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Perilla frutescens: a potential ingredient for the enhancement of white bread as a source of Omega-3

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ABSTRACT. *Perilla frutescens* seeds are rich in Omega-3 fatty acids which are important for human health. Intake of fatty acids depends on their presence in popular foods such as white bread. Current study evaluates the replacement of wheat flour by whole perilla at 1, 3 and 5% in white bread processing and its impacts on chemical and sensorial attributes, underscoring Omega-3 amounts. The use of whole perilla increases the Omega-3 content in white bread, balances the ratio n-6/n-3, decreases the specific volume, and maintains the concentration of phenolic compounds and antioxidant activity. The formulation with 1% whole perilla has a better acceptability and supplies 5.63 and 8.19% of the American recommended daily intake of alpha-linolenic acid for adult males and females, respectively.

Keywords: *Perilla frutescens*, white bread, Omega-3 fatty acids.

Perilla frutescens: ingrediente potencial para enriquecimento de pão de forma como fonte de ômega-3

RESUMO. As sementes de *Perilla frutescens* são ricas em ácidos graxos ômega-3, importantes para a saúde humana por serem considerados essenciais. A ingestão potencial desses ácidos graxos depende da sua presença em alimentos populares, como o pão de forma. O objetivo deste estudo foi avaliar a substituição da farinha de trigo por farinha integral de perilla a 1, 3 e 5%, no processamento de pães de forma, e o seu impacto nos atributos químicos e sensoriais, com ênfase na quantidade de ômega-3. O uso da farinha integral de perilla promoveu o aumento de ômega-3 nos pães de forma, balanceou a razão n-6/n-3, diminuiu o volume específico deles, e não alterou a concentração de compostos fenólicos e a atividade antioxidante. A formulação com 1% de farinha integral de perilla apresentou melhor aceitação, sendo capaz de suprir 5,63 e 8,19% da ingestão diária americana de ácido alfa-linolênico, para um homem e uma mulher adultos, respectivamente.

Palavras-chave: *Perilla frutescens*, pão de forma, ácidos graxos ômega-3.

Introduction

Perilla frutescens (perilla) is an aromatic perennial plant cultivated and consumed in northern India, China, Japan, Nepal, Burma, Bangladesh and Korea. Perilla seeds are small, but rich in oil (30-40%) and protein (16-24%). Perilla oil contains 63-70% of alpha-linolenic acid (LNA, 18:3 n-3), 14-23% of oleic acid (OA, 18:1 n-9), 16% of linoleic acid (LA, 18:2 n-6) and 12.6% of saturated fatty acids. Whilst perilla oil is locally used for edible purposes, it is a substitute for linseed oil in the United States of America (Ang, Liu, & Huang, 1999).

The fatty acid composition of perilla seeds stands out among other edible seeds due to their unsaturated fatty acids. In fact, it is mainly composed of LNA, an n-3 fatty acid family. The beneficial effects of perilla´s n-3 fatty acids family shown by Kinsella (1991) and Yu, Kosuna, and Haga (2004) comprise the evolution of brain activity and nervous system, suppression of carcinogenesis, metastasis, thrombosis and allergic reactions.

The LNA is produced by *de novo* synthesis through the activity of Δ15 and Δ12 desaturase on the OA in plants. It is precursor of the production of n-3 fatty acid family in animals. Advances in the cultivation of oilseeds have caused considerable disproportion in the natural balance of the contents of LA and LNA and the average consumption of LNA has fallen considerably during the last hundred years (Akoh & Min, 2008).

The optimal dose or ratio of fatty acids omega-6/omega-3 should range between 1:1 and 4:1,

depending on the health state. Since the intake of omega-6 should be reduced to prevent and monitor chronic diseases, the equilibrium between omega-6 and omega-3 is very important for homeostasis and normal human development (Simopoulos & Cleland, 2003).

Longvah, Deosthale, and Kumar (2000) concluded that histopathology tests in rats fed for 18 weeks with perilla oil did not show any toxic effects on the heart, lung, liver, spleen, kidneys, pancreas and gastrointestinal tract of the animals. The study confirmed that perilla oil may be safely consumed by humans. It is actually already part of the diet by the people of northeastern India. The authors suggest that perilla oil should be exploited for nutritional purposes in mixtures with other vegetable oils since the same study demonstrated decrease of cholesterol and triglycerides in the serum due to its high LNA contents.

Lin, Chou, Kuo, and Huang (2010) reported that the use of perilla seeds, leaves or stalks in the human diet may be a protection against oxidative damage due to phenolic compounds. According to Cuvelier, Richard, and Berset (1992), phenolic compounds act as antioxidants because of their ability to donate electrons or hydrogen and their stable radical intermediates which prevent the oxidation of various food ingredients, particularly fatty acids.

Since white bread is a highly popular food and perilla a rich source of omega-3 fatty acids, current assay focuses on the physical-chemical and sensory characterization of this type of bread formulated with different proportions of flour from perilla seeds, with special emphasis on omega-3 fatty acids.

Material and methods

Preparation of pan breads with different proportions of whole Perilla

The preparation of pan breads followed standard formulation and methodology proposed by El-Dash (1978). The standard formulation was modified by adding whole perilla, in different proportions (Table 1).

Table 1. Standard white bread formulation (F0) and with different proportions of whole perilla.

Ingredients	Bread formulations (% m m^{-1})			
	F0	F1	F3	F5
Refined wheat flour (WF)	100.00	99.00	97.00	95.00
Whole perilla (WP)	0.00	1.00	3.00	5.00
Commercial sodium chloride*	1.75	1.75	1.75	1.75
Commercial refined sucrose*	5.00	5.00	5.00	5.00
Fresh yeast*	3.00	3.00	3.00	3.00
Interesterified soybean oil (IS)*	3.00	3.00	3.00	3.00
Ascorbic acid*	0.10	0.10	0.10	0.10
Water*	53-57	53-57	53-57	53-57

*Percentages were based on total flour weight (WF plus WP).

The ingredients used to prepare the different formulations were purchased on the local market. Whole perilla was obtained after processing the seeds in a knife mill (Marconi, model MA630, Piracicaba, São Paulo State, Brazil).

A vertical mixer (G. Paniz, model AE25, Caxias do Sul, Rio Grande do Sul State, Brazil) was used to mix the ingredients, which were added in the proportions indicated in the test formulations and in the follow order: flour, whole perilla, fresh yeast, sugar, interesterified soybean oil, ascorbic acid, water and salt. Mixing time was five min and when the network gluten became homogenized, the dough was removed from the mixer, divided into three parts, each weighing 500 g, processed in a cylinder (G. Paniz, model CL390, Caxias do Sul, Rio Grande do Sul State, Brazil), modeled by a dough-shaping machine (Braesi, model MB350, Caxias do Sul, Rio Grande do Sul State, Brazil) and placed in standard-sized pans for white bread, weighing between 400 and 500 g.

The dough fermentation was conducted at a controlled temperature of 30° C, in an 80% wet environment for 90 min in a chamber fermentation (Venâncio Metalúrgica, model AC20T, Venancio Aires, Rio Grande do Sul State, Brazil). At the end of the fermentation, the dough was baked in a professional electric oven (Tedesco, FTT model 240E, Caxias do Sul, Rio Grande do Sul State, Brazil) at 165°C, for 20 min.

After natural cooling for 4 hours, the products were sliced by a professional slicing machine (G. Paniz, model FP12, Caxias do Sul RS Brazil) and packed in 440 x 110 mm plastic bags for white bread.

Proximate composition and fatty acids

Moisture, ash and raw protein were determined according to Cunnif (1998). The total lipid content and fatty acid composition were determined for WP, WF, IS and white bread. Total lipids were extracted following Bligh and Dyer (1959) and carbohydrates were estimated by the difference. Energy rate was calculated by the energy conversion factors reported by Brasil (2003a).

The fatty acids methyl esters were prepared according to method by Hartman and Lago (1973), modified by Maia and Rodriguez-Amaya (1993). The separation of fatty acids methyl esters was performed on a gas chromatograph (Thermo Fisher Scientific, model 3300 Ultra Trace, USA) with a flame ionization detector and fused silica capillary column CP – 7420 (Select FAME, 100 m long, 0.25 mm internal diameter and 0.25 mm in cyanopropyl). The flow of H_2 (carrier gas) was 1.2 and 30 mL min^{-1} of N_2 (make up), 35 and

300 mL min^{-1} for H_2 and synthetic air, for flame detector. The injected volume was approximately 2.0 μL using sample splitting of 1:80; the injector and detector temperatures were 220 and 240°C respectively. The column temperature at 185°C for 12.5 min was raised to 235°C at the rate of 4°C min^{-1} and maintained for 4.5 min. The peak areas were obtained by integration with Chromquest Software (Thermo Fisher Scientific, version 5, USA).

Fatty acids were quantified in mg g^{-1} of total lipids by internal standardization, using the tricosanoic acid methyl ester 1 mg g^{-1} (Sigma, USA). The fatty acids were identified by comparing retention times with those of standard methyl esters, and calculated following method by Joseph and Ackman (1992) and Visentainer (2012).

Characterization of antioxidant activity

Approximately 5.0 g of the sample were used for the solvent methanol extraction at a ratio 1:10 (m v^{-1}). The sample and solvent mixtures were stirred constantly for 4 hours, then filtered under vacuum and evaporated in a rotary evaporator at 40°C. The dried extracts were used to analyze total phenolic contents and antioxidant activity.

Total phenolic contents were determined by Folin-Ciocalteau method, described by Naczk and Shahidi (2004). Method by Buriol et al. (2009) was employed to analyze the content of flavonoids. The antioxidant activity was determined by the free radical DPPH, as described by Brand-Williams, Cuvelier, and Berset (1995), modified by Miliauskas, Venskutonis, and Van Beek (2004).

Instrumental color analysis and specific volume

The instrumental color evaluation was performed according to CIELab scale by colorimeter MiniScan EZ (HunterLab, model MSEZ-4000S, USA). L, a★ and b★ rates were measured, respectively corresponding to lightness (0 – white; 100 - black), green (a-), red (+), blue (b-) and yellow (b +). The experiment involved five repetitions for the analysis of crust and crumb of white bread with different formulations. The specific volume was determined by the displacement of millet seed, according to El-Dash, Camargo, and Diaz (1982).

Sensory evaluation of products

The sensorial analysis was approved by the Standing Committee on Ethics in Human Research of the State University of Maringá (CAAE - 0387.0.093.000-10).

The acceptance test of samples of sliced white bread followed Monteiro (2005) and the nine-point hedonic scale was applied. The sensory panel was composed of 40 untrained tasters, while tests were conducted in individual cabins.

Samples of bread, encoded by four random numbers, were distributed in a monadic way to tasters in white plastic dishes. The answer sheet attached to each sample comprised the scale of sensory evaluation and a scale on the purchase intention of the tasters.

Statistical analysis

The tests were conducted in triplicate, with the exception of the instrumental analysis of color, performed in five replications. Results of the different formulations, expressed as mean and standard deviation, were compared by analysis of variance (ANOVA) at 5% significance level. Mean rates were compared by Tukey's test by Statistica 5.1 (Statsoft Inc. Tulsa, OK USA, 1996).

Results and discussion

Proximate composition and fatty acids

The chemical composition of standard white bread with different proportions of WP is shown by Table 2.

Crude protein was 24.18% of WP, which is close to levels reported by Sharma, Hore, and Mondal (1989), which ranged between 15.7 and 23.7%. The total lipid content amounted to 40.12%, within the 35-50% range reported by Przybylski (2005).

Since the levels of ash, crude protein and carbohydrates were different ($p < 0.05$) between F0 and F5, the substitution of WF by WP at 5% decreased the similarity of the resulting product with the standard when the chemical composition was analyzed.

Table 2. Proximate composition (g 100 g^{-1}) and energetic rate (kJ 100 g^{-1}) of WP and different formulations of white bread types.

Proximate composition	WP	F0	F1	F3	F5
Moisture	6.35 ± 0.10	35.66^{ab} ± 0.52	36.20^{a} ± 0.37	36.28^{a} ± 0.02	35.05^{b} ± 0.07
Ash	3.34 ± 0.03	1.47^{b} ± 0.08	1.58^{ab} ± 0.06	1.60^{ab} ± 0.05	1.69^{a} ± 0.03
Crude protein	24.18 ± 0.08	10.20^{b} ± 0.08	10.32^{ab} ± 0.02	10.50^{ab} ± 0.52	11.02^{a} ± 0.16
Total lipid	40.12 ± 1.75	1.89^{d} ± 0.06	2.25^{c} ± 0.05	2.84^{b} ± 0.07	3.27^{a} ± 0.09
Carbohydrates	26.01 ± 0.49	50.78^{a} ± 0.45	49.65^{b} ± 0.36	48.7^{8b} ± 0.52	48.97^{b} ± 0.29
Energetic value	2265.13 ± 0.52	1104.27^{b} ± 0.53	1112.84^{b} ± 0.66	1112.78^{b} ± 0.17	1140.72^{a} ± 0.14

Means of three analytical replications ± standard deviation. Different letters in the line indicate statistically significant differences between samples ($p < 0.05$). WP: whole perilla; F0: white bread without WP; F1: flour with 1 WP and 99% refined wheat flour; F3: flour with 3 WP and 97% refined wheat flour; F5: flour with 5 WP and 95% refined wheat flour.

There were significant differences among the products, mainly with regard to total lipid rates. Since the lipid content of whole perilla is high (Table 2), the higher the replacement of wheat flour by the seed flour, the greater the value of total lipids of the resulting product of mixed flour. Although F1 and F3 had a greater percentage of total lipids, there were no significant differences ($p < 0.05$) in the energetic rates of these types of bread when compared to standard bread.

Each slice of bread obtained for the different formulations averaged 25 g. According to Brazilian legislation (Brasil, 2003a), a portion of packed bread, sliced or not, corresponds to 50 g, therefore, two slices of bread make up the portion for nutrition labeling. The energetic values of the portions of F0, F1, F3 and F5 corresponded, respectively, to 6.51, 6.56, 6.57 and 6.73% of the Recommended Daily Intake (RDI) or 2000 kcal by label standards (Brasil, 2003b).

Table 3 shows that there was a predominance of Pufa (78.00%) in WP lipids. Further, 62.25 and 16.01% of total Pufa corresponded respectively to LNA and LA. The n-6/n-3 ratio found in WP was 0.26. These rates are very similar to those reported by Przybylsk (2005) and by Dubois, Breton, Linder, Fanni, and Parmentier (2007).

Table 3. Fatty acid composition, in mg g^{-1} of total lipid, of the ingredients used for bread making.

Fatty acid	IS	WP	WF
14:0	3.85 ± 0.06	0.34 ± 0.04	1.17 ± 0.05
16:0	110.61 ± 2.66	67.44 ± 2.54	156.82 ± 0.23
16:1 n-7	0.51 ± 0.04	0.63 ± 0.05	0.68 ± 0.03
17:0	1.20 ± 0.05	0.20 ± 0.02	1.19 ± 0.01
17:1 n-9	0.23 ± 0.03	0.09 ± 0.01	0.41 ± 0.01
18:0	254.08 ± 5.71	15.98 ± 0.53	11.71 ± 0.17
18:1 n-9	197.49 ± 5.07	119.99 ± 8.10	80.29 ± 1.47
18:1 n-7	10.93 ± 0.22	9.71 ± 0.20	5.72 ± 0.05
18:2 t*	5.45 ± 0.17	nd	nd
18:2 n-6	345.67 ± 3.33	157.84 ± 3.31	479.46 ± 4.37
18:3 n-6	4.08 ± 0.15	nd	nd
20:0	3.81 ± 0,45	nd	nd
18:3 n-3	36.47 ± 2.12	613.81 ± 5.40	29.85 ± 0.03
20:1 n-9	3.40 ± 0.50	nd	3.64 ± 0.13
20:2 n-6	1.24 ± 0.07	nd	0.86 ± 0.03
22:0	3.45 ± 0.05	nd	1.89 ± 0.02
24:0	0.41 ± 0.71	nd	nd
SFA	377.41 ± 0.74	83.96 ± 3.17	172.78 ± 0.15
MUFA	212.56 ± 4.24	130.42 ± 8.47	90.74 ± 1.41
PUFA	387.46 ± 4.53	771.65 ± 8.65	510.17 ± 4.34
n-6	350.99 ± 3.01	157.84 ± 3.31	480.32 ± 4.39
n-3	36.47 ± 2.12	613.81 ± 5.40	29.85 ± 0.03
n-6/n-3	9.62 ± 0.22	0.26 ± 0.01	16.09 ± 0.16
TFA	5.45 ± 0.17	nd	nd

Means of three analytical replications ± standard deviation; IS: Interesterified soybean oil; WP: Whole perilla; WF: Wheat flour; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; TFA: *Trans* fatty acids; nd: Not detected; *Sums of *trans* isomers of 18:2 (9t, 12t; 9c,12t; 9t,12c).

The n-6/n-3 ratio for WF was 16.09. According to Simopoulos (2004), a high n-6/n-3 ratio triggers cancer, cardiovascular, inflammatory and immune system disease. When the ratios obtained for the two flours are taken into account, it becomes even more evident that the replacement of WF by WP is advantageous from a nutritional point of view. However, the technological and sensory aspects should not be discarded.

Brazilian legislation (Brasil, 2003b) determines a maximum level of 0.2 g of *trans* fatty acids in foods. Since fats may amount to 10 g (Brasil, 2003a), the maximum levels of TFA in the product may reach 2%. The interesterified soybean oil used in preparation of white breads had 0.5% of these fatty acids and thus within the legal standards.

Table 4 shows fatty acid composition of white bread with different formulations. Simopoulos and Cleland (2003) report that the best ratio of fatty acids omega-6/omega-3 should range between 1 and 4, depending on health status. The addition of WP in bread formulations positively affected the ratio n-6/n-3. The greater the mass of WF replaced by WP, the greater the content, in mg g^{-1} of LNA, that made the n-6/n-3 ratio change from 11.27 in F0 to 1.60 in F5.

Table 4. Fatty acid composition, in mg g^{-1} of total lipid, of white bread with different formulations.

Fatty acid	F0	F1	F3	F5
14:0	1.04^{a} ± 0.07	1.06^{a} ± 0.08	0.90^{a} ± 0.08	0.87^{a} ± 0.01
16:0	$128,41^{a}$ ± 5.90	122.74^{a} ± 3.69	112.35^{b} ± 2.92	108.85^{b} ± 0.99
16:1 n-7	1.07^{a} ± 0.07	1.00^{ab} ± 0.05	0.95^{ab} ± 0.04	0.93^{b} ± 0.03
17:0	1.21^{a} ± 0.02	$1,12^{a}$ ± 0,03	0.98^{a} ± 0.05	0.98^{a} ± 0.05
17:1 n-9	0.13 ± 0.02	nd	nd	nd
18:0	201.56^{a} ± 2.38	179.83^{b} ± 1.61	157.49^{c} ± 1.08	141.17^{d} ± 5.48
18:1 n-9	179.72^{a} ± 1.60	172.64^{a} ± 3.61	163.74^{b} ± 2.43	160.94^{b} ± 2.97
18:1 n-7	10.31^{a} ± 0.22	9.79^{b} ± 0.18	9.63^{b} ± 0.12	9.80^{b} ± 0.13
18:2 t*	4.32^{a} ± 0.07	4.10^{a} ± 0.06	3.51^{b} ± 0.05	3.25^{b} ± 0.04
18:2 n-6	395.92^{a} ± 9.94	374.69^{a} ± 11.67	337.59^{b} ± 5.14	317.45^{b} ± 3.33
18:3 n-6	2.98^{a} ± 0.03	2.97^{a} ± 0.03	2.97^{a} ± 0.04	3.02^{a} ± 0.03
20:0	2.78^{a} ± 0.45	1.94^{ab} ± 0.07	2.05^{ab} ± 0.54	1.53^{b} ± 0.08
18~~3~~n-3	35.63^{d} ± 0.33	81.74^{c} ± 3.22	144.51^{b} ± 1.51	201.59^{a} ± 6.15
20:1 n-9	3.27^{a} ± 0.03	2.92^{b} ± 0.08	2.67^{c} ± 0.01	2.43^{d} ± 0.13
20:2 n-6	2.83^{a} ± 0.12	2.44^{ab} ± 0.29	2.42^{ab} ± 0.03	2.11^{b} ± 0.21
22:0	3.60^{a} ± 0.05	3.11^{b} ± 0.03	2.69^{c} ± 0.05	2.37^{d} ± 0.20
24:0	1.38^{a} ± 0.04	1.21^{ab} ± 0.06	0.98^{ab} ± 0.04	0.60^{b} ± 0.03
SFA	339.98^{a} ± 4.29	311.01^{b} ± 5.46	277.44^{c} ± 3.16	256.37^{d} ± 7.20
MUFA	194.50^{a} ± 1.28	186.35^{b} ± 3.87	176.99^{c} ± 2.60	174.10^{c} ± 3.21
PUFA	437.36^{d} ±1 0.22	461.84^{c} ±1 4.90	487.49^{b} ± 6.77	$524,17^{a}$ ± 8.16
n-6	401.73^{a} ± 10.15	380.10^{b} ± 11.72	342.98^{c} ± 5.28	322.58^{d} ± 3.16
n-3	35.63^{d} ± 0.33	81.74^{c} ± 3.22	144.51^{b} ± 1.51	201.59^{a} ± 6.13
n-6/n-3	11.27^{a} ± 0.28	4.65^{b} ± 0.05	2.37^{c} ± 0.01	1.60^{d} ± 0.05
TFA	4.32^{a} ± 0.07	4.10^{a} ± 0.06	3.51^{b} ± 0.05	3.25^{b} ± 0.04

Means of three analytical replications ± standard deviation. Different letters in the line indicate statistically significant differences between samples ($p < 0.05$); IS: Interesterified soybean oil; WP: Whole perilla; WF: Wheat flour; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; TFA: *Trans* fatty acids; nd: Not detected; *Sums of *trans* isomers of 18:2 (9t, 12t; 9c,12t; 9t,12c); F0: white bread without WP; F1: flour with 1 WP and 99% WF; F3: flour with 3 WP and 97% WF; F5: flour with 5 WP and 95% WF.

According to Simopoulos (1999), it is highly relevant that the diet enhances low intake of saturated fats and amounts less than or equal to 2% of *trans* fatty acids so that cardiovascular risk factors and diabetes could be reduced. There was a

significant reduction ($p < 0.05$) in the contents of SFA among the four formulations of bread, due to the replacement of WF by WP, since WF has 2.6-fold higher content of palmitic acid (16:0) than WP. There was a slight TFA decrease, in mg g^{-1}, which may be due to the fact that the same amount of interesterified soybean oil was used in all formulations. However, levels of Pufa, due to WP, increased.

Rules for nutrition facts in Brazil (Brasil, 2003b) indicate that rates less than or equal to 0.2 g for SFA and TFA are tagged 'zero' or 'insignificant content'. SFA rates for each type of bread produced correspond to 0.32, 0.35, 0.39 and 0.42 g respectively for F0, F1, F3 and F5, which would make mandatory a declaration of the type of fat on the label of products. Since TFA contents were lower than 0.2 g, according to Brazilian legislation, the different types of bread produced did not contain significant amounts of TFA, and thus were 'zero *trans*'.

The sum of Pufa was statistically different ($p < 0.05$) among the formulations. The increase in PUFA content of the breads with WP may be due to high LNA contents, which is the main fatty acid in WP lipids.

According to the US Institute of Medicine, the RDI of LNA for adult males and females are respectively 1.6 and 1.1 g (Trumbo, Schlicker, Yates, & Poos, 2004). When serving 50 g, the levels obtained for LNA in F0, F1, F3 and F5 were 0.03, 0.09, 0.21 and 0.33 g, which corresponded, respectively, to 1.88, 5.63, 13.13 and 20.63% of the American recommended daily intake for male adults. In the case of female adults, the contents were 2.73, 8.19, 19.09 and 30.00%, respectively, for F0, F1, F3 and F5.

Color parameters and specific volume

The means obtained for instrumental color of whole perilla, using CIELab scale, L★, a★ and b★ were respectively 50.84 ± 0.52; 7.58 ± 0.08 e 22.66 ± 0.48. Table 5 shows rates for the different formulations of bread and their specific volume. The replacement of WF by WP gave bread loaves a darker crust, but no significant difference ($p < 0.05$) between F1, F3 and F5 was reported. The color of the crumb was similar between F0 and F1 and between F3 and F5.

The specific volume of bread types differed significantly and indicated that the higher the percentage of replacement of WF by WP, the lower the specific volume of the final products. The presence of perilla particles enhanced less resistance to the gluten formed and retained a smaller volume of gas, resulting in products with less volume. The substitution of WF by WP up to 3% level, albeit with a decrease in the products´ volume, provided a specific volume which was greater than that found by Esteller and Lannes (2005) in their study to fix the identity and quality of baked products, or rather, 4.10 cm^3 g^{-1} for white bread. The specific volume, corresponding to formulation F5, indicated low aeration and resulted in lower rate of acceptance to sensory attributes, such as appearance, texture and flavor.

Table 5. Means of instrumental color of the white breads crust and crumb and their specific volume.

Parameter		F0	F1	F3	F5
Crust[1]	L★	52.11^{a}±1.13	49.10^{b}±0.50	49.70^{b}±0.31	48.97^{b}±0.48
	a★	16.93^{c}±0.08	18.35^{a}±0.24	16.76^{c}±0.13	17.49^{b}±0.17
	b★	31.39^{a}±0.36	33.27^{b}±0.23	32.81^{b}±0.30	31.36^{a}±0.18
Crumb[1]	L★	77.66^{a}±0.50	77.67^{a}±0.84	75.63^{b}±0.43	74.48^{b}±0.33
	a★	0.21^{d}±0.02	0.61^{c}±0.05	1.17^{b}±0.13	1.39^{a}±0.09
	b★	16.52^{b}±0.44	18.58^{a}±0.36	19.49^{a}±0.33	19.35^{a}±0.36
Specific v.[2] (cm^3 g^{-1})		5.13^{a}±0.02	5.13^{a}±0.02	4.63^{b}±0.03	3.86^{d}±0.09

[1]Means of five analytical replications ± standard deviation; [2] Means of three analytical replications ± standard deviation. Different letters in the line indicate statistically significant differences between samples ($p < 0.05$); L★ = Lightness (0 = black; 100 = white); a★ = red-green component; b★ = yellow-blue component; WP: whole perilla; F0: white bread without WP; F1: flour with 1 WP and 99% WF; F3: flour with 3 WP and 97% WF; F5: flour with 5 WP and 95% WF.

Sensory evaluation

Appearance, color, texture, flavor, aroma and overall acceptance averages for the different formulations of white bread with perilla are shown in Table 6.

Table 6. Sensory evaluation of white bread with different formulations.

Attributes	F0	F1	F3	F5
Appearance	8.10^{a}±0.78	8.12^{a}±0.79	7.73^{ab}±1.18	7.28^{b}±1.45
Crumb color	8.20^{a}±0.72	8.10^{a}±0.71	7.40^{b}±1.38	7.30^{b}±1.44
Texture	7.95^{a}±1.22	8.00^{a}±0.96	7.18^{b}±1.55	7.18^{b}±1.36
Flavor	7.75^{a}±1.21	7.82^{a}±1.11	6.85^{b}±1.58	6.53^{b}±1.65
Aroma	8.05^{a}±0.96	8.03^{a}±0.89	7.08^{b}±1.95	7.00^{b}±1.96
Overall acceptance	7.80^{ab}±1.14	8.00^{a}±0.88	7.08^{bc}±1.59	6.73^{c}±1.75

Different letters in the line indicate statistically significant differences between samples ($p < 0.05$); WP: whole perilla; F0: white bread without WP; F1: flour with 1 WP and 99% WF; F3: flour with 3 WP and 97% WF; F5: flour with 5 WP and 95% WF.

Formulations F0 and F1 are similar ($p < 0.05$) in all attributes, as are F3 and F5, which reveal the correlation between sensory evaluation and instrumental color evaluation.

According to the tasters, formulations F3 and F5 showed perilla flavor remarkable, which decrease the acceptance of the products.

Lawless and Heymann (2004) report that an acceptability index is satisfactory when a minimum of 70% is achieved. The acceptability indexes were significantly different ($p < 0.05$) for all formulations. With 88.89%, formulation F1 had the highest acceptability index. The other formulations registered 86.67, 78.67 and 74.78% for F0, F3 and

F5, respectively. All formulations reached satisfactory levels. The frequency of favorable responses for purchase intent was equal to 88.00 for F1; 85.60 for F0; 71.00 for F3; and 69.00% for F5.

Sensory evaluation indicated that the replacement of 1% of WF by WP did not affect the sensory characteristics of bread when they are analyzed separately. The overall evaluation of the product showed that this substitution enhanced a better acceptance and therefore increased its purchase intent.

Total phenolic compounds and antioxidant activity

The content of total phenolic compounds in mg GAE^{-1} 100 g^{-1} of sample for whole perilla was 59.47 ± 1.50. Formulations F0, F1, F3 and F5 presented 8.24 ± 1.48; 9.94 ± 1.03; 9.99 ± 1.09; 10.78 ± 1.73; respectively in mg GAE^{-1} 100 g^{-1} of sample, and the addition of perilla in white bread formulations did not increased the contents of total phenolic compounds at level of 5 % of significance between these and the standard formulation. The content of flavonoid compounds present in whole perilla was 0.87 ± 0.26, in mg QE^{-1} 100 g^{-1}. Flavonoid compounds were not detected in the formulations of perilla white bread. Since antioxidant activity was not detected in whole perilla and white bread, the final products have not changed on this property, regardless of the level of inclusion of whole perilla in the formulations.

Conclusion

Whole perilla improved the fatty acid composition of white bread and increased the levels of omega-3 fatty acids, reducing n-6/n-3 ratio rates and making more nutritious products for human consumption.

The addition of perilla did not promote significant increase in phenolic compounds content and antioxidant properties in the final products.

Formulation with 1% perilla had better acceptability index, higher frequency of favorable responses to purchase intent and amount of omega-3 fatty acids capable of supplying 5.63 and 8.19% of the American-recommended daily intake, for adult male and adult female, respectively.

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