



## Scientific Research

### Stable and dynamic rheological characteristics of gluten-free cake batter enriched with whey protein concentrate, soy protein isolate and basil seed gum

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## ABSTRACT

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In recent years, the demand for gluten-free food products has been growing exponentially. Thus, research on the effects of various compounds for improving the quality of these products seems necessary. The current study aimed to enhance the rheological properties of a gluten-free batter and cake based on rice flour using whey protein concentrate (WPC), soy protein isolate (SPI) at three levels (0, 5, and 10%), and various amounts of basil seed gum (BSG) (0, 0.5, and 1%). The results revealed that the apparent viscosity in all the samples increased with protein and gum, so the highest apparent viscosity (27.9 Pa·s) related to the sample containing 10% SPI and 1% BSG. The flow behavior index of each sample was less than one. Hence, shear thinning behavior (pseudoplasticity) was seen in all batter samples. The storage modulus and loss modulus of the cake batter increased as the proportion of WPC, SPI, and BSG increased. The samples' elasticity was confirmed by the loss tangent value of batter samples with varying concentrations of WPC or SPI, with BSG being less than one. These findings showed that samples with 10% WPC, 10% SPI, and 1% BSG had greater stiffness than other samples at a frequency of 1 Hz. Their complex viscosity values were 277.268 and 523.299 Pa·s, respectively. In terms of rheological qualities, cake samples with 10% SPI and 1% BSG were recommended as an alternative to regular cakes for those with celiac disease.

## 1- Introduction

One of the major issues facing the food industry is manufacturing high-quality gluten-free goods, especially considering the growing number of people in society who are sensitive to gluten and the crucial role that gluten plays in bakery products. Wheat gluten is essential to the creation of bakery goods like bread and cakes, because it maintains the CO<sub>2</sub> created during fermentation and creates cohesiveness. Gas expansion increases the volume of these products and improves their texture [1, 2]. Cereals that do not contain or cereals that contain gluten less than 20 ppm are called gluten-free cereals. Such grains include rice, corn, sorghum, and millet [3]. Nonetheless, some individuals who consume gluten have inflammation of the small intestine villi, which lowers the absorption of a number of important minerals, such as calcium, iron, folic acid, and fat-soluble vitamins [4]. According to Google searches, the third most popular diet phrase is "gluten-free". However, gluten-free bakery products have a much more fragile structure, inappropriate appearance, and fewer flavors due to the replacement of wheat flour with gluten-free cereal flour. Thus, they have lower nutritional quality, weaker texture, and less acceptability regarding sensory characteristics [5, 6]. Therefore, it is necessary to conduct studies to use suitable substitutes for gluten, such as hydrocolloids, enzymes, and proteins in the preparation of gluten-free products to improve the characteristics of these products [7, 8]. Knowledge of the physical properties of agricultural foods and products is important for designing the equipment for processing, transportation, sorting, separation and storing [9].

Food hydrocolloids affect the final product's textural, rheological, and sensory properties. Hence, they are mostly used in low concentrations and as thickening, gelling, water retaining, dispersing, stabilizing, film forming, foaming agents, and texture modifiers in food products [10]. They primarily serve to enhance viscosity and emulsion stability, decrease weight loss, and promote cooking efficiency in food systems

and formulations. Better color, texture, symmetry, homogeneity, softening, and a reduction in the stickiness of the batter are all achieved by adding SPI to bread goods. Since this combination has a high protein content (85–90%), it is generally preferred over other types [11]. Rahim Monfared et al. (2023) reported that using protein isolates in gluten-free cakes caused an increase in batter viscosity, specific volume, and porosity and reduced baking loss [12]. All of the cake batter samples showed shear thinning behavior (pseudo-plastic) in the study by Yildiz et al. (2018), which looked at the impact of proteins, buckwheat flour, and gums on the rheological characteristics of gluten-free cake batter samples [13]. One byproduct of the making of cheese is whey protein. The main components of it include immunoglobulin, peptone, serum albumin, lactose, fat, immunoglobulin, and approximately 60% and 20% of  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin, respectively [14].

Some studies indicated that gluten-free cakes containing whey protein were more acceptable than other proteins, such as egg whites and peas [15]. Ammar et al. showed that adding whey protein to rice or corn flour can be a suitable substitute for wheat in cake preparation [16]. Under the scientific designation *Ocimum basilicum* L., basil is a member of the Lamiaceae family. In watery situations, the plant's seeds yield mucilage. The widespread use of these macromolecules in food as stabilizers, emulsifiers, and texture enhancers has been made possible by the hydrocolloid properties of basil seed mucilage. The incorporation of this mucilage into different food items enhances their ability to absorb oil, bind water, swell, froth, emulsify, texture, and flavour [17]. Pourmohammadi et al. (2020) reported that addition of basil seed gum increased the apparent viscosity of bread dough [18]. The review of literature indicates that no study has been conducted to evaluate stable rheological characteristics and dynamics of gluten-free cake batter containing WPC, SPI, and BSG. Therefore, the main objective of this work was

to ascertain how the addition of these animal and vegetable protein sources, along with a native gum (BSG), affects the rheological properties of rice flour-based gluten-free cake batter.

## 2- Materials and Methods

### 2-1- Materials

The rice used in this study was the half-grain type of Fajr cultivar (Gorgan, Iran), which was turned into flour after soaking and drying using a hammer mill. Then, it was passed through sieve No. 80 (with a pore size of 180 microns). Powdered sugar, sunflower oil, fresh eggs, baking powder, and vanilla were purchased from grocery stores (Gorgan, Iran). WPC was obtained from Lynn Company of USA, and SPI was obtained from Crown Company of China.

### 2-2- Gum extraction

The seeds of basil were obtained from the nearby market in Gorgan, Iran, and their contaminants were eliminated. BSG was created using a slightly modified version of

Razavi et al. (2009) [19]. The water-to-seed ratio in this trial was 20:1. The seeds were first inoculated for 20 minutes at 50°C and pH=7 in distilled water. An extractor device (Pars-Khazar, Iran) was used to extract BSG. Then, it was dried in a semi-industrial hot air dryer at a temperature of 45°C for 24 hours and then ground and stored in polyethylene packages impervious to moisture.

### 2-3- Preparation of cake batter

Raw materials include 100 g of rice flour, 72 g of sugar powder, 57 g of oil, 72 g of eggs, 0.5 g of vanilla, 2 g of baking powder, 30 g of water, SPI at 3 levels (0, 5, and 10%), WPC at 3 levels (0, 5 and 10%), and BSG was at 3 levels (0, 0.5, and 1%). The amount of the materials was considered based on 100 g of rice flour. The cake batter was prepared based on the method of Turabi et al. (2008) with some changes in the amount of raw materials [20]. Table 1 presents the treatments used in this study.

**Table 1- Treatments prepared and abbreviations used in the present research**

Treatment			
No	W*B (%)	No	S*B (%)
1	0, 0 (Control)	1	0, 0 (Control)
2	0, 0.5	2	0, 0.5
3	0, 1	3	0, 1
4	5, 0	4	5, 0
5	5, 0.5	5	5, 0.5
6	5, 1	6	5, 1
7	10, 0	7	10, 0
8	10, 0.5	8	10, 0.5
9	10, 1	9	10, 1

Whey protein concentrate (W), soy protein isolate (S), basil seed gum (B)

### 2-4- Common tests of rice flour, WPC, and SPI

The amounts of moisture (AACC 44-15.02), ash (AACC 08-01), protein (AACC 46-30), and fat (AACC 30-20) of these samples [21] with using an electric oven (Memmert, Germany), an electric furnace (Nabertherm,

Germany), an automatic Keldal device (Behr, Germany), and a Soxhlet (Peco, Iran).

### 2-5- Batter rheological tests

Rheological tests (static and dynamic) were performed by a rheometer (model MCR301, Anton Paar, Austria) using two parallel plates at a temperature of 25°C.

### 2-6- Stable rheological test

To create flow diagrams and ascertain variations in apparent viscosity, a rheological

test of the flow behaviour was carried out. The apparent viscosity measured at the shear rate in this test was reported at  $46.2 \text{ S}^{-1}$ , with a distance of 1 mm between the plates. The power law model with a better correlation coefficient was chosen to match the data in order to choose the best model to describe the flow behavior. The parameters of the chosen model were established for each sample. The model is shown in Equation 1.

$$\tau = k\dot{\gamma}^n$$

(1)

In this equation,  $\tau$  is shear stress (Pa),  $\dot{\gamma}$  is shear rate ( $\text{S}^{-1}$ ),  $k$  is consistency index ( $\text{Pa}\cdot\text{s}^n$ ), and  $n$  is flow behavior index (dimensionless) [22].

## 2-7- Dynamic rheological tests

### 2-7-1 Strain sweep test

The strain sweep test was performed in the strain range of 0.01-100% and a constant frequency of 1 Hz to determine the linear viscoelastic range [13].

### 2-7-2- Frequency sweep test

Storage modulus ( $G'$ ), loss modulus ( $G''$ ), loss tangent ( $\tan \delta$ ), and complex viscosity ( $\eta^*$ ) were among the data acquired from the frequency sweep test, which was conducted in the 0.001–40 Hz frequency range and imposed a constant strain of 0.1% in the linear viscoelastic range [23].

## 2-8- Statistical analysis

In this study, all tests were performed in three replicates. The data obtained from the tests were statistically analyzed using a completely random design in the form of factorial tests in SAS software. A 95% confidence level was applied when comparing means using Duncan's multiple range test. We analyzed the rheological data using Rheoplus 3.40 software.

## 3- Results and Discussion

### 3-1- Chemical compounds of rice flour, WPC, and SPI

Table 2 shows the chemical compounds in rice flour, WPC, and SPI. As shown, the moisture

in rice flour was more than in the other examined samples. However, the amounts of protein, fat, and ash were less than in the different samples. It was also found that the amount of protein in SPI was more than in other samples. As expected, protein was the most abundant compound in WPC and SPI, as it included 80% and 90% of the components of these materials, respectively.

**Table 2- Chemical composition of Rice flour, whey protein concentrate (WPC), and soy protein isolate (SPI)**

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Rice flour	9.97±0.10	8.33±0.20	0.51±0.09	0.81±0.10
WPC	6.17±0.17	80.00±1.90	4.28±0.96	4.00±0.97
SPI	5.97±0.30	90.00±2.17	0.49±0.17	4.27±0.24

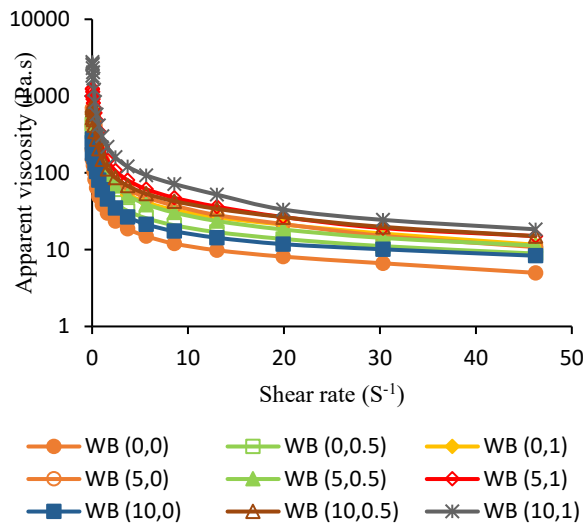
Data are the mean of three replicates ± standard deviation

### 3-2- The batter's apparent viscosity

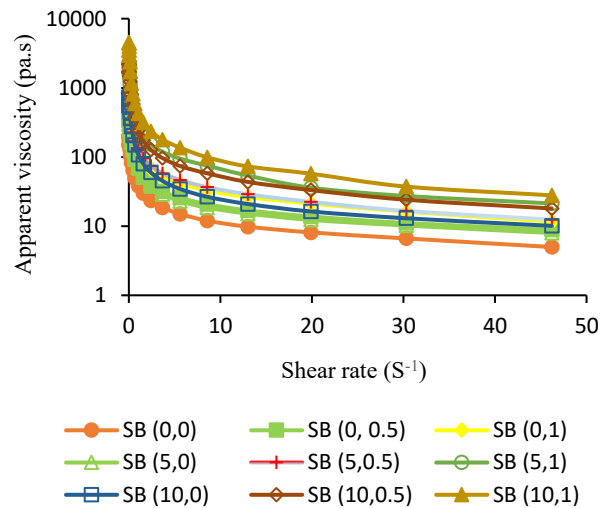
Batter viscosity is one of the most significant and effective characteristics of cake baking. A batter with low viscosity cannot keep trapped air bubbles, and these bubbles move to the surface of the batter and leave the cake in the oven, thus reducing the volume of the produced cakes [24, 25].

The effect of WPC, SPI, and BSG on the apparent viscosity of batter is depicted in Figure 1, where it is observed that all samples' apparent viscosities decreased as the shear rate increased. The batter samples' rheological characteristics were non-Newtonian, and they displayed shear thinning (pseudoplasticity) behavior. This reduction can be attributed to a potential breakdown of the batter's structure, wherein the macromolecular chains in the batter become cohesive in the direction of the shear rate rather than entangled [26]. Regarding the samples containing WPC and BSG (Figure a1), at a shear rate of  $46.2 \text{ (S}^{-1}\text{)}$ , the highest apparent viscosity ( $18.4 \text{ Pa}\cdot\text{s}$ ) belonged to the sample containing 10% WPC and 1% of the BSG, and the protein- and the gum-free sample had the lowest apparent viscosity ( $4.99 \text{ Pa}\cdot\text{s}$ ).

Figure 1b shows the apparent viscosity versus the shear rate of the samples containing SPI and BSG. The highest apparent viscosity (27.9 Pa.s) was the sample containing 10% SPI and 1% BSG. Thus, the apparent viscosity increased by increasing the percentage of WPC, SPI, and BSG. The increase of molecules that affect rheology, which results in a rise in intermolecular contacts and the participation of chains, maybe the reason for the increase in viscosity and consistency index [27].



a)



b)

**Fig. 1- The effect of (a) whey protein concentrate with basil seed gum (WB) and (b) soy protein isolate with basil seed gum (SB) on the apparent viscosity of batter samples**

### 3-3- Batter flow behavior

In order to ascertain the batter samples' flow behavior, the shear stress data was fitted against the shear rate using a power law model with a high coefficient of determination ( $R^2$ ). The parameter values of this model for batter samples are displayed in Tables 3 and 4, which also demonstrate that all samples had a flow behavior index of less than one. Thus, batter samples had a shear thinning behavior (pseudoplasticity). According to Dogan et al. (2005), hydrocolloids with a lower  $n$  index typically result in high viscosity and a pleasant mouthfeel [30]. As shown, all the studied batter samples showed a higher  $k$  index than the control sample, indicating an increase in the strength of the gel network. The results

also showed that the samples with lower  $n$  had higher  $k$  values. The highest consistency index (450.2 Pa.s <sup>$n$</sup> ) was related to the sample containing 10% SPI and 1% BSG, and the lowest  $k$  index (38.3 Pa.s <sup>$n$</sup> ) was related to the control sample. The increase in  $k$  index can be attributed to the reaction between different hydrocolloids and rice flour starch, the entanglement of these compounds with each other, and the reduction in the mobility of molecules [31]. Shaabani et al. (2018) examined the impact of pea protein isolate along with xanthan gum on gluten-free muffin batter and concluded that the consistency index value increased with increasing gum percentage [32]. Yildiz et al. (2018) reported that gum-containing cakes had a higher

consistency index, which may be related to the semi-solid molecules and the high water-binding capacity of the gums, which reduce the available free water and the movement of particles in the cakes [13]. Noorlaila et al. (2017) examined the impact of xanthan gum and hydroxyl-propyl methylcellulose on

sponge cake. They concluded that all cake batter samples had less than one flow index and shear thinning behavior. This flow behavior indicates that the viscosity of the batter decreases with the increase of the shear rate [33].

**Table 3- Power law model parameters in batter samples containing whey protein concentrate with basil seed gum (WB)**

Cake batter sample	k (Pa.s <sup>n</sup> )	n	R <sup>2</sup>
WB (0 - 0)	38.3	0.47	0.99
WB (0 - 0.5)	67.35	0.47	0.99
WB (0 - 1)	119.3	0.40	0.99
WB (5 - 0)	49.1	0.47	0.99
WB (5 - 0.5)	450.2	0.28	0.99
WB (5 - 1)	206.3	0.31	0.97
WB (10 - 0)	53.32	0.51	0.99
WB (10 - 0.5)	151.6	0.40	0.99
WB (10 - 1)	311.1	0.27	0.97

**Table 4- Power law model parameters in batter samples containing soy protein isolate with basil seed gum (SB)**

Cake batter sample	k (Pa.s <sup>n</sup> )	n	R <sup>2</sup>
SB (0 - 0)	38.3	0.47	0.99
SB (0 - 0.5)	67.35	0.47	0.99
SB (0 - 1)	119.3	0.40	0.99
SB (5 - 0)	65.87	0.44	0.99
SB (5 - 0.5)	130.2	0.40	0.99
SB (5 - 1)	278.9	0.33	0.98
SB (10 - 0)	104.9	0.38	0.99
SB (10 - 0.5)	257.2	0.31	0.98
SB (10 - 1)	450.2	0.28	0.98

### 3-4- Storage module and loss module

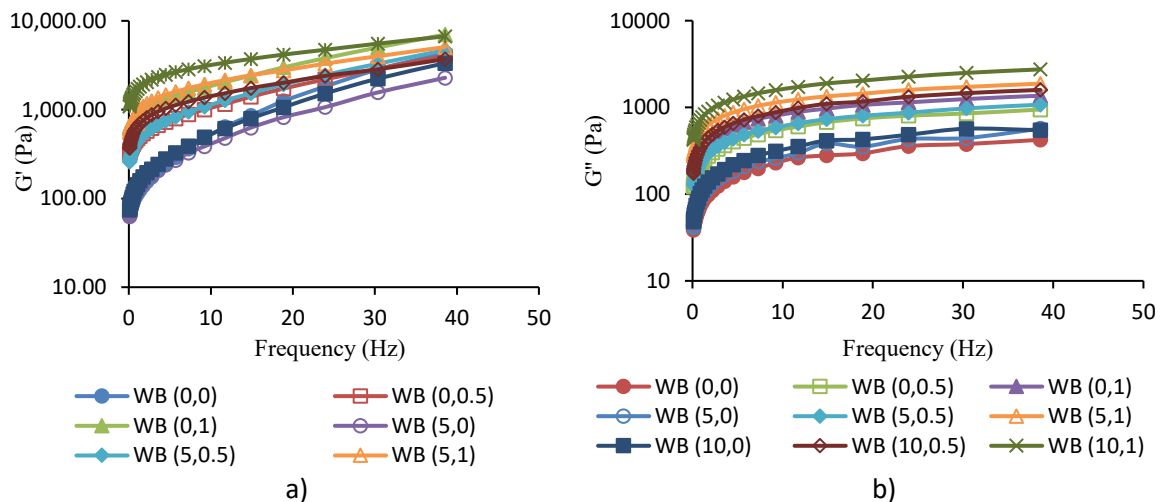
The quantity of elastic behavior and the amount of energy recovered per unit volume during each complete cycle of the strain wave are represented by the storage modulus. According to Gope et al. (2016), the loss modulus also shows how much flow behavior and energy are lost per unit volume throughout each complete cycle of the strain wave [34]. The variations in the loss and storage modulus of batter samples containing WPC and BSG

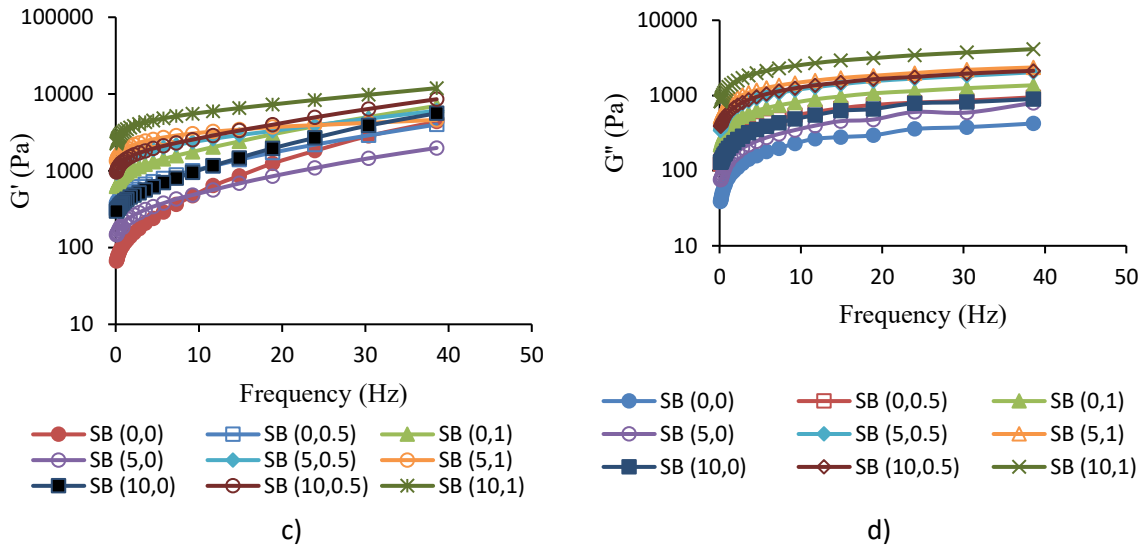
are shown in Figures a2 and b2. In every batter sample, the storage modulus was greater than the loss modulus, indicating that the elastic characteristic outweighed the viscosity.

The values obtained at a frequency of 1 Hz for samples containing WPC and BSG showed that the storage modulus was in the range of 115.144-1720.079 Pa and the loss modulus was in the range of 80.476-773.741 Pa. Regarding the samples containing SPI and BSG, the storage modulus was in the range of

115.144-3317.217 Pa and the loss modulus was in the range of 80.476-1291.316 Pa. Thus, the sample containing 10% SPI and 10% BSG had a higher storage modulus and loss modulus than other samples. The control sample also observed the lowest storage and loss modulus (Figure 2). Thus, increasing the percentage of WPC and SPI from 0 to 10%, and the percentage of BSG from 0 to 1% increased the storage and loss modulus. The reason for increasing the elastic modulus with the addition of whey protein may be the good solubility of this compound. Also, whey protein has a high content of total and free sulfhydryl groups, which have strong crosslinking tendencies with other components of the cake, resulting in an improvement in the viscoelastic properties of the batter [35]. Yildiz et al. (2018) ascribed the gum's innate elastic characteristics and the interaction of hydrocolloids with starch to the increase in elastic modulus in gluten-free cake batter with adding gum [13].

According to Matos et al. (2014), samples of muffin batter made with rice flour exhibited gel-like behavior and higher values of storage modulus [36]. The viscoelastic behavior of the batter was influenced by the addition of protein, with varying degrees of effect depending on the source of protein. The elastic and viscous components of the muffin batter made with rice flour exhibited modifications in the presence of all vegetable proteins, resulting in an increase in both the storage modulus and the loss modulus.  $G'$  and  $G''$  were greater in batters containing soy and pea proteins. Villanueva et al. (2018) found that enriching a gluten-free batter with soy protein or calcium caseinate increased the storage modulus and loss modulus. They demonstrated that the kind of protein added and the kind of starch used as the batter base affect the batter's rheological characteristics [37].



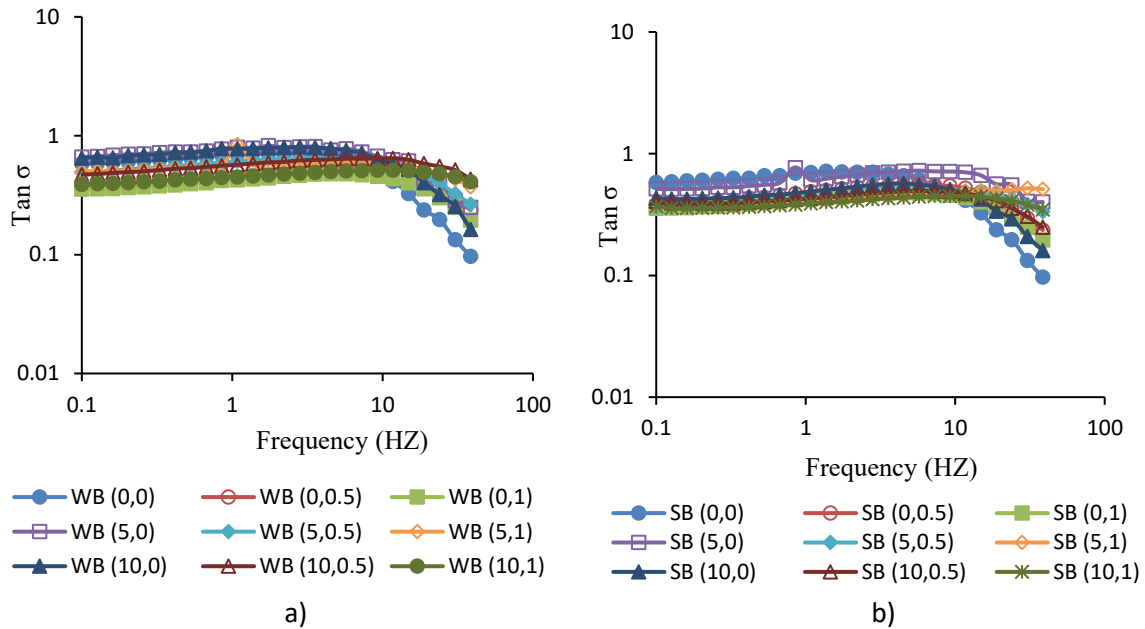


**Fig. 2- Storage modules of batter samples containing (a) whey protein concentrate with basil seed gum (WB) and (c) soy protein isolate with basil seed gum (SB) and loss modules of batter samples containing (b) whey protein concentrate with basil seed gum (WB) and (d) soy protein isolate with basil seed gum (SB)**

**3-5- Loss tangent**

Figure 3 showed that the loss tangent value of batter samples containing different levels of WPC or SPI with BSG was less than one, confirming the elasticity of the samples. The sample containing 10% WPC and 1% BSG and the sample containing 10% SPI, and 1% BSG with higher storage and loss modulus had a low loss tangent. The decrease in the tangent loss at higher amounts of gum and protein can be attributed to the better hydration of starch and protein, leading to the hardening of the cake batter [38]. Yildiz et al. (2018) reported that cake batter samples had a loss tangent of less than 1 [13]. Herranz et al. (2017) also

reported that reducing the amount of protein increases the loss tangent of gluten-free batter. However, the amount of this index decreased with increasing the amount of protein in the formulation. It was in line with the results of this section [39].



**Fig. 3- Loss tangent of batter samples containing (a) whey protein concentrate with basil seed gum (WB) and (b) soy protein isolate with basil seed gum (SB)**

### 3-6- The complex viscosity

The results revealed that the complex viscosity values of batter samples containing different levels of WPC or SPI with BSG decreased with increasing frequency, confirming the elasticity of the samples. Thus, the samples containing 1% BSG with 10% WPC or 10% SPI have more stiffness than other samples, and their complex viscosity is 277.268 Pa·s and 299.523 Pa·s, respectively, at the frequency of 1 Hz (Figure 4). In other words, by increasing the amount of gum and protein, the viscosity of the sample complex increased. This increase can be attributed to the high protein content of WPC and SPI. According to Patil et al. (2020), who examined the viscoelastic rheological characteristics of batter made from gluten-free flour, batter

viscosity rises as the protein concentration does. They claimed that the absence of a protein network results in reduced complex viscosity, which indicates less homogeneity of batter samples like rice batter [40]. It concurred with this section's conclusions. Benazir et al. (2019) demonstrated that the breakdown of inflated batter granules is the cause of the decreasing complex viscosity with increased frequency by examining the impact of adding hydrocolloids on the rheological characteristics of gluten-free maize flour batter. They also stated that the viscosity of the batter increases as a result of the creation of hydrogen bonds, which increases the amount of various hydrocolloids. It was consistent with this section's outcomes [41].

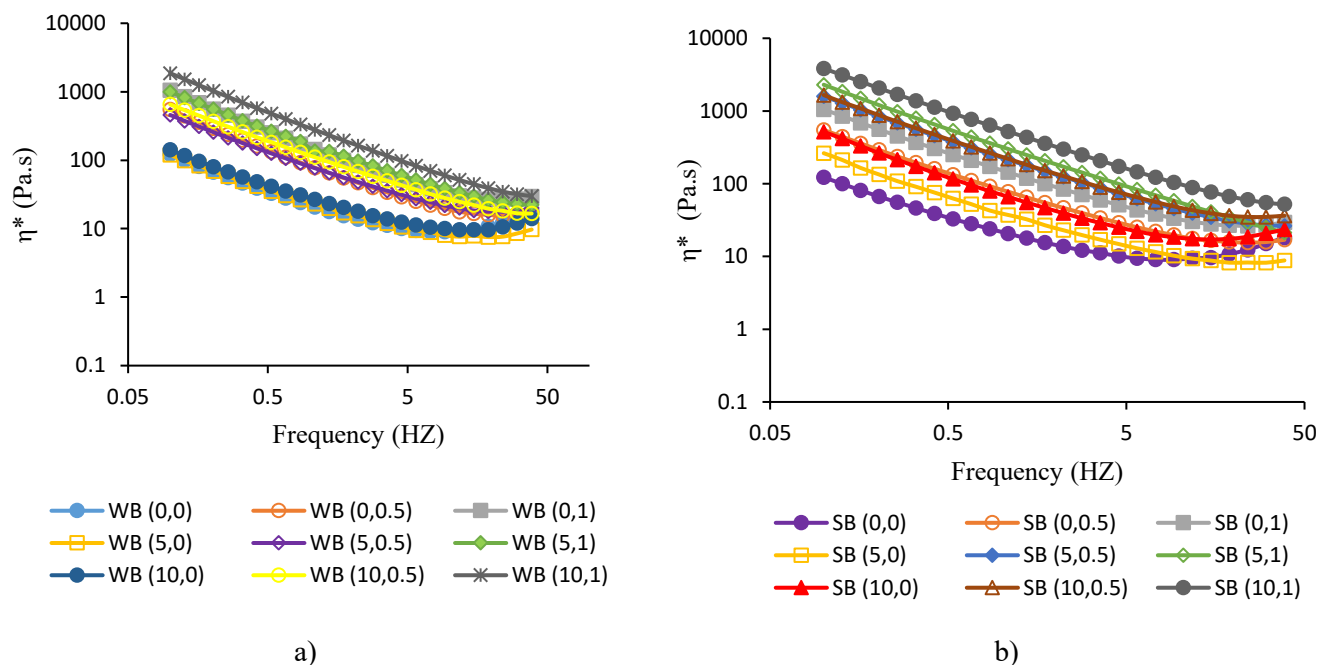


Fig. 4- The complex viscosity of batter samples containing (a) whely protein concentrate with basil seed gum (WB) and (b) soy protein isolate with basil seed gum (SB)

#### 4- Conclusion

The textural, rheological, thermal, structural, and microscopic characteristics of the batter should be examined to ensure the appropriate technological quality of the batter of bakery products since these parameters of the batter are related to the textural characteristics of the final product and significantly affect the sensory characteristics and consumer acceptability. This study showed that adding WPC, SPI, and BSG to the cake batter decreased the apparent viscosity in all samples with an increased shear rate. The produced batter samples exhibited shear thinning behavior (pseudoplasticity) and were rheologically non-Newtonian. All samples had flow behavior indices that were less than one. The oscillation test findings showed that in every batter sample, the elastic characteristic outweighed the viscous one, and the storage modulus was larger than the loss modulus. Also, the loss tangent values of batter samples containing different levels of WPC or SPI with BSG were less than one, confirming the elasticity of the samples. The viscosity of the

complex increased by increasing protein concentrate and SPI along with BSG. Based on these results, the samples containing 10% SPI and 1% BSG can be introduced as the optimal treatment that has the capability of industrial production. It can also compensate for some of rice's nutritional deficiencies while improving the batter's characteristics. To enhance the practical applicability of this formulation, further studies are recommended to evaluate the effects of the additives on the final product's characteristics, including flavor, shelf life, and nutritional value. Additionally, economic and operational assessments related to the industrial production of this formulation are essential to facilitate its market introduction.

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#### Author Contributions

All activities were carried out by the author.

## Competing Interests

The author confirms that he / she has no financial conflicts of interest or competing interests in this study.

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ویژگی‌های رئولوژیکی پایا و دینامیک خمیر کیک بدون گلوتن غنی شده با کنسانتره پروتئین آب پنیر و ایزوله پروتئین سویا به همراه صمغ دانه ریحان

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اطلاعات مقاله

چکیده

در سال‌های اخیر، تقاضا برای محصولات غذایی بدون گلوتن به‌طور چشمگیری افزایش یافته است. بنابراین، انجام پژوهش‌هایی درباره تأثیر ترکیبات مختلف به‌منظور بهبود کیفیت این محصولات ضروری به نظر می‌رسد. این مطالعه با هدف بهبود ویژگی‌های رئولوژیکی خمیر کیک بدون گلوتن بر پایه آرد برنج با استفاده از کنسانتره پروتئین آب پنیر و ایزوله پروتئین سویا در سه سطح (۰، ۵ و ۱۰ درصد) و سطوح مختلف صمغ دانه ریحان (۰، ۵/۰ و ۱ درصد) صورت پذیرفت. نتایج نشان داد که ویسکوزیته ظاهری در تمامی نمونه‌ها با افزایش میزان پروتئین و صمغ افزایش یافت به گونه‌ای که بالاترین ویسکوزیته ظاهری (۲۷/۹ پاسکال ثانیه) مربوط به نمونه حاوی ۱۰ درصد ایزوله پروتئین سویا و ۱ درصد صمغ دانه ریحان بود. شاخص رفتار جریان همه نمونه‌ها کمتر از یک بود، بنابراین تمامی نمونه‌های خمیر رفتار رقیق شونده با برش (سودپلاستیک) داشتند. افزایش درصد کنسانتره پروتئین آب پنیر، ایزوله پروتئین سویا و صمغ دانه ریحان در فرمولاسیون خمیر کیک منجر به افزایش مدول ذخیره و مدول افت گردید. مقدار تانژانت افت نمونه‌های خمیر حاوی سطوح مختلف کنسانتره پروتئین آب پنیر و یا ایزوله پروتئین سویا با صمغ دانه ریحان کمتر از یک بود که الاستیک بودن نمونه‌ها را تایید نمود. یافته‌ها نشان داد که نمونه‌های حاوی ۱ درصد صمغ دانه ریحان با ۱۰ درصد کنسانتره پروتئین آب پنیر یا ۱۰ درصد ایزوله پروتئین سویا دارای سفتی بیشتری نسبت به سایر نمونه‌ها بوده و در فرکانس ۱ هرتز میزان ویسکوزیته کمپلکس آن‌ها به ترتیب ۲۷۷/۲۶۸ و ۵۲۳/۲۹۹ پاسکال ثانیه بود. در نهایت، نمونه‌های کیک حاوی ۱۰ درصد ایزوله پروتئین سویا با ۱ درصد صمغ دانه ریحان از نظر ویژگی‌های رئولوژیکی به‌عنوان جایگزین کیک‌های معمولی برای افراد مبتلا به سلیاک پیشنهاد گردید.

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کلمات کلیدی:

صمغ دانه ریحان،  
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