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PHYSICO-CHEMICAL, RHEOLOGICAL AND BAKING PROPERTIES OF PROSO MILLET

Manjot Singh
University of Kentucky, manjot.singh@uky.edu
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Manjot Singh, Student

Dr. Akinbode A. Adedeji, Major Professor

Dr. Donald Colliver, Director of Graduate Studies

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THESIS

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By

Manjot Singh

Lexington, Kentucky

Co-Director: Dr. Akinbode A. Adedeji, Assistant Professor of Biosystems and Agricultural Engineering and Dr. Michael Montross, Professor of Biosystems and Agricultural Engineering

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ABSTRACT OF THESIS

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Due to climate change, water scarcity, increasing population and rising food prices, agriculture and food security has been affected worldwide. Cereal grains being a major part of world food supply also act as important energy source in human diet. In order to counter food insecurity, alternative grains are being explored, and millet being drought-resistant has the potential to serve as an alternative grain due to its comparable nutritional composition with other major cereals and its gluten free proteins. The evidence that gluten sensitivity is one of the increasing food intolerances is driving an increasing demand for gluten-free foods. However, gluten is a structure building protein essential for optimum dough development. Therefore, obtaining high-quality gluten-free bread (GFB) is a technological challenge. Due to lack of research about proso millet, this study investigates the physical properties of nine different cultivars to help in equipment design and significant difference was observed in dimensions, sphericity, volume, surface area, bulk density, porosity and angle of repose. This study also focused on characterization of proso millet starch and effect of acid and hydrothermal modification on native starch was observed. We were also interested in determining the rheological properties of millet based gluten free formulation with different hydrocolloids, and the quality attributes of bread made from them. Dough undergoes deformation during preparatory processes which was evaluated with the application of rheology. And the final baking parameters such as bread volume, texture, color allowed correlation between rheological and baking performance. This study has helped us to better understand millet potential in different industries including starch and bakery and in designing equipment and storage structures.

KEYWORDS: Proso millet, Gluten free, Physical Properties, Baking, Rheology, Starch

Manjot Singh

14-Nov-16

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Dr. Akinbode A. Adedeji

Co-Director of Thesis

Dr. Michael Montross

Co-Director of Thesis

Dr. Donald Colliver

Director of Graduate Studies

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Dedication

I dedicate this thesis to my family for their continued love and support.

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1. Introduction

In United States, millets are used mostly as feed for animals, birds especially, but very little is used as human food. Millet plays an important role in serving under-privileged groups in Africa, East-Asia and Indian sub-continent. Millet became a part of human diet about 10,000 years ago even before the *rise* of wheat and rice (Saleh et al., 2013). Millet is the 6th most important cereal crop in world agriculture production. The total world production of millet seeds was estimated as 762,712 tons in 2012 with India leading as the top producer (334,500 tons) followed by Niger (108,798 tons) and Nigeria (59,994 tons) (FAO, 2012).

Millet has many advantages over other cereals such as higher resistance to pests and diseases, adaptability to a wide range of climate conditions and grows well in higher temperatures and dry conditions compared to other cereals (Saleh et al., 2013). Millets are rich in fiber, iron, calcium, B vitamins and low in phytic acid, their nutritional value is comparable to other cereals like rice and wheat. However, millet is not consumed in major part of world as a staple food due to the presence of anti-nutritional factors of certain phytochemicals which interfere with mineral bioavailability, carbohydrate and protein digestibility (Pradeep and Sreerama, 2015). But these effects can be minimized with methods like cooking, soaking, and fermentation (Pradeep and Sreerama, 2015) and most of the anti-nutritional factors are present in husk and bran fractions and might be easily removed by dehulling and polishing. However, millet is reported to have beneficial effect in cancer and cardiovascular diseases prevention, lowering blood pressure due to the presence of phenolic compounds (Saleh et al., 2013).

1.1. Hypothesis

The hypothesis of this study was that millet has comparable quality characteristics as wheat, corn or other major cereals and possess similar functional properties that allow its application in gluten free foods. Furthermore, the addition of different starches and hydrocolloids will enhance the millet flour's viscoelastic properties.

1.2. Problem Statement

Physical characteristics of grains are important parameters for determination of proper standards for the design, processing, and packaging systems. Proso millet being an under-researched grain lacks processing equipment, although pearl millet has been studied in other parts of the world including China, India and African countries for its physical properties but the difference between both varieties is vast. So there is a need to investigate the physical properties of proso millet cultivars grown in USA to help in designing processing and storage equipment.

Starch is the second most abundant carbohydrate present in higher plants and it is a major ingredient used in food and non-food industries. Corn and potato starches are used extensively but millet also contain high amount of starch which can be used for various applications. Generally, modified starches are used in industries due to instability of native starches. There is limited information available on modified millet starch with most attention on pearl millet. Hence, modified proso millet starch was considered for study in this project, and two most common modification methods were selected, which are acid modification and hydrothermal modification.

Millet has comparable nutritional profile to wheat (Saleh et al., 2013), and has potential application in gluten free baking. Many different combinations of starches and hydrocolloids were used in recent studies to make the dough more viscoelastic. Previous studies focused on rice, corn and other cereal based gluten free breads which produced low quality bread. Therefore, it is important to investigate the effect of different hydrocolloids and starches with millet flour. It was hypothesized that the addition of both starches and hydrocolloids would improve the overall quality of millet based bread.

The following objectives were identified to address all the issues discussed above:

Objective 1. Understand the physical and functional properties of nine different proso millet cultivars

- Determine the proximate content and amylose content
- Determine physical properties including dimensions, equivalent diameter, sphericity, volume, surface area, bulk density, solid density, porosity and angle of repose
- Determine effect of amylose on thermal and pasting properties of the cultivars

Objective 2. Characterize modified starch from commercial proso millet

- Assess the effect of modifications (acid and hydrothermal) on physico-functional properties of starch
- Investigate the effect of thermal and pasting properties

Objective 3. Elucidate the rheological and baking properties of millet based gluten free formulations

- Determine the effects of starch on rheological and baking properties
- Determine the effects of hydrocolloids on gluten free formulations
- Investigate the correlation between rheological and baking properties

2. General literature review

2.1. Physical properties of millet

Physical characteristics of grains are important parameters for determination of proper standards for the design of grading, conveying, processing, and packaging systems (Tabatabaeefar and Rajabipour, 2005). Among these physical characteristics, mass, volume, surface area, sphericity, bulk density, porosity and angle of repose are the most important ones in determining sizing systems (Singh et al., 2010). Information regarding dimensional attributes is used in describing grain shape which is often necessary in designing processing equipment (Swami and Swami, 2010). During transportation of grains, the design of equipment is related to bulk density and porosity. Volumes and surface area of grains must be known for accurate modeling of heat and mass transfer during cooling and drying (Mohsenin, 1986). Determining a relationship between mass, dimensions and projected areas is useful and applicable in grading and sorting (Swami and Swami, 2010).

This literature review focuses on various studies conducted by researchers on different millet varieties. Adebowale et al. (2012) studied the effect of variety and initial moisture content on physical properties of improved Nigerian millet grains. Millet grains were conditioned to 10, 20 and 30% moisture content and physical properties such as length, width, thickness, arithmetic mean diameter, effective mean diameter, surface area, sphericity, volume, mass, bulk density, true density, porosity, angle of repose, static coefficient of friction and specific heat capacity were determined. The study showed that variety and moisture content had significant effect ($P<0.05$) on the physical properties of millet.

Mir and Bosco (2013) studied physical properties of seven rice cultivars commonly grown in temperate regions of India and found significant differences in the physical properties including length, width, thickness, equivalent diameter, surface area, sphericity, aspect ratio, volume, bulk density, true density, porosity, thousand kernel weight, angle of repose and coefficient of friction ($p \leq 0.05$). Similarly, Balasubramanian and Viswanathan (2010) studied influence of moisture content on physical properties of minor millets (foxtail, little, kodo, common, barnyard and finger millet) varying in moisture content from 11.1 to 25% db. Thousand kernel weight, angle of repose, coefficient of static and internal friction for minor millets were found to be directly proportional to moisture content however, bulk density, true density, porosity and grain hardness were found to be inversely proportional to moisture content. Baryeh (2002) studied physical properties of millet variety *P. gambiense* as a function of moisture. Baryeh (2002) reported that all linear dimensions of grain, grain surface area, grain volume, 1000 kernel weight, true density, angle of repose, coefficient of friction on plywood, mild steel and galvanized iron, and terminal velocity increased with an increase in grain moisture content with high correlation.

Ojediran et al. (2010) studied physical properties of pearl millet seeds (Penisetum glaucum) (Ex-Borno and SOSAT C88) as a function of moisture content in the range of 5-22% (db) and found similar results as reported by other researchers, sphericity changes with increase in moisture content. The estimated porosity, angle of repose and the thousand kernel weight increased with increase in seed moisture content for both varieties, while bulk density and solid density decreased.

A review of the literature showed that physical and nutritional properties of proso millet cultivars have not been determined. These properties are necessary for the design of equipment for harvesting, processing, transporting, sorting, separating and packing. Therefore, in this study the physical properties, namely, length, width and thickness, equivalent mean diameters, surface area, sphericity, thousand kernel weight, volume, bulk and solid densities, porosity, angle of repose, nutritional content, thermal and pasting properties were determined and compared.

2.2. Starch

Starch is a naturally occurring high-molecular weight polymer of α-D-glucose and serves not only as an energy reservoir of higher plants but also as a major supplier of energy in human and animal diets. Starch consists of two main components: amylose and amylopectin. Amylose is linear polymer with few side chains while amylopectin is a highly branched polymer. Amylose and amylopectin hold different properties and are therefore best suited for different applications (Zobel, 1988). Generally, non-waxy millet starch contains about 28% amylose and 72% amylopectin.

Amylose is an almost linear, water-soluble polysaccharide formed by α-D-1,4-anhydroglucose linkages. The molecular weight of amylose is 105-106 Da for most starch sources (Buléon et al., 1998; Whistler and Daniel, 1984). The molecular size depends on the source and it may contain anywhere from about 200 to 2000 anhydroglucose units. The amylose polymers have a tendency to orient themselves in a parallel fashion where hydrogen bonds can be formed between adjacent polymers, because of its linearity, mobility and hydroxyl groups. This phenomenon of intermolecular association is commonly known as retrogradation (Wurzburg, 1986) and as a result the amylose gels become opaque. The configuration of amylose is still ambiguous (Whistler and Daniel, 1984) but it is said that in water, amylose exists as a random coil, whereas in a good solvent (e.g. dimethyl sulphoxide) it exists as an extended coil. In the presence of a complexing agent (e.g. lipids) amylose exists as a helix (Banks and Greenwood, 1975). Due to its linear character, amylose can crystallize and films of amylose thus have better barrier properties and show higher modulus than amylopectin films (Stading et al., 2001).

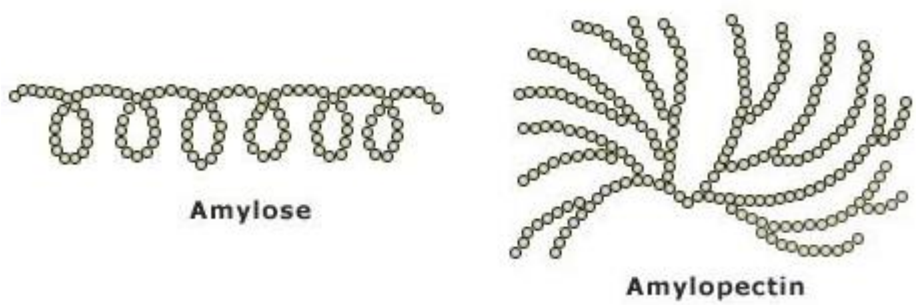


Figure 2.1. Structure of amylose and amylopectin (Starch, 2014)

Amylopectin is a highly branched polymer and contains mostly α-D-1,4- anhydroglucose linkages along with α-D-1,6-anhydroglucose linkages at the branch points. The molecular weight of amylopectin is 106-108 Da (Whistler and Daniel, 1984). Each branch contains about 20 to 30 anhydroglucose units and the degree of polymerization is about 2 million units. The large size and branched nature of the amylopectin polymer causes a reduction in its mobility and prevents the polymers from becoming oriented close enough to permit hydrogen bonding. As a result, aqueous solutions of amylopectin are clear and resistant to gelling upon ageing. Amylopectin supports the framework of the crystalline regions in the starch granule. It has been shown that branching points do not induce extensive defects in the double helical structure (Buléon et al., 1998). Properties of starch granules are majorly influenced by crystalline and non-crystalline structures (Zobel, 1988).

Native starches are unsuitable for most applications and are not widely utilized in food industry due to their poor functional properties such as, poor shear and thermal stability and high rates and extend of retrogradation (Hoover, 2000). Therefore, most starches are modified physically or chemically to enhance their positive characteristics and to minimize their limitations. Starch derivatives are used in food products as thickeners, gelling agents and encapsulating agents, in papermaking as wet-end additives for dry strength, surface sizes and coating binder (Hoover, 2000). The properties required for a particular application play an important role in selecting the type of modification. Therefore, it is necessary to understand how proso millet starch respond to these modifications (Dolmatova et al., 1998). Two modifications used in this study, hydrothermal and acid modifications are the most common type of physical and chemical modifications of starch, respectively.

2.2.1. Hydrothermal treatment (HMT)

Hydrothermal treatment involves thermal application in the presence of a limited amount of water (typically less than 35% w/w) and a process time typically between 15 min to 16 h (Jacobs and Delcour, 1998). This treatment alters morphological and physicochemical properties of starch granules including important changes in crystalline structure, swelling capacity, gelatinization, pasting properties and retrogradation (Hoover, 2010). Structural and physicochemical changes generated by HMT are directly influenced by the botanical source of the starch granule with respect to its composition and organization of amylose and amylopectin. HMT is also used as a pre-treatment because of the structural modification to amorphous and crystalline regions of the granules. These alterations make the granule susceptible to chemical and enzymatic modifications and acid hydrolysis (da Rosa Zavareze and Dias, 2011).

2.2.2. Acid modification (AM)

Acid modification is the oldest chemical modification technique. Products of acid modification have several applications and uses in the food, paper, textile and pharmaceutical industries (Hoover, 2000). Acid modification involves the application of acidic solutions (commonly HCl and H_2SO_4) to form a concentrated paste (35–40% of solids) at a temperature below glass transition for a specific duration depending on the desired viscosity or conversion degree (Thirathumthavorn and Charoenrein, 2005). The mechanism of acid modification is also known as acid hydrolysis. Hydrolysis is produced randomly, breaking the α-1,4 and α-1,6 links and shortening the polymeric chains. Acid hydrolysis of starch develops in two stages: an early stage in which hydrolysis preferentially attacks the amorphous regions of granules at a high reaction rate and a subsequent stage in which hydrolysis occurs in the crystalline region at a slower rate (Wang, L. and Wang, Y.-J., 2001). The hydrolysis rate and starch modification are in proportion to the amylose : amylopectin ratio, as well as to the size and conformation of granules (Hoover, 2000).

Balasubramanian et al. (2014) studied hydrothermal, acidic and enzymatic modifications of pearl millet starch. They found that hydrothermal modification caused an increase in swelling power and solubility. They also reported a significant reduction ($p<0.05$) in sediment value and water binding capacity for acid modified starch (AMS) and enzyme modified starch (EMS). However, an improved freeze-thaw stability and paste clarity was observed for AMS and EMS. Another study was performed on characterization of starches of proso, foxtail, barnyard, kodo, and little millets (Kumari and Thayumanavan, 1998). They used scanning electron microscopy and determined that proso millet contained small spherical and large polygonal granules while few large granules were present. Peak viscosity of proso millet was high when compared to other small millets but a low gelatinization temperature was noticed.

A similar study was conducted on white sorghum starch (Olayinka et al., 2008) and high solubility and swelling power of the starches was observed under alkaline conditions. An increase in gel formation and gel strength was also observed indicating potential use of modified starch for thickened sauces. Water absorption capacity, oil absorption capacity and alkaline water retention of the starches were improved after heat-moisture treatment. Effect of heat-moisture treatment and acid treatment on physico-chemical, pasting, thermal and morphological properties of horse chestnut starch was studied (Rafiq et al., 2016). Both heat-moisture and acid treatments reduced the swelling capacity of the native starch. Heat treatment caused an increase in amylose content, water absorption capacity and pasting temperature, suitable for food products like soup, noodles, dumpling and bread, while acid treatment promoted breakage of starch chains in amorphous regions, resulting in reduced peak viscosity, breakdown and final viscosity and thus desirable for formation of biodegradable films due to low viscosity and increased crystallinity.

2.3. Rheology and baking properties of gluten free breads

2.3.1. Role of hydrocolloids in gluten free baking

Gluten is the major component of wheat based bread which helps in ability to form thin gas-retaining films that trap gases, allowing dough to expand to become a softer, lighter and palatable food after baking (Cauvain and Young, 2007). Due to increasing gluten intolerance, development of healthier and better quality gluten-free products that would greatly improve the quality of life of celiac patients and those who develop sickness from wheat consumption is needed. A major challenge in producing bread without gluten is its inability to form viscoelastic dough and the resulting bread contains numerous quality defects including reduced volume, lack of cell structure, a dry, crumbly, grainy texture, a cracked crust, poor mouthfeel and flavor, and quick staling (Capriles and Arêas, 2014). Several additives, such as hydrocolloids, proteins, enzymes, antioxidants, emulsifiers and preservatives are used to improve dough properties, enhance quality and texture of breads (Capriles and Arêas, 2014).

Millet flour is often used to produce flat breads, porridges, beer and soup in countries of Africa, the Indian subcontinent and China (Saleh et al., 2013). Lorenz and Dilsaver (1980) used whole millet flour which produced low volume and dense texture breads but breads with blends of millet and wheat flour produced better results. Badi and Hoseney (1976) and Crabtree and Dendy (1979) made breads of optimum quality with 10% millet flour by adding 0.5% calcium stearoyl-2-lactylate to dough, and improved the baked bread quality significantly. Bread quality produced from composite flour of wheat and proso millet (50:50) is remarkably improved by the combined addition of emulsifiers and enzymes (xylanase and transglutaminase) at elevated dough moisture (Schoenlechner et al., 2013).

Millet use in producing gluten free bread (GFB) can be enhanced with the addition of hydrocolloids in bread making formulations. Hydrocolloids interact with water and produce a gel network structure that leads to increase in batter viscosity and increase in gas retention capability during proofing and baking, and improve texture, volume and structure of GFB (Anton and Artfield, 2008). Hydrocolloids showed promising results with other gluten free flours to produce high quality and consumer acceptable bread (Capriles and Arêas, 2014). Xanthan and hydroxypropyl methylcellulose (HPMC) are the most commonly used gums in GFB due to their favorable effects on the characteristics of the final product (Capriles and Arêas, 2014). Sabanis and Tzia (2011) evaluated the effect of HPMC and xanthan gum to gluten free formulations and results showed that gums helped in producing increased loaf volume and softer crumb. Demirkesen et al. (2010a) evaluated the effects of a combination of different hydrocolloids and emulsifiers on the quality of a rice-based GFB formula. Results showed that 0.5% DATEM (Diacetyl Tartaric Acid Esters of Monoglycerides) combined with 0.5% xanthan–guar blend provided the best final product, with good volume and crumb texture. Chestnut flour was tested as a raw material in GFB by Demirkesen et al. (2010b). They observed that breads containing 30% chestnut flour and 70% rice flour, in addition to a blend of xanthan–guar gum and DATEM emulsifier, had the best quality parameters (hardness, specific volume, color, and sensory values). Ahlborn et al. (2005) determined the blend of xanthan and HPMC which helped in improving moistness and overall freshness of a rice bread over that of the control rice bread and wheat bread. Imaging with SEM showed that the dough made with rice flour, cassava starch, HPMC, Xanthan, egg and milk protein created a bicontinuous matrix. Xanthan gum was investigated in this study because it forms high-viscosity pseudoplastic material and is very common in commercial GF loaves. Furthermore, xanthan mannose and glucuronic side chains are hydrophilic and are used to increase the water binding ability of GFB and increased moistness of the loaf. However, xanthan is never used alone but in combination with alternative proteins, hydrocolloids, or even supplemented with amino acids.

Recently many researchers investigated the effect of gluten-free doughs with the addition of various hydrocolloids, such as pectin, agarose, CMC and xanthan gum (Lazaridou et al., 2007; Sivaramakrishnan et al., 2004). They reported that water absorption of formulations containing hydrocolloids at 2% level (rice flour basis) varied between, 63.4% - 67%. Also, the dough development time in farinograph parameter increased with the addition of hydrocolloids from 4 minutes for the control to a range of 7.5-26.5 minutes, with the exception of xanthan, which decreased the dough development time to 2 minutes. The dough elasticity and cohesiveness when 500BU (Brabender Unit) of consistency is reached, was differently affected by each hydrocolloid with xanthan gum resulting to the highest elasticity values (100BU).

Gallagher (2009) studied that fundamental rheometry conducted on gluten-free doughs revealed an improvement in the viscoelastic properties of gluten-free doughs after supplementing the formulations with hydrocolloids. The addition of various hydrocolloids at 1 and 2% levels (rice flour basis) resulted in *rise* of elastic modulus, G' as well as an increase in the resistance to deformation. Xanthan gum, β-glucan and pectin addition resulted in firmer doughs (higher G' values) with increasing hydrocolloid concentration. Lazaridou et al. (2007) found that the elasticity and resistance to deformation of doughs followed in order of xanthan >CMC>pectin>agarose> oat β-glucan. The elasticity of the gluten-free doughs depended on water and hydrocolloid and increased by 65-75%, 45-50%, 35-40%, 25% and 8-15% when xanthan, pectin, agarose and oat β-glucan, respectively, were added.

2.3.2. Role of Starch in Bread-making

Starch plays a significant role in dough development and bread formation. Wheat flour used for bread-making contains about 80% starch and 12% protein (Petrofsky and Hoseney, 1995). Gluten formation and its viscoelastic behavior peaks the interest of researchers but the role of starch is not deeply investigated. However, starch also contributes abundantly in dough formation, playing different roles such as dilution of wheat gluten, provides surface for bonding with gluten, acts as substrate for amylase to produce fermentable sugars, flexibility for loaf expansion during partial gelatinization during baking, gives structural and textural properties to the final baked product, holds and retains water by acting as a water sink, and contributes to staling upon storage. Quality and shelf life of bread are restricted by staling which is a physico-chemical deterioration that leads to hard and crumbly texture bread upon storage (Eliasson, 2003). Bread staling starts immediately the product is taken out of the oven and begins to cool.

Many factors contribute to staling such as product formulation, baking process and storage conditions (BeMiller, 2007). It also reduces the shelf life of bread and major cause is the starch retrogradation which is gradual transition of amorphous starch (amylopectin) to a partially crystalline, retrograded state (Eliasson, 2003). Moreover, the bread firmness is affected by loss of moisture or redistribution which leads to bread hardening.

Bread-making is a complex process in which physical, chemical and biochemical changes takes place and results in formation of final product. Researchers have experimented with reconstituted flours in order to understand the starch behavior and gelatinization of starch granules is essential to the formation of a porous, elastic crumb. Kusunose et al. (1999) studied the role of starch granules in the expansion of doughs during baking, using artificial flours made from dry vital wheat gluten and wheat starch, potato starch, or tapioca starch. The authors concluded that the starch in bread doughs should gelatinize and set the dough after complete expansion. Tapioca starch gave the largest loaf volume whereas potato starch flour gave the smallest volume and the least shrinkage. Wheat starch, with its higher gelatinization temperature, allowed a longer time for the loaf to expand producing a larger loaf volume. Wheat starch granules gelatinized individually in the gluten matrix, which caused cracks in the cell membranes and prevented shrinkage upon cooling. Therefore, the gelatinization temperature of the different starches showed direct correlation to the expansion of the dough in the oven, the loaf volume, and the prevention of shrinkage of the loaves after baking.

Dennett and Sterling (1979) studied starch from different sources such as wheat, potato, tapioca, rice, maize, waxy maize, and high-amylose maize and assessed their effects on the properties of a bread-making. The authors concluded that as the amylose content decreases, the loaf volume increases, and the crumb gets softer and more hydrated. Amylose content showed positive effect on starch-gluten affinity and characteristics that affect the interaction between the starch and gluten components. Since as starches gelatinize during bread baking they hydrate from the neighboring gluten possibly causing gluten denaturation. The general reduction in bonding during baking might be essential to the formation of a softer more flexible crumb. Lastly, the reduced affinity also may produce weakening in the walls of gas cells, where rupture can occur and gases can enter during cooling, causing a collapse in the loaves.

Likewise, Goesaert et al. (2008) studied the role of the starch during bread production and storage. Authors used modified wheat starches in gluten−starch flour models to study the role of starch in bread making. Incorporation of hydroxyl-propylated starch in the formulation reduced loaf volume and initial crumb firmness and increased crumb gas cell size. The water uptake by the gelatinizing starch granule resulted in a loss of flexibility of the gelatinizing starch granules and of the gluten protein leading to destabilization of the gas cell walls, gas cell coalescence and ultimately their rupture as described by the previous studies mentioned. Starch swelling is already restricted due to limited water availability during baking, so cross-linking (additional restriction) had no-effect. Crumb firmness during storage depends upon amylopectin retrogradation and moisture loss after baking and during storage. These studies showed that starch plays a major role during bread production and affects loaf volume, expansion and firmness.

2.3.3. Effect of rheological properties on baking

Rheology, the science that studies the flow and deformation of matter when force is applied, can be used to analyze complex systems such as bread (Dobraszczyk and Morgenstern, 2003). Rheology tests allow opportunity to evaluate the performance of dough under various baking processes. It helps to determine the efficacy of processing aids and sufficient amount of water to make the best quality bread. The rheology of bread dough changes significantly between the mixing and the final product. Bread dough from wheat exhibits viscoelastic behavior, which is a combination of properties of both purely viscous fluids and purely elastic solids (Petrofsky and Hoseney, 1995). Rheology can be related to product functionality: many rheological tests have been used to determine hydration ratio, to predict final product quality such as mixing behavior, sheeting and baking performance (Dobraszczyk and Morgenstern, 2003). Several authors have studied the rheological properties such as elastic (Storage) modulus (G'), viscous (Loss) modulus (G''), and tan δ (G'/G'') of good and poor breadmaking flours, gluten, and sub-fractions of gluten. The rheological response of any material is expressed physically by stresses, strain and strain rate (Petrofsky and Hoseney, 1995). Particularly, dynamic oscillatory testing measures the elastic and viscous component of a material to assess the frequency-dependent properties of material that may provide important parameters of the behavior of food processing at a large scale. It is important to note that the validity of the calculated rheological parameters requires the samples to be linearly viscoelastic, so that the small deformation testing is not detrimental to the dough structure (Weipert, 1990).

Buresová et al. (2014) determined viscoelastic properties of gluten-free dough prepared from amaranth, chickpea, millet, corn, quinoa, buckwheat and rice flours using dynamic oscillation rheometry. Authors determined the relationship between storage modulus G´, loss modulus G´´ and phase angle tan(δ) with bread-making quality. In the conclusion, dynamic oscillation rheometry was found to be useful in differentiating the bread-making quality of gluten-free flour. Bread-making quality of gluten-free flour is best characterized by curve slope of storage modulus G´and phase angle tan(δ) while bread with larger volume was prepared from dough with nonlinear slope of storage modulus G´ and phase angle in the range of lower frequencies 0.01–0.10 Hz.

Zheng et al. (2000) examined deformations in dough during mixing and development. In order to monitor development of dough, both viscous and elastic behavior had to be monitored. Authors determined rheological changes, occurring during mixing, shear and extensional properties of dough prepared with two flours of different strength and various levels of mixing energy. Small deformation and large deformation, extensional flow and extrusion test were used to determine rheology of dough. In conclusion, results from small deformation shear tests exhibited large variability, particularly when non-mixed and underdeveloped doughs were tested. This variability was associated with poor water distribution in the sample due to insufficient mixing. Results of large deformation tests, including shear, planar extensional flow and the extrusion test, were less variable and showed that mixing and type of flour affect the rheological properties of dough.

Petrofsky and Hoseney (1995) studied the influence of dough rheological properties on starch-gluten interactions. The authors found that starches isolated from different wheat cultivars and mixed into dough with constant gluten concentration gave largely different rheological properties. Greater starch-gluten interactions in soft wheat and non-wheat starches produced higher moduli when compared to the hard wheat starch and commercial doughs. The source of gluten also had significant effect on dough rheology. Hard wheat gluten doughs had low G' and G'' values, indicating a greater extensibility and possibly less starch-gluten interaction. Soft wheat gluten doughs had higher G' and G'' values, possibly because of increased starch-gluten interactions.

Demirkesen et al. (2010a) studied the effect of rheological properties of gums and emulsifiers on rice bread dough. Different gums (xanthan gum, guar gum, locust bean gum (LBG), hydroxyl propyl methyl cellulose (HPMC), pectin, xanthan–guar, and xanthan–LBG blend) and emulsifiers (PurawaveTM and DATEM) were used. Shear thinning effect was observed in all formulations and elastic and loss module were obtained for rice dough samples containing xanthan gum, xanthan–guar and xanthan–LBG blend with DATEM. The viscoelastic properties of rice dough were found to be related to bread firmness. Both flow and oscillation measurements indicated that DATEM had more pronounced effect on rheological properties of dough.

3. Physical and functional properties of nine proso millet cultivars

3.1. Introduction

Millets are a group of small seeded cereal crops including many different species of Poaceae family. Major species in order of world production are pearl millet (*Pennisetum glaucum*), foxtail millet (*Setaria italic*), proso millet (*Panicum miliaceum*) and finger millet (*Eleusine coracana*) (Ojediran et al., 2010). Millet crops have unique ability to grow in regions with relatively low rainfall and can tolerate high temperatures and survive under drought conditions. Millets are widely grown in Africa and Asia and is the one of the major source of calories in developing countries with harsh natural environments (Saleh et al., 2013). In United States, proso millet is the major type of millet grown in a couple of states like Colorado, Nebraska, South Dakota and some limited production in Kansas, Kentucky, Minnesota and Wyoming (Baltensperger, 2002) with total production of 305,790 tons in 2014 (FAO, 2014). There is a growing interest in millet because of the technological potentials of its utilization in such industrial applications as gluten free foods, starch production and biofuels.

Proso millet is a warm season grass capable of producing seed 60 to 90 days after planting (Baltensperger, D et al., 1995). It grows best in full sun, moist to dry conditions, and can perform well in many soil types. Proso millet have higher protein contents compared to other varieties of millet and also nutritionally superior to major cereals like wheat, rice and corn (Saleh et al., 2013). A significant variation exists between proso millet cultivars' growth period, seed size, length of panicle, height of plant and straw strength necessitate evaluation of physical properties of the cultivars. *Panhandle* from Nebraska (1967), *Minco* from Minnesota (1976), are similar to the original common white millet but differ slightly in height, yielding ability, and maturity (Robinson, 1976). Nebraska released *Dawn* in 1976, a very short, very early variety, matures 7-10 days earlier than *Panhandle*, tight panicle, superior white grain but short in height (Nelson, 1976). A similar variety, *Rise* developed in 1984, is taller, better yielding, tight panicle and has smaller white seed. It is more stable under a wide range of production environments (Nelson, 1984). *Cope* was released by Colorado in 1978, which has medium size white seed and due to its maturity, *Cope* is likely best adapted to Colorado conditions and matures 5 days later than *Panhandle* (Hinze et al., 1978). Three cultivars *Huntsman*, *Earlybird* and Sun*rise* were released in 1994 and 1995, all having excellent lodging tolerance indicating stronger stems preventing bending or breakage during maturation. *Huntsman* is a large, white-seeded variety with excellent yield potential. It is late in maturity, has closed type panicle, good straw strength and was expected to replace *Cope* in most growing areas (Baltensperger, DD et al., 1995b). *Earlybird* is a large, white-seeded variety with excellent yield potential. It is early in maturity, and was expected to replace *Dawn* and *Rise* in most growing areas (Baltensperger, DD et al., 1995a). *Sunrise* is a large, white-seeded variety with excellent yield potential, intermediate in maturity, and has compact panicle (Baltensperger et al., 1997). *Plateau* is the latest cultivar released by Nebraska in 2014 a cross between *Huntsman* and a Chinese line that is high in waxy starches (Santra et al., 2015). *Plateau* demonstrated grain yields competitive with those currently grown cultivars and is the first waxy (amylose free) proso millet cultivar.

Therefore, evaluation and knowledge of physical and engineering properties of different proso millet cultivars is required for designing appropriate equipment for process operations like sorting, drying, heating, cooling, and milling (Sahay and Singh, 1996). Material quality indicators such as color, hardness, gelatinization, and pasting properties have significant importance in the food industry. The present study also investigates the effect of amylose/amylopectin on both pasting and gelatinization properties. This study will help provide new classification of proso millet cultivars based on their physical, thermal, pasting properties.

3.2. Materials and methods-

3.2.1. Raw materials

Nine proso millet cultivars namely *cope*, *earlybird*, *huntsman*, *dawn*, *rise*, sun*rise*, *plateau*, *panhandle* was obtained from Panhandle Research and Extension Center, University of Nebraska, Scottsbluff, USA. Cultivars were cleaned and sifted to remove foreign materials such as stones, straw and dirt. The cleaned grains were dehulled using modified disc mill (Glenn mills inc, Clifton, NJ). In the mill, the stationary disc was replaced with rubber disc to minimize the breaking and proper removal of hulls.

3.2.2. Proximate analysis

Samples were ground with a Quadrumat Junior mill (C.W. Brabender Instruments, Inc. South Hackensack, NJ) and AOAC standard methods were used to measure moisture, protein, crude fiber, crude fat, ash and carbohydrates(AOAC, 1990a, b, c). Amylose content was measured using official AACCI method 61-03.01 (AACCI, 1997).

3.2.3. Grain's physical properties

Millet grains were randomly selected and 100 grains of each cultivar were scanned using X-ray microCT-scanner (SkyScan 1173 x-ray microCT scanner, Bruker, Kontick, Belgium) and images were reconstructed using NRecon software (Bruker micro-CT, Belgium). CTAn software (Bruker micro-CT, Belgium) was used to measure the dimensions of the grains along three axis Length (L), Breadth (B), Thickness (T).

The equivalent diameter (De) considering spherical shape for a proso millet grain was determined using (Mohsenin, 1986).

$$D_e = (L * B * T)^{\frac{1}{3}}$$

The sphericity (Φ) and volume (V) were determined using following expressions (Mohsenin, 1986).

$$\Phi = \frac{(L * B * T)^{\frac{1}{3}}}{L}$$

$$V = \frac{\pi}{6}(L * B * T)$$

Surface area (S) was calculated using expression described by (Singh et al., 2010).

$$S = \pi * (D_e)^2$$

3.2.4. Bulk Density and True density

The bulk density (ρ_b) was determined by measuring weight of packed grains in a container of known volume.

$$\rho_b = \frac{measured\ weight}{volume\ of\ container}$$

The solid density (ρ_t) was determined using gas pycnometer (Model 1340 multivolume, Micromeritics Instrument Corporation, Norcross, GA, USA).

3.2.5. Grain Porosity

Grain porosity (ε) is defined as the ratio of intergranular void space volume and the volume of the bulk grain. Porosity was determined using an expression as described (Jain and Bal, 1997).

$$\epsilon = 1 - \frac{\rho_b}{\rho_t}$$

3.2.6. Thousand kernel weight

The thousand kernel weight was determined by randomly selecting one thousand grains from each cultivar and weighed in 10 replicates.

3.2.7. Angle of repose

The angle of repose (Θ) was determined by placing a hollow cylinder, filled with grains on a steel plate. The cylinder was raised gradually until it formed a cone and height (H) and diameter (D) was measured and Θ was calculated using following expression.

$$\Theta = \tan^{-1}\left(\frac{2H}{D}\right)$$

3.2.8. Hardness

Hardness was measured using a texture analyzer (TA-XT plus, Stable Micro Systems, UK). Force was measured in compression using following setting, return to start at 90% strain, with pre-test speed of 0.5 mm/s, test speed of 0.5 mm/s, post-test speed of 10.0 mm/s.

3.2.9. Color Characteristics

The color of millet cultivars was determined using digital colorimeter (Model CR400, Konica Minolta, Chiyoda, Tokyo, Japan). The color was determined on ***L***, ***a*** and ***b*** scale where L indicates the degree of lightness or darkness (black to white), ***a*** indicates degree of redness (+a) to greenness (-a) and b indicates the degree of yellowness (+b) to blueness (-b).

3.2.10. Gelatinization

Gelatinization properties were determined using differential scanning calorimetry (DSC – Q20 TA instruments, New Castle, Delaware, USA). Flour sample (10 mg, dry basis) was weighed into high volume stainless steel pans, followed by addition of 20 µl of distilled water. The pan was hermetically sealed and equilibrated at 4°C for 24 hrs. Samples were kept at room temperature for one hour prior to scanning from 10 to 150°C at 10°C/min (Krueger et al., 1987).

3.2.11. Pasting properties

Pasting characteristics were determined using Discovery Hybrid Rheometer (DHR-2, TA instruments, New Castle, Delaware, USA) with starch pasting cell. A mixture of 3.5 g starch (14% moisture) in 25 ml of distilled water was stirred at 160 rpm. Sample was held at 50°C for 1 min and then heated to 95°C at 4°C/min and held at 95°C for 5min. Subsequently, samples were cooled to 50°C at 4°C/min and held at 50°C for 5 min. A plot of viscosity (Pa.s) vs. time (s) was used to determine pasting temperature, peak and final viscosity, holding strength, setback and breakdown.

3.2.12. Statistical analysis

The data were analyzed statistically using SAS software and when there was significant effect of the model on variations observed the means were separated using the Duncan's multiple range test ($p \leq 0.05$). All the data are presented as the mean with the standard deviation. Correlation test was determined using Pearson correlation test.

3.3. Results

3.3.1. Physical properties

The mean values of proximate content and physical properties of nine cultivars are presented in Tables 3.1 and 3.2, respectively. Moisture content of cultivars varied from 9.40 to 10.71%. Among cultivars, protein content varied from 11.10 (*Rise*) to 13.72% (*Dawn*), whereas fiber and ash were less than 1% in all cultivars. *Cope* showed the lowest fat content of 1.80% whereas all other cultivars did not have significant ($P<0.05$) difference among their fat content and remained in the range of 2.91 to 3.45%. Carbohydrate content varied from 72.45 (*Panhandle*) to 74.34% (*Cope*). Cultivars had significantly ($P<0.05$) different amount of amylose content. *Plateau* being waxy millet had 3.10% amylose whereas *Minco* had highest amylose content of 34.60% and *Cope* (18.15%) had low amylose among all other cultivars.

Table 3.1. Proximate content of proso millet cultivars

	Moisture (%)	Crude protein (%)	Crude Fat (%)	Crude Fiber (%)	Ash (%)	Carbohydrate (%)	Amylose (%)
Cope	10.29 ± 0.02^{b}	12.90 ± 0.06^{c}	1.80 ± 0.08^{b}	0.57 ± 0.06^{a}	0.68 ± 0.02^{d}	74.34 ± 0.12^{a}	18.15 ± 1.06 ^{f}
Dawn	9.40 ± 0.08^{d}	13.72 ± 0.01^{a}	3.18 ± 0.03^{a}	0.59 ± 0.30^{a}	0.77 ± 0.01^{b,c}	72.93 ± 0.06^{b,c}	25.10 ± 0.28 ^{c}
Earlybird	10.18 ± 0.06^{b}	12.38 ± 0.05^{d}	3.23 ± 0.13^{a}	0.94 ± 0.35^{a}	0.83 ± 0.08^{b,c}	73.38 ± 0.10^{b}	30.20 ± 0.57 ^{b}
Huntsman	10.18 ± 0.15^{b}	12.11 ± 0.08^{e}	3.30 ± 0.11^{a}	0.90 ± 0.04^{a}	0.84 ± 0.02^{b,c}	73.57 ± 0.20^{a,b}	21.40 ± 0.57 ^{e}
Minco	9.64 ±0.11^{c,d}	12.10 ± 0.01^{e}	3.12 ± 0.23^{a}	0.71 ± 0.06^{a}	0.82 ± 0.00^{b,c}	74.33 ± 0.25^{a}	34.60 ± 0.28 ^{a}
Panhandle	10.35 ± 0.03^{b}	12.85 ± 0.03^{c}	3.45 ± 0.04^{a}	0.84 ± 0.28^{a}	0.90 ± 0.04^{a,b}	72.45 ± 0.02^{c}	26.40 ± 0.57 ^{c}
Plateau	9.71 ± 0.13^{c}	13.36 ± 0.02^{b}	3.28 ± 0.13^{a}	0.80 ± 0.08^{a}	0.74 ± 0.00^{c,d}	72.88 ± 0.11^{b,c}	3.10 ± 0.28 ^{g}
Rise	10.71 ± 0.01^{a}	11.10 ± 0.01^{f}	2.91 ± 0.76^{a}	0.93 ± 0.11^{a}	0.99 ± 0.02^{a}	74.27 ± 0.79^{a}	25.75 ± 0.07^{c,d}
Sunrise	9.45 ±0.09^{c,d}	12.86 ± 0.01^{c}	3.25 ± 0.07^{a}	0.81 ± 0.01^{a}	0.98 ± 0.01^{a}	73.45 ± 0.05^{b}	24.40 ± 1.41 ^{d}

The values are means ± standard deviations of two replicates. Means with different letter in a column differ significantly ($P<0.05$). %Carbohydrate = 100 - %(Moisture + protein + fat + ash)

Variation in length among the cultivars was observed from 2.27 (*Huntsman*) to 2.37 mm (*Minco*), whereas variation in width and thickness was from 2.08 (*Cope*) to 2.29 (*Panhandle*) and 1.59 (*Cope*) to 1.84 mm (*Earlybird*). These dimensions are important in equipment design for grain handling such as sieves, sorters, hullers and mills. Size and shape of perforations in these equipment are determined using dimensions of the seeds (Mohsenin, 1986). Different cultivars of pearl millet had their length (mm) in range from 3.16 to 3.87, width (mm) 2.30 to 2.93 and thickness (mm) 1.54 to 2.05 at 10% moisture content (Ojediran et al., 2010).

The D_e and Φ for cultivars differs significantly (P<0.05). The mean D_e and Φ varied from 1.96 (*Cope*) to 2.14 mm (*Earlybird*) and 0.86 (*Cope*) to 0.91 (*Earlybird*). Determination of D_e is important in estimating projected area and conveying pattern in pneumatic equipment, also helps in determining terminal velocity and drag coefficient. Higher sphericity of all cultivars indicate that proso millet grains have high rolling tendencies which is very important in designing hoppers and other processing equipment (Ghadge and Prasad, 2012). Jain and Bal (1997) studied pearl millet and reported it be more conico-spherical shape whereas proso millet is more round-shaped. Ojediran et al. (2010) also reported pearl millet have lower sphericity (70 – 72%) compared to proso millet (86-91%) and lower angle of repose, porosity, and solid density.

Among the cultivars, the volume (V) and surface area (S) varied significantly (P<0.05) from 3.97 (*Cope*) to 5.14 mm^3 (*Earlybird*) and 12.12 (*Cope*) to 14.39 mm^2 (*Earlybird*). Determination of surface area and volume play important role in calculating processing times and energy requirements for processes such as drying rates (Alonge and Adigun, 1999).

Thousand kernel weight (TKW) was found to be in the range of 4.97 (*Cope*) to 6.19 grams (*Sunrise*) and significantly ($P<0.05$) different among cultivars. It has high significance in determining seeding rates during planting (Miller and McLelland, 2001). The bulk density and solid density varied significantly ($P<0.05$) from 765.49 (*Plateau*) to 809.67 kg/m^3 (*Minco*) and 1371.86 (*Plateau*) to 1417.36 kg/m^3 (*Minco*), respectively. The values of porosity were found to be ranged from 42.87 (*Minco*) to 44.59% (*Dawn*). TKW, bulk, solid and porosity helps in determining transportation conditions, design of hoppers, cleaning and storage equipment. High solid density than water indicates wet cleaning can be used for cleaning grains, as grains will not float. Bulk density and porosity helps in designing storage bins as these properties help in determining space required to store specified amount of grains and void area present between grains. Swami and Swami (2010) determined physical properties of finger millet and reported true density to be around 1120 kg/m^3; bulk density 709 kg/m^3 and sphericity 96%. Pearl millet is reported to have higher porosity than proso millet indicating that pearl millet required larger space per unit mass than proso millet to store equal volume of grains (Jain and Bal, 1997).

Angle of repose varied among cultivars from 21.95° (*Huntsman*) to 26.68° (*Dawn*). It is the measure of internal friction between the grains, high cohesive forces between grains lead to higher angle of repose. It also provides maximum slope at which grains are stable. It is very important in designing the hoppers and silos for proper flow of grains. Hardness of a grains determines the milling yields and energy requirements for processing. Proso millet cultivar's hardness varied from 3.23 (*Huntsman*) to 4.05 kg (*Plateau*). Hardness or cracking force and strength of grains helps determining the seed resistance to cracking during harvesting and hulling. Balasubramanian and Viswanathan (2010) studied effect of moisture on physical properties of minor millets available in India and reported (at 10% moisture content) proso millet's bulk density to be 899.65 kg/m^3; true density 1838.5 kg/m^3; porosity 52.88 %, which are higher than values found in this study. However, angle of repose is in accordance with the current study.

Table 3.2. Physical properties of proso millet cultivars

Cultivar	#	*Cope*	*Dawn*	*Earlybird*	*Huntsman*	*Minco*	*Panhandle*	*Plateau*	*Rise*	*Sunrise*
L (mm)	100	$2.29 \pm 0.14^{c,d}$	$2.36 \pm 0.08^{a,b}$	2.34 ± 0.06^{b}	2.27 ± 0.07^{d}	2.37 ± 0.09^{a}	$2.36 \pm 0.08^{a,b}$	2.27 ± 0.13^{d}	2.30 ± 0.06^{c}	2.35 ± 0.07^{b}
W (mm)	100	2.08 ± 0.08^{d}	2.28 ± 0.08^{a}	2.28 ± 0.07^{a}	2.19 ± 0.10^{c}	2.27 ± 0.09^{a}	2.29 ± 0.08^{a}	2.18 ± 0.07^{c}	2.24 ± 0.07^{b}	2.28 ± 0.07^{a}
TH (mm)	100	1.59 ± 0.086^{f}	1.76 ± 0.09^{b}	1.84 ± 0.11^{a}	$1.73 \pm 0.07^{c,d}$	1.76 ± 0.08^{b}	1.76 ± 0.10^{b}	1.66 ± 0.07^{e}	$1.75 \pm 0.08^{b,c}$	1.72 ± 0.08^{d}
Φ	100	0.86 ± 0.04^{f}	$0.90 \pm 0.02^{c,d}$	0.91 ± 0.02^{a}	$0.90 \pm 0.02^{b,c}$	$0.89 \pm 0.02^{d,e}$	$0.90 \pm 0.02^{b,c}$	0.89 ± 0.03^{e}	0.91 ± 0.02^{b}	$0.89 \pm 0.02^{d,e}$
V (mm^3)	100	3.97 ± 0.37^{g}	$4.94 \pm 0.47^{b,c}$	5.14 ± 0.42^{a}	4.51 ± 0.40^{e}	4.97 ± 0.42^{b}	4.99 ± 0.50^{b}	4.30 ± 0.37^{f}	4.73 ± 0.39^{d}	$4.85 \pm 0.42^{c,d}$
D_e (mm)	100	1.96 ± 0.06^{g}	$2.11 \pm 0.07^{b,c}$	2.14 ± 0.06^{a}	2.05 ± 0.06^{e}	$2.12 \pm 0.06^{b,c}$	2.12 ± 0.07^{b}	2.02 ± 0.06^{f}	2.08 ± 0.06^{d}	$2.10 \pm 0.06^{c,d}$
S (mm^2)	100	12.12 ± 0.76^{g}	$14.01 \pm 0.90^{b,c}$	14.39 ± 0.79^{a}	13.19 ± 0.79^{e}	14.08 ± 0.79^{b}	14.11 ± 0.94^{b}	12.79 ± 0.74^{f}	13.62 ± 0.75^{d}	$13.84 \pm 0.81^{c,d}$
Bulk density (kg/m^3)	5	782.56 ± 5.02^{d}	774.30 ± 2.86^{e}	$790.38 \pm 9.45^{c,d}$	798.65 ± 6.20^{b}	809.67 ± 6.82^{a}	$788.98 \pm 3.62^{c,d}$	765.49 ± 5.47^{f}	$795.53 \pm 5.10^{b,c}$	$788.65 \pm 3.52^{c,d}$
Solid density (kg/m^3)	5	1397.72 ± 1.22^{e}	1397.28 ± 1.95^{e}	$1411.88 \pm 0.97^{b,c}$	1413.20 ± 1.15^{b}	1417.36 ± 1.53^{a}	1409.94 ± 1.53^{c}	1371.86 ± 0.94^{f}	1410.46 ± 1.853^{c}	1402.68 ± 1.931^{d}
Porosity (%)	5	$44.01 \pm 0.34^{a,b,c}$	44.59 ± 0.19^{a}	$44.02 \pm 0.67^{a,b,c}$	43.49 ± 0.46^{c}	42.87 ± 0.53^{d}	$44.04 \pm 0.29^{a,b,c}$	$44.20 \pm 0.39^{a,b}$	43.60 ± 0.41^{c}	$43.78 \pm 0.22^{b,c}$
Angle of repose (°)	5	$22.99 \pm 0.73^{c,d}$	26.68 ± 0.91^{a}	$22.68 \pm 0.72^{c,d}$	21.95 ± 0.90^{d}	$23.10 \pm 0.74^{c,d}$	$23.70 \pm 0.98^{b,c}$	25.74 ± 0.83^{a}	$22.96 \pm 0.63^{c,d}$	24.53 ± 0.79^{b}
TKW (g)	10	4.97 ± 0.09^{e}	5.79 ± 0.07^{c}	6.19 ± 0.04^{a}	6.01 ± 0.05^{b}	5.78 ± 0.04^{c}	5.58 ± 0.06^{d}	4.69 ± 0.06^{f}	6.06 ± 0.08^{b}	6.19 ± 0.05^{a}
Hardness (kg)	20	3.63 ± 0.52^{b}	$3.24 \pm 0.51^{c,d}$	$3.40 \pm 0.47^{b,c,d}$	$3.23 \pm 0.51^{c,d}$	$3.46 \pm 0.45^{b,c}$	$3.33 \pm 0.45^{b,c,d}$	4.05 ± 0.52^{a}	3.13 ± 0.44^{d}	$3.38 \pm 0.51^{b,c,d}$

The values are means ± standard deviation. Means with different letter in a row differ significantly ($P<0.05$). L:Length, W: Width, TH: Thickness, Φ : Sphericity, V: Volume, D_e: Geometric mean, S: Surface area, TKW: Thousand kernel weight, #: no of samples

The color of proso millet cultivars determined on ***L, a*** and ***b*** scale is presented in Table 3.3. Color of the cultivars is the important factor for utilization and can be used to design color sorter. *Rise* cultivar (***L***=71.80) is the darkest whereas *Plateau* (***L***=77.13) is the lightest. The ***a*** value is highest for *Sunrise* (-2.67) and lowest for *Plateau* (-4.56). However, the value of ***b*** was observed to be highest for *Sunrise* (43.46) and lowest for *Panhandle* (35.29). The color difference can be attributed to the differences in pigments, composition and genetic breeding of the cultivars.

Table 3.3. Color characteristics of proso millet cultivars

Cultivar	L	a	b
Cope	76.43 ± 1.19[a]	-4.47 ± 0.59[d]	38.12 ± 1.31[d]
Dawn	72.07 ± 1.81[e]	-2.57 ± 0.81[a]	41.27 ± 1.99[b]
Earlybird	73.31 ± 1.51[d]	-3.32 ± 0.55[b]	39.49 ± 1.33[c]
Huntsman	74.51 ± 0.94[c]	-3.52 ± 0.48[b,c]	39.38 ± 1.40[c]
Minco	74.31 ± 0.94[c]	-3.72 ± 0.50[c]	41.72 ± 1.74[b]
Panhandle	75.63 ± 0.91[b]	-3.50 ± 0.39[b,c]	35.29 ± 1.37[e]
Plateau	77.13 ± 1.17[a]	-4.56 ± 0.49[d]	38.73 ± 1.31[c,d]
Rise	71.80 ± 3.62[e]	-3.41 ± 0.41[b]	41.90 ± 2.01[b]
Sunrise	72.59 ± 1.32[d,e]	-2.67 ± 0.43[a]	43.46 ± 1.42[a]

The values are means ± standard deviation of 30 replicates. Means with different letter in a column differ significantly (P<0.05).

3.3.2. Pasting properties

Pasting properties of proso millet cultivars are presented in Table 3.4, and the pasting profiles of cultivars are shown in Figure 3.1. The cultivars showed significant ($P<0.05$) difference in pasting profiles. Proso millet cultivars can be classified in three different categories, i.e., low amylose or waxy millet (*Plateau*), medium amylose (*Cope*) and high amylose (*Dawn*, *Earlybird*, *Huntsman*, *Minco*, *Panhandle*, *Rise*, *Sunrise*). Waxy millet (*Plateau*) showed the lowest peak (0.92 Pa.s) and final viscosity (0.71 Pa.s) and medium amylose, *Cope* had peak (1.05 Pa.s) and final viscosity (1.49 Pa.s) which is significantly ($P<0.05$) lower than high amylose cultivars. Lower peak viscosities observed in waxy cultivar can be explained by the fact that starch granule swelling is a property of amylopectin, causing waxy starches to swell rapidly indicating that waxy cultivar develops viscosity but cannot maintain the stability of paste viscosity because at reduced amylose content, heating disrupts the structure of gel (Tester and Morrison, 1990). Pasting temperature varied from 76.76°C (*Plateau*) to 88.87°C (*Rise*). High amylose cultivars showed higher pasting temperatures compared to waxy and medium amylose cultivars indicating higher resistance to swelling (Singh et al., 2004).

Plateau had the lowest setback value of 0.28 Pa.s, also medium amylose cultivar, *Cope* provides lower setback compared to high amylose cultivars. Setback value reflects degree of retrogradation of paste. Waxy millet retrogrades to lesser extent as compared to cultivars having high amylose content. Three Korean proso millet cultivars including waxy millet showed similar setback and peak viscosities (Kim et al., 2012).

Figure 3.2, 3.3 and 3.4 illustrates strong positive correlation of amylose content with peak viscosity (r=0.84), final viscosity (r=0.91) and setback (r=0.90), respectively. Pasting temperature and setback values of *Plateau* and *Cope* were lower than high amylose cultivars and are in accordance with results reported for starches from different botanical sources (Jane et al., 1999). Wu et al. (2014) reported similar results for millet varieties grown in China and reported positive correlation of peak viscosity, final viscosity and setback with amylose content.

Table 3.4. Pasting properties of cultivars

Cultivar	Pasting temp (°C)	Peak viscosity (Pa.s)	Holding strength (Pa.s)	Final viscosity (Pa.s)	Breakdown (Pa.s)	Setback (Pa.s)
Cope	77.52 ± 0.10^{f}	1.05 ± 0.02^{e}	0.52 ± 0.01^{d}	1.49 ± 0.03^{d}	0.53 ± 0.02^{d}	0.97 ± 0.03^{e}
Dawn	82.05 ± 0.81^{c}	1.62 ± 0.01^{c}	0.74 ± 0.01^{c}	2.84 ± 0.04^{b}	0.88 ± 0.01^{b}	2.10 ± 0.04^{b}
Earlybird	79.06 ± 0.39^{e}	1.91 ± 0.03^{a}	0.83 ± 0.01^{b}	3.15 ± 0.01^{a}	1.08 ± 0.04^{a}	2.32 ± 0.01^{a}
Huntsman	77.31 ± 0.77^{f}	1.80 ± 0.01^{b}	0.77 ± 0.01^{c}	2.24 ± 0.01^{c}	1.03 ± 0.03^{a}	1.47 ± 0.02^{d}
Minco	80.56 ± 0.75^{d}	1.89 ± 0.04^{a,b}	0.84 ± 0.03^{b}	2.88 ± 0.04^{b}	1.05 ± 0.07^{a}	2.04 ± 0.01^{b}
Panhandle	80.49 ± 0.51^{d}	1.53 ± 0.01^{d}	0.87 ± 0.01^{a,b}	2.81 ± 0.01^{b}	0.66 ± 0.01^{c}	1.94 ± 0.01^{c}
Plateau	76.76 ± 0.03^{f}	0.92 ± 0.01^{f}	0.43 ± 0.01^{e}	0.71 ± 0.01^{e}	0.49 ± 0.01^{d}	0.28 ± 0.01^{f}
Rise	88.87 ± 0.23^{a}	1.93 ± 0.05^{a}	0.90 ± 0.02^{a}	3.22 ± 0.01^{a}	1.03 ± 0.04^{a}	2.32 ± 0.02^{a}
Sunrise	87.31 ± 0.16^{b}	1.62 ± 0.08^{c}	0.73 ± 0.04^{c}	2.80 ± 0.13^{b}	0.89 ± 0.05^{b}	2.06 ± 0.09^{b}

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly (P<0.05).

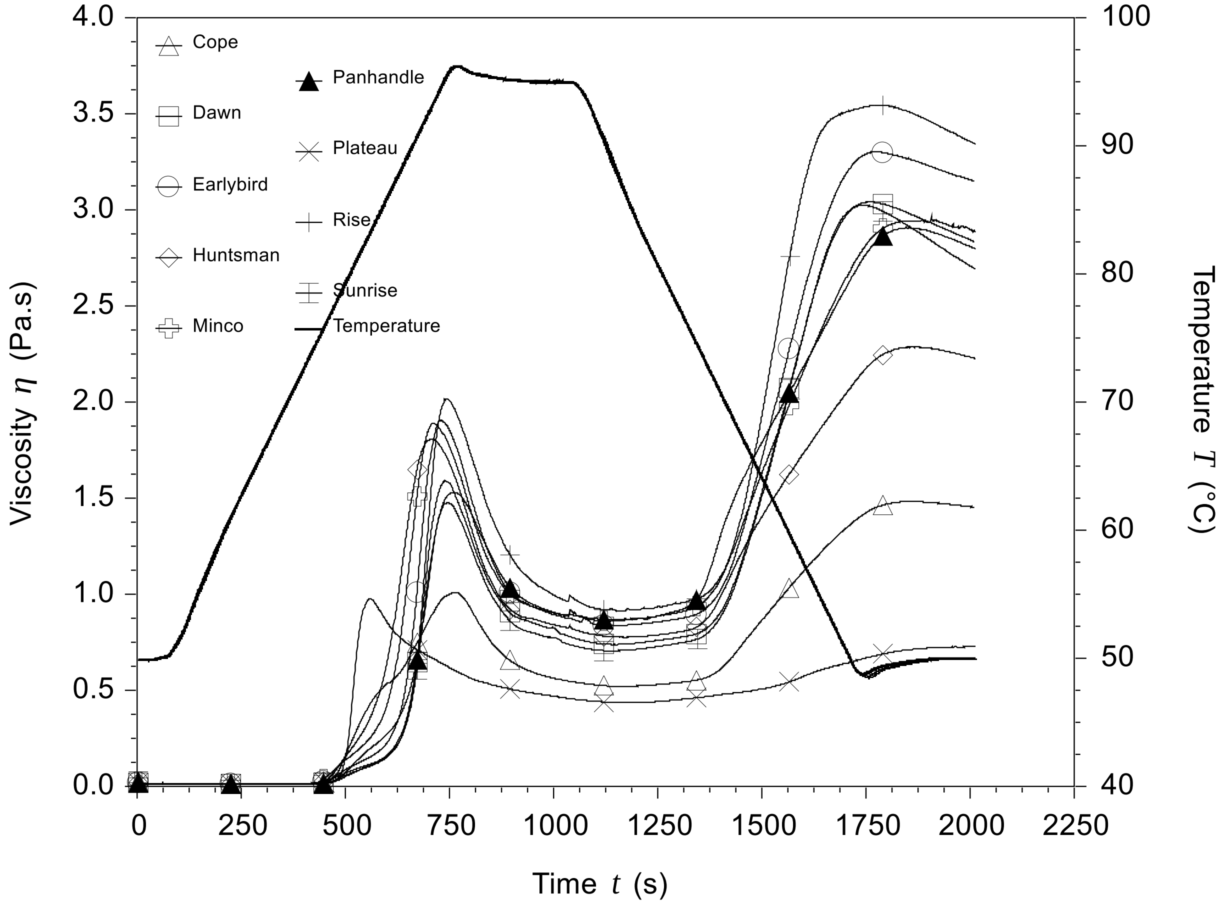


Figure 3.1. Pasting profile of different proso millet cultivars.

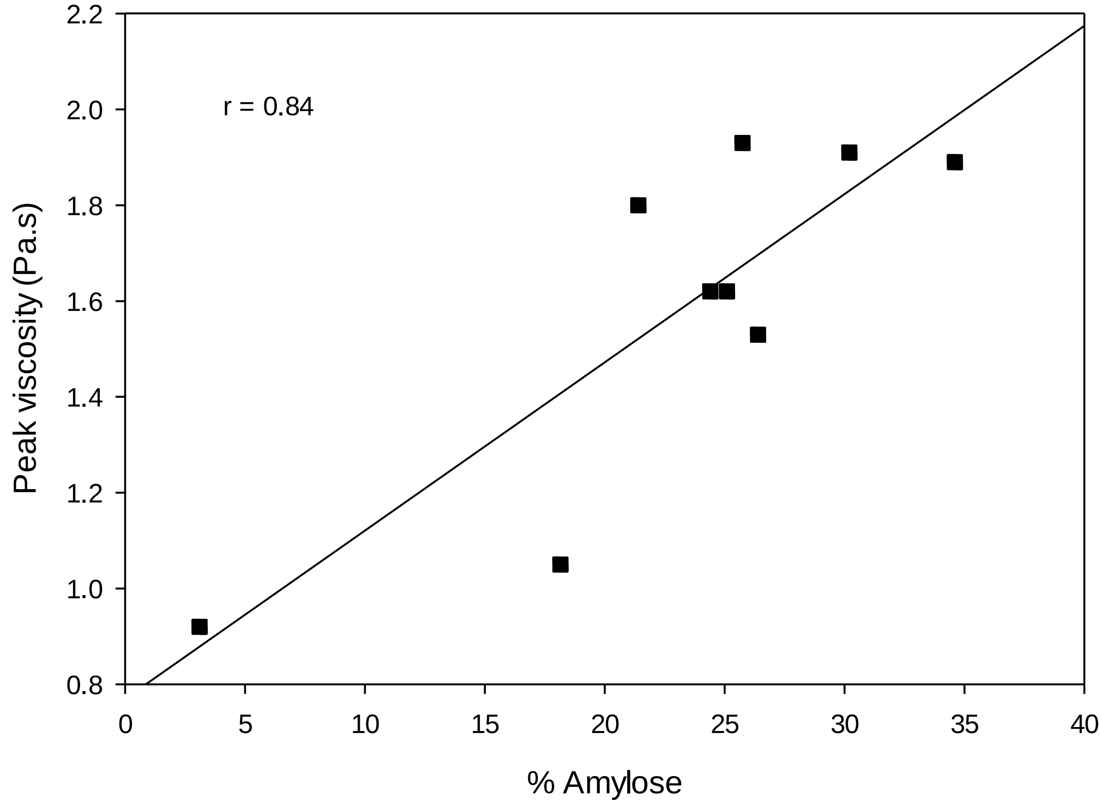


Figure 3.2. Relationship between % amylose and peak viscosity (Pa.s) for different proso millet cultivars.

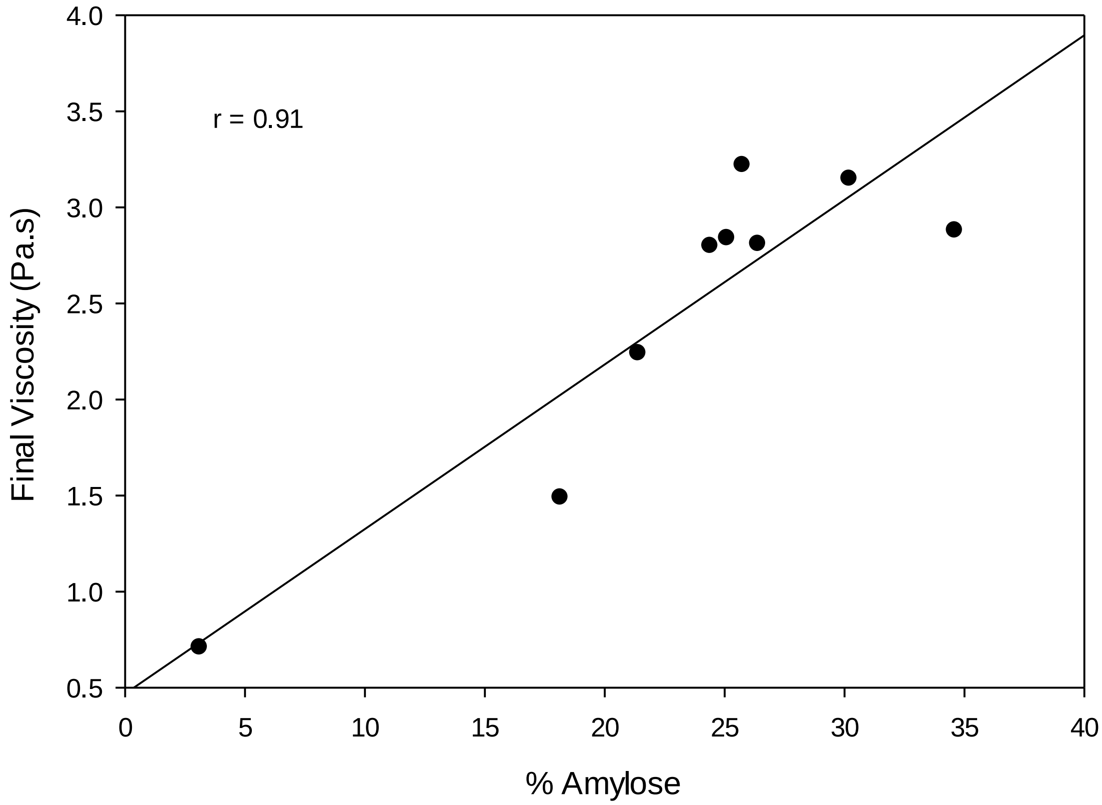


Figure 3.3. Relationship between % amylose and final viscosity (Pa.s) for different proso millet cultivars.

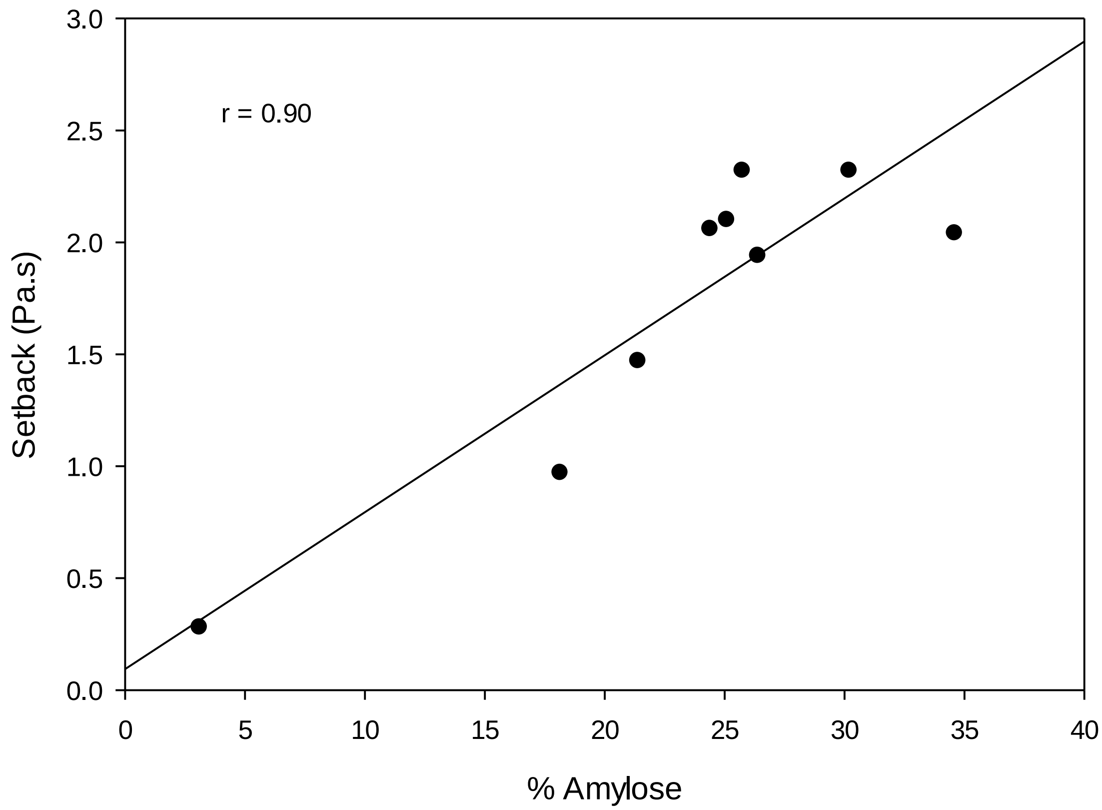


Figure 3.4. Relationship between % amylose and setback (Pa.s) for different proso millet cultivars.

3.3.3. Gelatinization

Gelatinization properties of proso millet cultivars are summarized in Table 3.5. Among cultivars, significant difference ($P<0.05$) in onset (T_O), peak temperature (T_P), conclusion temperature (T_C) and enthalpy (ΔH_G) was observed. T_O varied from 70.59°C (*Minco*) to 74.27°C (*Plateau*), T_P varied from 75.5 (*Minco*) to 79.29°C (*Plateau*) and ΔH_G ranged between 2.38 (*Sunrise*) to 3.45 J/g (*Plateau*).

Waxy millet, *Plateau* had higher T_O and T_P than other cultivars and showed strong negative correlation of T_O ($r = -0.94$) and T_P ($r = -0.94$) with amylose content as illustrated in Figure 3.5 and 3.6, respectively. Waxy barley showed similar results and higher T_P and ΔH_G was observed compared to other barley cultivars (Gudmundsson and Eliasson, 1992). Some authors reported negative correlation of T_P, T_C and ΔH_G with amylose content of wheat starches (Sasaki et al., 2000; Yasui et al., 1996). Amylopectin plays an important role in starch granule crystallinity, so with increase in amylose content, % crystallinity decreases and melting temperature of crystalline regions lowers resulting in lower energy requirements for gelatinization (Sasaki et al., 2000). The negative correlation of amylose content with onset and peak temperatures indicates that higher amylose implies more amorphous and less crystalline region. Wu et al. also reported higher T_P and ΔH_G for waxy millet compared to normal millet (Wu et al., 2014).

Table 3.5. Gelatinization properties of cultivars

Cultivar	Onset (°C)	Peak (°C)	Stop (°C)	Area (J/g)	Range (°C)
Cope	71.85 ± 0.01[c]	78.32 ± 0.17[b]	91.80 ± 0.49[a,b]	2.65 ± 0.39[b,c,d]	19.95 ± 0.51[a]
Dawn	71.62 ± 0.02[c,d]	77.22 ± 0.15[c]	91.78 ± 1.54[a,b]	2.51 ± 0.22[b,c,d]	19.17 ± 2.93[a]
Earlybird	71.32 ± 0.01[d]	76.49 ± 0.12[d]	88.95 ± 0.60[c]	2.41 ± 0.08[c,d]	17.63 ± 0.59[a]
Huntsman	72.57 ± 0.50[b]	77.84 ± 0.64[b,c]	91.92 ± 0.17[a,b]	2.43 ± 0.16[c,d]	19.36 ± 0.33[a]
Minco	70.59 ± 0.01[e]	75.66 ± 0.15[e]	89.38 ± 1.84[c]	2.91 ± 0.36[b]	17.79 ± 3.26[a]
Panhandle	71.90 ± 0.28[c]	77.20 ± 0.14[c]	92.13 ± 0.58[a,b]	2.88 ± 0.03[b,c]	20.23 ± 0.30[a,b]
Plateau	74.27 ± 0.09[a]	79.41 ± 0.01[a]	92.53 ± 1.58[a,b]	3.45 ± 0.09[a]	18.26 ± 1.67[a]
Rise	71.59 ± 0.01[c,d]	76.54 ± 0.15[d]	90.19 ± 0.18[b,c]	2.52 ± 0.08[b,c,d]	18.60 ± 0.19[a]
Sunrise	72.38 ± 0.21[b]	78.14 ± 0.42[b]	92.64 ± 0.67[a]	2.38 ± 0.18[d]	20.26 ± 0.88[a]

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($P<0.05$).

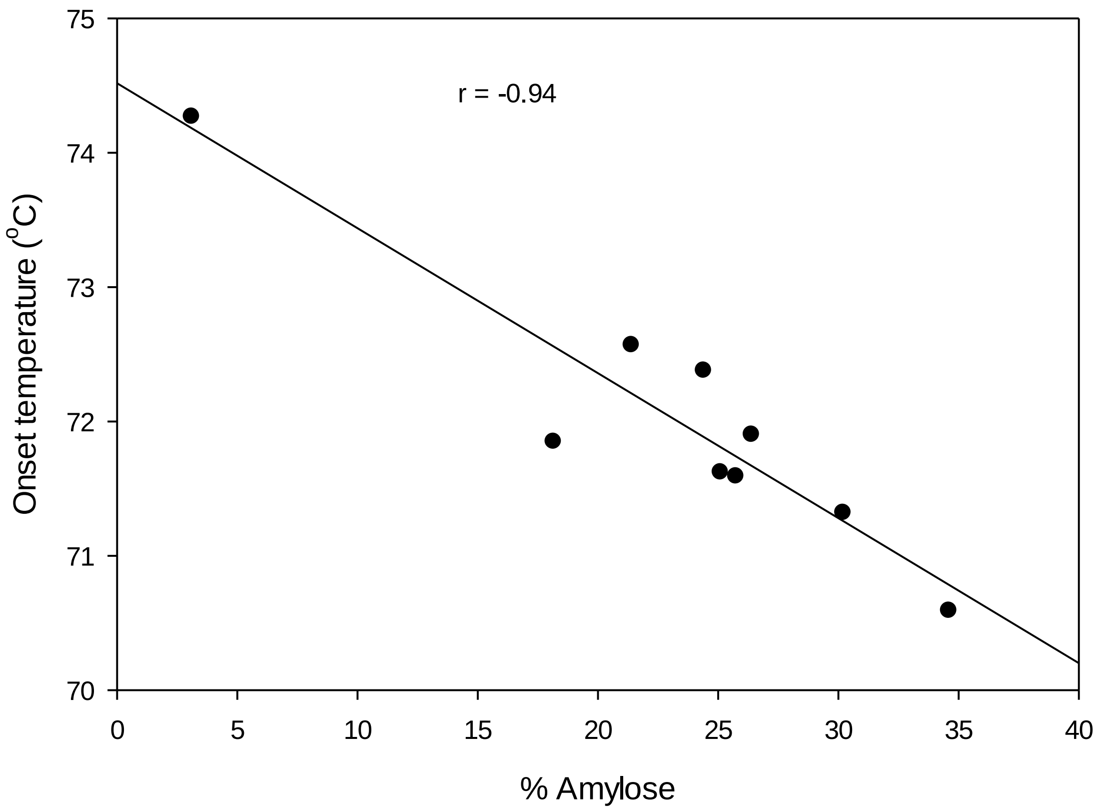


Figure 3.5. Relationship between % amylose and onset temperature (°C) for different proso millet cultivars.

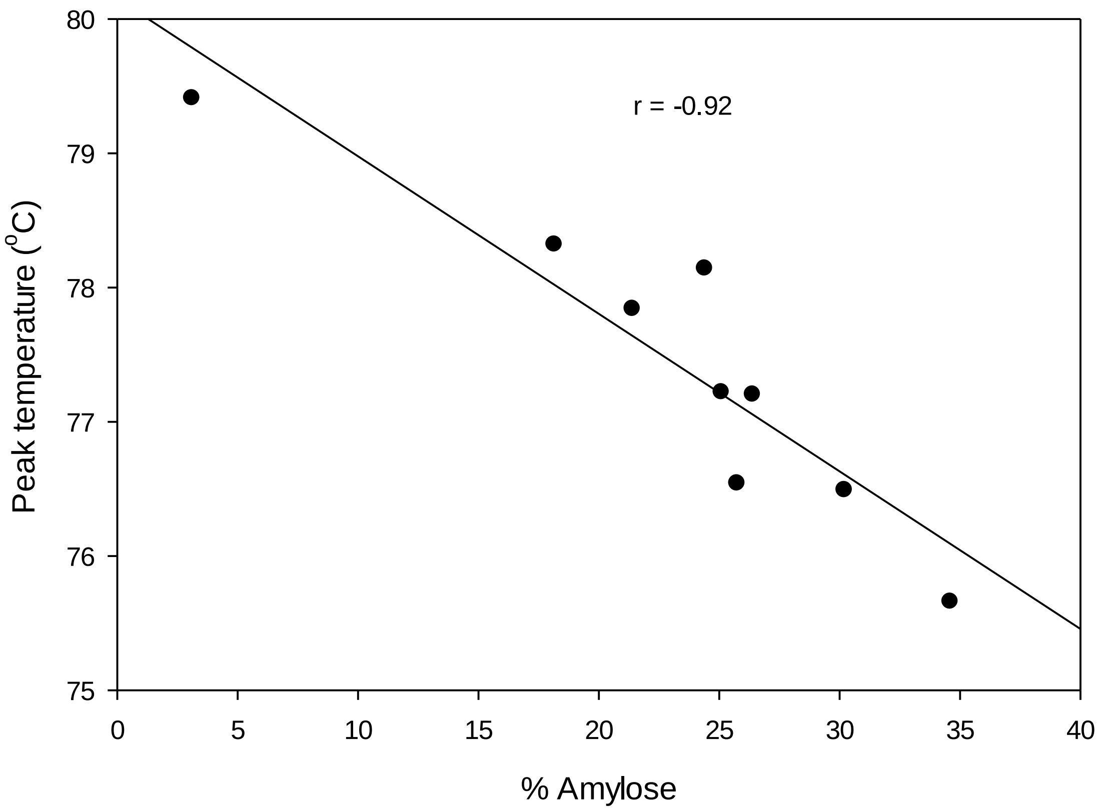


Figure 3.6. Relationship between % amylose and peak temperature (°C) for different proso millet cultivars.

3.3.4. Conclusion

The dimensions, sphericity, volume, surface area, equivalent diameter, bulk and solid density, porosity, angle of repose, hardness, weight and color was determined and significant ($P<0.05$) difference was observed among cultivars. Strong positive correlation of amylose content with peak viscosity ($r=0.84$), final viscosity ($r=0.91$) and setback ($r=0.90$) was observed. Negative correlation of onset temperature ($r = -0.94$) and peak gelatinization temperature ($r = -0.94$) with amylose content was observed. Evaluation of postharvest properties of different proso millet cultivars are mandatory to obtain the knowledge of their physical and engineering properties in order to design appropriate machineries for process operations like sorting, drying, heating, cooling, and milling.

Connecting text

In chapter 3, physical and functional properties of nine proso millet cultivars were evaluated. The effect of cultivar was found to be significant on different physical properties and amylose content showed correlation with gelatinization and pasting properties. In chapter 4, proso millet starch was isolated from commercial proso millet and modified to study its physico-functional properties.

4. Characterization of modified proso millet starch

4.1. Introduction

Interest in millet utilization has increased due to the various rediscovered health benefits and also due to its increasing use as non-gluten ingredient in food applications (Zhu, 2014). Millet has many advantages over other cereals such as higher resistance to pest and diseases, adaptability to a wide range of climatic conditions and grows well in high temperatures and dry conditions (Saleh et al., 2013). Besides having agronomic advantages, millets have better amino acid composition and high nutritive value which is comparable to that of major cereals such as wheat, corn and rice (Klopfenstein and Hoseney, 1995; Parameswaran and Sadasivam, 1994). Millet is widely consumed as food in African countries, China and Indian subcontinent, however it is not part of human diet in USA and mainly used for animal and bird feed (Lyon, 2008). Proso millet is the major variety of millet grown in the US with a total production of 418,145 tons in 2013 (FAO, 2014). Proso millet being underutilized grain in USA can be alternative source of starch as it has been reported to contain 60-67% starch (Santra, 2013). Due to the vast application of starches in food systems, different sources with good functional properties are being explored.

Starch is a naturally renewable, inexpensive and biodegradable material which is used to alter the textural properties of several foods (Radley, 1976). It has various industrial applications such as a thickener, binder, encapsulating agent, stabilizer and gelling agent (Radley, 1976). However, it is the modified starch that is used mostly in industrial applications due to undesirable characteristics of native starch upon cooking whereas modification improves gelling tendency, clarity and texture (Bemiller, 1997). Starch modification alters physical and chemical properties to improve functionality of native starch (Hermansson and Svegmark, 1996). Hydrothermal modification (HTM) involves controlled application of heat and moisture, which causes physical modification of starches without gelatinization and damage to the starch granules with respect to size, shape or birefringence (Stute, 1992). Acid modification (AM) of starch is a chemical modification process involving hydrolysis of starch using hydrochloric acid, which breaks the glycosidic linkages of α-glucan chains, changing the structure and characteristics of native starch (Hoover, 2000). AM is used to modify physicochemical properties of native starch for applications in various industries such as food and textile (Radley, 1976). Acid hydrolysis is widely used for production of starch gum candies, paper, cationic and amphoteric starches (Wurzburg, 1986). Understanding the properties, and potential uses of proso millet starch significantly contributes to the further expansion of millets as alternative functional crop (Zhu, 2014). The present study was undertaken to explore the behavior of native and modified starches as affected by different modifications methods. Most studies on millet starch have focused on pearl millet and other major millet varieties but no work has been done to investigate the effect of hydrothermal and acid modification on proso millet starch.

4.2. Materials and methods

4.2.1. Raw materials

Proso millet flour was purchased from Bob's Red mill (Milwaukie, OR, USA) and stored at ambient temperature (24-28°C). Information on type of cultivar of commercial proso millet flour was not known. All chemicals used for the analyses were of analytical grade (Sigma Aldrich, St. Louis, MO, USA).

4.2.2. Starch Isolation

Starch was isolated using alkaline steeping method (Sira and Amaiz, 2004; Wang, L. and Wang, Y.-J., 2001). Proso flour (100 g) was steeped in 200 mL of 0.1% NaOH for 18 hrs. Mixture was blended for 2 min using waring blender and passed through a sieve (U.S. 100 sieve size) and centrifuged at 2000 rpm for 15 min. The top layer was carefully decanted and the bottom layer was re-slurried and washed thrice with 0.1% NaOH, while removing the upper layer carefully every time. The starch was washed with deionized water, then neutralized with 0.1 N HCl to pH 6.5, and washed with deionized water four times, centrifuged, dried in an oven at 45°C for 48 hr.

4.2.3. Acid modification

Millet starch was modified according to the method described by Wang and Wang (Wang, L. and Wang, Y.J., 2001). HCl (0.14 N) was added to 40 g starch and kept in water bath for 8 h at 50°C and thereafter, 1 N NaOH was used to adjust the pH to 6.5. Starch slurry was washed thrice with deionized water and then dried in an oven at 45°C for 24 h.

4.2.4. Hydrothermal modification

Millet starch, conditioned to 30% moisture content (dry basis) was added in glass bottle and kept at 4°C for 12 h to equilibrate the moisture. Starch sample in sealed glass bottle was then heated for 3 h at 110°C. The bottle was occasionally shaken to distribute the heat evenly and then cooled and dried for 4 h at 45°C (Collado et al., 2001).

4.2.5. Physico-chemical analysis

Moisture, protein, fat, ash were determined using AOAC standard methods (AOAC, 2005). Amylose content were determined using AACCI method 61-03.01 (AACCI, 1997). Starch sample (100 mg) was mixed with 1 ml of 95% ethanol and 9 ml 1N NaOH and then transferred to 100 ml volumetric flask. Flasks were kept at room temperature for 10 min then heated in boiling water bath for 10 min and cooled for 2 h at room temperature. The resulting mixture was diluted to 100 ml using distilled water and mixed vigorously. An aliquot of starch solution (5 ml) was pipetted into 100 ml volumetric flask containing 50 ml distilled water. 1.0 mL of acetic acid (1N) and 2mL iodine solution were added and diluted to 100 ml. After 20 min, absorbance was measured at 620 nm using blank to zero the spectrometer (EVO 60 ThermoFisher scientific, Waltham, MA USA). Standard curve was developed using standard amylose and amylopectin blends and used to measure amylose content.

4.2.6. Thermal properties

Degree of gelatinization was determined using differential scanning calorimetry (DSC – Q20, TA instruments, New Castle, Delaware, USA). Starch sample (10 mg, dry basis) was weighed into high volume stainless steel pans, followed by addition of 20 µl of distilled water. The pan was hermetically sealed and equilibrated at 4°C for 24 h. Samples were kept at room temperature for one hour prior to scanning from 10 to 150°C at 10°C/min (Krueger et al., 1987).

After gelatinization, the samples were kept at 4°C for 10 days and then reheated at the rate of 10°C/min from 10°C to 150°C to determine retrogradation properties.

4.2.7. Pasting properties

Pasting characteristics were determined using Discovery Hybrid Rheometer (DHR) with starch pasting cell (DHR-2, TA instruments, New Castle, Delaware, USA). A mixture of 3.5 g starch (14% moisture) in 25 ml of distilled water was stirred at 160 rpm. Samples were held at 50°C for 1 min and then heated to 95°C at 4°C/min and held at 95°C for 5min. Subsequently, samples were cooled to 50°C at 4°C/min and held at 50°C for 5 min. A plot of viscosity (Pa.s) vs. time (s) was used to determine pasting temperature, peak and final viscosity, holding strength, setback and breakdown.

4.2.8. Solubility and swelling power

Solubility and swelling power was determined using Leach method (Leach et al., 1959) modified by Balasubramanian et al, Kusumayanti et al, and Subramanian et al (Balasubramanian et al., 2014; Kusumayanti et al., 2015; Subramanian et al., 1994). Starch (0.1 g) was heated in 10 ml of water at 70, 80, and 90°C for 30 min. Samples were stirred occasionally to avoid lump formation and then centrifuged at 3,000 rpm for 15 min. Supernatant was removed and starch sediment was weighed. Supernatant was dried for 2 h at 130°C and then weighed.

$$Solubility(\%) = (W_{ss} * 100) / W_s \quad (1)$$

Where, W_{ss} is the weight of soluble starch (g) and W_s is the weight of the sample (g).

$$Swelling\,power(\%) = (W_{sp} * 100) / (W_s * (100 - \%solubility)) \quad (2)$$

Where, W_{sp} is the weight of sediment paste (g) and W_s is the weight of sample (g).

4.2.9. Water binding capacity (WBC)

WBC was determined using the method described by Yamazaki (Yamazaki, 1953) . A mixture of 2.5 g (dry basis) starch in 25 mL distilled water was stirred for 30 min and centrifuged at 3,000 rpm for 10 min. Excess water was removed and then residue is weighed.

$$WBC(\%) = (W_{rs} * 100) / W_s \quad (3)$$

Where, W_{rs} is the weight of residual starch (g) and W_s is the weight of sample (g).

4.2.10. Paste clarity

Paste clarity was measured according to method described by Craig et al. (Craig et al., 1989). Starch (2% dry basis) aqueous mixture was heated and stirred in water bath for 30 min at 95°C. Samples were cooled and stored for 4 days at 4°C and percent transmittance was measured every day at 640 nm against water blank using UV–VIS Spectrophotometer (EVO 60 ThermoFisher scientific, Waltham, MA USA).

4.3. Results and discussion

4.3.1. Physico-chemical analysis

Proximate content of extracted proso millet starch is presented in Table 4.1. Starch extraction yield was 54.1% and it had low residual protein (1.21%), lipid (0.27%) and ash (0.62%) content. The amylose content and WBC properties of native, HTM and AM proso millet starches are presented in Table 4.2. The AM starch showed significant decline (P<0.05) in apparent amylose content from 28.51% in native starch to 25.78% in AM starch, which may be due to the attack of acid on amorphous section of starch. Hoover (Hoover, 2000) proposed that acid preferentially attacks the amorphous regions in the granules which leads to cleavage of amylose molecules causing reduction in amylose content. However, HTM starch with 29.08% amylose content showed no significant (P<0.05) change in amylose content. Rafiq et al. (2016) and Sun et al. (2014) reported similar results for acid modified and hydrothermal modified horse chestnut and sorghum starch respectively.

Table 4.1. Proximate content of extracted proso millet starch

	% Content
Starch yield	54.1 ± 0.11
Moisture	9.86 ± 0.08
Protein	1.21 ± 0.07
Ash	0.62 ± 0.02
Lipid	0.27 ± 0.01
Carbohydrates*	88.04 ± 0.04
Amylose	28.51 ± 0.22

The values are means ± standard deviation of three replicates. *Carbohydrates was calculated: 100 – (Moisture + Protein + Ash + Lipid)

4.3.2. Water binding capacity (WBC)

WBC of native starch showed significant ($P<0.05$) decline from 138.43 to 108.13% upon AM but HTM increased the WBC to 191.5%. WBC of HTM starch increased due to the increased hydrophilicity, which reduces the crystalline regions and improves the accessible binding sites in the amorphous region resulting in improved WBC. Similar reports for increased WBC due to hydrophilic affinity have been reported in white sorghum and chestnut starch (Olayinka et al., 2008; Rafiq et al., 2016). The AM starch showed low WBC than native proso starch which may be due to the reduced accessible binding sites caused by reduced amorphous region in starch granules. Balasubramanian et al. (2014) reported similar trend in AM and HTM pearl millet starch and Kaur et al. (2011) has reported a decrease in WBC of various acid treated starches from different botanical sources.

Table 4.2. Effect of HTM and AM on native proso millet starch's WBC and amylose content.

Type	WBC (%)	Amylose Content (%)
N	138.43^{c} ± 1.93	28.51^{a} ± 0.22
AM	108.13^{b} ± 0.76	25.78^{b} ± 0.25
HTM	191.65^{a} ± 1.94	29.08^{a} ± 0.38

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly (P<0.05). N: Native starch; AM: Acid modified starch; HTM: Hydrothermal modified starch.

4.3.3. Solubility and swelling power

The solubility and swelling power of native and modified starches are presented in Table 4.3. The solubility of native starch increased from 2.62 to 34.88% whereas increase in AM starch is significantly large (P<0.05) from 18.97 to 86.17% with *rise* in temperature from 70 to 90°C. However, HTM starch solubility increased from 1.71 to 12.45% but it is significantly (P<0.05) lower than native starch solubility. Swelling power also showed increase with increase in temperature. Native starch swelling power increased from 4.69 to 24.99%, AM starch solubility increased from 4.94 to 21.26% and HTM starch solubility also *rise*s from 5.29 to 10.37% from 70 to 90°C. According to Lawal and Adebowale (2005) and da Rosa Zavareze and Dias (2011), the decreased solubility and swelling power of HTM starch compared to native starch has been credited to starch granule's internal reordering providing higher interactions between starch functional groups, formation of more ordered double helical amylopectin clusters and the formation of amylose-lipid complexes within starch granules. In addition, the physical variations within the starch granules and unravelling of double helices of crystalline region, which reduced the granular stability might be accountable for the drop in swelling capacity and starch solubility at higher temperature (Leach et al., 1959; Olayinka et al., 2008). Balasubramanian et al. (2014) and Gunaratne and Hoover (2002) reported similar results of reduced swelling power and solubility with heat moisture treated pearl millet starch and cassava starch, respectively.

Solubility of AM starch was higher as compared to native proso starch and increased progressively with increase in temperature. AM leads to the structural weakening and de-polymerization of starch granules and similar effect on pearl millet was observed by Balasubramanian et al. (2014). However, swelling power showed no significant ($P<0.05$) change at 70°C, increase at 80°C but significant ($P <0.05$) reduction at 90°C was observed which may be due to amylose leaching, resulting in starch damage which limits swelling of starch (Jane et al., 1997). Acid hydrolysis causes an increase in percentage crystallinity as the crystalline region is not accessible to acid and the amorphous regions are being broken down. Rigidness of entangled amylopectin linkages in the crystalline area of the starch controls swelling and thus, increased crystallinity may cause reduction in swelling power of the AM (Kainuma and French, 1971).

Table 4.3. Effect of AM and HTM on native proso millet starch's solubility and swelling power at 70, 80 and 90°C.

Type	70°C		80°C		90°C	
	Solubility (%)	Swelling (%)	Solubility (%)	Swelling (%)	Solubility (%)	Swelling (%)
N	2.62[b] ± 0.17	4.69[b] ± 0.26	6.59[b] ± 1.07	11.65[b] ± 1.12	34.88[b] ± 0.77	24.99[a] ± 0.22
AM	18.97[a] ± 1.35	4.94[a,b] ± 0.41	67.98[a] ± 4.64	17.46[a] ± 2.48	86.17[a] ± 1.77	21.26[b] ± 1.68
HTM	1.71[c] ± 0.34	5.29[a] ± 0.23	7.25[b] ± 1.36	7.78[c] ± 0.63	12.45[c] ± 3.31	10.37[c] ± 1.06

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($P<0.05$). N: Native starch; AM: Acid modified starch; HTM: Hydrothermal modified starch.

4.3.4. Light transmittance

Figure 4.1 shows the native and modified proso millet starch light transmittance over a 4-day period. It is used to assess the level of starch paste retrogradation during storage, which depends on the swollen and non-swollen granules during gelatinization process (Craig et al., 1989; Sandhu et al., 2007). The transmittance of native, AM and HTM pastes showed a gradual decrease with storage but native and AM showed pronounced reduction over time, 42 to 2% and 85 to 47%, respectively, which is a result of retrogradation tendency. HTM improve flexibility of chains within amorphous area of granules and resulted in significant ($P<0.05$) low transmittance, less than 6% (Hoover and Vasanthan, 1994). AM starch showed higher transmittance which may be due to leaching of amorphous region causing better interactive bond formation between amylopectin molecules thus resulting in clear paste (Lawal, 2004). Corn and pinhao starch showed similar results on acid modification (Thys et al., 2013).

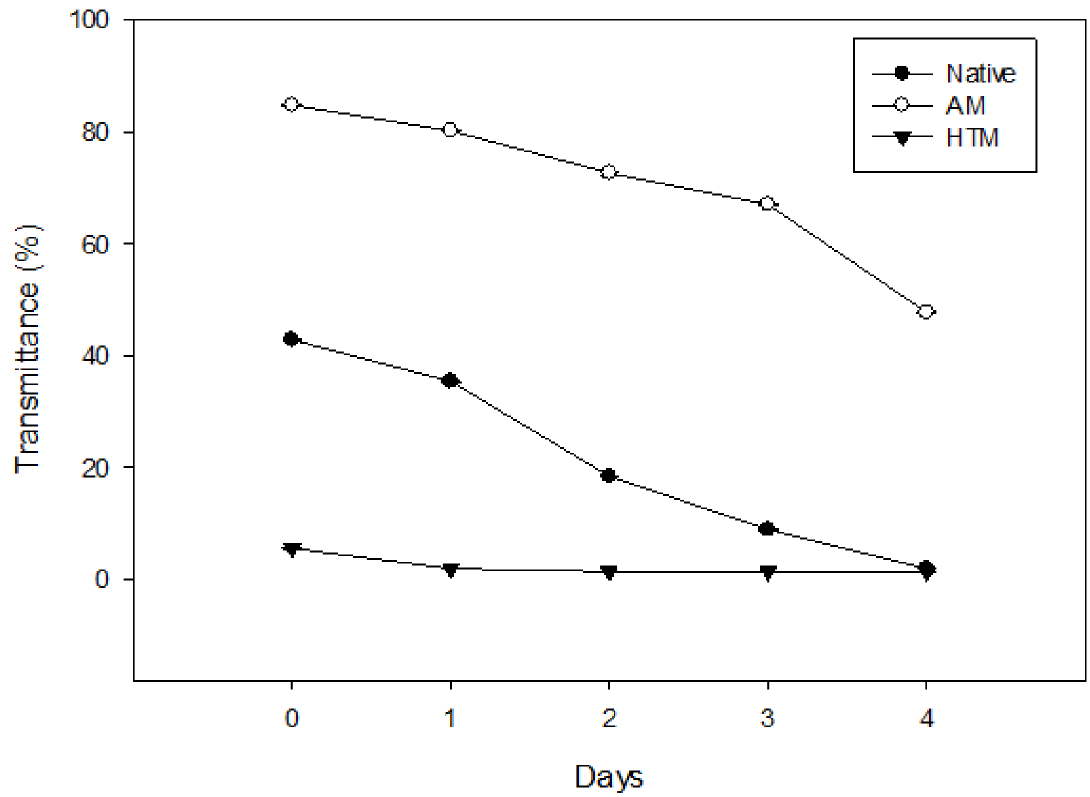


Figure 4.1. Effect of modifications on proso millet starch % light transmittance over 4-day period

4.3.5. Thermal properties

Degree of gelatinization of native, HTM and AM starches are shown in Table 4.4. HTM increased the onset (T_O), peak (T_P) and conclusion (T_C) temperatures to 79.13, 87.17 and 99.35°C, respectively compared to native starch T_O, T_P and T_C which were 72.93, 78.61 and 94.55°C, respectively. HTM starch needs more heat to break the bonds formed between freely moving amylose molecules and amylopectin chains present in crystalline regions, hence higher T_O and T_P (Sun et al., 2014). Moreover, high T_C is due to the reduced destabilization effect of amorphous regions on crystalline melting which is result of reduced swelling power (Gunaratne and Hoover, 2002). Li et al. (2011) and Sun et al. (2014) reported similar results for hydrothermal treated sorghum starch and mung bean starch, respectively. The decrease in enthalpy of gelatinization (ΔH_G) for HTM may be due to fewer double helices available to untangle. During modification some helices present in crystalline and non-crystalline regions are disrupted, which leads to reduction in relative crystallinity, hence reduction in enthalpy (Cooke and Gidley, 1992). Non-significant ($P<0.05$) change in gelatinization range was observed for HTM starches.

The acid treatment showed significant ($P<0.05$) decrease in the T_O (69.71 °C), T_P (77.26 °C) and increase in the range (26.56 °C). Acid modification focus on the amorphous region of the starch granules which no longer destabilizes the crystallites causing crystallites to melt at increased temperature resulting in wider range of gelatinization (Hoover, 2000). In addition, decreased in amorphous region results in an increase in relative crystallinity causing higher ΔH_G than HTM. Increase in enthalpy compared to HTM may also be due interaction between amylose-amylose and amylose-amylopectin causing formation of double helices which require more energy to break during gelatinization (Thirathumthavorn and Charoenrein, 2005).

Retrogradation properties of native, HTM and AM starches after 10 days of storage were measured and shown in Table 4.5. T_O, T_P and T_C of retrograded native, HTM and AM starches are significantly lower (P<0.05) when compared to T_O, T_P and T_C of gelatinization. ΔH_R (2.66 J/g) of native is lower than its ΔH_G (3.83 J/g) but HTM starch's ΔH_R (2.99 J/g) increased which may be due to the interplay between crystallinity changes and amylose-amylopectin interactions. The increase in ΔH_R was attributed to increased crystallinity on modification, which reduces the level of separation among the outer branches of adjacent amylopectin chain groups. Thus, during retrogradation, the formation and lateral association of double helices involving amylopectin chains would be stronger and occur more rapidly in HTM than in native starches (Hoover, 2010). AM starch showed similar ΔH_R (3.83 J/g) as ΔH_G (3.97 J/g) but higher than native starch ΔH_R (2.66 J/g) which is the result of high mobility of short chains and reduction in amylopectin branch points, consequently leading to high rate of realignment of chains during storage (Palma-Rodriguez et al., 2012).

Table 4.4. Effect of AM and HTM on native proso millet starch's degree of gelatinization.

Type	T_o Onset (°C)	T_P Peak (°C)	T_c Stop (°C)	ΔH_G (J/g)	Range (T_c-T_o) (°C)
N	$72.93^{b} \pm 0.62$	$78.61^{b} \pm 0.83$	$94.55^{b} \pm 1.34$	$3.83^{a} \pm 0.28$	$21.62^{b} \pm 1.54$
AM	$69.71^{c} \pm 1.67$	$77.26^{c} \pm 0.36$	$96.27^{b} \pm 2.93$	$3.97^{a} \pm 0.55$	$26.56^{a} \pm 1.44$
HTM	$79.13^{a} \pm 1.70$	$87.17^{a} \pm 1.46$	$99.35^{a} \pm 1.36$	$1.95^{b} \pm 0.09$	$20.21^{b} \pm 1.12$

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$).

N: Native starch; AM: Acid modified starch; HTM: Hydrothermal modified starch.

Table 4.5. Effect of AM and HTM on native proso millet starch's retrogradation.

Type	T_o Onset (°C)	T_P Peak (°C)	T_c Stop (°C)	ΔH_R (J/g)	Range(T_c-T_o) (°C)
N	$41.38^a \pm 0.88$	$55.05^{a,b} \pm 0.74$	$74.73^b \pm 1.49$	$2.66^b \pm 0.33$	$33.35^b \pm 1.26$
AM	$42.21^a \pm 1.90$	$56.58^a \pm 2.14$	$78.36^a \pm 2.09$	$3.83^a \pm 0.47$	$36.15^a \pm 1.53$
HTM	$41.49^a \pm 0.47$	$54.62^b \pm 0.52$	$75.05^b \pm 1.26$	$2.99^b \pm 0.08$	$33.55^b \pm 0.99$

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$). N: Native starch; AM: Acid modified starch; HTM: Hydrothermal modified starch.

4.3.6. Pasting properties

The pasting properties of the native, AM, HTM proso millet starches presented in Table 4.6 and pasting profiles of native and modified starches are illustrated in Figure 4.2. Both modifications showed significant changes ($P<0.05$) in pasting profile compared to native starch. HTM starch showed higher pasting temperature, 83.90°C compared to native starch 79.23°C, which may be due to additional heat requirements for degradation of starch granules and formation of paste caused by increased cross linking within starch granules (Singh et al., 2009). Low swelling power of HTM starch restricts amylose leaching and does not let the viscosity increase, resulting in low peak viscosity (2.29 Pa.s) (Hoover, 2010).

AM starches showed lower values for peak, breakdown, final viscosities and holding strength, which are 0.07, 0.03, 0.109 and 0.037 Pa.s respectively compared to native starch's 4.60, 2.60, 3.68 and 1.99 Pa.s respectively. AM disrupts the amorphous region and weakens the starch granule structure and limits the swelling during gelatinization as amorphous region is primarily associated with starch swelling. During pasting, starch is unable to achieve its maximum swelling power which results in reduced peak viscosity (Wang, L. and Wang, Y.J., 2001). The secondary *rise* during cooling in the pasting curve is known as setback, which is a measure of retrogradation was minimum for AM and no-significant ($P<0.05$) change in HTM (Thirathumthavorn and Charoenrein, 2005).

Table 4.6. Effect of AM and HTM on native proso millet starch's pasting properties.

Type	Pasting temperature (°C)	Peak Viscosity (Pa.s)	Holding strength (Pa.s)	Final Viscosity (Pa.s)	Setback (Pa.s)	Breakdown (Pa.s)
N	79.23 ^{b} ± 0.38	4.60 ^{a} ±0.15	1.99 ^{a} ±0.48	3.68 ^{a} ±0.37	1.69 ^{a} ±0.48	2.60 ^{a} ±0.64
AM	79.57 ^{b} ±0.04	0.07 ^{c} ±0.01	0.04 ^{b} ±0.01	0.11 ^{b} ±0.01	0.07 ^{b} ±0.01	0.03 ^{b} ±0.01
HTM	83.90 ^{a} ±1.94	2.29 ^{b} ±0.90	1.96 ^{a} ±0.74	3.21 ^{a} ±1.50	1.25 ^{a,b} ±0.76	0.32 ^{b} ±0.16

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$). N: Native starch; AM: Acid modified starch; HTM: Hydrothermal modified starch.

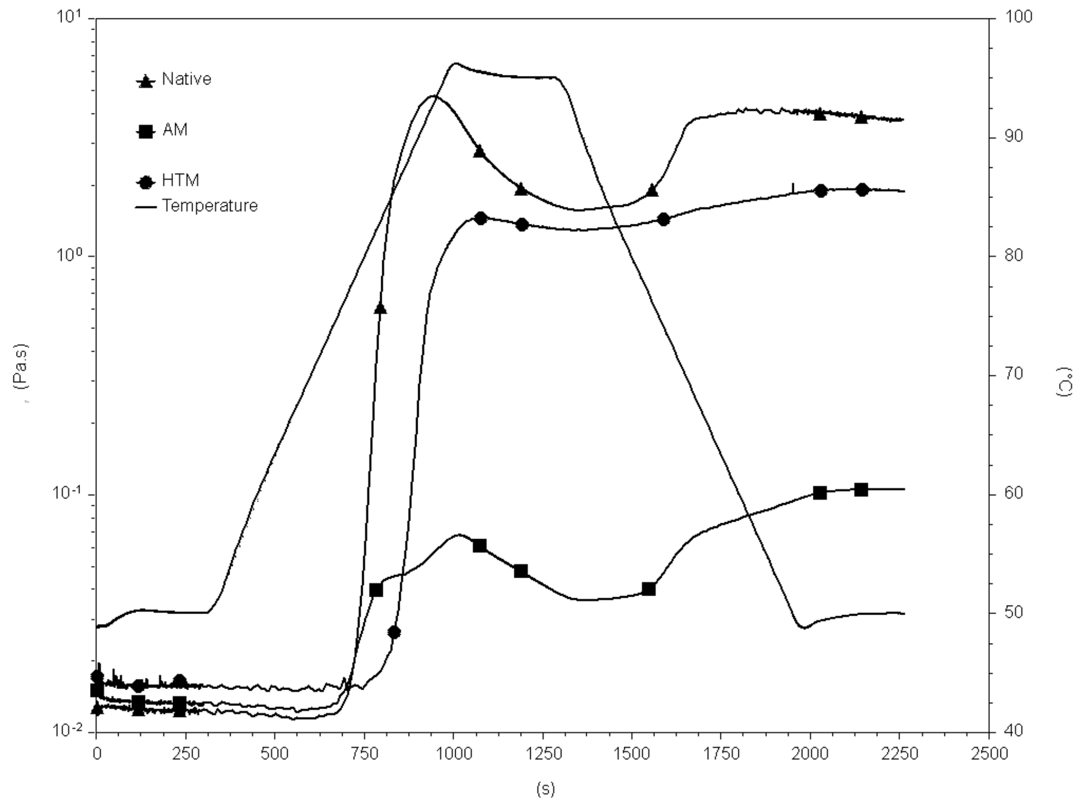


Figure 4.2. Effect of modifications on proso millet starch pasting profile

4.4. Conclusion

Proso millet starch modification by hydrothermal and acid resulted in a significant change in the physicochemical, functional, pasting and thermal properties. AM reduces the amylose content and WBC of starch, also improved the clarity of the paste whereas HTM had no effect on amylose content but increased the WBC and reduced the paste clarity. Both modifications significantly ($p<0.05$) change the swelling power and solubility of the native starch. The decrease in swelling is a desirable property for some food preparation such as noodle production. Modifications influenced the thermal and pasting properties, with low breakdown implying that starches were more stable during continued shearing and heating after modifications. Low setback viscosity after modification could enable the starches to be used in canned and frozen foods. The increased knowledge on the effects of modified proso millet starch presented in this study will help broaden the applications of proso millet starch in food and non-food industries.

Connecting text

Physico-functional properties of modified proso millet starch were determined. Modifications significantly ($p<0.05$) changed the native proso millet starch's WBC, swelling, solubility, thermal and pasting properties. In chapter 5, rheological and baking properties of different gluten free formulations based on proso millet, supplemented with different hydrocolloids and starches were studied.

5. Rheological and baking properties of millet based gluten free formulations

5.1. Introduction

Traditionally, bread products are made from wheat flour and are consumed worldwide, however, some consumers are intolerant to gluten or are allergic to wheat. Gluten related disorders are classified into three different classes namely autoimmune, allergic and non-autoimmune non-allergic. Celiac disease is a disorder associated with autoimmune response that compromises the small intestine on ingestion of gluten based foods (Lee and Newman, 2003). According to survey done by The National Health and Nutrition Examination Survey (NHANES), 1 in every 131 Americans, i.e. at least 3 million Americans are affected by this disease (Rubio-Tapia et al., 2012). There is also wheat allergy, which may or may not be related to gluten ingestion but it generates immune response in the body and can cause nausea, vomiting, diarrhea, and rash. Wheat is one of the eight identified food allergens which accounts for 90% of food allergies (FARE, 2014) and the last class of gluten disorder consist of people who are not allergic to gluten and do not have celiac disease, but are people diagnosed with non-celiac gluten sensitivity (NCGS), however it is less severe than celiac disease. The people diagnosed with NCGS experience abdominal pain, fatigue, headaches, tingling/numbness and foggy brain (Czaja-Bulsa, 2015). It is estimated that NCGS affects up to 6% of US population, i.e. about 18 million Americans and it is more common in adults whereas celiac disease can occur at any age but strongly linked with childhood and the only treatment available for all these diseases is to exclude gluten sources (wheat, rye, and barley) from their diet (Feighery, 1999).

Therefore, there is a need to find alternatives to wheat for production of bread for those who are intolerant to any of the above reasons.

Gluten is the major component of wheat based bread which helps in ability to form thin gas-retaining films that trap gases, allowing dough to expand to become a softer, lighter and palatable food after baking (Cauvain and Young, 2007). Due to increasing gluten intolerance, development of healthier and better quality gluten-free products that would greatly improve the quality of life of celiac patients and those who develop sickness from wheat consumption is needed. Major challenge in producing bread without gluten is its inability to form viscoelastic dough and the resulting bread contains numerous quality defects including reduced volume, lack of cell structure, a dry, crumbly, grainy texture, a cracked crust, poor mouthfeel and flavor, and susceptibility to quick staling (Capriles and Arêas, 2014). Several additives, such as hydrocolloids, proteins, enzymes, antioxidants, emulsifiers and preservatives are used to improve dough properties, enhance quality and texture of breads (Capriles and Arêas, 2014).

Millet has the potential to serve as an alternative to wheat in bread production. Millets proximate composition are similar to that of other major cereals like wheat, corn and rice. They are rich in fiber, iron, calcium, B vitamins, low in phytic acid, and have high protein content (11.8-12.5%) (Saleh et al., 2013). Millet flour is often used to produce flat breads, porridges, beer and soup in countries of Africa, Indian subcontinent and China (Saleh et al., 2013). Lorenz and Dilsaver (1980) used whole millet flour to produce breads, which had low volume and dense texture but breads with blends of millet and wheat flour produced better results. Badi and Hoseney (1976) and Crabtree and Dendy (1979) made breads of optimum quality with 10% millet flour and adding 0.5% calcium stearoyl-2-lactylate to dough, improved bread quality significantly. Bread quality produced from composite flour of wheat and millet (50:50) is remarkably improved by the combined addition of emulsifiers and enzymes (xylanase and transglutaminase) at elevated dough moisture (Schoenlechner et al., 2013).

Millet use in producing GFB can be encouraged with the addition of hydrocolloids in bread making formulations. Hydrocolloids showed promising results with other gluten free flours to produce high quality and consumer acceptable bread (Capriles and Arêas, 2014). Hydrocolloids interact with water and produce a gel network structure that leads to increase in batter viscosity and increase in gas retention capability during proofing and baking, and improve texture, volume and structure of GFB (Anton and Artfield, 2008). Xanthan and carboxymethyl cellulose (CMC) are the most commonly used gums in GFB due to their favorable effects on the characteristics of the final product (Capriles and Arêas, 2014). Sabanis and Tzia (2011) evaluated the effect of xanthan gum on gluten free formulations and the results showed that gums helped in producing increased loaf volume and softer crumb. Demirkesen et al. (2010a) evaluated the effects of a combination of different hydrocolloids and emulsifiers on the quality of a rice-based GFB formula. Results showed that 0.5% Diacetyl tartaric acid esters of monoglycerides (DATEM) combined with 0.5% xanthan–guar blend provided the best final product, with good volume and crumb texture. Chestnut flour was tested as a raw material in GFB and was observed that breads containing 30% chestnut flour and 70% rice flour, in addition to a blend of xanthan–guar gum and diacetyl tartaric acid ester of mono- and diglycerides

(DATEM) emulsifier, had the best quality parameters (hardness, specific volume, color, and sensory values) (Demirkesen et al., 2010b).

Rheology tests allows opportunity to evaluate the performance of dough under various baking processes. It helps to determine the efficacy of processing aids and sufficient amount of water to make the best quality bread. It is the science that studies the flow and deformation of matter when force is applied, and can be used to analyze complex systems such as bread (Dobraszczyk and Morgenstern, 2003). The rheology of bread change significantly between the mixing and the final product. Bread dough exhibits viscoelastic behavior which is a combination of properties of both purely viscous fluids and purely elastic solids (Petrofsky and Hoseney, 1995). Rheology can be related to product functionality: many rheological tests have been used to determine hydration ratio, to predict final product quality such as mixing behavior, sheeting and baking performance (Dobraszczyk and Morgenstern, 2003). The overall goal of this topic is to evaluate the rheological and baking properties of millet based gluten free formulations.

5.2. Materials and method

5.2.1. Raw materials

Gluten free formulations consisted of millet flour, corn starch, potato starch and nonfat dry milk purchased from Bob red mills (Milwaukie, OR), active dry yeast (Fleischmann, St. Louis, MO), shortening (Crisco, Ohio), sugar and salt. In addition, four different hydrocolloids were used in the formulation Xanthan VI (Xanthan gum), CMC 2500 (Carboxymethyl cellulose), Ticaloid 313 (Xanthan and Carboxymethyl cellulose), Ticaloid 345 (Xanthan, locust bean, carrageenan and sodium alginate) were purchased from TIC gums (White marsh, MD). Millet starch was isolated from proso millet flour using method described in chapter 3.

5.2.2. Bread formulation

Gluten free formulation used in this study are summarized in Table 5.1 and two different levels (2 and 3%) of hydrocolloid were used. Other ingredients are as follow: sugar (8.5%), shortening (4%), nonfat dry milk (4%), yeast (3%), salt (2%) and water (105%).

Table 5.1. Different gluten free formulations and their abbreviations

Abbreviations	Millet flour (%)	Corn starch (%)	Potato starch (%)	Millet starch (%)	Hydrocolloids
MG1	100				Xanthan gum
MG2	100				CMC
MG3	100				Ticaloid 313
MG4	100				Ticaloid 345
MCG1	50	50			Xanthan gum
MCG2	50	50			CMC
MCG3	50	50			Ticaloid 313
MCG4	50	50			Ticaloid 345
MPG1	50		50		Xanthan gum
MPG2	50		50		CMC
MPG3	50		50		Ticaloid 313
MPG4	50		50		Ticaloid 345
MMG1	50			50	Xanthan gum
MMG2	50			50	CMC
MMG3	50			50	Ticaloid 313
MMG4	50			50	Ticaloid 345

5.2.3. Dynamic oscillation measurements

Dynamic oscillation measurements are one of the most popular and widely used techniques to determine visco-elastic behavior in doughs and batters. It measures the response of a material by the application of sinusoidal oscillating stress or strain with time (Dobraszczyk and Morgenstern, 2003). The measurement has to be performed in the linear viscoelastic region in which the properties of the material are independent on the shear strain and stress and are only a function of time or frequency (Buresová et al., 2014).

In order to determine rheological properties, all ingredients except yeast were mixed in 100 g micro mixer (National mfg. co. Lincoln, NE). Rheological properties were measured using dynamic oscillation rheometer (DHR-2 TA, instruments, USA) with a 35 mm parallel plate geometry at 2 mm gap. Dough sample was poured between the plates and left for 20 min to relax and stabilize. Strain sweep test (0.01 to 100%) was performed at 25°C to determine linear viscoelastic region (LVR). Frequency sweep test was performed at 25°C from 0.1 Hz to 50 Hz using 0.05% strain value determined from the strain sweep test.

In creep-recovery measurements, stress is held constant and the deformation is measured. Removal of stress causes the material to recoil to its rest position which corresponds to dough's elasticity (Dobraszczyk and Morgenstern, 2003). It was performed using the same geometry as mentioned above. Stress of 50 Pa was applied for 60 s on the sample and then allowing strain recovery by sample in 180 s after removing the stress.

5.2.4. Bread making process

Optimized straight-dough bread making procedure AACC Method 10-10.03 (AACC, 1995a) was used for the baking experiments. A kitchen mixer (KitchenAid, Model KV25G0X, Benton Harbor, MI) was used to mix the bread dough. All the ingredients were mixed for 1 min at speed 1 and for 6 min at speed 2 while scrapping dough every 2 min. The dough was poured into pans and proofed for 35 min at 40°C and subsequently baked for 1 hr. at 375°F. Baked breads were kept for 1 hr. to cool before measurements. For storage effect, breads were packed in sealed poly bags and stored for 5 days at room temperature.

5.2.5. Bread Volume

Bread volume was determined according to AACC Method 10-05.01 (AACC, 2001) using seed displacement method.

5.2.6. Bake loss (%)

Moisture lost during baking was measured.

5.2.7. Color

Color of crust and crumb was determined with a chromameter (CR-400,Konica Minolta, USA) applying the ***L a b*** system. Crust color was measured at six different positions on top of the bread, then bread was sliced to obtain three uniform slices of 25 mm. Crumb color was measured in the center on both sides of each slice.

5.2.8. Texture profile test

The texture of GFB was determined according to the AACCI Method 74-09.01 (AACC, 1995b) using a texture analyzer (TA-XT plus, Stable Micro Systems, UK). A 25 mm thick slice was compressed up to 40% strain at 2.0 mm/s speed. Bread firmness was taken as the force required for compression of the bread sample by 25%. TPA was also performed using the following settings: test speed of 2.0 mm/s with trigger force 20 g to compress bread to 40% of its original height, and the following parameters were measured – hardness, gumminess, chewiness, resilience, springiness and cohesiveness.

5.3. Results and discussion

5.3.1. Frequency sweep

The viscoelastic behavior of all the formulations was determined using oscillatory and creep measurements. Dynamic oscillation measurements require elastic and viscous modulus to be independent of shear stress. Measurements under linear viscoelastic region assures the dough structure is not damaged. The linear viscoelastic region (LVR) was determined using strain sweep test and decline in elastic modulus above 0.6% strain limits LVR indicates breakdown of dough structure beyond this strain level. Similarly, it has been previously found that wheat flour doughs exhibit linear viscoelasticity at strain levels lower than 0.1–0.25% (Phan-Thien and Safari-Ardi, 1998; Weipert, 1990).

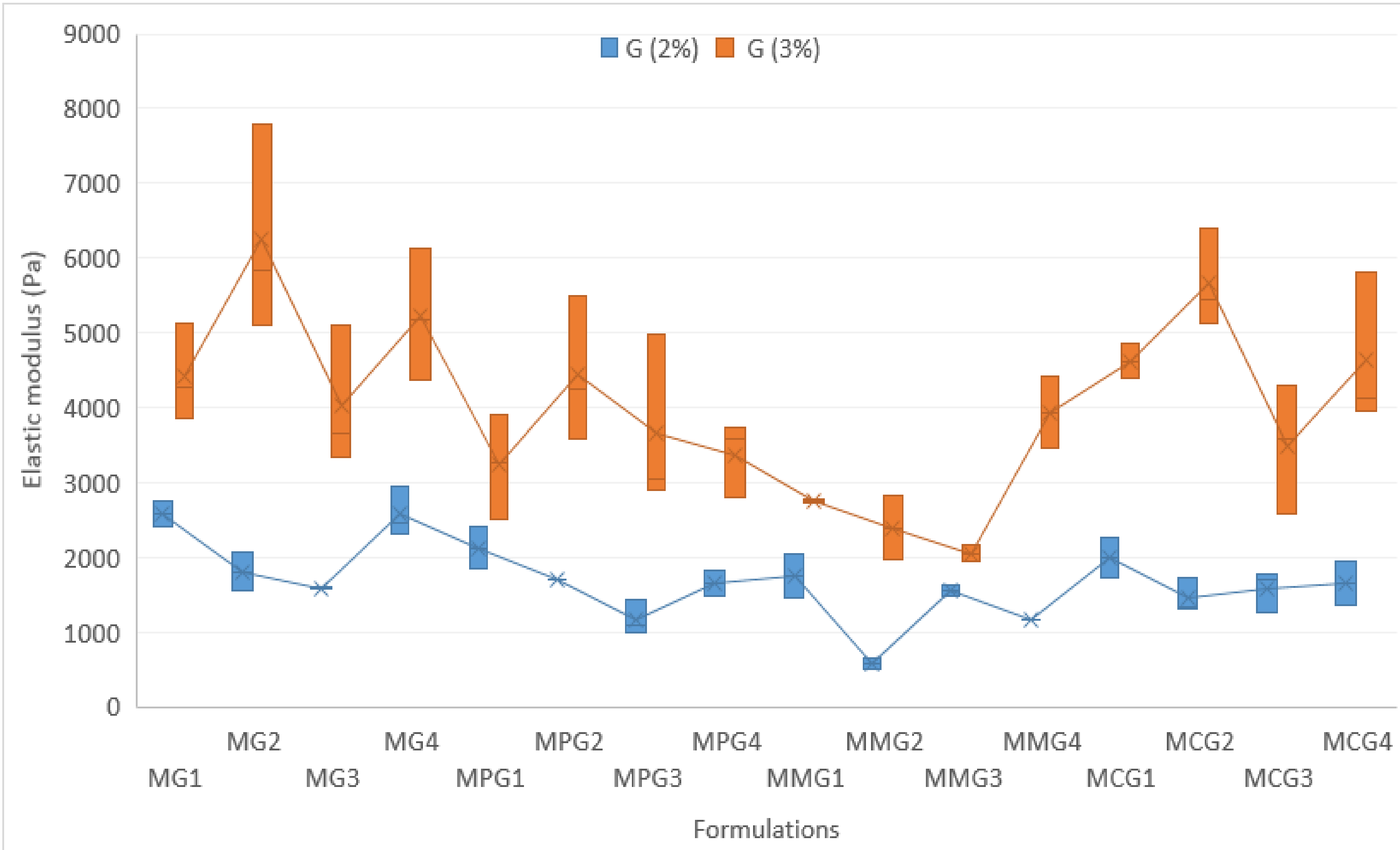


Figure 5.1a. Effect of gluten free formulations on elastic modulus (G') at 1 Hz

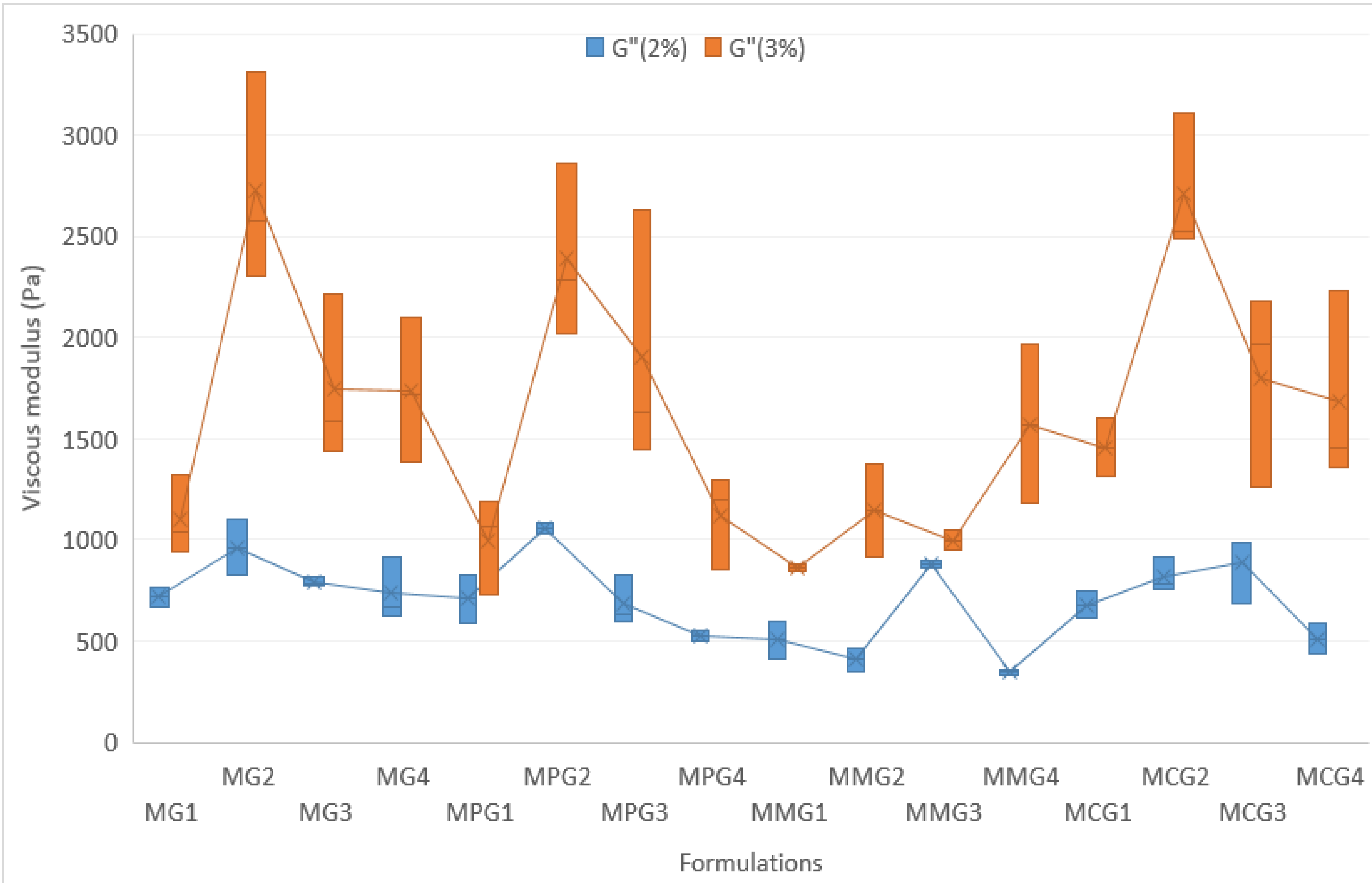


Figure 5.1b. Effect of gluten free formulations on viscous modulus (G'') at 1 Hz

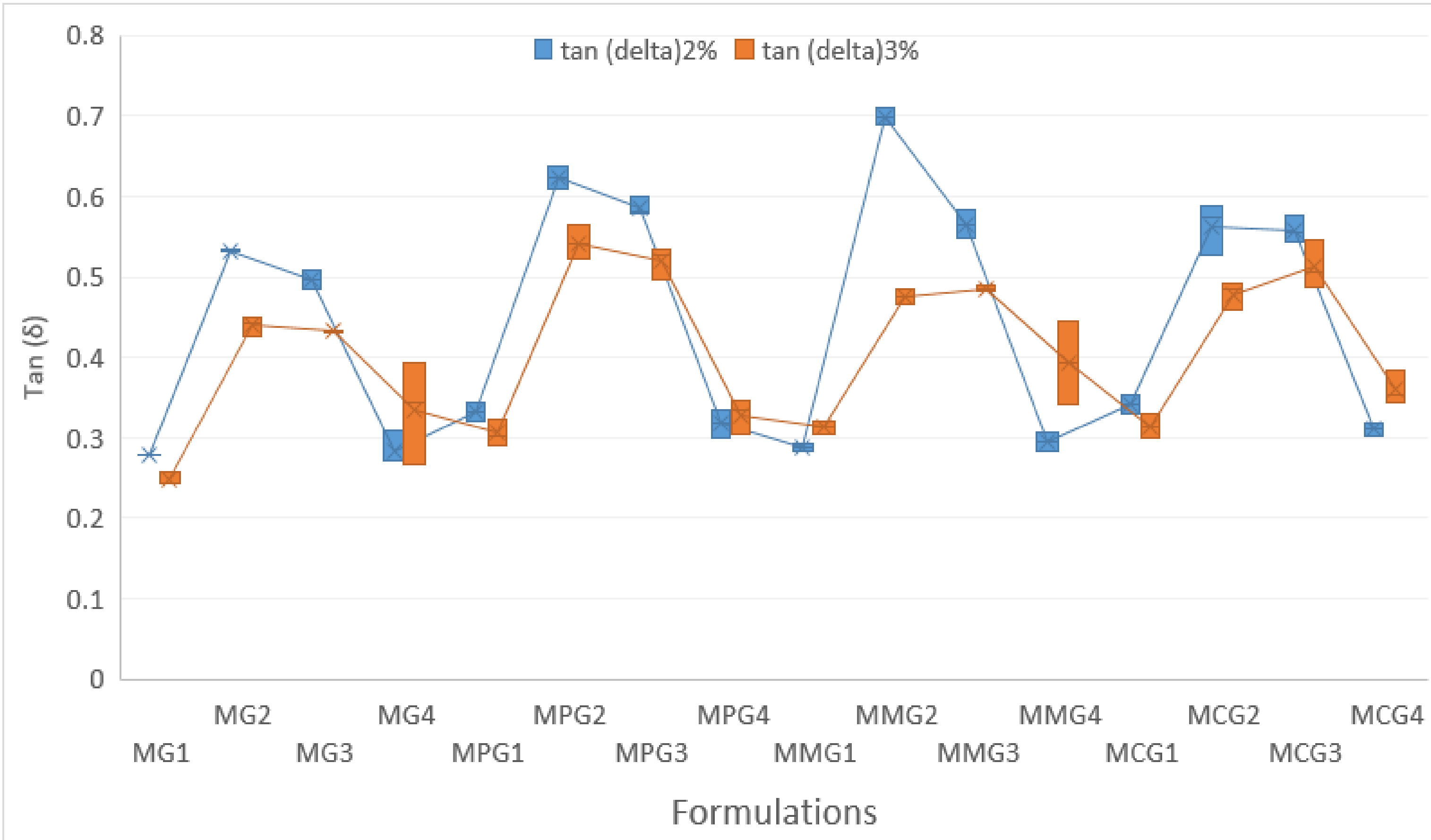


Figure 5.1c. Effect of gluten free formulations on tan(δ) at 1 Hz

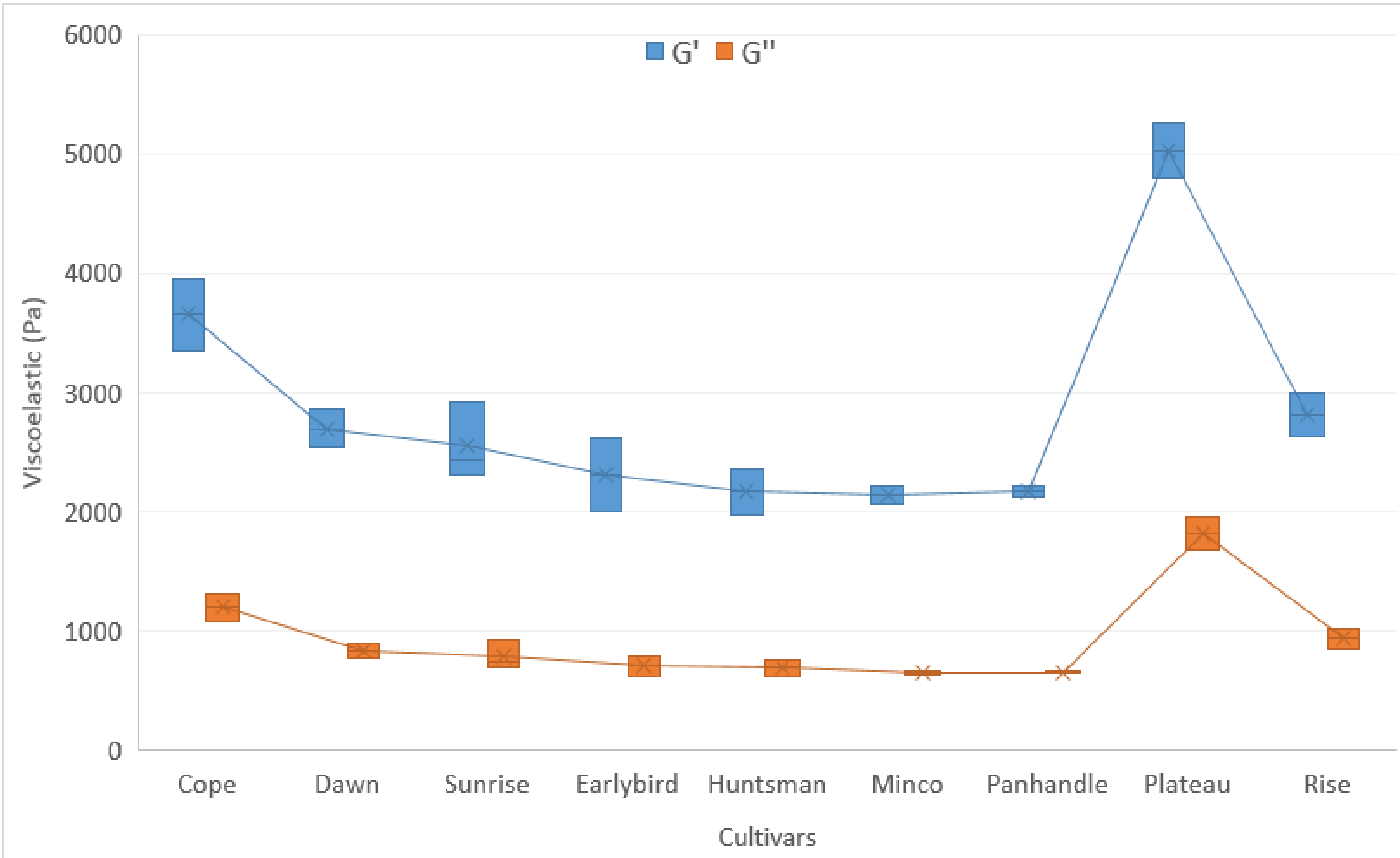


Figure 5.1d. Effect of proso millet cultivars on elastic (Gˈ) and viscous (Gˈˈ) modulus at 1 Hz

The elastic modulus (Gˈ), viscous modulus (Gˈˈ) and tan(δ) of all formulations in LVR at 1 Hz frequency are summarized in Figure 5.1a, 5.1b, 5.1c and 5.1d. All formulations showed high elastic modulus compared to viscous modulus which indicates solid like behavior of doughs. Significant effect ($p<0.05$) of starch, gum and level was observed on Gˈ, Gˈˈ and tan(δ). Interaction effect between gum and level was also significant ($p<0.05$) for all three parameters and interaction between starch and gum was observed for Gˈˈ and tan(δ).

Three different starches were used and compared with whole millet formulation. Whole millet flour formulation had high Gˈ and lower tan(δ) compared to other flour formulations indicating high elastic dough. The addition of starches led to the significant ($p<0.05$) decrease in Gˈ in all formulations but not much effect was observed on Gˈˈ. Hydrocolloid effect was also observed and G3 produced lower values of Gˈ compared to other gums whereas G1 and G4 showed lower values for Gˈˈ. Increase in tan(δ) values with addition of starches indicates higher viscous behavior and effect of gum was also significant ($p<0.05$) on tan(δ). The Gˈ and Gˈˈ both increased with increase in hydrocolloid level but opposite was observed for tan(δ) indicating significantly ($p<0.05$) high elastic behavior of dough at increased level.

Among hydrocolloids at 2% level, G1 made the dough more elastic but no significant difference was observed among them, similarly at 3% level, addition of hydrocolloids showed no significant difference except G3 producing lowest Gˈ. Higher level of elasticity in 3% formulations indicates stronger dough. Tan(δ) values for all the formulations were <1 which indicates that all gluten free formulations have higher elastic behavior in the whole range of frequencies (curves presented in appendix).

Similarly, an increase of Gˈ was reported when HPMC added to rice flour dough (Gujral et al., 2003). Edwards et al. (1999) reported rheological measurements as means of differentiating durum wheat cultivars according to dough strength; higher Gˈ for stronger and least extensible samples. Previous studies suggested that dynamic rheological parameters of dough show little relationship with the functionality during processing and end-use performance (Autio et al., 2001; Phan-Thien and Safari-Ardi, 1998; Wang and Sun, 2002). No significant correlation ($p<0.05$) was observed between baking parameters and frequency sweep test but after excluding data of 3% level hydrocolloids (Figure 5.1e), negative correlation was observed between Gˈ and specific volume ($r = -0.59$, $p<0.005$). Proso millet cultivars also showed higher elastic behavior but waxy starches showed highest Gˈ values which was due to higher water absorption of amylopectin compared to amylose consequently increasing dough elasticity (Hoover, 2000). High correlation was also observed between amylose content and Gˈ ($r = -0.84$) and Gˈˈ ($r = -0.88$).

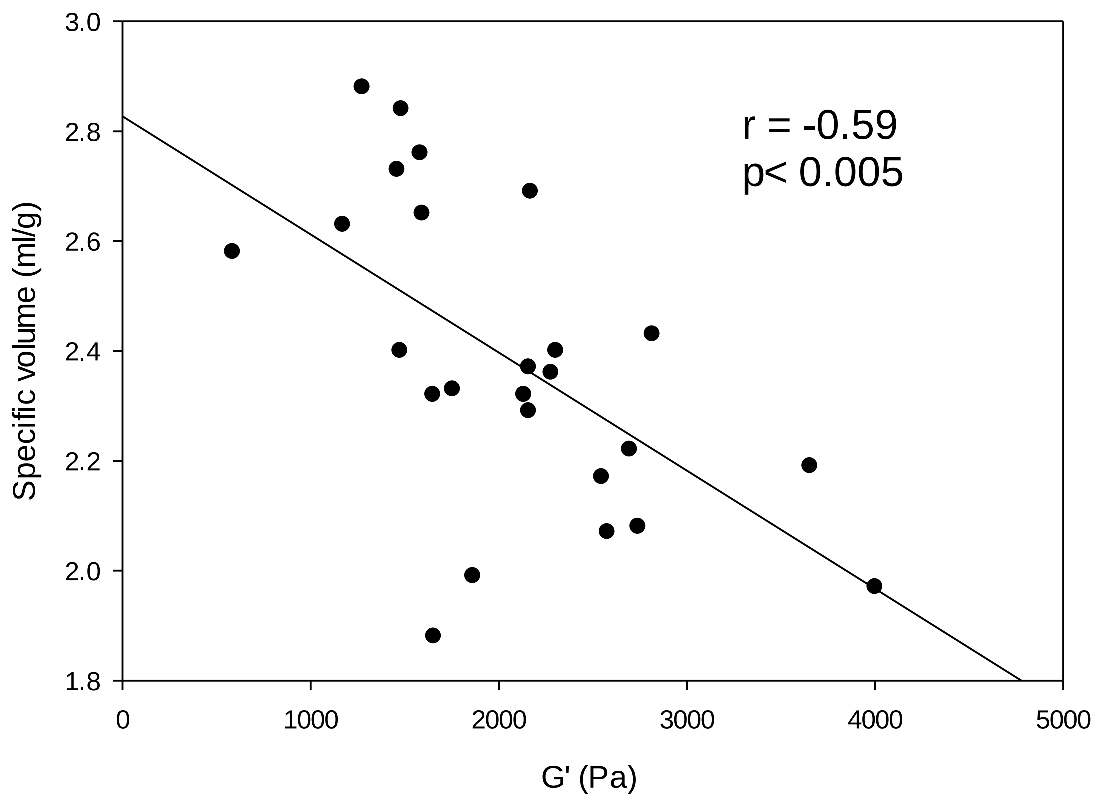


Figure 5.1e. Correlation between Gˈ and specific volume after excluding 3% hydrocolloid formulations data.

5.3.2. Creep and recovery

Creep recovery tests were conducted on all gluten free formulations and presented in Table 5.2a and 5.2b. Higher maximum creep% strain indicates reduced resistance of dough to deformations. The creep-recovery curves of gluten-free doughs showed viscoelastic behavior combining both viscous and elastic components (Steffe, 1996).

At 2% gum level, the addition of starches increased the maximum creep% strain indicating reduced resistance to deformation. With Increased level of hydrocolloids to 3%, decrease in maximum strain% for all the formulations was observed indicating higher resistance to deformation which is due to the increased water absorption capacity of dough with increased hydrocolloids. Hydrocolloid G3 with all formulations showed highest maximum creep% strain whereas G1 and G4 showed highest resistance to deformation. These results are in contrast to the findings of Sivaramakrishnan et al. (2004), where the addition of HPMC at different concentrations into rice flour doughs resulted in creep recovery curves which shifted to higher values compared to the control dough. Wang and Sun (2002) reported high correlation between maximum recovery strains and baking volumes.

Table 5.2a. Effect of proso millet cultivars on maximum creep and recovery %strain.

	Maximum creep (%) strain	Maximum recovery (%) strain
Cope	11.38 + 1.74^{d}	6.56 + 0.38^{f,g}
Dawn	11.40 + 1.25^{d}	7.40 + 0.43^{e}
Earlybird	15.21 + 2.97^{c,d}	8.28 + 0.22^{d}
Huntsman	18.01 + 0.72^{b,c}	9.48 + 0.51^{b,c}
Minco	25.55 + 1.34^{a}	10.81 + 0.47^{a}
Panhandle	21.45 + 4.32^{a,b}	10.08 + 0.13^{a,b}
Plateau	9.96 + 1.10^{d}	5.94 + 0.08^{g}
Rise	18.06 + 3.99^{b,c}	8.73 + 0.57^{c,d}
Sun*rise*	13.62 + 1.44^{c,d}	7.24 + 0.51^{e,f}

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$).

Table 5.2b. Effect of gluten free formulations on maximum creep and recovery %strain.

	2%		3%	
	Maximum creep (%) strain	Maximum recovery (%) strain	Maximum creep (%) strain	Maximum recovery (%) strain
MG1	21.77 + 8.26^{h}	9.10 + 2.31^{d}	10.87 + 0.68^{b,c}	3.60 + 0.06^{f}
MG2	99.02 + 10.50^{d-h}	33.88 + 4.12^{b}	9.82 + 1.17^{b,c}	6.22 + 0.43^{d-f}
MG3	195.15 + 67.79^{b,c}	32.80 + 2.76^{b}	22.93 + 7.47^{b,c}	11.22 + 1.27^{c}
MG4	46.80 + 17.39^{f-h}	11.13 + 1.80^{c,d}	5.06 + 0.25^{c}	3.48 + 0.64^{f}
MCG1	76.99 + 18.45^{d-h}	17.61 + 2.75^{c}	6.43 + 0.45^{c}	5.16 + 0.96^{e,f}
MCG2	133.91 + 35.37^{b-f}	45.62 + 2.09^{a}	14.04 + 1.18^{b,c}	8.81 + 2.02^{c-e}
MCG3	156.17 + 59.38^{b-d}	41.41 + 4.58^{a}	62.05 + 7.26^{a}	16.67 + 4.69^{b}
MCG4	115.42 + 26.83^{c-g}	17.11 + 1.93^{c}	9.55 + 3.09^{b,c}	7.01 + 0.76^{d-f}
MPG1	103.55 + 56.29^{d-h}	18.42 + 4.36^{c}	6.43 + 0.97^{c}	5.10 + 0.75^{e,f}
MPG2	141.83 + 68.16^{b-e}	49.01 + 9.87^{a}	14.88 + 0.07^{b,c}	9.90 + 0.87^{c,d}
MPG3	206.87 + 67.92^{b}	47.28 + 3.36^{a}	26.93 + 3.04^{b}	15.77 + 0.18^{b}
MPG4	28.24 + 10.17^{g,h}	12.64 + 2.09^{c,d}	6.51 + 0.71^{c}	4.98 + 0.29^{e,f}
MMG1	56.50 + 25.17^{e-h}	12.72 + 0.81^{c,d}	19.67 + 0.14^{b,c}	6.58 + 0.37^{d-f}
MMG2	90.99 + 5.46^{d-h}	30.18 + 1.16^{b}	26.49 + 11.64^{b}	17.57 + 6.66^{a,b}
MMG3	346.03 + 155.26^{a}	44.37 + 16.93^{a}	71.78 + 27.49^{a}	20.82 + 1.84^{a}
MMG4	54.13 + 43.05^{e-h}	13.56 + 3.82^{c,d}	13.73 + 5.01^{b,c}	7.62 + 1.15^{c-f}

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$) and a-e indicates a,b,c,d,e

5.3.3. Specific volume

Specific volume of all formulations were compared with whole wheat bread and presented in Table 5.3a and 5.3b. All the formulations showed significantly lower ($p<0.05$) volume compared to wheat bread. Addition of starches significantly ($p<0.05$) increased the volume compared to whole millet formulations except MP which showed significant ($p<0.05$) decrease. Among hydrocolloids, G3 produced the significantly ($p<0.05$) highest volume breads whereas G4 and G1 produced lowest. Increasing hydrocolloid level had no significant effect on volume of breads.

The volume of breads decreased with increase in hydrocolloid level from 2 to 3% excluding gum G1 and G4 in some formulation and significant ($p<0.05$) interaction between gum and level was observed. The volume of loaves ranged from 1.88 to 2.88 ml/g; the highest volume of breads were from MM and MC formulations. MP produced high volume breads when supplemented with G2 (2.84 ml/g) and G3 (2.63 ml /g) but reduced to 1.88 ml/g when G1 or G4 was added. Positive correlation was observed between specific volume and maximum creep% strain ($r = 0.54$) and also with maximum recovery% strain ($r = 0.63$). Effect of amylose was significant ($p<0.05$) on specific volume. Low amylose cultivars produced low volume breads and positive correlation was observed between amylose and specific volume ($r = 0.82$) which indicates that during baking, increased water absorption of amylopectin results in faster swelling of starch granules leads to poor structure holding capacity, hence produced low volume breads. Previously, McCarthy et al. (2005) reported decrease in loaf volume of a rice flour and potato starch based gluten-free bread with increasing levels of HPMC. Schober et al.

(2005) observed decrease in loaf volume of sorghum based gluten-free breads with increasing xanthan gum levels. Hydrocolloids can enhance dough development and gas holding by increasing dough viscosity (Rosell et al., 2001). Hydrocolloids such as CMC have hydrophilic nature enhancing water retention properties, but also contain hydrophobic groups which encourage further properties including increased interfacial activity within the dough system during proofing, and forming gel networks on heating during the bread-making process. Such network structures serve to increase viscosity and to further strengthen the boundaries of the expanding cells in the dough, thus increase gas retention through baking, and consequently lead to a better loaf volume (Bell, 1990).

5.3.4. Bake loss

Bake loss% for all gluten free formulation was observed to be in the range of 16.22 – 21.48%, which was significantly ($p<0.05$) higher than wheat based bread (10.89%). Higher bake loss was due to the high percentage of water used in the formulation.

Table 5.3a. Effect of gluten free formulations on specific volume and bake loss (%)

Sample	Specific volume (ml/g)	Bake loss (%)	Specific volume (ml/g)	Bake loss (%)
	2% level		3% level	
Wheat	3.58 ± 0.03[a]	10.89 ± 0.25[d]	3.58 ± 0.03[a]	10.89 ± 0.2[h]
M G1	2.08 ± 0.03[g]	18.52 ± 0.41[c]	2.34 ± 0.02[e,f]	17.06 ± 0.64[e,f,g]
M G2	2.69 ± 0.16[b,c]	18.94 ± 0.65[c]	2.28 ± 0.03[e,f]	16.25 ± 0.74[f,g]
M G3	2.65 ± 0.08[b,c]	18.98 ± 0.36[c]	2.43 ± 0.01[e,f]	18.36 ± 0.63[b-e]
M G4	2.07 ± 0.17[g]	18.48 ± 0.67[c]	2.29 ± 0.05[e,f]	18.44 ± 0.36[b-e]
MC G1	2.36 ± 0.04[e,f]	19.31 ± 1.36[c]	2.49 ± 0.08[d,e]	17.86 ± 0.76[d,e,f]
MC G2	2.73 ± 0.02[b,c]	18.94 ± 0.76[c]	2.49 ± 0.08[d,e]	17.64 ± 1.59[e,f,g]
MC G3	2.76 ± 0.12[b,c]	21.28 ± 1.70[a]	2.67 ± 0.05[c,d]	18.17 ± 0.83[c,d,e]
MC G4	2.32 ± 0.16[f]	19.01 ± 0.59[b,c]	2.33 ± 0.01[e,f]	17.86 ± 0.61[d,e,f]
MP G1	1.99 ± 0.10[g]	18.06 ± 0.42[c]	1.88 ± 0.23[g]	17.35 ± 0.59[e,f,g]
MP G2	2.84 ± 0.19[b]	18.39 ± 0.50[c]	2.27 ± 0.12[f]	16.14 ± 0.22[g]
MP G3	2.63 ± 0.02[b,c,d]	19.23 ± 1.44[a,b,c]	2.45 ± 0.04[e,f]	17.52 ± 0.95[e,f,g]
MP G4	1.88 ± 0.14[g]	17.75 ± 0.92[c]	1.90 ± 0.17[g]	17.52 ± 1.66[e,f,g]
MM G1	2.33 ± 0.13[f]	21.27 ± 0.52[a]	2.88 ± 0.07[b]	21.02 ± 0.69[a]
MM G2	2.58 ± 0.05[c,d,e]	21.48 ± 1.21[a]	2.42 ± 0.04[e,f]	19.27 ± 0.31[b,c,d]
MM G3	2.88 ± 0.03[b]	21.40 ± 0.83[a]	2.67 ± 0.07[c,d]	19.60 ± 0.26[a,b,c]
MM G4	2.40 ± 0.02[d,e,f]	20.75 ± 1.16[a,b]	2.71 ± 0.13[b,c]	19.89 ± 0.58[a,b]

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$) and a-e indicates a,b,c,d,e.

Table 5.3b. Effect of proso millet cultivars on specific volume and bake loss (%)

	Specific Volume (ml/g)	Bake Loss (%)
Wheat	3.58 ± 0.03^{a}	10.89 ± 0.25^{b}
Cope	$2.19 \pm 0.05^{d,e}$	17.55 ± 1.19^{a}
Dawn	$2.22 \pm 0.05^{c,d,e}$	16.22 ± 2.23^{a}
Earlybird	2.40 ± 0.05^{b}	18.43 ± 1.12^{a}
Huntsman	$2.29 \pm 0.08^{b-e}$	17.91 ± 1.02^{a}
Minco	$2.32 \pm 0.01^{b,c,d}$	18.15 ± 0.53^{a}
Panhandle	$2.37 \pm 0.08^{b,c}$	16.53 ± 1.74^{a}
Plateau	1.97 ± 0.01^{f}	17.96 ± 0.71^{a}
Rise	2.43 ± 0.17^{b}	17.86 ± 1.00^{a}
Sun*rise*	2.17 ± 0.02^{e}	17.21 ± 0.75^{a}

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$) and b-e indicates b,c,d,e.

5.3.5. Bread Color

Crust and crumb color of all the formulations were evaluated using ***L a b*** scale. The L scale measures lightness from dark (0) to white (100), the ***a*** scale extends from red hue (+) to green (-) and the ***b*** scale ranges from yellow (+) to blue (-). Table 5.4a, 5.4b, 5.4c summarizes the color of crust and crumb. Among the formulations, breads with MM formulation produced lighter color crust whereas breads produced from whole millet flour and MP were darker. The ***a*** value of crust were higher for whole millet bread and lowest for breads with millet starch in them. Potato starch formulations showed high ***a*** value compared to other starches. The yellowness of formulations ranged from 26.69 to 35.68 and significantly high for whole millet bread. Hydrocolloids had no significant effect on crust color but increasing the level from 2% to 3% decreased the ***L*** values in whole millet breads and significantly increased the ***a*** values for whole millet, MC and MP breads. Increasing hydrocolloid level showed no considerable effect on ***b*** values.

Starch effect on lightness of crumb color was not significant and ***L*** value ranged from 63.33 to 70.33 whereas G1 produced light color breads with MC and MM formulations 2% level. Both starch and hydrocolloid had no effect on ***a*** values at both levels whereas lower ***b*** value was observed for MC, MP and MM compared to whole millet bread. Increasing level from 2% to 3% increased the lightness values in M, MC and MM breads but no effect on ***a*** and ***b*** values.

Cultivar effect on crust and crumb color is summarized in Table 4c. No significant effect on crust color was observed. Crumb color lightness varied from 63.11 to 70.17, *Minco* producing lightest crumb among cultivars. The ***a*** value reduced for crumb compared to crust color and ***b*** value ranged from 18.85 to 23.23.

Table 5.4a. Effect of gluten free formulations on crust color

Sample	2% level			3% level		
	L	a	b	L	a	b
Wheat	43.86 + 2.02^{g}	9.41 + 0.39^{a}	25.35 + 1.61^{g}	43.86 + 2.02^{e}	9.41 + 0.39^{b}	25.35 + 1.61^{f}
MG1	56.70 + 2.99^{c,d}	6.71 + 0.29^{b,c}	34.82 + 1.29^{a,b}	52.72 + 5.00^{d}	6.93 + 1.46^{d}	33.74 + 2.09^{a,b}
MG2	52.67 + 1.85^{e,f}	7.20 + 1.55^{b}	32.58 + 3.04^{b-e}	44.14 + 2.89^{e}	11.27 + 0.64^{a}	33.21 + 1.42^{b,c}
MG3	51.36 + 2.64^{e,f}	7.69 + 1.25^{b}	32.65 + 2.10^{b-e}	46.68 + 1.74^{e}	9.94 + 0.90^{b}	30.51 + 1.26^{d,e}
MG4	55.10 + 3.54^{c-e}	6.42 + 0.64^{b,c}	34.27 + 1.51^{a,b}	53.33 + 2.18^{d}	8.39 + 0.84^{c}	33.93 + 1.45^{a,b}
MCG1	66.79 + 4.43^{b}	0.82 + 0.96^{g}	32.46 + 1.78^{b-e}	66.63 + 2.12^{c}	2.71 + 1.09^{f}	34.87 + 1.50^{a,b}
MCG2	65.13 + 5.22^{b}	0.45 + 0.84^{g,h}	30.27 + 3.47^{e,f}	64.65 + 1.92^{c}	3.32 + 1.02^{f}	34.69 + 1.83^{a,b}
MCG3	65.67 + 4.10^{b}	-0.21 + 2.38^{g,h}	29.87 + 4.18^{f}	65.14 + 3.41^{c}	2.72 + 1.91^{f}	33.98 + 2.01^{a,b}
MCG4	68.48 + 1.70^{b}	0.94 + 0.76^{g}	33.79 + 0.84^{a-c}	63.26 + 2.76^{c}	3.21 + 0.71^{f}	34.42 + 1.29^{a,b}
MPG1	57.56 + 4.88^{c,d}	2.96 + 1.50^{f}	31.74 + 2.94^{c-f}	55.98 + 3.25^{d}	6.06 + 0.60^{d,e}	35.40 + 1.98^{a}
MPG2	54.60 + 2.94^{d-f}	5.81 + 1.06^{c,d}	35.68 + 1.50^{a}	54.74 + 5.06^{d}	6.84 + 0.86^{d}	34.81 + 2.50^{a,b}
MPG3	51.15 + 5.69^{f}	5.08 + 1.70^{d,e}	31.32 + 2.33^{d-f}	53.62 + 5.86^{d}	6.75 + 1.08^{d}	34.92 + 2.46^{a,b}
MPG4	58.69 + 5.06^{c}	4.16 + 1.82^{e}	33.34 + 1.47^{a-d}	56.19 + 4.92^{d}	5.44 + 1.03^{e}	34.45 + 2.45^{a,b}
MMG1	73.17 + 2.00^{a}	-0.78 + 1.28^{h,i}	31.20 + 2.17^{d-f}	72.23 + 3.63^{b}	-0.14 + 0.70^{g}	31.22 + 1.01^{d}
MMG2	73.71 + 2.83^{a}	-2.09 + 0.92^{j}	27.28 + 2.53^{g}	75.19 + 3.13^{a,b}	-2.24 + 3.13^{i}	25.68 + 2.26^{f}
MMG3	73.79 + 3.84^{a}	-2.46 + 0.74^{j}	26.69 + 1.72^{g}	75.86 + 1.30^{a}	-1.19 + 0.97^{h}	29.14 + 1.73^{e}
MMG4	75.15 + 1.87^{a}	-1.53 + 0.74^{i,j}	29.61 + 1.37^{f}	73.81 + 0.93^{a,b}	0.13 + 0.33^{g}	31.80 + 0.41^{c,d}

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$) and a-e indicates a,b,c,d,e

Table 5.4b. Effect of gluten free formulations on crumb color

Sample	2% level			3% level		
	L	a	b	L	a	b
Wheat	57.81 + 4.10^{f}	2.52 + 0.46^{a}	26.59 + 1.34^{b}	57.81 + 4.07^{h}	2.52 + 0.47^{a}	26.59 + 1.34^{c}
MG1	68.50 + 2.55^{a-c}	-6.67 + 0.33^{c}	31.16 + 0.98^{a}	72.57 + 1.03^{a}	-7.15 + 0.24^{c,d}	31.21 + 0.52^{b}
MG2	68.37 + 1.82^{a-c}	-7.36 + 0.22^{c}	30.89 + 0.72^{a}	72.39 + 1.99^{a}	-7.30 + 0.31^{c,d}	32.34 + 1.43^{a}
MG3	66.45 + 4.21^{a-e}	-7.35 + 0.56^{c}	28.96 + 1.43^{a}	70.01 + 2.59^{a-d}	-7.14 + 0.27^{c,d}	30.40 + 1.05^{b}
MG4	67.66 + 3.72^{a-d}	-6.76 + 0.37^{c}	31.19 + 1.24^{a}	71.43 + 2.76^{a,b}	-6.68 + 0.41^{b}	30.43 + 1.66^{b}
MCG1	67.01 + 2.43^{a-e}	-7.12 + 0.44^{c}	25.63 + 1.31^{b,c}	68.90 + 3.87^{b-e}	-7.21 + 0.41^{c,d}	24.67 + 1.04^{d,e}
MCG2	63.33 + 4.62^{e}	-3.99 + 3.34^{b}	30.03 + 6.98^{a}	68.82 + 2.60^{b-e}	-7.69 + 0.31^{e-g}	25.45 + 1.13^{c,d}
MCG3	66.55 + 2.81^{a-e}	-7.56 + 0.32^{c}	24.24 + 1.42^{b-d}	65.64 + 1.68^{f,g}	-7.29 + 0.17^{c,d}	24.48 + 0.73^{d-f}
MCG4	64.44 + 5.56^{c-e}	-7.05 + 0.66^{c}	25.12 + 1.72^{b-d}	69.84 + 4.22^{a-d}	-7.39 + 0.45^{c-e}	25.87 + 1.59^{c}
MPG1	64.05 + 5.49^{d,e}	-6.78 + 0.62^{c}	24.66 + 2.20^{b-d}	64.73 + 4.60^{g}	-6.74 + 0.49^{b}	24.32 + 1.56^{d-f}
MPG2	66.42 + 3.19^{a-e}	-7.39 + 0.37^{c}	24.97 + 0.80^{b-d}	68.85 + 2.47^{b-e}	-7.47 + 0.28^{d-f}	26.06 + 1.64^{c}
MPG3	65.49 + 5.56^{b-e}	-7.54 + 0.54^{c}	22.85 + 2.17^{d}	68.13 + 2.76^{c-f}	-7.46 + 0.32^{d-f}	24.26 + 0.75^{d-f}
MPG4	64.15 + 3.11^{d,e}	-6.80 + 0.22^{c}	24.51 + 1.38^{b-d}	66.27 + 2.05^{e-g}	-7.08 + 0.29^{c}	23.75 + 0.99^{e,f}
MMG1	70.33 + 1.41^{a}	-7.59 + 0.13^{c}	24.51 + 0.67^{b-d}	71.27 + 2.92^{a-c}	-7.77 + 0.31^{g,f}	24.15 + 0.36^{e,f}
MMG2	67.13 + 3.07^{a-e}	-7.57 + 0.36^{c}	24.47 + 0.72^{b-d}	68.01 + 3.02^{d-f}	-7.75 + 0.30^{g,f}	23.85 + 0.47^{e,f}
MMG3	66.82 + 3.52^{a-e}	-7.73 + 0.39^{c}	23.69 + 0.97^{c,d}	69.10 + 3.57^{b-e}	-7.91 + 0.43^{g}	24.03 + 1.25^{e,f}
MMG4	69.36 + 1.49^{a,b}	-7.66 + 0.22^{c}	23.97 + 0.48^{c,d}	70.42 + 1.53^{a-d}	-7.66 + 0.21^{e-g}	23.21 + 0.95^{f}

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$) and a-e indicates a,b,c,d,e.

Table 5.4c. Effect of Cultivar on crust and crumb color

Cultivar	Crust		Crumb			
	L	a	b	L	a	b
Wheat	$43.86 + 2.02^{c}$	$9.41 + 0.39^{a}$	$25.35 + 1.61^{b}$	$57.81 + 4.10^{e}$	$2.52 + 0.46^{a}$	$26.59 + 1.34^{a}$
Cope	$65.89 + 3.73^{a,b}$	$0.36 + 1.79^{b,c}$	$31.41 + 4.56^{a}$	$69.93 + 1.91^{a,b}$	$-6.28 + 0.24^{b}$	$18.85 + 0.58^{f}$
Dawn	$63.82 + 4.94^{a,b}$	$1.76 + 1.21^{b}$	$33.02 + 2.94^{a}$	$68.54 + 1.66^{a-c}$	$-6.32 + 0.18^{b}$	$20.82 + 0.64^{d,e}$
Earlybird	$65.04 + 4.78^{a,b}$	$0.36 + 0.94^{b,c}$	$31.38 + 3.00^{a}$	$67.70 + 2.58^{a-c}$	$-6.78 + 0.23^{c}$	$21.37 + 0.75^{c,d}$
Huntsman	$61.89 + 6.93^{b}$	$1.47 + 2.48^{b}$	$31.84 + 4.63^{a}$	$66.16 + 2.58^{c}$	$-7.09 + 0.25^{d}$	$20.94 + 0.99^{d,e}$
Minco	$62.27 + 7.90^{b}$	$0.95 + 0.70^{b,c}$	$31.51 + 4.19^{a}$	$70.17 + 1.25^{a}$	$-7.67 + 0.15^{e}$	$23.23 + 0.80^{b}$
Panhandle	$65.31 + 4.68^{a,b}$	$0.53 + 1.42^{b,c}$	$31.42 + 3.01^{a}$	$68.97 + 2.77^{a,b}$	$-6.90 + 0.28^{c,d}$	$19.33 + 0.79^{f}$
Plateau	$68.35 + 5.29^{a}$	$-0.35 + 0.81^{c}$	$30.72 + 2.96^{a}$	$63.11 + 2.91^{d}$	$-7.08 + 0.33^{d}$	$23.48 + 1.08^{b}$
Rise	$62.78 + 6.67^{b}$	$1.17 + 1.31^{b}$	$31.20 + 3.50^{a}$	$68.54 + 2.38^{a-c}$	$-6.50 + 0.22^{b}$	$20.42 + 0.60^{e}$
Sun*rise*	$66.15 + 6.06^{a,b}$	$1.50 + 2.39^{b}$	$32.78 + 3.16^{a}$	$67.49 + 2.24^{b,c}$	$-7.00 + 0.29^{c,d}$	$21.85 + 1.11^{c}$

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$) and a-e indicates a,b,c,d,e.

5.3.6. Textural properties

Firmness of all the formulation were compared with whole wheat bread over 5 days' period and illustrated in Figures 5.2a, 5.2b and 5.2c. Firmness was found to be high compared to wheat bread for all formulations, and after 2 days sharp increase was observed in the firmness, which can be attributed to staling and increased retrogradation rate. Effect of starch was significant ($p<0.05$) on bread firmness as MC and MP bread produced low firmness value bread whereas MM had similar firmness compared to whole millet formulations. Hydrocolloids and their level showed no significant change in bread firmness, whereas interaction effect of starch-gum and starch-level of hydrocolloid is significant ($p<0.05$).

Among the formulations, G3 had lower firmness in M formulations, G2 had lower firmness in MC and MP formulations whereas G4 showed low firmness in MM formulations. High firmness values at day 5 indicates the low shelf life of product and high rate of staling which might be due to increased percentage of starch in formulation. Cultivar effect was also observed and illustrated in figure 5.2a. Waxy cultivar produced low firmness bread compared to high amylose cultivars and positive correlation between amylose and firmness (not significant) was observed ($r= 0.51$). In previous studies, it has been found that addition of some hydrocolloids, such as CMC, carrageenan, and alginate, causes crumb softening of wheat bread, while inclusion of xanthan results in an increase of crumb hardness (Bell, 1990; Rosell et al., 2001).

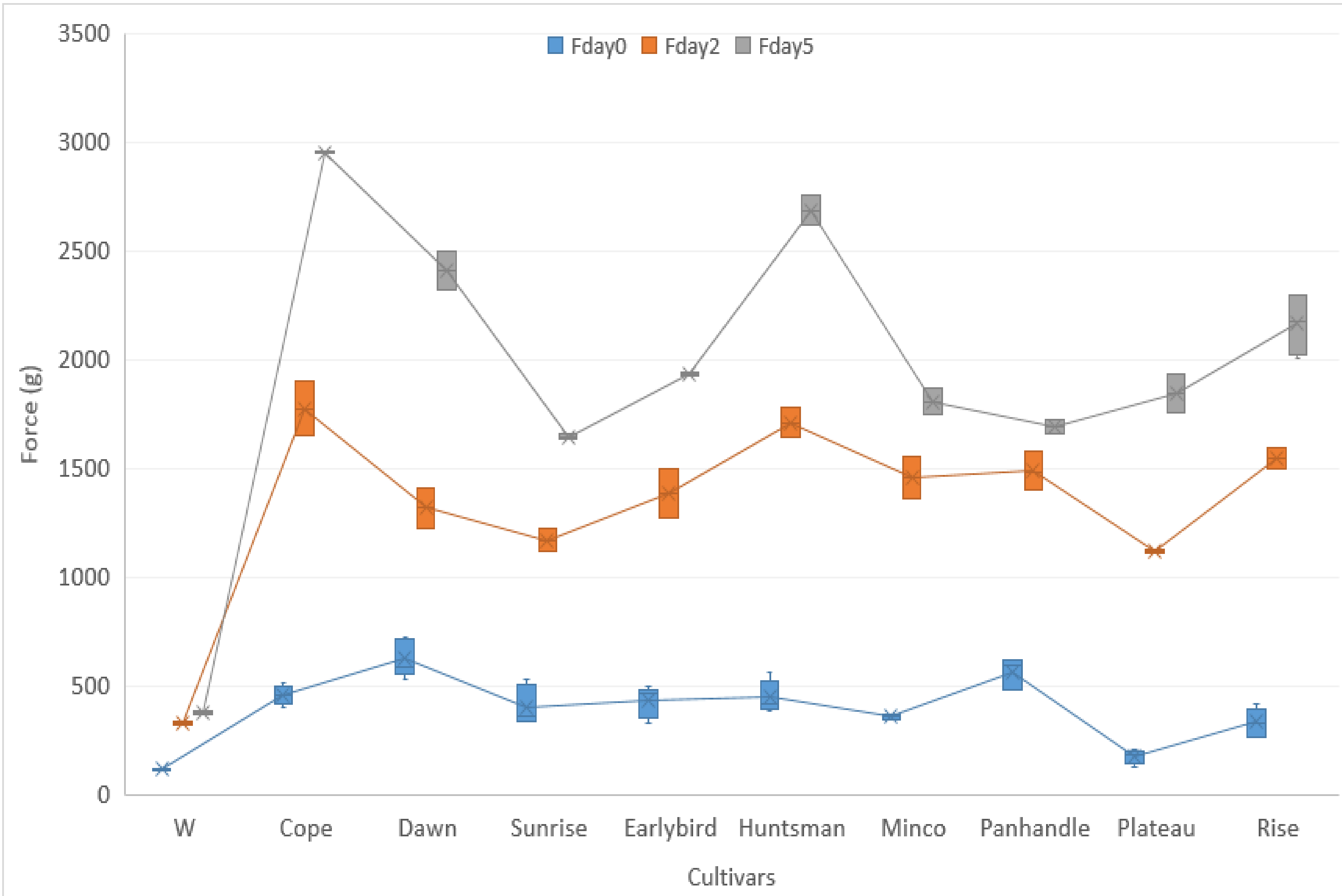


Figure 5.2a. Effect of proso millet cultivars on bread firmness

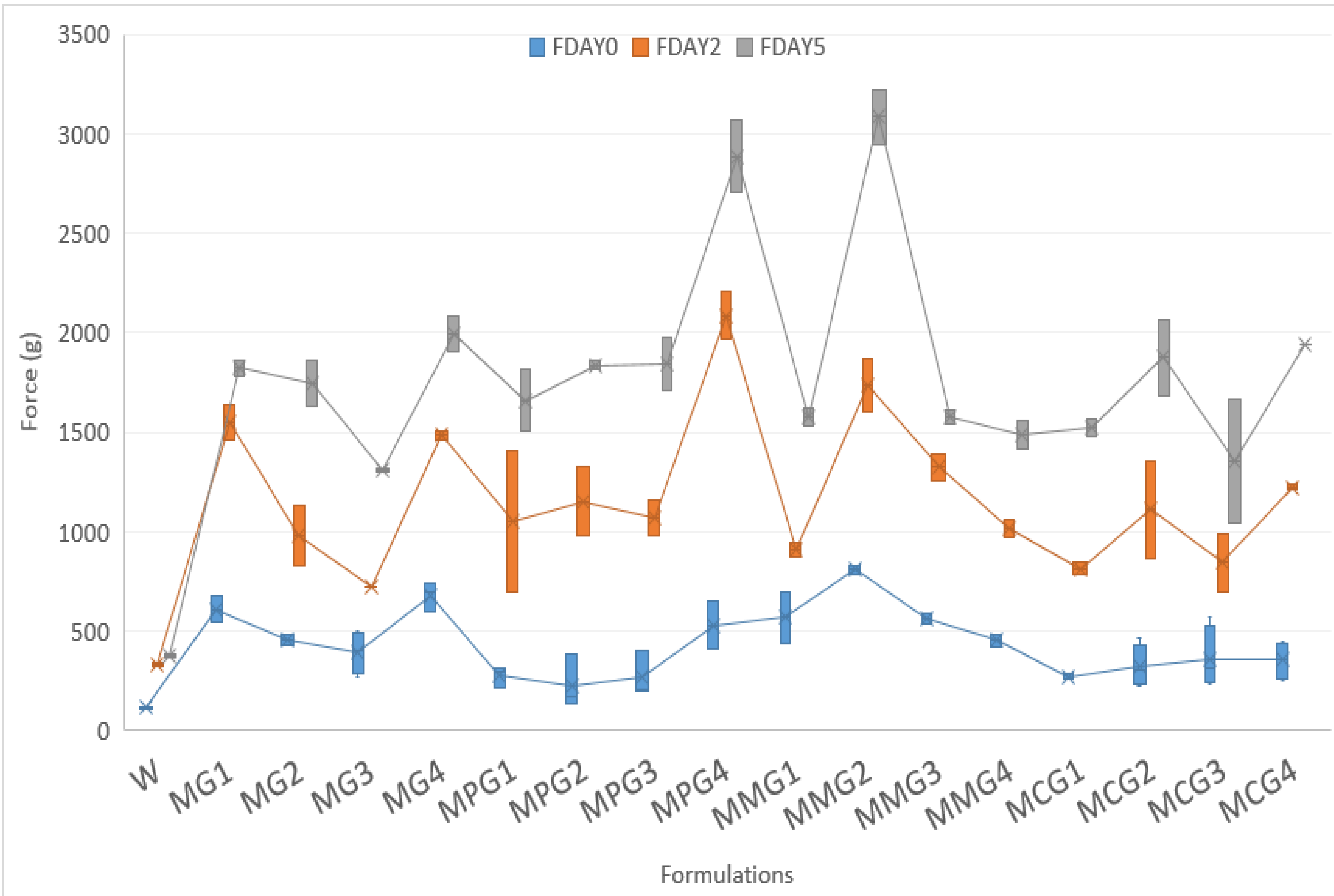


Figure 5.2b. Effect of gluten free formulations (at 2% level) on bread firmness

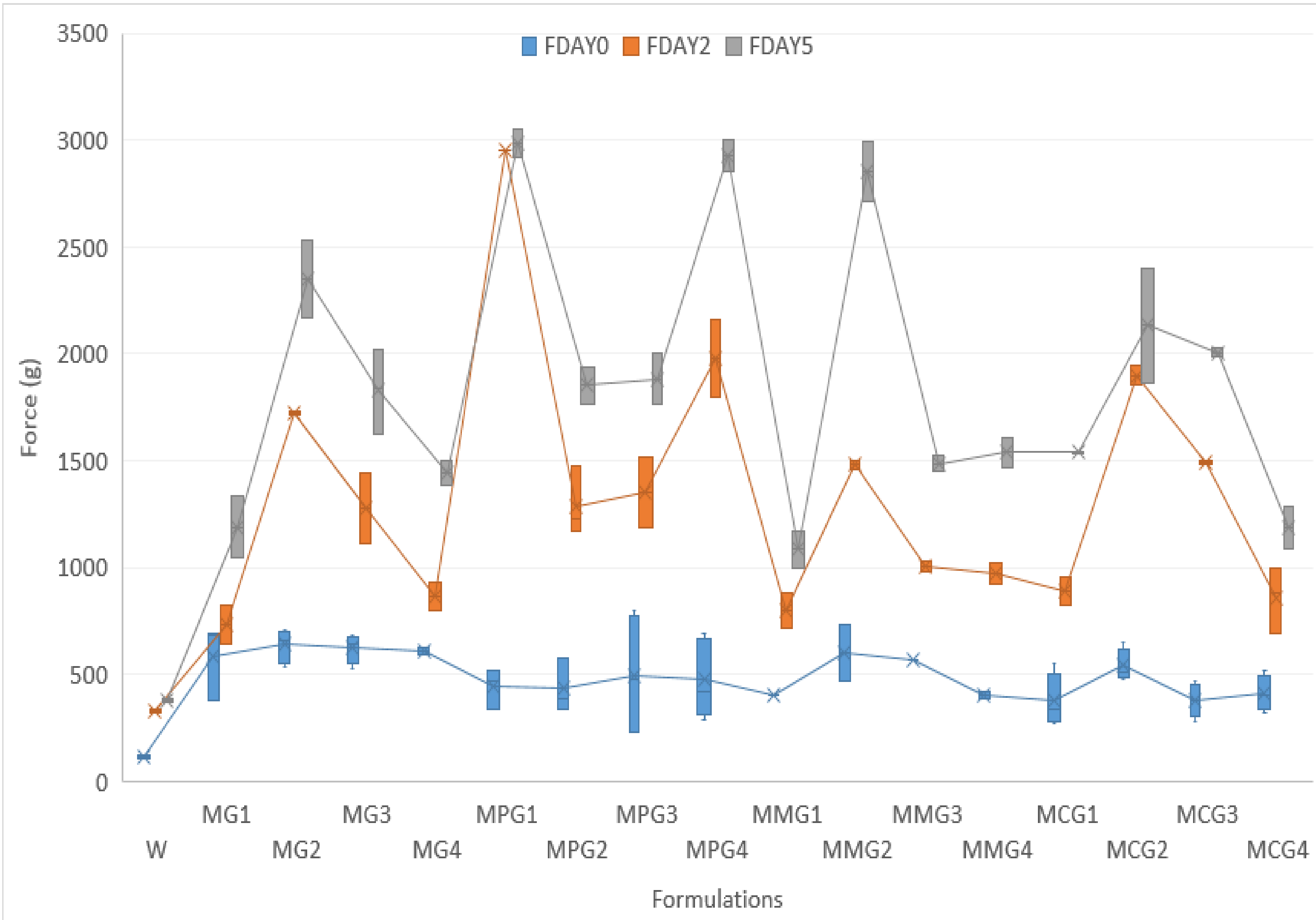


Figure 5.2c. Effect of gluten free formulations (at 3% level) on bread firmness

Texture parameters for bread from TPA, which include hardness, chewiness, gumminess, cohesiveness, springiness and resilience, are illustrated in Figures 5.5a-5.5i. All the formulations showed higher hardness, gumminess and chewiness values compared to wheat bread on all observed days (0,2,5). Effect of starch and gum was significant ($p<0.05$) on all TPA parameters whereas effect of level was observed only on hardness and gumminess.

On addition of starch, hardness values decreased significantly ($p<0.05$) except MM whereas increasing level of hydrocolloids showed significant ($p<0.05$) increase in hardness. Among hydrocolloids, G2 showed significantly higher hardness ($p<0.05$) compared to others. Hardness showed significant ($p<0.05$) time effect and increased upon storage whereas gumminess and chewiness had no time effect. Springiness of all formulations decreased with time whereas no change was observed in wheat bread, which indicates shorter shelf-life of gluten free breads compared to wheat. Sharp decrease in cohesiveness was also observed indicating the weak structure of bread. Unlike wheat bread, resilience decreased exponentially with time indicating the less elasticity of gluten free breads. Biliaderis et al. (1995) concluded that the effect of hydrocolloids on starch gel structure can be described by decrease in swelling of starch and limited amylose leaching from the granules resulting in increased rigidity of dough which determine the overall effect of hydrocolloids on mechanical properties of the bread structure. Cultivar effect was also observed to be significant with textural properties. Amylose content showed significant correlation with Gumminess ($r = 0.67$), Chewiness ($r = 0.73$), Resilience ($r = 0.83$). Waxy millet showed low hardness values compared to other cultivars. Similarly, Schober et al. (2005) reported an increase of crumb hardness with xanthan gum concentration in gluten-free breads from sorghum.

Table 5.5a. Effect of cultivars on different TPA parameters on day0

Cultivars	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)	Resilience
Wheat	226.87 ± 8.49^{d}	0.92 ± 0.01^{c}	0.74 ± 0.01^{a}	169.09 ± 7.07^{c}	158.02 ± 3.54^{b}	0.32 ± 0.02^{c}
Cope	606.30 ± 12.87^{a,b}	0.91 ± 0.07^{c}	0.66 ± 0.04^{b-e}	399.19 ± 65.82^{a}	362.21 ± 43.80^{a,b}	0.33 ± 0.03^{b,c}
Dawn	641.18 ± 45.78^{a}	0.95 ± 0.03^{b,c}	0.64 ± 0.02^{d,e}	413.07 ± 37.70 ^{a}	390.92 ± 42.93^{a}	0.34 ± 0.02^{b,c}
Earlybird	640.25 ± 66.79^{a}	0.98 ± 0.02^{a,b}	0.66 ± 0.03^{b-e}	424.67 ± 43.09^{a}	415.97 ± 43.58^{a}	0.36 ± 0.02^{a-c}
Huntsman	488.71 ±125.71^{b,c}	0.99 ± 0.01^{a,b}	0.62 ± 0.02^{e}	304.10 ± 89.75^{b}	299.65 ± 87.41^{b}	0.33 ± 0.02^{b,c}
Minco	650.89 ± 43.15^{a}	1.01 ± 0.03^{a}	0.68 ± 0.02^{b-d}	440.73 ± 15.29^{a}	446.07 ± 17.84^{a}	0.37 ± 0.02^{a,b}
Panhandle	499.41 ±100.43^{b,c}	1.02 ± 0.04^{a}	0.71 ± 0.03^{a-c}	353.20 ± 78.11^{a,b}	360.82 ± 89.63^{a,b}	0.40 ±0.03^{a}
Plateau	448.51 ± 47.45^{c}	0.98 ± 0.01^{a,b}	0.65 ± 0.03^{c-e}	292.15 ± 31.83	287.37 ± 30.05^{b}	0.27 ± 0.04^{d}
Rise	576.27 ± 82.53^{a-c}	0.97 ± 0.02^{a-c}	0.71 ± 0.04^{a,b}	409.42 ± 50.92^{a}	395.56 ± 49.49^{a}	0.39 ± 0.03^{a}
Sunrise	476.92 ± 53.32^{b,c}	0.95 ± 0.01^{b,c}	0.64 ± 0.01^{d,e}	306.59 ± 35.25^{b}	290.43 ± 35.15^{b}	0.34 ± 0.01^{b,c}

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$) and a-e indicates a,b,c,d,e.

Table 5.5b. Effect of cultivars on different TPA parameters on day2

Cultivars	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)	Resilience
Wheat	634.06 ± 88.77^{d}	0.86 ± 0.01^{a}	0.63 ± 0.05^{a}	370.79 ± 42.28^{a,b}	305.57 ± 12.12^{c,d}	0.25 ± 0.02^{a}
Cope	2563.78 ± 24.34^{a}	0.78 ± 0.08^{a,b}	0.31 ± 0.01^{c}	532.86 ± 50.69^{a}	548.71 ± 11.73^{a}	0.12 ± 0.01^{c,d}
Dawn	1600.18 ± 15.96^{b,c}	0.74 ± 0.08^{a,b}	0.29 ± 0.03^{c,d}	466.22 ± 48.99^{a,b}	345.37 ± 73.61^{b,c}	0.11 ± 0.01^{c,d}
Earlybird	1476.63 ± 82.93^{b,c}	0.68 ± 0.02^{a-c}	0.31 ± 0.01^{c}	450.94 ± 18.61^{a,b}	305.16 ± 1.34^{c,d}	0.12 ± 0.01^{b,c}
Huntsman	1810.37 ± 144.78^{b}	0.50 ± 0.01^{c}	0.20 ± 0.01^{e}	368.92 ± 7.16^{a,b}	188.47 ± 3.42^{e}	0.08 ± 0.01^{e}
Minco	1477.54 ± 151.26^{b,c}	0.79 ± 0.17^{a}	0.26 ± 0.01^{c,d}	380.09 ± 18.71^{a,b}	300.36 ± 49.32^{c,d}	0.10 ± 0.01^{d,e}
Panhandle	1824.04 ± 165.51^{b}	0.73 ± 0.15^{a,b}	0.30 ± 0.01^{c}	456.87 ± 171.14^{a,b}	318.09 ± 53.31^{c,d}	0.12 ± 0.01^{b-d}
Plateau	1345 ± 108.47^{c}	0.84 ± 0.01^{a}	0.38 ± 0.01^{b}	506.34 ± 58.75^{a,b}	424.59 ± 42.40^{b}	0.14 ± 0.01^{b}
Rise	1617.45 ± 369.72^{b,c}	0.58 ± 0.01^{b,c}	0.24 ± 0.01^{d,e}	391.86 ± 79.43^{a,b}	228.32 ± 41.49^{d,e}	0.09 ± 0.01^{e}
Sunrise	1240.94 ± 113.42^{c}	0.72 ± 0.01^{a,b}	0.30 ± 0.01^{c}	359.46 ± 5.17^{b}	252.70 ± 6.47^{c-e}	0.13 ± 0.01^{b,c}

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$) and a-e indicates a,b,c,d,e.

Table 5.5c. Effect of cultivars on different TPA parameters on day5

Cultivars	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)	Resilience
Wheat	870.93 ± 2.16^{e}	0.92 ± 0.01^{a}	0.66 ± 0.74^{a}	529.90 ± 1.43^{a}	480.45 ± 1.52^{a}	0.22 ± 0.01^{a}
Cope	3033.53 ± 159.80^{a}	0.70 ± 0.02^{b}	0.21 ± 0.01^{c,d}	599.37 ± 78.17^{a}	395.47 ± 69.89^{a,b}	0.09 ± 0.01^{c,d}
Dawn	2365.90 ± 353.83^{b,c}	0.71 ± 0.06^{b}	0.22 ± 0.02$^{b\text{-}d}$	533.14 ± 137.55^{a}	382.42 ± 126.93^{a,b}	0.09 ± 0.01^{c,d}
Earlybird	2107.35 ± 83.01^{c}	0.70 ± 0.06^{b}	0.24 ± 0.04$^{b\text{-}d}$	515.09 ± 109.30^{a}	364.80 ± 107.76^{a,b}	0.10 ± 0.01^{b,c}
Huntsman	2061.90 ± 72.11^{c,d}	0.71 ± 0.02^{b}	0.26 ± 0.01$^{b\text{-}d}$	502.11 ± 15.87^{a}	359.34 ± 6.28^{a,b}	0.12 ± 0.01^{b}
Minco	2491.70 ± 191.08^{b,c}	0.72 ± 0.10^{b}	0.22 ± 0.01$^{b\text{-}d}$	548.34 ± 4.12^{a}	394.84 ± 50.25^{a,b}	0.09 ± 0.01^{c,d}
Panhandle	2668.95 ± 137.10^{a,b}	0.67 ± 0.01^{b}	0.29 ± 0.02^{b}	682.92 ± 116.87^{a}	459.09 ± 88.38^{a}	0.12 ± 0.02^{b}
Plateau	2118.92 ± 74.79^{c}	0.77 ± 0.13^{a,b}	0.29 ± 0.03^{b,c}	609.83 ± 42.12^{a}	471.58 ± 110.06^{a}	0.11 ± 0.01^{b}
Rise	2374.20 ± 240.57^{b,c}	0.72 ± 0.12^{b}	0.22 ± 0.03$^{b\text{-}d}$	524.03 ± 27.24^{a}	379.51 ± 79.92^{a,b}	0.09 ± 0.01^{c,d}
Sunrise	1677.75 ± 102.99^{d}	0.70 ± 0.01^{b}	0.19 ± 0.01^{d}	312.17 ± 2.86^{b}	217.11 ± 8.31^{b}	0.08 ± 0.01^{d}

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$) and a-e indicates a,b,c,d,e.

Table 5.5d. Effect of different formulations at hydrocolloid level 2% on different TPA parameters on day0

Formulation	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)	Resilience
Wheat	226.87 ± 8.49[i]	0.92 ± 0.01[b,c]	0.74 ± 0.01[a,b]	169.09 ± 7.07[g]	158.02 ± 3.54[g,h]	0.32 ± 0.02[c-e]
MG1	900.55 ± 25.86[b]	0.93 ± 0.02[b,c]	0.57 ± 0.02[d-g]	517.64 ± 37.30[b]	481.30 ± 45.50[c-e]	0.29 ± 0.02[e,f]
MG2	790.55 ± 133.85[b-d]	1.76 ± 0.10[a]	0.68 ± 0.02[b,c]	539.37 ± 103.68[b]	954.02 ± 237.31[a]	0.39 ± 0.01[b]
MG3	535.95 ± 65.78[e,f]	1.03 ± 0.07[b,c]	0.69 ± 0.01[b,c]	367.27 ± 48.76[c-e]	381.56 ± 78.37[d-g]	0.40 ± 0.01[b]
MG4	850.89 ± 147.02[b,c]	0.89 ± 0.03[b,c]	0.56 ± 0.05[e-h]	470.79 ± 81.55[b-d]	419.42 ± 72.45[d-f]	0.27 ± 0.04[e-g]
MCG1	429.58 ± 39.68[f-i]	0.92 ± 0.06[b,c]	0.60 ± 0.06[d-f]	255.02 ± 7.91[e-g]	235.03 ± 7.13[f-h]	0.31 ± 0.05[d-f]
MCG2	642.28 ± 149.07[c-f]	1.43 ± 0.79[a,b]	0.75 ± 0.02[a]	481.75 ± 104.12[b,c]	635.88 ± 187.29[b,c]	0.46 ± 0.02[a]
MCG3	587.17 ± 188.99[d-f]	1.28 ± 0.33[a-c]	0.77 ± 0.02[a]	453.11 ± 139.64[b-d]	581.98 ± 242.95[b-d]	0.48 ± 0.02[a]
MCG4	532.45 ± 98.01[e,f]	0.94 ± 0.05[b,c]	0.61 ± 0.04[d,e]	322.21 ± 57.88[e]	301.99 ± 50.45[e-h]	0.32 ± 0.03[c-e]
MPG1	472.29 ± 49.93[e-h]	0.82 ± 0.12[c]	0.54 ± 0.02[f-h]	254.99 ± 21.16[e-g]	207.49 ± 26.25[f-h]	0.22 ± 0.02[h,i]
MPG2	519.39 ± 61.35[e-g]	0.98 ± 0.04[b,c]	0.59 ± 0.03[d-f]	306.50 ± 30.33[e,f]	301.69 ± 37.63[e-h]	0.29 ± 0.04[e,f]
MPG3	318.88 ± 16.86[g-i]	0.96 ± 0.03[b,c]	0.58 ± 0.02[d-f]	185.87 ± 8.60[f,g]	178.49 ± 9.68[g,h]	0.28 ± 0.01[e-g]
MPG4	277.63 ± 68.08[h,i]	0.94 ± 0.04[b,c]	0.52 ± 0.02[g,h]	143.29 ± 30.15[g]	134.18 ± 22.94[h]	0.20 ± 0.01[i]
MMG1	686.65 ± 215.83[b-e]	0.78 ± 0.10[c]	0.46 ± 0.04[i]	309.60 ± 69.11[e,f]	244.12 ± 85.14[f-h]	0.22 ± 0.03[g-i]
MMG2	1219.55 ± 89.71[a]	0.99 ± 0.01[b,c]	0.63 ± 0.05[c,d]	769.07 ± 3.00[a]	759.81 ± 6.91[b]	0.37 ± 0.04[b,c]
MMG3	886.56 ± 36.24[b]	0.99 ± 0.01[b,c]	0.60 ± 0.02[d-f]	528.14 ± 39.90[b]	522.27 ± 47.22[c-e]	0.36 ± 0.01[b-d]
MMG4	690.75 ± 81.94[b-e]	0.88 ± 0.06[c]	0.50 ± 0.02[h,i]	346.27 ± 31.92[d,e]	304.22 ± 34.49[e-h]	0.26 ± 0.02[f-h]

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$) and a-e indicates a,b,c,d,e.

Table 5.5e. Effect of different formulations at hydrocolloid level 2% on different TPA parameters on day2

Formulation	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)	Resilience
Wheat	634.06 ± 88.77^{i}	0.86 ± 0.01^{a}	0.63 ± 0.52^{a}	370.79 ± 42.28^{c-f}	305.57 ± 12.12^{c-f}	0.25 ± 0.02^{a}
MG1	1451.69 ± 207.13^{c-e}	0.63 ± 0.04^{d,e}	0.23 ± 0.01^{f}	333.31 ± 25.73^{d-g}	207.95 ± 3.40^{f-i}	0.09 ± 0.01^{g,h}
MG2	1101.43 ± 210.02^{f-g}	0.92 ± 0.02^{a}	0.41 ± 0.02^{b,c}	454.55 ± 94.45^{b-d}	406.62 ± 38.75^{b,c}	0.20 ± 0.04^{b,c}
MG3	745.21 ± 6.57^{h,i}	0.88 ± 0.01^{a}	0.43 ± 0.05^{b}	324.29 ± 30.63^{d-g}	281.93 ± 32.98^{d-g}	0.19 ± 0.04^{b-d}
MG4	1449.77 ± 255.18^{c-e}	0.67 ± 0.14^{c-e}	0.35 ± 0.03^{c,d}	509.13 ± 48.21^{b,c}	339.68 ± 36.03^{b-e}	0.16 ± 0.02^{d,e}
MCG1	995.71 ± 180.87^{g,h}	0.65 ± 0.03^{d,e}	0.24 ± 0.06^{f}	247.87 ± 103.54^{f,g}	162.78 ± 75.46^{h,i}	0.10 ± 0.02^{g,h}
MCG2	1076.33 ± 219.37^{f-h}	0.92 ± 0.01^{a}	0.45 ± 0.04^{b}	480.62 ± 135.78^{b-d}	432.06 ± 110.36^{b}	0.21 ± 0.01^{a,b}
MCG3	1366.06 ± 132.96^{d-f}	0.89 ± 0.01^{a}	0.40 ± 0.01^{b,c}	455.18 ± 67.15^{b-d}	425.91 ± 22.21^{b}	0.19 ± 0.01^{b-d}
MCG4	1276.61 ± 109.43^{d-g}	0.71 ± 0.07^{c,d}	0.26 ± 0.01^{e,f}	330.35 ± 16.56^{d-g}	233.18 ± 9.95^{e-h}	0.10 ± 0.01^{g,h}
MPG1	1930.35 ± 70.71^{b}	0.58 ± 0.01^{e}	0.16 ± 0.01^{g}	291.25 ± 7.07^{e-g}	172.95 ± 14.14^{g-i}	0.06 ± 0.01^{h}
MPG2	1361.42 ± 184.73^{d-f}	0.85 ± 0.04^{a}	0.33 ± 0.02^{d}	449.49 ± 87.09^{b-e}	374.79 ± 68.07^{b-d}	0.13 ± 0.01^{e-g}
MPG3	1743.45 ± 161.04^{b,c}	0.75 ± 0.01^{b,c}	0.31 ± 0.01^{d,e}	496.17 ± 121.05^{b,c}	386.37 ± 73.95^{b-d}	0.12 ± 0.02^{e-g}
MPG4	1441.95 ± 77.78^{c-e}	0.62 ± 0.01^{d,e}	0.14 ± 0.01^{g}	187.64 ± 3.55^{g}	117.18 ± 1.51^{i}	0.058 ± 0.01^{h}
MMG1	1044.37 ± 60.81^{f-h}	0.49 ± 0.01^{f}	0.24 ± 0.01^{f}	265.42 ± 7.90^{f,g}	134.26 ± 8.26^{h,i}	0.11 ± 0.01^{f,g}
MMG2	2379.55 ± 134.35^{a}	0.87 ± 0.01^{a}	0.31 ± 0.01^{d,e}	827.37 ± 70.71^{a}	733.60 ± 70.71^{a}	0.16 ± 0.01^{c-e}
MMG3	1465.97 ± 70.71^{c,d}	0.823 ± 0.01^{a,b}	0.33 ± 0.01^{d}	533.55 ± 28.28^{b}	445.03 ± 28.29^{b}	0.14 ± 0.01^{d-f}
MMG4	1350.82 ± 49.50^{d-f}	0.64± 0.01^{d,e}	0.23 ± 0.01^{f}	347.30 ± 25.32^{c-g}	230.83 ± 21.23	0.11 ± 0.01^{f,g}

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$) and a-e indicates a,b,c,d,e.

Table 5.5f. Effect of different formulations at hydrocolloid level 2% on different TPA parameters on day5

Formulation	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)	Resilience
Wheat	870.93 ± 2.16^{f}	0.92 ± 0.01^{a}	0.66 ± 0.74^{a}	529.90 ± 1.43^{c,d}	480.45 ± 1.52^{d,e}	0.22 ± 0.01^{a}
MG1	2269.22 ± 111.26^{c}	0.51 ± 0.03^{e,f}	0.22 ± 0.07$^{e\text{-}g}$	374.83 ± 38.87^{e,f}	193.18 ± 32.50^{g,h}	0.10 ± 0.04$^{d\text{-}g}$
MG2	1890.45 ± 82.29^{d}	0.82 ± 0.04^{a}	0.33 ± 0.01$^{b\text{-}d}$	630.35 ± 0.60^{c}	517.69 ± 24.72^{d,e}	0.14 ± 0.01$^{b\text{-}f}$
MG3	1747.55 ± 67.29^{d}	0.79 ± 0.01$^{a\text{-}c}$	0.30 ± 0.02$^{b\text{-}e}$	525.19 ± 17.55^{c,d}	416.76 ± 20.13^{e}	0.12 ± 0.01$^{b\text{-}g}$
MG4	2199.85 ± 189.09^{c}	0.53 ± 0.01^{e,f}	0.19 ± 0.01^{f,g}	420.34 ± 11.03$^{d\text{-}f}$	223.65 ± 11.48$^{f\text{-}h}$	0.08 ± 0.01^{f,g}
MCG1	1684.10 ± 159.89^{d}	0.63 ± 0.06$^{c\text{-}e}$	0.21 ± 0.07$^{e\text{-}g}$	443.10 ± 34.60$^{d\text{-}f}$	271.03 ± 61.95^{f,g}	0.09 ± 0.04$^{e\text{-}g}$
MCG2	1828.73 ± 114.19^{d}	0.89 ± 0.02^{a}	0.35 ± 0.03^{b,c}	642.98 ± 89.46^{c}	586.45 ± 77.60^{c,d}	0.16 ± 0.01^{b,c}
MCG3	1762.11 ± 178.25^{d}	0.76 ± 0.17$^{a\text{-}d}$	0.37 ± 0.03^{b}	623.46 ± 60.47^{c}	551.29 ± 77.18^{d}	0.17 ± 0.02^{b}
MCG4	1657.33 ± 198.54^{d}	0.64 ± 0.16$^{b\text{-}e}$	0.34 ± 0.08$^{b\text{-}d}$	473.17 ± 41.24^{d,e}	298.76 ± 48.43^{f,g}	0.16 ± 0.06$^{b\text{-}d}$
MPG1	2191.42 ± 96.84^{c}	0.45 ± 0.01^{f}	0.16 ± 0.01^{g}	341.49 ± 28.28^{e,f}	155.04 ± 14.10^{h}	0.07 ± 0.01^{g}
MPG2	2704.59 ± 66.89^{b}	0.85 ± 0.05^{a}	0.32 ± 0.02$^{b\text{-}d}$	868.93 ± 76.11 ^{b}	767.27 ± 67.12^{b}	0.14 ± 0.01$^{b\text{-}e}$
MPG3	2252.15 ± 188.56^{c}	0.79 ± 0.02$^{a\text{-}c}$	0.26 ± 0.05$^{c\text{-}f}$	541.38 ± 10.41^{c,d}	437.92 ± 12.27^{e}	0.12 ± 0.04$^{b\text{-}g}$
MPG4	2232.70 ± 147.42^{c}	0.54 ± 0.05^{e,f}	0.16 ± 0.05^{f,g}	361.20 ±92.99^{e,f}	197.29 ± 69.62$^{f\text{-}h}$	0.07 ± 0.02^{g}
MMG1	1360.73 ± 127.28^{e}	0.63 ± 0.01^{d,e}	0.24 ± 0.01$^{d\text{-}g}$	336.63 ± 21.21^{f}	212.92 ± 7.07$^{f\text{-}h}$	0.11 ± 0.01$^{c\text{-}g}$
MMG2	3014.57 ± 141.42^{a}	0.80 ± 0.13 ^{a,b}	0.34 ± 0.03$^{b\text{-}d}$	1112.92 ± 25.12^{a}	1003.04 ± 11.23^{a}	0.17 ± 0.01^{b,c}
MMG3	3277.56 ±141.42^{a}	0.85 ± 0.04^{a}	0.24 ± 0.01$^{d\text{-}g}$	852.81 ± 141.42^{b}	669.76 ± 70.70^{b,c}	0.14 ± 0.02$^{b\text{-}f}$
MMG4	2335.47 ± 72.73^{c}	0.64 ± 0.02$^{b\text{-}e}$	0.18 ± 0.01^{f,g}	453.68 ± 47.26$^{d\text{-}f}$	309.40 ± 63.64^{f}	0.08 ± 0.01$^{e\text{-}g}$

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$) and a-e indicates a,b,c,d,e.

Table 5.5g. Effect of different formulations at hydrocolloid level 3% on different TPA parameters on day0

Formulation	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)	Resilience
Wheat	226.87 ± 8.49^{f}	0.92 ± 0.01^{b}	0.74 ± 0.01^{a,b}	169.09 ± 7.07^{g}	158.02 ± 3.54^{g}	0.32 ± 0.02^{c,d}
MG1	711.53 ± 197.16^{c,d}	0.88 ± 0.02^{b}	0.52 ± 0.04^{f-h}	374.88 ± 108.09^{d,e}	326.08 ± 87.49^{e,f}	0.24 ± 0.03^{e}
MG2	998.82 ± 183.01^{a}	0.97 ± 0.02^{a,b}	0.65 ± 0.04^{c,d}	643.00 ± 88.96^{a,b}	621.75 ± 78.16 $^{a-c}$	0.37 ± 0.03$^{b.c}$
MG3	899.68 ± 65.62^{a-c}	0.98 ± .01^{a,b}	0.65 ±.02^{c,d}	583.40 ± 57.38^{b,c}	571.58 ± 54.70^{b,c}	0.37 ± 0.01^{b,c}
MG4	666.30 ± 88.96^{d}	0.85 ± 0.03^{b}	0.53 ± 0.02^{f-h}	356.20 ± 49.92^{d,e}	302.64 ± 50.21^{e-g}	0.24 ± 0.02^{c,d}
MCG1	602.81 ± 110.38^{d,e}	0.93 ± 0.05^{b}	0.60 ± 0.07^{c-f}	357.25 ± 27.98^{d,e}	330.07 ± 20.10^{e,f}	0.29 ± 0.05^{d,e}
MCG2	998.48 ± 151.87^{a}	1.41 ± 0.77^{a}	0.73 ± 0.02^{a,b}	733.28 ± 108.72^{a}	717.76 ± 114.90^{a}	0.44 ± 0.02^{a}
MCG3	635.20 ± 30.01^{d,e}	1.06 ± 0.10^{a,b}	0.75 ± 0.03^{a}	477.96 ± 31.87^{c,d}	505.33 ± 67.03^{c,d}	0.45 ± 0.02^{a}
MCG4	705.92 ± 80.67^{c,d}	1.16 ± 0.41^{a,b}	0.63 ± 0.05^{c-e}	442.17 ± 50.95^{d}	509.45 ± 176.80^{c,d}	0.31 ± 0.04^{e}
MPG1	761.03 ± 55.83^{b-d}	0.85 ± 0.09^{b}	0.46 ± 0.06^{h}	349.36 ± 68.16^{d,e}	296.23 ± 53.42^{e-g}	0.17 ± 0.02^{f}
MPG2	664.30 ± 135.24^{d}	0.98 ± 0.01^{a,b}	0.59 ± 0.09^{c-f}	384.77 ± 25.59^{d,e}	376.22 ± 26.41^{d,e}	0.29 ± 0.05^{d,e}
MPG3	681.98 ± 255.85^{c,d}	0.95 ± 0.01^{a,b}	0.56 ± 0.06^{e-g}	369.24 ± 109.06^{d,e}	351.17 ± 101.15^{e}	0.28 ± 0.03^{d,e}
MPG4	782.57 ± 48.44^{a-d}	0.88 ± 0.11^{b}	0.57 ± 0.06^{d-g}	445.57 ± 60.07^{d}	397.48 ± 93.90^{d,e}	0.28 ± 0.07^{d,e}
MMG1	429.85 ± 27.46^{e}	0.82 ± 0.04^{b}	0.50 ± 0.04^{g,h}	215.56 ± 2.08^{f,g}	176.97 ± 7.80^{f,g}	0.23 ± 0.04^{e}
MMG2	963.26 ± 28.34^{a,b}	1.06 ± 0.08^{a,b}	0.66 ± 0.01^{b,c}	640.34 ± 23.29^{a,b}	675.82 28.19^{a,b}	0.40 ± 0.01^{a,b}
MMG3	968.06 ± 115.74^{a,b}	0.99 ± 0.01^{a,b}	0.61 ± 0.01^{c-f}	590.86 ± 73.40^{b,c}	584.18 ± 70.30^{b,c}	0.36 ± 0.01^{b,c}
MMG4	552.38 ± 83.09^{d,e}	0.96 ± 0.04^{a,b}	0.56 ± 0.02^{d-g}	310.20 ±58.84^{e,f}	300.07 ± 68.56^{e-g}	0.27 ± 0.01^{d,e}

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$) and a-e indicates a,b,c,d,e.

Table 5.5h. Effect of different formulations at hydrocolloid level 3% on different TPA parameters on day2

Formulation	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)	Resilience
Wheat	634.06 ± 88.77^{i,j}	0.86 ± 0.01^{b-d}	0.63 ± 0.52^{a}	370.79 ± 42.28^{d,e}	305.57 ± 12.12^{e,f}	0.25 ± 0.02^{a}
MG1	800.39 ± 164.06^{h,i}	0.59 ± 0.03^{h,i}	0.27 ± 0.02^{g,h}	216.49 ± 37.94^{g,f}	126.09 ± 17.94^{h}	0.10 ± 0.01^{e}
MG2	2002.88 ± 133.38^{a}	0.84 ± 0.01^{b-e}	0.40 ± 0.03^{b}	801.44 ± 111.61^{a}	670.61 ± 87.65^{a}	0.18 ± 0.02^{b,c}
MG3	1560.56 ± 91.47^{c,d}	0.80 ± 0.06^{c-e}	0.34 ± 0.03^{c-f}	529.55 ± 77.87^{b,c}	428.25 ± 93.26^{c,d}	0.14 ± 0.02^{d}
MG4	917.99 ± 164.82^{h}	0.56 ± 0.06^{h,i}	0.28 ± 0.01^{f-h}	257.42 ± 43.61^{e,f}	142.00 ± 7.84^{g,h}	0.11 ± 0.01^{e}
MCG1	993.74 ± 59.94^{g,h}	0.65 ± 0.01^{g,h}	0.26 ± 0.01^{g,h}	259.21 ± 26.66^{e,f}	167.99 ± 16.42^{g,h}	0.09 ± 0.01^{e}
MCG2	1882.78 ± 84.21^{a,b}	0.92 ± 0.02^{a,b}	0.40 ± 0.01^{b}	757.63 ± 61.21^{a}	697.01 ± 44.23^{a}	0.18 ± 0.01^{b,c}
MCG3	1657.41 ± 53.48^{b,c}	0.87 ± 0.04^{b-d}	0.38 ± 0.02^{b,c}	622.18 ± 21.08^{b}	539.25 ± 3.65^{b}	0.16 ± 0.02^{c,d}
MCG4	860.88 ± 62.50^{h,i}	0.70 ± 0.11^{f,g}	0.29 ± 0.04^{f-h}	245.38 ± 20.45^{f}	171.71 ± 42.40^{g,h}	0.10 ± 0.01^{e}
MPG1	1042.89 ± 117.65^{f-h}	0.63 ± 0.08^{g-i}	0.26 ± 0.04^{g,h}	270.79 ± 68.44^{e,f}	172.35 ± 64.46^{g,h}	0.10 ± 0.02^{e}
MPG2	1373.91 ± 157.48^{d,e}	0.86 ± 0.02^{b-d}	0.36 ± 0.01^{b-e}	491.62 ± 64.73^{c}	420.85 ± 46.60^{c,d}	0.15 ± 0.01^{d}
MPG3	1509.26 ± 15.09^{c,d}	0.77 ± 0.04^{d-f}	0.30 ± 0.01^{d-g}	458.45 ± 10.49^{c,d}	354.70 ± 9.67^{d,e}	0.12 ± 0.01^{e}
MPG4	2078.62 ± 166.90^{a}	0.60 ± 0.01^{g-i}	0.27 ± 0.02^{g,h}	568.31 ± 80.63^{b,c}	341.29 ± 42.52^{d,e}	0.10 ± 0.01^{e}
MMG1	541.01 ± 42.43^{j}	0.54 ± 0.01^{i}	0.23 ± 0.01^{h}	125.96 ± 14.13^{g}	93.80 ± 45.25^{h}	0.09 ± 0.01^{e}
MMG2	1681.31 ± 77.78^{b,c}	0.97 ± 0.14^{a}	0.36 ± 0.03^{b-d}	639.41 ± 28.27^{b}	640.07 ± 7.07^{a}	0.18 ± 0.01^{b,c}
MMG3	1262.01 ± 63.69^{e,f}	0.89 ± 0.02^{a-c}	0.38 ± 0.01^{b,c}	527.53 ± 14.14^{b,c}	473.99 ± 12.15^{b,c}	0.19 ± 0.01^{b}
MMG4	1188.49 ± 91.92^{e-g}	0.75 ± 0.06^{e,f}	0.30 ± 0.03^{e-g}	335.23 ± 28.31^{e,f}	233.67 ± 10.36^{f,g}	0.12 ± 0.01^{e}

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$) and a-e indicates a,b,c,d,e.

Table 5.5i. Effect of different formulations at hydrocolloid level 3% on different TPA parameters on day5

Formulation	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)	Resilience
Wheat	870.93 ± 2.16[h]	0.92 ± 0.01[a]	0.66 ± 0.74[a]	529.90 ± 1.43[h,i]	480.45 ± 1.52[e,f]	0.22 ± 0.01[a]
MG1	1193.14 ± 220.62[h]	0.41 ± 0.03[d]	0.19 ± 0.01[f]	221.23 ± 38.53[j]	91.14 ± 22.47[j]	0.07 ± 0.01[d]
MG2	3041.86 ± 336.22[c]	0.83 ± 0.01[a]	0.38 ± 0.04[b-d]	1197.23 ± 177.84[b]	1010.80 ± 122.00[b]	0.17 ± 0.02[a-c]
MG3	2447.17 ± 4.71[d,e]	0.83 ± 0.15[a]	0.37 ± 0.03[b-d]	906.72 ± 64.88[d-f]	757.58 ± 185.87[c,d]	0.17 ± 0.02[a-c]
MG4	1822.48 ± 289.21[f,g]	0.44 ± 0.02[d]	0.19 ± 0.06[f]	291.12 ± 18.62[h,i]	127.01 ± 1.09[j]	0.07 ± 0.03[d]
MCG1	1205.84 ± 207.93[h]	0.60 ± 0.03[b,c]	0.22 ± 0.03[e,f]	260.54 ± 3.97[j]	157.30 ± 5.56[i,j]	0.08 ± 0.01[d]
MCG2	2758.40 ± 144.58[c,d]	0.89 ± 0.02[a]	0.40 ± 0.01[b,c]	1130.94 ± 83.00[b,c]	1002.22 ± 43.83[b]	0.19 ± 0.01[a,b]
MCG3	2239.76 ± 70.72[e]	0.92 ± 0.01[a]	0.43 ± 0.01[b]	975.82 ± 35.35[c-e]	912.41 ± 12.73[b,c]	0.22 ± 0.01[a]
MCG4	1656.17 ± 127.23[g]	0.60 ± 0.09[b,c]	0.23 ± 0.03[e,f]	370.72 ± 18.57[i,j]	223.43 ± 45.57[g-j]	0.09 ± 0.01[d]
MPG1	3416.88 ± 141.42[b]	0.51 ± 0.01[c,d]	0.23 ± 0.02[e,f]	774.97 ± 42.42[e-g]	371.46 ± 21.21[f-h]	0.08 ± 0.01[d]
MPG2	2282.76 ± 15.33[e]	0.90 ± 0.01[a]	0.36 ± 0.09[b-d]	823.46 ± 206.43[e-g]	738.18 ± 182.90[c,d]	0.17 ± 0.07[a-c]
MPG3	2169.60 ± 185.58[e,f]	0.88 ± 0.03[a]	0.35 ± 0.05[b-d]	748.90 ± 53.56[f,g]	660.41 ± 67.59[d,e]	0.15 ± 0.05[b,c]
MPG4	2331.30 ± 33.04[e]	0.68 ± 0.10[b]	0.22 ± 0.04[e,f]	509.96 ± 87.42[h,i]	340.17 ± 8.80[f-i]	0.09 ± 0.02[d]
MMG1	833.08 ± 77.78[h]	0.561 ± 0.01[b,c]	0.28 ± 0.01[d,e]	277.79 ± 70.72[j]	175.61 ± 60.72[h-j]	0.12 ± 0.01[c,d]
MMG2	4262.69 ± 282.84 [a]	0.87 ± 0.01[a]	0.36 ± 0.01[b-d]	1508.96 ± 141.42[a]	1320.01 ± 129.56[a]	0.18 ± 0.01[a-c]
MMG3	2922.80 ± 70.72[c]	0.89 ± 0.01[a]	0.37 ± 0.01[b-d]	1036.54 ± 65.31[b,d]	908.43 ± 68.42[b,c]	0.16 ± 0.01[b-c]
MMG4	2104.24 ± 114.55[e,f]	0.61 ± 0.01[b,c]	0.32 ± 0.01[c,d]	664.93 ± 63.64 [g,h]	378.42 ± 77.87[f,g]	0.13 ± 0.01[c,d]

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly ($p<0.05$) and a-e indicates a,b,c,d,e.

5.4. Conclusion

The study of dynamic oscillation and creep measurements showed that elasticity of dough and resistance to deformation of gluten free formulations supplemented with starches and hydrocolloids can be used to correlate to final bread volume. The extent of influence on bread quality produced were dependent on specific starch, hydrocolloid and its level. Corn or millet starch increased the bread volume and also produced low firmness bread whereas potato starch reduces the volume and produced high firmness bread. Among hydrocolloids G3 was able to produce highest volume breads. Millet starch resulted in light crust compared to other breads. Among cultivars, Plateau (waxy millet) showed lower volume compared to other cultivars but crumb firmness was similar to wheat bread. Starch and hydrocolloids showed significant ($p<0.05$) effect on all TPA parameters whereas effect of hydrocolloid level was observed only on hardness and gumminess.

6. General summary

6.1. General conclusion

Nine different cultivars of proso millet namely *Cope, Earlybird, Huntsman, Minco, Plateau, Sunrise, Rise, Dawn and Panhandle* were evaluated. Results showed significant (P<0.05) difference in their physical properties namely moisture content, sphericity, volume, bulk density, porosity and angle of repose, which range in values from 9.62 - 10.18%, 0.86 - 0.91, 3.94 - 5.141 (mm^3), 765.49 - 809.67 (kg/m^3), 42.49 - 44.20%, and 22.98°-25.74°, respectively. Cultivars were also evaluated for pasting and gelatinization properties and high correlation was found between amylose content and onset (r=-0.94) temperature, peak gelatinization temperature (r=-0.92), peak viscosity (r=0.84), final viscosity (r=0.91) and setback viscosity (r=0.90).

The current study also determined the effect of hydrothermal modification (HTM) at 30% moisture level and acid modification (AM) with HCl on extracted proso millet starch physicochemical and functional properties. Amylose content reduces with AM while HTM showed negligible effect. HTM starch had higher water binding capacity (WBC) whereas AM starch showed reduction in WBC. Additionally, the solubility and swelling power of HTM starch decreased with increase in temperature, and in AM starch solubility increased sharply but swelling power increases at 80°C but significantly (P<0.05) reduces at 90°C. HTM caused increase in gelatinization temperature with a mean value of 87.17°C compared to 78.61°C in native starch. AM reduced onset (69.71°C) and gelatinization temperature (77.26°C), and it increased the range (26.56°C) significantly (P <0.05) with no effect on ΔH_G. Pasting profiles of native proso millet starch changed significantly ($P < 0.05$) upon modifications and reduction in peak viscosity was observed in both modifications. AM reduced the holding strength, final viscosity, setback and breakdown whereas HTM reduced only breakdown and no change was observed in other parameters.

Proso millet based gluten free formulations supplemented with different starches and hydrocolloids showed correlation between specific volume and dynamic oscillation. Creep/recovery measurements also indicated the correlation with specific volume. Bread quality of gluten free formulation depend on type of starch or hydrocolloid or level of hydrocolloid used in the formulation. Mixture of xanthan and CMC was able to produce high volume breads and on the other hand corn and millet starches increased the bread volume and produced softer crumb bread.

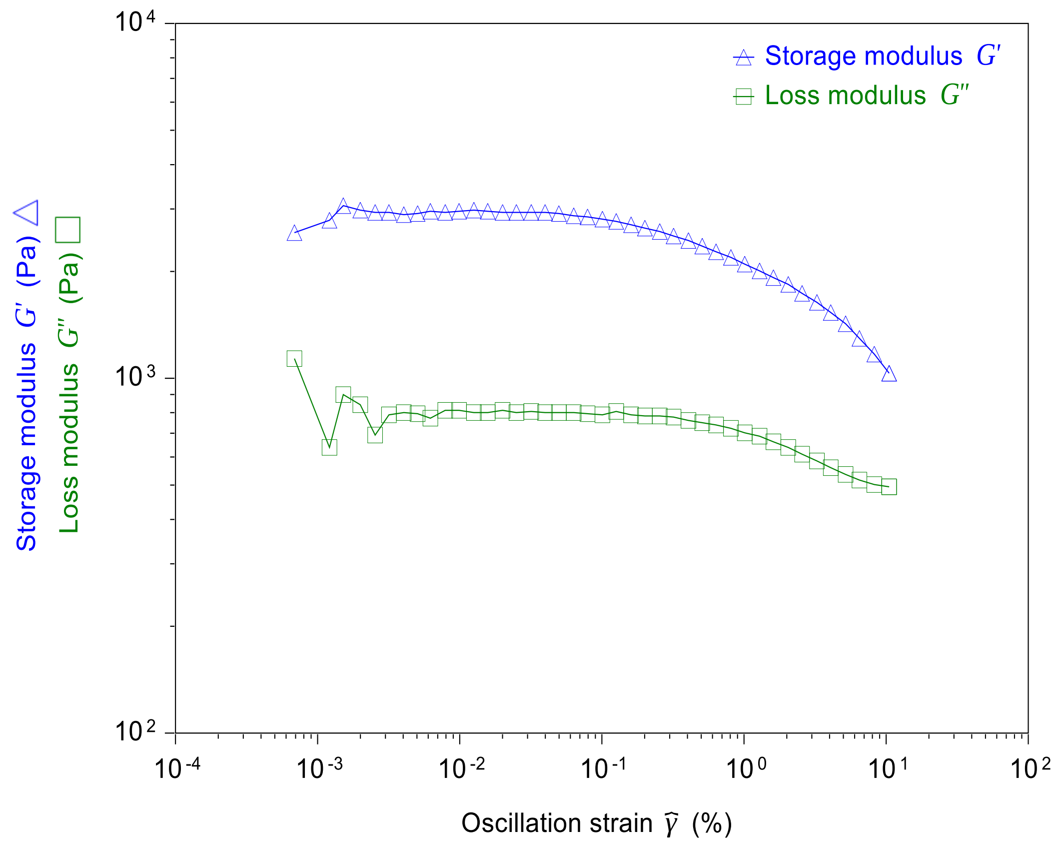
6.2. Recommendation

There is a need to further investigate the effect of different modifications of proso millet starch such as annealing and enzymatic modifications. Gluten free formulations with different hydrocolloids, starches, proteins, enzymes and emulsifiers can be explored using response surface methodology to optimize ingredients which might help reduce the limitations faced in this study such as higher retrogradation rates, lower shelf life and low volumes.

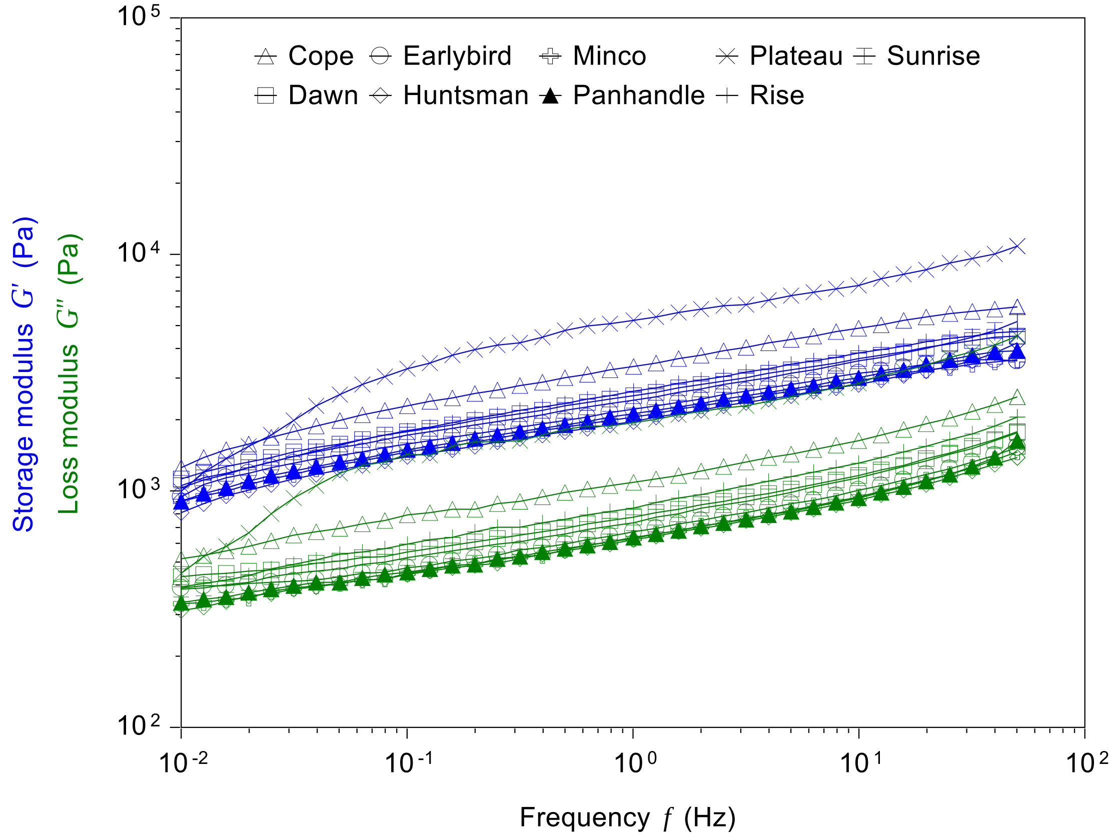
Another limitation of millet use in food industry is presence of anti-nutritional factors which affect starch and protein digestibility. So there is a need to evaluate the level of anti-nutritional factors present in different millet cultivars and effect of various processes like baking, frying, extrusion and fermentation on minimizing those factors.

Appendices

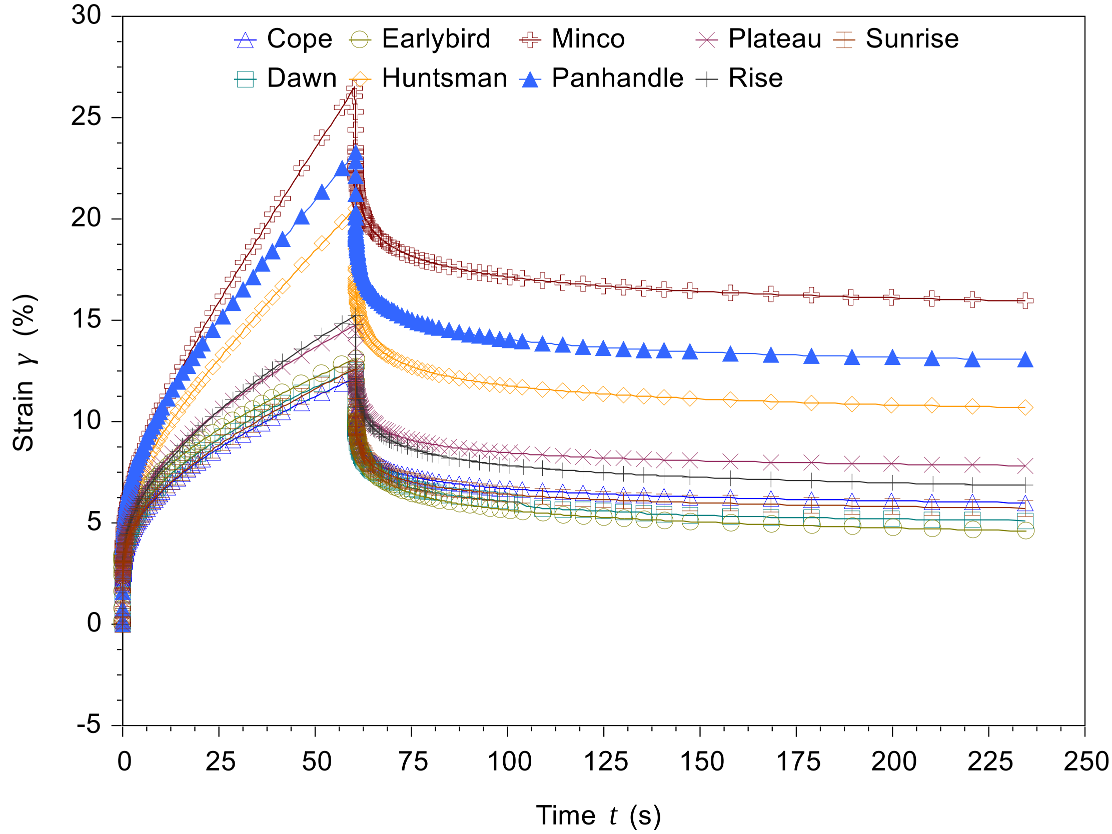
Appendix 1. Dynamic oscillation measurements curves



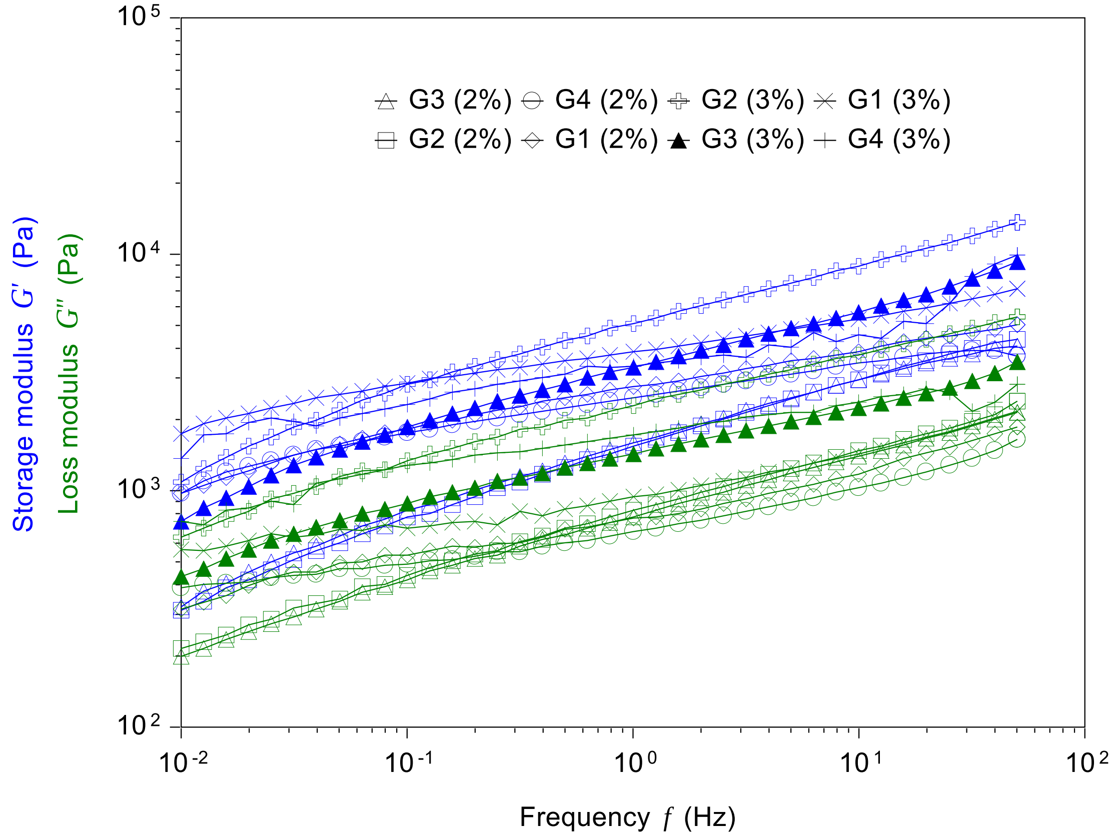
Strain sweep test to determine linear viscoelastic region



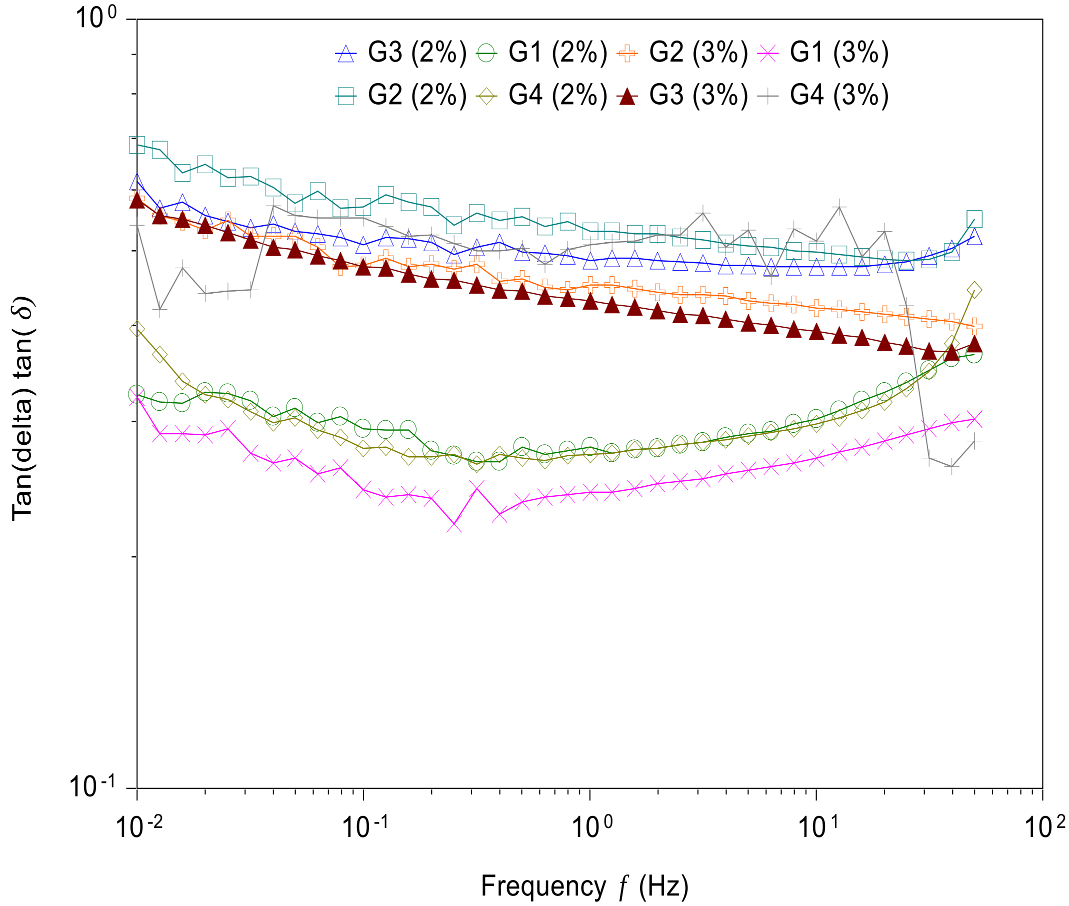
Frequency sweep test curves of different cultivars



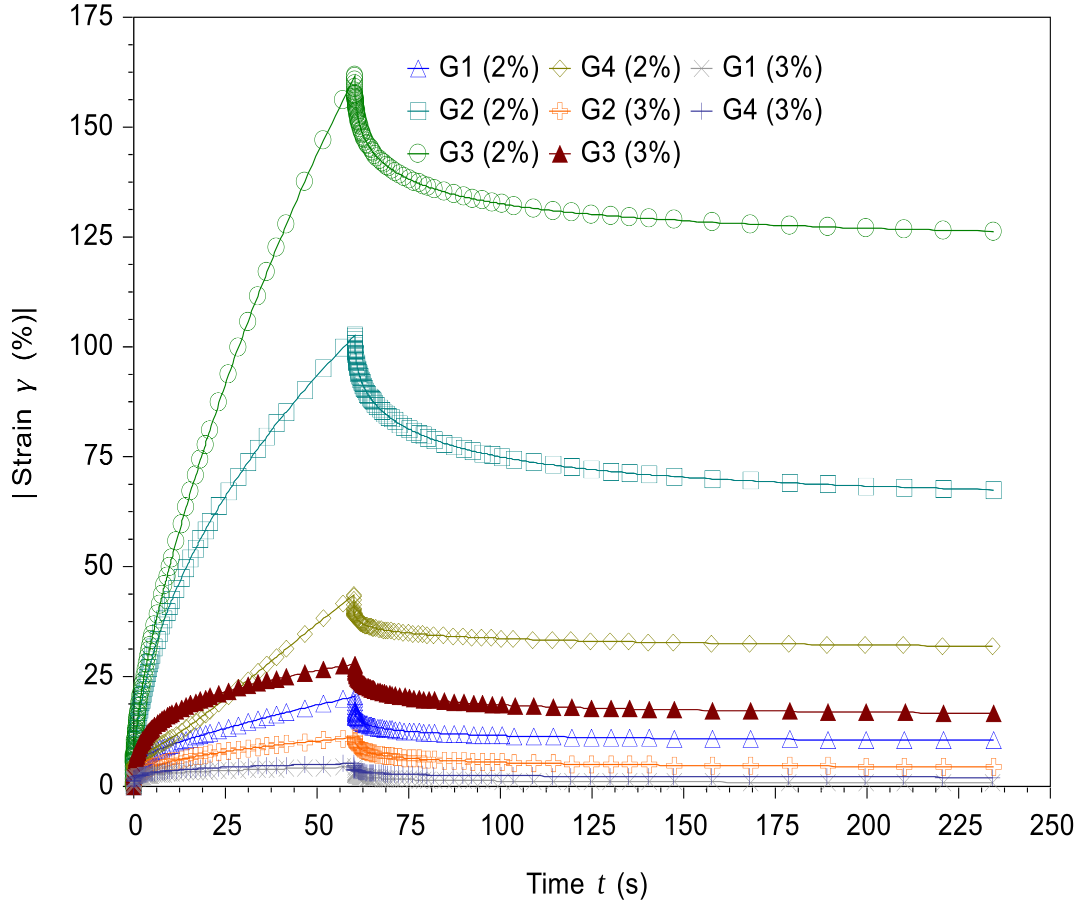
Creep recovery curves of different cultivars



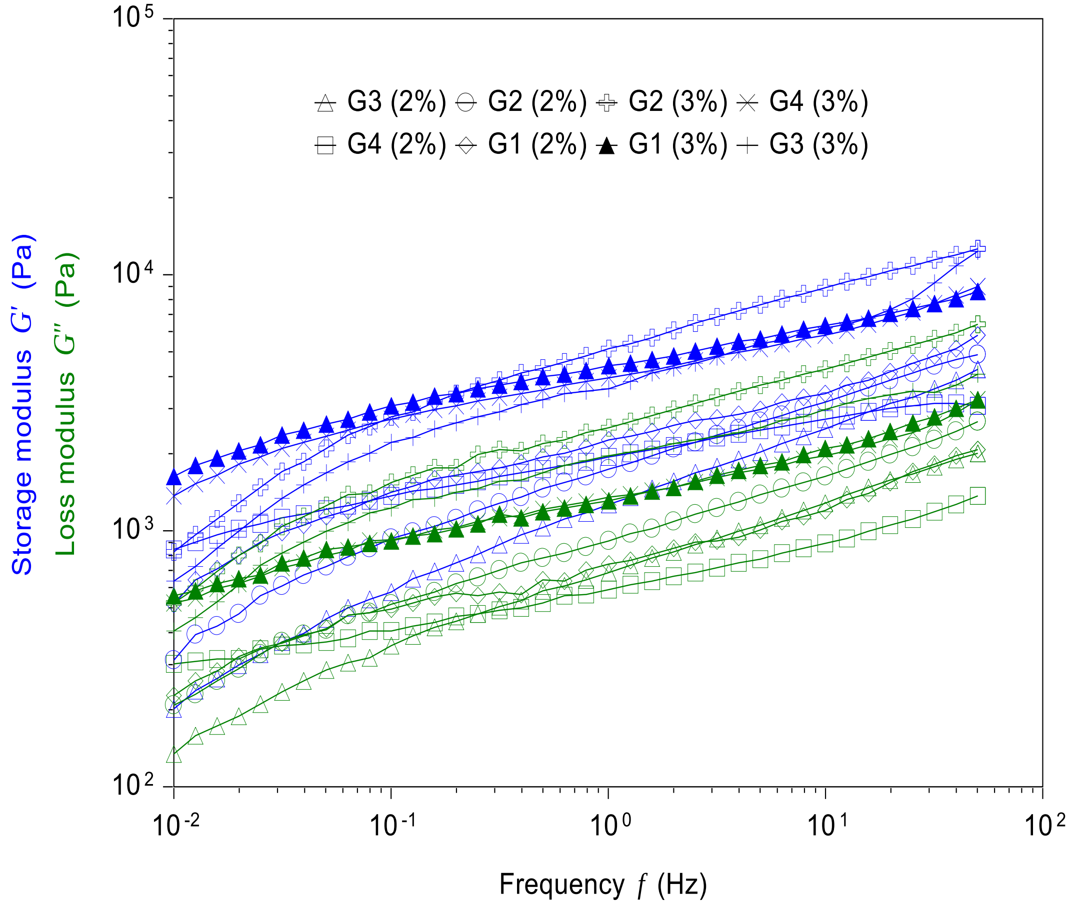
Frequency sweep curves of whole millet flour formulations



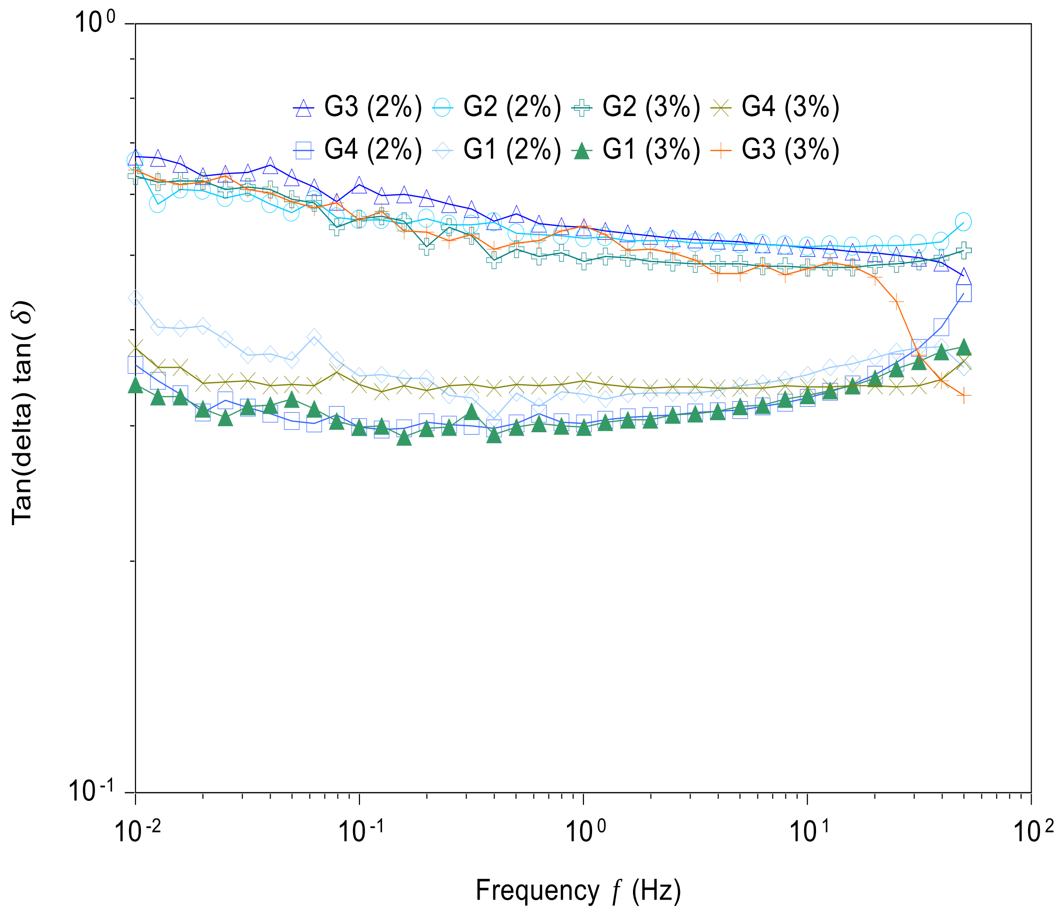
Tan(δ) curves of whole millet flour formulations



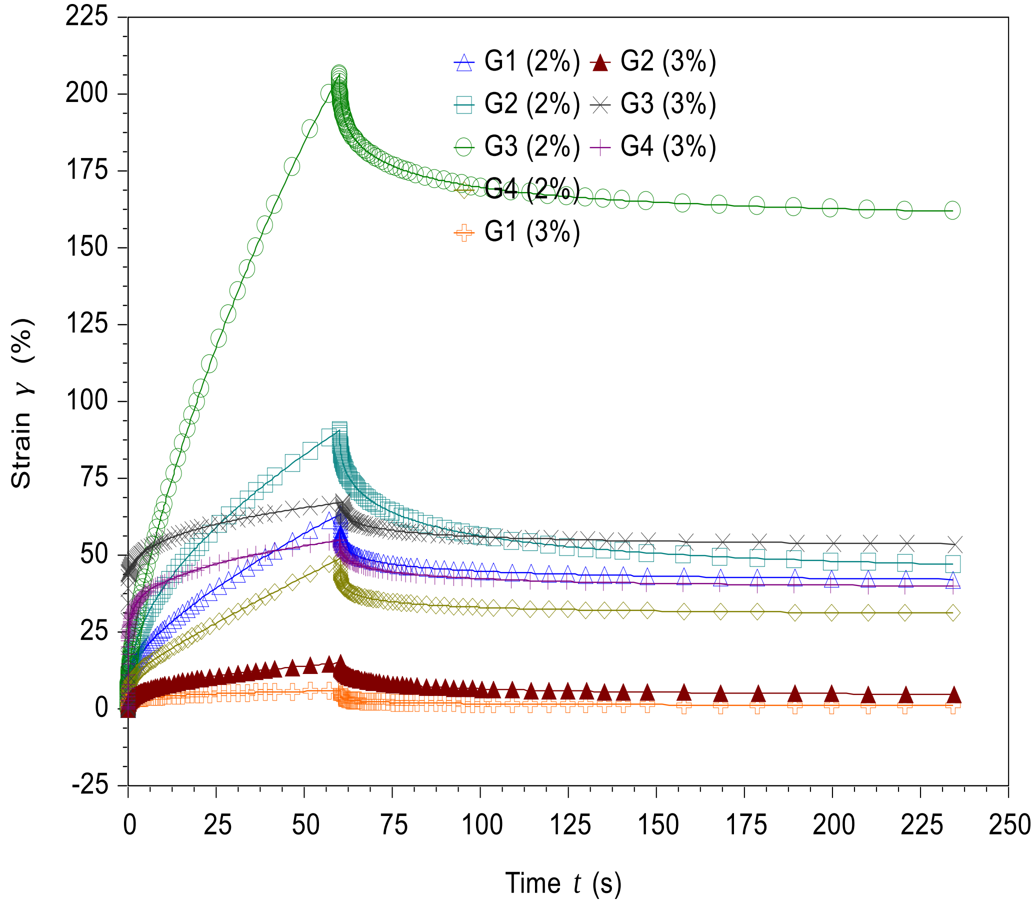
Creep and recovery curves of whole millet flour formulations



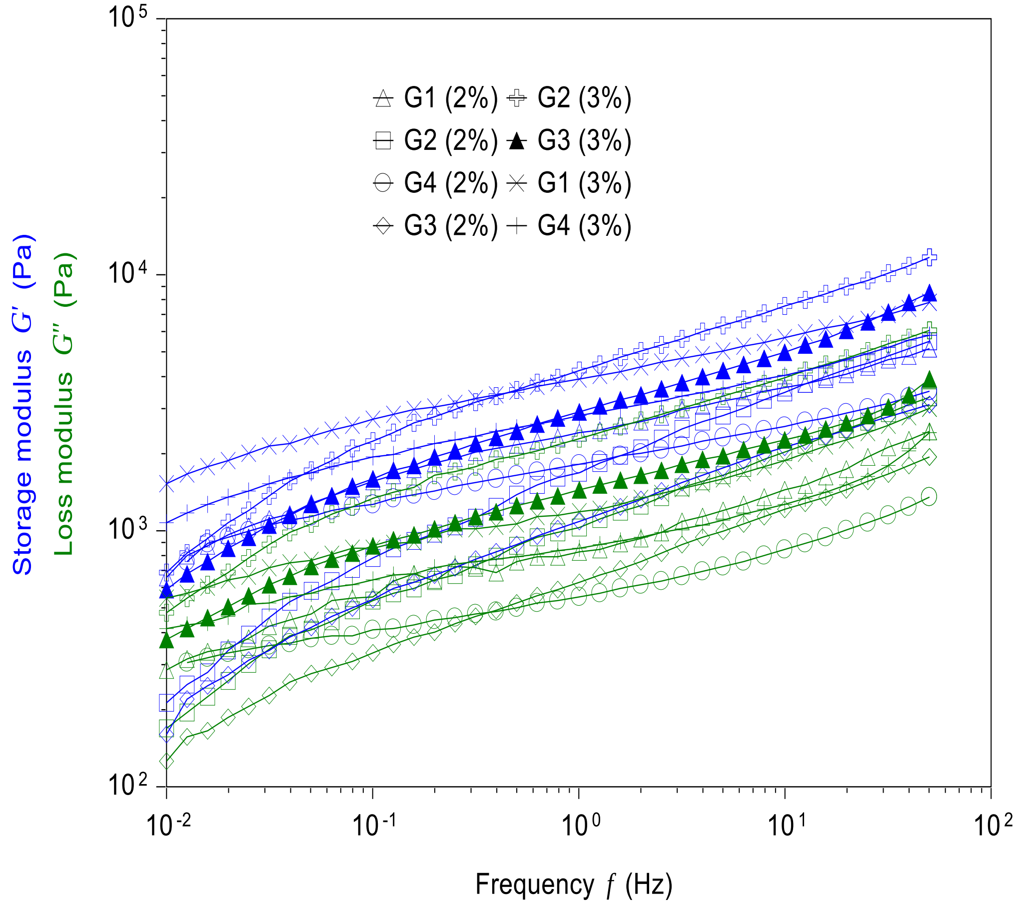
Frequency sweep curves of MC formulations



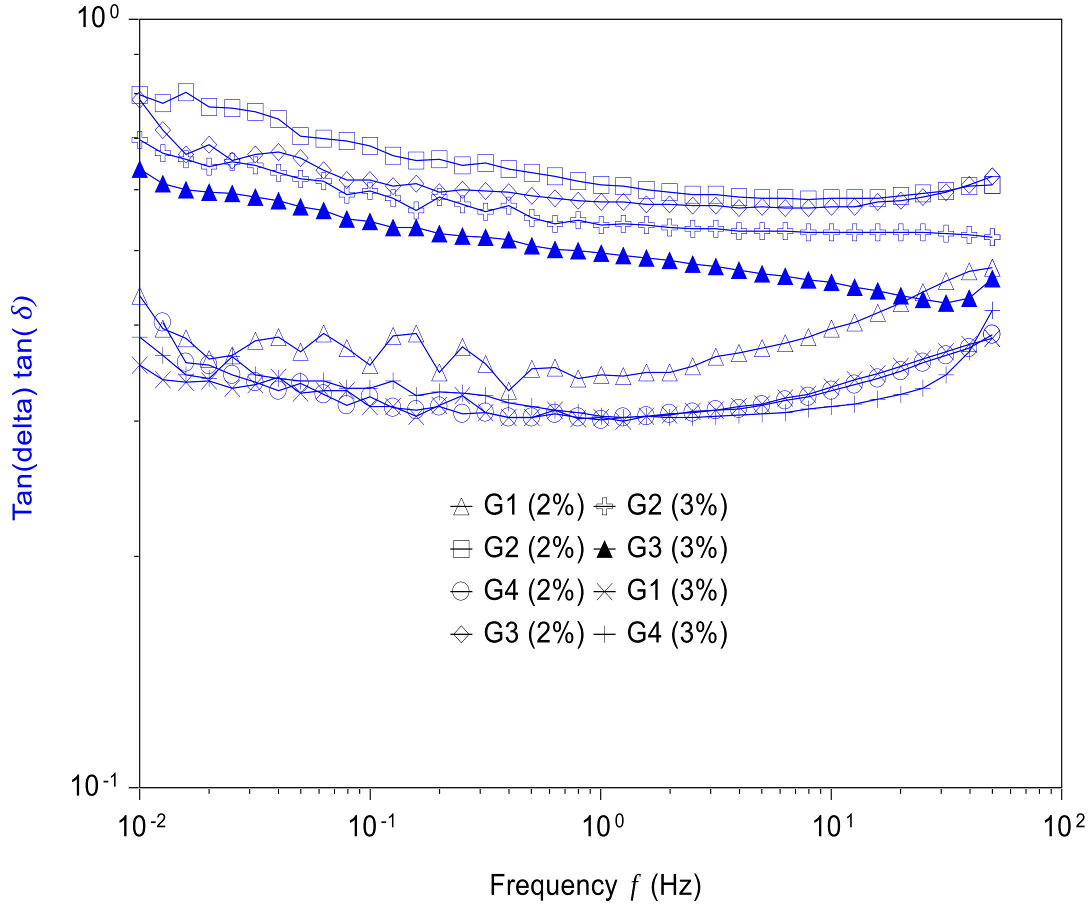
Tan(δ) curves of MC formulations



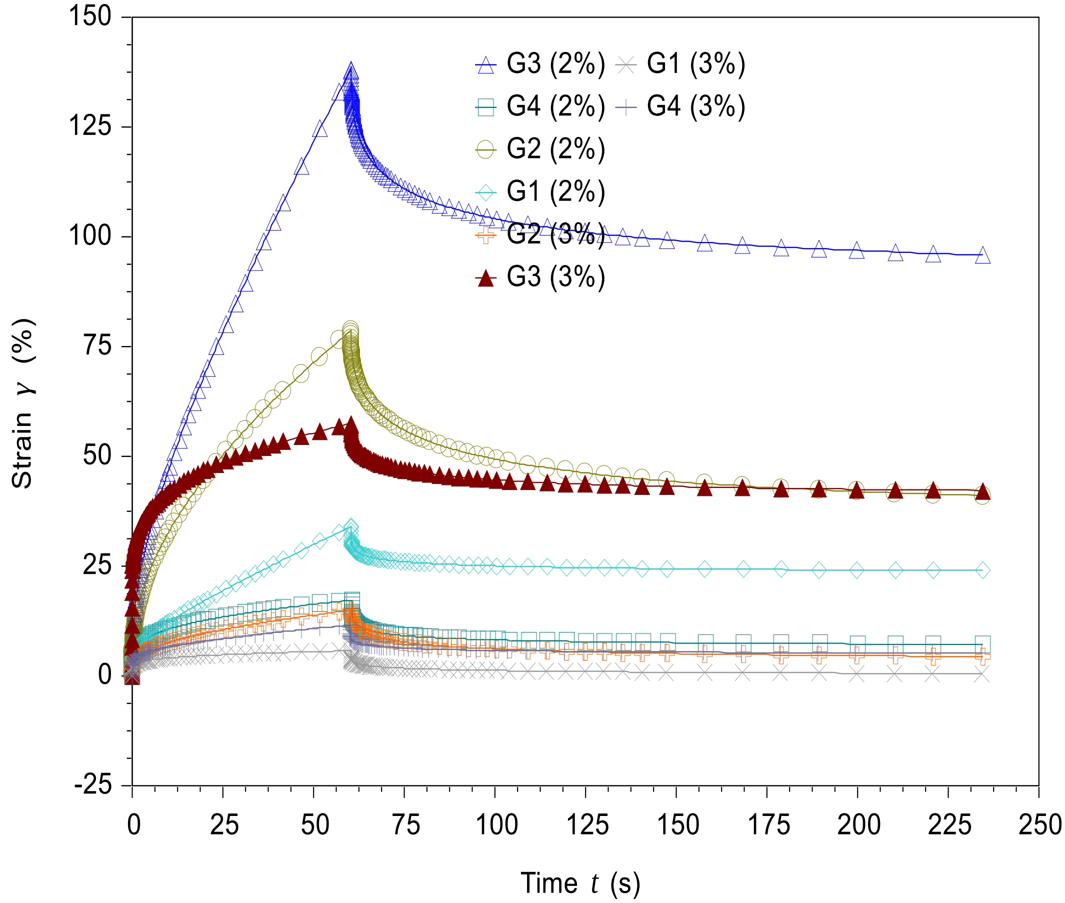
Creep and recovery curves of MC formulations



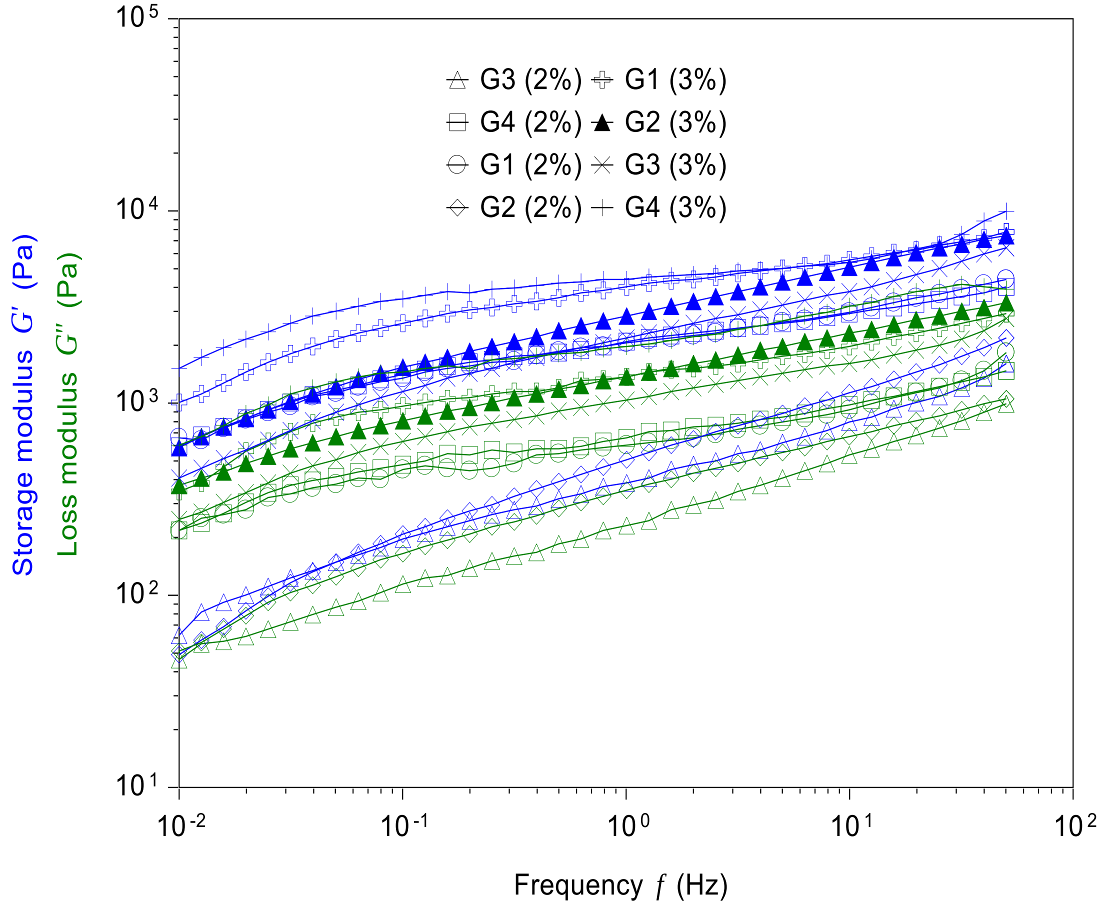
Frequency sweep curves of MP formulations



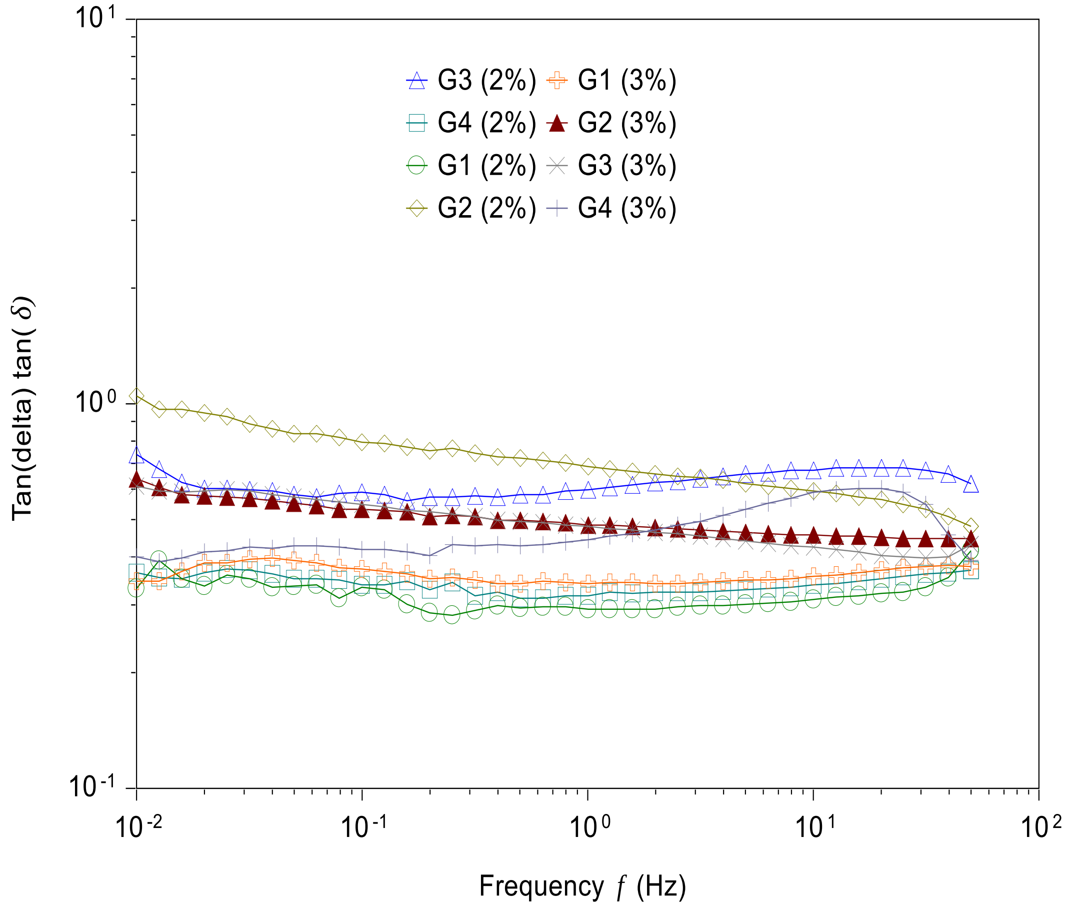
Tan(δ) curves of MP formulations



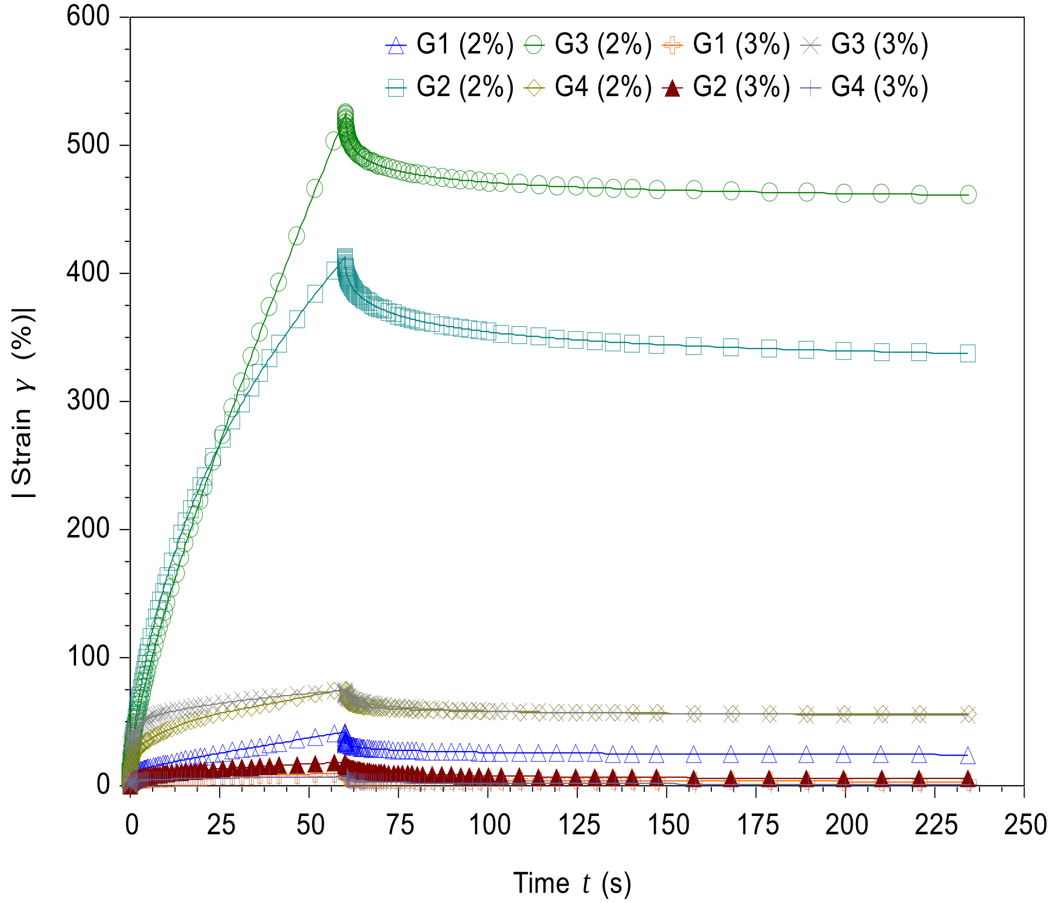
Creep and recovery curves of MP formulations



Frequency sweep curves of MM formulations



Tan(δ) curves of MM formulations



Creep and recovery curves of MM formulations

Appendix 2. Images of all the different formulations of breads in this study

Formulations	2%	3%
MG1		
MG2		
MG3		
MG4		
MCG1		

MCG2		
MCG3		
MCG4		
MPG1		
MPG2		
MPG3		

MPG4		
MMG1		
MMG2		
MMG3		
MMG4		

Minco

Sunrise

Rise

Earlybird

Panhandle

Dawn

Plateau

Cope

Huntsman

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Vita

Manjot Singh

EDUCATION

University of Kentucky (UK), Lexington, Kentucky, USA	Dec-2016
Masters in Biosystems and Agricultural Engineering	GPA- 4.0/4.0
Specialization in Food Process Engineering	
Kansas State University (KSU), Manhattan, Kansas, USA	Aug-2014
Bachelor of Science in Bakery Science and Management	GPA- 3.5/4.0
Punjab Agricultural University (PAU), Ludhiana, India	Aug-2014
Bachelor of Technology in Food Science and Technology	OCPA- 7.57/10.00

Transferred to KSU in July 2012 under Dual degree program between KSU and PAU.

PUBLICATIONS

PEER REVIEWED PAPER

Singh, Manjot, and Akinbode A. Adedeji. " Characterization of modified proso millet starch." *LWT Food Science and Technology*. **(under journal review)**

Singh, Manjot, Akinbode A. Adedeji and Dipak Santra. " Physico-functional properties of nine proso millet cultivars." Journal of Food Measurements and Characterization. **(under journal review)**

Singh, Manjot, Akinbode A. Adedeji and Paul Priyesh Vijayakumar. " Effect of hydrocolloids and different starches on rheological and baking properties of proso millet gluten free batter**." (under internal review)**

NON-PEER REVIEWED PAPER

Singh, Manjot, and Akinbode A. Adedeji. "Physicochemical and functional properties of proso millet starch." *2016 ASABE Annual International Meeting*. American Society of Agricultural and Biological Engineers (ASABE),July 17-20, 2016.

CONFERENCE ABSTRACTS

American Society of Agricultural and Biological Engineers (July 17 - 20, 2016)- Physicochemical and functional properties of proso millet starch

Conference of Food Engineering (Sept. 11 - 14, 2016)- Value-added Utilization of millet for Food, Feed, Fiber and Energy

American Association of Cereal Chemists International (Oct. 23-26, 2016)- Hydrocolloids effect on rheological and baking properties of proso millet composite dough

American Association of Cereal Chemists International (Oct. 23-26, 2016)- Physical, rheological and baking properties of proso millet cultivars

HONORS and AWARDS

Outstanding Graduate Student award recipient Fall-2016

Gamma Sigma Delta: Honor Society of Agriculture, UK (2016)

Vice President, Alpha Epsilon: Honor Society of Agricultural and Biological Engineers, UK (2016)