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EXTRATOS DO RESÍDUO DE AMORA-PRETA (*RUBUS FRUTICOSUS*): MICROENCAPSULAÇÃO POR SPRAY DRYER

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BIOGRAFIA

Suelen Siqueira dos Santos nasceu em 23 de janeiro de 1993 na cidade de Amambai, Mato Grosso do Sul.

Possui graduação em Engenharia de Alimentos pela Universidade Federal da Grande Dourados (UFGD).

Tem experiência nas áreas de tecnologia de produtos de origem animal e vegetal, atuando principalmente nos seguintes temas: extração de compostos e análises antioxidantes e microencapsulação.

Dedico

À minha mãe Nailza, meu exemplo de força e caráter, que em todas as fases da minha vida me apoiou e me deu total força para seguir todos os meus sonhos e objetivos, mesmo quando estes foram difíceis de realizar, e continua me incentivando a crescer mais e mais.

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APRESENTAÇÃO

Esta dissertação está apresentada na forma de TRÊS artigos científicos.

1. Suelen Siqueira dos Santos, Letícia Misturini Rodrigues, Silvio Cláudio da Costa, Rita de Cassia Bergamasco, Grasiele Scaramal Madrona. Microencapsulation of bioactive compounds from blackberry pomace (*Rubus fruticosus*) by spray drying technique.

Submetido a revista: Journal of the Science of Food and Agriculture. Qualis A2 em ciência de alimentos.

2. Suelen S. Santos, Letícia M. Rodrigues, Silvio C. Costa, Grasiele S. Madrona. Microcapsules of Blackberry Pomace (*Rubus fruticosus*): Light and Temperature Stability.

Submetido a revista: Chemical Engineering Transactions.
Qualis A2 em ciência de alimentos.

3. Suelen S. Santos, Letícia M. Rodrigues, Silvio C. Costa, Grasiele S. Madrona. Microencapsulação de extratos do resíduo de amora-preta (*Rubus fruticosus*) por spray dryer: estabilidade ao pH.

Submetido a revista: Pesquisa Agropecuária Brasileira.
Qualis B1 em ciência de alimentos.

GENERAL ABSTRACT

INTRODUCTION

The blackberry (*Rubus fruticosus*) is a fruit belonging to the family Rosaceae, genus *Rubus*, with 400 to 500 species, known as *berries.* The berries are small fruits, with sweet taste and rounded format, characterized by their high antioxidant potential due to the contents of total phenolic compounds and flavonoids. In addition, they have high content of anthocyanins, mainly anthocyanin cyanidin 3-glucoside. In fruit processing industries, about 20% of pomace is produced, basically composed of seeds and barks that still contain a large amount of phenolic compounds, such as anthocyanins. The limiting factor for the use of anthocyanins is their lower stability, being affected by several parameters such as pH, copigmentation, light, temperature and oxygen. In orther to minimize these adverse effects, microencapsulation can assist with many useful properties, the encapsulation provides a degree of stabilization for active compounds, avoiding deteriorating reactions.

AIMS

The aim of the present study was to microencapsulate and characterize the extracts (aqueous and hydroalcoholic) of the blackberry pomace by applying spray drying process, evaluating the stability against light, temperature and pH variations.

MATERIAL AND METHODS

On the blackberry pomace was realized analyzes of pH, titratable acidity, moisture, ashes, color using CIEL*a*b* system an hue (H) and chromaticity (C) angles were calculated. Two extracts of the blackberry pomace were prepared: aqueous extract and hydroalcoholic extract. Response surface methodology was used to evaluate the effect of the temperature and extraction time in the aqueous extraction of anthocyanins. The aqueous extract was obtained from the dilution of the blackberry pomace in distilled water (500 mg/mL) and mechanically shaken at 60°C for 45 minutes. The hydroalcoholic extract was obtained by diluting the pomace (500 mg/mL) in ethyl alcohol 80% (v/v), under mechanical stirring for 48 hours, filtration and rotation at 65°C until complete evaporation of the solvent. Afterwards, the extracts were filtered and submitted to drying in spray dryer. Maltodextrin (DE 10) was used as an encapsulating agent in the microencapsulation process. The carrier maltodextrin was added directly to the filtrates, in a ratio of 1:1 (w/w), by mechanical agitation. The aqueous and hydroalcoholic extracts mixed with maltodextrin were dried in mini spray dryer placed in plastic containers and stored under freezing (-18ºC). The determination of total phenolic compounds, total monomeric anthocyanins content, antioxidant activity by the radical sequestration method DPPH and Iron Reduction Method (FRAP), were determined by colorimetrical methods. Encapsulation yield was calculated, chromatographic analyzes by high performance liquid chromatography in a Shimadzu HPLC were performed and cyanidin-3-glucoside, galic acid, ellagic acid and quercetin concentrations

determined, the particle morphology was performed by scanning electron microscopy. Microcapsules were evaluated during 36 days for temperatures of 4 and 25 °C, light and no Light using two fluorescent lamps of 20W and a dark chamber. Color, total phenolic compounds and total monomeric anthocyanins were evaluated. Samples (extracts and microcapsules) were evaluated in different *McIlvaine* buffer (pH solutions 2.0, 3.5, 5.0 and 6.5), analyzes of color, total phenolic compounds, total monomeric anthocyanins and antioxidant activity by DPPH method. Color difference values were calculated to study color changes, the first-order reaction rate constants (k) and half-lives (t1/2), i.e. the times needed for 50% degradation of anthocyanins, were calculated. All analyzes were performed in triplicate and submitted to analysis of variance and Tukey's test ($p < 0.05$) for the minimum significant difference between the means using the statistical program STATISTICA version 7.0.

RESULTS AND DISCUSSION

The pomace has higher phenolic compounds contents and lower anthocyanins contents than the pulp. It is noted that dry aqueous extract are lighter than dry hydroalcoholic extract. When comparing a*, b* and H° parameters of microcapsules and dry extracts, the values are very close, this results demonstrate that encapsulating agent protected the color compounds of samples during the drying process. The phenolic compounds gallic acid followed by the ellagic were the major compounds in chromatographic analyzes, and the anthocyanins cyanidin was observed in higher concentration in all samples, followed by quercetin. Still analyzing the anthocyanins, hydroalcohol extraction was more efficient (1.5 times) in terms of encapsulation than aqueous extraction. However, for extraction of phenolic compounds the highest efficiency was when aqueous solution was used (1.2 times). In relation to the morphology, it was observed that the non-encapsulated extracts presented a more amorphous and irregular form than the microcapsules, indicating that the material that was encapsulated is actually protected. About temperature and light stability, it was observed that the use of temperature 4 °C kept the a* values constant, indicating greater red intensity in the samples and better stability in this condition. There was an increase for phenolic compounds in aqueous extraction microcapsule at 4 °C, whereas was observed at 25 °C, with light and without light, no significant difference during 36 days of storage. It was also observed that phenolic compounds were not influenced in hydroalcoholic extraction microcapsule under the different light and temperature conditions. Anthocyanins did not show degradation in aqueous extraction microcapsule at 4 °C and 25 °C during 36 days of storage, whereas in hydroalcoholic extraction the microcapsule manteined the anthocyanins content at 4 °C and at 25 °C low losses. Where observed light influenced the degradation of anthocyanins, with a loss in aqueous extraction microcapsule with light, in no light there was no losses. In the hydroalcoholic extraction microcapsule, the sample with light presented greater degradation as compared to no light. About pH stability, extracts presented higher color variations (ΔE) in pH 2.0, 3.5 and 5.0 as compared to the microcapsules, indicating that microencapsulation provided a higher color stability. When evaluating microcapsules as pH variations, the lower color variation was observed at pH 2.0, showing more anthocyanins stability at low pH. Regarding phenolic compounds, most samples presented an increse, while hydroalcoholic extract was degradated in pHs 3.5, 5.0 and 6.5 for

7 storage days. In anthocyanins it was observed that low pH kept all samples stable, the degradation increases with a pH increasement, the same relation was observed in antioxidant activity. About extractions, aqueous extraction has lower loss percentage during storage days in pHs (2.0, 3.5 and 5.0), thus use water as a solvent presented more advantageous than hydroalcoholic extraction. The degradation of the anthocyanins follow the first order reaction rate constants, and the half-lives of microcapsules were larger (2 to 7 times) than the extracts, and the largest half-lives were at low pH. Half-lives decreased and the degradation constants increased with pH increased.

CONCLUSIONS

The use of blackberry pomace is promising, since it has important antioxidant compounds, which when encapsulated may have technological applications by the food industry. Microencapsulation by spray dryer and with maltodextrin was efficient for the protection of phenolic compounds and anthocyanins during the studied storage period and the different conditions proposed to light, temperature and pH. Aqueous extraction was the only one that did not present anthocyanin loss in the temperature of 4 ºC, indicating its potential since this type of aqueous extraction is low cost and ecofriendly (does not use organic solvents). In general, higher stability was observed in samples stored at 4 °C and without light. At pH stability, the degradation of anthocyanins in the microcapsule was lower than the extracts, and the higher half-lives were in low pHs, until about 14 days.

Keywords: anthocyanins, phenolic compounds, antioxidant activity, HPLC, microencapsulation, spray dryer, maltodextrin.

RESUMO GERAL

INTRODUÇÃO

A amora-preta (*Rubus fruticosus*) é uma fruta pertencente à família Rosaceae, do gênero *Rubus*, para o qual se estimam existir entre 400 a 500 espécies, conhecidas como *berries* que são frutas pequenas, de sabor adocicado e formato arredondado. São caracterizadas pelo seu alto potencial antioxidante devido aos teores de compostos fenólicos totais e flavonoides, além disso, possuem elevado teor de antocianinas, principalmente a antocianina cianidina 3-glucosídeo. Nas indústrias de processamento de frutas, são gerados em torno de 20% de bagaço, compostos basicamente de cascas e sementes que ainda contêm uma grande quantidade de compostos fenólicos, como as antocianinas em amoras. O maior limitante para o uso das antocianinas é a sua baixa estabilidade, sendo afetada por diversos fatores como pH, copigmentação, luz, temperatura, metais, oxigênio. Para tentar minimizar esses efeitos adversos, a microencapsulação pode auxiliar com muitas propriedades úteis, conferindo um certo grau de estabilização para o composto ativo, uma vez que o material da parede funciona como uma barreira física evitando reações deteriorativas.

OBJETIVOS

O objetivo do presente estudo foi microencapsular os extratos aquosos e hidroalcoólicos do resíduo de amora-preta aplicando o processo de secagem por spray dryer, avaliando a estabilidade frente a variações de luz, temperatura, e pH.

MATERIAL E MÉTODOS

No bagaço de amora-preta foram realizadas análises de pH, acidez titulável, umidade, cinzas, cor usando o sistema CIEL*a*b* e foram calculados o ângulo hue (H) e a cromaticidade (C). Dois extratos de bagaço de amora: extrato aquoso e extracto hidroalcoólico, foram obtidos. A metodologia da superfície de resposta foi utilizada para avaliar o efeito da temperatura e do tempo de extração na extração aquosa de antocianinas. O extrato aquoso foi obtido a partir da diluição do bagaço de amora-preta em água destilada (500 mg/mL) e agitado mecanicamente a 60 °C durante 45 minutos. O extrato hidroalcoólico foi obtido por diluição do bagaço (500 mg/mL) em álcool etílico a 80% (v/v), sob agitação mecânica durante 48 horas, filtração e rotaevaporação a 65 ° C até completa evaporação do solvente. Posteriormente, os extratos foram filtrados e submetidos a secagem spray dryer. Utilizou-se maltodextrina (DE 10) como agente carreador no processo de microencapsulação. A maltodextrina foi adicionada diretamente aos filtrados, numa proporção de 1:1 (p/p), por agitação mecânica. Os extratos aquosos e hidroalcoólicos com maltodextrina foram secos em um mini spray dryer e armazenados em embalagens plásticas e estocados sob congelamento (-18 ºC). A determinação de compostos fenólicos totais, o teor de antocianinas totais monoméricas, a atividade antioxidante pelo método do sequestro de radicais (DPPH) e Método de Redução de Ferro (FRAP), foram determinados por métodos colorimétricos. O rendimento de encapsulação foi calculado, as análises cromatográficas por cromatografia

líquida de alta eficiência foram realizadas em HPLC Shimadzu, e determinadas as concentrações de cianidina-3-glucosídeo, ácido gálico, ácido elágico e quercitina e a morfologia das partículas foi realizada por microscopia eletrônica de varredura. As microcápsulas foram avaliadas durante 36 dias para temperaturas de 4 e 25 °C, com luz e sem luz utilizando duas lâmpadas fluorescentes de 20W e uma câmara escura. Avaliou-se a cor, os compostos fenólicos totais e as antocianinas monoméricas totais. As amostras (extratos e microcápsulas) foram avaliadas em diferentes tampões de *McIlvaine* (soluções de pH 2,0, 3,5, 5,0 e 6,5) e foram realizadas análises de cor, compostos fenólicos totais, antocianinas monoméricas totais e atividade antioxidante pelo método DPPH. Os valores de diferença de cor (ΔE) foram calculados para estudar as alterações de cor, assim como as constantes de velocidade degradação de reação de primeira ordem (k) e tempo de meia vida ($t_{1/2}$), isto é, os tempos necessários para a degradação de 50% das antocianinas, foram calculados. Todas as análises foram realizadas em triplicata e submetidas à análise de variância e teste de Tukey ($p < 0,05$) para a diferença mínima significativa entre as médias utilizando o programa estatístico STATISTICA versão 7.0.

RESULTADOS E DISCUSSÃO

O bagaço de amora apresentou teores de compostos fenólicos superiores e teores de antocianinas mais baixos do que a polpa. Observou-se que o extrato aquoso é mais claro do que o extrato hidroalcoólico, quando comparados os parâmetros a*, b* e H° das microcápsulas e dos extratos, os valores foram muito próximos, estes resultados demonstraram que o agente encapsulante protegeu os compostos corantes das amostras durante o processo de secagem. Os compostos fenólicos, ácido gálico seguido do elágico foram obtidos como os compostos principais na análise cromatográfica, para as antocianinas a cianidina foi observada em maior concentração em todas as amostras, seguida pela quercetina. Ainda analisando as antocianinas, a extração com álcool foi mais eficiente (1,5 vezes) em termos de encapsulação do que a extração aquosa. No entanto, para a extração de compostos fenólicos a maior eficiência foi quando a solução aquosa foi utilizada (1,2 vezes). Em relação à morfologia, observou-se que os extratos não encapsulados apresentaram uma forma mais amorfa e irregular do que as microcápsulas, indicando assim que o material que foi encapsulado estava protegido. Quanto à temperatura e estabilidade à luz, no parâmetro a*, a temperatura de 4 °C manteve os valores constantes, indicando maior intensidade de vermelho nas amostras e maior estabilidade nessa condição. Os compostos fenólicos apresentaram aumento na microcápsula de extração aquosa a 4 °C, enquanto que em 25 °C, com luz e sem luz, não apresentaram diferença significativa nos 36 dias de armazenamento. Observou-se também que os compostos fenólicos não foram influenciados na microcápsula de extração hidroalcoólica nas diferentes condições de luz e temperatura. As antocianinas não mostraram degradação na microcápsula de extração aquosa a 4 e 25 °C nos 36 dias de armazenamento, enquanto na microcápsula de extração hidroalcoólica 4 e 25 °C houve uma pequena perda. A luz influenciou a degradação das antocianinas, com uma perda na microcápsula de extração aquosa com luz, sem luz não houve perda. Na microcápsula de extração hidroalcoólica, a

amostra com luz apresentou maior degradação quando comparada com a ausência de luz. Quanto à estabilidade ao pH, os extratos apresentaram maiores variações de cor em pH 2,0, 3,5 e 5,0 quando comparados às microcápsulas, indicando que a microencapsulação influenciou em uma maior estabilidade da cor. Avaliando as microcápsulas em relação as variações de pH, observou-se menor variação de cor em pH 2,0, mostrando maior estabilidade de antocianinas em pH baixo. Quanto aos compostos fenólicos, a maioria das amostras do extrato hidroalcoólico foi degradada em pH 3,5, 5,0 e 6,5 em 7 dias de armazenamento. Nas antocianinas, observou-se que o pH baixo manteve todas as amostras estáveis, a degradação aumentou com o aumento do pH, a mesma relação foi observada na atividade antioxidante. Em relação aos tipos de extrações, a extração aquosa apresentou menor porcentagem de perda durante os dias de armazenamento em pH (2,0, 3,5 e 5,0), o uso de água como solvente foi mais vantajoso do que a extração hidroalcoólica. A degradação das antocianinas seguiu as constantes da velocidade de reação de primeira ordem, e os tempos de meia-vida das microcápsulas foram maiores (2 a 7 vezes) do que os extratos, os tempos de meia-vida maiores foram encontrados em pH baixo. Os tempos de meia-vida diminuíram e as constantes de degradação aumentaram com o aumento do pH.

CONCLUSÕES

A utilização do bagaço de amora é promissor, uma vez que tem importantes compostos antioxidantes, que quando encapsulados podem ter aplicações tecnológicas pela indústria de alimentos. A microencapsulação por spray dryer e com maltodextrina foi eficiente na proteção de compostos fenólicos e antocianinas durante o período de armazenamento estudado e nas diferentes condições propostas para a luz, temperatura e pH. A extração aquosa foi a única que não apresentou perda de antocianina na temperatura de 4 ºC, indicando seu potencial, uma vez que este tipo de extração é de baixo custo e é uma tecnologia limpa, que não usa solventes. Em geral, observou-se maior estabilidade em amostras armazenadas a 4 °C e sem luz. Na estabilidade ao pH, a degradação das antocianinas nas microcápsulas foi menor do que nos extratos, e os tempos de meia vida mais elevados foram encontrados em pHs baixos, chegando até cerca de 14 dias.

Palavras-chave: antocianinas, compostos fenólicos, atividade antioxidante, HPLC, microencapsulação, spray dryer, maltodextrina.

ARTICLE 1

Microencapsulation of bioactive compounds from blackberry pomace (*Rubus fruticosus*) by spray drying technique

Microcapsules of blackberry pomace

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Summary

Blackberry is highly appreciated for the enjoyable color and flavor. About 20% of the pomace is generated in its processing and often unused, presenting potential use by the food industry. Thus, the present study aimed to microencapsulate extracts of the blackberry pomace applying spray dryer process. Pure extracts (aqueous and hydroalcoholic solution) and the encapsulating agent (maltodextrin DE 10) in a ratio of 1:1 (w/w), were spray dried and analyzed for total anthocyanins, antioxidant activity, phenolics, HPLC-DAD chromatography, instrumental color and scanning electron microscopy. Hydroalcoholic extraction was more efficient (1.5 times) for anthocyanins encapsulation than aqueous extraction. However, for phenolic compounds the highest efficiency (1.2 times) was in the aqueous solution. The majority bioactive compounds were gallic acid and cyanidin. The encapsulating agent protected the coloring compounds of samples during the drying process. Considering that water is a low cost and ecofriendly solvent, it is indicated this type of extraction to obtain microcapsules of blackberry pomace mainly for future applications by food industry.

Keywords: anthocyanins, HPLC, microencapsulation, antioxidant activity.

Introduction

The blackberry (*Rubus fruticosus*) is a fruit belonging to the family Rosaceae, genus *Rubus*, with estimated 400 to 500 species, known as *berries.* The berries are small fruits, with sweet taste and rounded format (1,2). They are characterized by their

high antioxidant potential due to the contents of total phenolic compounds and flavonoids, and in addition, they have high content of anthocyanins, mainly anthocyanin cyanidin 3-glucoside (1).

Besides the possibility of exploitation for *in natura* consumption, fruits and their pomace such as seeds and barks can be exploited by the agro-industry through processing (3). It is known that about 20% of pomace is generated in the processing of these fruits, being composed basically of seeds and barks and still contain a great amount of phenolic compounds, as the anthocyanins (4).

An alternative is the extraction of extracts from fruit pomace for further processing. The quality of the extracts obtained depends on the extraction technique used and must be chosen according to the chemical profile of the product. The extraction of antioxidants as phenolic compounds and anthocyanins is usually performed with the aid of organic solvents, with stirring or heating. But this methodology is not feasible due to the amount of organic solvent used, the need to separate them from the extract and the properly disposal so that there is no damage to the environment, and in some cases may be prejudicial to human health (5,6). Thus, water extraction is a viable alternative for use in the food industry because it is a cheap and clean technology.

Are scarce are the reports that study the extraction of compounds of blackberry pomace, Machado and co-authors (2015), for example, evaluated the extraction with water, ethanol in combination with water, and acidified water in blackberry pomace with temperature variations and extraction methods (7). Reátegui and co-authors (2014) used supercritical extraction with ultrasound-assisted, CO_2 in water variations with ethanol combination (5).

The blackberries pomace, as its fruit, can be used in food industry being appreciated by consumers, not only because their high nutritional value, but also for their physical and mental health benefits; they also contains anthocyanins, that can be used as natural dyes (6). Besides presenting antioxidant activity these anthocyanins could be an alternative for the replacement of synthetic dyes by food industry. The limiting factor for the use of anthocyanins is their low stability, being affected by several parameters such as pH, copigmentation, light, temperature and oxygen (8). In order to minimize these adverse effects, microencapsulation can assist with many useful properties (9). Thus it is very important to evaluate the microencapsulation process of blackberry byproduct for future food applications, and therefore, this paper presenting innovative results, being the first report in the literature about that. The most used materials for microencapsulation are maltodextrins, which are obtained by acid hydrolysis of various starches (corn, potato, or others). In general, they have high water solubility, low viscosity, smooth taste and colorless solutions are widely used in the food industry (10,11).

Among the methods applied in microencapsulation, it is highlighted the pulverization (spray drying), which consists in the continuous processing of food from a fluid state in the form of dry particles by spraying the feed in a hot drying medium (12). It has been widely used in the microencapsulation of acids, bases, oils, vitamins, salts, gases, amino acids, essential oils, dyes, enzymes and microorganisms (13,14).

In this context, the objective of the present study was to microencapsulate and characterize different extracts of the blackberry pomace obtained by applying spray drying process.

Materials and methods

Materials

Blackberry pomace (*Rubus fruticosus*) belonging to the some lot, was obtained from a processing of blackberry pulp of a producer from the city of Paraibuna - São Paulo - BR and kept in freezing (-18ºC) until its use. Maltodextrin (DE10) was supplied by Cargil® (Campinas-SP). The other reagents used were of analytical grade, and the standards and reagents used in the chromatographic analysis were of Sigma Aldrich.

Pomace physical-chemical analysis

Analyzes of pH, titratable acidity, moisture and ashes were performed on the blackberry pomace, according to AOAC methodologies (15). Analyzes of total phenolic compounds, total monomeric anthocyanins and antioxidant activity were carried out by diluting the pomace with water.

The color of the pomace was evaluated by using a portable Minolta® CR400 colorimeter, with integration sphere and angle of view of 3^{o}, that is, d/3 illumination and D65 illuminant. The system used was CIEL*a*b*. The hue (H) and chromaticity (C) angles were calculated using equations (1) and (2), respectively. This analysis was also applied in the characterization of the capsules.

$$H(°) = tan^{-1} \frac{b^*}{a^*} \qquad (1)$$

$$C = \sqrt{(a^*)^2 + (b^*)^2} \qquad (2)$$

Extraction of the bioactive compounds

An experimental design (Central composite design) including eight points and three replicates at the central point, totalling eleven experiments in the total was appied. Response surface methodology was used to evaluate the effect of the temperature (X1=40 to 80 °C) and extraction time (X2=30 to 60 min) in the extraction of

anthocyanin from blackberry extract by using the statistical program STATISTICA version 7.0.

Two extracts of the blackberry pomace were prepared: aqueous extract (EA) and hydroalcoholic extract (HE). The aqueous extract was obtained from the dilution of the blackberry pomace in distilled water at a concentration of 500 mg/mL and mechanically shaken at 60°C for 45 minutes.

The hydroalcoholic extract was obtained by diluting the pomace (500 mg/mL) in ethyl alcohol 80% (v/v), under mechanical stirring for 48 hours, filtration and rotation at 65°C until complete evaporation of the solvent (16). Afterwards, the extracts were filtered and submitted to drying in spray dryer.

Microencapsulation of blackberry pomace by spray drying

Maltodextrin (DE 10) was used as an encapsulating agent in the microencapsulation process. The carrier maltodextrin was added directly to the filtrates, in a ratio of 1: 1 (w/w), by mechanical agitation (17).

The aqueous and hydroalcoholic extracts mixed with maltodextrin (MAE and MHE, respectively) were dried in Buchi B-191 mini spray dryer under the conditions: inlet drying air temperature 170°C and outlet 105°C; Atomization pressure: 4 bar; Average drying air flow: 3.5 m^3/h and average flow rate: 0.5L/h (18). The dried products were placed in plastic containers and stored under freezing (-18ºC).

Control samples were also prepared for comparison measurements. In this case, the extracts aqueous (AE) and hydroalcoholic (HE) were submitted to drying in spray drying (in the same conditions), without the presence of maltodextrin as carrier.

Microcapsules characterization

In this step the destruction of the capsules was performed by using water, agitation and centrifugation at 4000 rpm for 10 min (19).

Total phenolic compounds determination

The determination of the total phenolics (PCC) was performed using Folin-Ciocalteu reagent (50%) and sodium carbonate 3.79 M (Na_2CO_3). Results were expressed in µg of gallic acid equivalente (GAE).mg^{-1} of product (20,21).

Determination of the total monomeric anthocyanins content

To determine the total monomeric anthocyanin (TMA) content, the differential pH method was used (22). Results were in microgram of cyanidin-3-glucoside per miligram of sample (µg cyanidin-3-glucoside.mg^{-1}), according equations 3 and 4, respectively.

$$AT = (ABS\ 520nm - ABS\ 700nm)pH\ 1{,}0 - (ABS\ 520nm - ABS\ 700nm)pH\ 4{,}5 \quad (3)$$

$$Total\ monomeric\ anthocyanins\ (TMA) = \frac{(AT\ x\ MW\ x\ Dfx\ 10^3)}{\varepsilon\ x\ \lambda} \quad (4)$$

Where: MW = 449.2 g/mol (molar mass of cyanidin-3-glucoside); Df = dilution factor; 10^3 = conversion factor from g to mg; ε = 26900L/mol (molar absorptivity of cyanidin-3-glucoside); λ = 1 cm (optical path length of the cuvette).

Antioxidant activity by the radical sequestration method DPPH (2,2-diphenyl-1-picrylhydrazine)

The reduction of the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was determined according to the methodology described by (23), using the equation 5.

$$Efficiency\ of\ scavenging\ activity(\%) = \frac{(A_{control}\ - A_{sample}\ )}{A_{control}} x\ 100 \quad (5)$$

Where: Acontrol: Absorbance of negative control; Asample: Mean Sample Absorbance

The results were expressed as equivalent antioxidant capacity (EC50) μg.mL product^{-1} calculated by the software Graphpad Prism 5.

Antioxidant activity by the Iron Reduction Method (FRAP)

For this assay the extracts were mixed with distilled water and the FRAP reagent, soon after they were maintained at 37°C in a water bath and then sent to the spectrophotometer (595 nm) according to the methodology described by (24), results were expressed in μM eq Trolox.mg product^{-1}.

Encapsulation Yield

The encapsulation yield was determined as described by (25) and (26), using the equation 6.

$$EY\ (\%) = \frac{microcapsules\ \ phenolic\ or\ antocianin\ \ released}{phenolic\ or\ antocianin\ \ in\ initial\ \ extract} x\ 100 \qquad (6)$$

Chromatographic analyzes by HPLC-DAD

Cyanidin-3-glucoside, gallic acid, ellagic acid and quercetin concentrations were determined by high performance liquid chromatography in a Shimadzu HPLC (model LC-20A Prominence) equipped with a Discovery HS C18 column. Chromatographic analyses were carried out at 280 nm for gallic acid, 320 nm for cyaniding-3-glucoside and ellacig acid, 370 nm for quercetin at 40°C. A sample of 10 μL was automatically injected at a flow rate of 0.7 mL min^{-1}. The mobile phase consisted of 2% (v/v) acetic acid in water (eluent A) and 0.5% acetic acid in water and acetonitrile (50:50 v/v, eluent B) using a gradient program (27).

Microcapsules morphology

The study of the particle morphology was performed by scanning electron microscopy (SEM), using a scanning electron microscope (JEOL model JSM-6060 LV).

The samples were fixed in a metal support with a double-face tape of carbon, and covered with a thin layer of gold. Visualization was performed in increments of 100 to 2000 times, with an excitation voltage of 10 kV.

Statistical analysis

All analyzes were performed in triplicate and submitted to analysis of variance and Tukey's test ($p < 0.05$) for the minimum significant difference between the means using the statistical program STATISTICA version 7.0.

Results and discussion

Blackberry pomace characterization

Blackberry pomace was characterized by instrumental color, physicochemical analysis, phenolic compounds content (PCC), total monomeric anthocyanins (TMA) and antioxidants (EC_{50} e FRAP), and the results are showed in Table 1.

Value of pH and titratable acidity are higher than blackberry pulp, and moisture is lower than blackberry pulp, because of the amount of water in the pulp. Ferrari and co-authors found values of 3.31±0.02, 0.76±0.02 % citric acid, 91.96±0.14% for pH, titulable acidity and moisture, respectively. Ash content of blackberry pomace were three times greater than blackberry pulp, 0.20±0.00 %, because it is a pomace that has a higher content of inorganic matter (17).

The a* parameter represents chromatic value between green (-) and red (+), and the value of 19.82±2.07 indicates the red coloration of blackberry pomace. The value of b* parameter (chromatic value between blue and yellow) shows that the pomace has a tendency to yellow color.

Hue angle represents the rotation in a sphere of colors. The hue angle closer to 0 indicate the red color, so the value of 0.41±0.00 shows the red color of pomace. The values of L* and C parameters shows that the pomace has a dark color.

It was observed that the phenolic compounds content in the pomace was higher than in the pulp, according to results related by Ferrari and co-authors (2.10±0.42 µg GAE.mg^{-1}) (28), Ferreira and co-authors (2.41±0.08 µg.mg^{-1}) (1), and Zielinski and co-authors (1.69±0.09 µg.mg^{-1}) (29). Machado and co-authors extracted bioactives compounds from blackberry pomace by pressure liquid extraction, at 60°C. The results were 2.39±0.01 µg GAE.mg^{-1} for aqueous extraction and 5.23±0.83 µg GAE.mg^{-1} for hydroalcoholic extraction (50%) (7).

Generally, the pomace has higher phenolic compounds contents and lower anthocyanins contents than the pulp. Probably because of higher condensed tannins content in seeds and barks. However, this fact cannot be considering a limiting factor, because the anthocyanins are weakly bound to the antioxidant capacity (7). Anthocyanins found in the blackberry pomace were lower than those found in the literature for the pulp, 0.78±0.02 µg cyanidin-3-glucoside.mg^{-1} (17), 1.04±0.02 µg cyanidin-3-glucoside.mg^{-1} (1), 2.05±0.02 µg cyanidin-3-glucoside.mg^{-1} (29). It was observed that blackberry pomace had antioxidant activity higher than the pulp (more than 300 times), which may be related to the phenolic compounds present in the pomace, making viable the technological use of the pomace for the insertion of antioxidants compounds in other foods.

For blackberry pulp, it was reported in literature values of FRAP of 0.03±0.00 µM eq trolox.mg^{-1} (29), and 0.02 µM eq trolox.mg^{-1} (30). When blackberry is compared to the others fruits also presents higher antioxidant activity, 0.01±0.00 µM eq trolox.mg^{-1} in Japanese plum (31), and 0.07±0.00 µM eq. trolox.mg^{-1} in cranberry (32).

Antioxidant capacity value (EC_{50}) for blackberry pomace is closer to that found for cherries (*Cornus mas* L.), 520 µg.mL^{-1} (33).

Experimental desing for anthocyanin extraction

Figure 1 shows result for the extraction of anthocyanin indicating that high extractive values was obtained in a region at 60 °C and extraction time of 45 min, the extract obtained in these conditions was used in the next step (microencapsulation). Analyzing the equation obtained it is possible observed that no interactions between time and temperature was observed, and temperature (41.2 X1) presented more influence than time (16.7 X2).

Experimental design is very useful for the optimizing of bioactive compounds extraction from fruits. It is important to highlight that the solvent choice is certainly an important variable in order to increase the bioactive compounds extraction.

Encapsulation yield (EY) and characterization of microcapsules and dry extracts of blackberry pomace

Figure 2 shows encapsulation yield of MAE and MHE samples, in relation to PCC and TMA. MHE sample was more efficient in encapsulation of TMA, and MAE sample shows higher percentage of PCC.

Encapsulation yield for PCC did not reach 100% in MAE and MHE samples, while in relation to TMA, MHE sample obtained higher percentage. Thus, the aqueous extraction was more efficient to encapsulate phenolic compounds, and hydroalcoholic extraction was more efficient to encapsulate anthocyanins.

Microencapsulation of the anthocyanins in MAE and MHE samples can be associated to the complexation of *flavilium* cation of anthocyanins with dextrin, preventing its transformation into others less stable form (34,35). As MHE sample has greater availability of anthocyanins, due to the hydroalcoholic extraction, encapsulation yield was higher when compared to the MAE sample.

Some results reported in literature are similar to the results showed in the present study. In a study carried out with pomegranate juice encapsulated into maltodextrin, the authors obtained encapsulation yield of 53.5% of phenolic compounds and 86.6% of anthocyanins (36); in grape juice microencapsulated into a mix of isolated soy protein and maltodextrin, the authors found 100% of encapsulation yield of anthocyanins (35).

Table 2 shows analysis of color, total phenolic compounds, anthocyanins and antioxidant activity of dry extracts and microcapsules.

It is noted that dry aqueous extract (AE) are lighter than dry hydroalcoholic extract (HE), because of affinity anthocyanins extraction with organic solvent (Table 2). The use of maltodextrin provided a significant increase in the value of L*, when comparing microcapsules (MAE and MHE) and dry extracts (AE and HE), that is, the microcapsules are lighter than dry extracts. The same tendency was observed in chromaticity (C). This relationship is due to the white coloration of maltodextrin, and it was reported in others studies (37). Similar results were reported for blackberry pulp microencapsulated with maltodextrin, for the color parameters L*= 36.83 ± 1.04, a*= 23.45 ± 1.54, b*= 3.84 ± 0.31, C= 22.99 ± 1.73, h= 8.81 ± 0.21 (28).

It’s interesting to note that when comparing a*, b* and h parameters of microcapsules (MAE and MHE) and dry extracts (AE and HE), the values are very close. This results demonstrate that encapsulating agent protected the coloring

compounds of samples during the drying process, since the MAE and MHE presents only 50% of extract in it formulation.

Antioxidant activity determined by FRAP and DPPH methods was directly proportional to PCC and TMA values. In study of Azofeifa and co-authors with microfiltered blackberry extract (*R. adenotrichos* Schltdl), the authors found antioxidant activity of EC_{50} 5,05±0,35 µg.mL^{-1}, a value much higher than the present study, probably due to the use of a microfiltration process (38). Generally, high temperature process, such as spray drying, can increase the antioxidant activity due to the formation of phenolic compounds resulting from degradation of others compounds (39).

Table 3 shows chromatographic parameters of samples. As expected for anthocyanins, the concentration of cyanidin-3-glucoside was higher in hydroalcoholic extraction (HE) when compared to aqueous extraction (AE), consequently MHE and MAE followed the same trend.

Method of drying influenced on cyanidin-3-glucoside degradation in AE and HE, major compound in anthocyanins (40), and the encapsulation protected this compound in MAE e MHE, making viable the use of maltodextrin. Others studies found cyanidin concentration of 569.7±38.7mg.100g^{-1} in blackberry pomace subjected to supercritical extraction with CO_2 and water (5), and 549.1±9.7mg.100 g^{-1} for freeze dried blackberry (41).

In relation to quercetin concentration, it was not observed difference between the samples. The encapsulation did not influence on degradation of galic acid and ellagic acid in MAE and MHE, a fact expected, since the encapsulation yield of total phenolic compounds did not reach 100%. Datas reported in literature for freeze dried blackberry shows contents of ellagic acid of 24.4±1,6 mg.100 g^{-1} , and quercetin of 1.9±0.1 mg.100

g^{-1} (41), and in blackberry leaf extract 14.10±0.09 $mg.g^{-1}$ of ellagic galic and 0.65±0.01 $mg.g^{-1}$ for quercetin (42).

Figure 3 shows morphology of microcapsules analyzed by scanning electronic microscopy.

The dried extracts (AE and HE) showed amorphous and irregular shape when compared to microcapsules (MAE and MHE), with rounded outer structure and agglomerates of different sizes, a characteristics of spray dried powders (28). Roughness and cracking were also observed in microcapsules, as already mentioned by others authors (43,44). These irregularities are formed due to the fast evaporation of water in spray drying process (45).

When the solution are dried at lower inlet air temperature (110°C), there is a greater degree shrinkage during drying (46). Particles produced at inlet air temperature of 180°C (the present study) shows smooth surface and greater uniformity (17).

Conclusion

This work is innovative in that it is the first to report the process and characteristics of the blackberry pomace microencapsulation. The use of this pomace is promising, since it has important antioxidant compounds, which when encapsulated may have technological applications by the food industry. Among the phenolic compounds, gallic acid followed by the ellagic was obtained as the major compounds; for the anthocyanins cyanidin was observed in higher concentration in all samples, followed by quercetin.

Still analyzing the anthocyanins, the experimental design showed high extractive values at 60°C and 45 min in water. Hydroalcohol extraction was more efficient in terms of encapsulation than aqueous extraction (MHE was 1.5 times greater than MAE).

However, for extraction of phenolic compounds the highest efficiency was when aqueous solution was used, in this case MAE 1.2 times higher than MHE. In relation to the morphology, it was observed that the non-encapsulated extracts presented a more amorphous and irregular form than the microcapsules, thus indicating that the material that was encapsulated is actually protected, however later studies should be performed to evaluate the controlled release and stability to the storage of microcapsules obtained.

Finally, it is concluded that considering that the use of water as solvent for extraction is low cost, it is indicated this type of extraction to obtain microcapsules of blackberry by product mainly for future applications by food industry, therefore analyzing the results noticed it was found that it showed better efficiency for phenolic compounds and, even though the anthocyanins content was lower, this fact could be compensated by using higher concentrations of capsules, for example, so that this extraction process is more advantageous.

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Table 1. Blackberry pomace characterization

	Blackberry pomace
pH	3.46±0.01
Titulable acidity (% citric acid)	0.93±0.04
Moisture (%)	68.19±1.68
Ash (%)	0.68±0.05
L*	20.58±1.26
a*	19.82±2.07
b*	8.85±1.05
C	20.59±1.88
H	0.41±0.00
PCC (µg GAE.mg product^{-1})	8.36±0.18
TMA (µg cyanidin-3-glucoside.mg^{-1})	0.37±0.01
DPPH EC $_{50}$ (µg.mL product^{-1})	468.67±15.70
FRAP (µmol eq Trolox.mg product^{-1})	11.01±0.47

mean±standard deviation (results in dry base). L*= luminosity; a*= variations between green (-) and red (+); b*= variations between blue (-) and yellow (+); C= chromaticity; H= hue angle; PCC= phenolic compounds content; TMA= total monomeric anthocyanins. DPPH and FRAP= antioxidant capacity.

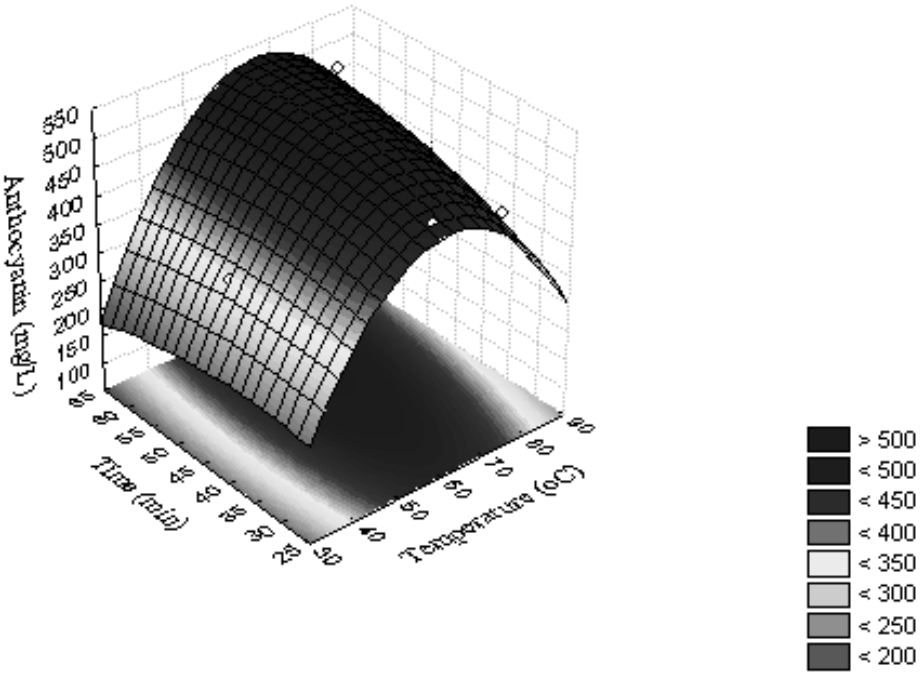

Figure 1. Response surface for anthocyanin extraction from blackberry ($Y=41.2X1-104.1X1^2+16.7X2-12.9X2^2$).

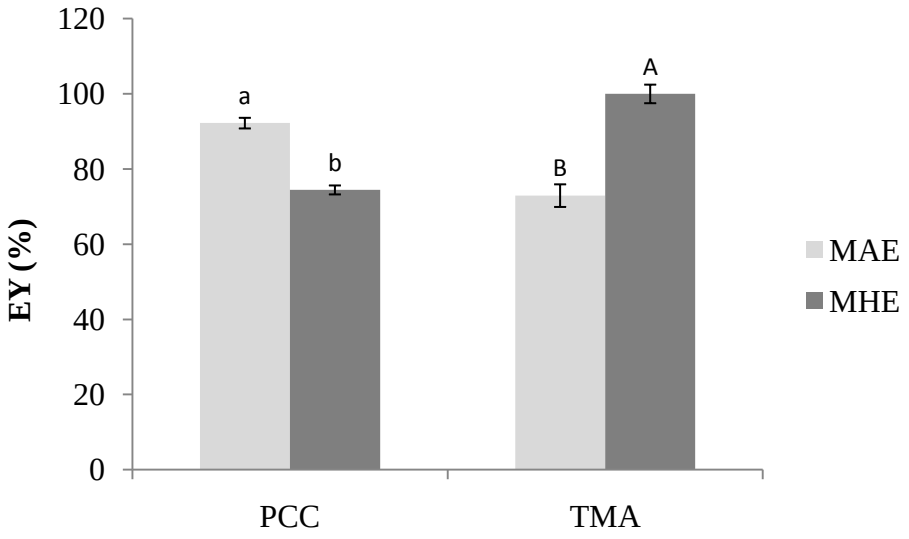

Figure 2. Encapsulation yield (EY) of MAE (microcapsule of aqueous extract) and MHE (microcapsule of hydroalcoholic extract) microcapsules in relation to PCC and TMA. Small letters in PCC and capital letters in TMA did not differ among themselves by the Tukey's test ($p<0.05$). PCC= phenolic compounds content; TMA= total monomeric anthocyanins.

Table 2. Analysis of color, total phenolic compounds, anthocyanins and antioxidant activity of AE, HE, MAE and MHE samples.

	EA	MAE	HE	MHE
L	27.51^{c}±1.43	45.05^{a}±1.41	14.18^{d}±1.45	38.17^{b}±2.55
a*	36.03^{c}±0.34	39.38^{b}±0.45	22.38^{d}±0.36	43.61^{a}±0.32
b*	12.03^{b}±0.15	9.58^{c}±0.15	7.80^{d}±0.17	13.32^{a}±0.24
C	37.98^{c}±0.37	40.52^{b}±0.46	23.70^{d}±0.39	45.60^{a}±0.36
H	0.32^{b}±0.00	0.24^{d}±0.00	0.34^{a}±0.00	0.30^{c}±0.00
PCC[1]	97.87^{b}±0.75	45.13^{d}±1.04	157.07^{a}±2.26	58.47^{c}±0.09
TMA[2]	4.54^{b}±0.33	1.66^{d}±0.97	6.36^{a}±0.17	3.53^{c}±0.12
DPPH EC_{50}[3]	34.33^{c}±0.58	67.33^{a}±1.15	17.00^{d}±0.00	42.33^{b} ±1.53
FRAP[4]	99.82^{b}±0.87	36.23^{d}±0.56	143.14^{a} ±2.26	61.21^{c}±1.42

Equal letters in the same line did not differ among themselves by Tukey's test ($p<0.05$). [1]PCC (phenolic compounds content)= µg GAE.mg $product^{-1}$; [2]TMA (total monomeric anthocyanins)= µg cyanidin-3-glucoside.mg^{-1}; [3]EC (antioxidant capacity) $_{50}$ = µg.mL $product^{-1}$; [4]FRAP (antioxidant capacity) = µM eq Trolox.mg $product^{-1}$. AE: 100% aqueous extract; MAE (microcapsule of aqueous extract): 50% aqueous extract/50% maltodextrin; HE: 100% hydroalcoholic extract; MHE (microcapusule of hydroalcoholic extract): 50% hydroalcoholic extract/50% maltodextrin.

Table 3. Chromatographic parameters of samples AE, HE, MAE and MHE.

Compounds	t_R (min)	Regression equation	R^2	AE(mg. 100 g^{-1})	MAE(mg. 100 g^{-1})	HE(mg. 100 g^{-1})	MHE(mg. 100 g^{-1})
Cyanidin-3-glucoside	12.1	y = 3e-07x + 0.019	0.9996	811.85[c] ±2.76	750.90[d] ±13.29	1482.45[a] ±3.18	1373.59[b] ±4.94
Galic acid	3.9	y = 3e-08x	0.9996	279.65[b] ±2.05	105.86[d] ±2.74	333.49[a] ±3.54	127.76[c] ±3.73
Ellagic acid	15.8	y = 7e-08x – 0.0754	0.9796	702.61[a] ±3.74	125.50[c] ±0.42	393.44[b] ±2.06	162.86[d] ±2.65
Quercetin	45.5	y = 2e-08x	0.9977	19.94[a] ±1.34	20.05[a] ±0.78	22.69[a] ±1.00	22.96[a] ±0.00

Means followed by the same letters on line did not differ among themselves by *Tukey's test* ($p<0.05$). AE= aqueous extract; MAE= microcapsule of aqueous extract; HE= hydroalcoholic extract; MHE= microcapsule of hydroalcoholic extract;

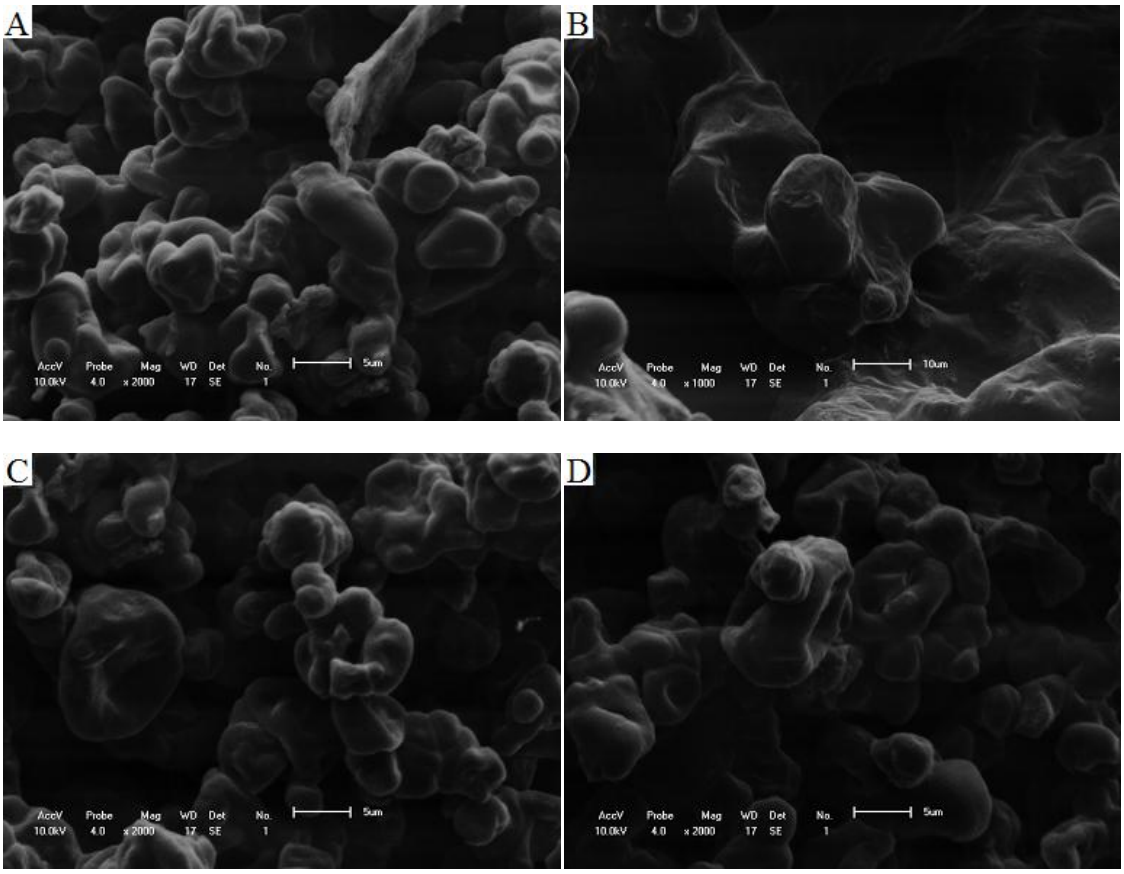

Figure 3. Samples dried in spray dryer with an increase of 1000x and 2000x. (A) AE= aqueous extract; (B) HE= hydroalcoholic extract; (C) MAE= microcapsule of aqueous extract; (D) MHE= microcapsule of hydroalcoholic extract.

ARTICLE 2

Microcapsules of Blackberry Pomace (*Rubus fruticosus*): Light and Temperature Stability

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Blackberries are appreciated for its high nutritional value and important source of healthy compounds. Blackberries' pomace represents 20% of the total fruit, mainly composed by seeds and barks which contains a significant amount of phenolic compounds and anthocyanins. This study aimed to microencapsulate blackberries' pomace extract with maltodextrin using the spray dryer technique. Also the stability against light and temperature variations was evaluated during 36 days. There were two types of extraction, aqueous (CA) and hydroethanol (CE), which were encapsulated with maltodextrin DE 10 and submitted to spray drying. Subsequently the samples were evaluated against different conditions of temperature (4 and 25 ° C), presence and absence of light, analyzing parameters of color, phenolic compounds and anthocyanins. The results were analyzed using ANOVA and Tukey's test ($p<0.05$). The intensity of red color in the samples, represented by a*, decreased during storage. Analyzing phenolic compounds there was no significant difference in all samples, indicating that these compounds in both (CA and CE) extraction were not affected by variations of light and temperature. For anthocyanins, on temperature variation, no degradation was observed for CA. For CE losses represented less than 10 %. Analyzing the influence of light in anthocyanins degradation, the highest loss was observed in the presence of light (for both CA and CE), in the absence of light the CA sample was not degraded, and the CE lost about 8 %. Therefore microencapsulation with maltodextrin was effective for the protection of phenolic compounds and anthocyanins during the storage time studied, under the different proposed conditions (light and temperature), noting that CA extraction had better results. It can be concluded that the aqueous extraction was successful, helping to stabilize encapsulated samples of blackberry pomace over 36 days of storage.
Keywords: microencapsulation, spray dryer, maltodextrin, phenolic compounds and anthocyanins.

1. Introdução

Blackberries (Rubus spp.) known as berries, which are small fruits with sweet taste and rounded format, are appreciated by consumers due to their high nutritional value and health benefits (Ferreira et al. 2009). This fruit is an important source of phenolic compounds, such as phenolic acids, tannins, elagitannins, flavonoids and anthocyanins (Ivanovic et al. 2014; Machado et al. 2015).

In fruit processing industries, about 20% of pomace is produced, basically composed of seeds and barks that still contain a large amount of phenolic compounds, such as anthocyanins (Ignat, Volf, and Popa 2011).

The extraction of antioxidants as phenolic compounds and anthocyanins is usually performed with the addition of organic solvents, with stirring or heating. Water extraction is a viable alternative for use in the food industry because it is a cheap and clean technology (Reátegui et al. 2014; Ivanovic et al. 2014).

Encapsulation provides a degree of stabilization for active compounds, since the wall material acts as a physical barrier to oxygen, light, and temperature, among other factors, avoiding deteriorating reactions (Madene et al. 2006).

The most used materials for microencapsulation are maltodextrins, which are obtained by acid hydrolysis of various starches (corn, potato, or others). In general, they have high water solubility, low viscosity, smooth taste and colorless solutions are widely used in the food industry (Saenz et al. 2009; Gibbs et al. 1999).

In this context, the present study aimed to microencapsulate by spray dryer the extracts (aqueous and hydroalcoholic) of blackberry pomace, evaluating the stability against light and temperature variations.

2. Material and Methods

Blackberry pomace (Rubus fruticosus) was locally purchased from a producer in the city of Paraibuna, SP-BR. Maltodextrin (DE10) was supplied by Cargil® (Campinas-SP). The reagents were of analytical grade.

2.1. Production of extracts and microcapsules by spray drying

An experimental design (central composite design) with eleven experiments in the total was applied, the response surface methodology was used to evaluate the temperature and time influence in anthocyanin extraction.

Two extracts from blackberry pomace were prepared: aqueous and hydroethanolic extract. The aqueous extract was obtained from the dilution of blackberry pomace in distilled water (500 mg / mL) and mechanically stirred at 60 ° C for 45 minutes, according to response surface. The hydroethanolic extract was obtained by diluting the pomace (500 mg / mL) in ethyl alcohol 80 % (v / v), under mechanical stirring for 48 hours, filtration and rotoevaporation at 65 ° C until total solvent evaporation (Shirahigue et al., 2011). Maltodextrin (DE 10) was added directly to the filtrates, in a ratio of 1 : 1 (w/w), using mechanical agitation (Ferrari et al., 2012). The aqueous and hydroethanolic extracts mixed with maltodextrin (CA and CE, respectively) were dried in a Buchi B-191 mini spray dryer, the inlet air temperature was 170 ° C and outlet 105 ° C; Atomization pressure: 4 bar; Average drying air flow: 3.5 m^3 / h; Average feed flow: 0.5 L / h (Valduga et al., 2008). After drying the powders were placed in plastic containers and stored under different temperature and light conditions for stability evaluation.

2.2. Microcapsule stability to light and temperature

The samples were evaluated during 36 days for temperatures of 4 and 25 ° C (CA 4 °, CA 25 °, CE 4 °, CE 25 °), Light and No Light (CA L, CA N. L, CE L and CE N. L) using two fluorescent lamps of 20W and a dark chamber. Color, total phenolic compounds and total monomeric anthocyanins were evaluated. The capsule disintegration was performed using water, agitation and centrifugation at 4000 rpm for10 min (Díaz et al., 2015).

The determination of total phenolic compounds (TPC) was performed using Folin-Ciocalteu reagent. The results were expressed in µg gallic acid equivalent (GAE).mg $^{-1}$ product (Pierpoint, 2004; Singleton and Rossi, 1965).

The total monomeric anthocyanins (TMA) content was measured using the differential pH method (Lee et al., 2005) according equation (1) and (2) with results expressed in µg cyanidin-3-glucoside.mg $^{-1}$.

$$AT = (ABS\ 520nm - ABS\ 700nm)pH\ 1{,}0 - (ABS\ 520nm - ABS\ 700nm)pH\ 4{,}5 \quad (1)$$

$$Total\ monomeric\ anthocyanins\ (TMA) = \frac{(AT\ x\ MW\ x\ Df x 10^3)}{\varepsilon\ x\ \lambda} \quad (2)$$

Where: MW = 449.2 g/mol (molar mass of cyanidin-3-glucoside); Df = dilution factor; 103 = conversion factor from g to mg; ε = 26900L/mol (molar absorptivity of cyanidin-3-glucoside); λ = 1 cm (optical path length of the cuvette).

The color was evaluated using a Minolta® CR400 portable colorimeter, using the CIEL*a *b* system, and the hue (H) angle and chromaticity (C) was calculated using equations (3) and (4), respectively.

$$H(°) = tan^{-1}\ \frac{b^*}{a^*} \quad (3)$$

$$C = \sqrt{(a^*)^2 + (b^*)^2} \quad (4)$$

The total loss percentage for anthocyanins and phenolic compounds during the storage period was calculated by the ratio between the concentration at the last storage day (d36), and the initial concentration (d0) (Souza et al., 2014).

The analyzes were performed in triplicate and evaluated by Anova and Tukey test ($p < 0.05$) using the statistical program Sisvar 5.6.

3. Results and discussion

Evaluating the color (L, a *, b *, H ° and C) it was observed (Figure 1) that for the luminosity (L), CA 4 ° C and CE 4 ° C had lower losses when compared to the CA and CE at the temperature of 25 ° C, the same pattern was observed in a *, b * and H °. The samples in the absence of light presented the greater loss of color in all parameters.

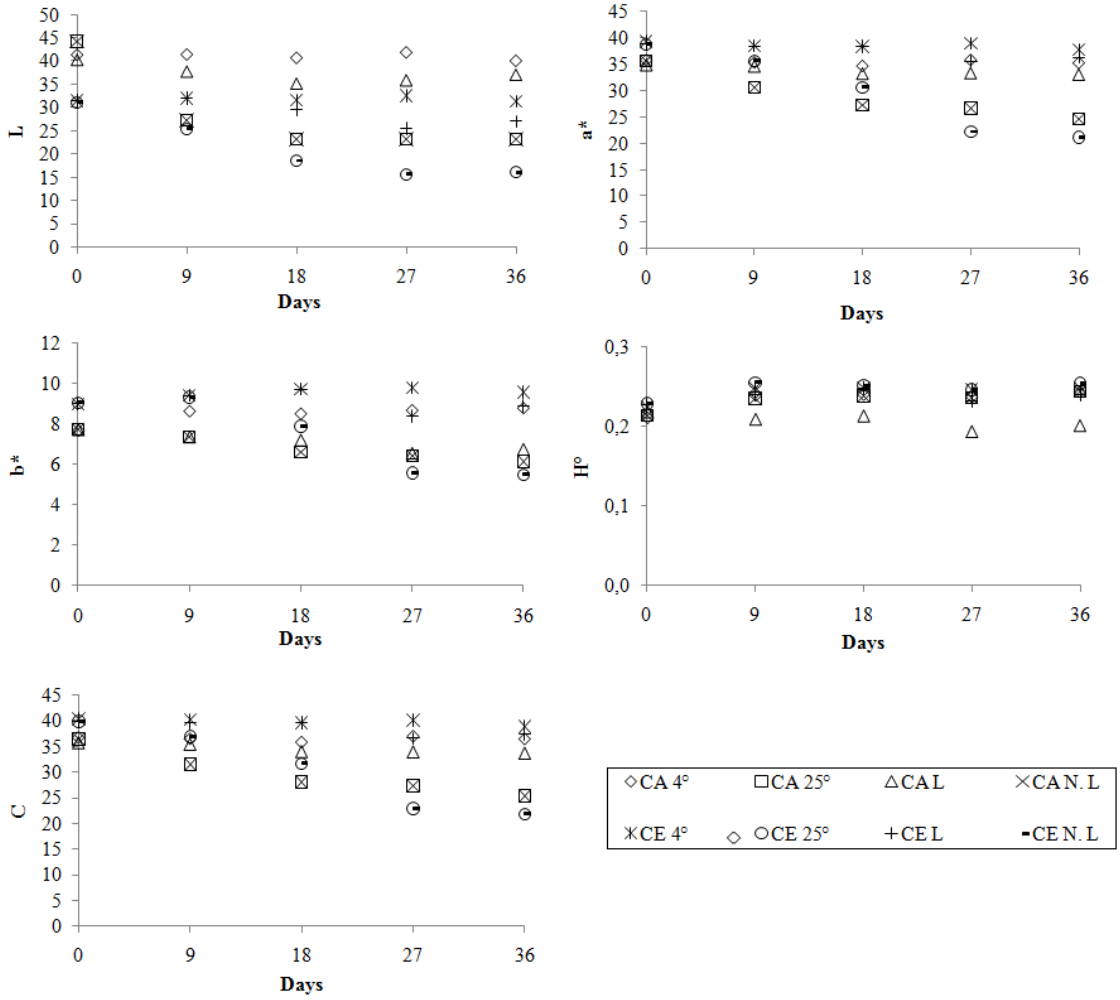

*Figure 1. Color parameters (L, a *, b *, H ° and C) of microcapsule solutions under light and temperature conditions. L = Light; N.L = No Light. L*= luminosity; a*= variations between green (-) and red (+); b*= variations between blue (-) and yellow (+); C= chromaticity; H= hue angle. CA= microcapsule aqueous; CE= microcapsule* hydroethanolic.

Regarding the parameter a *, 4 ° C temperature kept the values constant, indicating greater red intensity in the samples and better stability in this condition. CA 4 ° C had a loss of 1.53 % compared to 47.69 % in CA 25 ° C. In CE 4 ° C the loss was 4.21 % and 44.96 % in CE 25 ° C.
Another study observed the same pattern in a * and b * color parameters compared to higher temperatures for anthocyanins, where encapsulation with arabic gum protected the samples from heating (Guan and Zhong, 2015).
Goto and Kondo, reported that the decrease of red (a *) may be related to anthocyanins, due to the loss of the flavilium cation and hydrolysis of a double bond in the C-ring of the anthocyanin molecule (Goto and Kondo, 1991). In addition, glucose or sucrose from anthocyanins can be degraded during heat treatments producing glycol that reduces red Intensity (Brouillard and Delaporte, 1977).
Regarding the phenolic compounds (Table 1) there was an increase of 32.28 % in CA 4 ° C, whereas CA 25 ° C, with light and without light, showed no significant difference between 1 and 36 days of storage. It was also observed that TPC were not influenced in CE under the different light and temperature conditions.

Table 1. Total phenolic compounds and total monomeric anthocyanins in 36 days of storage

TPC (µg GAE.mg $^{-1}$ product)						
	d $_0$	d $_9$	d $_{18}$	d $_{27}$	d $_{36}$	%loss*
CA 4 °	23.13^{bB} ±1.11	23.60^{bC} ±0.07	23.29^{bC} ±1.04	18.29^{cC} ±0.39	30.60^{aB} ±1.51	+32.28
CA 25 ° C	25.64^{abB} ±0.62	22.38^{cC} ±0.20	24.53^{bC} ±0.12	19.86^{dB} ±0.43	26.67^{aC} ±1.11	+3.99
CA L	25.84^{aB} ±1.82	23.53^{aC} ±0.47	25.31^{aC} ±0.63	19.01^{bBC} ±0.37	25.13^{aC} ±0.13	2.75
CA N.L	25.64^{ab} ±0.62	22.38^{cC} ±0.20	24.53^{bC} ±0.12	19.86^{dB} ±0.43	26.67^{aC} ±1.11	+3.99
CE 4 °	37.78^{aA} ±0.54	38.00^{aAB} ±1.74	34.04^{bAB} ±0.77	28.82^{cA} ±0.50	38.58^{aA} ±0.68	+2.12
CE 25 ° C	38.18^{aA} ±1.37	39.56^{aA} ±1.01	32.44^{bB} ±1.01	28.42^{cA} ±0.50	38.40^{aA} ±0.35	+0.58
CE L	39.42^{aA} ±0.50	35.91^{bB} ±1.61	36.04^{bA} ±1.21	29.60^{cA} ±0.35	38.22^{abA} ±0.81	3.04
CE N.L	38.18^{aA} ±1.37	39.56^{aA} ±1.01	32.44^{bB} ±1.01	28.42^{cA} ±0.50	38.40^{aA} ±0.35	+0.58
TMA (µg cyanidin-3-glucoside.mg $^{-1}$)						
	d $_0$	d $_9$	d $_{18}$	d $_{27}$	d $_{36}$	%loss*
CA 4 °	1.18^{abB} ±0.02	0.98^{dC} ±0.02	1.12^{bcC} ±0.03	1.07^{cCD} ±0.02	1.22^{aD} ±0.03	+3.78
CA 25 ° C	1.19^{aB} ±0.04	0.84^{bD} ±0.04	1.02^{abD} ±0.03	1.21^{aC} ±0.21	1.05^{abE} ±0.03	11.16
CA L	1.13^{aB} ±0.05	0.78^{cD} ±0.02	0.99^{bD} ±0.01	0.83^{cD} ±0.03	0.79^{cF} ±0.02	30.04
CA N. L	1.19^{aB} ±0.04	0.84^{bD} ±0.04	1.02^{abD} ±0.03	1.21^{aC} ±0.21	1.05^{abE} ±0.03	11.16
CE 4 °	2.48^{abA} ±0.02	2.18^{dA} ±0.03	2.49^{aA} ±0.03	2.39^{bcA} ±0.06	2.38^{cdA} ±0.02	4.04
CE 25 ° C	2.46^{aA} ±0.02	2.18^{cA} ±0.03	2.20^{bcB} ±0.03	2.19^{bcAB} ±0.04	2.26^{bB} ±0.01	8.22
CE L	2.53^{aA} ±0.02	1.77^{dB} ±0.01	2.22^{bB} ±0.01	2.04^{cB} ±0.02	1.71^{dC} ±0.08	32.45
CE N. L	2.46^{aA} ±0.02	2.18^{cA} ±0.03	2.20^{bcB} ±0.03	2.19^{bcAB} ±0.04	2.26^{bB} ±0.01	8.22

Average followed by the same lowercase letter in the row and upper case in the column did not differ by Tukey's test ($P < 0.05$). d = days; L = Light; N.L = No Light; * Positive signs indicate increase. CA= microcapsule aqueous; CE= microcapsule hydroethanolic. TPC= total phenolic compounds; TMA= total monomerica anthocyanins.

Anthocyanins (Table 1) did not show degradation in CA 4 ° C and CA 25 ° C between 1 and 36 days of storage. In CE 4 ° C there was a loss of 4.04 % and 8.22 % in CE 25 ° C.

Another study observed a direct proportional relationship between the hydrolysis and the increased temperature, resulting in a lower stability of anthocyanins. The lower temperatures studied (- 20 ° C and 5 ° C) reduced the degradation of anthocyanins in extracts of Jamelao (Eugenia jambolana) throughout the storage (Sharma et al., 2016).

Light influenced the degradation of TMA, with a reduction of 30.04 % in CA Light. In CA No Light there was no loss. In the CE Light sample the degradation was greater 32.45 % as compared to 8.22 % in CE No Light. As previously studied (Weber et al., 2017) the samples stored in the dark presented greater stability, thus, the temperature had only a slightly accelerating effect on the anthocyanin degradation.

The limiting factor for the use of anthocyanins as a substitute for synthetic dyes is their low stability against factors such as light and temperature (Lopes et al., 2007). In general, it was observed that microencapsulation acts as a shield when the temperature is the variable in relation to color, and when light is the variable in relation to anthocyanins, thus minimizing degradation of samples under 4 ° C and no light.

Regarding the temperature at 25°C the loss was not great 11,26% in CA and 8,22% in CE. However, the microencapsulation can be used to storage in food industry at 25°C (ambient temperature). In relation to presence or absence of light, its indicated a packaging with materials which do not penetrate light.

4. Conclusions

Microencapsulation with maltodextrin was efficient for the protection of phenolic compounds and anthocyanins during the studied storage period and the different conditions proposed. This process can be used for technical applications in parallel with the re-propose of industrial waste, the use of this pomace is promising, since it has important antioxidant compounds, which when encapsulated may have technological applications by the food industry. Finally, is important to highlight that the light influency was more pronounced by light presence than temperature, the losses of bioactive compounds was higher in presence of light. The extraction with water, microcapsule CA was the only one that did not present anthocyanin loss in the temperature of 4 º C, indicating its potential since this type of aqueous extraction is low cost and ecofriendly (does not use organic solvents). In general, higher stability was observed in microcapsules with aquous extraction, stored at 4 ° C and without light, being the best stability condition for microcapsules of blackberry pomace. In the other hand, the microcapsules can be used for food industry for a variety of products, with food packaging without light penetration and storage at ambient temperature.

Acknowledgments

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ARTIGO 3

Microencapsulação de extratos de resíduo de amora-preta por spray-dryer: estabilidade ao pH

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Resumo - A extração de compostos antioxidantes do resíduo de amora-preta é interessante por utilizar um subproduto da indústria de processamento. O objetivo do presente estudo foi extrair compostos antioxidantes do resíduo de amora-preta por dois solventes, microencapsular por spray dryer e avaliar a estabilidade em diferentes pHs. Foram preparados dois extratos puros (aquoso e hidroalcoólico) e dois microencapsulados com maltodextrina, secos em spray dryer e analisados frente a estabilidade sob diferentes pHs (2,0, 3,5, 5,0 e 6,5) a 25°C, em relação a variação de cor (ΔE), compostos fenólicos totais, antocianinas, atividade antioxidante, e cinética de degradação de antocianinas durante 7 dias. Em relação a cor os extratos apresentaram maiores variações de cor quando comparados às microcápsulas, indicando a proteção das amostras. A microencapsulação com maltodextrina foi eficiente na redução da degradação de antocianinas frente ao aumento do pH, em pHs menores observou-se maior estabilidade das amostras encapsuladas, assim como os maiores tempos de meia vida das antocianinas, valores de 2 a 7 vezes maiores quando comparados aos extratos. A utilização de água no processo de extração resultou em menores percentuais de perdas no período de armazenamento de 7 dias, o que mostrou-se vantajoso em relação ao hidroalcoólico.

Termos para indexação: *Rubus fruticosus*, maltodextrina, compostos fenólicos, antocianinas, atividade antioxidante.

Microencapsulation of extracts of blackberry pomace by spray-dryer: pH stability

Abstract - The extraction of antioxidant compounds from the blackberry pomace is interesting because it uses a byproduct from the processing industry. This present study aimed to extract antioxidant compounds from the blackberry pomace by two solvents, microencapsulate by spray dryer and evaluate the stability at different pHs. Two pure extracts (aqueous and hydroalcoholic) and two microencapsulated with maltodextrin,were dried in spray dryer and analyzed for stability under different pHs with respect to color variation (ΔE), total phenolic compounds, anthocyanins, antioxidant activity, and kinetics degradation of anthocyanins in 7 days. Regarding the color, extracts presented greater color variations when compared to the microcapsules, indicating the protection of the samples. The microencapsulation with maltodextrin was efficient in reducing the degradation of anthocyanins against the increased pH, greater stability of the encapsulated samples was observed in lower pHs, as well as longer half-lives of anthocyanins, values from 2 to 7 times greater when compared to extracts. The use of water in the extraction process resulted in lower percentage losses in the storage period of 7 days, which was advantageous in relation to other types of extractions using organic solvents.

Index terms: *Rubus fruticosus*, maltodextrin, phenolic compounds, anthocyanins, antioxidant activity.

Introdução

As frutas silvestres, como a amora-preta (*Rubus fruticosus*), são altamente apreciadas pela combinação da sua cor e sabor agradável, bem como para os benefícios relatados sobre a saúde humana (D'AGOSTINO et al., 2015). Alguns estudos reportam a relação entra a atividade antioxidante e a presença de compostos fenólicos e antocianinas em amoras (ROSA et al., 2014). Os resíduos originados pela agroindústria de processamento de amoras geram em torno de 20% de cascas e sementes (IGNAT; VOLF; POPA, 2011).

Os compostos fenólicos contribuem para a redução do risco de doenças degenerativas, e os seus efeitos na saúde humana têm sido atribuídos principalmente à sua atividade antioxidante (MACHADO et al., 2015; SARIBURUN et al., 2010). Já as antocianinas são corantes naturais solúveis em água, responsáveis pela cor típica das amoras, e que têm sido consideradas potenciais substitutas dos corantes sintéticos na indústria de alimentos (HAMINIUK et al., 2012; LI et al., 2012).

Com relação à estabilidade, a principal desvantagem das antocianinas frente aos corantes sintéticos deve-se à mudança de coloração devido às reações químicas dos alimentos, visto que esse pigmento possui grupos cromóforos que são muito sensíveis às alterações do pH do meio (LOPES et al., 2007).

Em pH baixo as antocianinas apresentam-se na forma de cátion *flavilium* de coloração vermelha, com o aumento do pH ocorre uma desprotonação formando uma base quinoidal azul. As antocianinas também podem se apresentar nas formas de pseudo-base carbitol que é incolor, e chalcona levemente amarelada (HEREDIA et al., 1998).

A extração de compostos antioxidantes como fenólicos e antocianinas geralmente é realizada com o auxílio de solventes orgânicos, com agitação ou aquecimento, entretanto não pode ser considerada uma tecnologia limpa, enquanto a extração com água é uma alternativa viável para utilização na indústria de alimentos, por se tratar de uma tecnologia barata e limpa (IVANOVIC et al., 2014; REÁTEGUI et al., 2014).

Algumas técnicas podem melhorar a estabilidade dos compostos extraídos durante seu armazenamento, como por exemplo a microencapsulação, a qual pode ser definida como um processo em que pequenas partículas ou gotículas são cercadas por um revestimento, ou incorporadas numa matriz homogênea ou heterogênea, resultando em pequenas cápsulas com muitas propriedades úteis (GHARSALLAOUI et al., 2007). Vários são os revestimentos utilizados neste processo, sendo que os hidratos de carbono, tais como maltodextrinas, têm sido amplamente utilizados como agentes encapsulantes (CANO-HIGUITA et al., 2015; GOULA; ADAMOPOULOS, 2012).

Dentre as principais características desejáveis aos agentes microencapsulantes destacam-se a baixa higroscopicidade, baixa viscosidade a altas concentrações de sólidos, habilidade para emulsificar e estabilizar o material do núcleo, não reatividade, boa formação de filme, máxima proteção do núcleo conta luz, pH e oxigênio, ausência de sabor ou odor desagradável e baixo custo (CANO-HIGUITA et al., 2015; SHAHIDI; HAN, 1993)

O objetivo do presente estudo foi produzir microcápsulas a partir do resíduo de amora-preta aplicando extração hidroalcóolica e aquosa e avaliando-se a estabilidade das amostras em diferentes pHs.

Material e métodos

O resíduo de amora-preta (*Rubus fruticosus*) utilizado nesta pesquisa foi adquirido de um mesmo lote de um produtor da cidade de Paraibuna no estado de São

Paulo. A maltodextrina (DE10) foi cedida pela Cargil® (Campinas-SP). Os demais reagentes utilizados foram de grau analítico.

Os extratos obtidos foram secos nas mesmas condições das cápsulas para comparação. Assim denominou-se como extrato aquoso seco por spray dryer (EA), onde o resíduo de amora foi diluído em água em uma concentração de 500 mg/mL e agitado mecanicamente a 60°C por 45 minutos, conforme delineamento experimental realizado para avaliar a influência da temperatura e tempo na extração de antocianinas. E extrato hidroalcoólico seco em spray dryer (EE), onde o resíduo foi diluído na mesma proporção citada anteriormente em álcool etílico 80% (v/v) sob agitação mecânica por 48 horas, filtrado e rotaevaporado a 65°C graus até evaporação total do solvente (SHIRAHIGUE et al., 2011), logo após as amostras foram filtradas e submetidas a secagem em spray dryer.

Foram preparadas duas microcápulas, extrato aquoso encapsulado com maltodextrina (CA), onde o resíduo de amora-preta foi diluído em água, na concentração de 500 mg/mL, submetido à agitação por 45 minutos a 60°C e filtrado, o agente carreador maltodextrina DE 10 foi adicionado diretamente ao filtrado mediante agitação mecânica (1:1 p/p) de acordo com Ferrari et al. (2012). Para o extrato hidroalcoólico encapsulado com maltodextrina (CE), o resíduo foi diluído em álcool etílico 80% (v/v) sob agitação mecânica por 48 horas, filtrado e rotaevaporado a 65°C graus até evaporação total do solvente (SHIRAHIGUE et al., 2011). Posteriormente o agente carreador maltodextrina DE 10 foi adicionado (1:1 p/p) (FERRARI et al., 2012).

As amostras EA, EE, CA e CE foram submetidas a secagem em spray dryer (VALDUGA et al., 2008), sob as condições: temperatura do ar de secagem de entrada 170ºC e saída 105ºC; pressão de atomização: 4 bar; vazão média do ar de secagem: 3,5 m3/h; vazão média de alimentação: 0,5L/h, em equipamento Buchi B-191 Mini Spray-dryer. Os produtos secos foram colocados em embalagens plásticas e armazenados sob congelamento.

Foram preparadas soluções contendo as amostras (0,625 mg/mL) em tampões nos pHs 2, 3,5, 5 e 6,5. O ácido cítrico e o fosfato de sódio foram utilizados no preparo das soluções tampões de *Mcllvaine* (MORITA; ASSUMPÇÃO, 2001). A estas soluções foram adicionados 5% de sorbato de potássio, para evitar a contaminação por microrganismos.

A estabilidade das amostras nas soluções foi avaliada quanto ao teor de antocianinas, compostos fenólicos, atividade antioxidante e cor, frente a diferentes pHs,

no tempo inicial (t_0), após três dias (t_3) e sete dias (t_7), a temperatura constante de 25°C em BOD.

A determinação dos fenólicos totais (CFT) foi realizada utilizando os reagentes Folin-Ciocalteu (50%) e carbonato de sódio 3.79 M (Na_2CO_3) (PIERPOINT, 2004; SINGLETON; ROSSI, 1965). Os resultados foram expressos em µg de equivalente de ácido gálico (GAE).mg^{-1} de produto.

Para a determinação do teor de antocianinas totais monoméricas foi utilizado o método do pH diferencial (LEE; DURST; WROLSTAD, 2005). Os resultados foram expressos em µg cianidina-3-glucosídeo.mg^{-1}, conforme equação 1 e 2.

$$\mathrm{AT} = (\mathrm{ABS\ 520nm} - \mathrm{ABS\ 700nm})\mathrm{pH\ 1{,}0} - (\mathrm{ABS\ 520nm} - \mathrm{ABS\ 700nm})\mathrm{pH\ 4{,}5} \quad (1)$$

$$\mathit{A}\mathrm{ntocianinas\ (ACN)} = \frac{(\mathrm{ATxPMxdfx}\ \ 10^3)}{\varepsilon \mathrm{x}\ \lambda} \quad (2)$$

onde: PM= 449,2 g/mol (massa molar da cianidina-3-glucosídeo); df= fator de diluição; 10^3= fator de conversão de g para mg; Ɛ= 26900L/mol.(absortividade molar da cianidina-3-glucosídeo); ƛ= 1 cm (comprimento caminho óptico da cubeta).

A redução do radical estável DPPH (2,2-difenil-1-picrilhidrazila) foi determinada por colorimetria em espectrofotômetro (THAIPONG et al., 2006), a eficiência da atividade sequestradora foi calculada de acordo com a equação 3.

$$\text{Eficiência do sequestro dos radicais livres (\%)} = \frac{(\mathrm{A_{controle}} - \mathrm{A_{amostra}})}{\mathrm{A_{controle}}} \text{ x } 100 \quad (3)$$

Onde: $A_{controle}$: Absorbância do controle negativo; $A_{amostra}$: Absorbância média da amostra.

Os resultados foram expressos como capacidade antioxidante equivalente (EC50) µg.mL produto^{-1} calculados pelo software Graphpad Prism 5.

A cor foi avaliada por meio de um colorímetro portátil Minolta® CR400, com esfera de integração e ângulo de visão de 3^o, ou seja, iluminação d/3 e iluminante D65. O sistema utilizado foi o CIEL*a*b*.

Os valores de diferença de cor (ΔE) foram calculados de acordo com a equação 4, para estudar as alterações de cor (OBÓN et al., 2009).

$$\Delta E = [(L_7^* - L_0^*)^2 + (a_7^* - a_0^*)^2 + (b_7^* - b_0^*)^2]^{0,5} \quad (4)$$

Onde: L^*_0, a^*_0 e b^*_0 são os valores das amostras no tempo zero e L^*_7, a^*_7 e b^*_7 os valores após 7 dias de armazenamento.

O percentual (%) de perda total de antocianinas e compostos fenólicos no período de estocagem,foi calculado através da relação entre a quantidade no último dia de armazenamento (t7), pela quantidade inicial (t0) conforme a Equação (5) (SOUZA et al., 2014).

$$\text{Perda total (\%)} = \left(1 - \frac{\text{t7}}{\text{t0}}\right) \text{x } 100 \quad (5)$$

A degradação das antocianinas foi calculada pela equação 6. Onde: C é a concentração remanescente; C_0 é a concentração inicial; t é o intervalo de tempo entre C_0 e C; k é a constante de transformação de 1ª ordem (1/tempo).

$$\frac{dC}{dt} = -k.C \quad (6)$$

Numa plotagem de concentração (-ln C) versus tempo, a constante de transformação (k) é simplesmente a inclinação da reta.

O tempo de meia vida ($t_{1/2}$) para a reação é o tempo requerido para que a quantidade de antocianinas caia pela metade do seu valor inicial. Ele está diretamente relacionado com a constante da velocidade para uma reação de primeira ordem descrito pela Equação 7 (KIRCA; CEMEROĞLU, 2003).

$$t_{1/2} = \frac{\ln(2)}{k} \quad (7)$$

As análises foram submetidas à análise de variância e teste de *Tukey* para a diferença mínima significativa entre as médias utilizando o programa estatístico STATISTICA versão 7.0.

Resultados e discussão

A Figura 1 apresenta as variações de cor (ΔE) das amostras nos diferentes pHs estudados.

Os extratos EA e EE apresentaram maiores variações de cor em pH 2,0, 3,5 e 5,0 quando comparados com CA e CE (Figura 1), indicando que a microencapsulação influenciou em uma maior estabilidade de cor (L*, a* e b*).

Em geral avaliando-se as cápsulas em relação ao pH, para CA e CE as menores variações de cor foram observadas em pHs 2,0 e 5,0. Indicando a maior estabilidade das antocianinas em pH mais baixo (TÜRKER; ERDOG˘DU, 2006). A menor variação

de cor em pH 5,0 é importante para aplicação em alimentos, pois existe uma gama enorme de alimentos neste pH.

Considerando o tempo de armazenamento avaliado, segundo (OBÓN et al., 2009) variações significativas são encontradas em valores de ΔE maiores que 5,0, sendo assim observou-se que as microcápsulas mantiveram melhor a cor durante o tempo de armazenamento quando comparadas aos extratos.

A Tabela 1 apresenta os resultados das análises de compostos fenólicos totais, antocianinas e atividade antioxidante das soluções com as amostras nos diferentes pHs estudados.

Em pH 2,0 os compostos fenólicos totais aumentaram em todas as amostras durante os 7 dias de armazenamento, a mesma relação foi observada nos demais pHs para as amostras EA, CA e CE. Porém apenas a amostra EE foi degradada nos pHs 3,5, 5,0 e 6,5. O aumento de CFT pode estar relacionado à degradação das antocianinas em outros compostos fenólicos, como a cumarina 3,5-diglicosídeo (DAMODARAN; PARKIN; FENNEMA, 2010).

Avaliando as antocianinas totais monoméricas, os pHs mais baixos (2,0 e 3,5) mantiveram as amostras EA, EE, CA e CE, mais estáveis em relação aos outros pHs estudados.

Observou-se no pH 2,0, degradação de 77,69% de antocianinas em EA em t_7, e 78,54% em EE, nas microcápsulas a degradação foi bem menor, 27,72% em CA e 36,36% em CE. A degradação foi aumentando gradativamente em relação ao aumento do pH, no pH 6,5 a degradação foi de 100% em todas as amostras.

O pH influenciou na degradação das antocianinas, o aumento do pH pode resultar na redução da estabilidade do pigmento (TÜRKER; ERDOGˇDU, 2006). Estas podem sofrer reações que alteraram suas estruturas devido à deficiência de seus núcleos *flavilium*. A estabilidade das antocianinas aumenta com o número de metoxilas e diminui à medida que as hidroxilas aumentam, desta forma as antocianinas são mais estáveis a pH ácido (HOU et al., 2013).

A atividade antioxidante foi influenciada pelo pH, observou-se que não houve diferença significativa nos pHs 2,0 e 3,5 durante 7 dias de armazenamento. Com o aumento do pH (5,0 e 6,5), houve redução das antocianinas presentes nas amostras, observou-se uma redução geral da atividade antioxidante. Tal fato pode ser explicado,

pois possivelmente a degradação das antocianinas formou compostos fenólicos com menor atividade antioxidante, justificando assim o aumento de CFT e a redução na atividade antioxidante com o aumento do pH. Conforme reportado na literatura, a atividade antioxidante da amora está fortemente relacionada aos compostos fenólicos, assim como encontrado em outros estudos (GARCIA-MENDOZA et al., 2017; MACHADO et al., 2015).

Em relação ao tipo de extração utilizada, CA teve os menores percentuais de perda total no período de 7 dias nos pHs 2,0, 3,5 e 5,0, tornando-se vantajosa a utilização de água como solvente para extração, pelo baixo custo quando comparado à CE que utiliza solvente orgânico.

A Figura 2 apresenta os valores de tempo de meia vida e constante de degradação das amostras.

Observou-se que a degradação de antocianinas obedeceu uma cinética de primeira ordem, assim como reportado na literatura (KIRCA; CEMEROĞLU, 2003).

Em pHs 2,0, 3,5 e 5,0 os $t_{1/2}$ das microcápsulas (CA e CE) foram pelo menos de 2 a 7 vezes maiores quando relacionados aos extratos (EA e EE), os maiores $t_{1/2}$ foram encontrados nos pHs mais baixos para as microcápsulas, indicando a proteção das antocianinas pela encapsulação nestes pHs.

Os $t_{1/2}$ foram reduzindo gradativamente em relação ao aumento do pH, variando de 14 dias em pH 2,0 até 0,8 dias em pH 6,5, como as constantes de degradação são inversamente proporcionais ao $t_{1/2}$, as menores constantes foram encontradas nos pHs mais baixos. Escassos são os trabalhos que avaliam a microencapsulação de resíduo de amora preta, assim a estabilidade, bem como os parametros de degradação em relação ao pH ainda não foram reportados pela literatura.

Bustos-Garza et al., (2013) avaliando a microencapsulação de astaxanthina de *Haematococ cuspluvialis* a 25 °C com maltodextrina e goma arábica aplicando processo de spray dryer obtiveram tempos de meia vida entre 1,6 e 2,5 dias em diferentes pHs.

Conclusões

1. A extração de compostos antioxidantes do resíduo de amora-preta é interessante por utilizar um subproduto da indústria de processamento de frutas.

2. A microencapsulação com maltodextrina dos compostos antioxidantes provenientes desse resíduo foi eficiente na redução da degradação de antocianinas frente ao aumento do pH.
3. Em pHs mais baixos observou-se maior estabilidade das amostras encapsuladas, foram encontrados os maiores tempo de meia-vida e as menores constantes de degradação.
4. Como as microcápsulas foram eficientes na redução da degradação das amostras em pHs mais baixos até pH 5,0, indica-se a aplicação como corante e antioxidante em vários tipos de alimentos, como iogurtes e bebidas lácteas, sucos, molhos, refrigerantes e bebidas gasosas, doces, geleias entre outros.
5. O tipo de extração também influenciou, a utilização de água no processo de extração mostrou-se vantajoso em relação à outros solventes, por apresentar baixo custo e ser uma tecnologia limpa.

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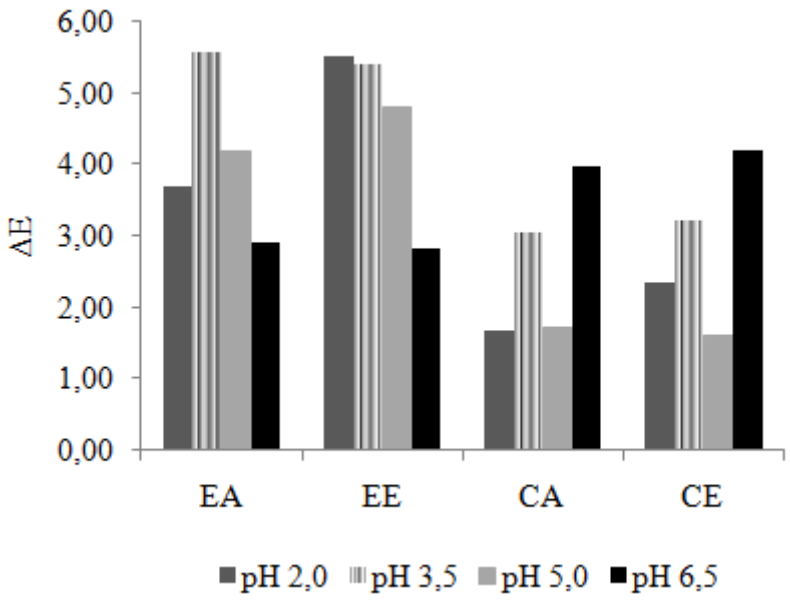

Figura 1. Variações de cor (ΔE) das soluções com as amostras nos diferentes pHs (2,0, 3,5, 5,0 e 6,5).

Tabela 1. Compostos fenólicos totais, antocianinas totais monoméricas e atividade antioxidante (EC_{50}) das soluções nos tempos iniciais (t_0) e finais (t_7)

pH 2,0						
	CFT (µg EAG.mg produto)		ACN (µg cianidina-3-glucosídeo.mg^{-1})		EC_{50} (µg.mL produto^{-1})	
	t_0	t_7	t_0	t_7	t_0	t_7
EA	52,53^{bB} ±0,00	66,62^{bA} ±2,53	1,21^{cA} ±0,00	0,27^{dB} ±0,05	44,33^{cA} ±0,58	43,67^{cA} ±1,15
EE	91,80^{aB} ±0,85	103,20^{aA} ±0,48	2,47^{aA} ±0,01	0,53^{cB} ±0,05	23,00^{dA} ±0,00	21,50^{dA} ±0,58
CA	23,61^{dB} ±0,18	29,47^{dA} ±0,81	1,01^{dA} ±0,01	0,73^{bB} ±0,04	91,67^{aA} ±1,53	94,33^{aA} ±2,52
CE	26,34^{cB} ±0,08	37,07^{cA} ±0,53	1,98^{bA} ±0,04	1,26^{aB} ±0,01	65,67^{bA} ±0,58	66,00^{bA} ±5,20
pH 3,5						
	CFT (µg EAG.mg produto)		ACN (µg cianidina-3-glucosídeo.mg^{-1})		EC_{50} (µg.mL produto^{-1})	
	t_0	t_7	t_0	t_7	t_0	t_7
EA	48,62^{bB} ±1,59	61,29^{bA} ±3,25	0,89^{bA} ±0,00	nd	44,00^{cA} ±0,00	46,67^{cA} ±2,52
EE	87,91^{aA} ±2,40	79,16^{aB} ±2,82	2,07^{aA} ±0,00	0,21^{cB} ±0,01	28,50^{dA} ±1,53	31,67^{dA} ±2,08
CA	22,24^{cB} ±0,43	30,51^{dA} ±1,00	0,88^{bA} ±0,04	0,31^{bB} ±0,00	95,50^{aA} ±9,07	98,67^{aA} ±0,58
CE	24,96^{cB} ±0,92	37,56^{cA} ±2,34	1,97^{aA} ±0,06	0,67^{aB} ±0,01	71,50^{bA} ±3,21	78,00^{bA} ±2,52
pH 5,0						
	CFT (µg EAG.mg produto)		ACN (µg cianidina-3-glucosídeo.mg^{-1})		EC_{50} (µg.mL produto^{-1})	
	t_0	t_7	t_0	t_7	t_0	t_7
E A	52,27^{bA} ±1,35	53,64^{bA} ±1,24	0,42^{cA} ±0,01	nd	46,50^{cA} ±0,58	50,00^{cA} ±3,61
EE	84,18^{aA} ±0,78	75,73^{aB} ±0,81	1,61^{aA} ±0,05	nd	28,00^{dB} ±0,58	34,67^{dA} ±0,58

C	21,29^{dB}	25,62^{dA}	0,65^{bA}	0,17^{bB}	174,00^{aA}	158,50^{aB}
A	±0,70	±0,38	±0,04	±0,01	±0,58	±6,36
CE	24,21^{cB}	37,80^{cA}	1,63^{aA}	0,33^{aB}	72,33^{bB}	100,50^{bA}
	±0,58	±3,24	±0,01	±0,01	±2,31	±7,78

pH 6,5

	CFT (µg EAG.mg produto)		ACN (µg cianidina-3-glucosídeo.mg^{-1})		EC_{50} (µg.mL produto^{-1})	
	t_0	t_7	t_0	t_7	t_0	t_7
EA	50,40^{bB}	53,16^{bA}	0,31^{dA}	nd	52,00^{cB}	93,00^{abA}
	±0,53	±1,37	±0,01		±1,41	±4,24
EE	82,76^{aA}	75,16^{aB}	1,26^{bA}	nd	52,00^{cA}	65,00^{bA}
	±2,01	±1,69	±0,01		±1,41	±19,80
CA	24,03^{cA}	23,22^{dA}	0,55^{cA}	nd	97,00^{aA}	105,67^{aA}
	±3,20	±0,67	±0,01		±7,51	±6,66
CE	25,94^{cB}	30,87^{cA}	1,37^{aA}	nd	72,50^{bB}	103,00^{abA}
	±0,76	±1,40	±0,02		±3,79	±2,83

Médias seguidas pela mesma letra minúscula na coluna e maiúscula na linha não diferiram entre si pelo teste de *Tukey* (P<0,05). nd: não detectado.

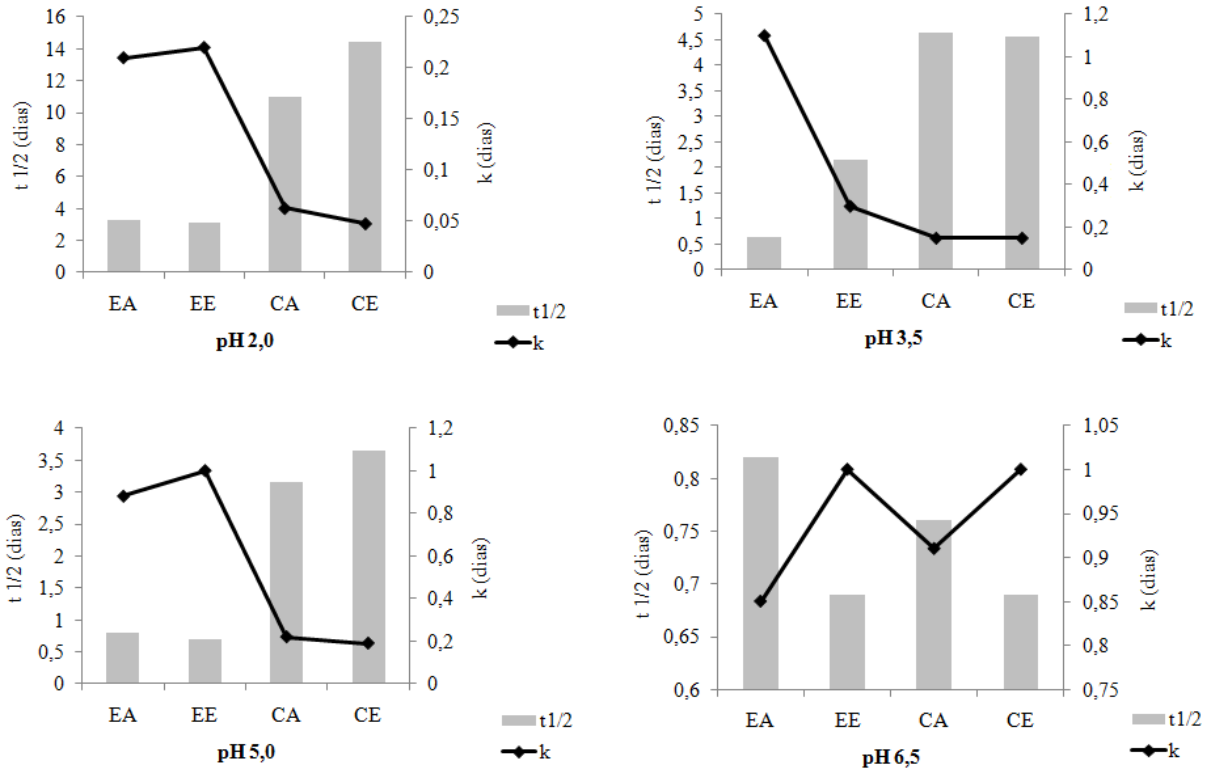

Figura 2. Tempo de meia vida ($t_{1/2}$) e constantes de degradação (k) das amostras EE, EA, CA e CE nos diferentes pHs estudados.