

## Research Article

# The Effect of Whey Protein Concentrate, Soy Protein Isolate, and Xanthan Gum on Textural and Rheological Characteristics of Gluten-Free Batter and Cake

### Parastoo Ghaemi<sup>(D)</sup>,<sup>1</sup> Saeedeh Arabshahi-Delouee<sup>(D)</sup>,<sup>1</sup> Mehran Aalami<sup>(D)</sup>,<sup>2</sup> and Seyyed Hossein Hosseini Ghaboos<sup>(D)</sup>

<sup>1</sup>Department of Food Science and Technology, Azadshahr Branch, Islamic Azad University, Azadshahr, Iran <sup>2</sup>Department of Food Sciences and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Correspondence should be addressed to Saeedeh Arabshahi-Delouee; saeedeh\_arabshahi@yahoo.com

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The market of gluten-free food products has been growing exponentially in recent years. Consequently, it is necessary to conduct research on the effects of various compounds to enhance the quality of these products. The present study is aimed at investigating the effects of adding whey protein concentrate (WPC) and soy protein isolate (SPI) at three levels (0%, 5%, and 10%) and xanthan gum (XG) (0%, 0.15%, and 0.3%) on the textural and rheological characteristics of gluten-free batter and cake based on rice flour. The results showed that increasing the levels of XG, WPC, and SPI improved the apparent viscosity of the batter samples. The effect of WPC on the apparent viscosity was more pronounced than that of SPI, as the highest value (21.9 Pa·s) was related to the sample containing 10% WPC and 0.3% XG. The loss tangent values of the batter samples, measured at a frequency of 1 Hz, ranged from 0.531 to 0.8 for different levels of WPC/XG and from 0.466 to 0.699 for SPI/XG, indicating a gel-like behavior of the batter samples. The sample containing 10% SPI and 0.3% XG displayed the strongest gel with higher storage modulus (982.112 Pa) and loss modulus (458.039 Pa) compared to other samples. Increasing the levels of XG, WPC, and SPI resulted in higher specific volume, porosity, firmness, and springiness of the samples compared to the control sample (P < 0.05). The addition of WPC, SPI, and XG improved the overall acceptability of the cake samples, with the samples containing WPC/XG combination being the most preferred. Ultimately, the cake sample containing 10% WPC along with 0.3% XG introduced as optimal sample. This formulation not only can enhance the characteristics of gluten-free cake but also meets parts of the nutritional needs of consumers, particularly those with celiac disease.

#### 1. Introduction

The substitution of gluten in gluten-free goods is considered to be a challenging issue for food industry experts, because gluten is the primary protein that forms the structure of baking goods and it gives batter the viscoelastic characteristics; therefore, removing gluten from the formulation leads to a poor texture, low volume, and a decrease in the quality of the product [1]. In contrast, as the number of cases of gluten sensitivity and celiac disease increased, the gluten-free goods market has grown significantly in the world over the past decade [2], and the only successful treatment for celiac disease at the moment is adhering to a permanent gluten-free diet [3]. Therefore, it is necessary to develop gluten-free goods with better quality characteristics and high nutritional value [4].

Cake is a widely accepted snack, so it has long been used as a basic formulation for production of functional goods such as gluten-free products. Among the gluten-free grains, rice is the most suitable grain for preparing gluten-free goods [5]. Furthermore, due to the importance of the proteins forming the viscoelastic network of gluten in creating the desired rheological characteristics of batter goods, the necessity of using compounds with similar characteristics

in order to create suitable qualitative characteristics in these goods is evident. In this regard, it has been suggested to add a combination of various gums, proteins, and fibers [6]. Gums such as agar, carrageenan, and xanthan are mainly added to the foods due to their thickening, gelling, and mouthfeel characteristics by changing the viscosity of solutions [7]. Whey proteins are highly soluble, foaming, waterholding, emulsifying, and capable of forming gels. They also have great nutritional and functional properties [8, 9]. Functional traits of soy proteins include their ability to hydrate, solubilize in water, maintain colloidal stability, gel, emulsify, foam, and adhere [10]. Several studies have been conducted regarding the use of different cereal flours and hydrocolloids, proteins, and enzymes in the production of gluten-free goods. Shaabani et al. [1] investigated the effect of using pea protein isolate, transglutaminase enzyme, and xanthan gum (XG) on the characteristics of gluten-free muffins prepared from millet flour, and they concluded that increasing the level of XG decreases firmness and increases specific volume and the porosity of the samples. Xian and Hu [11] investigated the effect of using corn flour and XG on the quality characteristics of sponge cake, and they reported that XG increased the firmness of the produced samples and decreased the specific volume of the samples. Jyotsna et al. [12] reported that substituting wheat flour for millet flour and whey protein isolate in the formulation of gluten-free muffins led to an increase in the volume and a decrease in the firmness of the produced samples. Ammar et al. [8] reported that the use of whey protein concentrate (WPC), corn, and rice flour can be a suitable substitution for wheat flour in high-quality gluten-free sponge cakes. They indicated that WPC had positive effects on the specific volume of the samples, and their combination increased the firmness. Maravić et al. [9] showed that the substitution of wheat flour with whey protein led to a decrease in the specific volume and an increase in the firmness of the sponge cake samples and it had a significant effect on the viscoelastic behavior of the batter. Lin et al. [13] investigated the effect of substituting eggs for soy protein isolate (SPI) and polysaccharides in yellow cake, and they reported that SPI with 1% mono and diglyceride have a higher batter viscosity than the control sample and it can be used as a substitute egg substitution in the cake. Azmoon et al. [14] reported that adding the combination of konjac and guar gums and SPI as a fat substitution in sugar-free muffin cake led to an increase in the specific volume and  $L^*$  value of the samples; however, it had a negative effect on the firmness and parameters  $a^*$  and  $b^*$ .

This research work was designed to evaluate the behavior of two commonly used proteins in the food industry, WPC and SPI along with XG (a pure gum of microbial origin), in gluten-free food formulations using cake batter as a realistic emulsion system. The specific objective was to accurately monitor the rheological properties of the batter and evaluate the impact of these protein-gum combinations. The novelty of this work lies in its innovative approach to understanding the behavior of these components, contributing to the development of new formulations and their potential industrial applications. It is worth noting that no similar research has been conducted in this area before.

#### 2. Materials and Methods

2.1. Materials. In this research, broken rice of the Fajr variety (Gorgan, Iran), WPC (Lynn, USA) containing 80% protein, SPI (Crown, China) containing 90% protein, xanthan gum, powdered sugar, fresh eggs, sunflower oil, baking powder, and vanilla were used. All other chemicals used were of analytical grade and purchased from chemical suppliers.

2.2. Batter and Cake Preparation. Ingredients were rice flour (100 g), powdered sugar (72 g), oil (57 g), egg (72 g), vanilla (0.5 g), baking powder (2 g), water (30 g), WPC (0%, 5%, and 10% flour basis), SPI (0%, 5%, and 10% flour basis), and xanthan gum (0%, 0.15%, and 0.3% flour basis). The amount of ingredients was considered based on 100 g of rice flour. The treatments used in this study are listed in Table 1. Cake batter was prepared using the method of Turabi et al. [15] with some modifications. To prepare the cake, 30 g of batter was poured into each cake mold and placed in the oven at 170°C for 25 minutes. After baking and cooling, the cakes were stored in polythene bags at room temperature.

#### 2.3. Batter Measurements

2.3.1. Apparent Viscosity. The apparent viscosity test was performed by a rheometer (Anton Paar, MCR301, Austria) using two parallel plates at a temperature of 25°C. In this test, the distance between the plates was 1 mm and the shear rate range was  $0.01-50 \text{ s}^{-1}$ , and the apparent viscosity obtained at the shear rate was reported as  $46.2 \text{ s}^{-1}$ .

2.3.2. Dynamic Rheological Tests. The oscillating rheological tests (strain sweep and frequency sweep) were performed by a rheometer (Anton Paar, MCR301, Austria) using two parallel plates at a temperature of 25°C. The strain sweep test was performed in the strain range of 0.01-100% and constant frequency (1 Hz) in order to determine the linear viscoelastic range [16]. The parameters obtained from the frequency sweep test included storage modulus (G'), loss modulus (G''), and loss tangent (tan  $\delta$ ). The frequency sweep test was conducted in the frequency range of 0.001-40 Hz by a constant strain of 0.1% within the viscoelastic range.

#### 2.4. Cake Measurements

2.4.1. Specific Volume. The specific volume of the cake was measured using the rapeseed displacement method (AACC) [17].

2.4.2. Porosity. In order to measure the porosity of the cake, the true volume of the cake was initially determined. For this purpose, the cake sample was compressed inside the graduated cylinder without any porosity. Then, the amount of porosity was calculated according to the following relationship [15]:

Porosity (%) = 
$$\left(1 - \frac{V_{\text{true}}}{V_{\text{bulk}}}\right) \times 100.$$
 (1)

2.4.3. Color Indices. HunterLab colorimeter (Lovibond, CAM-System 500, England) was used to evaluate the color

TABLE 1: Treatments prepared and abbreviations used in the present research.

Treatments					
No.	WPC*XG (%)	No.	SPI*XG (%)		
1	0, 0 (control)	1	0, 0 (control)		
2	0, 0.15	2	0, 0.15		
3	0, 0.3	3	0, 0.3		
4	5, 0	4	5, 0		
5	5, 0.15	5	5, 0.15		
6	5, 0.3	6	5, 0.3		
7	10, 0	7	10, 0		
8	10, 0.15	8	10, 0.15		
9	10, 0.3	9	10, 0.3		

WPC: whey protein concentrate; SPI: soy protein isolate; XG: xanthan gum.

of the cake. The color analysis was done by three indices:  $L^*$ ,  $a^*$ , and  $b^*$  [18].

2.4.4. Texture. The cake texture was evaluated on the day of baking using the texture profile analysis (TPA) test using a texture meter (Stable Micro Systems Ltd., TA-XT Plus, England) and Texture Expert software. In order to perform this test, after separating the cake crust, a cube piece with dimensions of  $20 \times 20 \times 20$  mm was cut from the central part of the cake. Then, 50% of the cake texture was compressed by an aluminum cylindrical probe (with a diameter of 25 mm). The speed of the force applied during the test was 2 mm/s, and the delay time between two cycles was also 30 s. The firmness and springiness indices were calculated using the force-time curve [19].

2.4.5. Sensory Evaluation. Evaluation of the sensory characteristics (overall acceptability) of the cake samples was done by 10 evaluators using a 5-degree hedonic scale [20].

2.5. Statistical Analysis. All experiments were performed in three repetitions, and results were expressed as mean values of triplicate  $\pm$  standard deviation. The data were subjected to statistical analysis using a completely random design in the form of factorial tests by SAS software. A comparison of means was also done with Duncan's multirange test at a 95% confidence level, and Excel (2010) was used to draw graphs.

#### 3. Results and Discussion

#### 3.1. Batter Properties

3.1.1. Apparent Viscosity. The effect of WPC and SPI and XG on the behavior of batter apparent viscosity at different shear rates is shown in Figure 1. As shown, the apparent viscosity in all batter samples decreased with increasing the shear rate. Therefore, the batter samples were rheologically non-Newtonian, and they showed shear thinning behavior (pseudoplastic). The results obtained at a shear rate of 46.2 (s<sup>-1</sup>) showed that among the samples containing WPC and XG (Figure 1(a)), the addition of gum and protein alone increased the apparent

viscosity of the samples, which was at the highest level. Their viscosity was 10.8 Pa·s and 8.3 Pa·s, respectively. The simultaneous use of gum and protein increased the apparent viscosity so that the highest amount of apparent viscosity (21.9 Pa·s) was observed in the sample containing 10% WPC and 0.3% XG. A similar trend was observed among samples containing SPI and XG (Figure 1(b)). Adding gum and protein separately and in combination increased the apparent viscosity compared to the control sample. In samples containing the highest levels of gum and protein separately, values of viscosity were 10.8 Pa·s and 7.95 Pa·s, respectively. The highest (18.8 Pa·s) and the lowest (4.99 Pa·s) amount of apparent viscosity belonged to the sample containing 10% SPI and 0.3% XG and the control sample, respectively. The increase in viscosity and consistency index can be attributed to the ability of XG to absorb water and its reaction with proteins and flour starch, which create a network that traps free water [21]. These results are consistent with the results of Shaabani et al. [1] that the gluten-free muffin batter incorporating a mixture of transglutaminase and XG showed shear thinning behavior. Yıldız et al. [16] also indicated that the cake batter samples containing buckwheat flour, gums (xanthan and guar), and proteins (soy protein and whey protein) had shear thinning behavior (pseudoplastic).

3.1.2. Storage and Loss Modulus. Important results from oscillation experiments include the storage or elastic modulus (G') and the loss or viscous modulus (G''). The quantity of elastic behavior and the amount of energy recovered per volume unit during each wave cycle are expressed by the storage modulus. The quantity of flow behavior and the amount of energy wasted per volume unit during each full cycle of the strain wave are indicated by the strain and the loss modulus. In the frequency sweep test, if the storage modulus (G') is greater than the loss modulus (G''), therefore, the behavior of the sample is solid viscoelastic, and if the loss modulus (G'') is greater than the storage modulus (G'), the behavior of the sample is liquid viscoelastic [22]. Generally, in gluten-free batters, if the rheological behavior moves towards viscoelasticity, it can play a more effective role in maintaining the cohesiveness of the batter as well as the ability to retain more carbon dioxide gas. Figures 2(a) and 2(c) show the storage modulus and loss modulus of cake batter samples containing different levels of WPC and XG, respectively. According to the results, the value of the storage modulus of batter samples was higher than the loss modulus. The amount of storage modulus at 1 Hz frequency was in the range of 115.144-441.544 Pa, and the amount of loss modulus was in the range of 80.476-272.238 Pa. The sample containing 10% WPC and 0.3% XG had a higher storage modulus and loss modulus than the control batter samples. Figures 2(b) and 2(d) show the storage modulus and loss modulus of batter samples containing SPI and XG, respectively. As it can be seen, the storage modulus value of the samples was higher than the loss modulus. At the 1 Hz frequency, the amount of storage modulus was within the range of 115.144-982.112 Pa and the amount of drop modulus varied from 80.476 to 458.039 Pa. Also, the sample containing

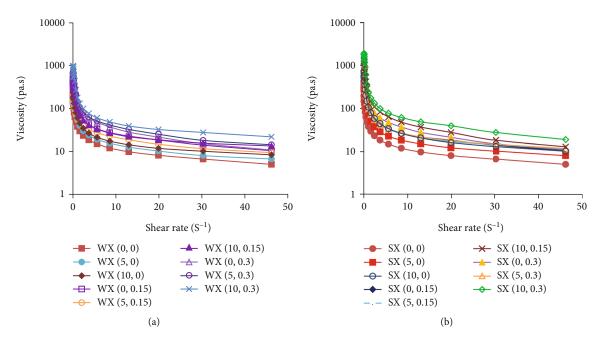


FIGURE 1: The effect of (a) whey protein concentrate and xanthan gum (WX) and (b) soy protein isolate and xanthan gum (SX) on behavior of batter apparent viscosity at different shear rates.

10% SPI and 0.3% XG had the highest storage modulus and loss modulus among all samples, the sample containing 10% SPI and 0.3% XG had a higher storage modulus and loss modulus than other samples, and the lowest amount of these parameters was observed in the control sample. In these samples, with the increase in the concentration of SPI and XG, the storage modulus increased compared to the control sample. The increase in the storage modulus and the loss modulus caused by XG can be attributed to its high water binding capacity, which reduces the available water and the starch-hydrocolloid reactions by attaching and encapsulating xanthan to starch granules [23]. The higher storage modulus of samples containing SPI than those of containing WPC is probably due to the function of two main globulins of SPI (beta-conglycinin and glycinin) in gel stabilization [24]. In accordance with these results, Yıldız et al. [16] and Singh et al. [25] found that adding hydrocolloids such as XG and guar gum increased the elasticity of gluten-free cake batter and also increased storage modulus more than loss modulus. Macedo et al. [26] also showed that the addition of WPC increased the storage modulus of bread batter.

3.1.3. Loss Tangent. The loss tangent is displayed by the relation of the loss modulus (G'') to the storage modulus (G')[27]. If the loss tangent values are less than 1, the elastic behavior is dominant, while values greater than 1 indicate viscous behavior. Loss tangent values greater than 0.1 and smaller than 1 mean that the samples are not true gels and have a structure between a high-concentration biopolymer and a true gel [28]. According to Figure 3, the value of the loss tangent of batter samples containing different levels of WPC and XG as well as SPI and XG at the frequency (1 Hz) was less than 1 and varied from 0.531 to 0.8 and 0.466 to 0.699, respectively, which indicates the gel-like behavior of the batter samples. These results of this research are consistent with the results of Sarabhai and Prabhasankar [29], who confirmed the solid-like behavior of gluten-free cake batter samples. Yıldız et al. [16] reported that the sample containing whey protein had a higher loss tangent indicating gel-like behavior, but the sample with guar gum had a lower loss tangent indicating solid-like behavior.

#### 3.2. Cake Charactristics

3.2.1. Specific Volume. Volume is regarded as a significant qualitative characteristic in cake assessment, with larger porous cake being more favorable and preferred by consumers [19]. The effect of WPC, SPI, and XG (alone and in combination) on cake specific volume is shown in Figure 4. As shown, the addition of XG increased the specific volume of the samples compared to the control sample. Adding WPC alone and in combination to XG caused a significant increase (P < 0.05) in the volume of the samples compared to the control sample. Similar results were also obtained by adding SPI alone and in combination to XG. However, adding WPC/XG caused greater increase (P < 0.05) in the volume of cake samples compared to the SPI/XG. According to the results, the highest amount of volume  $(79.93 \text{ cm}^3/\text{g})$  belonged to the sample containing 10% WPC and 0.3% XG and the lowest amount  $(69.07 \text{ cm}^3/\text{g})$ was observed in the control sample. The increase in the specific volume of cake samples produced can be attributed to the ability of protein and gum to maintain the gas bubbles formed during baking in the batter [9]. The results obtained are consistent with the research of Ammar et al. [8] on the volume of gluten-free cakes containing whey protein. The increase in volume can be caused by the increase in the viscosity of the batter samples. In fact, a higher apparent

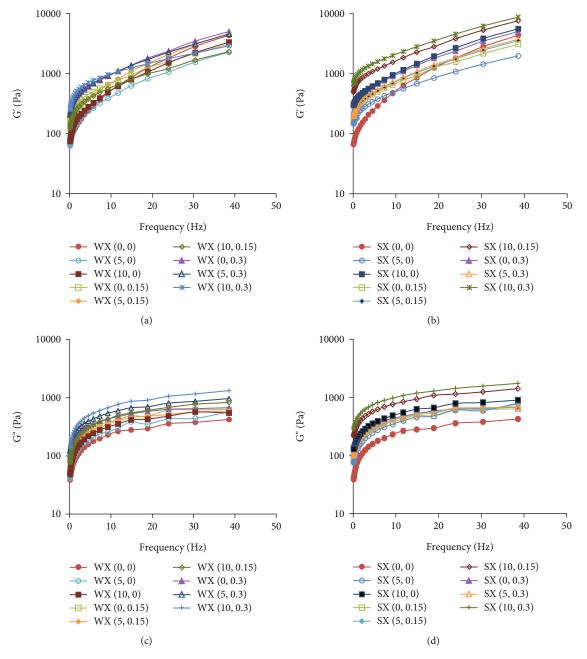


FIGURE 2: (a) Storage modulus of batter samples containing whey protein concentrate and xanthan gum (WX), (b) storage modulus of batter samples containing soy protein isolate and xanthan gum (SX), (c) loss modulus of batter samples containing whey protein concentrate and xanthan gum (WX), and (d) loss modulus of batter samples with soy protein isolate and xanthan gum (SX).

viscosity helps to trap air in the batter and creates a higher volume and porosity in the samples, and in the low viscosity of the batter, air bubbles can easily rise to the surface of the batter and leave the environment [30]. Statistical comparison of the results related to the two protein groups indicated that WPC at 10% level, along with 0.15% or 0.3% of XG, played a greater role in increasing the specific volume of the cake samples. The increase observed in specific volume in samples with higher levels of WPC can be attributed to the spherical structure of whey proteins and their ability to form a thermal gel. During cake baking, when temperatures exceed 70°C, these proteins undergo denaturation, causing the disruption of the bonds forming their tertiary structure. Consequently, the protein structure opens up, allowing for new protein-protein interactions or interactions with other components of the batter formula. This phenomenon ultimately leads to an increase in the specific volume of the produced samples [16, 31].

*3.2.2. Porosity.* Porosity is one of the important physical characteristics of cake that affects the quality of baked samples [32]. The effect of WPC, SPI, and XG (alone and in combination) on the porosity of cake samples is shown in Figure 5. As shown, adding XG and WPC separately and

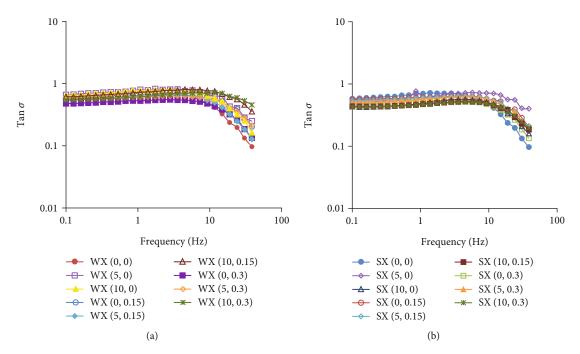


FIGURE 3: Loss tangent of batter samples containing (a) whey protein concentrate and xanthan gum (WX) and (b) soy protein isolate and xanthan gum (SX).

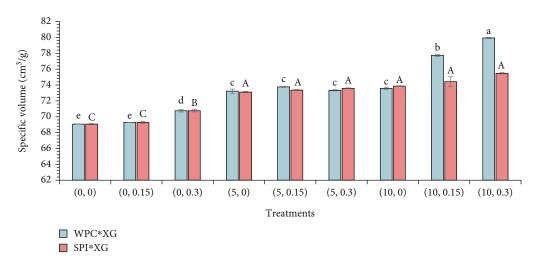


FIGURE 4: The average specific volume of cake samples containing whey protein concentrate and xanthan gum (WPC\*XG) and samples containing soy protein isolate and xanthan gum (SPI\*XG). Different letters (a–e) indicate significant differences among the samples of the WPC\*XG group (P < 0.05). Different letters (A–C) indicate significant differences among the samples of the SPI\*XG group (P < 0.05).

in combination increased the porosity of the samples significantly compared to the control sample (P < 0.05). A similar trend was observed for the porosity of samples containing SPI and XG. However, statistical comparison of the data obtained from the impact of two protein groups on the cake porosity showed that WPC had a greater ability than SPI to increase the porosity of cake samples. The highest porosity (59.93%) belonged to the sample containing 10% WPC and 0.3% XG. The texture porosity of baked goods is influenced by the number and distribution of holes inside their texture. The greater the number of cavities and gas cells inside the fabric and the more uniform its distribution, the

greater the porosity of the product [33]. In a study conducted by Meng and Kim [34], the addition of 1% WPC, 1% SPI, and 2% fish collagen increased the porosity of fermented rice cakes. Similarly, Shaabani et al. [1] found that using XG increased the volume and porosity of gluten-free muffins due to the increased viscosity.

3.2.3. Color Parameters. The crust color is one of the important qualitative characteristics of the cake due to its direct influence on the initial acceptance of the consumer [9]. Color is determined by a combination of  $L^*$ ,  $a^*$ , and  $b^*$ parameters, which are measured as lightness, red-green,

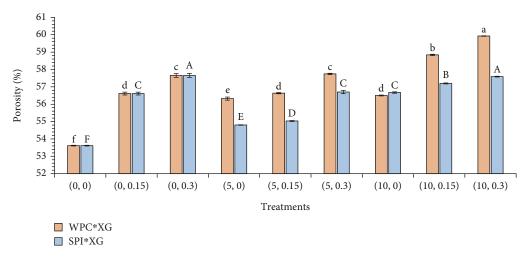


FIGURE 5: The average porosity of cake samples containing whey protein concentrate and xanthan gum (WPC<sup>\*</sup>XG) and samples containing soy protein isolate and xanthan gum (SPI<sup>\*</sup>XG). Different letters (a–f) indicate significant differences among the samples of the WPC<sup>\*</sup>XG group (P < 0.05). Different letters (A–F) indicate significant differences among the samples of the SPI<sup>\*</sup>XG group (P < 0.05).

and yellow-blue criteria for each sample, respectively [18]. The effect of WPC, SPI, and XG alone and in combinations on the crust color of the cake is shown in Table 2. The results indicated that the increase in the level of XG increased the L\* index of the cake samples compared to the control sample. The increase in the level of WPC decreased the lightness of the samples significantly compared to the control sample (P < 0.05). However, the simultaneous increase in WPC and gum levels caused a significant decrease in the  $L^*$  index of the cakes compared to the control sample (P < 0.05). A similar trend was observed in the samples containing SPI. The samples containing SPI along with XG were lighter than the control sample. Moreover, it was found that with the increase of XG from 0.15% to 0.3%, the  $a^*$  index of the cakes decreased significantly (P < 0.05). Besides, with the addition of WPC, the amount of redness increased compared to the control sample, and with the addition of XG to the WPC, the amount of redness of the samples decreased. With the addition of SPI, the  $a^*$  index showed an increasing trend compared to the control sample. The addition of XG to SPI up to 5% level led to a decrease in the red hue of  $a^*$  in the samples, but no significant changes were observed in higher amounts of gum and protein. By increasing the amount of XG, the  $b^*$  index of the samples showed a significant increase compared to the control sample, but with the addition of SPI, the  $b^*$  index had a significant decrease compared to the control sample (P < 0.05). This trend was also observed in WPC samples. The addition of SPI along with XG led to a decrease in  $b^*$  index compared to the control sample (Table 2). The decrease in the lightness of color sample of the cake crust is due to Maillard reactions and caramelization during baking [4]. The decrease in lightness and increase in redness in samples containing WPC can be attributed to the presence of amino acids and lactose in the structure of this compound, which causes Maillard browning reactions during cooking. SPI is also a rich source of lysine, which includes free amino groups. The reaction between free amino groups and reducing sugars during

TABLE 2: Color parameters  $(L^*a^*b^*)$  of crust of cake samples containing different amounts of whey protein concentrate (WPC), soy protein isolate (SPI), and xanthan gum (XG).

Treatment	$L^*$	<i>a</i> *	$b^*$
WX (0, 0)	$72.5 \pm 0.1^{b A}$	$14.1 \pm 0.1^{c A}$	$20.4 \pm 0.1^{c A}$
WX (0, 0.15)	$72.9 \pm 0.1^{ab}$ A	$14.1\pm0.0^{c~A}$	$21.2\pm0.0^{b~A}$
WX (0, 0.3)	$73.6 \pm 0.3^{a}$ <sup>A</sup>	$13.4\pm0.2^{d~A}$	$22.0\pm0.1^{a~A}$
WX (5, 0)	$68.4\pm0.3^{c~A}$	$15.7\pm0.1^{a~A}$	$21.2\pm0.1^{b~A}$
WX (5, 0.15)	$61.0 \pm 0.3^{e\ B}$	$14.9\pm0.2^{b~A}$	$21.2\pm0.0^{b~A}$
WX (5, 0.3)	$64.9 \pm 0.2^{d}$ A	$14.8 \pm 0.1^{b \ A}$	$21.2\pm0.1^{b~A}$
WX (10, 0)	$60.6 \pm 0.4^{e\ B}$	$15.7\pm0.1^{a~A}$	$21.2\pm0.3^{b~A}$
WX (10, 0.15)	$56.4\pm0.3^{g~B}$	$14.1\pm0.1^{c~A}$	$21.1\pm0.4^{b~A}$
WX (10, 0.3)	$58.4\pm0.1^{f~B}$	$14.1\pm0.1^{c~A}$	$18.9\pm0.1^{d~A}$
SX (0, 0)	$72.5 \pm 0.1^{b}$ A	$14.1 \pm 0.1^{b \ A}$	$20.4\pm0.1^{c~A}$
SX (0, 0.15)	$72.9\pm0.1^{a~A}$	$14.1\pm0.0^{b~A}$	$21.2\pm0.0^{b~A}$
SX (0, 0.3)	$73.6 \pm 0.3^{a}$ <sup>A</sup>	$13.4 \pm 0.2^{c}$ A	$22.0\pm0.1^{a~A}$
SX (5, 0)	$67.1 \pm 0.4^{cd}$ A	$14.9 \pm 0.0^{a}$ <sup>B</sup>	$19.7 \pm 0.1^{d B}$
SX (5, 0.15)	$69.0 \pm 0.3^{b}$ A	$14.1 \pm 0.0^{b \ B}$	$19.6 \pm 0.0^{d}$ <sup>B</sup>
SX (5, 0.3)	$58.5\pm0.3^{f~B}$	$13.2 \pm 0.1^{c B}$	$18.8\pm0.0^{e~B}$
SX (10, 0)	$62.6 \pm 0.3^{e}$ A	$14.1 \pm 0.1^{b \ B}$	$19.6 \pm 0.0^{d B}$
SX (10, 0.15)	$66.4 \pm 0.7^{d}$ A	$14.1\pm0.0^{b~A}$	$18.8 \pm 0.0^{e}$ <sup>B</sup>
SX (10, 0.3)	$68.2 \pm 0.1^{cb}$ A	$14.1\pm0.0^{b~A}$	$18.8\pm0.1^{e~A}$

Different superscript letters (a–g) within each column indicate significant differences among the samples of the same group (P < 0.05). Different superscript letters (A, B) within each column indicate significant differences of the same parameter among the samples of different groups (P < 0.05).

cooking can develop colored compounds through Maillard reactions, which causes the skin to get darken. Also, SPI has some natural pigments that participate in the crust color. In accordance with these findings, Meng and Kim [34] reported a reduction in the  $L^*$  and  $a^*$  values of gluten-free cake made from rice flour when supplemented with WPC

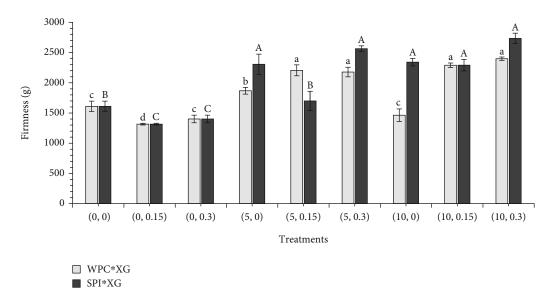


FIGURE 6: The average firmness of cake samples containing whey protein concentrate and xanthan gum (WPC\*XG) and samples containing soy protein isolate and xanthan gum (SPI\*XG). Different letters (a–d) indicate significant differences among the samples of the WPC\*XG group (P < 0.05). Different letters (A–C) indicate significant differences among the samples of the SPI\*XG group (P < 0.05).

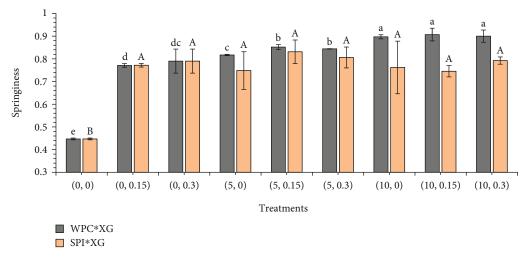


FIGURE 7: The average springiness of cake samples containing whey protein concentrate and xanthan gum (WPC\*XG) and samples containing soy protein isolate and xanthan gum (SPI\*XG). Different letters (a–d) indicate significant differences among the samples of the WPC\*XG group (P < 0.05). Different letters (A, B) indicate significant differences among the samples of the SPI\*XG group (P < 0.05).

and SPI. Similarly, a decrease in the  $L^*$  value of bread samples containing cassava and chickpea flour with the addition of whey protein has also been observed by Komeroski et al. [35].

3.2.4. Firmness. The firmness of the cake is one of the most important textural characteristics, and this factor has an important impact on the consumer acceptance, which is measured as the maximum force required to compress the cake to a certain length at a certain speed [19]. The effect of WPC, SPI, and XG alone and in combination on cake firmness is shown in Figure 6. By adding XG and WPC, the firmness of the samples decreased and increased, respectively, compared to the control sample. Adding WPC along

with XG as well as SPI along with XG increased the firmness of the samples significantly compared to the control sample (P < 0.05). The increased firmness of samples containing gum and protein can be attributed to the strengthening of the protein structure of the cake. Statistical comparison of the data obtained from the impact of two protein groups on the firmness of cake samples showed that, in general, SPI increased the firmness of the samples to a greater extent compared to WPC. These results are consistent with the results of porosity and specific volume tests, as an increase in porosity can be accompanied by a decrease in the firmness of the cake texture. The highest amount of firmness (2735.14 g) belonged to the sample containing 10% SPI and 0.3% XG. This can be attributed to the disulfide bonds of soy

Treatment	WPC*XG	Treatment	SPI*XG
WX (0, 0)	$2.45 \pm 0.36^{e}$ A	SX (0, 0)	$2.45 \pm 0.36^{d}$ A
WX (0, 0.15)	$3.05 \pm 0.27^{d \ A}$	SX (0, 0.15)	$3.05 \pm 0.27^{c}$ A
WX (0, 0.3)	$3.75 \pm 0.23^{c}$ A	SX (0, 0.3)	$3.75 \pm 0.23^{ab}$ A
WX (5, 0)	$3.02 \pm 0.58^{d}$ A	SX (5, 0)	$2.75 \pm 0.13^{cd}$ A
WX (5, 0.15)	$4.12 \pm 0.42^{bc}$ A	SX (5, 0.15)	$3.60 \pm 0.63^{b B}$
WX (5, 0.3)	$4.52 \pm 0.41^{ab}$ A	SX (5, 0.3)	$4.22 \pm 0.75^{a}$ <sup>A</sup>
WX (10, 0)	$4.20 \pm 0.37^{b}$ A	SX (10, 0)	$2.92 \pm 0.44^{cd B}$
WX (10, 0.15)	$4.50 \pm 0.50^{ab}$ A	SX (10, 0.15)	$3.67 \pm 0.62^{ab}$ B
WX (10, 0.3)	$4.72 \pm 0.31^{a}$ A	SX (10, 0.3)	$4.12 \pm 0.75^{ab}$ B

TABLE 3: Overall acceptability of cake samples containing whey protein concentrate and xanthan gum (WPC\*XG) and samples containing soy protein isolate and xanthan gum (SPI\*XG).

Different superscript letters (a–e) within each column indicate significant differences among the samples of the same group (P < 0.05). Different superscript letters (A, B) within each row indicate significant differences among the samples of different groups (P < 0.05).

proteins, which may reduce the level of hydrophobicity and stability of the foam and create a film with low flexibility between water and air, which results in less air contact with the batter [16]. The higher firmness value of samples containing WPC compared to the control sample can be attributed to the high solubility of whey proteins, which reduce the content of water available to dissolve sugar and crystallize during the baking process of the cake and affect the texture of the product [9]. These results are in agreement with the previous reports that the addition of proteins (whey protein, soy protein) and gums like XG increases the firmness of the various gluten-free products such as muffin, sponge cake, and bread [1, 8, 11, 16, 35].

3.2.5. Springiness. The effect of SPI, WPC, and XG on the springiness of cake samples is shown in Figure 7. Adding WPC and XG alone and in combination in the cake caused a significant increase (P < 0.05) in the sample's springiness compared to the control sample (without any additive). A similar trend was observed for SPI alone and in combination with gum. However, statistical comparison of the data obtained from the impact of two protein groups on the springiness of cake samples showed adding WPC specially at higher amounts (10%) (with or without XG) caused greater increase (P < 0.05) in springiness of the cake compared to the SPI at same amount. The highest amount of springiness (0.900) belonged to the sample containing 10% of WPC with 0.3% of XG. Due to the lack of a gluten network in the control sample, the batter becomes weak, and then during cooking, gases increase, and thus, the springiness decreases. The increase in springiness of cake samples containing gum and protein can be related to the increase in water absorption and its better distribution caused by these compounds. Jyotsna et al. [12] also showed that the addition of WPC to finger millet flour increased the springiness of the muffin samples. However, Gomez et al. [36] reported that the addition of XG did not have a significant effect on the springiness of yellow layer cakes compared to the control sample.

*3.2.6. Overall Acceptability.* The effect of SPI, WPC, and XG on the cake's overall acceptability is shown in Table 3. According to the scores given by the panelists, addition of

WPC, SPI, and XG improved the overall acceptability of the free-gluten cake samples, as the lowest score belonged to the control sample (without any additive) (P < 0.05). Among the samples containing WPC and XG, the highest score (4.72) belonged to the sample containing 10% of WPC with 0.3% of XG, although it was not significantly (P < 0.05) different from the sample containing 5% of WPC with 0.3% of XG. Among the samples containing SPI and XG, the highest score (4.22) was related to the sample containing 5% of SPI with 0.3% of XG, although it was not significantly different (P < 0.05) from the sample containing 10% of WPC with 0.3% of XG. The samples containing WPC/XG were more acceptable than those containing SPI/XG as they received higher scores (P < 0.05) by panelists. Moreover, addition of WPC was more effective than SPI in improving the sensory properties of the free-gluten cake samples. Jyotsna et al. [12] reported that muffins prepared with 90% finger millet flour and 10% WPC had the highest overall acceptance scores.

#### 4. Conclusion

In this research, the incorporation of WPC and SPI, along with XG, at various levels, resulted in significant improvements in the rheological and textural characteristics of gluten-free batter and cake samples. The findings revealed that all batter samples exhibited non-Newtonian flow behavior, displaying shear thinning (pseudoplastic) properties. The oscillation tests indicated that the storage modulus exceeded the loss modulus in all batter samples, indicating a prevalence of elastic behavior over viscous behavior. The simultaneous addition of WPC or SPI, in combination with XG, led to enhancements in apparent viscosity, specific volume, porosity, springiness, and sensory characteristics of the produced samples. Notably, the combination of WPC and XG demonstrated superior effectiveness in enhancing these properties compared to the combination of SPI and XG. Ultimately, the cake sample containing 10% WPC and 0.3% XG emerged as the optimal treatment, exhibiting the highest potential for improving the characteristics of gluten-free batter and cake. This formulation not only addresses the deficiency of rice proteins but also holds nutritional significance for consumers.

#### **Data Availability**

The data supporting the findings of this study are available upon request from the corresponding author.

#### **Conflicts of Interest**

The authors declare that they have no conflicts of interest.

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