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Physicochemical and rheological characterization of pan bread made with pumpkin seed flour

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Abstract

Several studies have focused on the production of lower cost, more nutritious, and profitable breads. From this perspective, the objective of this study was to characterize pumpkin seed flour (PSF) and evaluate the effects of its addition on the physicochemical and rheological characteristics of pan bread. Three formulations of pan bread were prepared containing 0 (T0), 30% shelled PSF (T1), and 30% unshelled PSF (T2). The PSFs and pan breads were subjected to physicochemical and rheological characterization. Rheological analysis showed a reduction in baking potential of the flour blends. On the other hand, the treatment T1 showed higher moisture, total dietary fiber, and carbohydrate levels, whereas T2 showed higher ash, lipids, protein, and caloric value. PSF enriched breads had darker color when compared to the control bread. The treatment T0 presented the highest brightness, T2 had the highest green hue, and T1 showed higher yellow hue. T1 and T2 were significant different from T0 for the parameters firmness, elasticity, and chewiness, while no significant difference was observed for the parameter cohesiveness. Thus, the unshelled PSF has proven to be the most suitable flour to be used along with wheat flour in pan bread making.

Introduction

Bread is a food consumed worldwide, with a high energy value and quite significant constituents for nutrition of an individual, meeting their energy needs. In Brazil, bread is a complete meal rather than a complementary food due to current consumers` habits (Vasconcelos *et al*., 2006).

Bread production has increased every year with a wide variety of formats and formulations. As the most of bakery products, your manufacture requires the use of flour with specific digestibility properties (Sluimer, 2005). In the 1960s, flour blends were used for partial substitution of wheat flour to reduce the import of cereal. Subsequently, the improvement of the nutritional quality of food products to meet consumers` needs was focus of various studies worldwide (Pierarski, 2009).

According to Mastromatteo (2012), bread made with durum wheat flour proportionally replaced by legume flour led to the development of a new viable formulation for the bakery industry, once the addition of functional nutrients has established new perspectives in baking. However, studies on flours from seeds and/or peels of fruits and vegetables mixed to wheat flour are needed, so that the properties of the product are not affected during processing.

The world production of pumpkins is estimated at 25.2 million tons per year. The production of pumpkins is prevalent in Asia (around 65% of world production) Europe (14%) and America (11%). China stands out as the largest producer (6,978,167 t), followed by India and Russia. In Brazil, the production is estimated at 384,916 tons per year (FAO, 2017). Pumpkin seed has been used as food and appetizer, or in the form of oil or flour. Besides having high dietary fiber levels, and antioxidant and anthelmintic effects, it is a good protein source (Esuoso *et al*., 1998). Thus, the incorporation of pumpkin seed in pan bread can increase the nutritional and functional value of the final product.

The consumption of pumpkin seeds in natura without heat treatment can reduce the bioavailability of nutrients (Del-Vechio *et al*., 2005). Studies on animals have shown no toxic effects of this type of seed, with beneficial effects on metabolism, physiology, and human nutrition (Trowell, 1976; Rodríguez *et al*., 2006). The objective of this study was to characterize pumpkin seed flour (PSF) and evaluate the effects of its addition on the physicochemical, rheological, and sensory characteristics of pan breads.

Material and Methods

Production of pumpkin seed flour (PSF)

Pumpkin seeds commercially known as

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"Chinese", "branca de neve" or "shine skin" were acquired in a wholesaler in Uberlândia-MG. Roast shelled (a) and unshelled (b) pumpkin seeds were selected for the study. Seeds were transported in their original packaging to the Bromatology Laboratory (IFTM-Campus Uberaba), and stored at room temperature until processing. Subsequently, the seeds were ground in an industrial blender (Vitalex LI-02) for 1 min and passed through a 24 mesh sieve. The respective flours were packaged in plastic bags, sealed in vacuum packaging machine (Sulpac -SVC200), and frozen stored (-18 ± 2 ° C) until bread making.

Formulation and production of pan breads

A control formulation and two formulations containing PSF were produced, as shown in Table 1. The proportions of wheat flour and PSF were chosen according to the results obtained in previous studies. The formulations T1 and T2 were adjusted to obtain dough consistency similar to T0. The amount of water in the formulations T1 and T2 were modified as follows: 10% water increase in T1 due to the seed coat in F1, and 10% water reduction in T2 due to the higher lipids levels in F2. The vegetable fat was removed from the formulations T1 and T2, since the PSFs were able to provide more fat than the amount in formulation T0.

The dry ingredients were placed in slow trough (Skymsen AMB-25) and mixed for 1 minute at low speed. Then, water and the liquid ingredients were added and mixed at low speed for further 9 minutes. The dough was kept at rest in closed environment for 35 minutes and then kneaded and opened manually. Finally, it was divided into 600 g pieces and placed in forms for pan bread. Dough fermentation was carried out at 35°C for 75 min, protected by plastic bags to prevent drying. Subsequently, pan bread was baked at 200°C for 20 minutes in combination furnace (Prática - EC6). After removal from the forms, breads were cooled at room temperature for at least an hour, cut into 25 g slices of about 1.50 cm thickness, packaged, and stored for about 24 h until the time of analysis.

Physicochemical characterization

The pH of the samples was determined by direct readings in digital pH meter calibrated and operated according to the manufacturer's instructions (Hanna/HI 221). The total soluble solids (TSS) was determined by reading on a digital refractometer with automatic temperature compensation at 25°C (Reichert/ AR200), and expressed as ºBrix (IAL, 2008). Water activity (a_w) was measured by Aqualab (S4TE) dew point water activity meter.

Table 1. Pan bread formulations containing pumpkin seed flour (PSF)

Ingredients	Formulation*(%)		
	T0	T1	T2
Wheat flour	100	100	100
Shelled PSF	0	30	0
Unshelled PSF	0	0	30
Water	56.5	66.5	46.5
Cristal sugar	4.25	4.25	4.25
Glucose	2.5	2.5	2.5
Salt	2.0	2.0	2.0
Vegetable fat (65% fat)	4.5	0	0
Powdered milk	1.25	1.25	1.25
Biological yeast	1.0	1.0	1.0
Enzyme alpha amylase	1.0	1.0	1.0
Mold inhibitor calcium propionate	0.2	0.2	0.2

* % related to wheat flour weight on a wet basis.
T1 = Pan bread with 30% shelled pumpkin seed flour, T2 = Pan bread with 30% unshelled pumpkin seed flour

Moisture was determined by gravimetric method at 105°C; ash by incineration in a muffle at 600ºC and 550°C for PSF and pan breads, respectively; crude protein was determined by the Kjeldahl method, multiplying the nitrogen content by the factor 5.30 and 6.25 for PSF and pan breads, respectively; total lipids were determined by the Soxhlet method (AOAC, 2005). Dietary fiber was determined according to the method of Prosky *et al*. (1985) as reported by the IAL (2008). Total available carbohydrates were determined by the difference between the value of 100 and the sum of protein, lipids, dietary fiber, moisture, and ash levels. Calories were calculated using the Atwater conversion factors as follows: 4 kcal / g (proteins), 4 kcal / g (carbohydrates), and 9 kcal / g (lipids) as reported by Osborne and Voogt (1978).

Rheological analysis

The rheological properties of dough were determined in the wheat flour without PSF (F0), and in the flour blends containing shelled (F1) and unshelled PSF (F2).

The flour mixing characteristics were analyzed according to the method of AACC 54-21.01 (2010), using a farinograph Brabender with 300 g bowl. The parameters used to interpret the farinogram were water absorption (WA), dough development time (DDT), stability (ST) and mixing tolerance index (MTI).

The flour samples were subjected to Alveograph Chopin, according to the AACC 54-30.02 (2010) to determine their viscoelastic properties. The alveogram parameters were dough tenacity (P), which measures the maximum pressure exerted for dough expansion (mm); extensibility (G) that measures the length of the curve (mm), and deformation energy (W), which corresponds to the mechanical work necessary for rupture the bubbles, expressed as 10-4 J.

Color measurement

The color of the pan breads was measured by colorimeter CR-400 MINOLTA, according to the *L* * *a* * *b* * CieLab system. The color parameters were L^*, a^*, b^* and Chroma (C^*) as reported by Minolta (2006).

Texture profile

The firmness of the breads was evaluated by Stable Micro System TA.XT. plus texture analyzer, with a flat-ended cylindrical aluminum probe 36 mm diameter (P / 36R), and the force applied to the samples was measured in Newton. A compression of 7.5 mm corresponding to a deformation of 75% of the sample was used. The configuration parameters were: compression distance: 5.0 mm, pre-test speed: 5.0 mm / s, test speed: 2.0 mm / s, post-test speed: 5.0 mm / s.

Statistical analysis

The formulations were analyzed in five replicates and the results of physicochemical analyses were submitted to a completely randomized design. The effects of the treatments were subjected to analysis of variance (ANOVA) and the means analyzed by Tukey test at 5% probability for identifying the differences. The results were statistically analyzed using the Assistat 7.7 software (SILVA, 2014).

Results and Discussion

Pumpkin seed flour

Table 2 shows the results of the physicochemical characteristics and color parameters of pumpkin seed flours. No significant differences were observed between F1 and F2 for the parameters pH, TSS, and Aw, thus the presence or absence of the seed coat did not affect these parameters.

With respect to the pH values, Santangelo (2006) evaluated PSF and obtained pH of approximately 6.16, while Amorim *et al.* (2012), and Silva (2012) found pH values of 6.22 and 6.31, respectively. Thus, the present results are in accordance with the pH results in literature. Alves *et al.* (2012) found different Aw values in PSF when compared to this study, which was approximately 0.68 in roast PSF. The discrepancy in results may be due to several factors including kind of the pumpkin, time and temperature of drying the seeds, and the processing technique for obtaining the flour. Low Aw value allows the preservation of products for longer periods, once it inhibits bacteria growth and oxidation and browning reactions (Vitalli, 1987).

Table 2. Physicochemical parameters and color determination of pumpkin seed flours

Physicochemical properties		
	F1	F2
pH	6.56ª±0.05	6.59ª±0.04
TSS (° Brix/ 20° C)	2.71ª± 0.01	2.78ª±0.03
Aw	0.37ª±0.13	0.38ª±0.39
Color		
L*	71.93ª±3.63	71.33ª±1.87
a*	-1.73ᵇ±0.41	-0.53ª±0.19
b*	20.61ª±2.12	20.96ª±0.44
Croma	20.68ª±2.14	20.97ª±0.44

* Means followed by the same letter do not differ by Tukey test p <0.05.

No differences were observed between F1 and F2 for the color parameters L^*, b^* and Chroma. The high L^* value indicates clear color of both flours. Negative a^* values demonstrate greater tendency to green, while b* suggests more prone to yellow. The flour F1 had more intense green color than F2. Chroma represents color saturation (Minolta, 2006), thus the higher the chroma value, the greater the saturation. Silva (2012) found opposite results, which were L^* 64.12; a^* 0.51; and b^* 32.81. The flours of the present study exhibited similar color saturation and were lighter, with greater tendency to green color, and less yellow color.

Table 3 shows the physicochemical composition of the PSFs. The flour F1 had higher moisture, total dietary fiber, and total carbohydrates levels, while F2 presented higher ash content, total lipids, protein, and caloric value ($p<0.05$). The higher moisture content of F1 can be due to the presence of the tegument, which acts as an insulator, preventing water loss from the seed into the environment. The higher lipids and protein levels in F2 can be explained by Pumar *et al.* (2008), who reported higher lipids and protein levels in whole and sieved PSFs due to the presence of the endosperm. In contrast, the residual flour with higher fiber content from the tegument had lower lipids and protein levels when compared with F1.

Naves *et al.* (2010) studied the chemical composition of PSFs subjected to different processes, and obtained protein, lipids, and ash levels similar

Table 3. Physicochemical composition and caloric value of pumpkin seed flour in 100 g of dry matter.

Parameter	F1	F2
Moisture (%)	3.13^a±0.11	2.27^b±0.19
Ash (%)	3.62^b±0.24	4.42^a±0.02
Lipids (%)	39.60^b±0.97	48.68^a±0.89
Crude protein (%)	25.56^b±2.74	28.78^a±0.51
Dietary fiber (%)	52.56^a±0.17	44.74^b±2.37
Available carbohydrates (%)	N/S*	N/S*
Caloric value (kcal)	484.78^b±2.14	556.71^a±1.66

*N/S= not significant
Means followed by the same letter do not differ by Tukey test $p<0.05$

to this study. Silva *et al.* (2011) obtained lipids, total fiber, total carbohydrates contents, and caloric value close to those observed in this study. Borges *et al.* (2006) found high protein and lipid levels in PSFs, while Silva (2012), Cerqueira *et al.* (2008), and Santagelo (2006) found high caloric values. The caloric value of F1 was close to that found in the study of Franzen *et al.* (2014), which evaluated PSFs for use in cupcakes.

Rheological characteristics of the blends

The Brabender farinograph parameters of the wheat flour with and without addition of PSF are presented in Table 4. The flour F0 presented higher values for water absorption, development time, and stability time. Although no differences were observed between F1 and F2 for water absorption, and development time, significant differences were observed for stability time and mechanical tolerance index.

According to Germani (2003), for bread making, flour should provide water absorption from 60 to 64%, which is close to the WA observed in F0 (control), whereas the flours containing PSF presented lower water absorption values. For Guarienti (1996), the development time is the time in minutes for development of the dough's maximum consistency, i.e., the time required to determine the percentage of water absorption so that flour reaches the consistency required for bread making.

According to Carvalho (1999), the development time is an important parameter to evaluate dough fermentation, once an adequate time is required for gas retention. From this perspective, the flour F0 presented development time greater than F1 and F2, thus the addition of PSF reduced the development time and gas retention capacity.

Table 4. Farinograph and alveograph parameters of control wheat flour, and flour blends with PSF.

Farinograph parameters			
	F0	F1	F2
WA (%)	57.67^a±0.06	53.47^b±0.57	54.00^b±0.17
DT. (min.)	14.17^a±1.04	6.00^b±0.00	5.67^b±0.29
ST (min.)	19.67^a±1.04	4.67^c±0.29	7.33^b±0.76
MTI (BU)	30.00^b±10.00	56.67^a±5.77	23.33^b±5.77
Alveograph parameters			
	F0	F1	F2
P (mm)	125.67^a±1.53	46.67^b±0.58	34.67^c±0.58
L (mm)	61.00^a±1.73	29.33^c±1.15	48.67^b±6.03
P/L	2.06^a±0.07	1.59^b±0.08	0.72^c±0.10
W (X10^{-4} J)	330.00^a±0.00	63.33^c±2.89	75.00^b±5.00

WA. = Water absorption, DT= developing time; ST = stability time; MTI= Mechanical tolerance index; BU = Brabender units. P = overpressure; L = average abscissa; P / L = Curve setting Index; W = deformation energy of dough.
*Means followed by the same letter do not differ by Tukey test $p<0.05$.

Dough stability is a parameter indicative of mechanical resistance and fermentation time, while the MTI indicates dough tolerance during mixing (Guarienti, 1996). El-Dash and Germani (1994) have suggested minimal stability time of 7.5 minutes to produce pan breads. In this study, only the formulation T1 showed a lower stability time when compared to the value suggested by those authors.

The addition of PSF provided a decrease in flour stability. F1 had a higher MTI, probably due to its high fiber level, while the higher lipids levels in F2 led to the lowest MTI in this flour.

According to the classification proposed by Wiilams *et al.* (1988), the flours of this study were classified into very strong (F0), and medium weak flour (F1 and F2), thus they are suitable for production of bread, cakes and biscuits, respectively (Benassi and Watanabe, 1997).

The Chopin alveograph parameters of the wheat flour samples with and without PSF are presented in Table 4. Guarienti (1996) defines "P" as the maximum tenacity limit and dough stability index, which indicates dough resistance to deformation. Significant differences were observed for the flours in this study, and F0 showed greater tenacity, thus, it is more resistant to mechanical work, followed by F1 and F2. The "L" represents a measure of dough extensibility, and the higher the L value, the higher

Table 5. Physicochemical characteristics, color measurements, and texture profile of pan breads

	T0	T1	T2
Physicochemical parameters			
pH	5.53^{b}±0.04	5.61^{a}±0.01	5.57^{ab}±0,02
Aw	0.92^{a}±0.01	0.92^{a}±0.01	0.94^{a}±0,00
Color			
L*	65.40^{a}±2.91	57.00^{c}±2.25	63.65^{b}±2.59
a*	-0.89^{b}±0.25	-0.41^{a}±0.34	-1.49^{c}±0.24
b*	17.87 ^{c}±1.72	23.58^{a}±1.37	21.35^{b}±1.52
Chroma	17.89^{bc}±1.71	23.59^{a}±1.37	21.41^{b}±1.52
Texture			
Firmness (N)	6.07^{b}±2.953	9.88^{a}±1.174	9.64^{a}±3.233
Elasticity	0.00899^{a}±0.001	0.00673^{b}±0.003	0.00647^{b}±0.002
Cohesiviness	0.00641^{a}±0.001	0.00590^{ab}±0.001	0.00519^{b}±0.001
Chewiness (N.mm)	0.03^{b}±2.044	4.30^{a}±0.008	3.29^{a}±1.786
Physicochemical composition			
Moisture (%)	26.69^{b}±0.77	27.49^{b}±0.49	30.51^{a}±0.49
Ash (%)	2.66^{c}±0.06	2.99^{b}±0.05	3.15^{a}±0.04
Lipids (%)	6.17^{c}±0.32	7.72^{b}±0.15	9.39^{a}±0.25
Crude protein (%)	12.26^{b}±0.60	17.47^{a}±0.15	17.15^{a}±0.19
Total dietary fiber (%)	8.26^{c}±0.05	11.82^{a}±0.16	10.49^{b}±0.01
Available carbohydrates[1] (%)	43.94^{a}±0.75	32.50^{b}± 0.41	29.30^{c}±0.67
Caloricvalue[2] (Kcal)	280.40^{a}±5.17	269.40^{b}±2.22	270.33^{b}±1.30

* Average of 5 repetitions ± standard deviation, followed by the same letter do not differ by Tukey test p <0.05.
1Available carbohydrates calculated by difference; 2 Calculated using the Atwater conversion factor: 4 (proteins and carbohydrates) and 9 (lipids).

bread volume. The flour F0 provided pan breads with a tendency to higher volume, followed by F2 and F1 (Guarienti, 1996).

W is the overall baking strength of gluten. The P / L, along with W indicate the baking potential of a product. The flour F2 showed P / L between 0.61 and 1.20, which is considered balanced gluten flour. Both the flours F0 and F1 had P / L above 1.21, therefore, they are considered tenacious gluten flour. According to the classification of Willams *et al.* (1988), F0 has strong gluten, while F1 and F2 have weak gluten.

According to Germani (2003), flour should provide W values between 150-280 x 10^{-4} J, and P / L between 0.5 to 1.7 mm H_2O.mm-1 for the production of pan breads. Therefore, the F0 appears more appropriate when considering W values, while F1 and F2 are more suitable in relation to P / L values, with significant differences between them ($p < 0.05$).

Pan breads

The physicochemical characterization, color measurements, and texture profile of pan breads are presented in Table 5. The lowest pH value was found in the formulation T0, while no significant differences were observed for T2 when compared to T0 and T1. T1 had the highest pH of all samples.

Gorgonio *et al*. (2011) studied cakes containing PSF and starch, and found pH close to neutral. In contrast, Santagelo (2006) found pH of 5.34 in panettone samples, and Silva (2012) found pH of 6.31 in cereal bars. Therefore, the pH of the product depends not only on pH of PSF, but also on the other ingredients used in bread making. No significant difference was observed for water activity of the pan bread containing PSF.

The formulation T0 presented the highest L^* value followed by T2 and T1. The formulations T1 and T2 had L^* similar to the values found by Silva (2012) who used 12.5 and 25% PSF in cereal bars. Moura *et al.* (2010) evaluated the color of cookies with replacement of 30% wheat flour by different levels of pumpkin seed flour, and found that all cookies containing PSF had L^* values lower than the cookies produced only with wheat flour. Similarly, Borges *et al.* (2011) have studied breads fortified with flaxseed flour, which were darker when compared to control breads.

The formulation T2 had a higher green hue, followed by T0 and T1. The shelled PSF in formulation T1 may have led to an increase in a^* values. T1 had lower green hue among all treatments. The formulation T1 had a higher yellow hue, followed by T2 and T0. In general, breads are more likely to yellow. Bitencourt *et al.* (2014) produced cakes and found a higher yellow color when 30% PSF were used. Silva *et al.* (2009) have also reported an increase in yellow color when okara flour was incorporated into pan bread formulations.

The formulation T1 presented more saturated hue of 23.59 when compared to T0 (17.89) and T2 (21.41). Significant differences were observed for the treatments T1 and T2 regarding the parameters firmness, elasticity, and chewiness, while T1 did not differ statistically from T0 and T2 for the attribute cohesiveness.

Bitencourt *et al.* (2014) observed that cakes with higher PSF levels presented higher hardness. Borges *et al.* (2011) found high firmness in linseed enriched breads when compared to the control bread. Silva *et al*. (2009) produced pan bread with addition of okara, and also observed that the increase in okara flour levels led to an increase in hardness.

The elasticity of the pan breads of formulations T1 and T2 was lower when compared to T0, but not statistically different, indicating that the presence of gluten in wheat flour may have contributed to dough elasticity. Salmenkallio-Marttila *et al.* (2001) have reported that the addition of bran or fibers in bread formulation can weaken dough structure, reducing thickness and elasticity of the crumb. For Gomez *et al*. (2004), this effect is due to the interaction between fiber and gluten, reducing the gas holding capacity.

For the attribute cohesiveness, no significant difference was observed in T1 when compared to T0 and T2, while T0 and T2 differ from each other. Bitencourt *et al*. (2014) have reported that low cohesiveness may lead to a susceptibility of the bread to fracture or crumble, which was observed in the formulation containing PSF.

No significant differences were observed for the attribute chewiness between the formulations T1 and T2, which was lower in formulation T0. This parameter is directly proportional to dough strength, so bread with PSF required high level of energy to swallow. The high total dietary fiber and protein contents may have contributed to increased hardness in breads, thus requiring higher chewiness index.

Table 5 shows the physicochemical composition and caloric value of pan breads in 100 g of dry matter. The formulation T2 had higher moisture, ash, and total lipids levels when compared to the other samples. No significant differences were observed for crude protein between the formulations T1 and T2, which were higher when compared to T0. Silva *et al.* (2010) also found that the addition of PSF in cookies is suitable to increase the dietary fiber levels of the product. In the present study, the formulation T1 had the highest total dietary fiber content, followed by T2 and T0. The formulations T1 and T2 can be classified as products with high dietary fiber, because they have more than 5 g dietary fiber per 100 g product. With respect to the formulation T0, the dietary fiber content was increased by more than 25% (BRASIL, 2012).

The moisture contents are close to those described by Esteller *et al*. (2004), who found around 30% moisture in traditional breads. Alves (2012) found an increase in moisture content of breads produced with the addition of PSF. The increase in protein content is due to the high protein level in PSF. Lima and Lima (1987) added 15% PSF into manioc flour, and found an increase in protein content.

Bitencourt *et al.* (2014) found higher lipids, fiber, protein, and ash levels in cakes made with PSF. Silva (2012) produced cereal bars with partial and total replacement of oats by PSF, and observed an increase in protein, lipids, and fiber levels. Moura *et al*. (2010) studied the effect of addition of different pumpkin seed flour levels in cookies, and found that the cookies with PSF presented higher protein, lipids, and ash contents.

Gorgonio *et al.* (2011) evaluated cakes made with blends of PSF and starch, and found that the formulations with higher PSF showed higher ash, lipids, proteins, and insoluble dietary fiber Levels. Silva *et al.* (2010) produced biscuits with PSF, and found significant levels of fiber, lipids, and proteins in all formulations.

The formulation T0 presented higher carbohydrate content, thus the addition of PSF led to an increase in fiber levels and consequently a reduction in the available carbohydrates. In addition, this formulation had a higher caloric value, while T1 and T2 were not statistically different from each other. The main role of fiber incorporation in bread formulations is to increase the dietary fiber and decrease the caloric value of the final product (Stauffer, 1990). Silva (2012) produced cereal bars containing PSF and found a reduction in the caloric value. Alves *et al.* (2012) produced breads containing pumpkin pulp and PSF, and observed a reduction in caloric value and carbohydrate levels with increasing PSF levels. Gorgonio *et al.* (2011) evaluated cakes made with PSF and starch blends, and found a reduction in carbohydrates and calorie.

Conclusion

The formulation F2 exhibited more suitable characteristics for use as an ingredient in bread making. Despite the alveograph and farinograph analyses have demonstrated a decrease in baking potential, the addition of PSF led to an increase in protein, lipids, and dietary fiber levels, and a decrease in carbohydrate content and caloric values of the pan breads.

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