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Effect of Whey Protein Concentrate on Rheological Properties of Gluten-Free Doughs and Their Performance in Cookie Applications

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Abstract: Gluten-free foods continue to be a hot topic and trend in the food market because more people are being diagnosed with gluten intolerance. Whey is a by- or co-product of the dairy industry and is considered a waste stream. In this study, whey protein concentrate (WPC), one of the whey products, was added at 8, 9, 10, 11 and 12% levels to sorghum and corn flours to make gluten-free products in the form of cookies. Mixograph and subjective evaluation showed that optimal water absorption (corn: 50–55%; sorghum: 55–60%) increased with increasing WPC level in both sorghum and corn flour dough systems. Increasing WPC from 8 to 12% resulted in a decrease in storage modulus (G′) and loss modulus (G″) for both sorghum and corn doughs. Corn dough rheological properties were less affected by WPC addition as compared to sorghum. The diameter of gluten-free sorghum and corn cookies significantly increased with the fortification of WPC. The color of sorghum and corn cookies became darker as the WPC level increased. Cookies prepared with 10% WPC addition showed the most hardness and brittleness, probably due to the gelling property of WPC. This study contributes to the sustainable utilization of whey product and helps understand the performance of WPC during the processing of gluten-free products and its potential for making food snacks such as cookies in food manufacturing.

Keywords: gluten-free; sorghum; corn; whey protein concentrate; rheology; cookie

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1. Introduction

Gluten-free foods are increasingly gaining acceptance as a desirable option for consumers suffering from celiac disease. Corn and sorghum are two examples of gluten-free options for making food products, where wheat is widely used. Corn is the largest crop in U.S. in terms of total production. Grain sorghum is the fifth most cultivated cereal crop in the world, and the fourth largest in the U.S. Sorghum is used primarily for livestock feed and ethanol production; however, recently it is becoming popular in the human food sector. Sorghum and corn are non-allergenic food grains, which make them a good alternative for people with gluten intolerance. However, proteins in sorghum and corn are different from wheat gluten in structure and amino acid composition. Proteins from sorghum and corn are difficult to hydrate, and they are more hydrophobic than gluten [1–3] Flours based on these grains have poor rheological properties and limited applications in dough-based food products. Supplementation with additives such as gums and functional proteins are often required to improve the performance of these gluten-free ingredients [4]. Sorghum and corn proteins if processed well with other functional proteins might participate in a dough-forming process similar to wheat [5].

Whey is a by- or co-product of the dairy industry, such as cheese making and casein manufacture, and is considered a waste stream [6], which has led to considerable environmental problems due to its high organic matter content [7]. Over the past decades, several biotechnological approaches and process technologies developed to convert this side product into a source of high-value nutritional components, and whey protein concentrate (WPC) is one of these valuable nutritional components. It contains as high as 80% protein and it possesses almost all the essential amino acids, including lysine [6,8,9]. Apart from being nutritious, whey proteins exhibit particular functional properties, such as solubility, viscosity, water binding, whipping, emulsification and gelation that are desirable in a food system [10–15]. Past research has shown that the thickening effect of whey proteins is similar to hydrocolloids and starches [16].

Dynamic rheological testing is a powerful tool for examining the deformation and flow of matter under a wide range of testing conditions. This technique simultaneously measures the viscous and elastic behavior of the dough [17,18] and can be used as a quality indicator for the final quality of cereal products [19]. There are lots of studies focused on the rheological behavior of wheat-based dough [20–22] or gluten-free bread dough [23–25]. However, there is lack of published research on the use of WPC on the rheological behavior of sorghum- and corn-based gluten-free cookie dough.

In this study, WPC was used as a functional ingredient in developing gluten-free product, which is in high demand by celiac disease patients in the food market. The effect of WPC on the rheological properties of the sorghum- and corn-based dough system was investigated, as well as evaluation of gluten-free system's performance in cookie applications. We determined water absorption and dough-forming characteristics of sorghum and corn flours with WPC (8–12%) using mixograph and subjective evaluation. The detailed subjective evaluation provides extra information describing the performance of these gluten-free doughs. Dynamic rheological properties of the sorghum- and corn-based dough system with 8–12% WPC were also tested. The successful conversion of whey proteins into high-demand food products is a great and potential pathway for reducing the negative impact of whey disposal into the environment. This study contributes to sustainable utilization of whey products and helps understand the performance of WPC during the processing of gluten-free products and its potential for making food snacks such as cookies in food manufacturing.

2. Materials and Methods

2.1. Flours

Decorticated sorghum flour, whole corn flour and WPC were purchased from NuLife Market LLC (Scott City, KS, USA), People's Grocery (Manhattan, KS, USA) and Davisco Foods International Inc., (Eden Prairie, MN, USA), respectively.

2.2. Mixograph and Dynamic Rheometer

Dough was prepared using a 35 g bowel–capacity mixograph (National Manufacturing Co., Lincoln, NE, USA) according to the AACC method 54-40 A mixograph standard [26] to determine the optimum water absorption of the flour mixtures. In this study, dough quality properties such as stickiness, cohesiveness, and firmness were also evaluated subjectively to validate the optimum water absorption obtained by mixograph and better describe the dough's performance. Scales for subjective evaluations on dough stickiness, cohesiveness and firmness are shown in Table 1. Optimum water absorption from the mixograph as well as scores from the subjective test are shown in Table 2.

Dynamic oscillatory tests were performed in a P25 serrated plate dynamic oscillatory rheometer (StressTech, ATS Rheosystems, Bordentown, NJ, USA). Optimum water absorption data from subjective evaluation were used for the sample test on rheometry. The assay was executed at 22.4 ± 0.1 °C, using a serrated plate–plate sensor system with a 1.0 mm gap between plates. Before measurement, each dough was allowed to rest for 20 min between plates to relax in an air-tight container. To prevent sample dehydration during the assay, silicon oil was applied around the sample between the plates. Strain sweep was performed with a stress ranging from 1 to 10,000 Pa to determine the linear viscoelastic region of each dough. Then, a frequency sweep (from 0.01 to 100 Hz) was performed at a constant stress (100 Pa) within the linear viscoelastic range. Dynamic moduli G′ (elastic or storage modulus), G″ (viscous or loss modulus) and G* (complex shear modulus) were obtained as a function of frequency. Doughs were prepared in duplicate, and three measurements were performed on each dough.

Table 1. Scales for subjective evaluations of dough stickiness, cohesiveness and firmness.

Stickiness Scale				
1	3	5	7	10
-Not sticky at all; falls off hands	-Sticking to hands and itself a little	-Sticking to hands and to itself, but weak dough that easily breaks	-Sticking well to hands and itself	-Extremely sticky and bind well with itself
Cohesiveness Scale				
1	3	5	7	10
-Falls apart easily	-Did not stretch when elongated, just broke with a little shaking	-Stretches a little when elongated	-Stretched a few inches when elongated	-Stretches seemingly indefinitely
Firmness Scale				
1	3	5	7	10
-Falls apart when pressed	-Hard and cracks appeared quickly with little press	-Little soft, press to see cracks	-Fairly visco-elastic when pressed	-Good visco-elastic, little to no cracks appear when pressed

Table 2. Optimum water absorption of flour mixtures and subjective evaluations of dough stickiness, cohesiveness and firmness.

Flour Type	% WPC	Mixograph	Subjective Evaluation		
		Optimum Water Absorption, %	Stickiness	Cohesiveness	Firmness
Sorghum Flour	0	70.00	-	-	-
	8	55.00	5.5	7	5
	9	57.50	5.5	5	6
	10	57.50	5.5	6	6.5
	11	57.50	5	6.5	6.5
	12	60.00	7	8	8
Corn Flour	0	75.00	-	-	-
	8	50.00	5	4	3
	9	50.00	4.5	4.5	4
	10	52.50	4	5.5	5
	11	52.50	5	5.5	6
	12	55.00	6	5.5	5.5
Wheat Flour	0	60.00	10	10	10

"-" means too weak dough, cannot be tested.

2.3. Cookie Preparation and Properties Evaluation

The cookies were produced according to AACC International Method 10-50.05 [26]. Cookie doughs of 100% wheat flour were prepared as control samples. Cookie doughs were baked at 205 ± 2 °C for 10 min and they were allowed to cool for 10 min and packed. Measurements of the weights and diameters of the cookies from each sample set were tested using a balance scale and caliper, respectively, with three randomly selected cookies. Also, thickness measurement was tested on a stack of 3 cookies, then divided the thickness by 3 for each cookie thickness. All tests were performed in triplicate.

The hardness and brittleness/flexibility of each cookie was tested using a Texture Analyzer (TA-XT2, Texture Technologies Corp., Scarsdale, NY, USA). The standard 3-point bending method with a macro run was used to test the breaking strength and distance. The sample was placed centrally on the supports placed 32 mm apart and subjected to force until it fractured and crumbled into pieces. Analyses were made on 4 randomly selected cookies for each sample set. Lightness (L*), redness (a*), and yellowness (b*) color values of the cookies were determined by a hand-held Chroma Meter (Model CR-210, Minolta, Japan) according to the procedure described by Gajula, Alavi, Adhikari, and Herald [27]. Triplicates were performed on the color test.

2.4. Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) (SPSS version 13.0, SPSS Inc., Chicago, IL, USA), and Tukey's HSD test was used to examine the differences when ANOVA was significant. Results with a corresponding probability value of $p < 0.05$ were considered to be statistically significant.

3. Results and Discussion

3.1. Optimal Water Absorption of Flour Mixtures

Mixograph tests mixing properties of dough, in which dough development time and peak dough resistance were used to assess the dough's strength, and it is largely a function of protein content and the environment. In this study, results showed that mixograph behavior of the polymeric proteins in sorghum and corn with WPC mixtures were unique, which lacked a defined peak, indicating the polymeric proteins in these mixtures did not behave as predicted or behaved similarly to mixograms from wheat flour. With an increase in WPC, the optimal water absorption of the flour mixtures increased, and the subjective values of stickiness, cohesiveness and firmness increased overall as well (Table 2), which means the subjective tests correlate with the objective tests using the mixograph. Other studies have also reported that dairy ingredients increase water absorption and could improve dough-handling properties [28–30].

3.2. Rheological Properties of Sorghum and Corn Doughs with 8–12% WPC

The results for the dynamic mechanical rheology of corn and sorghum doughs with 8–12% WPC are presented in Figure 1. G' (storage modulus), G'' (loss modulus) and G^* (complex shear modulus) were recorded during the experiment. The results show that the addition of WPC resulted in a reduction in the values of moduli, G', G'' and G^*. The more WPC was added, the lower the values of the dynamic moduli, both in sorghum and corn doughs, which indicated that WPC addition resulted in softening behavior in the dough system during the frequency sweep measurements. Balestra et al. [31] proposed that water molecules in high-moisture doughs behave as inert fillers. When water content in the dough system increased, elastic modulus and viscous modulus decreased, resulting in the softening of the dough [32–34]. In this study, the addition of WPC resulted in an increase in optimal water absorption in the flour mixture (Table 2), and thus increased the water content in the dough and subsequently decreased the moduli values. This result is in agreement with the findings of Asghar, Anjum, Allen, Daubert, and Rasool [35], who found lower moduli values with the addition of 5% modified WPC in wheat flour. Patil and Arya [36] concluded that whey protein might confer a protective effect on the gluten network's dough system and render it more stable. Whey protein was reported to have good solubility and a high emulsifying property, and it acts as a thick film covering the starch granules when whey protein was added in the gluten-free system [37] and that might contribute to increasing the viscoelasticity of the gluten-free network system. Waziiroh, Bender Jäger and Schönlechner [38] also found that whey protein possesses high contents of total and free sulfhydryl groups, which exhibited strong crosslinking tendencies and protein addition indicated a minor dilution effect on the starch content, which changes the batter viscosity profile.

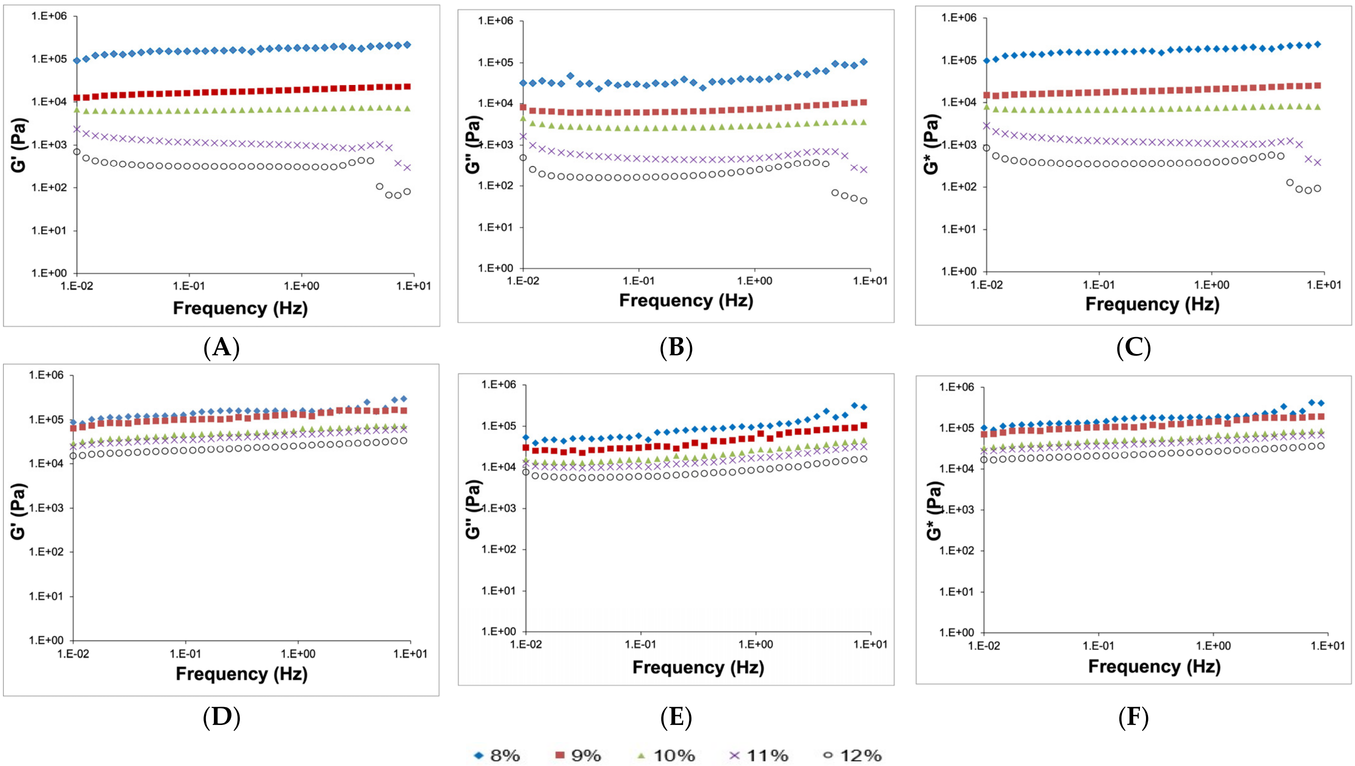


Figure 1. Rheological properties of sorghum (**A**–**C**) and corn (**D**–**F**) doughs with 8–12% WPC. G′, elastic or storage modulus; G″, viscous or loss modulus; G*, complex shear modulus.

In the present study, the decrease in G′, G″ and G* was less in the case of corn than sorghum, indicating the lower impact of WPC addition on the rheological properties of the former. Aprodu and Banu [39] observed that the type of starch also affects the efficiency of whey protein on the thermo-mechanical properties of the bread crumb. The different behavior between sorghum and corn might be due to their different starch structures and properties. The values of G′ are larger than the value of G″, both for corn and sorghum, which indicates that the doughs were more elastic than viscous [40]. Also, an increase in the values of the moduli was observed with an increase in oscillation frequency during testing, except for sorghum flour with 10–12% WPC. This finding is in close agreement with Asghar, Anjum, Allen, Daubert, and Rasool's [35] findings, who also reported that increasing oscillation frequency led to an increase in the values of G′ and G″ of flour doughs [41–45]. The higher dynamic moduli at higher frequencies implies more bonds involved in the mechanical response of the system due to a stress or strain applied over a shorter time [46]. In this study, sorghum flour with 10–12% WPC seems to be less dependent on frequency. It was previously observed that high elastic modulus values with low frequency dependence are related to good quality gluten [47]. Thus, according to this observation, sorghum flour with 10–12% WPC addition might be a good quality dough as WPC exerted a softening effect on the doughs. The high subjective values (7, 8, 8) for sorghum flour with 12% WPC mixtures (Table 2) support this finding.

3.3. Evaluation of Cookies from Sorghum and Corn Flour with 8–12% WPC

Figure 2 shows cookies made from corn (A) and sorghum (B) with 8–12% WPC and Table 3 shows the quality parameters for the cookies from sorghum and corn flour with 8–12% WPC. The result shows a significant increase in cookie diameters for both sorghum and corn flour with 8–12% WPC addition as compared to the control cookies with sorghum and corn only (0% WPC). Cookie diameters for sorghum flour with 11–12% WPC and corn flour with 8–12% WPC were even significantly ($p < 0.05$) higher than the pastry control. This phenomenon indicated the weakening of the dough matrix and lower intermolecular network interactions in the corn and sorghum dough system compared to pastry dough. The resulting dough was easy to spread with minimal shrink-back during baking. Sahagún and Gómez [48] found that animal proteins (egg white and whey) led to a more pronounced decrease in dough consistency (given by a reduction in the rheological parameters G′ and G″) compared to vegetable (pea and potato) proteins, and therefore led to a large dough expansion during baking. An increase in cookie diameter by fortification with whey protein was also reported by Sarabhai and Prabhasankar [49], Wani, Gull, Allaie, and Safapuri [50], and Sinthusamran, Benjakul, Kijrrongrojana, and Prodpran [51].

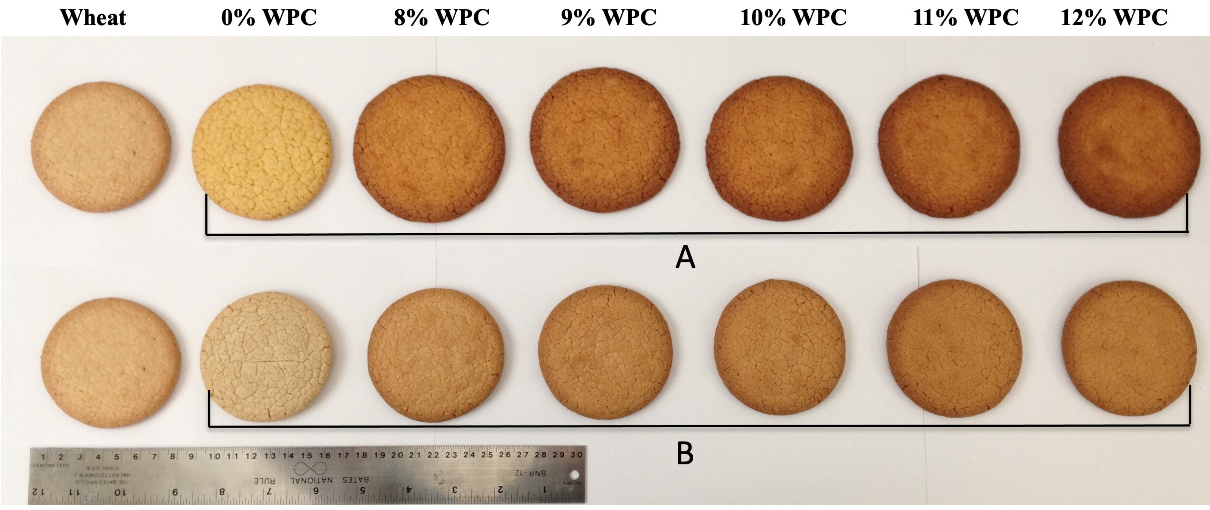


Figure 2. Cookies made from corn (**A**) and sorghum (**B**) with 8–12% WPC.

Table 3. Evaluation of cookies from sorghum and corn flour with 8–12% WPC.

Sample	Diameter, cm	Thickness, cm	D/T *	Weight, g	Color Values		
					L	a	b
Wheat Control	7.59 ± 0.03 ^{bc}	0.90 ± 0.03 ^{a}	8.45 ± 0.29 ^{e}	19.46 ± 0.67 ^{a}	61.6 ± 1.2 ^{f}	6.4 ± 0.6 ^{c}	21.3 ± 0.2 ^{f}
Sorghum	7.12 ± 0.03 ^{a}	1.26 ± 0.04 ^{e}	5.67 ± 0.16 ^{a}	21.30 ±0.10 ^{a}	63.4 ± 0.4 ^{g}	2.9 ± 0.2 ^{a}	18.1 ± 0.1 ^{g}
Sorghum + 8% WPC	7.46 ± 0.01 ^{b}	1.20 ± 0.03 ^{de}	6.22 ± 0.17 ^{b}	20.15 ± 0.40 ^{a}	52.3 ± 0.8 ^{e}	9.2 ± 0.3 ^{d}	19.6 ± 0.6 ^{e}
Sorghum + 9% WPC	7.66 ± 0.08 ^{c}	1.14 ± 0.02 ^{cde}	6.69 ± 0.10 ^{c}	20.36 ± 0.42 ^{a}	50.9 ± 1.1 ^{e}	9.6 ± 0.4 ^{d}	19.6 ± 0.3 ^{e}
Sorghum + 10% WPC	7.73 ± 0.03 ^{cd}	1.12 ± 0.02 ^{cde}	6.89 ± 0.13 ^{c}	19.43 ± 0.26 ^{a}	48.7 ± 0.9 ^{d}	10.4 ± 0.2 ^{e}	19.1 ± 0.4 ^{d}
Sorghum + 11% WPC	7.84 ± 0.09 ^{d}	1.12 ± 0.04 ^{cde}	6.99 ± 0.17 ^{c}	19.83 ± 1.09 ^{a}	48.1 ± 1.1 ^{d}	10.4 ± 0.2 ^{e}	18.9 ± 0.4 ^{d}
Sorghum + 12% WPC	7.81 ± 0.05 ^{d}	1.16 ± 0.02 ^{de}	6.76 ± 0.15 ^{c}	20.27 ± 0.65 ^{a}	47.3 ± 1.3 ^{d}	10.8 ± 0.2 ^{e}	18.9 ± 0.6 ^{d}
Corn	7.68 ± 0.08 ^{cd}	1.02 ± 0.04 ^{bc}	7.52 ± 0.22 ^{d}	20.91 ± 0.82 ^{a}	64.0 ± 1.0 ^{g}	4.2 ± 0.6 ^{b}	28.1 ± 0.5 ^{g}
Corn + 8% WPC	8.63 ± 0.10 ^{e}	0.91 ± 0.02 ^{a}	9.48 ± 0.09 ^{f}	21.00 ± 0.74 ^{a}	47.5 ± 1.2 ^{d}	12.1 ± 0.4 ^{f}	21.5 ± 0.7 ^{d}
Corn + 9% WPC	8.64 ± 0.03 ^{e}	0.91 ± 0.02 ^{a}	9.48 ± 0.17 ^{f}	21.02 ± 0.43 ^{a}	45.4 ± 1.0 ^{c}	12.5 ± 0.2 ^{fg}	20.3 ± 0.7 ^{c}
Corn + 10% WPC	8.62 ± 0.07 ^{e}	0.92 ± 0.04 ^{ab}	9.36 ± 0.31 ^{f}	21.30 ± 1.07 ^{a}	44.6 ± 1.1 ^{bc}	12.6 ± 0.2 ^{fg}	19.9 ± 0.7 ^{bc}
Corn + 11% WPC	8.66 ± 0.01 ^{e}	0.96 ± 0.02 ^{ab}	9.06 ± 0.18 ^{f}	21.05 ± 0.46 ^{a}	43.3 ± 1.2 ^{b}	12.7 ± 0.2 ^{g}	19.2 ± 0.7 ^{b}
Corn + 12% WPC	8.64 ± 0.06 ^{e}	0.91 ± 0.02 ^{a}	9.48 ± 0.14 ^{f}	20.70 ± 0.80 ^{a}	41.5 ± 1.5 ^{a}	12.9 ± 0.1 ^{g}	18.1 ± 0.9 ^{a}

* D/T = Diameter/Thickness Ratio; data are means of triplicates ($n = 3$); means with the same superscript in the same column are significantly different ($p < 0.05$).

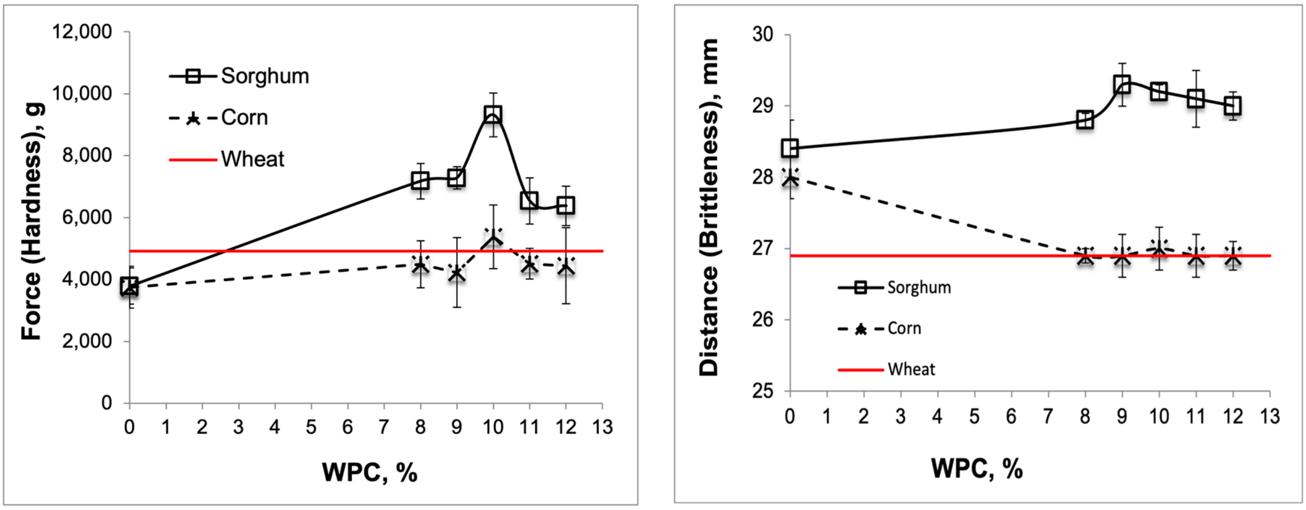
The thickness of cookies made from sorghum with 8–12% WPC showed no statistical difference as compared to the control sorghum-only cookies. Cookies made from corn with

8–12% WPC became thinner when compared to the control corn-only cookies, but were not significantly different from the pastry control. The spread ratio (diameter/thickness, D/T) was significantly ($p < 0.05$) increased for both sorghum and corn with 8–12% WPC as compared to the controls of sorghum- and corn-only cookies. The D/T for sorghum cookies was significantly lower than the pastry control, owing to the large thickness of sorghum cookies. The D/T for corn cookies, except the corn-only control, was significantly higher than the pastry control, owing to the increase in diameter for corn cookies. The addition of WPC for both sorghum and corn showed a positive effect on the D/T of the cookies.

The surface color of the cookie is one of the most important elements for initial acceptability by consumers. With increasing WPC levels, the lightness value (L) for both cookies from sorghum and corn with WPC addition significantly decreased as compared to the controls of pastry, sorghum- and corn-only cookies. There was a significant increase in positive 'a' (redness) values of the cookie made from sorghum and corn flour with 8–12% WPC. For sorghum with 8–10% WPC, the positive 'b' (yellowness) values significantly increased as compared to sorghum-only cookies, and for corn with 8–12% WPC, the 'b' value was significantly decreased as compared to corn-only cookies. The 'b' values for the sorghum- and corn-based cookies were decreasing with increasing WPC additions. Žilić, Kocadaugli, Vančetović, and Gökmen [52] indicated that the main cause of color development was due to the high degree of Maillard browning during baking, although sugar caramelization may have had some influence. Sarabhai and Prabhasankar [49] also reported that the addition of WPC favors the development of the cookie's color. Pérez, Matta, Osella, de la Torre, and Sánchez [53] asserted that color variations of cookies may be explained as a result of the development of colored compounds through the Maillard reaction between the residual WPC lactose and the free amino groups from the lysine incorporated with the protein ingredients. The increase in the relative contribution of the Maillard reaction to the total color of the cookies results in an increment of the excitation purity or saturation of the samples. Pico, Reguilón, Bernal and Gómez [37] reported that animal proteins yield a more pronounced effect on crust color than the vegetal ones (rice and pea) due to their higher solubility which may induce their contact and reactivity with reducing sugars. Sahagún and Gómez [54] also explained that the high lysine content that whey protein contains triggered the dark color, since it is the primary reactive amino group that reacts with the reducing sugars. Both Pico, Reguilón, Bernal and Gómez [37] and Komeroski et al. [55] found a similar trend that, in general, the higher the whey protein addition level, the darker the product color.

3.4. Textural Characteristics of Cookies

The effect of WPC addition on the textural characteristics of sorghum- and corn-based cookies is presented in Figure 3. Compared to pastry cookies, sorghum cookies with WPC were consistently harder and more brittle, but corn cookies with WPC were similar to the pastry cookies in textural properties. Parate, Kawadkar, and Sonawane [56] also reported that the texture of WPC-fortified biscuits was found hard as compared to control biscuits. In this study, the hardness and brittleness of cookies increased up until 10% WPC addition due to the gelation/thermosetting properties of whey proteins, and decreased on further addition of WPC, probably due to greater spread and D/T. Sarabhai and Prabhasankar [49] and García-Solís, Bello-Pérez, Agama-Acevedo, and Flores-Silva [57] asserted that the hardness of cookies was probably caused by the interaction of proteins and starch due to hydrogen bonding.



(**a**) (**b**)

Figure 3. Hardness (**a**) and brittleness (**b**) data for cookies from sorghum and corn flour with 8, 9, 10, 11 and 12% whey protein concentrate. (n = 4).

4. Conclusions

In general, WPC can be used in gluten-free doughs as a functional ingredient. The addition of WPC to sorghum and corn flour at a concentration of 8–12% resulted in an increase in optimal water absorption, which helped the binding of more moisture, thus softening the dough system. The dynamic and mechanical properties of sorghum and corn flour doughs were also affected by WPC addition, which lowered moduli values during the rheological measurements. However, the decrease in moduli values was less in the case of corn than sorghum, indicating a lower impact of WPC addition on rheological properties of the former. With increasing WPC levels, cookie diameter and the D/T value increased in both sorghum- and corn-based cookies. Also, the addition of WPC had a significant effect on color development due to the Maillard reaction and probably caramelization, which makes the cookies darker than the control. The cookies with 10% WPC added showed the highest hardness and flexibility both in sorghum and corn. Compared to pastry cookies, sorghum cookies with WPC were consistently harder but corn cookies with WPC were comparable to pastry cookies.

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