

INCLUSION OF PROTEIN CONCENTRATES FROM MARINE AND FRESHWATER FISH PROCESSING RESIDUES IN CEREAL BARS

Maria Luiza Rodrigues de Souza^{1*}, Kelly Cristina Vitorino², Ana Paula Sartorio Chambo³, Melina Franco Coradini³, Jane Martha Graton Michka⁴, Eliane Gasparino¹, Alex Augusto Gonçalves⁵

¹Departamento de Zootecnia, Universidade Estadual de Maringá (UEM), Maringá, PR, Brazil

²Graduação em Zootecnia, Universidade Estadual de Maringá (UEM), Maringá, PR, Brazil

³Programa de Pós-Graduação em Zootecnia, Universidade Estadual de Maringá (UEM), Maringá, PR, Brazil

⁴Departamento de Análises Químicas e Biomedicina, Universidade Estadual de Maringá (UEM), PR, Brazil

⁵Centro de Ciências Agrárias (CCA), Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró, RN, Brazil

*Corresponding author: mlrsouza@uem.br

Abstract - The consumer's demand for healthy food is increasing in recent years, and the cereal bars becomes an interesting food product for practical use, diversity of flavors and the possibility to include new functional ingredients. In order to address this consumer's demand, cereal bars with fish protein concentrate (FPC) inclusion, obtained from fish processing waste were developed. The proximate composition, caloric value, microbiological and sensorial this new product were evaluated. Four cereal bars formulation were prepared with 4.5 % fish protein concentrate inclusion: Treatment 1 (control, no FPC addition), Treatment 2 (Nile tilapia protein concentrate) Treatment 3 (Nile tilapia and salmon protein concentrate), and Treatment 4 (Nile tilapia and tuna protein concentrate). The microbial results for FPC and cereal bars were in accordance with the microbiological standard for human consumption. The inclusion of 4.5% FPC from different fish species did not affect the proximate composition and biological value of the cereal bars, but interference in the evaluated sensory attributes (color, texture, flavor and overall impression) of the cereal bars was observed. In conclusion, according to the best results, the addition of protein concentrate from Nile tilapia is the most appropriate.

Keywords- processing residue; protein concentrate; new products; quality; safety.

1. INTRODUCTION

The demand for healthy foods has increased in recent years, and this can be attended by multi-component products, such as cereal bars, which complement each other in flavor, texture, and physical properties. It is an affordable product due to the reduced price, presents a higher practicality and suitable for ingestion of required nutrients such as minerals, fatty acids, proteins, different high-quality fiber sources, among others. An option to address the lack of time for consumers, and enjoy the many benefits of the fibers present in the product as, improving intestinal transit and increased satiety, in addition to be a product easier to buy and transport, the cereal bar is considered as a quick way to restore the energy spent in intense physical activities as part of the menu as nutritional aids. [1]

In order to develop cereal bars that allow the use of healthier ingredients, but without causing any damage in the sensory attributes valued by consumers, researchers have been seeking alternatives for the development of cereal bars with new food ingredients, nutritional and functional. [2] It has been quite common to include different types of fiber in the cereal bars to evaluate the acceptance and nutritional quality; however, studies with the inclusion of ingredients from the animal source are scarce.

In this context, the fish processing industries, that generate large amounts of residues, can be significant sources of

excellent quality nutrients. However, the use of such residues still too small, where around 50-60% of this biomass is discarded during the processing lines, i.e., filleting, canning, etc.[3]. Thus, it is very important to develop ways to use these residues (heads, scales, skin, viscera, carcass or spine after the fillets withdrawal), improving the efficiency in an attempt the integral use of fish, providing economic sustainability in the sector.

An important factor is the increasing interest of the nutritional quality of this residue; in particular to the polyunsaturated fatty acids (among them, the omega-3 series), minerals (calcium, phosphorus and iron) [4], vitamins A, D and B complex, especially B12, which makes the product with a high nutritional value [5,6], besides the great biological value, i.e. balanced amino acid composition, usually rich in methionine and cysteine, limiting in many food. [7].

Considering these information, the development of the protein concentrate from fish processing residues, and its inclusion in food products commonly eaten daily, may be an alternative food source, with economic potential and social application, once that residue, besides being common and generated in large quantities, are not utilized in food industries (for human consumption), and have excellent nutritional quality. However, the quantities (percentage) to be used for each type of processed product should be indicated,

and if a fish protein concentrate (FPC) mixture of two or more species of fish is used, the proportions to be used for each species should be verified. Thus this work aimed to develop cereal bars, with the inclusion of protein concentrate fish of different species (Nile tilapia, salmon, and tuna), and to evaluate their nutritional, microbiological and sensory characteristics.

2. MATERIAL E METHODS

2.1 Fish residues and protein concentrate development

The different fish protein concentrates were developed in the Seafood Technology Laboratory of the State University of Maringá (UEM), located at the Iguatemi Experimental Farm (FEI/UEM). Residues from Tuna (*Tunnus* sp) processing (GDC Alimentos SA Company, Itajaí, SC, Brazil), salmon (*Salmo salar*) processing (Taiyo Company, Maringá, PR, Brazil), and Nile tilapia (*Oreochromis niloticus*) processing (Smartfish Company, Rolândia, PR, Brazil) were used for protein concentrated recovering. The residues were washed, weighed, cooked in a pressure cooker with the antioxidant butylated hydroxytoluene (BHT at 0.5 mg kg⁻¹) for 60 minutes, drained, pressed (hydraulic press - 10 tons capacity) and milled. The protein concentrate, separated by species, was dehydrated in a drying oven (60°C, 24h) ground in a knife mill Willye type (TE-650, Tecnal, Piracicaba, SP, Brazil), vacuum packed, and frozen (-18°C) for use in the cereal bars development. For the preparation of protein concentrate, 90% of Nile tilapia concentrate and 10% of salmon or tuna concentrate were used.

2.2 Cereal bars development

The cereal bars were developed in the Seafood Technology Laboratory (FEI/UEM). The ingredients used in the cereal bars development were divided into basic and syrup ingredients (Table 1). The fish protein concentrate – FPC (4.5%) was included in the cereal bar (CB) formulation based on the weight of the ingredients, and four formulations was developed, resulting in a total of four treatments: Treatment 1 = without the FPC addition (CB-C, control), Treatment 2 = Nile tilapia protein concentrate (CB-TPC), Treatment 3 = Nile tilapia and salmon protein concentrate (CB-TSPC) and Treatment 4 = Nile tilapia and tuna protein concentrate (CB-TTPC) with four replicates per treatment.

Table 1. The ingredients used for the cereal bars development with the inclusion of fish protein concentrate.

Basic ingredients	CB-C	CB-TPC	CB-TSPC	CB-TTPC
	Quantity (g)	Quantity (g)	Quantity (g)	Quantity (g)
Brazilian' nuts (Castanha-do-Pará)	95.25	95.25	95.25	95.25
Oat flour (fine)	95.25	95.25	95.25	95.25
Oat flour (thick)	95.25	95.25	95.25	95.25
Sugarless granola	47.60	47.60	47.60	47.60
Rice flakes	47.60	47.60	47.60	47.60
Integral flour	71.50	71.50	71.50	71.50
Dehydrated banana	47.60	47.60	47.60	47.60
Raisins (dried) grape	44.65	44.65	44.65	44.65
Peanut	125.00	125.00	125.00	125.00
Tilapia protein concentrate	-	30.50	-	-
Tilapia + salmon protein concentrate	-	-	30.50	-
Tilapia + tuna protein concentrate	-	-	-	30.50
Syrup ingredients	Quantity (g)	Quantity (g)	Quantity (g)	Quantity (g)
Brown sugar	107.5	107.5	107.5	107.5
Honey	89.25	89.25	89.25	89.25
Margarine	133.9	133.9	133.9	133.9

CB-C (control, cereal bar without the FPC); CB-TPC (cereal bar with Nile tilapia protein concentrate); CB-TSPC (cereal bar with Nile tilapia and salmon protein concentrate); CB-TTPC (cereal bar with Nile tilapia and tuna protein concentrate)

2.3 Microbiological analysis

The cereal bars were evaluated microbiologically (by most probable number (MPN) of coliforms at 35°C and 45°C, *Staphylococcus coagulase* positive (CFU g⁻¹) and *Salmonella* spp.) following the standards recommended by Brazilian legislation [8] and according to the official's methods[9,10]. The analyses were performed in the Laboratory of Microbiology and Food Microscopy, Clinical Analysis Department, State University of Maringá (UEM).

2.4 Proximate composition and caloric value analysis

The proximate composition was performed following the official methodology [11] for moisture and ash content; the semi-micro Kjeldahl method for the determination of nitrogen/crude protein [12]; the Bligh and Dyer method [13]for the lipid extraction. The carbohydrate was accomplished by the difference between components. The caloric value was calculated by multiplying the values of total carbohydrate, lipid, and protein by the factors 4, 9 and 4 respectively, taking the sum of the products and expressing the result in kilocalories. [14]

2.5 Sensory analysis

The sensory analysis was performed 12 hours after the cereal bars development. Samples of cereal bars were cut into pieces of 25g, wrapped in aluminum foil, identified with three random numbers and offered 50 untrained panelists. The sensorial attributes evaluated following the hedonic scale of 9 points (from 1 – dislike very much, to 9 – liked very much) were: aroma, color, taste, overall impression, according to the hedonic scale. [15] The intention to purchase [16]using the 5 points hedonic scale, where 5 represent the highest score "definitely buy" and 1 represent the minimum score "certainly would not buy", was also evaluated. The acceptability index (AI) was employed to determine how much a person like or dislike the samples. It has been calculated considering the average measure and maximum measure achieved for examined product according to the follow equation: AI (%) = [mean value/higher score] x 100. Products reaching a percentage ≥70% were considered accepted by consumers. [15]

2.6 Experimental design and statistical analysis

The experimental design was completely randomized, with four treatments and four repetitions, whose averages were submitted to analysis of variance and compared by Tukey test at 5% probability. [17] For the statistical analyses of sensory analysis data (n = 50), the computer system SAS Proc GENMOD was used considering the distribution of variables as areverse link with gamma function. The effect of treatment and panelists was considered, testing the FPC inclusion against the control, by means of testing and the behavior of the panelist's notes and according to the notes for cereal bars with the inclusion of different protein concentrates used.

3. RESULTS AND DISCUSSION

Accordingly to the Table 1, the main goal was to evaluate the FPC inclusion made with different species (i.e., Nile tilapia, Nile tilapia+salmon, and Nile tilapia+ tuna) at only one single level (30.5 g) in the overall sensory acceptance following the levels suggested by several authors. Goes et al.

[18] used the inclusion of up to 15% of the FPC mixture (20% of salmon protein concentrate and 80% of tilapia protein concentrate) in spinach cake.

Considering the sensory results, the maximum inclusion recommended was 10% of the dehydrated mixture of fish in the spinach cake. Kimura et al. [19] to prepare a dehydrated fish mix, used 10% salmon flour and 90% tilapia flour to include different levels (0%, 5%, 10% and 15%). The authors reported that for alfajor, up to 15% can be used without changing the level of acceptance of the product. Also, other authors have been used the inclusion of only one specie, i.e. of tilapia protein concentrates in sweet products.

Fuzinato et al. [20] used 10% of the tilapia flour in the inclusion of sweet cookies with excellent results regarding the physical characteristics, chemical composition, technological and sensorial. It may be noted that the inclusion of this type of ingredient in the preparation of a food product is variable depending on the type of product to be made and especially if it has other ingredients which may further mask the possibility of the appearance of fish aroma and taste.

Franco et al. [21] developed cookies and homemade biscuits with the fishmeal inclusion. The authors used protein concentrate from a freshwater species (Nile tilapia) and due to the flavor and aroma quality, smoother than a marine species, it was possible to include up to 30% in chocolate cookies and only 12% in homemade biscuits, because above this level the aroma became compromised. According to those authors, the ideal should be the inclusion of some ingredients to improve the aroma and even the flavor, such as chocolate, cinnamon and clove powder, among others.

The results of microbiological analysis of cereal bars (Table 2) is consistent with the microbiological standards set by Brazilian law [8] and are being apt for human consumption. The addition of fish protein concentrate in the preparation of cereal bars did not influence significantly ($p < 0.05$) the moisture, protein, fat, ash, carbohydrates and consequently in calorific value, as shown in Table 3. The average values for the respective parameters were 21.32%, 11.36%, 10.84%, 2.38%, 54.25%, 359.37%. The initial idea was to enhance the nutritional properties of the new product developed, and although the results showed higher protein levels with FPC inclusion, there was no significant interference between the groups. The protein quality is not evaluated by biological method because this was not the focus of this research experiment.

These results differ from those found by Brito et al. [22], where developed and evaluated cereal bars (with cornstarch cookie, raw sugar, and skimmed milk powder). They reported average values of moisture (7.63%), lipid (0.68%) and protein (6.27%) lower than those found in this study (10:38% and 16.11%, respectively, for lipids and protein). Bauet et al. (2010) developed food bars with high protein value and obtained 15.8% of protein, which is higher than the present study, but the ash values (2.74%) were similar.

For moisture values, the mentioned authors found levels of 12.5%, which is much lower than observed in the present study (Table 3). The moisture content of cereal bars with FPC ranged from 21.54% to 21.13%, however, the moisture content for cereal products established by Brazilian legislation [23] is 15%.

Table 2. Microbiological analysis of fish protein concentrates and cereal bars with FPC.

Treatments	Microbiological analysis			
	Coliforms (35°C) MPN g ⁻¹	Coliforms (45°C) MPN g ⁻¹	<i>Staphylococcus aureus</i> positive (CFU g ⁻¹)	<i>Salmonellasp.</i> (absence in 25g)
CB-C	<3	<3	10 ²	Absence
CB-TPC	<3	<3	10 ²	Absence
CB-TSPC	<3	<3	10 ²	Absence
CB-TTPC	<3	<3	10 ²	Absence
FPC (tilapia, salmon and tuna)	<3	<3	10 ²	Absence

Table 3. Centesimal composition analysis of cereal bars with FPC.

Nutrients	Treatments				P*	CV (%)
	CB-C	CB-TPC	CB-TSPC	CB-TTPC		
Moisture	21.10±1.89 ^a	21.54±2.65 ^a	21.30±2.56 ^a	21.13±2.15 ^a	0.99	10.84
Protein	9.56±1.79 ^a	9.76±2.11 ^a	11.54±1.66 ^a	12.13±1.33 ^a	0.13	16.21
Lipids	10.15±0.49 ^a	10.30±1.00 ^a	10.51±1.77 ^a	11.72±1.92 ^a	0.42	13.35
Ash	2.49±1.22 ^a	1.85±0.27 ^a	2.77±0.73 ^a	2.54±0.74 ^a	0.46	34.01
Carbohydrate	56.75±5.15 ^a	56.55±1.73 ^a	53.88±0.23 ^a	52.33±0.91 ^a	0.12	5.02
Caloric value	356.58±11.33 ^a	357.93±10.28 ^a	356.29±21.90 ^a	363.89±20.45	0.91	4.69

The inclusion of fish protein concentrate interfered in the sensory attributes of the cereal bars (Table 4), except for the aroma attribute, whose average was 5.94. The score is given by the panelists for the color attribute, the bars receiving the concentrated fish were lower than the control (cereal bar without FPC), despite not having differed significantly from this treatment. For the color attribute, the CB-TTPC showed the worst visual aspect and statistically different only control (cereal bar without FPC) whose score was 7.40. Panelists did not observe differences between treatments for the aroma attribute (6.17), but this characteristic (attribute) received a satisfactory evaluation by the hedonic scale of 5 points. For texture, flavor and overall impression it was possible to verify that the treatments that received the Nile tilapia protein concentrate +marine fish protein concentrate had lower values than treatments with the inclusion of only Nile tilapia concentrate or without FPC inclusion.

The purchase intention evaluation can be inferred that the CB-TTPC did not have a good acceptance among consumers, indicating that this product would certainly not be purchased if you were in the market. However, the cereal bar with Nile tilapia protein concentrate showed excellent results, indicating that the consumer would purchase that product.

The acceptance of values obtained in this study are lower than those reported by Brito et al. [22], whose develop bars with different fiber sources and starch, observed higher acceptance values than the trademark product tested, as well as, the study reported by Arevalo et al. [24], which was develop bars with dry nut babassu flour, which showed higher levels of acceptance. In the present study, the acceptance value was in average of 3.13%.

Several authors have studied the inclusion of fish derivatives in food products for the purpose of nutritional enrichment and have obtained good results, both for the chemical composition and for the sensory acceptance. For example, Veit [25] developed and characterized chocolate and carrot cakes with Nile tilapia fillet. Goeset et al. [18] conducted sensory analysis for spinach cake with the inclusion of different mix levels of dehydrated fish. Tavares et al. [26] tested the replacement of 5% wheat flour by matrinxã (*Brycon lundii*) flour. Centenaro et al. [27] used dried fish pulp (DFP) and moist fish pulp (MFP) for the

enrichment of bread and obtained satisfactory acceptance for bread with 3% DFP, 5% DFP, and 50% MFP.

CONCLUSION

The inclusion of 4.5% of a protein concentrate from different species of fish (freshwater or marine) during the development of cereal bar did not affect the nutrient content and biological value, however, interfered in color, texture, flavor and overall impression. The inclusion of Nile tilapia protein concentrate in cereal bars was the best choice. Thus, the increase of fish protein concentrates inclusion, to improve the nutritional value, should be evaluated the amounts and types of ingredients to be used in the syrup, primarily to improve the taste.

REFERENCES

1. Carvalho, M.G. (2008). Barras de cereais com amêndoas de chichá, sapucaia e castanha-do-gurguéia, complementadas com casca de abacaxi. Dissertação (Mestrado em Tecnologia de Alimentos). Faculdade de Engenharia de Alimentos, Universidade Federal do Ceará, Fortaleza, 93p.
2. Palazzolo, G. (2003). Cereal bars: they're not just for breakfast anymore. *Cereal Foods World*, 48(2): 70-72.
3. Kubitzka, F. (2006). Aproveitamento dos subprodutos do processamento de pescados. *Panorama da Aqüicultura*, 16(94): 23-29.
4. Oliveira, M.V.M.; Sanchez, L.M.B.; Vargas Junior, F.M.; Gomes Neto, A.N.; Pires, C.C.; Haygert Velho, I.M.P.; Frizzo, A.; Lana, R.P. (2002). Avaliação das farinhas de peixe e pena, no confinamento de bezerras leiteiras desmamadas, através de dietas calculadas em termos de proteína bruta ou de proteína metabolizável. *Revista Brasileira de Zootecnia*, 31(3): 1571-1581.
5. Simões, D.R.S.; Queiroz, M.I.; Volpato, G.; Zepka, L.Q. (2004). Desodorização de base proteica de pescado (BPP) com ácido fosfórico. *Ciência e Tecnologia de Alimentos*, 24(1): 23-26.
6. Vila Nova, C.M.V.M.; Godoy, H.T.; Aldrigue, M.L. (2005). Composição química, teor de colesterol e caracterização dos lipídios totais de tilápia (*Oreochromis niloticus*) e pargo (*Lutjanus purpuraceus*). *Ciência e Tecnologia de Alimentos*, 25(3): 430-436.
7. Neiva, C.R.P. (2008). Instituto de Pesca desenvolve alimentos adequados para refeições em instituições. Available: <<http://www.saopaulo.sp.gov.br/spnoticias/lenoticia.php?id=97688>>. Access: March 4, 2015.
8. Brazil – Ministério da Saúde (MS), Agência Nacional de Vigilância Sanitária (ANVISA). Resolução RDC nº12, de 02 de janeiro de 2001. Aprova o regulamento técnico sobre padrões microbiológicos para alimentos. *Diário Oficial da União*, 2001.
9. Brazil – Ministério da Agricultura, Pecuária e Abastecimento (MAPA). Instrução Normativa Nº 62, de 26 de agosto de 2003. Oficializa os Métodos Analíticos Oficiais para Análise Microbiológicas para Controle de Produtos de Origem Animal e Água. *Diário Oficial da União*, Brasília, 26 de agosto de 2003.
10. American Public Health Association – APHA (2001). *Compendium of methods for the microbiological examination of foods*. 4. ed. Washington, DC, 2001. 1219p.
11. Association of Official Analytical Chemists – AOAC (1995). *Official Methods of Analysis of AOAC International*, 16th Ed., Gaithersburg, MD (USA), Official Method for Fish and Other marine Products, chap. 35, p. 1-36.
12. Silva, D.J.; Queiroz, A.C. (2002). *Análise De Alimentos: Métodos Químicos e Biológicos*. 3^a.ed. Viçosa, MG: Universidade Federal De Viçosa, 235p.
13. Bligh, E.G.; Dyer, W.J.A. (1959). Rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37(8): 911-917.
14. Souci, S.W.; Fachman, H.; Kraut, E. (2000). *Foods Composition and Nutrition Tables*. 6thed., MedpharmScientific Publishers, 1182p.
15. Dutcosky, S.D. (2013). *Análise sensorial de alimentos*. 4th ed. Curitiba: Champagnat, 531 p.
16. Damásio, M.H.; Silva, M.A.A.P. (1996). *Curso de treinamento em análise sensorial*. Apostila. Campinas: Fundação Tropical de Tecnologia "André Tosello", 57 p.
17. SAS user's guide: Statistics. Statistical Analysis System Institute Inc, Cary, NC. 2005.
18. Goes, E.S.R.; Souza, M.L.R.; Kimura, K.S.; Franco, M.C.; Verdi, R.; Mikcha, J.M.G. (2016). Inclusion of dehydrated mixture made of salmon and tilapia carcass in spinach cakes. *Acta Scientiarum. Technology*. 38(2): 241-246.
19. Kimura, K.S.; Souza, M.L.R.; Verdri, R.; Coradini, M.; Mikcha, J.M.G.; Goes, E.S.R. (2017). Nutricional, microbiological and sensorial characteristics of alfajor prepared with dehydrated mixture of salmon and tilapia. *Acta Scientiarum. Technology*, 39 (1): *in press*.
20. Fuzinato, M.M.; De Lima, D.P.; Andretto, A.P.; Menezes, L.A.; Souza, A.H.P.S.; Franco, M.L.R.; Steinmacher, N.C.; De Mendonça, S.N.T.G.; Vargas, L. (2015). Influence of a homeopathic product on performance and on quality flour and cookie (Grissini) of Tilapia. *African Journal of Pharmacy and Pharmacology*. 9(27):675-683.
21. Franco, M.L.R.; Abreu, B.B.; Saccomani, A.P.O.; Del Vesco, A.P.; Vieira, V.; Mikcha, J.M.G.; Gasparino, E.; Adelbem, A. (2013). Elaboración de cookies y galletas con inclusión de harina de pescado. *Infopesca Internacional*, 53: 30-33.
22. Brito, I.P.; Campos, J.M.; Souza, T.F.L.; Wakiyama, C.; Azeredo, G.A. (2004). Elaboração e avaliação global de barra de cereais caseira. *Boletim do Ceppa*, 22(1): 35-50.
23. Brazil – Ministério da Saúde (MS), Agência Nacional de Vigilância Sanitária (ANVISA). Resolução RDC nº 263, de 22 de setembro de 2005. Aprova o Regulamento técnico para produtos de cereais, amidos, farinhas e farelos. *Diário Oficial [da] República Federativa do Brasil*, Brasília, 22 set. 2005, Seção 1, pt. 1.
24. Arévalo-Pinedo, A.; Arévalo, Z.D.S.; Beserra, N.S.; Zuniga, A.D.G.; Coelho, A.F.S.; Pinedo, R.A. (2013). Desenvolvimento de barra de cereais à base de farinha de amêndoa de babaçu. *Revista Brasileira de Produtos Agroindustriais*, 15(4): 405-411.
25. Veit, J. C.; Freitas, M.; Reis, E. S.; Moore, O.; Finkler, J.K.; Boscolo, W.R.; Feiden, A. (2013). Desenvolvimento e caracterização de bolos de chocolate e de cenoura com filé de tilápia do Nilo (*Oreochromis niloticus*). *Alimentos e Nutrição Araraquara*, 23(3): 427-433.
26. Tavares, T.S.; Bastos, S.C.; Pimenta, M.E.S.G.; Pinheiro, A.C.M.; Fabricio, L.F.F.; Leal, R.S. (2010). Perfil sensorial de pão de forma enriquecido com farinha de matrixã (*Brycon lundii*). In: XIX Congresso de Pós-Graduação DA UFPA. Anais.
27. CENTENARO, G.S.; FEDDERN, V.; BONOW, E.T.; SALAS-MELLADO, M. (2007). ENRIQUECIMENTO DE PÃO COM PROTEÍNAS DE PESCADO. *Ciência e Tecnologia de Alimentos*, 27(3): 663-668.