a]	71.0[a]	0.07[b]	17.6[a]	106[c]	249[d]
20% SD	3.75[c]	32.8[c]	50.7[c]	10.9[d]	23.4[b]	64.5[d]	1.50[a]	16.3[d]	65.4[d]	1.27[a]	16.6[b]	304[a]	806[a]
20% Yst	5.22[b]	34.6[b]	55.4[a]	12.3[b]	28.2[a]	69.8[b]	0.62[c]	17.5[b]	69.9[b]	0.83[a]	17.4[a]	132[c]	348[c]
SE	0.04	0.08	0.35	0.13	0.14	0.25	0.04	0.06	0.2	0.14	0.08	8.08	25.6

SV = specific volume, MC = moisture content (%db), HD = highly digestible [protein], LD = low digestibility [protein], Nml = normal starch, Wx = waxy starch, SD = sourdough starter, Yst = yeast, values are means of triplicate measurements and those with different superscript letters are significantly different ($p<0.05$), SE = Standard error of means.

Table 21: Bread characteristics as influenced by three-way interaction of different variables

Variables	SV (ml/g)	MC (%)	Crust			Crumb						Firmness (g)	
			L*	a*	b*	Day1			Day2			Day 1	Day 2
						L*	a*	b*	L*	a*	b*		
Protein by starch by formulation													
HD, Nml, 10%	4.21^{ef}	34.3^{ab}	53.0^{b}	13.1^{a}	27.1^{a}	69.6^{a}	0.28^{e}	18.1^{a}	69.9^{a}	0.21^{c}	17.5^{a}	257^{a}	542^{abc}
HD Nml, 20%	4.09^{f}	34.4^{a}	55.3^{ab}	11.8^{c}	26.8^{a}	66.5^{c}	0.77^{bc}	17.0^{cd}	66.3^{d}	0.72^{abc}	16.8^{c}	257^{a}	682^{a}
HD, Wx, 10%	4.76^{c}	33.5^{cd}	53.4^{b}	12.6^{abc}	26.6^{ab}	69.3^{ab}	0.64^{bcd}	17.5^{b}	67.2^{cd}	1.15^{ab}	16.9^{c}	180^{bc}	593^{ab}
HD, Wx, 20%	4.43^{de}	33.1^{de}	56.0^{a}	10.8^{d}	25.9^{bc}	67.5^{c}	1.37^{a}	16.5^{e}	68.9^{ab}	1.33^{a}	17.4^{ab}	185^{b}	667^{a}
LD, Nml 10%	5.11^{a}	32.9^{e}	50.6^{c}	12.4^{abc}	25.8^{bc}	67.8^{bc}	0.36^{de}	17.2^{cd}	67.3^{cd}	0.42^{bc}	16.9^{bc}	132^{c}	277^{d}
LD, Nml, 20%	4.64^{cd}	33.5^{cd}	50.4^{c}	11.9^{c}	25.6^{cd}	66.8^{c}	1.21^{a}	17.3^{bc}	67.0^{d}	1.09^{abc}	17.1^{abc}	263^{a}	517^{abc}
LD, Wx, 10%	5.03^{ab}	32.9^{e}	48.4^{c}	12.8^{ab}	24.6^{e}	69.1^{ab}	0.54^{cde}	17.2^{bcd}	69.9^{a}	0.33^{bc}	16.8^{c}	168^{bc}	380^{cd}
LD, Wx, 20%	4.78^{bc}	33.8^{bc}	50.4^{c}	12.1^{bc}	24.9^{de}	67.8^{bc}	0.9^{b}	16.9^{d}	68.3^{bc}	1.07^{abc}	16.6^{c}	167^{bc}	442^{bcd}
SE	0.06	0.12	0.5	0.18	0.2	0.35	0.06	0.08	0.28	0.19	0.12	11.43	36.22

SV = specific volume, MC = moisture content (%db), HD = highly digestible [protein], LD = low digestibility [protein], Nml = normal starch, wx = waxy starch, SD = sourdough starter, Yst = yeast, values are means of triplicate measurements and those with different superscript letters are significantly different ($p<0.05$), SE = Standard error of means.

Table 21 Continued

Variables	SV (ml/g)	MC (%)	Crust			Crumb						Firmness (g)	
						Day1			Day2				
			L*	a*	b*	L*	a*	b*	L*	a*	b*	Day 1	Day 2
Protein by starch by starter													
HD, Nml, SD	3.51^{e}	32.4^{d}	52.1^{bc}	11.5^{bc}	24.1^{d}	65.3^{cd}	0.93^{b}	17.3^{c}	65.8^{cd}	0.85^{abc}	16.7^{cd}	405^{a}	982^{a}
HD, Nml, Yst	4.79^{c}	36.4^{a}	56.3^{a}	13.5^{a}	29.8^{a}	70.8^{ab}	0.12^{e}	17.8^{ab}	70.4^{b}	0.08^{c}	17.6^{a}	108^{c}	242^{d}
HD, Wx, SD	3.76^{de}	32.2^{d}	53.4^{b}	11.3^{bc}	24.7^{d}	67.0^{c}	1.45^{a}	16.6^{d}	66.4^{c}	1.54^{a}	16.7^{cd}	240^{b}	808^{b}
HD, Wx, Yst	5.42^{b}	34.3^{c}	56.1^{a}	12.0^{b}	27.8^{b}	69.9^{b}	0.56^{c}	17.4^{c}	69.7^{b}	0.94^{abc}	17.6^{ab}	125^{c}	452^{c}
LD, Nml, SD	3.95^{d}	31.4^{e}	48.6^{d}	11.0^{c}	23.2^{e}	65.1^{d}	1.14^{b}	16.5^{d}	65.1^{d}	1.15^{ab}	16.4^{d}	267^{b}	525^{c}
LD, Nml, Yst	5.81^{a}	34.9^{b}	52.4^{bc}	13.3^{a}	28.1^{b}	69.5^{b}	0.43^{cd}	18.0^{a}	69.2^{b}	0.36^{bc}	17.7^{a}	128^{c}	268^{d}
LD, Wx, SD	4.00^{d}	32.8^{d}	47.9^{d}	11.5^{bc}	22.6^{e}	65.2^{d}	1.17^{ab}	16.6^{d}	65.7^{cd}	0.96^{abc}	16.4^{d}	222^{b}	590^{c}
LD, Wx, Yst	5.80^{a}	33.9^{c}	50.9^{c}	13.3^{a}	26.9^{c}	71.7^{a}	0.27^{de}	17.6^{bc}	72.5^{a}	0.44^{bc}	17.1^{bc}	113^{c}	232^{d}
SE	0.06	0.12	0.5	0.18	0.2	0.35	0.06	0.08	0.28	0.19	0.12	11.43	36.22

SV = specific volume, MC = moisture content (%db), HD = highly digestible [protein], LD = low digestibility [protein], Nml = normal starch, wx = waxy starch, SD = sourdough starter, Yst = yeast, values are means of triplicate measurements and those with different superscript letters are significantly different ($p<0.05$), SE = Standard error of means.

Table 21 Continued

Variables	**SV (ml/g)**	**MC (%)**	**Crust**			**Crumb**						**Firmness (g)**	
						Day1			**Day2**			**Day 1**	**Day 2**
			L*	**a***	**b***	**L***	**a***	**b***	**L***	**a***	**b***		
Protein by formulation by starter													
HD, 10%, SD	3.64^{e}	31.6^{d}	52.0^{cd}	12.0^{bc}	24.5^{d}	68.0^{b}	0.89^{b}	17.8^{a}	66.2^{c}	1.20^{a}	16.6^{c}	335^{a}	837^{a}
HD, 10%, Yst	5.33^{b}	36.2^{a}	54.5^{b}	13.7^{a}	29.2^{a}	70.9^{a}	0.03^{c}	17.9^{a}	70.9^{a}	0.16^{b}	17.8^{a}	102^{c}	298^{cd}
HD, 20%, SD	3.64^{e}	32.9^{c}	53.5^{bc}	10.7^{e}	24.3^{d}	64.3^{c}	1.49^{a}	16.2^{d}	66.0^{c}	1.20^{a}	16.8^{bc}	310^{a}	953^{a}
HD, 20%, Yst	4.88^{c}	34.5^{b}	57.8^{a}	11.9^{cd}	28.4^{ab}	69.8^{a}	0.64^{b}	17.3^{b}	69.2^{b}	0.86^{ab}	17.4^{a}	132^{c}	395^{c}
LD, 10%, SD	4.09^{d}	31.5^{d}	48.6^{ef}	11.3^{cde}	23.3^{e}	65.6^{c}	0.81^{b}	16.5^{cd}	66.1^{c}	0.76^{ab}	16.3^{c}	190^{b}	457^{c}
LD, 10%, Yst	6.05^{a}	34.3^{b}	50.3^{de}	13.8^{a}	27.1^{c}	71.4^{a}	0.09^{c}	17.9^{a}	71.2^{a}	-0.02^{b}	17.5^{a}	110^{c}	200^{d}
LD, 20%, SD	3.86^{de}	32.6^{c}	47.8^{f}	11.2^{de}	22.5^{e}	64.8^{c}	1.50^{a}	16.5^{c}	64.7^{d}	1.34^{a}	16.5^{c}	298^{a}	658^{b}
LD, 20%, Yst	5.56^{b}	34.6^{b}	53.0^{bc}	12.8^{b}	27.9^{bc}	69.8^{a}	0.6^{b}	17.7^{ab}	70.5^{a}	0.81^{ab}	17.3^{ab}	132^{c}	300^{cd}
SE	0.06	0.12	0.5	0.18	0.2	0.35	0.06	0.08	0.28	0.19	0.12	11.43	36.22

SV = specific volume, MC = moisture content (%db), HD = highly digestible [protein], LD = low digestibility [protein], Nml = normal starch, wx = waxy starch, SD = sourdough starter, Yst = yeast, values are means of triplicate measurements and those with different superscript letters are significantly different ($p<0.05$), SE = Standard error of means.

Table 21 Continued

Variables	SV (ml/g)	MC (%)	Crust			Crumb						Firmness (g)	
						Day1			Day2				
			L*	a*	b*	L*	a*	b*	L*	a*	b*	Day 1	Day 2
Starch by formulation and starter													
Nml, 10%, SD	3.83^{de}	31.3^{f}	50.6^{d}	11.4^{e}	23.8^{c}	65.8^{c}	0.69^{c}	17.3^{b}	66.2^{c}	0.71^{ab}	16.7^{cd}	275^{b}	605^{b}
Nml, 10%, Yst	5.49^{b}	35.9^{a}	53.1^{bc}	14.1^{a}	29.1^{a}	71.6^{a}	-0.05^{d}	18.0^{a}	71.0^{a}	-0.08^{b}	17.7^{a}	113^{d}	213^{c}
Nml, 20%, SD	3.63^{e}	32.4^{e}	50.0^{d}	11.0^{ef}	23.5^{c}	64.7^{c}	1.38^{a}	16.5^{c}	64.7^{d}	1.29^{a}	16.3^{d}	397^{a}	902^{a}
Nml, 20%, Yst	5.10^{c}	35.4^{a}	55.7^{a}	12.7^{c}	28.9^{a}	68.6^{b}	0.60^{c}	17.8^{a}	68.6^{b}	0.52^{ab}	17.6^{ab}	123^{d}	297^{cd}
Wx, 10%, SD	3.9^{d}	31.9^{e}	50.0^{d}	12.0^{d}	24.0^{c}	67.8^{b}	1.00^{b}	17.0^{b}	66.1^{c}	1.25^{a}	16.2^{d}	250^{bc}	688^{b}
Wx, 10%, Yst	5.88^{a}	34.5^{b}	51.8^{cd}	13.4^{b}	27.3^{a}	70.7^{a}	0.18^{d}	17.7^{a}	71.0^{a}	0.23^{b}	17.5^{ab}	98^{d}	285^{cd}
Wx, 20%, SD	3.86^{de}	33.1^{d}	51.3^{cd}	10.8^{f}	23.3^{c}	64.3^{c}	1.62^{a}	16.2^{c}	66.0^{c}	1.25^{a}	16.9^{c}	212^{c}	710^{b}
Wx, 20%, Yst	5.34^{bc}	33.7^{c}	55.2^{ab}	12.0^{d}	27.5^{a}	71.0^{a}	0.65^{c}	17.2^{b}	71.2^{a}	1.15^{a}	17.2^{bc}	140^{d}	398^{c}
SE	0.06	0.12	0.5	0.18	0.2	0.35	0.06	0.08	0.28	0.19	0.12	11.43	36.22

SV = specific volume, MC = moisture content (%db), HD = highly digestible [protein], LD = low digestibility [protein], Nml = normal starch, wx = waxy starch, SD = sourdough starter, Yst = yeast, values are means of triplicate measurements and those with different superscript letters are significantly different ($p<0.05$), SE = Standard error of means.

Table 22: Bread characteristics as influenced by four-way interaction of different variables

Variables	**SV (ml/g)**	**MC (%)**	**Crust**			**Crumb**						**Firmness (g)**	
						Day 1			**Day 2**			**Day 1**	**Day 2**
			L*	**a***	**b***	**L***	**a***	**b***	**L***	**a***	**b***		
HD, Nml, 10%, SD	3.50^{f}	31.6^{hi}	51.6^{bcd}	11.9^{cde}	24.0f^{gh}	66.9^{efg}	0.71^{def}	18.4^{a}	67.7^{cd}	0.56^{a-d}	17.3^{abc}	410^{a}	880^{ab}
HD, Nml, 10%, Yst	4.92^{cd}	36.9^{a}	54.5^{ab}	14.3^{a}	30.2^{a}	72.2^{a}	-0.140^{h}	17.9^{ab}	72.1^{a}	-0.14^{d}	17.7^{a}	103^{e}	203^{g}
HD, Nml, 20%, SD	3.53^{f}	32.9^{fg}	52.6^{bc}	11.0^{ef}	24.2^{fgh}	63.8^{i}	1.15^{bcd}	16.3^{fg}	64.0^{f}	1.14^{a-d}	16.1^{e}	400^{a}	1083^{a}
HD, Nml, 20%, Yst	4.65^{d}	35.9^{ab}	58.0^{a}	12.6^{bcd}	29.4^{ab}	69.3^{bcde}	0.38^{fg}	17.7^{bc}	68.7^{bcd}	0.30^{bcd}	17.5^{a}	113^{e}	280^{fg}
HD, Wx, 10%, SD	3.78^{ef}	31.6^{hi}	52.3^{bc}	12.1^{cde}	25.0^{ef}	69.2^{cde}	1.07^{cde}	17.2^{cde}	64.8^{f}	1.84^{a}	16.0^{e}	260^{b}	793^{bc}
HD, Wx, 10%, Yst	5.74^{ab}	35.4^{bc}	54.5^{ab}	13.0^{bc}	28.3^{bc}	69.5^{bcd}	0.21^{gh}	17.9^{ab}	69.6^{bc}	0.46^{a-d}	17.8^{a}	100^{e}	393^{efg}
HD, Wx, 20%, SD	3.75^{ef}	32.9^{fg}	54.4^{ab}	10.4^{f}	24.3^{fg}	64.7^{ghi}	1.83^{a}	16.1^{g}	68.0^{bcd}	1.25^{a-d}	17.5^{a}	220^{bc}	823^{abc}
HD, Wx, 20%, Yst	5.11^{c}	33.2^{f}	57.6^{a}	11.1^{ef}	27.4^{cd}	70.3^{abcd}	0.90^{de}	17.0^{de}	69.7^{bc}	1.41^{abc}	17.4^{ab}	150^{cde}	510^{def}
SE	0.08	0.17	0.71	0.26	0.28	0.5	0.09	0.11	0.39	0.27	0.17	16.16	51.23

SV = specific volume, MC = moisture content (%db), HD = highly digestible [protein], LD = low digestibility [protein], Nml = normal starch, wx = waxy starch, SD = sourdough starter, Yst = yeast, values are means of triplicate measurements and those with different superscript letters are significantly different ($p<0.05$), SE = Standard error of means.

Table 22 Continued

Variables	**SV (ml/g)**	**MC (%)**	**Crust**			**Crumb**						**Firmness (g)**	
						Day 1			**Day 2**			**Day 1**	**Day 2**
			L*	**a***	**b***	**L***	**a***	**b***	**L***	**a***	**b***		
LD, Nml, 10%, SD	4.15^{e}	30.9^{i}	49.6^{cde}	10.9^{ef}	23.6^{f-i}	64.6^{ghi}	0.68^{ef}	16.2^{fg}	64.7^{f}	0.85^{a-d}	16.1^{de}	140^{cde}	330^{rfg}
LD, Nml, 10%, Yst	6.06^{a}	34.9^{cd}	51.6^{bcd}	13.9^{ab}	27.9^{c}	71.0^{abc}	0.04^{gh}	18.1^{ab}	70.0^{b}	-0.02^{d}	17.8^{a}	123de	123^{g}
LD, Nml, 20%, SD	3.74^{ef}	31.9^{h}	47.5^{e}	11.0^{ef}	22.8^{hi}	65.6^{fghi}	1.60^{ab}	16.7^{ef}	65.5^{ef}	1.44^{ab}	16.6^{b-e}	393^{a}	720^{bcd}
LD, Nml, 20%, Yst	5.55^{b}	35.0^{bcd}	53.3^{b}	12.8^{bc}	28.3^{bc}	68.0^{def}	0.81^{def}	17.9^{ab}	68.5^{bcd}	0.74^{a-d}	17.7^{a}	133de	313^{fg}
LD, Wx, 10%, SD	4.02^{e}	32.2^{gh}	47.7^{e}	11.8^{cde}	23.0^{ghi}	66.5^{fgh}	0.93^{de}	16.8^{ef}	67.4^{de}	0.67^{a-d}	16.4^{cde}	240^{b}	583^{cde}
LD, Wx, 10%, Yst	6.03^{a}	33.6^{ef}	49.1^{cde}	13.8^{ab}	26.2^{de}	71.8^{ab}	0.14^{gh}	17.6^{bc}	72.4^{a}	-0.01^{cd}	17.2^{a-c}	97^{e}	178^{g}
LD, Wx, 20%, SD	4.00^{e}	33.3^{ef}	48.1^{de}	11.3^{def}	22.2^{i}	63.9^{hi}	1.40^{abc}	16.3^{fg}	64.0^{f}	1.25^{a-d}	16.3^{de}	203^{bcd}	597^{cde}
LD, Wx, 20%, Yst	5.58^{b}	34.2^{de}	52.7^{bc}	12.9^{bc}	27.5^{cd}	71.6^{abc}	0.39^{fg}	17.5^{bcd}	72.6^{a}	0.88^{a-d}	17.0^{a-d}	130de	287^{fg}
SE	0.08	0.17	0.71	0.26	0.28	0.5	0.09	0.11	0.39	0.27	0.17	16.16	51.23

SV = specific volume, MC = moisture content (%db), HD = highly digestible [protein], LD = low digestibility [protein], Nml = normal starch, wx = waxy starch, SD = sourdough starter, Yst = yeast, values are means of triplicate measurements and those with different superscript letters are significantly different ($p<0.05$), SE = Standard error of means.

Table 23: Breads with sorghum substitutions compared to wheat-based controls

Variables	SV (ml/g)	MC (%)	Crust			Crumb						Firmness	
						Day 1			Day 2			Day 1	Day 2
			L*	a*	b*	L*	a*	b*	L*	a*	b*		
CHD 10%, SD	3.98^{fgh}	32.6^{ghi}	49.3^{e-h}	11.9^{f-i}	23.9^{ab}	66.9^{i-l}	1.89^{ef}	16.8^{kl}	66.5^{f-j}	1.79^{c-f}	16.5^{j-m}	207^{d-i}	733^{def}
CHD 20%, SD	3.97^{fgh}	34.2^{de}	58.2^{a}	9.5^{k}	25.6^{ab}	60.8^{n}	3.26^{c}	15.5^{o}	61.7^{m}	3.13^{b}	15.2^{n}	257^{de}	577^{d-j}
HD Nml 10%, SD	3.50^{i}	31.6^{ijk}	51.6^{c-g}	11.9^{f-i}	24.0^{ab}	66.9^{jkl}	0.71^{klm}	18.4^{efg}	67.7^{d-i}	0.56^{g-j}	17.3^{g-k}	410^{c}	880^{cd}
HD wx 10%, SD	3.78^{ghi}	31.6^{jk}	52.3^{c-f}	12.1^{e-h}	25.0^{ab}	69.2^{g-j}	1.07^{h-k}	17.2^{ijk}	64.8^{jkl}	1.84^{cde}	16.0^{mn}	260^{de}	793^{cde}
HD Nml 20%, SD	3.53^{i}	32.9^{fgh}	52.6^{cde}	11.0^{hij}	24.2^{ab}	63.8^{m}	1.15^{hij}	16.3^{lmn}	64.0^{kl}	1.14^{c-h}	16.1^{mn}	400^{c}	1083^{c}
HD wx 20%, SD	3.75^{hi}	32.9^{fgh}	54.4^{bc}	10.4^{jk}	24.3^{ab}	64.7^{lm}	1.83^{ef}	16.1^{no}	68.0^{c-h}	1.25^{c-g}	17.5^{f-i}	220^{d-h}	823^{cde}
HG 100%, SD	4.24^{f}	30.4^{l}	43.1^{k}	12.8^{c-f}	20.3^{b}	73.1^{ab}	0.05^{no}	18.8^{e}	72.9^{a}	-0.06^{ijk}	18.3^{f}	277^{d}	497^{e-k}
LD Nml 10% SD	4.15^{fg}	30.9^{kl}	49.6^{d-h}	10.9^{ij}	23.6^{ab}	64.6^{lm}	0.68^{klm}	16.2^{mn}	64.7^{jkl}	0.85^{e-j}	16.1^{lm}	140^{f-j}	330^{g-l}
LD wx 10%, SD	4.02^{fgh}	32.2^{hij}	47.7^{hij}	11.8^{f-i}	23.0^{ab}	66.5^{kl}	0.93^{i-l}	16.8^{klm}	67.4^{e-i}	0.67^{f-j}	16.4^{klm}	240^{d-g}	583^{d-i}
LD Nml 20%, SD	3.74^{hi}	31.9^{hij}	47.5^{hij}	11.0^{g-j}	22.8^{b}	65.6^{klm}	1.60^{fg}	16.7^{klm}	65.5^{i-l}	1.44^{c-g}	16.6^{j-m}	393^{c}	720^{def}
LD wx 20%, SD	3.98^{fgh}	33.3^{efg}	48.1^{ghi}	11.3^{g-j}	22.2^{b}	63.9^{m}	1.40^{gh}	16.3^{lmn}	64.0^{kl}	1.25^{c-g}	16.3^{lm}	203^{d-i}	597^{d-i}
SE	0.07	0.18	0.69	0.22	1.93	0.43	0.07	0.11	0.41	0.21	0.16	20.25	62.86

SV = specific volume, MC = moisture content (%db), CHD = control HD, HD = highly digestible [protein], LD = low digestibility [protein], Nml = normal starch, wx = waxy starch, HG = high gluten bread flour UG = ultra-ground whole wheat flour, WW = whole wheat flour, SD = sourdough starter, Yst = yeast, values are means of triplicate measurements and those with different superscript letters are significantly different ($p<0.05$), SE = Standard error of means.

Table 23 Continued

Variables	SV (ml/g)	MC (%)	Crust			Crumb						Firmness	
			L*	a*	b*	Day 1			Day 2			Day 1	Day 2
						L*	a*	b*	L*	a*	b*		
UG WW 10%, SD	3.75^{hi}	31.6^{jk}	45.1^{ijk}	12.8^{c-f}	21.3^{b}	69.4^{fgh}	1.17^{hi}	20.5^{c}	70.0^{bc}	1.04^{d-i}	20.6^{cd}	253de	633^{d-h}
UGWW 20%, SD	3.77^{ghi}	32.9^{fgh}	44.1^{jk}	12.1^{e-i}	20.8^{b}	65.3^{lm}	2.12^{de}	21.1^{c}	66.1^{h-k}	2.06^{bcd}	21.3^{c}	247^{def}	650^{d-g}
UGWW 100% SD	2.54^{j}	37.0^{a}	48.7^{f-i}	12.3^{efg}	33.6^{a}	56.0^{o}	6.24^{a}	22.8^{b}	55.7^{n}	6.23^{a}	22.9^{b}	1597^{a}	2467^{a}
CHD 10%, Yst	5.81^{bc}	33.8^{ef}	51.1^{c-h}	13.3^{a-e}	27.6^{ab}	65.1^{lm}	2.39^{d}	17.1^{ijk}	68.5^{c-g}	0.79^{e-j}	16.8^{h-m}	67^{j}	143^{l}
CHD 20%, Yst	4.97^{de}	34.2^{de}	52.7^{cde}	12.8^{c-f}	27.5^{ab}	70.5^{c-g}	0.64^{lm}	16.6^{klm}	66.3^{g-j}	2.29^{bc}	16.7^{i-m}	133^{g-j}	257^{i-l}
HD Nml 10%, Yst	4.92^{de}	36.9^{a}	54.5^{abc}	14.3^{a}	30.2^{ab}	72.2^{a-d}	-0.14^{o}	17.9^{gh}	72.1^{ab}	-0.14^{jk}	17.7^{fg}	103^{ij}	203^{kl}
HD wx 10%, Yst	5.74^{bc}	35.4^{bc}	54.5^{abc}	13.0^{b-f}	28.3^{ab}	69.5^{e-h}	0.21^{no}	17.9^{gh}	69.6^{cde}	0.46^{g-k}	17.8^{fg}	100^{ij}	393^{f-l}
HD Nml 20%, Yst	4.65^{e}	35.9^{bc}	58.0^{ab}	12.6^{def}	29.4^{ab}	69.3^{f-i}	0.38^{mn}	17.7^{hi}	68.7^{c-f}	0.30^{g-k}	17.5^{f-i}	113^{hij}	280^{i-l}
HD wx 20%, Yst	5.11^{d}	33.2^{efg}	57.6^{ab}	11.1^{g-j}	27.4^{ab}	70.3^{d-h}	0.90^{i-l}	17.0^{jk}	69.7^{cd}	1.41^{c-g}	17.4^{g-j}	150^{e-j}	510^{e-k}
HG 100%, Yst	6.33^{a}	32.6^{g-j}	42.1^{k}	13.6^{a-d}	21.6^{b}	74.3^{a}	0.76^{j-m}	18.5^{ef}	74.2^{a}	-0.61^{k}	18.3^{f}	113^{hij}	210^{kl}
LD Nml 10%, Yst	6.06^{ab}	34.9^{cd}	51.6^{c-g}	13.9^{abc}	27.9^{ab}	71.0^{b-g}	0.04^{no}	18.1^{fgh}	70.1^{bc}	-0.02^{ijk}	17.8^{fg}	123^{hij}	223^{kl}
SE	0.07	0.18	0.69	0.22	1.93	0.43	0.07	0.11	0.41	0.21	0.16	20.25	62.86

SV = specific volume, MC = moisture content (%db), CHD = control HD, HD = highly digestible [protein], LD = low digestibility [protein], Nml = normal starch, wx = waxy starch, HG = high gluten bread flour UG = ultra-ground whole wheat flour, WW = whole wheat flour, SD = sourdough starter, Yst = yeast, values are means of triplicate measurements and those with different superscript letters are significantly different ($p<0.05$), SE = Standard error of means.

Table 23 Continued

Variables	SV (ml/g)	MC (%)	Crust			Crumb						Firmness	
						Day 1			Day 2			Day 1	Day 2
			L*	a*	b*	L*	a*	b*	L*	a*	b*		
LD wx 10%, Yst	6.03^{ab}	33.6^{ef}	49.1^{e-h}	13.8^{a-d}	26.2^{ab}	71.8^{b-e}	0.14^{no}	17.6^{hi}	72.4^{a}	-0.01^{h-k}	17.2^{g-k}	97^{ij}	177^{kl}
LD Nml 20%, Yst	5.55^{c}	35.0^{cd}	53.3^{cd}	12.8^{c-f}	28.3^{ab}	68.0^{h-k}	0.81^{i-l}	17.9^{gh}	68.5^{c-g}	0.74^{e-j}	17.7^{fgh}	133^{g-j}	313^{g-l}
LD wx 20%, Yst	5.58^{c}	34.2^{de}	52.7^{cde}	12.9^{c-f}	27.5^{ab}	71.6^{b-f}	0.39^{mn}	17.5^{hij}	72.6^{a}	0.88^{e-j}	17.0^{g-l}	130^{g-j}	287^{h-l}
UGWW 10%, Yst	6.28^{a}	33.6^{ef}	50.1^{d-h}	14.1^{ab}	27.6^{ab}	73.2^{ab}	0.06^{no}	19.6^{d}	73.5^{a}	0.01^{h-k}	19.2^{e}	113^{hij}	180^{kl}
UGWW 20%, Yst	5.87^{bc}	36.0^{ab}	50.5^{d-h}	13.9^{abc}	27.6^{ab}	72.7^{abc}	0.91^{i-l}	20.9^{c}	73.2^{a}	0.95^{d-j}	20.4^{d}	127^{hij}	230^{jkl}
UGWW 100% Yst	2.74^{j}	34.9^{cd}	53.4^{cd}	12.9^{b-f}	27.6^{ab}	63.5^{m}	5.31^{b}	25.3^{a}	63.2^{lm}	5.32^{a}	25.1^{a}	883^{b}	1607^{b}
SE	0.07	0.18	0.69	0.22	1.93	0.43	0.07	0.11	0.41	0.21	0.16	20.25	62.86

SV = specific volume, MC = moisture content (%db), CHD = control HD, HD = highly digestible [protein], LD = low digestibility [protein], Nml = normal starch, wx = waxy starch, HG = high gluten bread flour UG = ultra-ground whole wheat flour, WW = whole wheat flour, SD = sourdough starter, Yst = yeast, values are means of triplicate measurements and those with different superscript letters are significantly different ($p<0.05$), SE = Standard error of means.

Crust L*a*b* color parameters

As the proportion of sorghum incorporation increased from 10 to 20%, crust got darker regardless of other variables (Table 19). The darker color of the higher level of sorghum addition is due to the increased bran level from the whole grain sorghum flour. Comparing the sorghum with HD and LD protein types, the samples with the HD lines were characterized by lighter crust both on days 1 and 2. A lighter crust was observed for the sourdough breads at both 10 and 20% levels of sorghum addition compared to the yeast breads regardless of the protein and starch types. The lighter color might be due the formation of blisters under the thin crust of the sourdough fermented breads.

Crumb L*a*b* color parameters

There were no clear differences in the crumb color for the different sorghum treatments (Table 19). A darker crumb color was observed for the sourdough breads at both 10 and 20% levels of sorghum addition compared to the yeast breads regardless of the protein and starch types. The darker crumb of the sourdough fermented bread is due to denser structures with higher moisture retained (section 4.3.4.1.). The crumb color was significantly and positively correlated with the specific volume ($r = 0.73$ for day 1 and 0.77 for day 2, Appendix A- 3). As the proportion of sorghum incorporation increased from 10 to 20%, crumb color got darker. The overall implication is that the increased level of sorghum addition might contribute to poor crumb structure by the dilution effect on the gluten, giving denser and a darker crumb.

The whole wheat flour controls (20 and 100%, Table 23) showed higher yellowness/redness (a* = 2.06 for the 20% and 6.24 for the 100% whole wheat flour, respectively), compared to all the other samples (a* ranging from -0.61 to 1.84). It seems that the pigment of whole wheat bran (ultra-ground) greatly influenced the color of both yeast and sourdough leavened bread samples. The yellowish-red color characteristics was further enhanced by extremely dense crumb structure. The high density (least specific volume) was due partly to the interference of the bran particles into the gluten network together with the dilution effect and also due partly to the high moisture absorption and holding by the bran that limits complete hydration of gluten and starch.

Crumb firmness

The sourdough bread samples were generally characterized by significantly firmer crumb texture compared to their yeast counter parts due to less gas production by the sour dough culture. As the proportion of sorghum incorporation increased from 10 to 20%, crumb firmness increased due to the dilution effect on the gluten network by the sorghum flour. Comparing the sorghum with HD and LD protein types (Table 19), the samples with the HD lines were generally characterized by firmer crumb both on day 1 (220 versus 183 g for the LD) and day 2 (621 versus 404 g for the LD). The increased firmness for the samples with HD protein mutants are likely due to starch hydration with the water absorbed and retained by the protein mutants in the improved HD lines. As expected, crumb firmness and specific volume were significantly and negatively correlated (r = -0.67 for day1 and -0.78 for day 2, Appendix A-3). The other overall observation (Table 19) was that values of crumb firmness were higher for the sourdough (283 g) than the yeast breads (119 g) and for those having 20% level of sorghum (218 g) incorporation than the ones with only 10% (184 g). The sourdough bread crumb firmness for the HD-normal (257 g) was higher compared to ($p<0.05$) those made of HD-waxy (183 g). in a similar trend the crumbs from LD-normal (198) had higher firmness than that of LD-waxy (168) sorghum although the LD-normal/waxy (Table 20) are comparable to that of the HD-waxy. The HD-normal is close in firmness to the high gluten flour control (277 g, Table 23). The softer crumbs for the samples from the HD and LD-waxy might be due to sticky mass formed by the waxy starch that is not gelling faster like the amyloses in the normal starch. The LD-normal might be softer due to limited water uptake by the wild protein types and the amyloses in normal starch limiting the degree of starch cooking resulting in soft and crumbly texture.

The higher crumb firmness for the samples from the HD-normal (Tables 20) is due to the better interaction with and retention of moisture by the more functional irregularly shaped protein mutants as well as the stronger gel formed by the normal starches where the amylose partially and rapidly re-associates. On the contrary, the LD protein bodies might have limited the absorption of water into the matrix that might have limited swelling and gelatinization of the starches resulting into less firm crumb.

Comparing the HD and LD proteins in waxy versus normal starch backgrounds, there was significant differences in the firmness values where those with the HD mutant proteins had firmer crumb regardless of the starch type (Table 23). The firmness of fresh bread samples with HD sorghum incorporations were higher than the 100% high gluten and 20% whole wheat flour incorporated samples. The bread crumb from the HD-normal sorghum traits (Table 23) for the 20% addition and sourdough starter was higher in firmness (400 g on day 1 and 1083 g on day 2) the high gluten bread flour control (277 –

day 1, 497 – day 2) as well as all other protein and starch treatment combinations in sorghum (140 – 410 g on day 1 and 330 – 880 g on day 2). The highest magnitudes of firmness were observed for the whole wheat samples (UGWW) with ranges of firmness from 1597 g on day 1 to 2467 g on day 2. The data shows that all the sorghum additions are closer to the high gluten flour-based controls than to the whole wheat-based ones, showing the potential of sorghum as ingredient in baked goods. The HD-normal were seen to give better bread properties looking at the specific volume and moisture levels (sections 4.3.4.1.). The crumb firmness (Table 23), however, shows that the HD-normal combination has higher firmness compared to the HD-waxy and LD-waxy and normal starch types.

The overall observation regarding the incorporation of the improved sorghum lines in breads was that the HD protein trait coupled with yeast fermentation had better stability (less firmness, Tables 19.- 23), compared to the sourdough fermented samples. For instance, the firmness values of the 20% sorghum incorporated samples with yeast fermentation (Table 20) was lower (132 g on day 1 and 348 g on day 2) compared to the sourdough counterpart (304 g on day 1 and 806 g on day 2) regardless of the protein and starch types.

Changes in crumb firmness

As expected, in a matter of 24 h of storage, the firmness (g) of the crumb showed an average increase from 304 to 806 for the sourdough bread samples (Figure 7A&B). The increase in the crumb firmness was more pronounced (Figure 7A) for the breads incorporated with sorghum having the HD protein trait (slope = 643) than that for LD (slope = 360) ones regardless of the starch type. There was no difference in the rate of change in firmness between the waxy and normal starch types. Looking at the rate of firming for the HD versus LD proteins with the waxy versus normal starch backgrounds (Figure 7B), samples from the normal starch had higher fresh crumb firmness (400 g for HD and 393 g for LD) compared to the waxy ones (220 for HD and 203 for LD). Over the storage period of 24 h, the firmness increased for all samples with higher rate of firming for the HD lines (slope = 603 for waxy and 683 for normal), compared to the LD lines (slope = 393 for the waxy and 327 for the normal). The general observation (Tables 20-23, Figures 7A&8A) was that the increase in firmness was higher for the samples with the HD than LD proteins. This implies that the improved lines containing HD protein retained more water that might have migrated over the storage time, resulting in higher rate of starch retrogradation that in turn affected shelf stability of breads.

Better water holding characteristic was also shown by significantly higher moisture content of the HD based samples (Table 19) and significant correlations with other parameters (loaf density, r = 0.90; specific volume, r = -0.78). The pronounced firming of the sourdough breads (Figure 7A) made from HD-sorghum may be due to its better interaction with water and the starch hydrolysis by the acidification of the sourdough fermentation (Arendt, Ryan, & Dal Bello, 2007) compared to the yeast fermented counterparts (Figure 8A). The higher rate of firming may suggest that the sourdough bread should be consumed fresh or suitability and effect of different improvers and stabilizers such as hydrocolloids (Rosell, Rojas, & De Barber, 2001) and emulsifiers (commonly monoacylglycerols) (Kohajdová, Karovičová, & Schmidt, 2009) should be investigated.

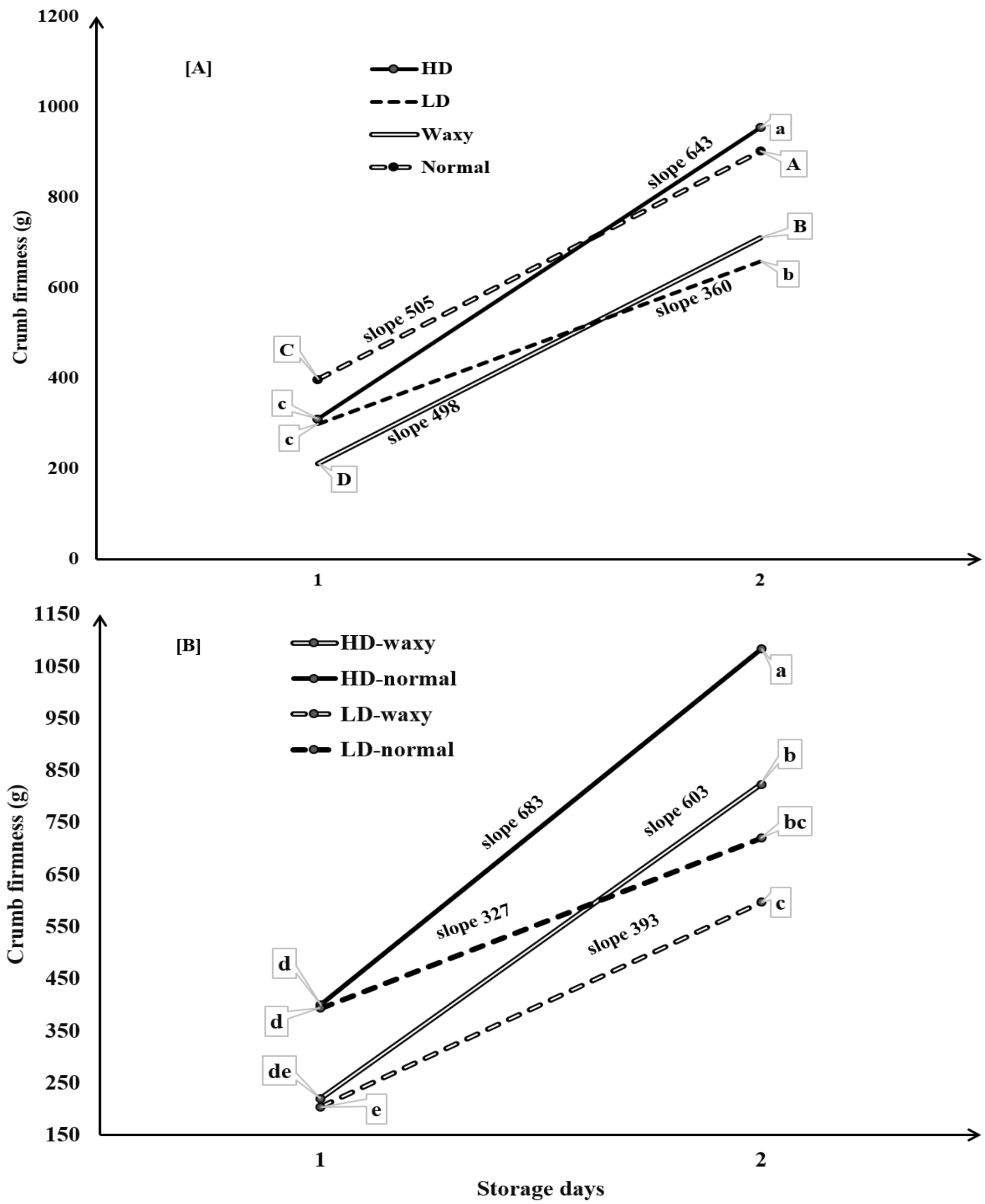

Figure 7: Change in crumb hardness (firmness) of 20% sorghum incorporated sourdough breads over storage, [A] the HD versus LD as well as waxy versus normal; [B] HD versus LD in waxy versus normal starch backgrounds. Points with different letters of the same style on the same line are significantly different ($p<0.05$).

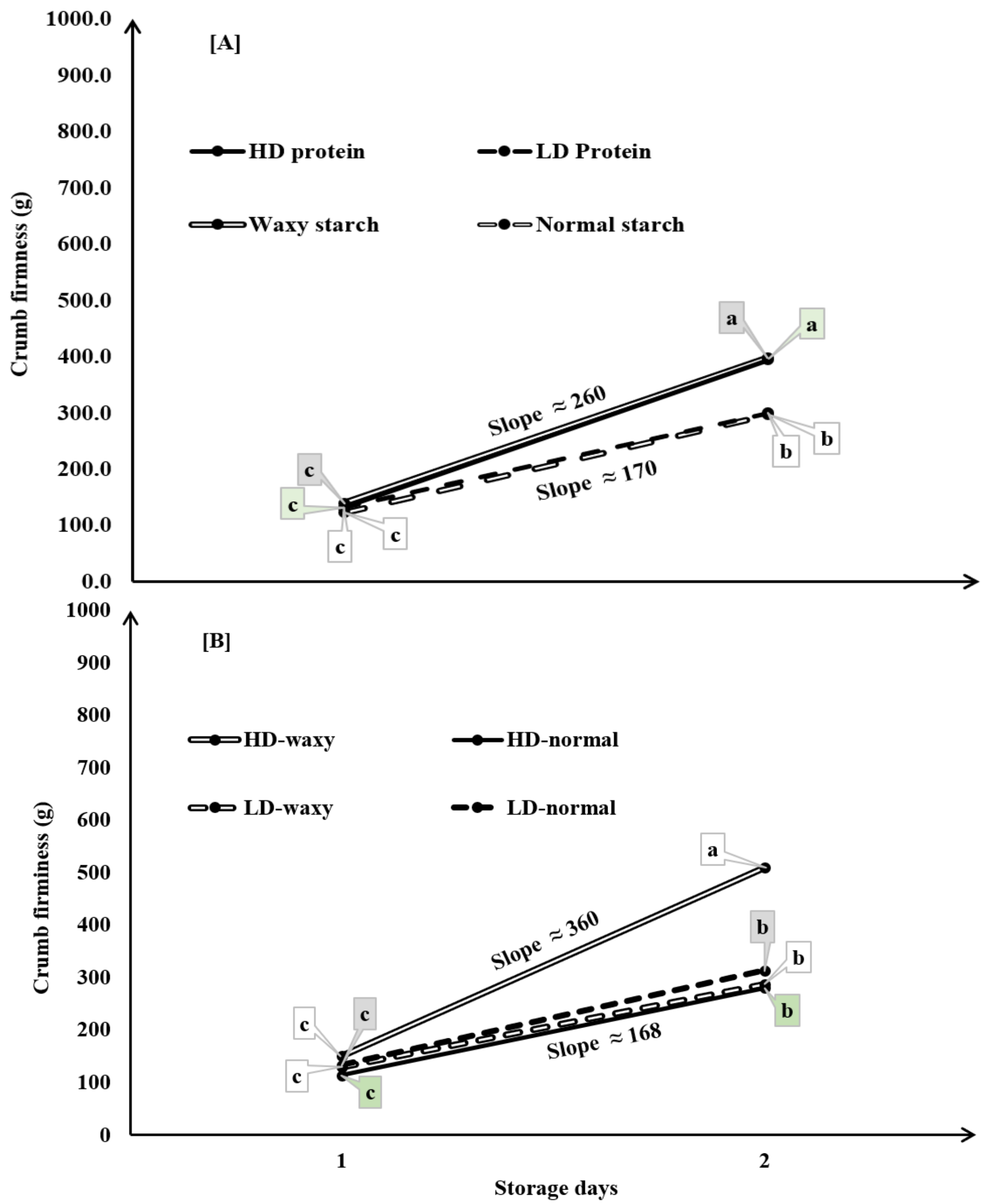

Figure 8: Change in crumb hardness (firmness) of 20% sorghum incorporated yeast breads over storage, [A] the HD versus LD as well as waxy versus normal; [B] HD versus LD in waxy versus normal starch backgrounds. Points with different letters of the same style on the same line are significantly different ($p<0.05$).

Comparing the sorghum incorporated bread samples with the whole and refined wheat flour controls, it was generally noted that the highest crumb firmness (1597 g, day 1) was associated with the sourdough fermented 100% UGWW control. The firmness of the 100% UGWW was increased to 2467 g on the second day. The yeast fermented version of the same formula had the second highest (883 g) on day 1, which also increased to 1607 g on day 2. This indicates that the whole sorghum flour incorporated samples had lower magnitude and rate of firmness and firming compared to the whole wheat controls and slightly higher high gluten refined wheat control, making the improved sorghum a potential option for healthy whole grain-incorporated bread making with respect to improved functionality for processing and nutritional quality (lysine content) described in Chapter 3. However, shelf stability and crumb firmness could be improved using bread additives such as emulsifiers and hydrocolloids.

Chapter Summary

The HD-waxy sorghum traits resulted in low cohesiveness and springiness of the pancakes which are indications of less spongy and more sticky mass showing poor structural integrity of the pancake crumb. The HD and LD-normal combinations at 50% substitution gave pancakes with more spongy and fluffy textural attributes that are comparable to that of pastry flour-based control. The HD-normal at 100% formulation also gave pancakes with better desirability compared to other combinations. The implication is that the HD-normal combination at 100% level can be a potential ingredient in gluten-free and healthy whole grain-based applications with respect to the better processing functionality and nutritional quality. The pancakes from HD-normal sorghum also showed higher thickness and moisture levels indicating better fluffiness and desirability compared to the HD-waxy and the LD-waxy/normal combinations. The descriptive sensory data showed that the 100% HD-normal combination had higher scores of bitterness, denseness and grittiness, but the scores are still on the lower end of the scale and are not affecting the desirability of the products.

The cookies from HD-waxy were seen to have undesirable texture as it had higher hardness and rate of hardening over storage due to slow but continuous retrogradation of the waxy starch over storage. The LD-normal combination also gave cookies of highest hardness due to the limited interaction of both the globular wild proteins and the amylose-rich normal starches that resulted into a drier mass by extensive crystallization of sugar. The HD-normal combination, on the other hand, seemed to produce cookies of desirable properties due to higher moisture retention that resulted softer texture. The HD-normal was the best combination of sorghum traits for making the softest and stable sugar snap cookie with desirable physical properties. This sorghum type can potentially be used as ingredient for a gluten-free and whole grain applications. The descriptive sensory analysis however, showed similar textural scores such as hardness, fracturability, surface roughness and crispiness for all the 100% sorghum containing cookies. Consumer sensory testing may be necessary to determine if the different traits influence consumer preference.

Bread samples with the HD-normal treatment were observed to have lower specific volume, higher moisture contents, lower magnitude and rate of firming with values fairly comparable to the high gluten flour control. The HD-waxy combination resulted in higher rate of firming that might be due to higher moisture absorption and retention by the HD proteins and waxy starch. The results suggest that suitability and effect of different additives such as hydrocolloids and emulsifiers should be tested. However, breads with up to 20% sorghum incorporated may be acceptable for fresh consumption better than those containing same level of whole wheat flour (ultra-ground).

CHAPTER V

CONCLUSIONS, RECOMMENDATIONS AND LIMITATIONS

Conclusions

Protein digestibility analysis using *in vitro* assay was inconsistent in establishing the presence of the HD protein mutation in sorghum and thus not recommended for definitive trait identification. The *in vitro* assay appears to be greatly influenced by unrelated factors such as growing environment and kernel hardness. FE-SEM on the other hand was seen to be a definitive qualitative method to identify the morphological differences between the HD and LD protein bodies.

The HD protein body mutation in hard endosperm is associated with improved endosperm quality such as better protein digestibility, higher lysine content and better starch pasting properties. The improved endosperm quality was observed without changes to the phenolic content as well as phenolic acid and flavonoid profiles, which are known to have health beneficial effects. This indicates that the improved endosperm functionality and protein quality can potentially enhance the suitability of the improved lines for food applications.

The sorghum lines with HD protein mutant were observed to have better interaction with water and better functionality in batter-based food models. Generally, HD-normal lines were observed to have better performance in pancakes (higher thickness, lower stickiness) and cookies (higher moisture, lower hardness and higher fracturability) at 100%

formulations. This makes the improved sorghum with the HD protein trait a compelling healthy as a gluten-free whole grain ingredient.

Bread samples from both the sourdough and yeast fermentations having HD-waxy traits exhibited higher magnitude and more pronounced rate of firming over storage period of 24 h compared to HD-normal. The reason for the higher firming is most likely due to the fact that both the HD protein and waxy starch traits absorb and retain higher moisture that migrated during storage and caused staling. The moisture level and both the magnitude and rates of firmness for the sorghum incorporated bread samples were higher compared to the high gluten refined bread flour control, although they performed better than the whole wheat (ultra-ground) control.

Recommendations

Looking at the *in vivo* digestibility of the HD proteins to establish if protein digestibility depends on protein structure or kernel hardness is important to see if the apparent protein digestibility of the improved lines is comparable to that of the original HD mutant. The outcome of the *in vivo* digestibility may also dictate the nomenclature of the improved HD sorghum. If the digestibility is not necessarily high, the new lines may be called quality protein sorghum (QPS) similar to the quality protein maize (QPM) as the functionality and the lysine content are better than the LD counterparts.

Regarding the product development, it is important that effects of additives like hydrocolloids on the textural and shelf stabilities of different categories of products are checked. It is also important that the performance of the 100% HD-normal sorghum in pancakes and cookies is checked in comparison with popular gluten free formulations for the respective products. The use of the improved HD-normal sorghum in mixture with the HD-waxy and optimization of the proportions is also interesting as the HD-waxy showed better water interactions in the dough and batter systems. It is also recommended that the improved HD sorghum is tested in other forms of cookies other than the sugar snap type, other quick products like muffins, other gluten-free products and multi-grain breads.

Isolation of the protein bodies for both the HD and LD types and checking their suitability for other technological applications such as encapsulation of drugs, edible films and coatings is another dimension that worth exploring. Checking suitability of the LD kafirins for hydrophobic coating and other similar applications in other non-edible consumer goods may unveil new horizon of sorghum uses.

Limitations

We demonstrated that the HD protein mutants are heritable into a hard endosperm hybrid with the desirable nutritional and functional properties maintained. However, we were not able to confirm if the improved HD lines are really "highly digestible" *in vivo* or if the protein digestibility rather depends on the kernel hardness. The *in vivo* digestibility of the mutant proteins in the hard endosperm is not known and this might change the naming of the mutant or the improved sorghum lines.

The field emission scanning electron microscope (FE-SEM) technique for the identification of HD proteins is expensive. There is still a need for a definitive but cost-effective technique. The use of selective protein staining coupled with confocal scanning microscopy may be checked if there is difference in the degree of absorption of the pigments between the irregularly shaped HD mutant and the regular protein bodies in sorghum endosperm. Identification of genetic markers associated with the HD mutation and developing a simpler molecular way of screening may be more practical.

The 100% sorghum formulations for pancakes and cookies could have been compared to a gluten-free control. Use of a standardized recipe for the formulation and having the HD sorghum compared to rice-based gluten-free versions for the different products could have given some idea of the suitability of the improved HD sorghum for gluten-free applications.

The percentage waxiness varied (50-100%) in some lines used for the cookies and breads. We suspect that the variation in the percent waxiness might have masked some of the influences of waxy starch particularly in the cookies where the sorghum incorporation levels were high.

In all the product trials, only basic ingredients were used in the formulations (model products). It is not clear if the HD traits in different starches may deviate in performance from what was observed when other optional ingredients are added or changed.

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APPENDIX A

CORRELATION ANALYSIS

Table A- 1: Significant pairwise correlations for pancake parameters (n=36)

Variable	by Variable	Correlation	Signif Prob	Variable	by Variable	Correlation	Signif Prob
Diameter	Height	-0.8836	<.0001*	Cohesiveness	Springiness	0.9456	<.0001*
Hardness	Height	0.3719	0.0255*	Gumminess	Hardness	0.9646	<.0001*
Hardness	Diameter	-0.4475	0.0062*	Chewiness	Hardness	0.8125	<.0001*
Adhesiveness	Moisture	-0.3452	0.0392*	Chewiness	Gumminess	0.9315	<.0001*
Springiness	Height	-0.8147	<.0001*	Resilience	Height	-0.7203	<.0001*
Springiness	Diameter	0.9211	<.0001*	Resilience	Diameter	0.8673	<.0001*
Springiness	Hardness	-0.3904	0.0186*	Resilience	Hardness	-0.3936	0.0175*
Cohesiveness	Height	-0.7329	<.0001*	Resilience	Springiness	0.9488	<.0001*
Cohesiveness	Diameter	0.8874	<.0001*	Resilience	Cohesiveness	0.9773	<.0001*
Cohesiveness	Hardness	-0.4393	0.0073*				

*significant at α = 0.05

Table A- 2: Significant pairwise correlations for cookie parameters (n=52)

Variable	by Variable	Correlation	Signif Prob	Variable	by Variable	Correlation	Signif Prob
Thickness	Spread	0.6502	<.0001*	Hardness W1	Moisture	-0.6639	<.0001*
Spread ratio	Thickness	-0.6450	<.0001*	Hardness W1	Hardness W0	0.7644	<.0001*
Color L*	Spread	0.4954	0.0002*	Fracturability, W0	Spread	0.6992	<.0001*

*significant at α = 0.05

Table A- 2 Continued

Variable	by Variable	Correlation	Signif Prob	Variable	by Variable	Correlation	Signif Prob
Color L*	Thickness	0.3661	0.0076*	Fracturability, W0	Thickness	0.5396	<.0001*
Color a*	Color L*	-0.8686	<.0001*	Fracturability, W0	Color L*	0.3440	0.0125*
Moisture	Thickness	-0.4003	0.0033*	Fracturability, W0	Hardness, W0	-0.4386	0.0011*
Moisture	Color L*	-0.4433	0.0010*	Fracturability, W1	Spread	0.6182	<.0001*
Moisture	Color a*	0.4129	0.0023*	Fracturability, W1	Thickness	0.5732	<.0001*
Hardness, W0	Color b*	0.3674	0.0074*	Fracturability, W1	Color L*	0.3713	0.0067*
Hardness, W0	Moisture	-0.6242	<.0001*	Fracturability, W1	Hardness, W0	-0.3464	0.0119*
Hardness, W1	Color b*	0.4535	0.0007*	Fracturability, W1	Fracturability, W0	0.7299	<.0001*

*significant at α = 0.05

Table A- 3: Significant pairwise correlations for bread parameters (n=84)

Variable	by Variable	Correlation	Signif Prob	Variable	by Variable	Correlation	Signif Prob
Loaf density	Loaf vol.	-0.9538	<.0001*	Crumb a D2	Loaf density	0.7676	<.0001*
specific vol.	Loaf vol.	0.9956	<.0001*	Crumb a D2	specific vol.	-0.6565	<.0001*

*significant at α = 0.05

Table A- 3 Continued

Variable	by Variable	Correlation	Signif Prob	Variable	by Variable	Correlation	Signif Prob
specific vol.	Loaf density	-0.9619	<.0001*	Crumb a D2	Moisture	0.263	0.0156*
Moisture	Loaf vol.	0.2633	0.0155*	Crumb a D2	Crust a	-0.3194	0.0031*
Moisture	Loaf weight	0.3445	0.0013*	Crumb a D2	Crust b	0.2358	0.0308*
Moisture	specific vol.	0.2300	0.0353*	Crumb a D2	Crumb L D1	-0.7384	<.0001*
Crust L	Moisture	0.4642	<.0001*	Crumb a D2	Crumb a D1	0.9087	<.0001*
Crust a	Loaf vol.	0.6161	<.0001*	Crumb a D2	Crumb b D1	0.4924	<.0001*
Crust a	Loaf weight	0.3622	0.0007*	Crumb a D2	Crumb L D2	-0.7964	<.0001*
Crust a	Loaf density	-0.4585	<.0001*	Crumb b D2	Loaf weight	0.5329	<.0001*
Crust a	specific vol.	0.5824	<.0001*	Crumb b D2	Loaf density	0.4302	<.0001*
Crust a	Moisture	0.3947	0.0002*	Crumb b D2	specific vol.	-0.2522	0.0206*
Crust a	Crust L	-0.2351	0.0313*	Crumb b D2	Moisture	0.3317	0.0021*

*significant at α = 0.05

Table A- 3 Continued

Variable	by Variable	Correlation	Signif Prob	Variable	by Variable	Correlation	Signif Prob
Crust b	Moisture	0.6524	<.0001*	Crumb b D2	Crust L	-0.2261	0.0386*
Crust b	Crust L	0.4738	<.0001*	Crumb b D2	Crust a	0.3481	0.0012*
Crust b	Crust a	0.2459	0.0241*	Crumb b D2	Crumb a D1	0.5647	<.0001*
Crumb L D1	Loaf vol.	0.7410	<.0001*	Crumb b D2	Crumb b D1	0.9694	<.0001*
Crumb L D1	Loaf density	-0.7636	<.0001*	Crumb b D2	Crumb a D2	0.5314	<.0001*
Crumb L D1	specific vol.	0.7292	<.0001*	Hardness D1	Loaf vol.	-0.6584	<.0001*
Crumb L D1	Crust a	0.6272	<.0001*	Hardness D1	Loaf density	0.8243	<.0001*
Crumb a D1	Loaf vol.	-0.6225	<.0001*	Hardness D1	specific vol.	-0.6658	<.0001*
Crumb a D1	Loaf density	0.7641	<.0001*	Hardness D1	Moisture	0.2256	0.0391*
Crumb a D1	specific vol.	-0.6292	<.0001*	Hardness D1	Crust b	0.27	0.0130*
Crumb a D1	Moisture	0.2392	0.0284*	Hardness D1	Crumb L D1	-0.6696	<.0001*

*significant at α = 0.05

Table A- 3 Continued

Variable	by Variable	Correlation	Signif Prob	Variable	by Variable	Correlation	Signif Prob
Crumb a D1	Crust a	-0.2943	0.0066*	Hardness D1	Crumb a D1	0.8312	<.0001*
Crumb a D1	Crumb L D1	-0.7955	<.0001*	Hardness D1	Crumb b D1	0.5971	<.0001*
Crumb b D1	Loaf weight	0.5255	<.0001*	Hardness D1	Crumb L D2	-0.7023	<.0001*
Crumb b D1	Loaf density	0.4078	0.0001*	Hardness D1	Crumb a D2	0.8139	<.0001*
Crumb b D1	specific vol.	-0.2271	0.0378*	Hardness D1	Crumb b D2	0.6007	<.0001*
Crumb b D1	Moisture	0.3074	0.0045*	Hardness D2	Loaf vol.	-0.7738	<.0001*
Crumb b D1	Crust L	-0.2586	0.0175*	Hardness D2	Loaf density	0.9008	<.0001*
Crumb b D1	Crust a	0.4217	<.0001*	Hardness D2	specific vol.	-0.7824	<.0001*
Crumb b D1	Crumb a D1	0.5287	<.0001*	Hardness D2	Crust a	-0.3198	0.0030*
Crumb L D2	Loaf vol.	0.7783	<.0001*	Hardness D2	Crumb L D1	-0.7306	<.0001*
Crumb L D2	Loaf density	-0.7939	<.0001*	Hardness D2	Crumb a D1	0.8183	<.0001*

*significant at $\alpha = 0.05$

Table A- 3 Continued

Variable	by Variable	Correlation	Signif Prob	Variable	by Variable	Correlation	Signif Prob
Crumb L D2	specific vol.	0.7659	<.0001*	Hardness D2	Crumb b D1	0.4995	<.0001*
Crumb L D2	Crust a	0.5834	<.0001*	Hardness D2	Crumb L D2	-0.7537	<.0001*
Crumb L D2	Crumb L D1	0.9128	<.0001*	Hardness D2	Crumb a D2	0.8133	<.0001*
Crumb L D2	Crumb a D1	-0.7607	<.0001*	Hardness D2	Crumb b D2	0.5187	<.0001*
Crumb a D2	Loaf vol.	-0.6478	<.0001*	Hardness D2	Hardness D1	0.9345	<.0001*

*significant at $\alpha = 0.05$

APPENDIX B

SUMMARIES OF ANOVA OUTPUTS

Table B- 1: Pancake batter viscosity

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Sorghum %	1	1	2318816.7	78.1185	<.0001*
Protein type	1	1	1278816.7	43.0820	<.0001*
Sorghum %*Protein type	1	1	260416.7	8.7732	0.0092*
Starch type	1	1	7912016.7	266.5474	<.0001*
Sorghum %*Starch type	1	1	1066816.7	35.9399	<.0001*
Protein type*Starch type	1	1	5626016.7	189.5345	<.0001*
Sorghum %*Protein type*Starch type	1	1	2124150.0	71.5604	<.0001*

Table B- 2: Pancake height/thickness (mm)

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Sorghum %	1	1	64.092017	561.7590	<.0001*
Protein type	1	1	0.936150	8.2052	0.0112*
Sorghum %*Protein type	1	1	5.606667	49.1418	<.0001*
Starch type	1	1	3.760417	32.9596	<.0001*
Sorghum %*Starch type	1	1	0.021600	0.1893	0.6693
Protein type*Starch type	1	1	1.995267	17.4883	0.0007*
Sorghum %*Protein type*Starch type	1	1	1.653750	14.4949	0.0015*

Table B- 3: Pancake diameter (mm)

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Sorghum %	1	1	1115.8884	547.1774	<.0001*
Protein type	1	1	9.3126	4.5664	0.0484*
Sorghum %*Protein type	1	1	0.3197	0.1568	0.6974
Starch type	1	1	1.9210	0.9420	0.3462
Sorghum %*Starch type	1	1	17.2212	8.4444	0.0103*
Protein *Starch types	1	1	0.3978	0.1951	0.6646
Sorghum %*Protein *Starch types	1	1	0.9401	0.4610	0.5069

Table B- 4: Pancake moisture content (%)

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Sorghum %	1	1	0.340817	1.0298	0.3253
Protein type	1	1	67.402017	203.6545	<.0001*
Sorghum %*Protein type	1	1	3.728817	11.2666	0.0040*
Starch type	1	1	39.168150	118.3462	<.0001*
Sorghum %*Starch type	1	1	30.150417	91.0992	<.0001*
Protein *Starch types	1	1	37.150817	112.2508	<.0001*
Sorghum %*Protein *Starch types	1	1	6.427350	19.4202	0.0004*

Table B- 5: Pancake hardness

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Sorghum %	1	1	47.586176	3.4210	0.0829
Protein type	1	1	8.019750	0.5766	0.4587
Sorghum %*Protein type	1	1	6.708251	0.4823	0.4974
Starch type	1	1	0.802090	0.0577	0.8133
Sorghum %*Starch type	1	1	4.036450	0.2902	0.5975
Protein *Starch types	1	1	18.358379	1.3198	0.2675
Sorghum %*Protein *Starch types	1	1	13.361607	0.9606	0.3416

Table B- 6: Pancake adhesiveness

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Sorghum %	1	1	55.262798	26.1023	0.0001*
Protein type	1	1	35.301940	16.6742	0.0009*
Sorghum %*Protein type	1	1	0.085502	0.0404	0.8433
Starch type	1	1	4.898325	2.3136	0.1478
Sorghum %*Starch type	1	1	3.091349	1.4601	0.2445
Protein *Starch types	1	1	5.302070	2.5043	0.1331
Sorghum %*Protein *Starch types	1	1	0.000319	0.0002	0.9904

Table B- 7: Pancake springiness

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Sorghum %	1	1	0.26062504	616.3170	<.0001*
Protein type	1	1	0.00604838	14.3030	0.0016*
Sorghum %*Protein type	1	1	0.00001504	0.0356	0.8528
Starch type	1	1	0.00403004	9.5301	0.0071*
Sorghum %*Starch type	1	1	0.00000704	0.0167	0.8989
Protein *Starch types	1	1	0.00537004	12.6989	0.0026*
Sorghum %*Protein *Starch types	1	1	0.00001504	0.0356	0.8528

Table B- 8: Pancake cohesiveness

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Sorghum %	1	1	0.12384067	103.8424	<.0001*
Protein type	1	1	0.00322017	2.7002	0.1198
Sorghum %*Protein type	1	1	0.00013067	0.1096	0.7449
Starch type	1	1	0.00749067	6.2810	0.0234*
Sorghum %*Starch type	1	1	0.00660017	5.5343	0.0318*
Protein s*Starch types	1	1	0.00540000	4.5280	0.0492*
Sorghum %*Protein s*Starch types	1	1	0.00070417	0.5905	0.4534

Table B- 9: Pancake gumminess

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Sorghum %	1	1	4.1110204	1.0086	0.3302
Protein type	1	1	4.5614320	1.1191	0.3058
Sorghum %*Protein type	1	1	3.3160100	0.8136	0.3804
Starch type	1	1	0.0400984	0.0098	0.9222
Sorghum %*Starch type	1	1	0.4037820	0.0991	0.7570
Protein type*Starch type	1	1	3.3727504	0.8275	0.3765
Sorghum %*Protein type*Starch type	1	1	2.4263400	0.5953	0.4516

Table B- 10: Pancake chewiness

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Sorghum %	1	1	0.2810170	0.2796	0.6042
Protein type	1	1	1.8598234	1.8505	0.1926
Sorghum %*Protein type	1	1	0.8782200	0.8738	0.3638
Starch type	1	1	0.1228370	0.1222	0.7312
Sorghum %*Starch type	1	1	0.2434120	0.2422	0.6293
Protein *Starch types	1	1	0.4409170	0.4387	0.5172
Sorghum %*Protein *Starch types	1	1	0.3888760	0.3869	0.5427

Table B- 11: Pancake resilience

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Sorghum %	1	1	0.02801667	164.9657	<.0001*
Protein type	1	1	0.00114817	6.7605	0.0193*
Sorghum %*Protein type	1	1	0.00015000	0.8832	0.3613
Starch type	1	1	0.00312817	18.4190	0.0006*
Sorghum %*Starch type	1	1	0.00091267	5.3739	0.0340*
Protein s*Starch types	1	1	0.00268817	15.8283	0.0011*
Sorghum %*Protein s*Starch types	1	1	0.00000067	0.0039	0.9508

Table B- 12: Pancake treatments versus controls

Parameter	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Batter viscosity	11	11	34771918	155.1503	<.0001*
Height/thickness	11	11	116.60794	96.4267	<.0001*
Diameter	11	11	1466.5808	89.5165	<.0001*
Moisture	11	11	245.15841	79.4942	<.0001*
Hardness	11	11	234.68090	1.4904	0.1992
Adhesiveness	11	11	12898.336	8.1017	<.0001*
Springiness	11	11	0.41980675	93.0646	<.0001*
Cohesiveness	11	11	0.21424208	22.9241	<.0001*
Gumminess	11	11	57.054470	1.0348	0.4484
Chewiness	11	11	19.820555	0.9500	0.5134
Resilience	11	11	0.05533008	36.5523	<.0001*

Table B- 13: Cookie spread

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Formulation	1	1	246.75311	386.6051	<.0001*
Protein type	1	1	13.59811	21.3051	0.0001*
Starch type	1	1	9.28805	14.5522	0.0008*
Formulation*Protein type	1	1	0.85151	1.3341	0.2594
Formulation*Starch type	1	1	9.03125	14.1499	0.0010*
Protein *Starch types	1	1	0.00005	0.0001	0.9930
Formulation*Protein*Starch types	1	1	7.33445	11.4914	0.0024*

Table B- 14: Cookie spread ration

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Formulation	1	1	0.03993280	0.7412	0.3978
Protein type	1	1	0.11194625	2.0779	0.1624
Starch type	1	1	0.03187170	0.5916	0.4493
Formulation*Protein type	1	1	0.27397105	5.0853	0.0335*
Formulation*Starch type	1	1	0.02721210	0.5051	0.4841
Protein *Starch types	1	1	0.53728084	9.9727	0.0043*
Formulation*Protein*Starch types	1	1	0.32141873	5.9660	0.0223*

Table B- 15: Cookie thickness

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Formulation	1	1	10.822878	39.3790	<.0001*
Protein type	1	1	0.003828	0.0139	0.9070
Starch type	1	1	0.010878	0.0396	0.8440
Formulation*Protein type	1	1	1.660753	6.0427	0.0216*
Formulation*Starch type	1	1	0.065703	0.2391	0.6293
Protein *Starch types	1	1	2.392578	8.7054	0.0070*
Formulation*Protein *Starch types	1	1	2.826253	10.2833	0.0038*

Table B- 16: Cookie color L*

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Formulation	1	1	105.99680	2812.208	<.0001*
Protein type	1	1	0.29261	7.7633	0.0103*
Starch type	1	1	1.42805	37.8877	<.0001*
Formulation*Protein type	1	1	0.27751	7.3627	0.0121*
Formulation*Starch type	1	1	1.80500	47.8886	<.0001*
Protein *Starch types	1	1	1.81451	48.1409	<.0001*
Formulation*Protein*Starch types	1	1	2.74951	72.9475	<.0001*

Table B- 17: Cookie color a*

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Formulation	1	1	1.6290125	305.6787	<.0001*
Protein type	1	1	0.0760500	14.2705	0.0009*
Starch type	1	1	7.0876125	1329.966	<.0001*
Formulation*Protein type	1	1	0.0004500	0.0844	0.7739
Formulation*Starch type	1	1	0.0028125	0.5278	0.4746
Protein *Starch types	1	1	0.3280500	61.5575	<.0001*
Formulation*Protein *Starch types	1	1	0.1458000	27.3589	<.0001*

Table B- 18: Cookie color b*

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Formulation	1	1	2.116653	49.7122	<.0001*
Protein type	1	1	2.536878	59.5817	<.0001*
Starch type	1	1	29.013153	681.4098	<.0001*
Formulation*Protein type	1	1	0.008778	0.2062	0.6539
Formulation*Starch type	1	1	3.093828	72.6624	<.0001*
Protein*Starch types	1	1	3.941028	92.5599	<.0001*
Formulation*Protein*Starch types	1	1	1.474903	34.6399	<.0001*

Table B- 19: Cookie moisture content

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Formulation	1	1	1.1973781	102.2581	<.0001*
Protein type	1	1	4.5075031	384.9482	<.0001*
Starch type	1	1	0.2000281	17.0827	0.0004*
Formulation*Protein type	1	1	0.0712531	6.0851	0.0212*
Formulation*Starch type	1	1	0.0124031	1.0592	0.3136
Protein *Starch types	1	1	6.4171531	548.0355	<.0001*
Formulation*Protein *Starch types	1	1	0.0101531	0.8671	0.3610

Table B- 20: Cookie hardness (week 0)

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Formulation	1	1	9445553	58.2281	<.0001*
Protein type	1	1	8156942	50.2844	<.0001*
Starch type	1	1	815096	5.0248	0.0345*
Formulation*Protein type	1	1	1683007	10.3751	0.0036*
Formulation*Starch type	1	1	955950	5.8931	0.0231*
Protein*Starch types	1	1	13203581	81.3949	<.0001*
Formulation*Protein *Starch types	1	1	448447	2.7645	0.1094

Table B- 21: Cookie hardness (week 1)

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Formulation	1	1	271784	1.8518	0.1862
Protein type	1	1	13602650	92.6840	<.0001*
Starch type	1	1	3760132	25.6203	<.0001*
Formulation*Protein type	1	1	2067053	14.0842	0.0010*
Formulation*Starch type	1	1	268102	1.8268	0.1891
Protein*Starch types	1	1	5096699	34.7272	<.0001*
Formulation*Protein *Starch types	1	1	31992	0.2180	0.6448

Table B- 22: Cookie breaking strength (Fracturability, week 0)

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Formulation	1	1	9.4503781	46.7684	<.0001*
Protein type	1	1	0.0331531	0.1641	0.6890
Starch type	1	1	1.3243781	6.5541	0.0172*
Formulation*Protein type	1	1	0.2261281	1.1191	0.3007
Formulation*Starch type	1	1	0.7472531	3.6980	0.0664
Protein*Starch types	1	1	0.0166531	0.0824	0.7765
Formulation*Protein*Starch types	1	1	0.0000031	0.0000	0.9969

Table B- 23: Cookie breaking strength (Fracturability, week 1)

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Formulation	1	1	10.998050	27.8715	<.0001*
Protein type	1	1	0.082012	0.2078	0.6526
Starch type	1	1	1.117512	2.8320	0.1054
Formulation*Protein type	1	1	1.548800	3.9250	0.0591
Formulation*Starch type	1	1	0.460800	1.1678	0.2906
Protein *Starch types	1	1	0.002112	0.0054	0.9423
Formulation*Protein *Starch types	1	1	0.151250	0.3833	0.5417

Table B- 24: Sorghum based cookies compared with different controls

Response	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Spread	12	12	512.13434	80.0009	<.0001*
Thickness	12	12	21.130605	8.2733	<.0001*
Spread ration	12	12	2.1856473	4.5921	0.0001*
Color L*	12	12	682.23024	694.4526	<.0001*
Color a*	12	12	112.48375	1359.162	<.0001*
Color b*	12	12	364.37230	758.7411	<.0001*
Moisture content	12	12	24.198817	149.8539	<.0001*
Hardness (week 0)	12	12	62182937	37.2281	<.0001*
Hardness (week 1)	12	12	59301379	31.6934	<.0001*
Fracturability (week 0)	12	12	20.424356	9.7702	<.0001*
Fracturability (week 1)	12	12	18.742030	5.3657	<.0001*

Table B- 25: Bread specific volume

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Starter	1	1	32.636723	1635.059	<.0001*
Formulation	1	1	1.009709	50.5852	<.0001*
Protein type	1	1	3.208192	160.7264	<.0001*
Starch type	1	1	0.663908	33.2609	<.0001*
Starter*Formulation	1	1	0.364903	18.2812	0.0002*
Starter*Protein type	1	1	0.404457	20.2628	<.0001*
Formulation*Protein type	1	1	0.052134	2.6119	0.1159
Starter*Starch type	1	1	0.082775	4.1469	0.0501
Formulation*Starch type	1	1	0.000019	0.0010	0.9755
Protein *Starch types	1	1	0.524969	26.3003	<.0001*
Starter*Formulation*Protein type	1	1	0.028564	1.4310	0.2404
Starter*Formulation*Starch type	1	1	0.072286	3.6214	0.0661
Starter*Protein *Starch types	1	1	0.145879	7.3083	0.0109*
Formulation*Protein *Starch types	1	1	0.137635	6.8953	0.0131*
Starter*Formulation*Protein *Starch types	1	1	4.7073e-6	0.0002	0.9878

Table B- 26: Bread moisture content

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Starter	1	1	89.134752	1089.972	<.0001*
Formulation	1	1	0.850669	10.4023	0.0029*
Protein type	1	1	3.624502	44.3217	<.0001*
Starch type	1	1	2.362969	28.8952	<.0001*
Starter*Formulation	1	1	10.332352	126.3478	<.0001*
Starter*Protein type	1	1	1.829102	22.3669	<.0001*

Table B- 26 Continued

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Formulation*Protein type	1	1	2.362969	28.8952	<.0001*
Starter*Starch type	1	1	14.641252	179.0386	<.0001*
Formulation*Starch type	1	1	0.051352	0.6280	0.4339
Protein *Starch types	1	1	4.374169	53.4889	<.0001*
Starter*Formulation*Protein type	1	1	3.450769	42.1973	<.0001*
Starter*Formulation*Starch type	1	1	0.181302	2.2170	0.1463
Starter*Protein *Starch types	1	1	0.091002	1.1128	0.2994
Formulation*Protein *Starch types	1	1	0.474019	5.7965	0.0220*
Starter*Formulation*Protein *Starch types	1	1	0.423752	5.1818	0.0297*

Table B- 27: Bread crust color L*

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Starter	1	1	143.41710	96.1720	<.0001*
Formulation	1	1	33.85200	22.7003	<.0001*
Protein type	1	1	243.31510	163.1612	<.0001*
Starch type	1	1	0.93242	0.6253	0.4349
Starter*Formulation	1	1	20.97485	14.0652	0.0007*
Starter*Protein type	1	1	0.00092	0.0006	0.9804
Formulation*Protein type	1	1	6.65285	4.4612	0.0426*
Starter*Starch type	1	1	4.29005	2.8768	0.0996
Formulation*Starch type	1	1	4.96010	3.3261	0.0775
Protein type*Starch type	1	1	8.19227	5.4935	0.0255*
Starter*Formulation*Protein type	1	1	2.31880	1.5549	0.2215
Starter*Formulation*Starch type	1	1	0.82950	0.5562	0.4612

Table B- 27 Continued

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Starter*Protein *Starch types	1	1	0.29297	0.1965	0.6606
Formulation*Protein *Starch types	1	1	2.78885	1.8701	0.1810
Starter*Formulation*Protein *Starch types	1	1	0.13975	0.0937	0.7615

Table B- 28: Bread crust color a*

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Starter	1	1	36.296408	182.9053	<.0001*
Formulation	1	1	13.910533	70.0981	<.0001*
Protein type	1	1	0.594075	2.9937	0.0932
Starch type	1	1	0.816408	4.1141	0.0509
Starter*Formulation	1	1	1.235208	6.2245	0.0180*
Starter*Protein type	1	1	1.400833	7.0591	0.0122*
Formulation*Protein type	1	1	2.793675	14.0779	0.0007*
Starter*Starch type	1	1	2.557633	12.8885	0.0011*
Formulation*Starch type	1	1	0.357075	1.7994	0.1892
Protein *Starch type	1	1	3.564300	17.9613	0.0002*
Starter*Formulation*Protein type	1	1	0.124033	0.6250	0.4350
Starter*Formulation*Starch type	1	1	0.464133	2.3389	0.1360
Starter*Protein *Starch types	1	1	0.350208	1.7648	0.1934
Formulation*Protein *Starch types	1	1	0.034133	0.1720	0.6811
Starter*Formulation*Protein *Starch type	1	1	0.004408	0.0222	0.8825

Table B- 29: Bread crust color b*

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Starter	1	1	245.43608	1080.879	<.0001*
Formulation	1	1	0.77013	3.3916	0.0748
Protein type	1	1	23.91363	105.3135	<.0001*
Starch type	1	1	8.21707	36.1873	<.0001*
Starter*Formulation	1	1	0.79053	3.4814	0.0713
Starter*Protein type	1	1	0.09013	0.3969	0.5331
Formulation*Protein type	1	1	1.00341	4.4189	0.0435*
Starter*Starch type	1	1	7.41041	32.6348	<.0001*
Formulation*Starch type	1	1	0.00163	0.0072	0.9329
Protein *Starch type	1	1	0.12000	0.5285	0.4725
Starter*Formulation*Protein type	1	1	3.70741	16.3271	0.0003*
Starter*Formulation*Starch type	1	1	0.57203	2.5192	0.1223
Starter*Protein *Starch types	1	1	2.82270	12.4309	0.0013*
Formulation*Protein *Starch types	1	1	0.60301	2.6556	0.1130
Starter*Formulation*Protein *Starch types	1	1	0.00068	0.0030	0.9569

Table B- 30: Bread crumb color L* Day 1

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Starter	1	1	278.30701	374.1922	<.0001*
Formulation	1	1	38.95203	52.3722	<.0001*
Protein type	1	1	1.53367	2.0621	0.1607
Starch type	1	1	6.81013	9.1564	0.0049*
Starter*Formulation	1	1	2.57613	3.4637	0.0719
Starter*Protein type	1	1	4.67501	6.2857	0.0174*

Table B-30 Continued

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Formulation*Protein type	1	1	4.41653	5.9382	0.0206*
Starter*Starch type	1	1	0.05880	0.0791	0.7804
Formulation*Starch type	1	1	0.50021	0.6725	0.4182
Protein *Starch types	1	1	1.70253	2.2891	0.1401
Starter*Formulation*Protein type	1	1	9.29280	12.4945	0.0013*
Starter*Formulation*Starch type	1	1	24.05501	32.3427	<.0001*
Starter*Protein *Starch types	1	1	16.28670	21.8980	<.0001*
Formulation*Protein *Starch types	1	1	1.92801	2.5923	0.1172
Starter*Formulation*Protein *Starch types	1	1	0.30401	0.4087	0.5272

Table B- 31: Bread crumb color a* Day 1

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Starter	1	1	8.2502083	353.2334	<.0001*
Formulation	1	1	4.3923000	188.0567	<.0001*
Protein type	1	1	0.0024083	0.1031	0.7502
Starch type	1	1	0.5125333	21.9442	<.0001*
Starter*Formulation	1	1	0.0225333	0.9648	0.3334
Starter*Protein type	1	1	0.0070083	0.3001	0.5876
Formulation*Protein type	1	1	0.0000333	0.0014	0.9701
Starter*Starch type	1	1	0.0533333	2.2835	0.1406
Formulation*Starch type	1	1	0.0444083	1.9013	0.1775
Protein *Starch type	1	1	0.8856333	37.9185	<.0001*
Starter*Formulation*Protein type	1	1	0.0261333	1.1189	0.2981
Starter*Formulation*Starch type	1	1	0.0090750	0.3885	0.5375

Table B- 31 Continued

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Starter*Protein *Starch types	1	1	0.0075000	0.3211	0.5749
Formulation*Protein *Starch types	1	1	0.4144083	17.7429	0.0002*
Starter*Formulation*Protein *Starch types	1	1	0.0014083	0.0603	0.8076

Table B- 32: Bread crumb color b* Day 1

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Starter	1	1	10.687969	290.0891	<.0001*
Formulation	1	1	4.042602	109.7229	<.0001*
Protein type	1	1	0.256669	6.9664	0.0127*
Starch type	1	1	1.445602	39.2360	<.0001*
Starter*Formulation	1	1	0.637102	17.2920	0.0002*
Starter*Protein type	1	1	1.162519	31.5527	<.0001*
Formulation*Protein type	1	1	3.005002	81.5607	<.0001*
Starter*Starch type	1	1	0.025669	0.6967	0.4101
Formulation*Starch type	1	1	0.084169	2.2845	0.1405
Protein *Starch types	1	1	0.397852	10.7984	0.0025*
Starter*Formulation*Protein type	1	1	1.310102	35.5583	<.0001*
Starter*Formulation*Starch type	1	1	0.055352	1.5023	0.2293
Starter*Protein *Starch types	1	1	0.427519	11.6036	0.0018*
Formulation*Protein *Starch types	1	1	0.222769	6.0463	0.0195*
Starter*Formulation*Protein *Starch types	1	1	1.452552	39.4247	<.0001*

Table B- 33: Bread crumb color L* Day 2

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Starter	1	1	265.22102	580.3206	<.0001*
Formulation	1	1	11.47585	25.1099	<.0001*
Protein type	1	1	0.03255	0.0712	0.7913
Starch type	1	1	10.68797	23.3860	<.0001*
Starter*Formulation	1	1	0.43892	0.9604	0.3344
Starter*Protein type	1	1	7.37117	16.1286	0.0003*
Formulation*Protein type	1	1	0.01367	0.0299	0.8638
Starter*Starch type	1	1	1.41797	3.1026	0.0877
Formulation*Starch type	1	1	11.83060	25.8861	<.0001*
Protein *Starch types	1	1	12.23110	26.7624	<.0001*
Starter*Formulation*Protein type	1	1	3.09575	6.7737	0.0139*
Starter*Formulation*Starch type	1	1	1.10110	2.4093	0.1305
Starter*Protein *Starch types	1	1	11.16505	24.4298	<.0001*
Formulation*Protein *Starch types	1	1	31.93172	69.8686	<.0001*
Starter*Formulation*Protein *Starch type	1	1	16.72060	36.5857	<.0001*

Table B- 34: Bread crumb color a* Day 2

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Starter	1	1	5.4203521	24.1733	<.0001*
Formulation	1	1	3.3232687	14.8209	0.0005*
Protein type	1	1	0.1938021	0.8643	0.3595
Starch type	1	1	1.5516021	6.9197	0.0130*
Starter*Formulation	1	1	0.6697688	2.9870	0.0936
Starter*Protein type	1	1	0.0038521	0.0172	0.8965

Table B-34 Continued

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Formulation*Protein type	1	1	0.3870021	1.7259	0.1983
Starter*Starch type	1	1	0.1441021	0.6427	0.4287
Formulation*Starch type	1	1	0.0540021	0.2408	0.6270
Protein *Starch type	1	1	2.0708521	9.2354	0.0047*
Starter*Formulation*Protein type	1	1	0.1552687	0.6925	0.4115
Starter*Formulation*Starch type	1	1	0.6233521	2.7800	0.1052
Starter*Protein *Starch types	1	1	0.0072521	0.0323	0.8584
Formulation*Protein *Starch types	1	1	0.1150521	0.5131	0.4790
Starter*Formulation*Protein *Starch types	1	1	0.4504688	2.0090	0.1660

Table B- 35: Bread crumb color b* Day 2

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Starter	1	1	11.126502	135.0270	<.0001*
Formulation	1	1	0.024752	0.3004	0.5874
Protein type	1	1	0.888352	10.7807	0.0025*
Starch type	1	1	0.280602	3.4053	0.0743
Starter*Formulation	1	1	0.490052	5.9471	0.0205*
Starter*Protein type	1	1	0.045019	0.5463	0.4652
Formulation*Protein type	1	1	0.018802	0.2282	0.6361
Starter*Starch type	1	1	0.379852	4.6097	0.0395*
Formulation*Starch type	1	1	0.600769	7.2907	0.0110*
Protein *Starch types	1	1	0.325052	3.9447	0.0556
Starter*Formulation*Protein type	1	1	0.009352	0.1135	0.7384
Starter*Formulation*Starch type	1	1	1.200169	14.5648	0.0006*

Table B- 35 Continued

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Starter*Protein *Starch types	1	1	0.239419	2.9055	0.0980
Formulation*Protein *Starch types	1	1	2.013102	24.4302	<.0001*
Starter*Formulation*Protein *Starch types	1	1	2.310019	28.0335	<.0001*

Table B- 36: Bread crumb hardness Day 1

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Starter	1	1	325052.08	414.9601	<.0001*
Formulation	1	1	13668.75	17.4495	0.0002*
Protein type	1	1	16502.08	21.0665	<.0001*
Starch type	1	1	32552.08	41.5559	<.0001*
Starter*Formulation	1	1	752.08	0.9601	0.3345
Starter*Protein type	1	1	20418.75	26.0665	<.0001*
Formulation*Protein type	1	1	11718.75	14.9601	0.0005*
Starter*Starch type	1	1	33602.08	42.8963	<.0001*
Formulation*Starch type	1	1	12352.08	15.7686	0.0004*
Protein *Starch type	1	1	5852.08	7.4707	0.0101*
Starter*Formulation*Protein type	1	1	15052.08	19.2154	0.0001*
Starter*Formulation*Starch type	1	1	27552.08	35.1729	<.0001*
Starter*Protein *Starch types	1	1	17252.08	22.0239	<.0001*
Formulation*Protein *Starch types	1	1	14352.08	18.3218	0.0002*
Starter*Formulation*Protein *Starch types	1	1	11102.08	14.1729	0.0007*

Table B- 37: Bread crumb hardness Day 2

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Starter	1	1	2197352.1	279.1027	<.0001*
Formulation	1	1	198918.8	25.2662	<.0001*
Protein type	1	1	565502.1	71.8288	<.0001*
Starch type	1	1	3168.8	0.4025	0.5303
Starter*Formulation	1	1	11102.1	1.4102	0.2438
Starter*Protein type	1	1	174002.1	22.1013	<.0001*
Formulation*Protein type	1	1	5852.1	0.7433	0.3950
Starter*Starch type	1	1	59502.1	7.5578	0.0097*
Formulation*Starch type	1	1	45018.7	5.7182	0.0228*
Protein *Starch types	1	1	52.1	0.0066	0.9357
Starter*Formulation*Protein type	1	1	5002.1	0.6354	0.4313
Starter*Formulation*Starch type	1	1	69768.8	8.8619	0.0055*
Starter*Protein *Starch types	1	1	176418.8	22.4083	<.0001*
Formulation*Protein *Starch types	1	1	9352.1	1.1879	0.2839
Starter*Formulation*Protein *Starch types	1	1	6302.1	0.8005	0.3776

Table B- 38: Bread parameters compared to different controls

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Specific volume	27	27	2569517.6	261.4572	<.0001*
Moisture content	27	27	245.00947	92.3181	<.0001*
Crust color L*	27	27	1424.5068	36.7582	<.0001*
Crust color a*	27	27	116.20617	28.7739	<.0001*
Crust color b*	27	27	813.75307	2.6874	0.0009*
Crumb color L* day 1	27	27	1459.5535	95.6229	<.0001*

Table B- 38 Continued

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Crumb color a* day 1	27	27	180.43760	415.7925	<.0001*
Crumb color b* day 1	27	27	392.32727	429.3713	<.0001*
Crumb color L* day 2	27	27	1431.3333	104.9598	<.0001*
Crumb color a* day 2	27	27	187.31822	52.1376	<.0001*
Crumb color b* day 2	27	27	405.57871	198.8998	<.0001*
Hardness day 1	27	27	7598308.3	228.8401	<.0001*
Hardness day 1	27	27	19844433	61.9986	<.0001*

APPENDIX C

DESCRIPTIVE SENSORY EVALUATION BALLOT

C- 1: Pancakes

Line code	Order	Code Day I	Order	Code Day II	Hardness	Fracturability	Surface roughness	Crispiness
		W/U		W/U				
65[1]	1	296	8	106				
65[2]	2	918	7	214				
77[1]	3	824	6	872				
66[2]	4	148	5	566				
Break								
77[2]	5	642	4	475				
09[1]	6	471	3	319				
66[1]	7	341	2	721				
09[2]	8	567	1	907				

[1]100% sorghum addition; [2]50% sorghum addition; sorghum line codes and categories: 09 = HD-waxy; 65 = HD-normal; 66 = LD-normal; 77 = HD-waxy

C- 2: Cookies

Line code	Order	Code Day I	Order	Code Day II	Hardness	Fracturability	Surface roughness	Crispiness
		W/U		W/U				
65[1]	1	541	2	407				
65[2]	2	766	5	526				
77[1]	3	424	7	903				
66[2]	4	915	6	105				
		Break		Break				
77[2]	5	843	1	129				
64[1]	6	397	8	821				
66[1]	7	128	3	670				
64[2]	8	672	4	279				

[1]100% sorghum addition; [2]50% sorghum addition; sorghum line codes and categories 64 = HD-waxy; 65 = HD-normal; 66 = LD-normal; 77 = HD-waxy