



Orange-flavored soft drink with the addition of isolated whey protein

Mirian Souza Prado¹, Débora Francielly de Oliveira², Ivane Benedetti Tonial², Isabel Craveiro Moreira¹, Lúcia Felicidade Dias¹ and Alexandre Rodrigo Coelho^{1*}

¹Departamento de Tecnologia de Alimentos, Universidade Tecnológica Federal do Paraná, Avenida dos Pioneiros, 3131, 86036-370, Londrina, Paraná, Brazil. ²Departamento de Tecnologia de Alimentos, Universidade Tecnológica Federal do Paraná, Linha Santa Barbara, s/n, 85601-970, Francisco Beltrão, Paraná, Brazil. *Author for correspondence. E-mail: arcoelho@utfpr.edu.br

ABSTRACT. Current assay developed an orange-flavored soda pop with the addition of isolated whey protein, bottled in a 2L-polyethylene terephthalate container and stored at room temperature for 90 days. Physical, chemical, microbiological and sensorial analyses were conducted periodically on the product. The physicochemical analysis showed pH 3.53, 11.5°Brix and 224 mg of citric acid per 100 mL of the drink and the following proximal composition: protein 0.501%, humidity 88.9%, ash 0.084% and carbohydrates 10.5%. Microbiological analyses detected no microorganisms during the storage period of the drink. Sensorial analysis results had good acceptability. Results showed that the product is stable when stored at room temperature for 90 days. This beverage contains higher nutritional rates and the same calorie rates when compared to sodas and some oranges juices found on the consumer market.

Keywords: beverage, protein source, sensorial profile, shelflife.

Elaboração de um refrigerante, sabor laranja com adição de isolado proteico de soro de leite

RESUMO. Este trabalho teve como objetivo elaborar um refrigerante, sabor laranja com adição de isolado proteico de soro de leite (IPS), envasado em PET (poli tereftalato de etileno), com capacidade de 2 L armazenado em temperatura ambiente por 90 dias. Foram realizadas, periodicamente durante o armazenamento em temperatura ambiente, análises físico-químicas, microbiológicas e sensoriais do produto. Os resultados da análise sensorial tiveram boa aceitabilidade, situando-se entre os termos hedônicos 'gostei moderadamente' e 'gostei muito'. A análise físico-química apresentou pH 3,53, 11,5°Brix e 224 mg de ácido cítrico por 100 mL de bebida e com a seguinte composição proximal: proteínas 0,501%, umidade 88,9%, cinzas 0,084% e carboidratos 10,5 %. Durante o período de armazenamento a bebida obteve ausência de micro-organismo. Os resultados mostraram que este produto é estável, quando armazenado em temperatura ambiente por 90 dias. Desse modo, conclui-se que a bebida elaborada constitui uma alternativa na introdução de proteína de soro de leite no refrigerante, tornando assim uma bebida com o valor nutricional superior e com os mesmos valores de calorias comparados com refrigerante e alguns sucos de laranjas encontrados no mercado consumidor.

Palavras-chave: bebida, fonte proteica, perfil sensorial, vida de prateleira.

Introduction

Since Brazil is the third largest producer of soft drinks in the world, ranking only next to the United States and Mexico, the local soft drinks industry has been seeking alternatives to meet the demands of consumers for healthier products, such as the development of 'low calorie', 'diet', 'light' and the 'zero calorie' formulas. According to Lof and Weiderpass (2006), another highly promising area is the demand for functional foods, which are products that reduce the risk of chronic degenerative diseases.

Ready-to-drink beverages are one of the several segments within this functional food market. Following European and North American trends, sports drinks, such as ALLPROX[®], ISOPURE[®] and MY WHEY[®], claim an applicability of whey as a supplement which would directly act on protein skeletal muscle synthesis and consequently improve physical performance (BALDISSERA et al., 2011). According to Smithers (2008), the soluble proteins are the most important components in the serum since they contain all the essential amino acids, considered to be of high nutritional value.

Until the 1960s, milk whey was thought to be an industrial waste by cheese manufacturers (SMITHERS, 2008) and large quantities of this byproduct were disposed of in lakes and rivers, with great ecological concern due to the pollutant's high level of Biochemical Oxygen Demand-BOD (PINTADO et al., 1999). Indeed, one of the several proposals suggested in scientific studies was the possibility of using whey for the development of carbonated drinks to minimize the environmental impact (SMITHERS, 2008).

Current assay develops and sensorially evaluates an orange-flavored soda added with whey protein isolate, stored at room temperature for three months.

Material and methods

Formulation and processing of the soft drink

The basic formulation for the carbonated beverage comprised drinking water, Whey Protein Isolate (WPI), sucrose, simple syrup, orange juice (65°Brix, emulsion of essential orange oil with twilight yellow dye, citric acid, natural aroma of orange, anti-foaming dimethylpolysiloxane 20%, antioxidant ascorbic acid, preservatives sodium benzoate and potassium sorbate. Maximum and minimum limits established by Brazilian legislation were complied with (BRASIL, 2009).

Whey Protein Isolate (WPI) (Alibra®, Campinas, Brazil) contained the following ingredients: whey protein isolate powder with 80% protein (dry base), phosphoric acid and citric acid acidulants, and soy lecithin as emulsifier.

To understand the physicochemical characteristics of WPI, a solution was prepared with 1% protein (g 100 mL⁻¹) in 250 mL distilled water and the following analyses were performed: Total Soluble Solids, pH and titratable acidity. Further, turbidity and color were evaluated by comparing them to distilled water (AOAC, 1995).

Assay was carried out on a pilot scale, using the facilities and equipment from a beverages company situated at Astorga, Paraná State, Brazil.

Initially, the simple syrup was prepared from the dissolution of 62.5 Kg of crystal sugar in 100 L of water at 85-90°C, by means of continuous and moderate agitation. At this stage, the heating system in the dissolution tank was operated by water vapor circulation passing through a serpentine tube. Activated carbon was then added for the removal of color and strange smells in the syrup. The dissolving tank was closed and kept at 85°C for 15 minutes.

After pasteurization, the simple syrup was filtered onto plates of stainless steel coated with

diatomaceous earth, which acted as a filter bed. After filtration, the syrup was cooled to 26°C by a heat exchanger and transferred to a storage tank for corrections of total soluble solids, until it reached 58.5°Brix.

At the same time, 0.65% (g 100 mL⁻¹) of WPI and 0.005% (g 100 mL⁻¹) of anti-foaming agents dimethylpolysiloxane 20% were added in a 1000 L-stainless steel tank containing 40 L of water, and stirred with moderate but continuous agitation for 30 minutes at 26°C, for hydration. Immediately after the hydration period, 41.7 L of simple syrup and the following components (g 100 mL⁻¹) were added: orange juice (1.75%), potassium sorbate (0.013%), sodium benzoate (0.043%), citric acid (0.056%), sodium citrate (0.0085%), essential oil emulsion (0.09%), orange aroma (0.045%) and the antioxidant ascorbic acid (0.010%). The final volume of the beverage produced with water was 300 L at 14°C.

Finally, carbonation was initiated by injecting carbon dioxide at a pressure of 50 psi. The product was bottled in 2 L-polyethylene terephthalate (PET) containers and stored at room temperature for 90 days. A total of 100 samples of PET bottles were obtained.

Physicochemical properties

The physical and chemical analysis performed comprised Total Soluble Solids (TSS), pH, titratable acidity (as citric acid) and the volume of CO₂ (AOAC, 1995). Analyses were carried out every 7 days of storage, at room temperature. All analyses were performed in triplicate.

Moisture contents (105°C inside a hood), ash (muffler incinerator at 550°C) and total protein (f = 6.38) were analyzed by the micro-Kjeldahl method, following the Association of Official Analytical Chemists techniques (AOAC, 1995), while total lipids were extracted following Bligh and Dyer (1959).

Carbohydrate was calculated as the difference in percentage between the sums of the other nutrients: protein, lipids, and ash and moisture contents.

Energy rates of the beverage were calculated by the following conversion factors: carbohydrates 4 kcalg⁻¹ = 17 kJg⁻¹; proteins 4 kcalg⁻¹ = 17 kJg⁻¹; lipids 9 kcalg⁻¹ = 37 kJg⁻¹ (BRASIL, 2003).

Microbiological analysis

The Most Probable Number (MPN) of coliforms at 35°C, as established by Brazilian current legislation (BRASIL, 2001), was determined following AOAC (1995). The MPN of coliforms at 35°C was determined in the freshly prepared product and after 15, 30, 60 75

and 90 days of storage at room temperature. All analyses were performed in triplicate.

Sensorial analysis

The sensorial analysis was performed at the Laboratory of Sensorial Analysis of the Federal University of Technology-Parana, Londrina, Paraná State, Brazil, and included the following attributes: appearance, aroma, flavor and overall aspect.

A group of panelists with 50 untrained volunteers was selected for the acceptance test. Following ABNT (1998), the panelists recorded their scores on answer cards with a 9-point structured hedonic scale (1 = 'I dislike it very much' to 9: 'I like it very much'). The beverage samples were provided in a randomized complete block design and served individually at a temperature of 8-10°C, in 50 mL-disposable cups. A 5-point scale was used for the purchase intention test to verify whether people would buy the formulations developed (1 = 'I certainly would not buy this product', to 5 = 'I would definitely buy this product') (LAWLESS; HEYMANN, 2010). The soft drink was subjected to sensorial analysis after 2, 45 and 90 days of storage, at room temperature.

Statistical analysis

Statistical analyses were performed with Statistica 7.0 (STATSOFT, 2005). The results of the sensorial analyses were submitted to analysis of variance (ANOVA) and the means were compared using the 'post-hoc' Tukey's test. The significance level for rejection of the null hypothesis adopted in this analysis was 5% ($p < 0.05$).

Results and discussion

Physicochemical properties

The results of the physicochemical analysis of the protein isolate solution containing 1.0% protein ($\text{g } 100 \text{ mL}^{-1}$) in distilled water were: TSS $1.40 \pm 0.00^\circ\text{Brix}$; pH 3.30; titratable acidity $0.193 \pm 0.007 \text{ g}$ of citric acid 100 mL^{-1} ; turbidity $32.62 \pm 0.16 \text{ NTU}$ (Nephelometric Turbidity Unit); absence of color.

The presence of citric and phosphoric acid in WPI composition justifies acidic pH and high acidity. The low turbidity and the absence of color proved to be favorable for the development of a carbonated beverage. Thus, the whey protein isolate showed satisfactory physicochemical characteristics and suitable for the preparation of the soda pop.

The results of the proximal composition analysis for the orange flavored carbonated beverage with the addition of WPI were: lipids 0.00%; protein 0.50%;

ash 0.08%; moisture 88.90%; carbohydrates 10.50% and energy value 44 kcal. When the results of the drink with the rates on the labels of two samples of soda and one sample of orange-flavored juice bought on the market were compared, current formulated beverage proved to be nutritionally superior (data not shown). The drink prepared with the addition of WPI differentially contained 0.50% protein, unlike the commercial beverages with no protein content.

Table 1 shows that when 355 mL (can measurement) of the prepared beverage are taken, the child (1 to 10 years) and adult consumer would be ingesting, respectively, 13.8 to 5.3 and 3.6% of proteins, proportional to the percentage of the Recommended Daily Intake (RDI) of protein established by Brazilian legislation (BRASIL, 2005). Consequently, the WPI-added beverage and the significant percentage of IDR of protein would stimulate the preference for this type of soft drink by adults and children, when compared to soft drinks currently on the market, with no nutritional appeal.

Table 1. Recommended daily intake (RDI) of protein for consumption of a 355 mL can of the prepared soda pop.

	Consumer			
	Children			Adults
	Age 1-3	Age 4-6	Age 7-10	
Quant. Daily protein (g)*	13	19	34	50
% RDI of protein in 355 mL beverage	13.8	9.5	5.3	3.6

*Source: (BRASIL, 2005).

Thus, above data underscore the importance of the beverage when compared to that on the market. Coupled to the refreshing property of soda pops, the beverage would actually provide protein, an essential component to human diet, to the consumer.

The results of the physicochemical analysis of the drink during storage at room temperature for 90 days are shown in Figures 1, 2 and 3.

The total titratable acidity of the beverage increased during storage (Figure 1). Initially acidity was 224 mg of citric acid per 100 mL of the beverage and remained unchanged up to 7 days of storage. After this period there was a brief increase in acidity, with a rate of $301 \text{ mg } 100 \text{ mL}^{-1}$ of the beverage after 90 days of storage. Although the beverage presented an increase of $77 \text{ mg } 100 \text{ mL}^{-1}$ of drink after 90 days of storage, the results comply with current Brazilian legislation, which sets a minimum rate of 100 mg citric acid per 100 mL of soda pop.

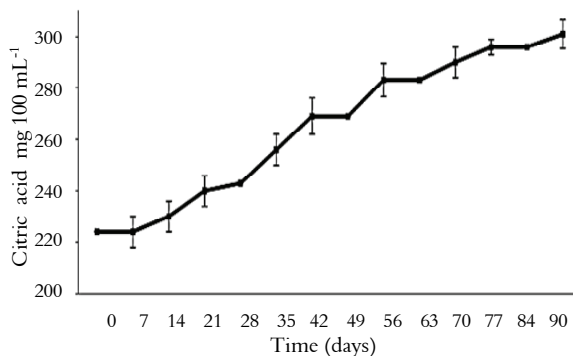


Figure 1. Total acidity (as citric) of orange soft drink during 90 days storage at room temperature. Standard deviations of each point are represented by error bars.

Figure 2 shows the results of pH changes during the storage period for the carbonated beverage. The results, where pH rates ranged from 3.52 to 3.59, were expected. According to and Correa and Faria (2003), pH rates in pasteurized orange juice showed no variation after storage in PET bottles during 28 days at 4°C.

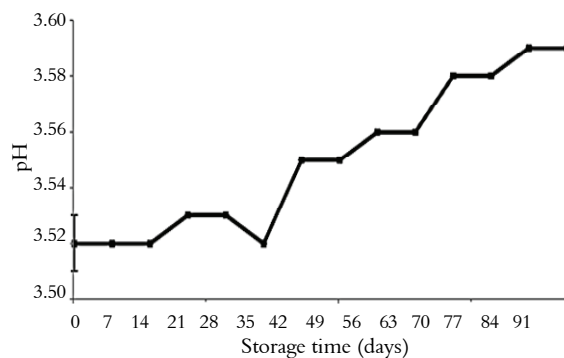


Figure 2. Variation of pH in carbonated beverage during 90 days of storage at room temperature.

The volume of carbon dioxide is defined as the amount of gas dissolved in a volume of water at atmospheric pressure (760 mmHg) at 15.5°C. According to Brazilian Legislation, carbon dioxide should be industrially pure and in dissolved minimum quantity of 1.0 volume (V) in soft drink (BRASIL, 2009).

In the beverage currently developed, the volume of carbon dioxide after the preparation of the product was 3.8 V, decreasing to 3.7 after 42 days and reaching 2.7 V after 90 days of storage (Figure 3). The loss of carbon dioxide in the drink during storage is an important factor to be considered in quality control. This fact may be due to the PET bottle used for bottling of the beverage.

According to Ashurst (2010), the PET packaging tends to lose CO₂ within a shorter storage period when compared to glass or aluminum containers.

This fact is due to its greater porosity which causes the gases to escape through the container's pores, commonly known as effusion.

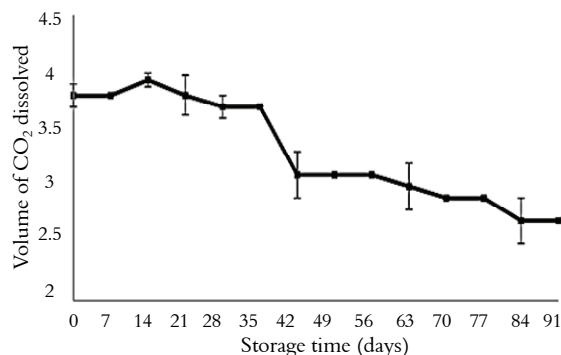


Figure 3. Volume of dissolved CO₂ in the beverage in a 90-day-storage period at room temperature. Standard deviations of each point are represented by error bars.

Siqueira et al. (2009) analyzed the loss of CO₂ in cola beverages bottled in aluminum cans and in PET bottles after 70 days of storage. The authors reported a greater loss of CO₂ from sodas bottled in PET than from those in aluminum cans. At the start of storage, the cola beverage in the PET container had 4.2 mL CO₂ L⁻¹ and ended up at 3.4 mL CO₂ L⁻¹. On the other hand, the cola beverage in the aluminum can began storage at 3.8 mL CO₂ L⁻¹ and ended at 3.52 mL CO₂ L⁻¹.

Although the volume of CO₂ has decreased to 3.8 at time zero to 2.7 after 90 days of storage, the product still complied with Brazilian legislation, which establishes the minimum quantity of dissolved CO₂ 1.0 volume (BRASIL, 1998).

Since the total soluble solids of 11.5°Brix of the beverage did not change during storage time, this fact indicated satisfactory quality control and hygienic conditions of the product.

Microbiological analysis

Microbiological analysis indicated the absence of coliforms at 35°C during the 90 days of storage. Thus, the beverage was within the standards established by Brazilian legislation (BRASIL, 2001). The beverage's microbiological quality indicated that the health-sanitary conditions of the thermal processing of the syrup, the employment of quality crude material, appropriate handling conditions and additives, sodium benzoate and potassium sorbate, and carbonation (3.8 V) were effective for the preservation of the product for a period of 90 days, kept at room temperature.

Sensorial analysis

Table 2 shows the profile of the non-trained tasters who participated in the Test of Acceptability for the beverage during the 3 storage times under analysis. Tasters were predominantly females, 18 – 27 years age bracket, for stages 2 and 90 days of storage; males were predominant with 56 % and females with 44% in stage 45 days of storage.

Table 2. Percentage (%) of gender and age group of untrained tasters at 3 storage times.

Profile of Tasters	Storage Time		
	2 Days	45 Days	90 Days
Female	58	44	54
Male	42	56	46
Age 18 - 27	76	90	94
Age 28 - 37	12	8	4
Age 38 - 47	12	2	2

The results from the sensorial analysis of the beverage at days 2, 45 and 90 of storage, performed by non-trained tasters, are given in Table 3. In this context, acceptability tests were carried out with a 9-point hedonic scale with the following sensorial attributes: appearance, aroma, flavor and overall impression. The consumer purchasing attitude was also evaluated by means of a 5-point scale (Table 4).

Table 3. Average rates and standard deviation of sensorial attributes and beverage during the storage period.

Sensorial Attributes	Storage Time		
	2 Days	45 Days	90 Days
Appearance	8.18±0.7197 ^a	8.0±0.7284 ^a	8.12±0.8722 ^a
Aroma	7.64±1.0451 ^a	7.05±1.2930 ^b	7.22±1.1800 ^{ab}
Flavor	7.42±1.1082 ^a	6.68±1.7313 ^b	7.04±1.3242 ^{ab}
Overall Impression	7.68±0.9134 ^a	7.34±1.0806 ^a	7.22±1.0160 ^a

Mean ± standard deviation. The same small letters in the same line are not significantly different by Tukey's test ($p > 0.05$).

In the evaluation carried out at storage times, the overall impression and appearance did not have any significant difference, indicating that the product did not undergo any changes in the overall aspect of the stored product. A comparative analysis between times 2 and 45 of storage, the averages for the attributes aroma and flavor showed a statistically significant decline ($p < 0.05\%$); however in the sensorial evaluation at 90 days of storage, the averages of the attributes aroma and flavor did not differ statistically from the evaluations performed at 2 and 45 days of storage.

Table 4. Average rates and standard deviation of the purchase attitude for the product during the storage period.

Storage Time /	2 Days	45 Days	90 Days
Purchase Attitude	4.22±0.6158 ^a	3.38±0.9852 ^b	4.02±0.1443 ^a

Mean ± standard deviation. The same small letters in the same line are not significantly different by Tukey's test ($p > 0.05$).

The significant difference in averages for aroma and flavor during 2 and 45 days of storage may be explained by a larger number of male tasters (56%) in the acceptability test on the 45 days of storage, whereas tasters were predominantly female for the tests of days 2 and 90 of storage (Table 4).

The product developed presented an average higher than 7.0 in the analysis for all sensorial attributes, keeping to the hedonic terms - 'I moderately liked it' and 'I liked it very much', except for the flavor attribute on day 45 of storage. The attribute appearance remained between the terms 'I quite enjoyed it' and 'I liked it very much', with an average higher than 8.

As a rule, averages for all sensorial attributes remained within the acceptance range, with no drastic reduction on averages for the final storage time. Consequently, the product may be sold after 90 days of storage without any modifications in its sensorial characteristics. Further studies are suggested on the acceptance of the product after longer storage periods, following the conventional expiry dates for soft drinks.

The consumer purchasing attitude provided significant differences during storage (Table 5). The average of 3.38 on purchasing attitude at period 45 days of storage, in which the tasters were predominantly male, lies between the terms 'I am not sure if I would buy this product' and 'I would probably buy this product'. As for periods 2 and 90 days of storage the average were respectively 4.22 and 4.02, between the terms 'I would probably buy this product' and 'I would certainly buy this product'.

Conclusion

The physicochemical and microbiological analyses carried out throughout 90 days of storage complied with the established standards for soft drinks. The soft drink showed good acceptability by the sensorial analysis along the 90-day-storage and revealed that it would probably be easily accepted by consumers.

Finally, the soft drink prepared in current assay is a viable and cost-effective alternative for the introduction of whey protein in sodas. In fact, it constitutes a beverage with a higher nutritional and differentiated value from those commonly available on the market.

Acknowledgements

The authors would like to thank the Federal University of Technology-Parana, Londrina, the Directors of Research and Post-graduate studies -

DIRPPG-Londrina, and the Post-graduate Program in Food Technology-PPGTAL, for their financial support.

References

- ABNT-Associação Brasileira de Normas Técnicas. **NBR 14141**. Escalas utilizadas em Análise sensorial de alimentos e bebidas. Rio de Janeiro: ABNT, 1998.
- AOAC-Association of Official Analytical Chemists. Method 985.35. **Official methods of analysis of AOAC international**. 16th ed. Washington, D.C.: AOAC, 1995.
- ASHURST, P. R. Packaging and the shelf life of water and carbonated drinks. In: ROBERTSON, G. L. (Ed.). **Food packaging and shelf life: a practical guide**. London: CRC Press; Boca Raton, 2010. p. 157-178.
- BALDISSERA, A. C.; BETTA, F. D.; PENNA, A. L. B.; LINDNER, J. D. Alimentos funcionais: uma nova fronteira para o desenvolvimento de bebidas protéicas a base de soro de leite. **Semina: Ciências Agrárias**, v. 32, n. 4, p. 1497-1512, 2011.
- BLIGH, E. G.; DYER, W. J. A rapid method of total lipid extraction and purification. **Canadian Journal of Biochemistry and Physiology**. v. 37, n. 8, p. 911-917, 1959.
- BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Decreto 6871, de 4 de junho de 2009. Padronização, classificação, registro, inspeção, produção e fiscalização de bebidas. **Diário Oficial da União**, Brasília, 4 de junho de 2009.
- BRASIL. Ministério da Saúde, Agência Nacional de Vigilância Sanitária. Resolução RDC n.º 269. Aprova o regulamento técnico sobre a ingestão diária recomendada (IDR) de proteína, vitaminas e minerais. **Diário Oficial da União**, Brasília, 22 de setembro de 2005.
- BRASIL. Ministério da Saúde, Agência Nacional de Vigilância Sanitária. Resolução RDC n.º 360, Regulamento técnico sobre rotulagem nutricional de alimentos embalados. **Diário Oficial da União**, Brasília, 23 de dezembro de 2003.
- BRASIL. Ministério da Saúde, Agência Nacional de Vigilância Sanitária. Resolução RDC n.º 12. Aprova o regulamento técnico sobre padrões microbiológicos para alimentos. **Diário Oficial da União**, Brasília, 2 de janeiro de 2001.
- BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Portaria n.º 544. Aprova os Regulamentos técnicos para fixação dos padrões de Identidade e qualidade, para refresco, refrigerante, preparado ou concentrado líquido para refresco ou refrigerante, preparado sólido para refresco, xarope e chá pronto para o consumo. **Diário Oficial da União**, Brasília, 16 de novembro de 1998.
- CORREA NETO, R. S.; FARIA, J. A. F. Alterações químicas e enzimáticas em suco de laranja pasteurizado. **Revista Higiene Alimentar**, v. 17, n. 114/115, p. 60-67, 2003.
- LAWLESS, H. T.; HEYMANN, H. **Sensory evaluation of food: principles and practices**. 2nd ed. Berlin: Springer, 2010.
- LOF, M.; WEIDERPASS, E. Epidemiologic evidence suggests that dietary phytoestrogen intake is associated with reduced risk of breast, endometrial, and prostate cancers. **Nutrition Research**, v. 26, n. 12, p. 609-619, 2006.
- PINTADO, M. E.; PINTADO, A. E.; MALCATA F. X. Controlled whey protein hydrolysis using two alternative proteases. **Journal of Food Engineering**, v. 42, n. 1, p. 1-13, 1999.
- SIQUEIRA, M. I. D.; PACHECO, A. R.; COBUCCI, R. M. A. Influência da carbonatação no sabor de refrigerante tipo cola. **Estudos**, v. 36, n. 5, p. 765-774, 2009.
- SMITHERS, G. W. Whey and whey proteins: from 'gutter to gold'. **International Dairy Journal**, v. 18, n. 7, p. 695-704, 2008.
- STATSOFT. **Data Analysis Software**. Version 7. Tulsa: Statsoft. Inc., 2005.

Received on December 9, 2014.

Accepted on January 9, 2015.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.