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PERILLA FRUTESCENS: INGREDIENTE POTENCIAL NA ELABORAÇÃO DE PÃO DE FORMA COMO FONTE DE ÁCIDOS GRAXOS ÔMEGA-3

ANDRÉIA VIEIRA DA SILVA

Maringá

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Dissertação apresentada ao Programa de Pós Graduação em Ciência de Alimentos da Universidade Estadual de Maringá como parte dos requisitos para obtenção do título de Mestre em Ciência de Alimentos.

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Orientador

Prof. Dr. Jesuí Vergílio Visentainer

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BIOGRAFIA

Andréia Vieira da Silva, natural de Campo Mourão, estado do Paraná, graduou-se em Tecnologia em Alimentos pelo Centro Federal de Educação Tecnológica do Paraná (CEFET-PR), Unidade de Campo Mourão, atual Universidade Tecnológica Federal do Paraná, *campus* Campo Mourão, no ano de 2003, e especializou-se em Tecnologia e Qualidade de Alimentos de Origem Vegetal pela Universidade Federal de Lavras, em 2005.

De 2003 a 2005 foi professora colaboradora do Curso Superior de Tecnologia em Alimentos do CEFET-PR, Unidade de Campo Mourão, atuando nas disciplinas de Higiene Industrial e Legislação, Microscopia, Microbiologia, Controle e Segurança Alimentar.

De 2005 a 2007 foi servidora do Estado do Paraná, como professora padrão no Colégio Estadual Agrícola de Campo Mourão, ministrando a disciplina de Agroindústria I e II para o Curso Técnico em Agropecuária.

Desde 2007 é servidora da Universidade Tecnológica Federal do Paraná, *campus* Campo Mourão, atuando como Assistente em Administração, inicialmente na Diretoria de Graduação e Educação Profissional e, atualmente, na Diretoria de Relações Empresariais e Comunitárias.

Tem experiência em análises físico-químicas de alimentos, com ênfase em ácidos graxos, e controle de qualidade.

Crom Alimentos
Grupo de Pesquisa

DEDICO...

Ao meu filho Davi,

fonte de vida e pureza, que esteve comigo em todos

os momentos desta etapa.

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APRESENTAÇÃO

Esta dissertação de mestrado está apresentada na forma de um artigo científico:

Andréia Vieira da Silva, Marcos Vieira da Silva, Elton Guntendorfer Bonafé, Paula Fernandes Montanher, Jesuí Vergílio Visentainer. *Perilla frutescens*: potential ingredient for enhancement of pan bread as omega-3 source. International Journal of Food Science and Technology.

GENERAL ABSTRACT

INTRODUCTION. *Perilla frutescens* (perilla) is a plant grown and consumed in several Asian countries, whose seeds are rich in lipids and proteins. The major fatty acid present in the total lipids of perilla is alpha-linolenic acid, which is an essential fatty acid to the human body, belonging to the omega-3 family. Benefits related to the consumption of alpha-linolenic acid correspond to the evolution of brain activity and nervous system, suppression of carcinogenesis, metastasis, thrombosis, and allergic reactions. The optimal dose of fatty acids ômega-6/omega-3 should range between 1:1 to 4:1, depending on the health condition. It is essential to reduce the intake of omega-6 to prevent and monitor chronic diseases, therefore, the balance of omega-6 and omega-3 is very important for human development and homeostasis. The potential intake of omega-3 depends on the presence of these in popular products such as bread. This paper will evaluate the physical and chemical characteristics and fatty acids compostion of this type of bread made with wholemeal perilla, emphasizing omega-3 fatty acids contents.

AIMS. The aim of this study was to evaluate the proximate composition, fatty acids and antioxidants in wholemeal perilla and transferring of constituents to the pan bread formulated with different proportions of wholemeal perilla. Assess the impact of substitution of wheat flour by wholemeal perilla through the analysis of instrumental color, specific volume and sensory attributes.

MATERIAL AND METHODS. The development of pan breads with different proportions of wholemeal perilla, analysis of the chemical composition and instrumental color of that flour and final products were performed in the laboratories of Bakery and Food Chemistry of the Federal University of Technology – Paraná, Campo Mourão. The breadmaking process followed El-Dash (1978) methodology. Three formulations were developed with wholemeal perilla instead of wheat flour: in 1% of substitution (F1), 3% (F3) and 5% (F5); the standard formulation without wholemeal perilla was called F0. Analyses of moisture, ash and raw protein were performed according to Cunnif (1998). The instrumental color evaluation followed CIELab scale. The determination of specific volume of breads was made based on the method of El-Dash *et al.* (1982). Nifext fraction was determined by difference. Total lipids were determined according to Bligh and Dyer (1959). The transesterification of lipids and chromatographic analysis of fatty acid methyl esters were performed at the Laboratory of Food Chemistry, State University of Maringá. The transesterification of lipids was performed according to Hartman and Lago (1973), modified by Maia and Rodriguez-Amaya (1993).The separation of fatty acids methyl esters was performed by gas chromatography. Fatty acids were quantified by internal standardization and the calculations were made according to Joseph and Ackman (1992); the identification of fatty acids was performed by comparison of retention times with standards and according to Visentainer and Franco (2006) methodology. The pan breads have been evaluated according to Monteiro (2005) about the flavor, aroma, crumb color, texture, appearance and overall acceptance; the acceptability indexes calculations were made according to Lawless and Heyman (2004); the tests were conducted in the Laboratory of Sensory Analysis, State University of Maringá. The content of natural antioxidants was performed using the methanol extract of wholemeal perilla and breads

prepared. Total phenolic compounds were analyzed by the Folin-Ciocalteau method, described by Naczk and Shahidi (2004). The method of Eberlin (2009) was used to analyze the content of flavonoids. The antioxidant activity was determined by the free radical DPPH, as described by Brand-Williams *et al.* (1995), modified by Miliauskas *et al.* (2004). The tests were conducted in triplicate, with the exception to instrumental analysis of color, which was done in five replicates. The results were expressed as mean and its standard deviation. Data were subjected to analysis of variance and Tukey's test at 5% significance level using Statistica software, version 5.1 (1996).

RESULTS AND DISCUSSION. The levels of raw protein and total lipids found in wholemeal perilla were 24.18 and 40.12%; that values are close to those reported by Sharma *et al*. (1989) e Przybylsk (2005). The use of wholemeal perilla in developing of pan breads had little effect on the ash (1.47, 1.58, 1.60 and 1.69% for F0, F1, F3 and F5, respectively); raw protein content (10.20, 10.32, 10.05 and 11.02% for F0, F1, F3 and F5, respectively) and promoted a significant increase in total lipid products (1.89, 2.25, 2.84 and 3.27% for F0, F1, F3 and F5, respectively). There was an increase of the caloric value of bread, but statistical difference was shown only for the formulation F5. The calories per serving of 50 g (two slices) of F0, F1, F3 and F5 correspond, respectively, 6.51, 6.56, 6.57 and 6.73% of the Recommended Daily Value, which is 2000 kcal. As for the content of fatty acids, there was a predominance of polyunsaturated fatty acids of wholemeal perilla: 62.25% corresponded to omega-3 and 16.1% to omega-6 fatty acids, and the n6/n3 ratio reason found was 0.26. To the flour n6/n3 ratio found was 16.09. Considering the ratios obtained for the two flours, it was evident that the replacement of wheat flour by wholemeal perilla is advantageous to nutritional aspects. The interesterified soyabean oil used to manufacture the products presented 0.5% of *trans* fatty acids and was within the legal standards required for "zero *trans*" products. The addition of wholemeal perilla to bread formulations positively affected the ratio n-6/n-3, whose value improved from 1.60 to 11.27 in F0 to F5. There was a significant reduction in the concentration of saturated fatty acids among the four formulations of bread; this reduction was due to the fact that wholemeal perilla has 2.6-fold less palmitic acid content of the wheat flour. The values of saturated fatty acids per serving for each type of bread produced correspond to 0.32, 0.35, 0.39 and 0.42 g to F0, F1, F3 and F5. The levels found to *trans* fatty acids corresponded, per serving, to values lower than 0.2 g, therefore, the Brazilian law classifies the products obtained as "zero *trans*". Significant increase in the amount of polyunsaturated fatty acids in bread due to the increased content of alpha-linolenic acid, the main fatty acid present in the total lipids of wholemeal perilla. Per serving, the levels found of omega-3 fatty acids in F0, F1, F3 and F5 were 0.03, 0.09, 0.21 and 0.33 g, which corresponded, respectively, for adult male 1.88%, 5.63%, 13.13% and 20.63% of the American recommended daily intake. For adult female, the contents were 2.73, 8.19, 19.09 and 30.00%, respectively, for F0, F1, F3 and F5. As for the instrumental assessment of color, the replacement of wheat flour by wholemeal perilla resulted in darker crust, but there were no significant difference between F1, F3 and F5. The color of the crumb part is similar between F0 and F1 and between F3 and F5. The specific volume of breads differed significantly (5.13, 4.63, 4.41 and 3.86 $cm^3.g^{-1}$ to F0, F1, F3 and F5, respectively), indicating that the higher the percentage of replacement of wheat flour by wholemeal perilla, the lower the specific volume of the resulting products. The sensory evaluation showed that the formulations F0 and F1 are equal, as well as F3

and F5 in all attributes. The formulation with the highest acceptability index was F1 (88.89%). The other formulations showed 86.67, 78.67 and 74.78% for F0, F3 and F5, respectively. The frequency of favorable responses for intention to purchase was equal to 88.00% for F1, 85.60% for F0, 71.00% for F3, and 69.00% for F5. As for the content of total phenolic compounds, there were no significant difference between F0 and other formulations and antioxidant activity was not detected in the products produced.

CONCLUSIONS. The replacement of wheat flour by wholemeal perilla in bread developing promoted changes in chemical composition, mainly on the content of total lipids. There were significant changes in fatty acid composition between the formulations, especially in the content of saturated fatty acids, polyunsaturated, omega-6 and omega-3. Was observed significantly increase on the levels of omega-3 fatty acid in formulations with wholemeal perilla, decrease on the levels of omega-6 fatty acids and reducing the values of ratio n-6/n-3, making products more nutritious for human consumption . The addition of perilla in the form of wholemeal, did not cause significant increase in phenolic compounds and antioxidant properties in the final products. The formulation with substitution of wheat flour at 1% showed the better acceptability index and the higher frequency of favorable responses to purchase intent. This formulation did not differ from the standard in sensory attributes, instrumental color and possessed amount of omega-3 enough to supply 5.63 and 8.19% of the American recommended daily intake of alpha-linolenic acid, for adult male and adult female, respectively.

Key words: pan bread, perilla, omega-3 fatty acids

RESUMO GERAL

INTRODUÇÃO. *Perilla frutescens* (perilla) é uma planta cultivada e consumida em diversos países asiáticos, cujas sementes são ricas em lipídios e proteínas. O principal ácido graxo presente nos lipídios totais da perila é o ácido alfa-linolênico, que é um ácido graxo essencial ao organismo humano e pertencente à família ômega-3. Os benefícios relacionados ao consumo de ácido alfa-linolênico correspondem à evolução da atividade cerebral e do sistema nervoso, supressão da carcinogênese, metástase, trombose e reações alérgicas. A dose ideal de ácidos graxos ômega-6/ômega-3 deve variar entre 1:1 a 4:1, dependendo do estado de saúde. É essencial reduzir a ingestão de ômega-6 para prevenção e monitoramento de doenças crônicas, portanto, o balanço de ácidos graxos ômega-6 e ômega-3 é muito importante para homeostase e desenvolvimento humano. A ingestão potencial de ácidos graxos ômega-3 depende da presença destes em produtos populares, como o pão de forma. Este trabalho abordará a caracterização físico-química e de ácidos graxos deste tipo de pão formulado com a farinha integral de perila, com ênfase em ácidos graxos ômega-3.

OBJETIVOS. O objetivo deste estudo foi avaliar a composição centesimal, de ácidos graxos e antioxidantes na farinha integral de perila e a transferência de constituintes para o pão de forma formulado com diferentes proporções de farinha integral de perila. Avaliar o impacto da substituição da farinha de trigo por farinha integral de perila por meio da análise de cor instrumental, de volume específico e atributos sensoriais.

MATERIAL E MÉTODOS. A elaboração de pães de forma padrão e com diferentes proporções de farinha integral de perila, bem como as análises da composição centesimal e de cor instrumental da referida farinha e dos produtos finais foram desenvolvidas nos Laboratórios de Panificação e de Química de Alimentos da Universidade Tecnológica Federal do Paraná, campus Campo Mourão. O processamento dos pães de forma seguiu metodologia de El-Dash (1978). Foram desenvolvidas três formulações com farinha integral de perila em substituição à farinha de trigo: substituição em 1% (F1), 3% (F3) e 5% (F5); a formulação padrão, sem farinha integral de perila foi denominada F0. As análises de umidade, cinzas e proteína bruta foram realizadas segundo Cunniff (1998). A avaliação de cor instrumental seguiu a escala CIELab. A determinação de volume específico dos pães foi feita com base no método de El-Dash *et al.* (1982). A fração Nifext foi determinada por diferença. Lipídios totais foram determinados segundo Bligh e Dyer (1959). A transesterificação de lipídios e análise cromatográfica de ésteres de ácidos graxos foram realizadas no Laboratório de Química de Alimentos da Universidade Estadual de Maringá. A transesterificação dos lipídios foi realizada segundo Hartman e Lago (1973), modificado por Maia e Rodriguez-Amaya (1993). A separação dos ésteres metílicos de ácidos graxos foi realizada por cromatografia em fase gasosa. Os ácidos graxos foram quantificados por padronização interna e os cálculos foram feitos segundo Joseph e Ackman (1992); a identificação dos ácidos graxos foi realizada por comparação do tempo de retenção com padrões e segundo a metodologia de Visentainer e Franco (2006). Os pães de forma foram avaliados sensorialmente segundo Monteiro (2005) quanto aos atributos sabor, aroma, cor do miolo, textura, aparência e aceitação global; cálculos de índice de aceitabilidade foram feitos segundo Lawless e Heyman (2004); os testes foram conduzidos no

Laboratório de Análise Sensorial da Universidade Estadual de Maringá. Para avaliação do conteúdo de antioxidantes naturais foi utilizado o extrato metanólico da farinha integral de perila e dos pães elaborados. Compostos fenólicos totais foram analisados pelo método de Folin-Ciocalteau, descrito por Naczk e Shahidi (2004). O método de Eberlin (2009) foi utilizado para analisar o teor de flavonóides. A atividade antioxidante foi determinada pelo método via radical livre DPPH, conforme descrito por Brand-Williams *et al.* (1995), modificado por Miliauskas *et al.* (2004). Os testes foram conduzidos em triplicada, com exceção da análise instrumental de cor, que foi feita em cinco repetições. Os resultados foram expressos como média e respectivo desvio padrão. Os dados foram submetidos à análise de variância e ao Teste de Tukey ao nível de 5% de significância utilizando o software Statistica, versão 5.1 (1996).

RESULTADOS E DISCUSSÃO. Os teores de proteína bruta e lipídios totais encontrados na farinha integral de perila foram de 24,18 e 40,12%, valores próximos aos reportados por Sharma *et al*. (1989) e Przybylsk (2005). A utilização de farinha integral de perila na elaboração de pães de forma pouco afetou o valor do teor de cinzas (1,47, 1,58, 1,60 e 1,69% para F0, F1, F3 e F5, respectivamente) e proteína bruta (10,20, 10,32, 10,5 e 11,02% para F0, F1, F3 e F5, respectivamente), mas promoveu aumento significativo de lipídios totais nos produtos (1,89, 2,25, 2,84 e 3,27% para F0, F1, F3 e F5, respectivamente). Houve aumento do valor calórico dos pães, no entanto, diferença estatística foi apresentada apenas para a formulação F5. O valor calórico por porção de 50 g (duas fatias) de F0, F1, F3 e F5 correspondeu, respectivamente a 6,51, 6,56, 6,57 e 6,73% do Valor Diário Recomendado, que é de 2000 kcal. Quanto ao conteúdo de ácidos graxos, houve predominância de ácidos graxos poliinsaturados nos lipídios totais da farinha integral de perila. Do total destes, 62,25% correspondeu a ácidos graxos ômega-3 e 16,01% a ácidos graxos ômega-6, e a razão n-6/n-3 encontrada foi de 0,26. Para a farinha de trigo a razão n6/n3 encontrada foi de 16,09. Considerando as razões obtidas para as duas farinhas, ficou evidente que a substituição de farinha de trigo por farinha integral de perila é vantajosa do ponto de vista nutricional. A gordura vegetal interesterificada utilizada na elaboração dos produtos apresentou 0,5% de ácidos graxos *trans* e esteve dentro dos padrões legais exigidos para produto “zero *trans*”. A adição de farinha integral de perila às formulações de pão de forma afetou positivamente a razão n-6/n-3, cujo valor passou de 11,27 em F0 para 1,60 em F5. Houve redução significativa da concentração de ácidos graxos saturados entre as quatro formulações de pão de forma; considerou-se que tal redução tenha sido devido ao fato de que a farinha integral de perila possui 2,06 vezes menos teor de ácido palmítico que a farinha de trigo. Os valores de ácidos graxos saturados, por porção, para cada tipo de pão produzido correspondem respectivamente, para F0, F1, F3 e F5 a 0,32, 0,35, 0,39 e 0,42 g, o que obrigaria a declaração do referido tipo de gordura no rótulo dos produtos. Os teores encontrados para ácidos graxos *trans* corresponderam, por porção, a valores inferiores a 0,2 g, portanto, segundo a legislação brasileira, os produtos obtidos são “zero *trans*”. Houve aumento significativo da quantidade de ácidos graxos poliinsaturados nos pães devido ao aumento do teor de ácido alfa-linolênico, principal ácido graxo presente nos lipídios totais da farinha integral de perila. Por porção, os teores obtidos de ácidos graxos ômega-3 em F0, F1, F3 e F5 foram 0,03, 0,09, 0,21 e 0,33 g, que corresponderam, respectivamente, para um homem adulto a 1,88, 5,63, 13,13 e 20,63% da ingestão diária recomendada americana de LNA. Para uma mulher adulta, os teores correspondem a 2,73, 8,19, 19,09 e 30,00% da ingestão diária americana recomendada, respectivamente, para F0, F1, F3 e F5. Quanto à avaliação instrumental de cor, a substituição de farinha de trigo por farinha integral de perila resultou em pães com casca mais escura, mas não houve diferença significativa entre F1, F3 e F5. A cor do miolo é similar entre F0 e F1, bem como entre F3 e F5. O volume específico dos pães diferiu significativamente (5,13, 4,63, 4,41 e 3,86 $cm^3.g^{-1}$ para F0, F1, F3 e F5, respectivamente), indicando que quanto maior for o percentual de substituição da farinha de trigo por farinha integral de perila, menor será o volume especifico dos produtos resultantes. A análise sensorial mostrou que as formulações F0 e F1 são iguais, assim como F3 e F5, em todos os atributos avaliados. A formulação com maior índice de aceitabilidade foi F1 (88,89%). As demais formulações apresentaram 86,67, 78,67 e 74,78%, para F0, F3 e F5, respectivamente. A freqüência de respostas favoráveis para intenção de compra foi igual a 88,00% para F1, 85,60% para F0, 71,00% para F3, and 69,00% para F5. Quanto ao conteúdo de compostos fenólicos totais, não houve diferença significativa entre F0 e as demais formulações e não foi detectada atividade antioxidante nos produtos elaborados.

CONCLUSÕES. A substituição de farinha de trigo por farinha integral de perila na elaboração de pão de forma promoveu mudanças na composição centesimal, principalmente quanto ao teor de lipídios totais. Houve mudança significativa na composição em ácidos graxos entre as formulações, especialmente no teor de ácidos graxos saturados, poliinsaturados, ômega-6 e ômega-3. Foi observado aumento significativo do teor de ácido graxo ômega-3 nas formulações com farinha integral de perila e diminuição dos níveis de ácidos graxos ômega-6 e, portanto, redução dos valores da razão n-6/n-3, tornando os produtos mais nutritivos para o consumo humano. A adição de perila, na forma de farinha integral, não promoveu aumento significativo de compostos fenólicos e de propriedades antioxidantes nos produtos finais. A formulação com substituição da farinha de trigo em 1% apresentou melhor índice de aceitabilidade e maior freqüência de respostas favoráveis quanto à intenção de compra. Tal formulação não diferiu da padrão nos quesitos sensoriais e de cor instrumental e possuiu quantidade de ácidos graxos ômega-3 capaz de suprir 5,63 e 8,19% da ingestão diária recomendada americana de ácido alfa-linolênico, para homens e mulheres adultos, respectivamente.

Palavras chaves: pão de forma, perilla, ácidos graxos ômega-3

ARTIGO CIENTÍFICO COMPLETO

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TITLE PAGE

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Perilla frutescens: potential ingredient for enhancement of pan bread as omega-3 source

Running title:

Omega-3 fatty acids in perilla pan bread

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KEYWORDS: *Perilla frutescens*, pan bread, omega-3 fatty acids.

SUMMARY

Perilla frutescens seeds are rich in omega-3 fatty acids, important for human health because they are essential fatty acids. The potential intake of these fatty acids depends on the presence of them in popular foods such as pan bread. The aim of this study was to evaluate the replacement of wheat flour by wholemeal perilla in 1, 3 and 5% in pan bread processing and its impacts on chemical and sensorial attributes, emphasizing omega-3 contents. The use of wholemeal perilla promoted an increase of omega-3 content in pan breads and balanced the ratio n-6/n-3. The use of wholemeal perilla decreased the specific volume of pan breads prepared, didn't increased the content of phenolic compounds and antioxidant activity. The formulation with 1% wholemeal perilla presented better acceptability and supplied 5.63 and 8.19% of the American recommended daily intake of alpha-linolenic acid, for adult male and adult female, respectively.

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. Perilla oil is locally used for edible purposes. In the United States of America, is considered as a substitute for linseed oil (Ang *et al.*, 1999).

The fatty acid composition of perilla seed stands out among other edible seeds for the content of unsaturated fatty acids, it is mainly composed of LNA, a fatty acid n-3 family. The beneficial effects on fatty acids n-3 family of perilla shown by Kinsela

(1991) and Yu *et al.* (2004) correspond to 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 action of Δ15 and Δ12 desaturase on the OA in plants. In animals, it is precursor of production of fatty acids n-3 family. Advances in the cultivation of oilseeds have caused considerable disproportion in the natural balance of the contents of LA and LNA and in the last 100 years the average consumption of LNA has fallen considerably (Akoh and Min, 2008).

The optimal dose or ratio of fatty acids omega-6/omega-3 should range between 1:1 to 4:1, depending on the health state. It is essential to reduce the intake of omega-6 to prevent and monitor chronic diseases, therefore, the balance of omega-6 and omega-3 is very important for homeostasis and normal human development (Simopoulos and Cleland, 2003).

Longvah *et al.* (2000) concluded through histopathology exams in rats fed for 18 weeks with perilla oil, there was no toxic effect on the heart, lung, liver, spleen, kidneys, pancreas and gastrointestinal tract of animals. The study confirmed that perilla oil may be safely consumed by humans and is already practiced by the people of northeast India. The authors suggest that perilla oil should be exploited for nutritional purposes in mixtures with other vegetable oils, because it has shown in the same study, the decrease of cholesterol and triglycerides in the serum due to its high content of LNA.

Lin *et al.* (2010) report that the use of perilla seeds, leaves or stalks in the human diet may act as protection against oxidative damage due to the content of phenolic compounds, especially rosmarinic acid. According to Cuvelier *et al.* (1992), phenolic compounds act as antioxidants not only for their ability to donate electrons or hydrogen, but also because of its stable radical intermediates, which prevent the oxidation of various food ingredients, particularly fatty acids.

Data from ABIMA (2010) show that in 2010 the sales of industrial bread (white and whole bread, "bisnaguinha", among others) increased 5.8% from a year earlier. Brazilian per capita consumption had an increase of 2.2% and the production of approximately 4.7%.

Being the pan bread a popular food and perilla a rich source of omega-3 fatty acids, this study focused the physical-chemical and sensory characterization of this type of bread formulated with different proportions of flour from the seed in question, with emphasis on fatty acids omega-3.

MATERIAL AND METHODS

Preparation of pan breads with different proportions of wholemeal perilla

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

The ingredients used to prepare the different formulations were purchased from the local traders and processing was conducted at the Laboratory of Baking, Federal University of Technology – Paraná, *campus* Campo Mourão, Paraná, Brazil. The wholemeal perilla was obtained after processing the seeds in a knife mill (Marconi, model MA630, Piracicaba, São Paulo, Brazil).

Table 1: Standard pan bread formulation (F0) and with different proportions of wholemeal perilla

Ingredients	**Bread formulations (% m/m)**			
	F0	F1	F3	F5
Refined wheat flour (WF)	100.00	99.00	97.00	95.00
Wholemeal 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 soyabean oil (IS)*	3.00	3.00	3.00	3.00
Ascorbic acid*	0.10	0.10	0.10	0.10
Water*	53 – 57.00	53 – 57.00	53 – 57.00	53 – 57.00

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

It was used a vertical mixer (G. Paniz, model AE25, Caxias do Sul, Rio Grande do Sul, Brazil) to mix the ingredients, which were added in the proportions indicated in the test formulations and in the follow order: flour, wholemeal perilla, fresh yeast , sugar, interesterified soyabean oil, ascorbic acid, water and salt. The mixing time was five minutes. When it was reached the homogeneity of the network gluten, the dough was removed from the mixer, divided in three parts of 500 g, processed in cylinder (G. Paniz, model CL390, Caxias do Sul, Rio Grande do Sul, Brazil), modeled with a dough shaping machine (Braesi, model MB350, Caxias do Sul, Rio Grande do Sul, Brazil) and placed in forms of standard size for pan bread between 400 and 500 g.

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

After natural cooling for 4 hours, the products were sliced by a professional slicing machine (G. Paniz, model FP12, Caxias do Sul, Rio Grande do Sul, Brazil) and then packed in plastic bag for pan breads, measuring 440 x 110 mm.

Proximate composition and fatty acids

The analysis concerning the proximate composition of the WP and products originated from different formulations of pan bread were performed in Laboratory of Food Chemistry, Federal University of Technology – Paraná, Campo Mourão, Paraná, Brazil.

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 the pan breads. The total lipids were extracted according to the method of Bligh and Dyer (1959). Carbohydrates were estimated by difference. The energy value was calculated assuming the energy conversion factors reported by Brasil (2003a).

The procedures of lipid esterification and chromatographic analysis to determine the fatty acid composition of the samples were performed at Laboratory of Food Chemistry, State University of Maringá, Paraná, Brazil.

The fatty acids methyl esters were prepared according to the method of Hartman and Lago (1973), modified by Maia and Rodriguez-Amaya (1993). The separation of fatty acids methyl esters was performed on 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 mL.min^{-1} 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 ℃ respectively. The column temperature of 185 ^{o}C for 12,5 min was elevated to 235 ℃ with a rate of 4 ℃.min^{-1}, 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 comparison of retention times with those of standard methyl esters. The calculations were performed according to the method of Joseph and Ackman (1992) and Visentainer and Franco (2006).

Characterization of antioxidant activity

The tests to characterize the antioxidant activity of different bread formulations were conducted at the Laboratory of Food Chemistry, State University of Maringá, Brazil.

Approximately 5.0 g of sample were used for the solvent methanol extraction in the ratio 1:10 (m.v^{-1}). The sample and solvent mixtures were agitated constantly for 4 h, then filtered under vacuum and evaporated in a rotary evaporator at 40 ℃. The dried extracts were used for analysis of total phenolics and antioxidant activity.

The total phenolics were determined by Folin-Ciocalteau method, described by Naczk and Shahidi (2004).

The Eberlin (2009) method was used to analyze the content of flavonoids.

The antioxidant activity was determined by the free radical DPPH, as described by Brand-Williams *et al.* (1995), modified by Miliauskas *et al.* (2004).

Instrumental color analysis and specific volume

Measurements of instrumental color and specific volume of wholemeal perilla and bread samples were performed at the Laboratory of Bakery, at Federal University of Technology – Paraná, Campo Mourão, Paraná, Brazil.

The instrumental color evaluation was performed according to CIELab scale, using colorimeter MiniScan EZ (HunterLab, model MSEZ-4000S, USA). It was measured the values of L, a* and b*, which respectively correspond to the lightness (0 - white, 100 - black), green (a-), red (+), blue (b-) and yellow (b +). The experiment involved five repetitions of the analysis for the crust and crumb of pan bread with different formulations.

The specific volume was determined by displacement of millet seed, according to El-Dash *et al.* (1982).

Sensory evaluation of products

Samples of sliced pan bread were subjected to acceptance test described by Monteiro (2005). The acceptance test was applied using a hedonic scale of nine points. The sensory panel was composed of 40 untrained tasters, recruited from the students and employees of State University of Maringá, Paraná, Brazil.

The tests were conducted in individual cabins in the Laboratory of Sensory Analysis, in State University of Maringá, Paraná, Brazil.

Samples of bread were presented in monadic way to tasters in white plastic dishes, encoded by four random numbers. Each sample was presented with an answer sheet that included in addition to scale of sensory evaluation, a scale to investigate the purchase intent by the tasters.

Statistical analysis

The tests were conducted in triplicate, except for instrumental analysis of color, which were performed five repetitions. The results were expressed as mean and standard deviation. The results for the different formulations were compared by analysis of variance (ANOVA) with the 5% level of significance. Mean values were compared by Tukey's test with the software Statistica 5.1 (Statsoft Inc.Tulsa, OK, USA, 1996).

RESULTS AND DISCUSSION

Proximate composition and fatty acids

The chemical composition of standard pan bread and with different proportions of WP is shown in Table 2.

The raw protein content of WP was 24.18%, which is close to levels reported by Sharma *et al*. (1989), which ranged from 15.7 to 23.7%. The total lipid content was 40.12%, which is within the range cited by Przybylsk (2005), which varied from 35 to 50%.

The levels of ash, raw protein, and the carbohydrates were different ($p \leq 0.05$) between F0 and F5, indicating that the substitution of WF by WP at 5%, decreased the similarity of the resulting product with the standard when is considered the chemical composition.

Table 2 – Proximate composition (g.100g^{-1}) and energetic value (kJ.100g^{-1}) of WP and different formulations of pan breads

Proximate composition	WP	F0	F1	F3	F5
Moiusture	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
Raw 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	48.78^{b}±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 repetitions ± standard deviation. Different letters in the line indicate statistically significant differences between samples (p≤0.05). WP: wholemeal perilla; F0: pan 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 on the value of total lipids. As the lipid content of wholemeal 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 the F1 and F3 had a greater percentage of total lipids, there were no significant differences (p ≤ 0.05) on the energetic values of these breads when compared to standard bread.

Each slice of bread obtained for the different formulations weighed, on average, 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), which is 2000 kcal, considered by labeling standards (Brasil, 2003b).

The results at Table 3 show that there is predominance of PUFA (78.00%) in the lipids of WP. Of total PUFA, 62.25% corresponded to LNA and 16.01% to LA.

The n-6/n-3 ratio found in WP was 0.26. These values are close to those reported by Przybylsk (2005) and Dubois *et al*. (2007).

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

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 repetitions ± standard deviation. IS: Interesterified soyabean oil; WP: Wholemeal 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).

For WF, the n-6/n-3 ratio found was 16.09. According to Simopoulos (2004), elevated n-6/n-3 ratio promotes cancer, cardiovascular, inflammatory and immune system disease. Considering the ratios obtained for the two flours, became even more evident that the replacement of WF by WP is advantageous from a nutritional point of view, however, it must be also considered the technological and sensory aspects.

The Brazilian legislation (Brasil, 2003b) states, per serving, the maximum level of 0.2 g of *trans* fatty acids in foods. As for the portion of fats corresponds to 10 g (Brasil, 2003a), it can be asserted that the maximum level of TFA in the product may reach 2%. The interesterified soyabean oil used in preparation of pan breads had 0.5% of these fatty acids and was within legal standards required.

The fatty acid composition of pan breads with different formulations is presented in Table 4.

Simopoulos and Cleland (2003) assert that the optimal ratio of fatty acids omega-6/omega-3 should range between 1 to 4, depending on the state of health. The addition of WP in the bread formulations positively affected the reason n-6/n-3. The greater the mass of WF replaced by WP, the greater the content, in $mg.g^{-1}$ of LNA, which made the n-6/n-3 ratio change from 11.27 in F0 to 1.60 in F5.

According to Simopoulos (1999) it is essential that the diet promotes low intake of saturated fats and amounts less than or equal to 2% of *trans* fatty acids in order to produce the effect of reducing cardiovascular risk factors and diabetes. There was a significant reduction ($p \leq 0.05$) in the contents of SFA among the four formulations of bread. It was considered that this reduction was due to replacement of WF by WP, since WF has 2.6-fold higher content of palmitic acid (16:0) then WP. There was a slight decrease, in $mg.g^{-1}$, of the TFA. This can be explained by the fact that in all formulations was used the same amount of interesterified soyabean oil, but there were increased levels of PUFA, from WP.

Table 4: Fatty acid composition, in $mg.g^{-1}$ of total lipid, of pan breads 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} ± 10.22	461.84^{c} ± 14.90	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 repetitions ± standard deviation. Different letters in the line indicate statistically significant differences between samples ($p \leq 0.05$). IS: Interesterified soyabean oil; WP: Wholemeal 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: pan 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.

Labeling rules in Brazil (Brasil, 2003b) indicate that, per serving, values less than or equal to 0.2 g for SFA and TFA are declared as "zero" or "insignificant content". SFA values per serving for each type of bread produced correspond, respectively, for F0, F1, F3 and F5 to 0.32, 0.35, 0.39 and 0.42 g, which would force the declaration of that type of fat on the label of products. The TFA content found corresponded to levels lower than 0.2 g per serving, therefore, according to Brazilian legislation, the different breads produced didn't contain significant amounts of TFA, so they are "zero *trans*".

The sum of PUFA was statistically different ($p \leq 0.05$) among all formulations. The increase in PUFA content of the breads with WP can be explained by the higher content of LNA, which is the main fatty acid in WP lipids.

According to the Institute of Medicine of the United States, the RDI of LNA for an adult male is 1.6 g, and an adult female, 1.1 g (Trumbo *et al.*, 2004). Per serving of 50 g, the levels obtained to LNA in F0, F1, F3 and F5 were 0.03, 0.09, 0.21 and 0.33 g, which corresponded, respectively, for adult male 1.88, 5.63, 13.13 and 20.63% of the American recommended daily intake. For adult female, 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 wholemeal perilla, using CIELab scale, L*, a * and b* were respectively 50.84±0.52; 7.58±0.08 e 22.66±0.48. The values obtained for the different formulations of bread, as well as the specific volume are shown in Table 5.

The replacement of WF by WP resulted in breads with darker crust, but there were no significant difference ($p \leq 0.05$) between F1, F3 and F5. The color of the crumb was similar between F0 and F1 and between F3 and F5.

Table 5 – Means of instrumental color[1] of the pan breads crust and crumb and its specific volume[2]

Parameter		F0	F1	F3	F5
Crust	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	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 volume (cm^3g^{-1})		5.13^{a}±0.02	4.63^{b}±0.03	4.41^{c}±0.08	3.86^{d}±0.09

[1] Means of five analytical repetitions ± standard deviation. [2] Means of three analytical repetitions ± 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: wholemeal perilla; F0: pan 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.

The specific volume of breads differed significantly, indicating that the higher the percentage of replacement of WF by WP, the lower the specific volume of the final products. The presence of particles of perilla promoted less resistance to the gluten formed and retaining a smaller volume of gas, resulting in products with less volume.

The substitution of WF by WP until the level of 3%, although had decreased the volume of products, presented specific volume higher than that found by Esteller and Lannes (2005) in study to fix the identity and quality of baked products, which was 4.10 $cm^3.g^{-1}$ for pan bread. The specific volume corresponding to the formulation F5 indicated low aeration, which resulted in lesser acceptance to sensory attributes like appearance, texture and flavor.

Sensory evaluation

The means for appearance, color, texture, flavor, aroma and overall acceptance for the different formulations of pan breads with perilla are showed in the Table 6.

Table 6: Sensory evaluation of pan breads 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: wholemeal perilla; F0: pan 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.

The formulations F0 and F1 are similar (p ≤ 0.05) in all attributes as well as F3 and F5. This result shows the correlation between sensory evaluation and instrumental color evaluation.

The formulations F3 and F5 showed perilla flavor remarkable, according to the tasters, which damaged the acceptance of products.

Lawless and Heyman (2004) assert that an acceptability index is satisfactory when achieves the minimum of 70%. The acceptability indexes were significantly different (p ≤ 0.05) for all formulations. The formulation F1 showed the higher acceptability index, 88.89%. The other formulations showed 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% to F0, 71.00% to F3, and 69.00% to F5.

The sensory evaluation indicated that the replacement of 1% of WF by WP didn't affect the sensory characteristics of bread when they are analyzed separately. The global evaluation of the product showed that this substitution promoted the better acceptance and therefore increased the purchase intent.

Total phenolic compounds and antioxidant activity

The content of total phenolic compounds in mgGAE.100g^{-1} of sample, for wholemeal perilla was 59.47 ± 1.50. The formulations F0, F1, F3 and F5 respectively presented in mgGAE.100g^{-1} of sample, 8.24 ± 1.48, 9.94 ± 1.03, 9.99 ± 1.09, 10.78 ± 1.73. The addition of perilla in pan bread formulations promoted increase in content of total phenolic compounds, but there were no significant difference between these and the standard formulation.

The content of flavonoid compounds present in wholemeal perilla was 0.87 ±0.26, in mgQE.100g^{-1}. Flavonoid compounds weren't detected in the formulations of perilla pan bread.

Antioxidant activity wasn't detected in wholemeal perilla and pan bread prepared, indicating that the perilla as flour didn't contribute to the increase of natural antioxidants in the final products.

CONCLUSIONS

The replacement of wheat flour by wholemeal perilla in bread developing promoted changes in chemical composition, mainly on the total lipids content.

There was significant change in fatty acid composition between the formulations, especially in the content of SFA, PUFA, omega-6 and omega-3 fatty acids. It was observed significantly increased in the levels of omega-3 fatty acid in formulations with wholemeal perilla, decreased in the levels of omega-6 fatty acids and therefore reducing the n-6/n-3 ratio values, making products more nutritious for human consumption.

The addition of perilla in the form of flour, didn't promote significant increase in phenolic compounds content and antioxidant properties in the final products.

The formulation with substitution of wheat flour at 1% showed a 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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