Powdered beverage mix with acerola pulp, whey and maltodextrin¹

Bebida composta em pó de polpa de acerola, soro lácteo e maltodextrina

Natalia Duarte de Lima², Marcos Rodrigues Amorim Afonso^{3*}, José Maria Correia da Costa³ and Juliane Doering Gasparin Carvalho³

ABSTRACT - Mixing acerola pulp with whey, a by-product of the dairy industry, led to this study, whose aim was the development of a powdered beverage mix of acerola pulp, whey and maltodextrin (DE20). To determine the proportions of the mixture, a simplex-centroid mixture design was used. Different proportions of the mixture were dehydrated in a spray dryer at $175\,^{\circ}$ C. The results were evaluated in relation to hygroscopicity, moisture and ascorbic acid. Higher proportions of whey and pulp increased hygroscopicity and moisture, while maltodextrin reduced these parameters. An increase in pulp content raised the levels of ascorbic acid. The proportions (m m⁻¹) of 50% acerola pulp, 25% whey and 25% maltodextrin resulted in significantly lower hygroscopicity (8.25 g $100\,\mathrm{g}^{-1}$) and moisture (1.66%), and appreciable levels of ascorbic acid (1492.49 g $100\,\mathrm{g}^{-1}$). The results indicated that the powder obtained shows good stability after 75 days of storage, with 9.20%, 1.79% and $1146.13\,\mathrm{g}\,100\,\mathrm{g}^{-1}$ of hygroscopicity, moisture and ascorbic acid respectively.

Key words: Spray dryer. Mixture design. Stability.

RESUMO - A mistura de polpa de acerola com soro lácteo, subproduto da indústria de laticínios, conduziu a base deste trabalho, cujo objetivo foi desenvolver uma bebida composta em pó de polpa de acerola, soro lácteo e maltodextrina (dextrose equivalente 20). Para determinação das proporções da mistura foi utilizado um delineamento de misturas de tipo Simplex Centroide. Diferentes proporções da mistura foram desidratadas em secador tipo spray dryer à 175 °C. Os resultados foram avaliados em relação a higroscopicidade, umidade e ácido ascórbico. Maiores proporções de soro e polpa, elevaram a higroscopicidade e umidade, enquanto que a maltodextrina reduziu estes parâmetros. O aumento do teor de polpa elevou os valores de ácido ascórbico. Proporções (m m⁻¹) de 50% polpa de acerola, 25% soro lácteo e 25% maltodextrina, resultaram em higroscopicidade (8,25 g 100g⁻¹) e umidade (1,66 %) significativamente baixos e valores apreciáveis de ácido ascórbico (1492, 49 g 100g⁻¹). Os resultados indicaram que o pó obtido possui boa estabilidade após 75 dias de armazenamento com 9,20 %, 1,79 % e 1146,13 g 100 g⁻¹ de higrocopicidade, umidade e ácido ascórbico, respectivamente.

Palavras-chave: Spray dryer. Delineamento de misturas. Estabilidade.

DOI: 10.5935/1806-6690.20190068

^{*}Author for correspondence

Received for publication 06/12/2016; approved on 23/04/2019

Parte da Dissertação do primeiro autor, apresentado ao Curso de Pós-Graduação Ciências e Tecnologia de Alimentos, Universidade Federal do Ceará/ UFC

²Universidade Federal do Ceará/UFC, Campus do Pici, Fortaleza-CE, Brasil, nataliaduartelima@gmail.com (ORCID ID 0000-0003-2191-6715)

³Departamento de Engenharia de Alimentos, Universidade Federal do Ceará/UFC, *Campus* do Pici, Fortaleza-CE, Brasil, m.r.a.afonso@gmail.com (ORCID ID 0000-0002-8149-6276), correiacostaufc@gmail.com (ORCID ID 0000-0002-6266-6778), julianedgc@gmail.com (ORCID ID 0000-0002-0199-7864)

INTRODUCTION

The challenge facing the food industry today is to seek alternatives for producing healthier foods, with no resulting price increase for consumers. Acerola is known for its ascorbic acid content, which varies from 2164 mg 100 g⁻¹ to 1074 mg 100 g⁻¹ depending on maturation stage (VENDRAMINI; TRUGO, 2000).

Whey is a by-product of the dairy industry that has aroused the interest of many researchers around the world due to its nutritional, functional and economic potential (ANTUNES; CAZETTO; BOLINI, 2004). According to Baldasso, Barros and Tessaro (2011), whey can be obtained by processing cheese through the action of rennin or acid precipitation (it contains 4 to 6 g of protein per litre). Nowadays, it can be found in the form of powder, protein concentrates, whey protein (PELEGRINE; CARRASQUEIRA, 2010; PERRONE *et al.*, 2013), or added to beverages (CRUZ *et al.*, 2009; LUIZ *et al.*, 2014).

Spray drying, when applied under ideal conditions, has proved to be effective in obtaining various products. However, optimisation is vital in order to obtain powdered products with better sensory and nutritional characteristics, as well as achieving good yields from the process (ROCHA *et al.*, 2014).

The idea of mixing acerola pulp, a tropical product, with whey, a by-product of the dairy industry, led to this study. The aim was to develop a powdered beverage mix of acerola pulp, whey and maltodextrin, using a mixture design to determine the best proportions of the three components, in relation to hygroscopicity, moisture and ascorbic acid. In addition, the stability of the powder was checked for the best proportions that were determined.

MATERIAL AND METHODS

Acerola pulp, without the addition of preservatives, was obtained from local markets in Fortaleza, Ceará, and the liquid whey from a dairy in the metropolitan area. Both were transported in isothermal boxes to the Refrigeration Laboratory of the Federal University of Ceará, kept in a vertical freezer at -18 °C, and thawed in a refrigerator (5 °C) for 18 h. Corn maltodextrin DE20 was used as a drying aid.

To evaluate the powdered mix, an augmented simplex-centroid mixture design was adopted. The proportions of the mixture were defined following this mixture-design methodology (Table 1), and adopting a restriction on the minimum and maximum limits, of 80 g and 233.33 g for the acerola pulp and whey, and

20 g and 200 g for the maltodextrin. Each test totalled 400 g of sample added to the acerola pulp, whey and maltodextrin. The hygroscopicity, moisture and ascorbic acid of the powder were evaluated as response variables. Samples of the mixture were placed in a model LM MSD 1.0 spray dryer (Labmaq do Brasil), which had a 1.2-mm diameter nozzle, hot-air flow parameters of 3.7 m³ min⁻¹, air-inlet temperature of 175 °C, a feed rate of 0.50 L h⁻¹, and compressed-air flow of 3.0 L min⁻¹.

The regression models obtained for the response variables from the mixture design were evaluated by analysis of variance using the F-test (p<0.05) and coefficients of determination (R²). Ternary diagrams were used to analyse the behaviour of each variable as a function of the proportions of the three components of the mixture.

The powders were evaluated in the Food Quality Control and Drying Laboratory of the Federal University of Ceará. The hygroscopicity was analysed as per Goula and Adamopoulos (2008), with modifications. About 1 g of the powder was added to a petri dish, which was placed for 90 minutes in a desiccator at 23 °C and a relative humidity of 76%, using saturated NaCl solution instead of saturated HNO₃ solution, and weighed at 10-minute intervals. Values were expressed as a percentage, in grams of water absorbed per gram of solids from the sample. The moisture and ascorbic-acid content of the powders were determined as per the Association of Official Analytical Chemists (2005), with values expressed as percentage moisture and mg of ascorbic acid per 100 g of sample.

To study the stability, samples of the powders were vacuum sealed in laminated aluminium and PET containers (180 x 80 cm), and stored at a mean ambient temperature of 25 °C and a relative humidity of 75%. The samples were analysed every 15 days during the 75-day storage period. Tukey's test was used to verify differences between the mean values during the period of analysis.

RESULTS AND DISCUSSION

Values for hygroscopicity ranged from 13.69 to 6.21%. The maximum value for this variable was obtained in the test containing the greatest amount of liquid whey. The mixture showing the minimum value for hygroscopicity contained 100 g of acerola pulp, 100 g of whey and 200 g of maltodextrin.

The analysis of variance (ANOVA) for the regression model (Eq.1) describing the hygroscopic behaviour of the mixture was significant (p<0.05) by F-test, with a coefficient of determination (R²) of 0.62. All

the coefficients shown in Equation 1 were significant (p<0.05).

$$H = 0.0232a + 0.0314s - 0.00111m \tag{1}$$

where:

H - hygroscopicity (%)

a - acerola pulp (g)

s - whey (g)

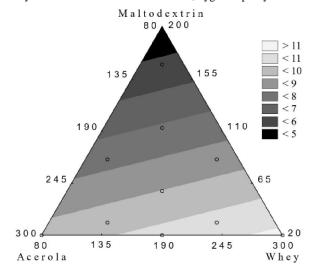
m - maltodextrin (g)

The ternary diagram (Figure 1) was generated from the regression model (Eq. 1) for hygroscopicity. Figure 1 shows that lower values for hygroscopicity in the powders are obtained with increases in the concentration of maltodextrin. This behaviour was similar to that found by Oliveira, Costa and Afonso (2014) in powdered freezedried cajá pulp; by Ferrari, Ribeiro and Aguirre (2012) in powdered blackberry pulp; and by Canuto, Afonso and Costa (2014) in powdered papaya pulp.

From Figure 1 it can be seen that the highest values for hygroscopicity were obtained in the trials with the greatest proportions of whey. This can be explained since, according to Perrone *et al.* (2013), the drying process of dairy products involves the formation of lactose in the amorphous state, which is highly hygroscopic. It was found that the greater the number of whey and acerolapulp aggregates in the mixture, the higher the values of this variable.

The results for the moisture content of the powdered beverage mix ranged from 1.56 to 2.21% (Table 1), similar to the work of Rocha *et al.* (2014), whose values varied from 1.36 to 3.35% for powdered cashew juice obtained with a spray dryer. The ANOVA

Figure 1 - Ternary diagram of the powdered mix of acerola pulp, whey and maltodextrin for the variable, hygroscopicity



for the regression model (Eq. 2) that describes the moisture behaviour of the mixture showed a significant F-test (p<0.05) and an R^2 of 0.71. The coefficients represented in Equation 2 were significant (p<0.05). As such, the moisture response due to interaction between the components of the mixture can be seen in Figure 2.

$$U = 0.00441a + 0.00563s - 0.00252m \tag{2}$$

where:

U - moisture (%)

a - acerola pulp (g)

s - whey (g)

m - maltodextrin (g)

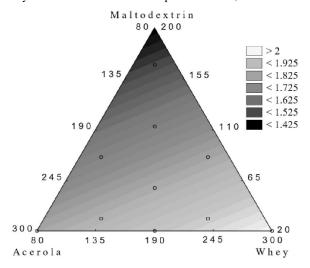
Table 1 - Mean results for hygroscopicity, moisture and ascorbic acid of the powdered mix of acerola pulp, whey and maltodextrin

Acerola Pulp (g)	Whey (g)	Maltodextrin (g)	Hygroscopicity (%)	Moisture (%)	Ascorbic Acid (mg 100 g ⁻¹)*
300.00	80.00	20.00	10.31 ± 0.34	1.86 ± 0.01	3299.96 ± 5.78
80.00	300.00	20.00	13.69 ± 0.28	2.21 ± 0.07	1176.43 ± 0.66
100.00	100.00	200.00	6.21 ± 0.16	1.65 ± 0.02	401.77 ± 0.04
190.00	190.00	20.00	10.32 ± 0.21	2.08 ± 0.07	2533.18 ± 5.70
200.00	100.00	100.00	7.61 ± 0.63	1.65 ± 0.05	1238.62 ± 2.82
100.00	200.00	100.00	7.44 ± 0.12	1.74 ± 0.03	760.32 ± 0.83
166.67	166.67	66.67	6.60 ± 0.53	1.88 ± 0.06	1337.09 ± 2.69
233.33	133.33	33.33	10.25 ± 0.20	1.82 ± 0.04	2628.56 ± 5.45
133.33	233.33	33.33	8.60 ± 0.02	1.80 ± 0.01	1350.43 ± 1.60
133.33	133.33	133.33	7.48 ± 0.15	1.56 ± 0.05	850.41 ± 0.50

^{*}Dry basis values

In Figure 2, it can be seen that the greater the concentration of whey and acerola pulp, the higher the values for moisture. An increase in the concentration of maltodextrin resulted in a powder with less moisture. These results are similar to those found by Oliveira, Costa and Afonso (2014) and Ferrari, Ribeiro and Aguirre (2012), when drying cajá pulp in a freeze dryer and blackberry pulp in a spray dryer respectively.

Figure 2 - Ternary diagram of the powdered mix of acerola pulp, whey and maltodextrin for the response variable, moisture



Mean values for ascorbic acid (Table 1) ranged from 3299.96 to 401.77 mg 100 $\rm g^{-1}$ powder (db). Ribeiro, Costa and Afonso (2015) found 2448.24 mg 100 $\rm g^{-1}$ (db) in powdered acerola pulp containing 19.1% maltodextrin. The maximum value was obtained in the test with the maximum concentration of acerola pulp, and the minimum value in the test with the maximum concentration of maltodextrin, both as defined by the mixture design. This behaviour was expected, due to the acerola pulp being the only source of ascorbic acid in the mixture.

The quadratic regression model for the analysis of variance (Eq. 3) that predicts the behaviour of ascorbic acid in the mixture presented a significant F-test (p<0.05) and an R^2 of 0.98. All the coefficients shown in Equation 3 were significant (p<0.05). Figure 3 shows the behaviour of this variable for the proportion of components in the mixture. A region with high values for ascorbic acid can be seen. In this region, the acerola pulp is found at the greatest concentrations, whereas high proportions of whey and maltodextrin have a reducing effect.

$$AA = 11,57a + 0,1189b + 7,633m + 0,01249a.s$$
$$-0,08969a.m - 0,02860b.m \tag{3}$$

where:

AA - ascorbic acid content (mg 100 g⁻¹)

a - acerola pulp (g)

s - whey (g)

m - maltodextrin (g)

The behaviour seen in Figure 3 agrees with that of Faraoni *et al.* (2012) in their study of mixture design in a mixture of mango, acerola and guava juice. Acerola can be used in powdered beverage mixes to increase the vitamin C content of these products.

For the three variables under study, it was found that there was no coincident region that presented a minimum value for hygroscopicity and moisture with a maximum value for ascorbic acid. As such, the test containing 200 g of acerola pulp, 100 g of whey and 100 g of maltodextrin was defined to study the stability. This formulation showed a high ascorbic acid content (1238.62 mg 100 g⁻¹) and low values for hygroscopicity (7.61%) and moisture (1.65%), favouring the physical and chemical conditions of the powder during storage. In addition, the formulation meets the minimum limits specified for a powdered beverage mix, which should contain at least 50% products of plant origin (BRAZIL, 2013).

The mixture with the chosen proportions was redried, and a study made of the stability of the powder (Table 2). The values obtained for hygroscopicity were close to those found by Jaya and Das (2004), and Oliveira, Costa and Afonso (2014), of between 5.13 and 9.38% for powdered mango, and between 8.51 and 12.93% for powdered cajá respectively.

Figure 3 - Ternary diagram of the powdered mix of acerola pulp, whey and maltodextrin for the response variable, ascorbic acid

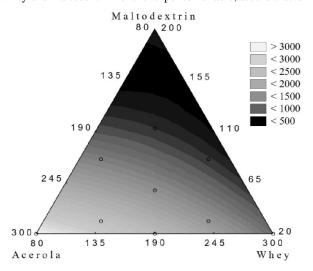


Table 2 - Stability of the powdered mix of acerola pulp, whey and maltodextrin for hygroscopicity, moisture and ascorbic acid

Time (days)	Hygroscopicity (%)	Moisture (%)	Ascorbic Acid (mg 100 g ⁻¹)*
0	$8.25a \pm 0.39$	$1.66b \pm 0.01$	1492.49 a ± 57.11
15	$8.83a \pm 0.57$	$1.78a \pm 0.06$	$1404.78 \text{ b} \pm 22.09$
30	$8.63a \pm 0.90$	$1.77a \pm 0.02$	$1340.77 \text{ b} \pm 22.31$
45	$9.01a \pm 0.53$	$1.77a \pm 0.00$	$1339.05 \text{ b} \pm 22.11$
60	$9.54a \pm 0.83$	$1.84a \pm 0.01$	$1158.76 c \pm 20.19$
75	$9.20a \pm 0.93$	$1.79a \pm 0.04$	$1146.13 \text{ c} \pm 22.69$

^{*}Dry basis values. Mean values followed by the same letter in a column do not differ statistically (p<0.05)

Higher values for hygroscopicity were found by Ferrari, Ribeiro and Aguirre (2012) when drying blackberry in a spray dryer, with values of between 18.77 and 29.51%. The amount, composition and structure of the different sugars in the pulps, the moisture content and the presence of drying aids, such as maltodextrin, explain the different values for hygroscopicity found in the literature for powdered fruit products.

Values for hygroscopicity of the powdered mix of acerola pulp, whey and maltodextrin showed no statistical difference (p<0.05) by Tukey's test for the number of days of storage (Table 2). Fernandes *et al.* (2014) explained that the addition of albumin to the foam mat when drying powdered tomato pulp reduced the influence of sugars and organic acids, which are highly hydrophilic and contribute to hygroscopicity. In this research, the maltodextrin in the powder may have been a determining factor in stabilising hygroscopicity.

There was a difference (p>0.05) in the moisture content of the powder during storage (Table 2) between the first day and the remaining days only, with a 7.83% increase in moisture after 75 days. The moisture content was stable from day 15, which may have had an effect on stability with respect to the hygroscopicity of the powder. Gomes, Figueirêdo and Queiroz (2004) saw an increase of 51.31% in the moisture content of powdered acerola after 60 days packed in transparent polyethylene film.

Endo *et al.* (2007) saw an increase in moisture of 1.16 to 1.66% in the powder obtained with a spray dryer from passion-fruit juice with added sucrose after 60 days of storage at 30°C in *metallised biaxially oriented polypropylene*. The authors proved the efficiency of using sucrose, which is less hygroscopic than the sugars naturally present in the juice, to reduce any increase in the moisture level of the powder during storage. Alexandre *et al.* (2014) found an increase in moisture of between 20.11 and 23.21% in powdered pitanga stored for 60 days in PET-PP plastic film at 25 °C and an average relative humidity of 75%. The increase in moisture of these

products depends, among other factors, on the sugars present in the powders, the addition of drying aids and the permeability of the packaging. Aluminium-PET composite laminated packaging was used in this research, as it has low permeability and is recommended for use in dehydrated foods, despite possible variations in the efficiency of the welding.

Mean values for the ascorbic acid content of the powder (Table 2) showed a significant difference (p<0.05) by Tukey's test for days of storage, with a reduction of 23.21% after 75 days. Gomes, Figueiredo and Queiroz (2004) saw a reduction of 29.73% in the ascorbic acid content of powdered green acerola after 60 days. Whereas, Alexandre *et al.* (2014), found a loss of 16.46% ascorbic acid in powdered pitanga stored for 60 days in PET-PP plastic film.

This loss of ascorbic acid can be explained by its instability, the result of variations in pH, temperature, moisture content, oxygen and light (MARQUES; FERREIRA; FREIRE, 2007). This parameter can therefore be used as a nutritional quality index during food processing and storage.

In view of the values obtained for ascorbic acid (Table 2), and knowing that for adults the recommended daily intake of vitamin C is 45 mg (BRASIL, 2005), it can be seen that despite losses after 75 days of storage, a 5 g serving of the powdered beverage mix still provides the established RDI, characterising this product as a good source of that vitamin.

CONCLUSIONS

- 1. The powdered beverage mix with the best characteristics for hygroscopicity, moisture and ascorbic acid contained a 50, 25 and 25% (m m⁻¹) proportion of acerola pulp, whey and maltodextrin;
- 2. A higher proportion of whey and pulp increased hygroscopicity and moisture, while an increase in

- maltodextrin reduced these parameters, favouring product stability;
- 3. The powdered beverage mix has nutritional appeal and presents good stability during the 75 days of storage, showing a slight increase in moisture, a reduction in ascorbic acid content, and hygroscopic stability.

REFERENCES

ALEXANDRE, H. V. *et al.* Armazenamento de pitanga em pó. **Comunicata Scientiae**, v. 5, n. 1, p. 83-91, 2014.

ANTUNES, A. E. C.; CAZETTO, T. F.; BOLINI, H. M. A. Iogurtes desnatados probióticos adicionados de concentrado protéico do soro de leite: perfil de textura, sinérese e análise sensorial. **Alimentos e Nutrição**, v. 15, n. 2, p. 107-114, 2004.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Official methods of analysis of the Association Analytical Chemists. 18. ed. Washington, 2005. 1298 p.

BALDASSO, C., BARROS, T. C., TESSARO, I. C. Concentration and purification of whey proteins by ultrafiltration. **Desalination**, v. 278, n. 3, p. 381-386, 2011.

BRASIL. Agência Nacional de Vigilância Sanitária. Resolução RDC nº 269, de 22 de setembro de 2005. Dispõe sobre o Regulamento Técnico sobre a ingestão diária recomendada (IDR) de proteína, vitaminas e minerais. **Diário Oficial da União**, Poder Executivo, Brasília, 23 set. 2005.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 17, de 19 de junho de 2013. Padrões de Identidade e Qualidade para refresco, refrigerante, bebida composta, chá pronto para consumo e soda. **Diário Oficial da União**, Poder Executivo, Brasília, 20 jun. 2013.

CANUTO, H. M. P.; AFONSO, M. R. A.; COSTA, J. M. C. Hygroscopic behavior of freeze-dried papaya pulp powder with maltodextrin. **Acta Scientiarum Technology**, v. 36, n. 1, p. 179-185, 2014.

CRUZ, A. G. *et al.* Milk drink using whey butter cheese and acerola juice as a potential source of vitamin C. **Food Bioprocessing Technology**, v. 2, p. 368-373, 2009.

ENDO, E. *et al.* Avaliação da vida de prateleira do suco de maracujá (*Passiflora edullis f. flavicarpa*) desidratado. **Revista Ciência e Tecnologia de Alimentos**, v. 27, n. 2, p. 382-386, 2007

FARAONI, A. S. *et al.* Desenvolvimento de um suco misto de manga, goiaba e acerola utilizando delineamento de misturas. **Ciência Rural**, v. 42, n. 5, p. 911-917, 2012.

FERNANDES, R. V. B. *et al.* Estudo da adição de albumina e da temperatura de secagem nas características de polpa de tomate

em pó. **Semina: Ciências Agrárias**, v. 35, n. 3, p. 1267-1278, 2014

FERRARI, C. C.; RIBEIRO, C. P.; AGUIRRE, J. M. Secagem por atomização de polpa de amora-preta usando maltodextrina como agente carreador. **Brazilian Journal Food Technology**, v. 15, n. 2, p. 157-165, 2012.

GOMES, P. M. A.; FIGUEIRÊDO, R. M. F.; QUEIROZ, A. J. M. Armazenamento de polpa de acerola em pó a temperatura ambiente. **Ciência e Tecnologia de Alimentos**, v. 24, n. 3, p. 384-389, 2004.

GOULA, A. M.; ADAMOPOULOS, K. G. Effect of maltodextrin addition during spray drying of tomato pulp in dehumidified air: II. powder properties. **Drying Technology**, v. 26, p. 726 -737, 2008.

JAYA, S.; DAS, H. Efect of maltodextrin, glycerol monostearate and tricalcium phosphate on vaccum dried mango powders properties. **Journal of Food Engineering**, v. 63, p. 125-134, 2004.

LUIZ, L. M. P. *et al.* Conservação à temperatura ambiente de uma bebida à base de soro de leite envasada a quente. **Ciência Rural**, v. 44, n. 11, p. 2090-2094, 2014.

MARQUES, L. G.; FERREIRA, M. C.; FREIRE, J. T. Freezedrying of acerola (*Malpighia glabra* L.). **Chemical Engineering and Processing: Process Intensification**, v. 46, p. 451-457, 2007.

OLIVEIRA, G. S.; COSTA, J. M. C.; AFONSO, M. R. A. Caracterização e comportamento higroscópico do pó da polpa de cajá liofilizada. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 18, p. 1059-1064, 2014.

PELEGRINE, D. H. G.; CARRASQUEIRA, R. L. Aproveitamento das proteínas do soro do leite no enriquecimento nutricional de sucos vitaminados. **RECEN - Revista Ciências Exatas e Naturais**, v. 10, n. 1, p. 103-114, 2010.

PERRONE, Í. T. *et al.* Influência das condições de operação em spray-dryer piloto sobre a umidade e a atividade de água do leite em pó integral. **Revista Instituto Lacticínios "Cândido Tostes"**, v. 68, n. 393, p. 5-9, 2013.

RIBEIRO, L. C.; COSTA, J. M. C.; AFONSO, M. R. A. Pó da polpa de acerola liofilizada. **Higiene Alimentar**, v. 29, p. 4877-4882, 2015.

ROCHA, E. M. F. F. *et al.* Obtenção de suco de caju atomizado através do controle das condições de secagem. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 18, n. 6, p. 646-651, 2014

VENDRAMINI, A. L.; TRUGO, L. C. Chemical composition of acerola fruit (*Malpighia puncifolia* L.) at three stages of maturity. **Food Chemistry**, v. 71, p. 195-198, 2000.



This is an open-access article distributed under the terms of the Creative Commons Attribution License