Effect of Soy Concentrate, Oat (*Avena sativa*) Flour and Chia Seeds (*Salvia hispanica*) as a Partial Substitute of Wheat Flour (*Triticum aestivum*) on Protein Content, Dietary Fiber Content, Textural Shelf Life and Organoleptic Properties of Breadsticks

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Abstract

Few studies have examined the effects on nutrient contents and organoleptic properties of substituting wheat flour by protein dense ingredients as are soy protein concentrate, oat and chia in bakery formulas. The objective of the study was to assess the effect of adding soy protein concentrate, oat and chia to two breadsticks formulas proposed to provide at least 10 % of the daily recommended value of protein for an adult. Thirty three percentage of wheat flour were substituted by 3:1 oat:chia (BO) and 1:1 oat:chia (BC) composite flours. The analyzed parameters were wheat-meal fermentation time, moisture, protein, insoluble and soluble dietary fiber, firmness, organoleptic acceptance, and preference. Results revealed that both formulas contributed the minimum wished protein content, had higher dietary fiber content than only wheat flour breadsticks and had an acceptable texture, flavor, and appearance. It is recommended to continue formula BO because it turned out to be the favorite in the preference test.

Keywords: soy protein concentrate, oat, chia, protein content, dietary fiber, breadstick

1. Introduction

In recent years obesity, diabetes, and other chronic diseases have been increasing among the poor population of Mexico, this is partially due to an important growth in sugar consumption, refined carbohydrates and fat, most likely found in higher concentrations in cheap convenience foods. On the other hand, protein intake has declined in all regions (Rivera et al., 2002), especially in those with less economic resources.

There are many definitions of functional foods, but this category encompasses potentially helpful products including any modified food or food ingredients that may provide a health benefit beyond that of the traditional nutrient it contains. In this respect, the addition of dietary fiber at a determined concentration in a food product could turn it into a functional food since there is a growing number of studies proving the beneficial effects of dietary fiber consumption. It has been found that this type of fiber may participate in the regulation of the gastrointestinal motility, influence glucose, and lipid metabolism, promote faecal output, stimulate bacterial metabolic activity and contribute towards maintaining the equilibrium of the colon ecosystem and integrity of intestinal mucosa (Gibson & Williams, 2001).

Oat (*Avena sativa*) differentiates itself from other cereals because it contains β -glucan, a type of dietary fiber, and it contains the best amino acid composition profile among all the cereal grains in addition to overall high protein content (Salehifar & Shahedi, 2007). In the same way, chia (*Salvia hispanica L*) is a seed that has been gaining attention for its high content of soluble and insoluble dietary fiber, and its high protein content which also has a complete amino acid profile (Inglett, Chen, Liu & Lee, 2014). Coorey, Tjoe and Jayasena (2014) studied the effect of chia in chips and observed that until 10% generated a good quality dough with important effects on protein content, dietary fiber and calcium content. Likewise, soy (*Glycine max*) protein is well known for supplying all nine essential amino acids, being easily digested by humans, and equaling the protein quality of

milk, meat, and eggs (Singh, Kumar, Sabapathy & Bawa, 2008).

Bearing all this in mind, it can be concluded that there is the opportunity to develop new affordable snacks that fight hunger deliciously and conveniently, but that act as functional foods by providing less refined carbohydrates, substituting them with dietary fiber, and provide more protein to the diet of the Mexican population.

Thus, the objective of the study was to evaluate the effect of incorporating soy concentrate, oat, and chia in breadsticks formulation that could serve as healthy snacks by providing 10% of the daily recommended protein consumption value for an adult with an average weight of 70 kg (5.6 g of proteins) per serving size (60 g) (Committee on Dietary Reference Intakes, 2005). The present study pretends to reach that protein while keeping texture, flavor, and appearance considered as acceptable by potential consumers.

2. Materials and Methods

2.1 Materials

Soy protein concentrate 65%, was given by Nutrigrains[®]. The other ingredients were obtained at the local market: Medium strength commercial soft and white wheat flour (Selecta[®], Guadalupe, N.L., Mexico), oat flakes (Granvita[®], Zapopan, Jal., Mexico), oat flour (Granvita[®], Zapopan, Jal., Mexico), chia (O3 Ch á Premium[®], Guadalajara, Jal., Mexico), jalape ño pepper (San Ambrosio[®], Ciudad Mante, Tamps., Mexico), sun dried tomatoes in olive oil (Bella Sun Luci[®], Chico, CA), salt (La Fina[®], Ciudad de Mexico, D.F., Mexico), honey (Vitareal[®], Azcapotzalco, D.F., Mexico), olive oil (Carbonell[®], Madrid, España), skimmed milk (Alpura Light[®], Cuautitl án Izcalli, Edo. Mex., Mexico), calcium propionate (Alkem, Monterrey, N.L., Mexico), instant yeast (Tradi-Pan[®], Toluca, Edo. Mex., Mexico), and dried basil (McCormick[®], Mexico City, D.F., Mexico).

2.2 Methods

2.2.1 Breadsticks Preparation

Breadsticks were prepared as recommended by Atkinson (2004). The formulations were three, control and two treatments, the first substituting white wheat flour in 35% by oat and chia in 3:1, respectively (BO), and the second treatment substituting white wheat flour in 35% by oat and chia in 1:1 (BC) (Table 1). For the treatments, jalape ño peppers and sun-dried tomatoes were previously chopped with a kitchen knife in tiny pieces. Likewise, oat was slightly minced in a food processor (Traditions Mod.72588R, China) for 40 s or until getting small pieces. All the ingredients for the control were mixed in a Hobart mixer (Hobart, Mod. 8-120T, Troy, OH) at low speed (60 rpm) for 3.5 min. A similar mixing procedure was used for both treatments, except that all the ingredients (excluding jalape ño pepper, sun-dried tomato and dried basil) were mixed at low speed in the same Hobart mixer for 2.5 min. Then, jalape ño pepper, sun-dried tomato and dried basil were added to the mixer and the dough was mixed at low speed for 1 min more.

Each dough was formed into a ball and placed in a lightly oiled bowl. It was covered with plastic wrap and left to rise for 15 min at room temperature and 85% relative humidity (RH) in a fermentation cabinet (National MFG. Co. Lincoln, NE). After that, the dough was stretched and thinned with a roller pin until reaching a thickness around 1.0 cm and cut with a kitchen knife in 1.3 cm x 28.0 cm pieces. Then, each piece was slightly twisted and placed on a pan covered with a wax baking sheet. Pans were introduced to the fermentation cabinet, and pieces were left to rise for another 10 min at room temperature and 85% relative humidity (RH). Immediately, the pans were taken to a convection oven (Electrolux, Stockholm, Sweden) and baked for 20 min and 150 °C. Finally, breadsticks were left to cool down for about an hour or until reaching room temperature and packaged in cellophane bags.

2.2.2 Flour Mixes Properties Measurement

Flour mixes (including white wheat flour, minced oat, oat flour and chia) were evaluated using Pelshenke assay as determined by American Association of Cereal Chemists (AACC, 2000, Method 56-50.01, 7).

2.2.3 Chemical Composition of Breadsticks

Moisture content (n = 3 per treatment) was analyzed as determined by AACC (2000, Method 44-15.02, 8). Crude protein (n = 3 per treatment) was analyzed by the Kjeldahl test as determined by AACC (1999, Method 46-12.01, 9). Total dietary insoluble and soluble fibers (n = 3 per treatment) were determined using the commercial kit Total Dietary Fiber provided by Megazyme®, following approved methods 32-45-01 (2000) and 32-50.01 (2010) of the AACC.

Table 1. F	ormulations	employed to	produce of	control	100%	white	wheat	flour (Control),	65%	white	wheat	flour
and 35% c	oat-chia subs	stitution (3:1)	(BO), and	d 65%	white	wheat	flour a	and 35%	oat-chia	subst	titution	(1:1)	(BC)
breadstick	S												

Ingredients	Control	BO	BC
Wheat flour	100	65.65	65.65
Oat Flake	-	10.10	4.04
Soy concentrate	-	10.10	10.10
Oat flour	-	8.08	8.08
Chia	-	6.06	12.12
Jalape ño pepper	6.94	6.94	6.94
Sun dried tomato	6.24	6.24	6.24
Salt	0.51	0.51	0.51
Honey	1.05	1.05	1.05
Olive oil	1.05	1.05	1.05
Fluid skim milk	-	1.00	1.00-
Calcium propionate	0.75	0.75	0.75
Instant yeast	0.51	0.51	0.51
Dried basil	0.21	0.21	0.21

2.2.4 Measurement of Firmness during Shelf Life

Firmness of breadsticks was measured after baking at days 0, 1 and 8 using TA.XT plus texture analyzer (Stable Micro Systems Ltd, Surry GU7 1JG, England) and a modified version of the American Institute of Baking (AIB) Standard Procedure for White Pan Bread Firmness Measurement (AIB, 2015). Firmness was measured on five breadsticks per treatment. The measures were taken on the widest parts of the breadsticks not containing blisters. A texture profile analysis (compression test) was executed with a flat-ended cylindrical probe, a 5 mm distance, and pre-test, test and post-test speeds of 10 mm/s, 1 mm/s, and 10 mm/s, respectively.

2.2.5 Organoleptic Evaluation

Two organoleptic tests were applied the same day that breadsticks were baked, the first test evaluated the acceptance of the breadsticks' attributes, while the second was a preference test. Both organoleptic tests were applied at room temperature to the same 30 bread consumers (18 women and 12 men) whose ages were between 18 and 35 years old. In the acceptance test, consumers assigned a value from 1 (dislike extremely) to 7 (like extremely) for each one of the following attributes: texture, crunchiness, flavor, and appearance. In the preference test, consumers chose their favorite formulation (Control, BO or BC).

2.2.6 Statistical Analysis

It was used to analyze the characteristics of the breadsticks with oat and chia concentration as the independent variables, and the protein content and firmness, after 0, 1 and 8 days of storage as the dependent variables. The JMP 5 software (SAS, SAS Campus Drive Building T Cary, NC) was used for the analysis of variance (ANOVA) at a constant significant effect (p < 0.05). Differences in the acceptability of the treatments' attributes caused by the different oat and chia concentrations were assessed by ANOVA using the same statistical program. Preference differences were evaluated by a binomial test carried out in Microsoft Excel 2013 software (Microsoft Corporation, Redmond, WA). Both organoleptic tests had as null hypothesis that there was no difference in the acceptance and preference of the treatments, while the alternative was that a difference does exist in their acceptance and preference.

3. Results and Discussion

3.1 Characterization of Flour Mixes

The Pelshenke values decreased as the wheat flour concentration was reduced and the content of chia was increased. The Pelshenke values for control, BO, and BC were 50 ± 6.6 min, 45.7 ± 4.5 min, and 23.7 ± 6.5 min, respectively. The Pelshenke test is a simple fermentation-based assay that measures gluten quality (Serna-Sald var, 2013) since part of the wheat flour was replaced for more water-binding substances in the treatments it could be expected that their Pelshenke values would be lower. Fibers and proteins are water-binding substances present in higher proportion in some ingredients such as soy concentrate, oat (flour and grain) and chia. Comparing the control against the treatments, control has a higher Pelshenke value than the treatments. This is due to the partial replacement of wheat flour with soy concentrate, oat, and chia. Soy protein concentrates

contain polysaccharides that absorb a significant amount of water. Adding soy proteins, either in the form of flour or concentrate, to wheat flour dilutes the gluten proteins and starch while exhibiting a strong water-binding power that provides some resistance to dough expansion (Jideani, 2018).

Likewise, the higher Pelshenke value for BC in comparison with BO is due to a higher content of chia instead of oat. De-husked oat meal contains a considerable amount of crude fiber (2.1% dry weight basis) in comparison with wheat flour (0.4% dry weight basis) (Chang & Sosulski, 1985), but chia has even a bigger content of crude fiber (25.55% dry weight basis) (Coorey et al., 2014) which translates into a higher water binding capacity, and thus a faster precipitation of the dough ball in the Pelshenke test. This have effect on water absorbin of mixes and control absorbed 22.66% (14% basis) until BO and BC absobed 29.30 and 29.01%, respectively.

3.2 Composition

3.2.1 Moisture

Moisture content is higher in treatments in comparison with control (Table 2) as it was expected since, as discussed above, soy concentrate, oat and chia act as water-binders that reduce the water released during baking. Numerous authors report an increase in moisture content in baked goods that include soy proteins (Mishra & Chandra, 2012; Zhang, Albrecht, Bomser, Schwartz & Vodovotz, 2003) oat products (Kurek, Wyrwisz, Piwińska & Wierzbicka, 2016; Lee & Inglett, 2006), and chia seeds (Rend ón-Villalobos, Ort ź-S ánchez, Solorza-Feria & Trujillo-Hern ández, 2012; Silveira Coelho & Salas-Mellado, 2015).

3.2.2 Protein

Protein content was also significantly higher (p<0.05) in treatments than in control due to the addition of soy concentrate, oat, chia and skim milk (Table 2). Protein content increased from 5.80% in control to 9.73% and 10.4%, in BO and BC treatments, respectively. According to Ndife, Abdulraheem and Zakari (2011) substituting 7% of the whole wheat flour with soy flour (39.4% protein) resulted in a bread with 9.44% protein. Addition of soybean protein to cereal products could not only be effective to increase their protein content but also their protein quality since it contains a considerable concentration of lysine, an essential amino acid missing in cereals (Singh et al., 2008). Oat differentiates itself from other cereals by its high relative content of lysine, according to Chang and Sosulski (1985) meal from domestic groats contains 15.3% protein and 4.1 g of lysine per 100 g of groats. Likewise, chia is a seed that characterizes for being a good source of protein, since its protein content is higher than that of other traditional crops such as wheat and corn, and its amino acid profile has no limiting factors in the adult diet (Ayerza & Coates, 2011).

Table 2. Moisture, protein and dietary fiber composition (%) of 100% white wheat flour (Control), 65% white wheat flour and 35% oat-chia substitution (3:1) (BO), (3) 65% white wheat flour and 35% oat-chia substitution (1:1) (BC), dry basis breadsticks

Treatment	Moisture ²	Protein ^{1,3}	Total Dietary Fiber ¹	Insoluble Dietary Fiber ¹	Soluble Dietary Fiber ¹
Control	$5.33 \pm 0.01^{\circ}$	$5.80 \pm 0.50^{ m b}$	3.99	$2.56 \pm 0.05^{\circ}$	$1.43 \pm 0.17^{\circ}$
BO	9.00 ± 0.01^{b}	9.73 ± 0.52^{a}	7.39	3.04 ± 0.04^{b}	4.35 ± 0.05^{b}
BC	9.70 ± 0.05^{a}	10.4 ± 0.51^{a}	12.50	5.92 ± 0.07^{a}	6.58 ± 0.07^{a}

Means with different letters in each column are statistically different (p < 0.05). Each value is the average and standard deviation of three observations.

¹ Values provided in dry weight basis.

² Moisture taken on storage day 0.

³ N*6.5.

3.2.3 Insoluble and Soluble Dietary Fiber

Incorporating soy concentrate, oat and chia in the treatments increased (p<0.05) their insoluble and soluble dietary fiber content (Table 2) in comparison with control as it was expected, especially for BC since it includes the highest chia concentration. According to Bednar, Patil, Murray, Grieshop, Merchen and Fahey (2001) soy flour contains 15.4 g/100 g of total dietary fiber (TDF), 14.7 g are insoluble dietary fiber (IDF), and 0.7 g are soluble dietary fiber (SDF), it could be expected that TDF for soy concentrates would be lower since in its production process carbohydrates, mainly sugars and soluble carbohydrate material, are extracted from defatted soy flour in order to increase its protein content (Jideani, 2018). In the same way, oat has a TDF content of 37.7

g/100 g, 33.9 g are IDF and 3.8 are SDF (Bednar et al., 2001). Likewise, V ázquez -Ovando, Rosado-Rubio, Chel-Guerrero and Betancur-Ancona (2009) reported that defatted chia flour obtained by dry processing contains 56.46 g/100 g of TDF, most of its content represented by 53.45 g of IDF and 3.01 g of SDF. Results presented in Table 2 show that SDF content was higher than IDF in both treatments, obtaining 4.35 g and 6.58 g of SDF for BO and BC, respectively. Several authors have reported an increase in the TDF content of baked goods by incorporating oat products (Kurek et al., 2016; Gularte, de la Hera, Gómez & Rosell, 2012) and chia seeds (Rendón-Villalobos et al., 2012; Silveira Coelho & Salas-Mellado, 2015).

3.3 Firmness

Table 3 shows the values for breadstick firmness on storage days 0, 1 and 8. On day 0 there were not a significant difference in firmness among control, BO, and BC (p>0.05). On day 1 a significant difference in firmness (p<0.05) is detected among control, BO and BC, being this last the one with the highest firmness and BO the treatment with less firmness. This trend continued on day 8, in which the difference (p<0.05) among treatments increases especially for BC.

Table 3. Firmness (N) of breadsticks 100% white wheat flour (Control), (2) 65% white wheat flour and 35% oat-chia substitution (3:1) (BO), (3) 65% white wheat flour and 35% oat-chia substitution (1:1) (BC) stored at room temperature for 8 days. Values correspond to mean \pm standard deviation

	Day 0	Day 1	Day 8
Control	5.44 ± 0.68^{a}	$5.50 \pm 0.58^{a,b}$	5.44 ± 0.68^{b}
BO	2.16 ± 0.24^{b}	4.22 ± 0.79^{b}	4.24 ± 1.11^{b}
BC	2.16 ± 0.40^{b}	6.77 ± 0.88^{a}	10.35 ± 0.82^{a}

Means with different letters in each column are statistically different (p < 0.05). Each value is the average and standard deviation of three observations.

Less firmness or greater softness of treatments in comparison with control on day 0 is due to the addition of soy concentrate, oat, and chia. As previously discussed, the higher protein and dietary fiber content of BO and BC contributed to a stronger water binding capacity that trapped more moisture inside the product. BO kept a relative low firmness through time due to its higher content of oat; it has been reported that oat starch has a higher water absorption than other cereals, thus keeping bread fresher for longer periods of time (Salehifar & Shahedi, 2007). Also, oat has a high content of β -glucan, a hydrocolloid that at certain concentrations (around 1% flour basis) prevents changes in the water activity (aw) values of the crumb, a reduction in aw values is associated with an increase in firmness of crumb (Lazaridou, Duta, Papageorgiou, Belc & Biliaderis, 2007).

On the other hand, surprisingly BC firmness increased through time, even though in the formulation chia seeds replaced starch, main component that causes staling of bakery products through retrogradation, probably this ingredient interfered with the size and distribution of the air cells within the breadsticks structure, a similar result was reported by Luna Pizarro, Lopes Almeida, Samm án and Chang (2013).

3.4 Organoleptic Evaluation

3.4.1 Acceptance

In this study, attributes acceptance of products after tasting them was evaluated using a seven-point hedonic scale. Results of organoleptic evaluation (Figure 1) for overall texture acceptance show that control, BO, and BC were accepted since all of them were given a score above 4.0. Also, they reveal that there is a significant difference (p<0.05) in this attribute among treatments being control (6.27) the most accepted, followed by BC (5.83) and BO (5.50). A decrease in texture acceptance because of increasing the substitution levels of wheat flour with buckwheat-chia flours has been reported by Divyashree, Ashwath Kumar, Sharma, Semwal and Umesha (2016).



Figure 1. Comparison of organoleptic acceptance of breaksticks made with 100% white wheat flour (Control), (2) 65% white wheat flour and 35% oat-chia substitution (3:1) (BO), (3) 65% white wheat flour and 35% oat-chia substitution (1:1) (BC)

Results for crunchiness (Figure 1) indicate that all the treatments had an acceptable crunchiness, but that there is a significant difference (p<0.05) among them. BO got the highest score (6.5) for crunchiness acceptance, control obtained the second-best score (6.3), and finally, BC was evaluated with the lowest score (5.5). Comparing these results with overall texture acceptance, it's concluded that crunchiness didn't play an important role in texture acceptance. Probably other texture attributes such as firmness, cohesiveness or grainy are more decisive on consumers texture acceptance. Flavor acceptance values (Figure 1) reveal that consumers accepted control, BO, and BC and that there is a significant difference for this attribute (p<0.05). According to consumers, BO had a slightly most acceptable flavor (6.83), followed by control (6.5), and lastly by BC (5.80). No adverse effect on flavor by incorporating oat flour at levels of 10% and 20% in bread has been reported by Salehifar and Shahedi (2007). Likewise, lower scores in flavor acceptance of bread incorporating whole chia flour in comparison with only wheat flour bread have been reported by Luna Pizarro et al. (2013). Scores for appearance acceptance (Figure 1) indicate that the consumers accepted all the breadsticks, and that there is not a noticeable difference (p>0.05) in their appearance. Results obtained for this attribute were 6.70, 6.30 and 6.27 for control, BC, and BO, respectively.

3.4.2 Preference

Organoleptic evaluation revealed a statistical preference of breadsticks prepared with oat-chia substitution 3:1 (70%), over control (17%) and breadsticks including oat-chia substitution 1:1 (13%) (Figure 2).



Figure 2. Preference results (%) for 100% white wheat flour (Control), 65% white wheat flour and 35% oat-chia substitution (3:1) (BO) and 65% white wheat flour and 35% oat-chia substitution (1:1) (BC) breadsticks

4. Conclusion

Results of this study indicate that substitution of white wheat flour with oat-chia blend and soy protein

concentrated improve protein content by 67.75%. Breadstick with 10% soy concentrate, 6% chia seed, and 18% oat improve total dietary fiber content by 85.21%, interestingly soluble dietary fiber was improved twice. The breadstick produced with this grains blend has the highest preference, even higher than 100% white wheat flour breadstick. According to the results obtained, it is recommended to follow up with BO since it remained softer longer, it received the best score in flavor and crunchiness acceptance, and was preferred over BC. Further studies are needed to determine fat content. The evaluated breadsticks could serve as the base for the development of functional breadsticks, or other baked goods, that could help to diminish the increasing incidence of chronic diseases in Mexico.

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Disclosure statement

The authors declare no conflict of interest.

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