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Novel Gluten-Free Amaranth and Oat Flour Cookies Fortified with Soybean Hulls

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Abstract

Soybean hulls, an abundant byproduct of soybean processing, contain rich phytochemicals, fibers, proteins, and minerals. Currently soybean hulls are primarily used as animal feeds. For value-added soybean hull utilization, 25% soybean hulls were substituted for amaranth or whole oat flour (WOF) in novel gluten-free cookies. Composition, nutritional values, water-holding capacities, correlation between properties, and pasting and rheological properties of soybean hulls, amaranth, and WOF were appraised in comparison to wheat flour. Water loss, cookie texture, and geometrical properties of the cookies were examined. The results disclosed that soybean hulls, amaranth and WOF contain higher protein content, minerals, fiber, special amino acids, and critical vitamins (C and K) than wheat flour. Considerably higher total amino acid content was found in soybean hulls (18.33%) than wheat flour (12.77%). Water-holding capacities increased by replacing amaranth and WOF with soybean hulls. Soybean hulls exhibited higher rheological elastic properties than amaranth, WOF and wheat flours. The soybean hulls utilized in amaranth or WOF cookies greatly improved their nutritional value, the water retention and moisture content along with acceptable physical properties when compared to wheat flour cookies. This study explored the feasibility and potential of utilizing soybean hulls with amaranth and WOF in gluten-free bakery products and other food applications.

Keywords

Amaranth, Nutrition, Oat, Protein, Rheology, Soybean Hulls, Water-Holding

1. Introduction

Soybean hulls, a residual product of soybean dehulling, contain proteins, minerals, soluble sugars, and amino acids as well as phenolic compounds with antioxidant activities that are higher than soybean flour [1]. Soybean hulls show substantially higher ($P < 0.05$) antioxidant activity (3.06 μmol/g) than soybean flour (0.80 μmol/g) based on total phenolic content [2]. Phenolic compounds with antioxidant activities may promote anti-inflammatory conditions in your body when people eat them regularly. Soybean hulls have inspired new interest as a functional food ingredient source. Recent attention has been focused on the conversion of soybean hulls to generate value-added input feedstock. Potential health benefits gained by incorporating phytochemicals, increasing fiber and mineral content, and utilizing the addition of a low-value byproduct have prompted investigation of soybean hulls as an additive to wheat flour [2]. Other similar studies involved looking into water-holding capacities (WHC) and rheological properties for the dough made with soybean hulls and wheat flour [3]. Soybean hulls were also used as a bread additive to increase iron availability; soybean hulls up to 5% were added to bread formulations without decreasing acceptance [4].

Bakery products in many parts of the world have recently witnessed a shift of consumer attitude from traditional snack foods towards healthier, zero-allergen, and nutrient-intense meal substitutes or supplements. Cookies are an important category of food products in the American food market. There are many publications of studies that focusing on the improvement of physicochemical, functional, and sensory properties (e.g., [5]). Information on the application of soybean hulls on food was limited. No literature was found on cookies containing soybean hulls. Although soybean hulls have outstanding nutritional profile and relatively bland flavor, structural compatibility with other food ingredients is relatively low, making the material difficult to work and process in many commercial food processing. Therefore, other gluten-free ingredients need to be selected to incorporate with soybean hulls for desired nutrition and texture.

Amaranth grains (*Amaranthus caudatus*) contain significant sources of thiamine, niacin, riboflavin, and folate, and especially comprise an remarkably rich source of the essential amino acid lysine, which is low in other cereal grains [6]. Amaranth also has vitamin E in comparable amounts to that of olive oil which may benefit people with high blood pressure and cardiovascular disease through lowering blood pressure and cholesterol levels; whereas enhancing antioxidant potency and some immune responses [7]. Amaranth and oat contain higher dietary minerals such as calcium, iron, magnesium, and phosphorus than those of wheat flour [8]. Amaranth grain flour has been assessed as a supplement to wheat flour. Several portions of amaranth grain flour were blended with wheat flour and other ingredients that were mixed with yeast, proved in the pan, and baked. The loaf volume of the bread is disproportional to the amount of amaranth substitution of wheat flour in the bread formulation. There were significant differences in the use of 15% amaranth grain flour in evaluated sensory qualities [9]. On the other hand, whole oat flour (WOF) was also successfully used with amaranth for gluten-free cookies since WOF contains β-glucan known for suppressing blood cholesterol and thwarting heart disease [10] [11]. In addition to β-glucans, oat phenolics and other antioxidant compounds also deliver health benefits [12]. Besides, whole oat products comprising β-glucan have many functional food applications to decrease fat content and calories in foodstuffs, as well as regulate the rheology and texture of products and provide freezing/thawing stability [13]. Thus, amaranth and WOF were selected to be combined with soybean hulls to develop gluten-free cookies with improved nutritional values and acceptable texture in this exploratory study.

The objective of this research was to identify important data on characteristics of gluten-free amaranth flour and WOF cookies comprising soybean hulls. This research involved applying 25% soybean hulls to amaranth flour or WOF, respectively, to make nutritious gluten-free cookies with good texture. Our preliminary study indicated that twenty-five percentage of substitution with soyhulls is the maximum amount can be achieved without negative impact on dough integrity and handling. To incorporate soybean hulls into the food formulations, the water-holding capacity, the correlation between properties of ingredients, pasting and rheological properties of the starting ingredients must be comprehensively understood beforehand. Also, the impact of soyhull presence on cookie physicochemical properties, e.g., moisture reduction, thickness, diameter, and hardness, should be studied and compared to wheat flour cookie. This study is important for providing important scientific information to developers of nutritious gluten-free snack foods since two million people in America alone are considered as gluten intolerant. The results from this research may also have important bearing on variety of food research topics including dietary fiber inclusion in various food formulations.

2. Materials and Methods

2.1. Ingredients

Organic soybean hulls (OSH) were received from Keystone Mills (Ephrata, PA, USA). Soybean hulls were milled employing a Fritsch rotor speed-mill (Idar-Oberstein, Germany) and went through U.S. sieve 50 mesh with sizes of 297 μm.

Ingredients for cookies: whole wheat flour (Hy-Vee, Inc., West Des Moines, IA, USA); organic whole oat flour, colloidal fine (WOF, Grain Millers, Eugene, OR, USA); organic amaranth flour, gluten-free (Dakota Prairie Organic Flour Co., Harvey, ND, USA); sugar (C&H Sugar Company, Crockett, CA, USA); nonfat dry milk (Carnation, Nestlé, Vevey, Switzerland); shortening (Crisco, the J.M. Smucker Company, Orrville, OH, USA); baking powder (Calumet, Kraft Foods Group, Inc., Northfield, IL, USA); salt (Morton Salt, Inc., Chicago, IL, USA).

2.2. Compositions

The total solid, moisture, protein and ash content were determined using the modified methods in a previous study [14]. Crude fat content was determined with hexane as solvent using a Soxhlet system following AOAC methods (1998) [15].

2.3. Amino Acid Analysis

Amino acid analysis was performed on ground powder of each tested sample based on the method described in a previous study [14]. It was revised according to the AOAC method 94.12 [16]. Three samples (5 - 15 mg) were first hydrolyzed in 10 µl of 1 mM solution of aminoisobutyric acid (AIB) and hydrochloric acid for 22 h at 110˚C, neutralized, and derivatized with 6-aminoquinolyl-*N*-hydroxylsuccinimyl carbamate (AQC) [17] [18]. Liquid chromatography was employed with an HPLC system (Thermo Scientific Dionex Ultimate 3000, San Jose, CA, USA) on a reverse phase C18 column (Waters, Milford, MA, USA) with a PDA detector (260 nm) for 10 min at 55˚C. Amino acid standard H (Product #20088, Thermo Scientific, Rockford, IL, USA) was employed for computation from extinction coefficients obtained from linear standard curve. For each sample, the derivatization was made in triplicate, resulting in nine analytical HPLC runs for each sample. The result was reported as % w/w amino acid in sample.

2.4. Water-Holding Capacity

Water-holding capacity (WHC) was measured according to an earlier technique with modifications [19]. Flour samples (2 g, dry weight) were mixed with 25 g of deionized water and energetically blended by employing a Vortex stirrer to form a suspension, held for 2 h, followed by centrifugating at 1590 g for 15 min using a Beckman centrifuge (Allegra 6R, Beckman Inc., Indianapolis, IN, USA). Water was dispatched by pipette and determined. Each treatment was duplicated.

Water-holding capacity (WHC) was calculated as follows:

$$\text{WHC (\%)} = [\text{Water added (g)} - \text{decanted water (g)}]/\text{dry sample weight g} * 100$$

2.5. Pasting Properties

The pasting properties of materials were performed according to a modified method using a Rapid Visco Analyzer (RVA-4, Perten Scientific, Springfield, IL, USA) [1]. Samples from each tested flour (3.92 g d.b.) were prepared to be composed of a total weight of 28 g with distilled water in an RVA canister (14% solids, w/w) forming suspensions. The viscosity of the suspensions in the RVA was recorded during the heating and cooling phases. Suspensions were equilibrated at 50˚C for 1 min, heated to 95˚C at a rate of 6.0˚C/min, maintained at 95˚C for 5 min, cooled to 50˚C at a rate of 6.0˚C/min and held at 50˚C for 2 min. For all measurements, a constant paddle rotating speed (160 rpm) was used throughout the entire testing except for 920 rpm in the first 10 s to disperse samples. Each sample was analyzed in duplication. The results were designated in centipoises (mPa s).

2.6. Rheological Properties

The rheological properties of food materials are measured using a rheometer. The methods were used according toa previous study [20]. In general, the samples from the RVA study above were cooled to 25˚C, equilibrated overnight and loaded on a rheometer (AR 2000, TA Instruments, New Castle, DE, USA) with a 6 cm-diameter parallel acrylic plate with 1 mm gap to the surface. The outer edge of the plate was sealed with a thin layer of mineral oil (Sigma Chemical Co., St Louis, MO, USA) to prevent dehydration during the measurement. All rheological measurements were carried out at 25˚C using a water circulation system within ±0.1˚C. A strain sweep test was performed initially to ascertain the bounds of linear viscoelasticity; then a frequency sweep test was conducted to acquire storage modulus (G') and loss modulus (G") at frequencies from 0.1 to 100 rad/s using a fresh sample. A strain of 0.5%, which was within the linear viscoelastic range, was used for the dynamic experiments. The steady shear viscosity of the sample was assessed as a function of shear rate from 1 to 100 s^{-1}.

2.7. Cookie Preparation

Cookies were prepared using the AACCI method 10 - 52 for sugar cookie with some adjustments [21]. Mixture of 22.5 g brown sugar, 2.3 g nonfat dry milk, 2.8 g salt, 2.3 g sodium bicarbonate and 72 g sugar were first blended in a bowl with a whisk. Then 100 g palm-oil based shortening was added to the mixture and blended with a paddle beater in the bowl of a KitchenAid mixer (St Joseph, MI, USA) at Speed 2 for 3 min, scraping down the mixture from the wall of the bowl every minute. The solution of 1.1 g ammonium bicarbonate in 50 g water was added to the mixture and mixed for another 1 min at Speed 2. After scraping the bowl, the mixture was mixed again for 1 min at Speed 2. The 225 g flour, or amaranth/WOF composites containing 25% soybean hulls, were added to the bowl while mixing at Speed 1, and mixing continued for 2 min at Speed 2 with scraping the bowl every 30 s. The cookie dough from the mixer was placed on a cutting board and flattened by a rolling pin and cut using a cookie cutter. Cookies were baked at 205˚C in a convection oven (XAF-113 LineChef Stefania, Cadco, Ltd., Winsted, CT, USA) for 10 min and cooled. The cookies were stored in a sealed plastic zipped bag before further measurements were taken. All measurements were performed in 3 replications.

2.8. Water Loss and Cookie Moisture

Water losses of cookies during baking were determined by the weight difference before and after baking at room temperature. Prior to the measurements, samples were stored and cooled in a sealed container to minimize the fluctuation of moisture content of the samples. Moisture contents of cookies were measured by drying 5 g of ground cookie sample at 105˚C to a constant weight (about 3 - 4 h); the difference in weight before and after drying is the moisture content.

2.9. Geometrical Properties

Cookies were positioned next to each other, and the total diameter was measured four times with rotating the cookies by 90˚. The average of four measurements was used for cookie diameter. Cookies were stacked and measured for height; then the cookies were restacked in a different order and measured again. The average cookie height was the mean of readings of heights resulting from different stacking of cookies. The spread ratio was calculated by dividing diameter by height.

2.10. Cookie Texture Analysis

Cookie hardness was measured using the recommended method for TA-XT2 Texture Analyzer (Texture Technology Corp., Scarsdale, NY, USA) furnished with 30 kg loading cell. The cookie hardness measurement was performed by a cutting force using a three-point bending method with sharp-blade probe (6 cm long and 1 mm thick). The hardness of the cookies was indicated by the maximum peak force required to break the cookies. The slotted inserts of the texture analyzer were adjusted and secured on a heavy-duty platform to fit the sample size and ensure the sample is positioned centrally under the knife edge. The cookie was placed on two supporting beams spaced at 3 cm apart. The analyzer was set to the "return to start" cycle, a pre-test speed of 1.5 $mm\cdot s^{-1}$, test speed of 2.0 $mm\cdot s^{-1}$, post-test speed of 10 $mm\cdot s^{-1}$, and the distance was 5.0 mm.

2.11. Statistical Analysis

Statistical data analysis was conducted for comparisons of variances with Duncan's multiple, and Pearson correlation analysis between properties of ingredients for cookies at significant differences ($P < 0.05$) using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

3.1. Compositions

Table 1 shows the protein, ash, fat, and carbohydrate contents of the starting ingredients. Amaranth and WOF contain about 30% more protein (13.56%, 13.70%) than wheat flour (9.61%). Unlike the protein found in wheat flour, amaranth and WOF contain an important source of proteins that do not cause allergic reaction to gluten intolerant people. Soybean hulls had the highest ash content (4.05%), followed by amaranth (2.60%), and WOF (2.15%), and they are all higher than that of wheat flour (0.66%). The highest carbohydrate by difference (75.73%) was found in soybean hulls. Carbohydrate fraction was estimated by subtracting the measured protein, fat, ash, and water from the total weight, which in fact is the sum of the digestible carbohydrates (dextrins, starches, and sugars), undigestible carbohydrates (pentosans, pectins, hemicelluloses, and cellulose) and non-carbohydrates (such as organic acids and lignins) [22]. Moreover, Jaworski *et al.* (2017) reported soybean hulls contain extremely high dietary fiber (67.46%) as shown in **Table 1** [23]. Dietary fiber adds bulk to diet and help control weight. It also helps digestion and prevents constipation.

Ash is a mixture of substances left after the burning of materials. It is mostly mineral, but generally still contains some combustible organic or other oxidizable residues. High ash content indicates high mineral content. Tremendously higher calcium and potassium contents were found for amaranth flour (159, 508 mg/100g) and soybean hulls (600, 1700 mg/100g) than wheat flour (33, 394 mg/100g) (**Table 2**). Higher magnesium and phosphorus contents were found for amaranth (248 and 557 mg/100g, respectively) and WOF (270 and 458 mg/100g, respectively) than wheat flour (117 and 323 mg/100g, respectively). Also, higher iron content was found in soybean hulls (32.4 mg/100g), amaranth (7.61 mg/100g) and WOF (4.64 mg/g) compared to wheat flour (3.71 mg/100g).

It is noteworthy that amaranth flour is the only flour that has high vitamin C (4.2 mg/100g) among the materials tested in **Table 1**. Vitamin C (ascorbic acid) possesses antioxidant property that can also function in countering bacterial infections [24]. Some experts suggest taking 200 mg of vitamin C daily for COVID-19 prevention or 1 - 2 grams daily for COVID-19 treatment [25]. Whole oat flour contains high vitamin K (3.2 µg/100g). Vitamin K is important for blood coagulation and in the prevention and attenuation of bone and vascular disease [26]. In addition, the folate content for amaranth flour is considerably higher (82 µg/100g) than wheat flour. Folate is one of the B vitamins essential for the human body to build DNA and RNA and metabolized amino acids essential for cell division [27].

Results show that soybean hulls, amaranth and WOF contain higher dietary fiber and mineral contents, such as calcium, iron, magnesium, and phosphorus, along with critical vitamins for health compared to wheat flour. In addition, the potential source of gluten-free proteins is essential for those who are allergic to gluten.

3.2. Amino Acids

Amino acids, as the building blocks of all proteins, are compounds critical to the

Table 1. Proximate compositions of starting ingredients.

Sample	Moisture %	Protein %	Ash %	Fat %	Carbohydrate, by difference %	Fiber total dietary %
Amaranth	7.51	13.56 ± 0.05^{a}	2.60 ± 0.01^{b}	5.93 ± 0.01^{a}	65.25^{d}	6.7*
Whole oat	8.37	13.70 ± 0.01^{a}	2.15 ± 0.01^{c}	5.39 ± 0.02^{b}	68.18^{c}	9.4*
Wheat	8.85	9.61 ± 0.15^{c}	0.66 ± 0.02^{d}	1.88 ± 0.01^{c}	74.48^{b}	3*
Soybean hulls	6.56	10.41 ± 0.25^{b}	4.05 ± 0.21^{a}	1.54 ± 0.02^{d}	75.73^{a}	67.46**

Means followed by the same letter within the same column were not significantly different ($P > 0.05$). *USDA FoodData Central (usda.gov) [8]; **Jaworski, *et al.*, 2017 [23].

Table 2. Minerals and vitamins of starting ingredients.

Nutrient (Per 100 g)	Unit	Whole wheat flour	Amaranth	Whole oat flour	Soybean hulls
Minerals					
Calcium, Ca	mg	33.00	159.00	47.00	600*
Iron, Fe	mg	3.71	7.61	4.64	32.4*
Magnesium, Mg	mg	117.00	248.00	270.00	-
Phosphorus, P	mg	323.00	557.00	458.00	220*
Potassium, K	mg	394.00	508.00	358.00	1700*
Sodium, Na	mg	3.00	4.00	3.00	10.00*
Zinc, Zn	mg	2.96	2.87	3.20	2.40*
Vitamins					
Vitamin C, total ascorbic acid	mg	0.00	4.20	0.00	-
Thiamin	mg	0.30	0.12	0.54	-
Riboflavin	mg	0.19	0.20	0.12	-
Niacin	mg	5.35	0.92	0.82	-
Vitamin B-6	mg	0.19	0.59	0.10	-
Folate, DFE	µg	28.00	82.00	32.00	-
Vitamin A, IU	IU	9.00	2.00	0.00	-
Vitamin E (alpha-tocopherol)	mg	0.53	1.19	0.70	-
Vitamin K	µg	1.9	0	3.2	

Data were selected from USDA Food Data Central (2021) [8]. *Kansas State University Agricultural Experiment Station and Cooperative Extension Service (2021) [28].

human health. Considerably higher total amino acid content was found in soybean hulls (18.22%) than in wheat (12.77%), amaranth (12.725), and WOF (12.5%) (**Table 3**). The highest arginine (4.55%), asparagine/aspartic acid (1.20%), cysteine (0.55%), histidine (0.82%), isoleucine (0.74%), leucine (1.16%), lysine (1.01%), phenylalanine (1.28%), serine (1.17%), threonine (0.69%), tyrosine (1.32%) and valine (0.765) contents were found in soybean hulls among the four ingredients tested. Arginine has garnered more publicity recently for its possible heart benefits which are significant since millions of Americans have some forms of cardiovascular illness [29]. Phenylalanine and serine are amino acids used by human body to build proteins and other vital biomolecules; it has been researched for its effects on illness that is as diverse as depression, pain, and skin disorders [30]. Tyrosine is an amino acid that has gained popularity as dietary supplement for uses in improving mental wellbeing. It produces important brain chemicals that help nerve cells communicate and may even regulate mood [31]. Results show that considerably higher lysine was found in soybean hulls (1.01%), amaranth flour (0.69%) and oat flour (0.59%) compared to wheat flour

Table 3. Amino acids contents of starting ingredients.

Name %	Wheat	Amaranth	whole oat flour	Soybean hull
Alanine %	0.31^{d}	0.40^{c}	0.64^{a}	0.60^{b}
Arginine %	1.65^{b}	1.46^{c}	0.92^{d}	4.55^{a}
Asparagine/aspartic acid %	0.38^{c}	0.20^{d}	1.16^{b}	1.20^{a}
Cysteine %	0.53^{b}	0.20^{d}	0.44^{c}	0.55^{a}
Glutamine/glutamic acid %	3.18^{a}	1.62^{c}	2.96^{b}	1.63^{c}
Glycine %	0.34^{d}	1.73^{a}	0.69^{b}	0.58^{c}
Histidine %	0.24^{d}	0.70^{b}	0.33^{c}	0.82^{a}
Isoleucine %	0.47^{c}	0.53^{b}	0.54^{b}	0.74^{a}
Leucine %	0.87^{c}	0.78^{d}	1.08^{b}	1.16^{a}
Lysine %	0.25^{d}	0.69^{b}	0.59^{c}	1.01^{a}
Methionine %	0.04^{c}	0.05^{b}	0.24^{a}	0.05^{b}
Phenylalanine %	1.22^{b}	1.19^{c}	0.72^{d}	1.28^{a}
Proline %	1.55^{a}	0.58^{d}	0.79^{c}	0.87^{b}
Serine %	0.75^{c}	1.07^{b}	0.64^{d}	1.17^{a}
Threonine %	0.36^{d}	0.52^{b}	0.47^{c}	0.69^{a}
Tyrosine %	0.63^{c}	1.00^{b}	0.29^{d}	1.32^{a}
Valine %	0.56^{c}	0.59^{c}	0.74^{b}	0.76^{a}
Total %	12.77	12.72	12.50	18.22

Means followed by the same letter within the same row were not significantly different ($P > 0.05$).

(0.25%). Lysine is an amino acid that sometimes used as supplement for cold sores, athletic performance, and diabetes. Unlike some other amino acids, the human body cannot make lysine, therefore it must be consumed in the diet, such as consuming meat, fish, dairy products, eggs, and legumes [32]. Among ingredients tested, amaranth flour had tremendously higher glycine amino acid content (1.73%), a building block for protein used for schizophrenia, stroke, and memory and thinking skills [33]. Significant higher amounts of asparagine/aspartic acid were observed for soybean hulls (1.20%) and oat flour (1.16%) than amaranth (0.20%) and wheat flour (0.38%). Higher glutamine and glutamic acid amounts were observed for wheat flour (3.18%) and WOF (2.96%), which are part of the numerous metabolic processes for physical and mental stress and plays a pivotal role in maintaining a balanced acid-base ratio, and derivatives of glutamic acid are used for possible prevention of cancers [34]. The highest methionine content (0.24%) was found in WOF among samples tested. The importance of methionine oxidation in aging has been reported [35] bean hulls, amaranth, and WOF provided important essential amino acids that are lower in wheat flour, along with gluten-free proteins which will complement each other as nutritious ingredients for functional food. The replacement of amaranth and WOF by 25% soybean hulls in gluten-free recipes can greatly improve nutritional value.

3.3. Water-Holding Capacity (WHC)

The water-holding capacity (WHC) of all starting inputs for cookies is shown in Figure 1. The WHC of soybean hulls exhibited the highest value (488%) among the constituents for cookies whereas wheat flour was the lowest (107.5%). WHC is linked to many physical properties including structural morphology and fiber content. As reported by Jaworski *et al.* (2017) [23], soybean hulls contained 67.46% total dietary fiber (Table 1), including 5.31% soluble fiber and 62.25% insoluble fiber. The water may attach to cellulose fibrils through hydrogen bonding or reside inside fiber pores. The high WHC may be responsible for the loose fibril arrangement, branchy structures, large pore size and high surface area or hydrophilicity [36]. The WHC of amaranth (143.1%) and WOF (157.4%) was 40% - 50% higher than wheat flour (107.5%) and may be caused by higher protein and mineral content than that of wheat flour (Table 1 and Table 2). In addition, the higher WHC of WOF versus wheat flour could be associated with its β-glucan content. Beta-glucan is a type of hydrocolloids with the abundance of hydroxyl groups that facilitates hydrogen bonding with water, enabling beta-glucan to hold water in both soluble and insoluble states [37]. The increasing trends were observed from 143.1 to 220.2 g/100g and 157.4 to 218.5 g/100g for amaranth and WOF flour containing 25% soybean hulls, respectively (Figure 1). The WHC of WOF-soybean hull (3:1) composite (220.2%) and amaranth-soybean

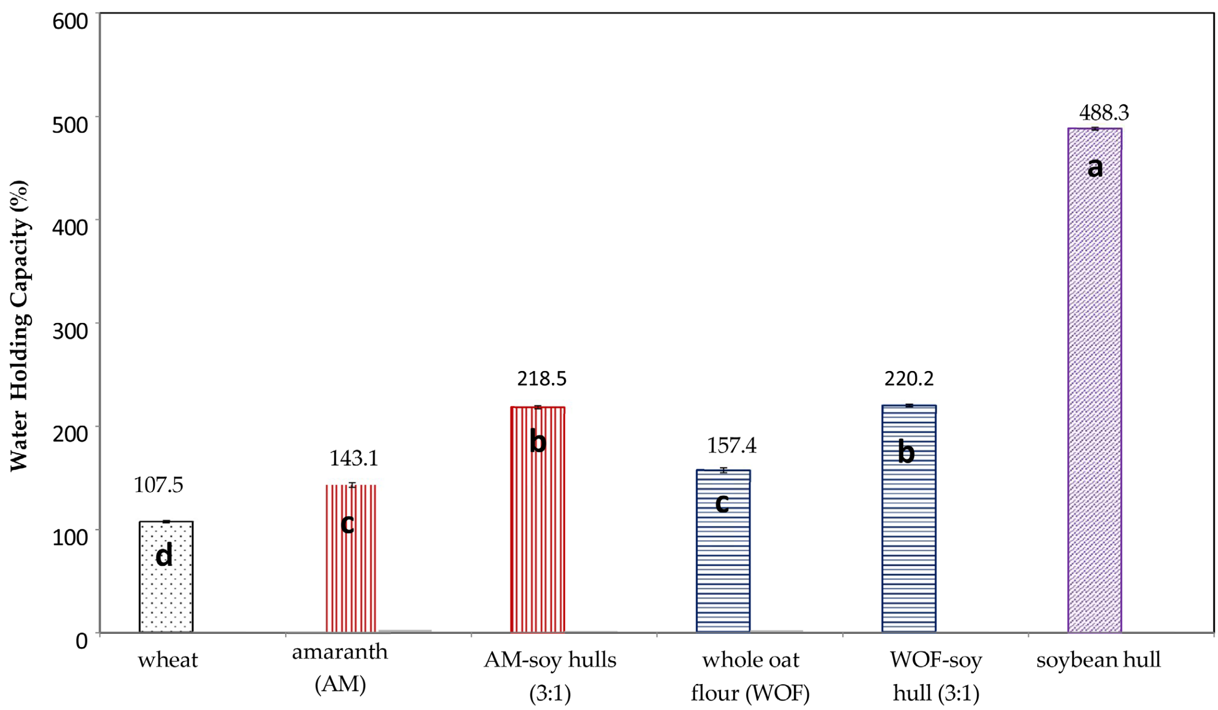

Figure 1. Water-holding capacities of starting ingredients and mixtures with soybean hulls.

hull (3:1) composite (218.3%) are almost twice that of wheat flour (107.5%). Clearly, the improved high WHC of oat or amaranth composites with soybean hulls may be attributed to higher WHC from soybean hulls. The elevated WHC can enhance water retention and moisture content for products using amaranth and WOF containing 25% soybean hulls.

3.4. Correlation of Properties of Materials for Cookies

The Pearson correlations between selective properties of ingredients are showed in **Table 4**. A significantly positive correlation was found between protein and fat ($P = 0.05$). This indicated that proteins are likely accompanying with fat. Also, the significantly positive correlations were observed for total dietary fiber with both total amino acids content ($P = 0.01$) and WHC ($P = 0.01$). It agrees with a previous study that WHC is affected by physical features of fiber including pore size, surface area, structure and arrangement, and hydrophilic nature [36]. Furthermore, a significantly positive contraption existed between total amino acids with water holding capacity ($P = 0.01$). Amino acids (hydroxy serine) induced 200% greater moisture uptake due to efficacy associated to the molecular structure and capability to attach to water molecules [38]. These results discovered the water holding capacities were attributed by both total dietary fiber and amino acids. It clarified that the higher water holding capacity from soybean hulls is accredited to its high total amino acids and dietary fiber (**Figure 1**.).

In contrast, the correlation tests disclosed a significantly inverse relationship for carbohydrate content with fat content ($P = 0.01$) (**Table 4**). Carbohydrates can be classified by molecular size into sugars, oligosaccharides, and polysaccharides [39]. Fat can be broken down by hydrolysis and release triglycerol and fatty acids, and then being converted into glucose through a process called gluconeogenesis [40]. The likelihood of a conversion of fatty acids to carbohydrate was mentioned as early as 1896 and by 1905, suggesting that fat was the principal

Table 4. Pearson correlation coefficients for selective properties of ingredients (wheat, amaranth, oat, and soybean hulls).

	Protein	Ash	Fat	Carb	Fiber	TAA	WHC
Protein	1.000						
Ash	0.158						
Fat	0.967*	−0.039					
Carb	−0.932	0.061	−0.989**				
Fiber	−0.372	0.842	−0.564	0.590			
TAA	−0.407	0.829	−0.589	0.608	0.998**		
WHC	−0.338	0.860	−0.534	0.561	0.999**	0.997**	1.000

Carb: carbohydrate by difference; TAA: total ammino acids; WHC: water-holding capacity; fiber: total dietary fiber. *=0.05, **=0.01 level of significance.

source of energy in diabetes [41]. Gluconeogenesis can take place in plants, animals, fungi, bacteria, and other microorganisms. This may explain the significantly inverse relationship between carbohydrate content and fat in grains and legumes.

3.5. Pasting Properties

Rapid Visco-Analyser pasting data revealed viscosity changes during heating and cooling which provides valuable information for material handling and food product formulation. The pasting curves are showed in **Figure 2**. The pasting curves were markedly different amongst the samples, indicating dissimilar viscosity properties. The viscosities of amaranth flour increased sharply (60 cP/min) to ~600 cP at 10 min during heating and shearing, reduced slightly to ~500 cP after the peak, displayed a flat pasting viscosity curve, and then increased a little (~100 cP) to a final peak viscosity (~600 cP) during cooling. The viscosities of WOF increased rapidly (~50 cP/min) to the initial peak (~590 cP) after the temperature increased to 95˚C at 13 min during heating and shearing, viscosities reduced to ~400 cP and increased quickly (60 cP/min) to the highest final peak (~1000 cP), which was seemingly higher than amaranth flour (~600 cP). The initial peak usually contributed to starch gelatinization while the elevated final viscosity indicated an entanglement of molecules. The high final viscosities usually related to the great stability of products under heat and shear. The tremendously high final viscosity (~1000 cP) of WOF was possibly caused by the interaction of protein with *β*-glucan from WOF. Enhancement in the textural characteristics of food using oat *β*-glucan hydrocolloids can be found in

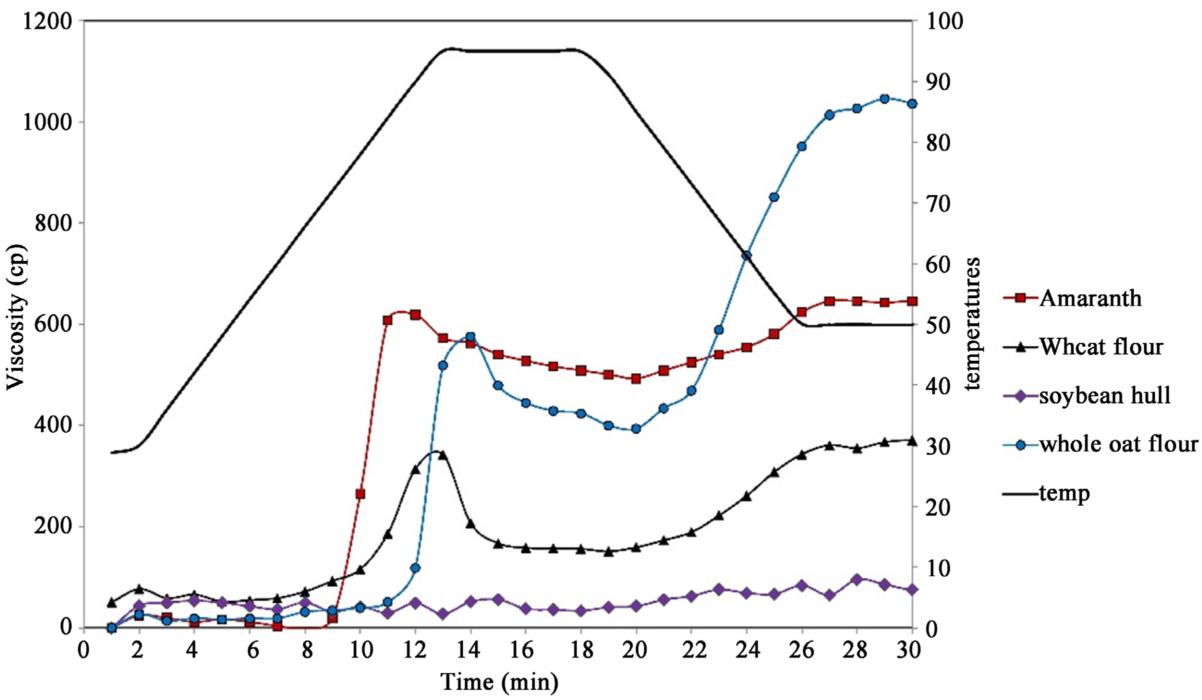

Figure 2. Rapid Visco-Analyser pasting curves of starting ingredients.

the literature [20]. A sharp peak was observed at 12 min (~380 cP) for wheat flour, quickly reduced to ~150 cP, and then the viscosity increased gradually to 380 cP during cooling. The initial sharp viscosity peak detected during the heating phase is generally attributed to starch swelling and gelatinization as temperatures risen from 75˚C to 95˚C. No visual distinguishing peaks were observed for the viscosity of soybean hulls during heating, shearing, and cooling, displaying a lowest final peak (70 cP). This may be caused by the size and coarse texture of soybean hulls influencing starch gelatinization. Also, the soybean hulls were heated during the dehulling process. It is recognized that the pasting viscosity of fully gelatinized starch granules previously would not rise during heating [42]. The pasting curves of the tested samples displayed disparate patterns, demonstrating dissimilar pasting properties. Results from this part of the research suggest that the use of amaranth and WOF will increase the pasting viscosities and stability of the product.

3.6. Rheological Measurements

Dynamic viscoelastic properties at various frequencies for pasting of the starting ingredients are illustrated in **Figure 3**. The dynamic viscoelastic properties of food materials are often referenced in the food production for material handling and textural consistency of final products. The elastic (storage) modulus (G') signifies the non-dissipative constituent of the mechanical properties of a substance and indicates elastic characteristics. In contrast, the viscous (loss) modulus (G") symbolizes the dissipative component of the mechanical properties and characterizes the viscous property of the substance. The values of elastic

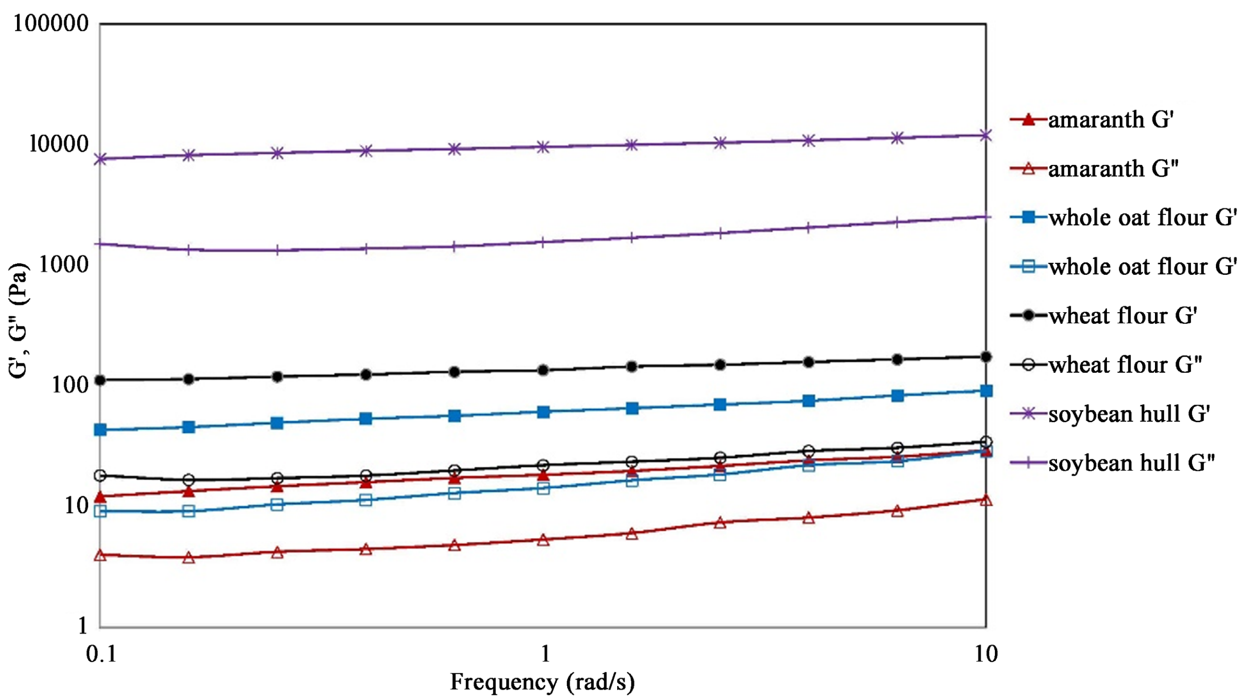

Figure 3. Dynamic viscoelastic properties for starting ingredients.

(storage) G' and viscous (loss) G" for all samples were nearly frequency- independent throughout the frequency range tested. Furthermore, elastic moduli G' were greater than viscous moduli G" throughout the frequency range (**Figure 3**) for inputting samples with diverse levels, suggesting more solid-like profiles. Large variances between G' and G" were detected, especially for soybean hulls, indicating that the material might be considered as viscoelastic solid materials [14]. The storage modulus G' at 1 rad/s for soybean hulls, wheat, WOF, and amaranth flour were ~10,000, 100, 75, and 10 (Pa), respectively. The lowest value of the elastic G' was observed for amaranth flour while the highest G' value was detected for soybean hulls, indicating high intermolecular interactions and entanglements to form a stable gel, possibly owing to the entanglement of protein and fibers from soybean hulls. Results suggested the addition of soybean hulls to amaranth and WOF will enhance the elastic property of the products.

The value of tan δ (G"/G') is a quantitative measurement for relative strength between energy lost and energy stored during a test cycle. The tan δ is useful for food materials to show the strong connection between viscous behavior and degree of hydrolysis. Tan δ was somewhat increased with increasing the frequency of all tested samples, thereby displaying elastic property (**Figure 4**). The loss tangent value of amaranth flour was the highest among all tested samples. The loss tangents for soybean hulls and wheat flour were comparable and lower than those of amaranth and WOF. The tan δ values indicate greater interactions of molecular chains [43]. The low value of tan δ (tan $\delta < 1$) indicates a largely elastic tendency, while tan $\delta > 1$ shows a mostly viscous pattern. All measured samples showed tan (δ) value < 1, signifying solid-like viscoelastic behaviors (**Figure 4**). The loss tangent value at frequencies of 1 rad/s was 0.3, 0.23, 0.16, 0.16 for amaranth flour, WOF, soybean hull, and wheat flour, respectively. Tan δ value >

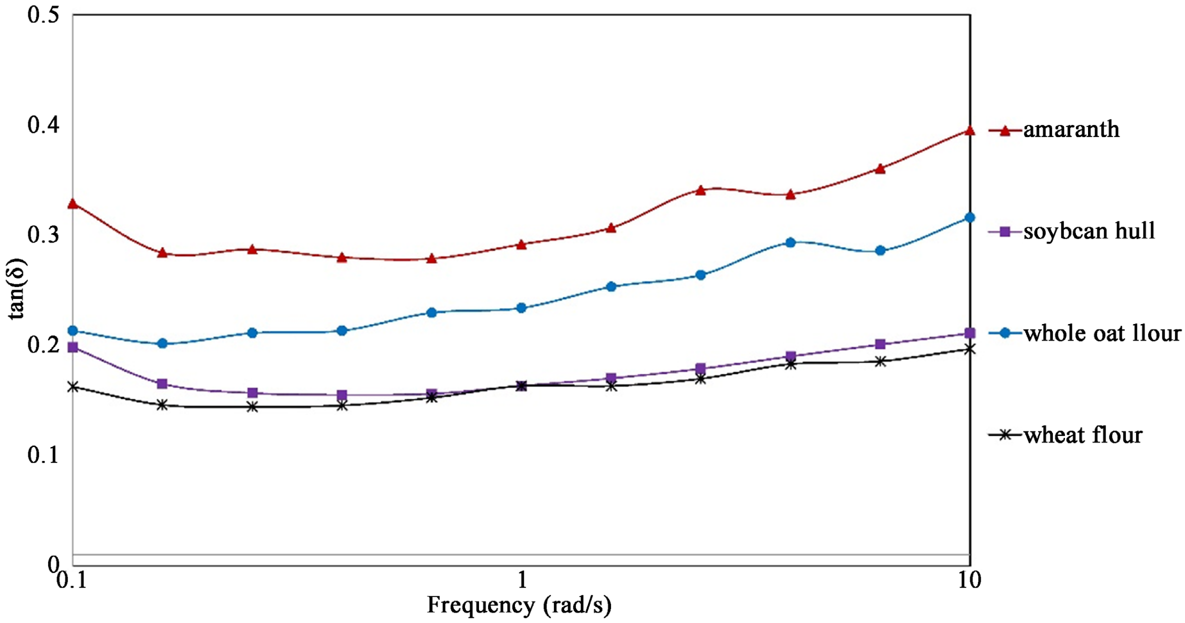

Figure 4. Values of tan (δ) versus frequency (rad/s) for starting ingredients.

0.1 shows the sample was not a real gel and the structure is something between a high concentrated biopolymer and a true gel [44]. Tan δ values of the tested samples were smaller than 1, nevertheless higher than 0.1, which shows the existence of an elastic structure in a weak biopolymer gel [14]. Results suggested that soybean hulls would be good contenders for using as food ingredients food products with desirable viscoelastic properties.

The viscosity during the pasting test is plotted against shear rate in **Figure 5**. All samples displayed shear-thinning behavior over the entire shear rates at 25˚C. Shear-thinning behavior of a material is significant in industrial food manufacturing such as mixing and pumping. Shear-thinning also contributes to a light and non-slimy mouth feel when food materials are masticated. Shear properties for WOF and wheat flour were identical, representing similar viscoelastic properties during shearing. Soybean hulls exhibited the highest apparent viscosity, indicating the improved elastic characteristics when undergoing deformation; this could offer better shape retention during material handling and thermomechanical processing. Majority of food processing and mastication in mouth is carried out at a shear rate range of 1 - 100/s, same as those used in this investigation. Subsequently the experimental conditions were like those in an actual processing environment; the information obtained from this study will no doubt facilitate the adoption of soybean hulls and other underutilized food materials in commercial food production.

3.7. Water Loss during Baking, Cookie Moisture and Textures

Water losses during baking ranged from 9.20% to 10.62% (**Table 5**). Cookies with wheat flour had the highest water loss (10.62%) among all cookies tested,

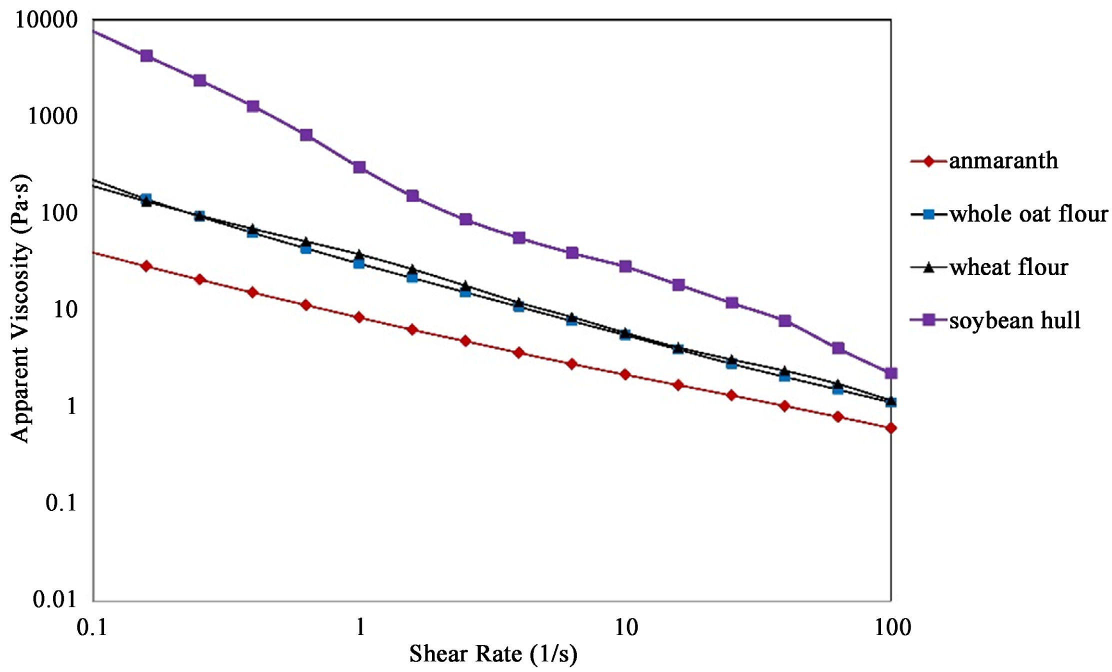

Figure 5. Apparent viscosity versus shear rate for pastes of starting ingredients.

Table 5. Water loss during baking, cookie moisture and cookie hardness.

Sample	Water loss during baking %	Cookie moisture %	Cookie hardness Cutting force (kg)
Wheat	10.62 ± 0.01[a]	3.95 ± 0.01[c]	5.34 ± 0.01[b]
Whole oat flour (WOF)	10.12 ± 0.03[b]	3.70 ±0.05[c]	5.10 ± 0.05[b]
WOF-Soy hulls (3:1)	9.33 ±0.12[c]	4.56 ±0.12[a]	4.60 ± 0.60[c]
Amaranth (AM)	9.82 ± 0.01[b]	3.95 ±0.01[c]	6.01 ± 0.60[a]
AM-Soy hulls (3:1)	9.20 ± 1.24[c]	4.31 ± 0.08[b]	5.44 ± 0.01[b]

Means followed by the same letter within the same column were not significantly different ($P > 0.05$).

probably due to the lowest WHC of wheat flour (107.5%) (**Figure 1**) among all flours tested in this study. It was observed that water losses during baking were reduced due to the addition of soybean hulls, comparing WOF cookies (10.12%) to WOF-soybean hull cookies (9.22%), and amaranth flour cookies (9.82%) to amaranth-soybean hull cookies (9.20%). Moisture content is an important aspect of food properties. The moisture contents of all cookies tested were in the range of 3.70% to 4.56%. The moisture content of cookies was increased by the addition of soybean hulls, when comparing WOF cookies (3.70%) to WOF-soybean hull cookies (4.56%), and amaranth cookies (3.95%) to amaranth-soybean hull cookies (4.31%). In general, the starting ingredients with higher WHC resulted in lower water loss during baking and higher moisture content in cookies. Results suggest that soybean hulls can be well blended with amaranth and WOF resulting in improved water retention for food products.

Cookie hardness is an essential character of cookies. The hardness was not considerably influenced by using amaranth, WOF or their composites with soybean hulls when compared to cookies made from wheat flour. Adding soybean hulls in the formulations somewhat decreased the hardness when compared to amaranth or WOF cookies (5.10 to 4.60, 6.01 to 5.34). The reduced hardness was probably due to improved WHC and moisture contents by using soybean hulls. Hardness of WOF cookies (5.10 kg) was similar to wheat flour cookies (5.34 kg). In general, no substantial differences were found among cookie hardness tested by the addition of soybean hulls.

3.8. Geometrical Properties

Cookie geometrical properties comprise width, thickness and cookie spread factor. The largest diameter observed for wheat flour cookies (6.35 cm) and the smallest diameter for WOF cookies (6.08 cm) (**Table 6**) indicated that the cookie width was not greatly influenced by the presence of soybean hulls. The cookies using wheat flour had the highest thickness (1.26 cm) while the other cookies had similar thicknesses ranging from 1.13 - 1.16 cm (**Table 6**). The baking powder used in the preparation of the cookies released carbon dioxide gas into a

Table 6. Geometrical properties of cookies.

Sample	Width		Thickness		Spread factor
	Before bake Cm	Before bake cm	Before bake cm	Before bake cm	width/thickness cm
Wheat	6	6.35 ± 0.03[a]	0.7	1.26 ± 0.02[a]	5.04 ± 0.03[c]
WOF	6	6.08 ± 0.01[c]	0.7	1.14 ± 0.02[b]	5.33 ± 0.04[b]
WOF-soy hull (3:1)	6	6.25 ± 00.1[b]	0.7	1.13 ± 0.01[b]	5.53 ± 0.02[a]
Amaranth (AM)	6	6.09 ± 0.01[c]	0.7	1.16 ± 0.02[b]	5.25 ± 0.03[b]
AM-soy hulls (3:1)	6	6.27 ± 0.03[b]	0.7	1.15 ± 0.01[b]	5.45 ± 0.02[a]

Means followed by the same letter within the same column were not significantly different ($P > 0.05$).

dough as a result of an acid-base reaction, producing bubbles in the wet mixture to increase the volume. The smaller diameter value and the slightly lower thickness of the cookies may be resulted from high protein and fiber content as well as high WHC, which could make the dough less spreadable and expandable compared to wheat flour cookies. However, it was obvious from the results that diameter and thickness were not greatly affected by using amaranth or oat composites with soybean hulls compared to wheat flour cookies.

The dissimilarity was also present in the spread factor (width/thickness). Wheat flour cookies showed the lowest spread ratio (5.04) followed by cookies comprising amaranth flour (5.25) and WOF (5.33). The spread factor increased from 5.33 to 5.53 for WOF cookies and WOF-soy hull cookies, while the spread factor increased from 5.25 to 5.45 for amaranth cookies and amaranth-soybean hull cookies. The results demonstrated that diameter, thickness and spread factor were influenced by the presence of amaranth flour, WOF and their blends with soybean hulls compared to wheat flour cookies. However, no remarkable differences were found among all cookies using amaranth flour, WOF and their composites with soybean hulls compared with wheat flour cookies. Results from this study demonstrated soybean hulls may be excellent ingredients to combine with other conventional ingredients, such as amaranth and WOF in food products.

4. Conclusion

This study found that like amaranth and whole oats, soybean hulls contain higher protein and fiber content, minerals, amino acids, and critical vitamins (C and K) than wheat. The addition of soybean hulls enhanced the water-holding capacity in the starting ingredients. The significantly positive correlations were found between protein and fat ($P = 0.05$), total dietary fiber with both total amino acids content ($P = 0.01$) and water holding capacity properties ($P = 0.01$), and between total amino acids with water holding capacity ($P = 0.01$), respectively. In contrast, the correlation tests revealed a significantly negative relationship for carbohydrate content with fat content ($P = 0.01$). Differential pasting and rheological properties were observed in the starting materials. Soybean hulls also improved the water retention and moisture content of cookies. No significant difference was found in the geometrical and textural properties of gluten-free cookies fortified with soybean hulls when compared to wheat flour cookies. In general, soybean hulls are very good ingredients to incorporate with amaranth and oat flour to make gluten-free cookies. All cookies tested were acceptable in terms of textural qualities and geometrical properties along with improved nutritional value in comparison to the wheat flour cookies. These results indicate that the dietary fibers in soybean hulls do not significantly affect the physicochemical and rheological properties that were reflected on textural and geometrical measurements. This study explored the feasibility and great potential of utilizing soybean hulls with amaranth and WOF in functional gluten-free bakery products and potential industrial applications.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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