UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS
Programa de Pós-Graduação em Ciência de Alimentos

AVALIAÇÃO DO ARMAZENAMENTO NA CONSERVAÇÃO DE COMPOSTOS BIOATIVOS DA POLPA DE ACEROLA (*Malpighia* spp), ATRAVÉS DE MICROENCAPSULAÇÃO E LIOFILIZAÇÃO.

BRUNO HENRIQUE FIGUEIREDO SAQUETI

Maringá
2021

BRUNO HENRIQUE FIGUEIREDO SAQUETI

“AVALIAÇÃO DO ARMAZENAMENTO NA CONSERVAÇÃO DE COMPOSTOS BIOATIVOS DA POLPA DE ACEROLA (*MALPIGHIA* SPP), ATRAVÉS DE MICROENCAPSULAÇÃO E LIOFILIZAÇÃO”.

Dissertação apresentada à Universidade Estadual de Maringá, como parte das exigências do Programa de Pós-graduação em Ciência de Alimentos, para obtenção do grau de Mestre em Ciência de Alimentos.

Prof. Dr. Jesuí Vergílio Visentainer

Profa. Dra. Suelen Pereira Ruiz Herrig

Prof. Dr. Oscar de Oliveira Santos Júnior
Orientador

Maringá – 2021

Dados Internacionais de Catalogação-na-Publicação (CIP)
(Biblioteca Central - UEM, Maringá - PR, Brasil)

S242a

Saqueti, Bruno Henrique Figueiredo

Avaliação do armazenamento na conservação de compostos bioativos da polpa de acerola (*Malpighia* spp), através de microencapsulação e liofilização / Bruno Henrique Figueiredo Saqueti. -- Maringá, PR, 2021.

53 f.: il. color., figs., tabs.

Orientador: Prof. Dr. Oscar de Oliveira Santos Junior .

Dissertação (Mestrado) - Universidade Estadual de Maringá, Centro de Ciências Agrárias, Departamento de Ciências Agronômicas, Programa de Pós-Graduação em Ciência de Alimentos, 2021.

1. Microencapsulação. 2. Liofilização. 3. Acerola (Malpighia spp). I. Santos Junior , Oscar de Oliveira, orient. II. Universidade Estadual de Maringá. Centro de Ciências Agrárias. Departamento de Ciências Agronômicas. Programa de Pós-Graduação em Ciência de Alimentos. III. Título.

CDD 23.ed. 664

Marinalva Aparecida Spolon Almeida - 9/1094

Orientador

Prof. Dr. Oscar de Oliveira Santos Junior

BIOGRAFIA

Bruno Henrique Figueiredo Saqueti nasceu no estado do Paraná, na cidade de Pérola. Possui graduação em Tecnologia em Processos Gerenciais pela Faculdade Alfa de Umuarama e Tecnologia em Alimentos pela Universidade Estadual de Maringá. Tem experiência na área de Ciência de Alimentos atuando principalmente nos seguintes temas: Tecnologia de alimentos, alimentos funcionais e compostos bioativos, desenvolvimento de novos produtos alimentícios, cromatografia em fase gasosa, microencapsulação e ensaios antioxidantes.

Dedico

A minha família em especial minha mãe Elza Ryska Figueiredo e meus avós Leocadia Ryska Figueiredo e Florisvaldo Alves Figueiredo, pelo exemplo de coragem e simplicidade em suas metas, e com muito carinho me ensinou o caminho da justiça, e a meus queridos amigos que sempre confiaram em meu potencial.

AGRADECIMENTOS

Com grande satisfação, agradeço sinceramente todos àqueles que me acompanharam e contribuíram para o meu crescimento pessoal e profissional ao longo do curso de Pós-Graduação, em especial agradeço:

A Deus;

Ao meu orientador Prof. Dr. Oscar de Oliveira Santos Júnior, pela oportunidade em me escolher como seu orientado, pelo apoio e principalmente pela confiança;

Aos meus amigos e companheiros de pesquisa que contribuíram para o desenvolvimento e elaboração deste mestrado;

Aos membros do grupo de pesquisa APLE-A, pela colaboração, união, incentivo e amizade;

Ao Programa de Pós-Graduação em Ciência de Alimentos pela oportunidade de realizar esta etapa da vida acadêmica;

Aos docentes do programa, que sempre estiveram dispostos a ajudar e contribuir com seus ensinamentos.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), à Fundação Araucária de Apoio ao desenvolvimento Científico e Tecnológico do Estado do Paraná e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pelo auxílio, suporte financeiro e apoio concedido.

APRESENTAÇÃO

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

Autores: Bruno Henrique Figueiredo Saqueti; Eloize da Silva Alves; Matheus Campos Castro; Patrícia Daniele da Silva Santos; Nayane Braga Mattos Sinosaki; Carlos Eduardo Rubio Senes; Jesuí Vergilio Visentainer; Oscar de Oliveira Santos Júnior.

Revista: Journal of the Brazilian Chemical Society.

Título: Evaluation of the storage in the conservation of bioactive compounds of the acerola pulp (*Malpighia* spp.) through microencapsulation and lyophilization.

GENERAL ABSTRACT

INTRODUCTION. Acerola (Malpighia spp) is a tropical fruit originally from Central America, its industrial interest is due to its organoleptic characteristics and its high levels of bioactive compounds (BC), such as ascorbic acid (AA), phenolic compounds, flavonoids and anthocyanins. The presence of bioactive compounds has been frequently studied due to its health benefits. Its processing consists of products, such as pulps, juices, concentrates, jellies and jams, obtained from industrial processing suitable for each segment. Processing to obtain frozen fruit pulp consists of removing the edible part of the fruit by pulping followed by preservation by freezing. Emerging methods of preserving fruit pulps are studied, in order to guarantee the bioavailability of nutrients and prevent their loss by freezing and / or other applied treatments. Microencapsulation is a technique that traps solid, liquid or volatile particles within some matrix, remaining covered by this material (encapsulating agent), which can guarantee BC protection and stability. Different coating agents are used, which can be natural, semi-synthetic or synthetic polymers. In the microencapsulation technique, drying processes such as lyophilization are associated, this promotes the removal of water from frozen mixtures by sublimation at low temperatures and under vacuum, and a secondary drying phase by desorption. Lyophilization guarantees superior quality to other techniques, maintaining the biofunctionality and a longer useful life to BC, without affecting the sensory properties. Therefore, the stabilization of BC can be improved using microencapsulation technology, associated with freeze drying.
AIMS. The objective of this work is to evaluate the effect of storage on the conservation of BC of acerola pulp by microencapsulation and lyophilization techniques, based on physical-chemical analyzes, antioxidant tests and mass spectrometry with electrospray ionization (ESI-MS).
MATERIAL AND METHODS. The acerolas were obtained at an open market, located in the city of Maringá (Paraná, Brazil), and were subjected to the stages of hygiene, weighing and pulping, carried out at the Plant Processing Laboratory of the State University of Maringá. They were submitted to three treatments obtaining the following samples: frozen acerola pulp (FAP), lyophilized acerola pulp (LAP) and microencapsulated and lyophilized acerola pulp (MLAP). The microencapsulation performed in the MLAP sample was performed by a cavitation system in an ultrasonic bath for 20 minutes, 25 ° C at 80 kHz, adding 10% maltodextrin as an encapsulating agent to the pulp, submitted to the MLAP sample. For drying operation used in the LAP and MLAP samples, a bench freeze dryer was used. The treatments were stored in a freezer (-18 ° C) until the analyzes were performed. To obtain the extract, the FAP treatment was thawed at room temperature (25 ° C), while LAP and MLAP were resuspended in distilled water, using the yield in the lyophilization process. The extracts were prepared according to Rezende, Nogueira & Narain (2017) under conditions optimized for the extraction of bioactive compounds in acerola pulp. DPPH assay analyzes, pH, soluble solids, colorimetric analysis (L *, a * and b *), total phenolic compounds (TPC), total flavonoids (TF), total anthocyanins (TA) and identification of ascorbic acid (AA) by mass spectrometry with direct electrospray infusion (ESI-MS). The data obtained were submitted to statistical analysis of variance (ANOVA), and the means were compared using the Tukey test with a 95% significance level.

RESULTS AND DISCUSSION. The concentration data of the DPPH assay were evaluated every two weeks for 180 days. The highest concentrations at time 0 were obtained in the FAP (199.39 ± 1.63 μM TE g-1) and MLAP (200.93 ± 2.42 μM TE g-1) treatments, with no significant difference (p> 0.05) however, the FAP treatment (184.31 ± 2.17 μM TE g-1) obtained a lower concentration, differing significantly (p ≤ 0.05). In 150 days the samples had a significant reduction, after being stable until the storage time of 180 days, the MLAP sample was the one that obtained the highest final concentration (161.58 μM TE g-1). The antioxidant activity is influenced by the concentration of BC present in the sample, that is, if the sample undergoes a degradation in its phytochemicals, consequently this will influence the antioxidant activity. The data from the analysis of pH and soluble solids were evaluated at 0, 90 and 180 days. The pH results showed differences according to the treatment applied, that is, the samples that were lyophilized LAP and MLAP obtained lower values 3.66 ± 0.00 and 3.72 ± 0.04 respectively, obtaining significant difference (p ≤ 0.05). The storage time implied changes in pH, and the FAP and LAP samples had a significant reduction in the two analyzed times, being possible to verify an instability of the compounds in these samples. The MLAP sample from 90 days (3.58 ± 0.01) to 180 days (3.54 ± 0.02) showed no significant difference (p> 0.05), verifying stability, in this case microencapsulation served as a barrier to sample stability. The soluble solids in the FAP and LAP samples showed the values of 5.97 ± 0.04 and 6.13 ± 0.04 ° Brix in the time of 0 days, not significantly differing (p> 0.05), with the passage of time storage it was possible to observe an increase in the content of soluble solids in the same proportion. For the MLAP sample at 0 days it showed a value of 10.93 ± 0.49 ° Brix, being higher among the other treatments, this is justified by the addition of maltodextrin as an encapsulating agent in the sample. The results of the colorimetric analysis were evaluated at 0, 90 and 180 days. For L * it refers to luminosity, in the time of 0 days the values were 42.97 ± 0.15 (FAP), 48.92 ± 0.20 (LAP) and 63.71 ± 0.31 (MLAP) differing statistically (p ≤ 0.05) among themselves, this due to the lyophilization process and the addition of maltodextrin. The two processes will contribute to the increase in luminosity and to a greater extent the addition of maltodextrin than being a white powder, when dissolved in the acerola pulp and acting with an encapsulant, the sample tended to clear. For a * refers to the green / red chromaticity. The samples showed values that varied between 0.56 and 13.49, with positive values and their color tending to the red color spectrum. For b * it refers to the blue / yellow chromatic intensity, the samples presented values that varied between 22.64 and 31.23, being positive values and their color tending to the yellow color spectrum. For TPC the results show that there was a significant difference (p ≤ 0.05) in the concentration of the storage time of 0 days, with the highest concentration for the MLAP sample (494.25 ± 1.79 mg GAE / 100g), followed by LAP (478, 36 ± 2.13 mg GAE / 100g) and FAP (444.83 ± 2.07 mg GAE / 100g). After 90 days of storage, the samples behaved differently, with FAP (481.84 ± 0.65 mg GAE / 100g) increasing, LAP (455.17 ± 8.74 mg GAE / 100g) reducing and MLAP (493.16 ± 3.31 mg GAE / 100g) remained stable and there was no significant difference (p> 0.05), with 180 days of storage the FAP samples (501.78 ± 0.31 mg GAE / 100g) and MLAP (535.56 ± 0.56 mg GAE / 100g) had an increase in concentration while LAP (460.34 ± 0.57 mg GAE / 100g) remained stable. The TF results in the storage time of 0 days had no significant difference (p> 0.05) in the concentrations for the FAP samples (161.55 ± 2.45 mg QE / 100g) and MLAP (160.40 ± 1.99 mg QE / 100g), differing only for the LAP sample (155.81 ± 0.84 mg QE / 100g), with 90 days and 180 days of storage the samples remained stable, with no significant difference (p>

0.05) in relation to the storage time. The sample that obtained the highest concentration in the storage time of 180 days was MLAP (160.53 ± 1.04 mg QE / 100g). For the results of TA in the storage period of 0 days, the FAP sample (8.46 ± 0.07 mg 100 g-1) showed a higher concentration, differing statistically ($p \leq 0.05$) from LAP (7.61 ± 0, 07 mg 100 g-1) and MLAP (7.57 ± 0.18 mg 100 g-1), that is, lyophilization and microencapsulation affected the sample concentrations. In the storage period of 180 days, the samples that had reduced concentrations were FAP (5.08 ± 0.03 mg 100 g-1) and LAP (4.25 ± 0.01 mg 100 g-1), decreasing 21, 4% and 43.2% respectively, while MLAP (5.70 ± 0.03 mg 100 g-1) remained stable, with the treatment having the highest concentration at the end of the storage time. The ESI-MS analysis was performed in order to verify the variations in the ascorbic acid concentration. It can be noted that there was variation in the concentration of ascorbic acid in the different treatments over the days. In addition, in the first 90 days, there was an increase in the concentration of AA, with an increase of 39.2% for the FAP, 8.1% for LAP and 20.3% for MLAP. However, after 90 days, it is noticeable that there was a decrease in the concentration in the CAP, in contrast the other two forms of treatment of the acerola pulp LAP and MLAP had a significant increase in concentration, of 39.9% and 60.6% respectively. This factor is due to the AA's intrinsic characteristics, mainly due to its high solubility in water that directly influences oxidation and reduction reactions. For the frozen sample, after 90 days, its concentration was reduced, possibly due to the AA molecules interacting with water, because the hydrogen bonds reduce the movement of the molecules, mainly in solid materials, besides, the particle size and the concentrations, directly influence oxidation reactions by reducing the concentration.

CONCLUSIONS. The microencapsulation technique associated with lyophilization that was used in the acerola pulp was efficient to obtain a stable product and with superior quality to the other applied treatments. Higher values were found in the concentrations of bioactive compounds and antioxidant activity at the end of the storage period of 180 days. In addition, the MLAP treatment obtained greater stability in pH during the storage period evaluated. It was observed that the soluble solids had an increase, due to the addition of the encapsulating agent (maltodextrin). For color obtained higher values of luminosity (L*), tendency to red color (a*), but with less intensity and tendency to yellow color (b*), with greater intensity in relation to the FAP and LAP treatment. A significant increase in the concentration of AA was observed in the LAP and MLAP treatments, that is, the samples that underwent drying (lyophilization) were able to preserve and concentrate the AA content. The study showed the feasibility of using the microencapsulation technique to obtain a powder product with stability of its bioactive compounds, which can be used in the development of new functional products and / or in their reconstitution in water.

Key words: Antioxidants; Phenolic compounds; Anthocyanins; Flavonoids, DPPH.

RESUMO GERAL

INTRODUÇÃO. Acerola (*Malpighia* spp) é uma fruta tropical originária da América central, seu interesse industrial é decorrente das suas características organolépticas e seus altos níveis de compostos bioativos (CB), como ácido ascórbico (AA), compostos fenólicos, flavonoides e antocianinas. A presença de compostos bioativos tem sido frequentemente estudada devido aos seus benefícios à saúde. Seu beneficiamento consiste em produtos, como polpas, sucos, concentrados, geleias e compotas, obtidos de processamento industrial adequado para cada segmento. O processamento para obtenção de polpa de frutas congeladas consiste na remoção da parte comestível da fruta realizando o despolpamento seguido da preservação por congelamento. Métodos emergentes de conservação de polpas de frutas são estudados, a fim de garantir a biodisponibilidade dos nutrientes e evitar a perda dos mesmos pelo congelamento e/ou outros tratamentos aplicados. A microencapsulação é uma técnica que aprisiona partículas sólidas, líquidas ou voláteis dentro de alguma matriz, permanecendo revestidas por esse material (agente encapsulante), podendo garantir proteção e estabilidade dos CB. Diferentes agentes de revestimento são utilizados, podendo ser polímeros naturais, semi-sintéticos ou sintéticos. Na técnica de microencapsulação associam-se processos de secagem como a liofilização, esta promove a remoção de água de misturas congeladas por sublimação em baixas temperaturas e sob vácuo, e uma fase de secagem secundária por dessorção. A liofilização garante qualidade superior a outras técnicas, mantendo a biofuncionalidade e uma vida útil mais longa aos CB, não afetando as propriedades sensoriais. Portanto, a estabilização dos CB pode ser melhorada usando tecnologia de microencapsulação, associada com secagem por liofilização.

OBJETIVOS. O objetivo deste trabalho é avaliar o efeito do armazenamento na conservação dos CB da polpa de acerola por técnicas de microencapsulação e liofilização, a partir de análises físico-químicas, ensaios antioxidantes e espectrometria de massas com ionização por *electrospray* (ESI-MS do inglês *electrospray ionization – Mass spectrometry*).

MATERIAL E METODOS. As acerolas foram obtidas em feira livre, localizada na cidade de Maringá (Paraná, Brasil), foram submetidas às etapas de higienização, pesagem e despolpamento, realizada no Laboratório de Processamento Vegetal da Universidade Estadual de Maringá. Foram submetidos a três tratamentos obtendo as seguintes amostras: polpa de acerola congelada (PAC), polpa de acerola liofilizada (PAL) e polpa de acerola microencapsulada e liofilizada (PAML). O microencapsulamento feito na amostra PAML foi realizado por sistema de cavitação em banho ultrassônico por 20 minutos, 25 °C a 80 kHz, adicionando na polpa 10% de maltodextrina como agente encapsulante, submetido a amostra PAML. Para operação de secagem utilizada nas amostras PAL e PAML foi utilizado um liofilizador de bancada. Os tratamentos foram armazenados em um freezer (-18 ° C) até realização das análises. Para a obtenção do extrato o tratamento PAC foi descongelado em temperatura ambiente (25 °C), enquanto PAL e PAML foram ressuspensos em água destilada, utilizando o rendimento no processo de liofilização. Os extratos foram preparados segundo Rezende, Nogueira & Narain (2017) em condições otimizadas para extração de compostos bioativos em polpa de acerola. Foram realizadas análises de ensaio de DPPH, pH, sólidos solúveis, análise colorimétrica (L*, a* e b*), compostos fenólicos totais (CFT), flavonoides totais (FT), antocianinas totais (AT) e identificação de ácido ascórbico (AA) por

espectrometria de massa com infusão direta por electrospray (ESI-MS). Os dados obtidos foram submetidos à análise estatística de variância (ANOVA), e as médias comparadas pelo teste de Tukey com nível de significância de 95 %.

RESULTADOS E DISCUSSÃO. Os dados de concentração do ensaio de DPPH foram avaliados quinzenalmente por 180 dias. As maiores concentrações no tempo 0 obtidas foram nos tratamentos PAL (199,39 ± 1,63 μM TE g^{-1}) e PAML (200,93 ± 2,42 μM TE g^{-1}) não apresentando diferença significativa ($p > 0.05$), porém o tratamento PAC (184,31 ± 2,17 μM TE g^{-1}) obteve menor concentração, diferindo significativamente ($p \leq 0.05$). Em 150 dias as amostras tiveram uma redução significativa, após permaneceu estável até o tempo de estocagem de 180 dias, a amostra PAML foi a que obteve a maior concentração final (161,58 μM TE g^{-1}). A atividade antioxidante é influenciada pela concentração de CB presentes na amostra, ou seja, se a amostra sofre uma degradação em seus fotoquímicos, consequentemente isso influenciará na atividade antioxidante. Os dados da análise de pH e sólidos solúveis foram avaliados nos tempos de 0, 90 e 180 dias. Os resultados de pH, apresentaram diferenças de acordo com o tratamento aplicado, ou seja, as amostras que foram liofilizadas PAL e PAML obtiveram menores valores 3,66 ± 0,00 e 3,72 ± 0,04 respectivamente, obtendo diferença significativa ($p \leq 0.05$). O tempo de estocagem implicou em mudanças no pH, sendo que as amostras PAC e PAL tiveram uma redução significativa nos dois tempos analisados, sendo possível verificar uma instabilidade dos compostos nestas amostras. Já a amostra PAML de 90 dias (3,58 ± 0,01) a 180 dias (3,54 ± 0,02) não obtiveram diferença significativa ($p > 0.05$), verificando uma estabilidade, nesse caso a microencapsulação serviu como barreira para estabilidade da amostra. Os sólidos solúveis as amostras PAC e PAL apresentaram os valores de 5,97 ± 0,04 e 6,13 ± 0,04 °Brix no tempo de 0 dias, não diferindo significativamente ($p > 0.05$), com o passar do tempo de estocagem foi possível observar um aumento no teor de sólidos solúveis em mesma proporção. Para amostra PAML no tempo de 0 dias apresentou um valor de 10,93 ± 0,49 °Brix sendo superior entre os outros tratamentos, isso é justificado pelo acréscimo da maltodextrina como agente encapsulante na amostra. Os resultados da análise colorimétrica foram avaliados nos tempos de 0, 90 e 180 dias. Para L* refere-se à luminosidade, no tempo de 0 dias os valores foram 42,97 ± 0,15 (PAC), 48,92 ± 0,20 (PAL) e 63,71 ± 0,31 (PAML) diferindo estatisticamente ($p \leq 0.05$) entre si, isso devido ao processo de liofilização e na adição de maltodextrina. Os dois processos contribuirão para o aumento da luminosidade e em maior proporção a adição da maltodextrina que por ser um pó branco, quando dissolvido na polpa de acerola e agindo com encapsulante tendeu-se a clarear a amostra. Para a* refere-se à cromaticidade verde/vermelho. As amostras apresentaram valores que variaram entre 0,56 e 13,49, sendo valores positivos e sua cor tendendo ao espectro de coloração vermelho. Para b* refere-se à intensidade cromática azul/amarelo, as amostras apresentaram valores que variaram entre 22,64 e 31,23, sendo valores positivos e sua cor tendendo ao espectro de coloração amarelo. Para CFT os resultados demonstram que houve diferença significativa ($p \leq 0.05$) na concentração do tempo de estocagem de 0 dias, sendo a maior concentração para amostra PAML (494,25 ± 1,79 mg GAE/100g), seguida PAL (478,36 ± 2,13 mg GAE/100g) e PAC (444,83 ± 2,07 mg GAE/100g). Após 90 dias de estocagem as amostras tiveram comportamento diferente, sendo que PAC (481,84 ± 0,65 mg GAE/100g) obteve um aumento, PAL (455,17 ± 8,74 mg GAE/100g) houve redução e PAML (493,16 ± 3,31 mg GAE/100g) se manteve estável e não havendo diferença significativa ($p > 0.05$), com 180 dias de estocagem as amostras PAC (501,78 ± 0,31 mg GAE/100g) e PAML (535,56 ± 0,56 mg GAE/100g)

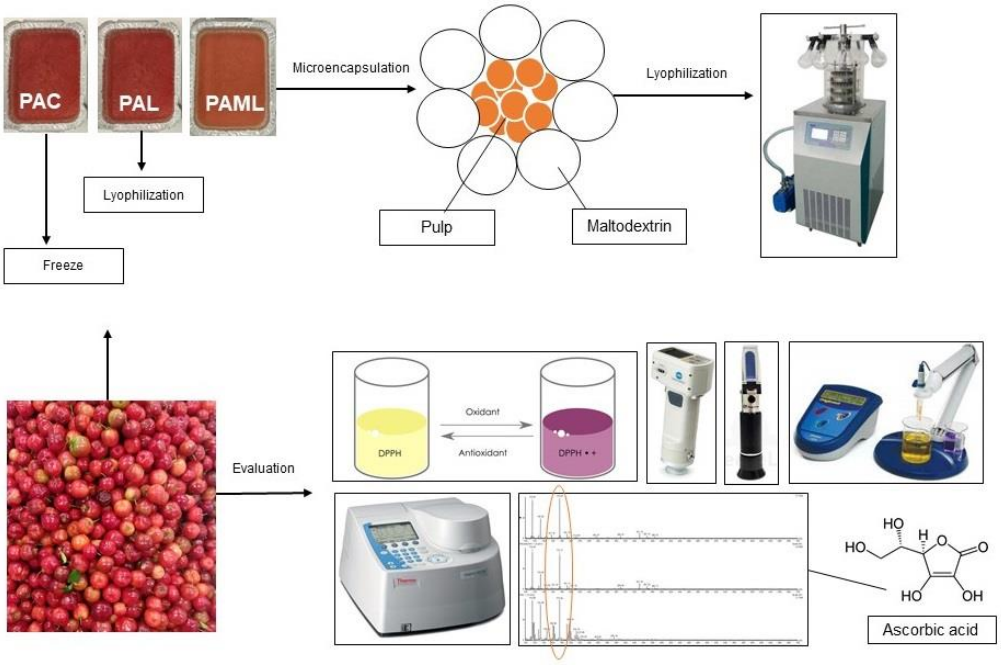
teve um aumento na concentração enquanto PAL (460,34 ± 0,57 mg GAE/100g) manteve-se estável. Os resultados de FT no tempo de estocagem de 0 dias não tiveram diferença significativa ($p > 0.05$) nas concentrações paras as amostras PAC (161,55 ± 2,45 mg QE/100g) e PAML (160,40 ± 1,99 mg QE/100g), se diferenciando apenas para a amostra PAL (155,81 ± 0,84 mg QE/100g), com 90 dias e 180 dias de estocagem as amostras mantiveram-se estáveis, não havendo diferença significativa ($p > 0.05$) em relação ao tempo de armazenamento. A amostra que obteve maior concentração no tempo de estocagem de 180 dias foi a PAML (160,53 ± 1,04 mg QE/100g). Para os resultados de AT no tempo de estocagem de 0 dias, a amostra PAC (8,46 ± 0,07 mg 100 g^{-1}) apresentou maior concentração, diferindo estatisticamente ($p \leq 0.05$) de PAL (7,61 ± 0,07 mg 100 g^{-1}) e PAML (7,57 ± 0,18 mg 100 g^{-1}), ou seja, a liofilização e microencapsulação afetou nas concentrações das amostras. No tempo de estocagem de 180 dias as amostras que tiveram redução nas concentrações foram PAC (5,08 ± 0,03 mg 100 g^{-1}) e PAL (4,25 ± 0,01 mg 100 g^{-1}), diminuindo 21,4% e 43,2% respectivamente, enquanto PAML (5,70 ± 0,03 mg 100 g^{-1}) se manteve estável, sendo o tratamento que obteve maior concentração no final do tempo de estocagem. A análise de ESI-MS foi realizada com o intuito de verificar as variações na concentração de ácido ascórbico. Pode-se notar que houve variação na concentração do ácido ascórbico nos diferentes tratamentos ao longo dos dias. Além disto, nos primeiros 90 dias, houve um aumento da concentração de AA, sendo que para o PAC sucedeu um aumento de 39.2%, o PAL de 8.1% e PAML de 20.3%. Todavia após 90 dias, é perceptível que houve um decréscimo da concentração na PAC, em contrapartida as outras duas formas de tratamento da polpa de acerola PAL e PAML tiveram um aumento expressivo da concentração, de 39.9 % e 60.6 % respectivamente. Este fator é decorrente das características intrínsecas do AA, principalmente devido sua alta solubilidade em água que influencia diretamente em reações de oxidação e redução. Para a amostra congelada, após 90 dias sua concentração foi reduzida possivelmente devido as moléculas do AA interagir com à água, pois, as ligações de hidrogênio reduz o movimento das moléculas, principalmente em materiais sólidos, além do mais, o tamanho das partículas e as concentrações, influenciam diretamente para que aconteça reações de oxidação reduzindo a concentração.

CONCLUSÕES. A técnica de microencapsulação associada com liofilização que foi empregada na polpa de acerola foi eficiente para obtenção de um produto estável e com qualidade superior aos outros tratamentos aplicados. Foram encontrados valores maiores nas concentrações de compostos bioativos e atividade antioxidante no final do tempo de armazenamento de 180 dias. Além disso, o tratamento PAML obteve uma maior estabilidade no pH durante o período de armazenamento avaliado. Observou-se que os sólidos solúveis tiveram um aumento, devido a adição do agente encapsulante (maltodextrina). Para cor obteve maiores valores de luminosidade (L*), tendência a cor vermelho (a*), mas com menos intensidade e tendência a cor amarelo (b*), com maior intensidade em relação ao tratamento PAC e PAL. Observou-se aumento expressivo na concentração de AA nos tratamentos PAL e PAML, ou seja, as amostras que passaram por operação de secagem (liofilização) conseguiram preservar e concentrar o conteúdo de AA. O estudo mostrou a viabilidade do emprego da técnica de microencapsulação para obtenção de um produto em pó com estabilidade de seus compostos bioativos, podendo ser empregado no desenvolvimento de novos produtos funcionais e/ou na sua reconstituição em água.

Palavras chaves: Antioxidantes; Compostos fenólicos; Antocianinas; Flavonoides, DPPH.

ARTICLE

Graphical Abstract (GA)



GA Text: Evaluation of conservation methods (freeze, lyophilization, and microencapsulation and lyophilization) in the antioxidant activity, bioactive compounds, and storage stability of acerola pulp.

Evaluation of the storage in the conservation of bioactive compounds of the acerola pulp (*Malpighia* spp.) through microencapsulation and lyophilization

Bruno H. F. Saqueti, [a] ***Eloize S. Alves,*** [a] ***Matheus C. Castro,*** [b] ***Patrícia D. S. dos Santos,*** [b] ***Nayane B. M. Sinosaki,*** [b] ***Carlos E. R. Senes,*** [b] ***Jesuí V. Visentainer,*** [a, b] ***and Oscar O. Santos*** [a, b] *******

[a] *Departamento de Ciência de Alimentos, Universidade Estadual de Maringá (UEM), 87020-900 Maringá-PR, Brasil*

[b] *Departamento de Química, Universidade Estadual de Maringá (UEM), 87020-900 Maringá-PR, Brasil*

* corresponding author:

Phone: + 554430113663

e-mail: oliveirasantos.oscardeoliveira@gmail.com

ORCID ID https://orcid.org/0000-0002-9631-8480

Abstract

This work reports the use of conservation methods in the study of antioxidant activity, bioactive compounds, and storage stability for 180 days of acerola pulp. Three treatments were conducted: Frozen acerola pulp (FAP), lyophilized acerola pulp (LAP), and microencapsulated and lyophilized acerola pulp (MLAP). The MLAP sample achieved higher concentrations of antioxidant activity, phenolic compounds, flavonoids, and anthocyanins when compared to other treatments in 180 days. The FAP and LAP treatments experienced a significant decrease in pH, while MLAP remained stable. MLAP showed a higher value of soluble solids due to the addition of the encapsulating agent (maltodextrin). The LAP and MLAP samples stood out in the analysis of ascorbic acid with obtained concentrations greater than the FAP sample around 40% and 60%, respectively. The study indicated that microencapsulation was an efficient method for the chemical stability of acerola pulp, ensuring greater conservation of bioactive compounds, moreover it is an important conservation method to reduce import-related expenses concerning no need for refrigeration during transportation.

Keywords: Antioxidants, Phenolic compounds, Anthocyanins, Flavonoids, DPPH.

Introduction

Acerola (*Malpighia* spp.) is a tropical fruit native from Central America, and its industrial interest is due to its organoleptic characteristics and high levels of bioactive compounds (BC), such as ascorbic acid, phenolic compounds, flavonoids, and anthocyanins.[1,2] The presence of BC often has been studied due to its health benefits since researches show several biological activities such as antioxidant, antimutagenic, antidiabetic, antitumor, and hepatoprotective activity. [3-6]

Industrial products suitable for each segment are obtained from acerola such as pulps, juices, concentrates, jellies, and jams.[7,8] Processing to obtain frozen fruit pulp consists of removing the edible part of the fruit by pulping followed by preservation by freezing.[6,9] Obtaining frozen pulps results in a reduction in losses due to perishability, as well as offering fruits with low seasonality.[10]

Emerging methods of preserving fruit pulps are studied to ensure the bioavailability of nutrients and prevent their loss by freezing and/or other applied treatments.[11-15] Techniques described in the literature has shown positive results to conserve BC using microencapsulation in extracts and/or fruits such as blackberry, blueberry, cherry, jabuticaba, and acerola. [16,17]

Microencapsulation is a technique that traps solid, liquid, or volatile particles within some matrix, remaining covered by this material (encapsulating agent), which can guarantee BC protection and stability.[18] Different coating agents are used, which can be natural polymers (proteins or polysaccharides), semi-synthetic (modified natural polymers), or synthetic.[19] Important characteristics are attributed to encapsulating agents such as biocompatibility, biodegradability, non-toxicity, and low-cost.[20]

In the microencapsulation technique drying processes such as lyophilization are associated, which promotes the water remotion from frozen mixtures by sublimation at low temperatures and under vacuum, and a secondary drying phase by desorption.[21] Lyophilization ensure a higher quality than other techniques maintaining the bio-functionality and a longer lifespan to BC without affecting the sensory properties.[17] Therefore, the stabilization of BC can be improved using microencapsulation technology associated with freeze-drying.

The BC stability of the acerola pulp shows to be affected by pH, light exposure, oxygen, and temperature.[16,22,23] Among the main BC is the ascorbic acid (AA), a water-soluble and thermolabile vitamin that is characterized as an unstable, easily oxidizable antioxidant, whose bonds can be broken by oxygen, alkaline substances, and high temperatures.[24] Microencapsulation of chemical sensitive substances such as AA by spray drying is feasible for obtaining dehydrated juices, concentrating typical active substances, and protecting them in a polymeric matrix against oxidation.[25] Therefore, it is necessary to evaluate the useful life of the acerola pulp when applying different treatments.

This work aimed to evaluate the effect of storage to conserve BC from acerola pulp by microencapsulation and lyophilization techniques, based on physical-chemical analyzes, antioxidant assays, and mass spectrometry with electrospray ionization.

Materials and methods

Chemicals, reagents, and encapsulating agents

2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid, Folin-Ciocalteu phenol reagent, quercetin, and maltodextrin (encapsulating agent) were obtained from Sigma-Aldrich (Darmstadt, Germany). Methanol p.a., ethanol p.a., hydrochloric acid, formic acid, and buffer solution (pH 4 and pH 10) were acquired from Synth (São Paulo, Brazil). Potassium chloride, sodium acetate, sodium carbonate, and aluminum chloride were purchased from Dinâmica (São Paulo, Brazil). Ultrapure water was obtained from a Milli-Q® purification system (Millipore, United States). All chemicals used were analytical grade or HPLC grade.

Sample Preparation and Treatments

Acerolas (*Malpighi*a spp.) were acquired in a local street market in the city of Maringá (23°25′S, 51°57′W), Paraná, Brazil. The samples were subjected to the steps of hygiene, weighing, and pulping in a fruit pulper (APITEC, DF-100), carried out at the Vegetable Processing Laboratory of the State University of Maringá. They were submitted to three treatments obtaining the following samples: frozen acerola pulp (FAP), lyophilized acerola pulp (LAP), and microencapsulated and lyophilized acerola pulp (MLAP). The microencapsulation performed on the MLAP sample was performed by a cavitation system in an ultrasonic bath (ELMA, Elmasonic P), for 20 min, at 25 °C and 80 kHz adding 10% of maltodextrin as an encapsulating agent to the pulp. For drying operation used in the LAP and MLAP samples, a bench freeze dryer (Liotop, L-101) was used. The treatments were vacuum-packed in polyethylene bags and stored in a freezer (-18 °C) until analyzes.

Preparation of the extracts

The FAP treatment was thawed at room temperature (25 °C), while LAP and MLAP were resuspended in distilled water using the yield in the lyophilization process. The extracts were prepared according to Rezende *et al.*,[16] under conditions optimized for the extraction of bioactive compounds. A 46.5% ethanol solution acidified with hydrochloric acid (HCl) at pH 2 was used as the extractor solvent, the solvent/pulp ratio was 8.66 mL g^{-1}. The extraction was performed in an ultrasonic bath (ELMA, Elmasonic P) working at frequency of 80 kHz and temperature of 30 °C for 50 min. The extract obtained was filtered through Whatman N °3 filter paper and later the liquid was concentrated on a rotary evaporator (Fisatom 802) at 55 °C until removal of about 95% of the extraction solvent, then the concentrated extract was resuspended in acidified water at pH 2. The extracts were stocked in an amber flask and stored in a freezer (-18 °C) for further analysis.

DPPH assay

The DPPH radical scavenging assay was performed according to the official method [26] with modifications.[27] The wavelength (λ) of 517 nm was used by a spectrophotometer (Genesys 10-S UV/Vis). To calculate the antioxidant activity, it was used a standard curve with Trolox (0-0.3 mg/mL), and the result was expressed as μMol TE g^{-1} of sample. The DPPH assay was carried out every 15 days for 180 days after obtaining the treatments.

pH and soluble solids analysis

The pH values were measured by a potentiometer at room temperature using a pH meter (Digimed DM-22), and the refractive index (soluble solids) was evaluated in a refractometer (Abbe RTA-100), expressed in °Brix.[28] The evaluation was carried out at the storage times of 0, 90, and 180 days.

Colorimetric analysis

Color analysis was performed using a digital colorimeter (Konica Minolta CR-400), obtaining the values of L* (lightness), chromaticity a* (intensity of red/green), and b* (intensity of yellow/blue) according to the model of the Commission International lluminant (CIE).[29] The evaluation was carried out at storage times of 0, 90, and 180 days.

Total Phenolic compounds (TPC)

The TPC content was determined using the Folin-Ciocalteu reagent according to the method described by Singleton & Rossi.[30] The TPC content was calculated using a standard curve prepared from aqueous solutions of gallic acid (0.1-1 mg mL^{-1}) and the result was expressed in mg of gallic acid equivalent (GAE) / 100 g of sample. The evaluation was carried out at storage times of 0, 90, and 180 days.

Total Flavonoids (TF)

The TF content was determined following the method described by Moo-Huchin *et al.*[31] The absorbance was measured at 415 nm using a spectrophotometer (Genesys 10-S UV / Vis). The TF content was calculated using a standard curve prepared with quercetin (0.05-0.5 mg mL^{-1}) and the result expressed in mg of quercetin equivalent (QE) / 100 g of sample. The evaluation was carried out at storage times of 0, 90, and 180 days.

Total Anthocyanins (TA)

The TA content was determined by the pH difference method.[32] The concentration of monomeric pigments in the extract was calculated and represented in cyanidin-3-glycoside mg 100 g^{-1}. The evaluation was carried out at storage times of 0, 90, and 180 days.

AA identification by direct infusion electrospray ionization mass spectrometry (ESI-MS)

The AA identification present in the extracts of the treatments was carried out in the storage times of 0, 90, and 180 days using direct infusion electrospray ionization mass spectrometry (ESI-MS) (XevoAcquity^{TM}, Waters, Milford, MA, USA), operating in negative mode. The spectra were acquired in scanning mode using *m/z* ranging from 100 to 700 Da. For the extract infusion, 50 µL of the extract was dissolved in 950 µL of ultrapure water, then 1 mL of this solution was transferred to a vial and 20 µL of 1% formic acid solution was added. The sample was infused at a flow rate of 10 µL min^{-1}. The working conditions of the ionization source were as follows: capillary voltage of 3.06 kV, source temperature of 150 °C, cone flow of 40 L h^{-1}, gas flow (Nitrogen) for

desolvation of 400 L h^{-1}, desolvation temperature of 350 °C, and the gas flow cone of 30 L Hr. Data acquisition was performed using MassLynx v 4.1 software.

Statistical analysis

The analyzes were performed in triplicate and the results obtained were evaluated by analysis of variance (ANOVA) using the Tukey test with a significance level of 5% ($\alpha = 0.05$) through the software Assistat 7.7.[33]

Results and discussion

DPPH assay

The concentration data from the DPPH assay evaluated every two weeks for 180 days are shown in Figure 1. The highest concentrations at time zero were achieved in LAP (199.39 ± 1.63 μM TE g^{-1}) and MLAP (200.93 ± 2.42 μM TE g^{-1}) treatments, with no significant difference ($p > 0.05$). However, the FAP treatment (184.31 ± 2.17 μM TE g^{-1}) obtained a lower concentration, differing significantly ($p \leq 0.05$). The data obtained corroborate those presented by Rezende *et al.*,[16] who found similar values in the optimization of the ultrasound-assisted extraction of the acerola residue, which the greatest value found was 181.78 μM TE g^{-1}. After 150 days, the samples decreased significantly and remained stable until the storage time of 180 days. The MLAP sample obtained the highest final concentration (161.58 μM TE g^{-1}). The antioxidant activity is influenced by the concentration of bioactive compounds present in the sample, i.e., if the sample is photochemically degraded, the antioxidant activity will be affected. The

decrease in antioxidant activity in fruits during storage was observed in the work of Michalczyk *et al.*,[34] who used freeze-drying operation, and even with high levels of polyphenols and anthocyanins, this reduction was observed.

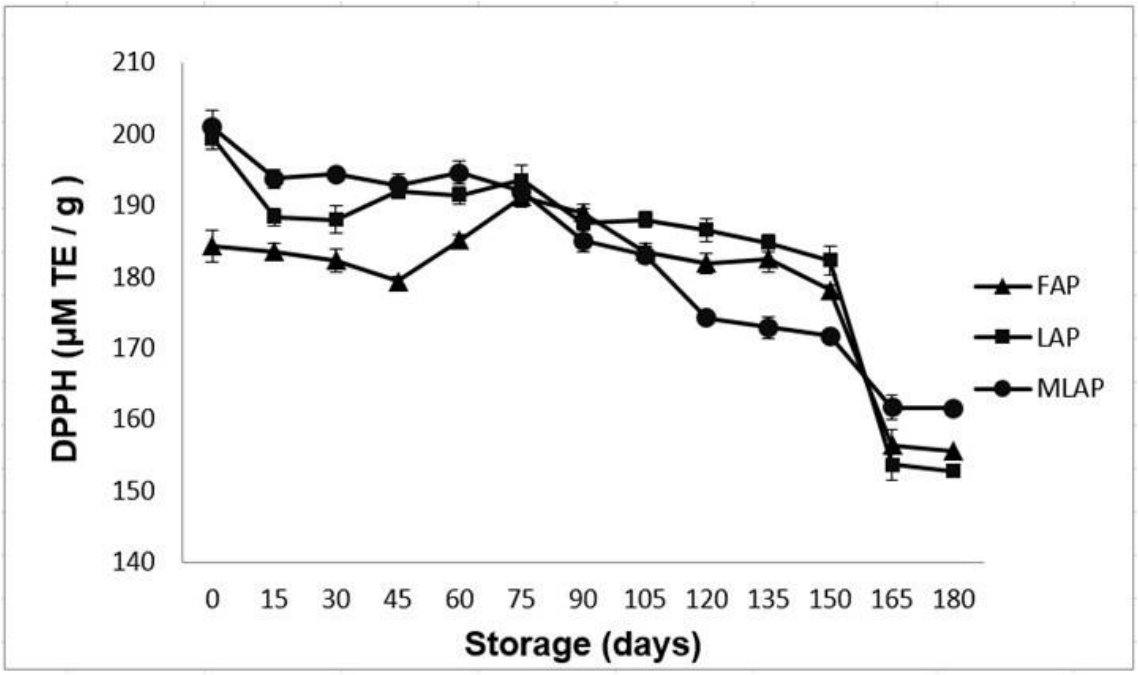


Figure 1. DPPH (radical scavenging activity) assay carried at every 15 days of storage times in different treatments. FAP: Frozen acerola pulp; LAP: Lyophilized acerola pulp; MLAP: Microencapsulated and lyophilized acerola pulp. Values are expressed as mean ± triplicate standard deviation.

pH and soluble solids analysis

The data from the analysis of pH and soluble solids are exhibited in Figure 2. The results of pH (A) showed differences according to the treatment applied, that is, the freeze-dried samples LAP and MLAP attained lower values, 3.66 ± 0.00 and 3.72 ± 0.04, respectively, presenting a significant difference ($p \leq 0.05$). The storage time

involved changes in pH, and the FAP and LAP samples showed a significant reduction in both analyzed times, which allowed the stability of the compounds in these samples to be verified. From 90 days (3.58 ±0.01) to 180 days (3.54± 0.02) MLAP sample showed no significant difference ($p > 0.05$), verifying the stability of the sample into which it was microencapsulated. In this instance, the microencapsulation served as a barrier to sample stability. When analyzed *in vitro*, pH levels affect the antioxidant properties of phenolic compounds, which are more sensitive to changes in pH than the numbers and positions of surrogate groups. In this case, increased pH resulted in increased antioxidant activity, such that samples that showed a decrease in pH tended to decrease antioxidant activity.[35]

Soluble solids (B) are total solids dissolved in water, in which sugars, organic acids, soluble proteins and salts are counted and measured as the sum of those solids and expressed in in °Brix. FAP and LAP samples showed the values of 5.97 ± 0.04 and 6.13 ± 0.04 °Brix in the time of zero days not differing significantly ($p > 0.05$), and with the passage of the storage time it was possible to note an increase in the content of soluble solids in the same proportion. For the MLAP sample at zero days, it showed a value of 10.93 ± 0.49 °Brix, which is higher than other treatments, justifying the addition of an encapsulation agent to the sample. The MLAP sample behaved differently during the storage period because after 90 days it had a small reduction in insoluble solids and after 180 days of analysis an increase, showing a significant difference ($p \leq 0.05$). Jeong *et al.*[36] reported a gradual increase in soluble solids in stored chilled kiwi cultivars due to cell wall degradation, which makes organic acids more accessible. The values of the soluble solids found in FAP and LAP are close to the values obtained by Souza *et al.*,[37] which obtained values ranging from 6.8 to 8.2 º Brix in immersion treatment of acerola.

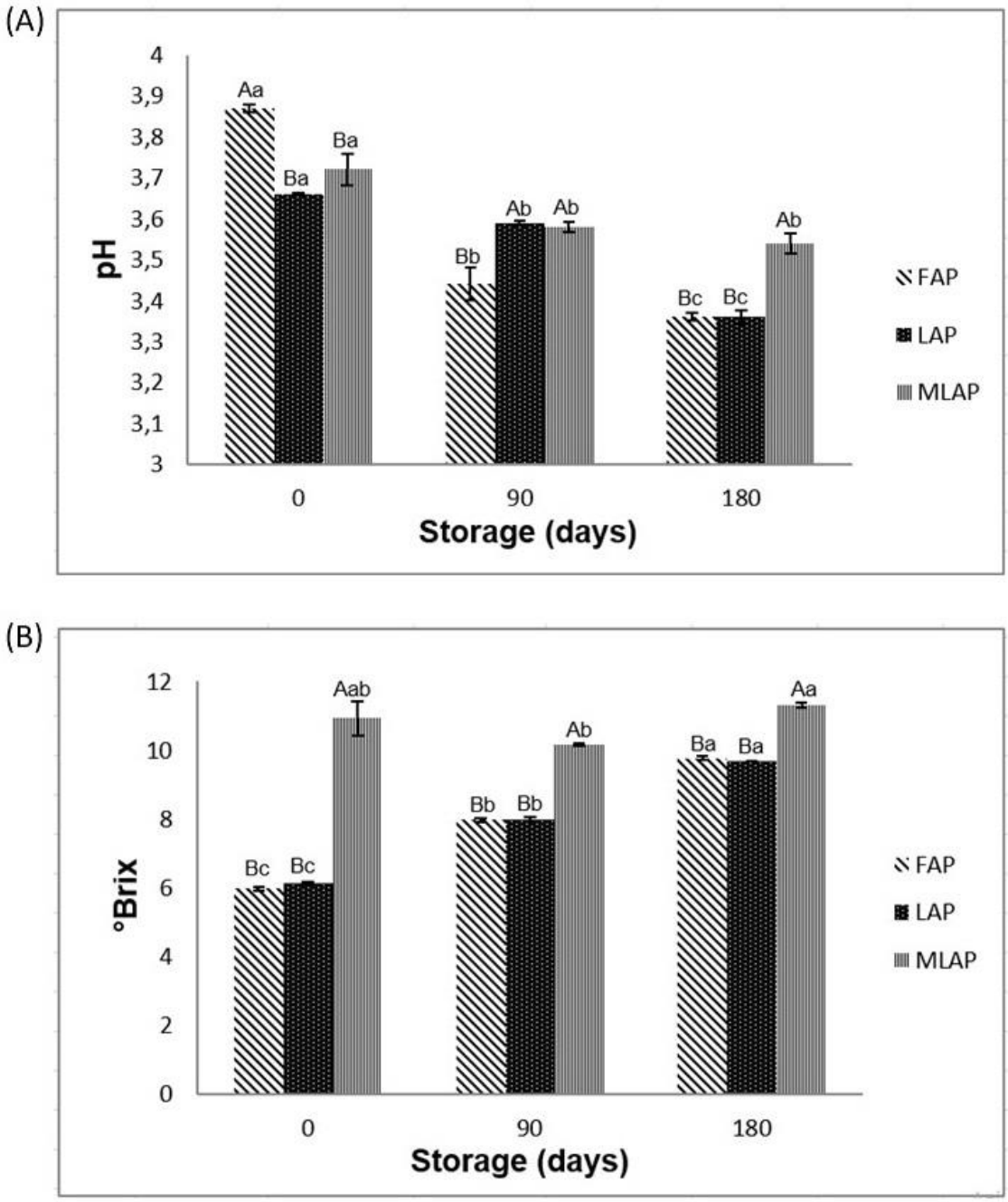


Figure 2. (A) pH (hydrogen potential) analysis; (B) Soluble solids analysis. FAP: Frozen acerola pulp; LAP: Lyophilized acerola pulp; MLAP: Microencapsulated and lyophilized acerola pulp, in storage times of 0, 90, and 180 days in different treatments. Data are expressed as mean ± triplicate standard deviation. Equal capital letters mean

that the treatments do not differ significantly by the Tukey test at 5% probability, equal lower letters mean that the storage times (days) do not differ significantly by the Tukey test at 5% probability.

Colorimetric analysis

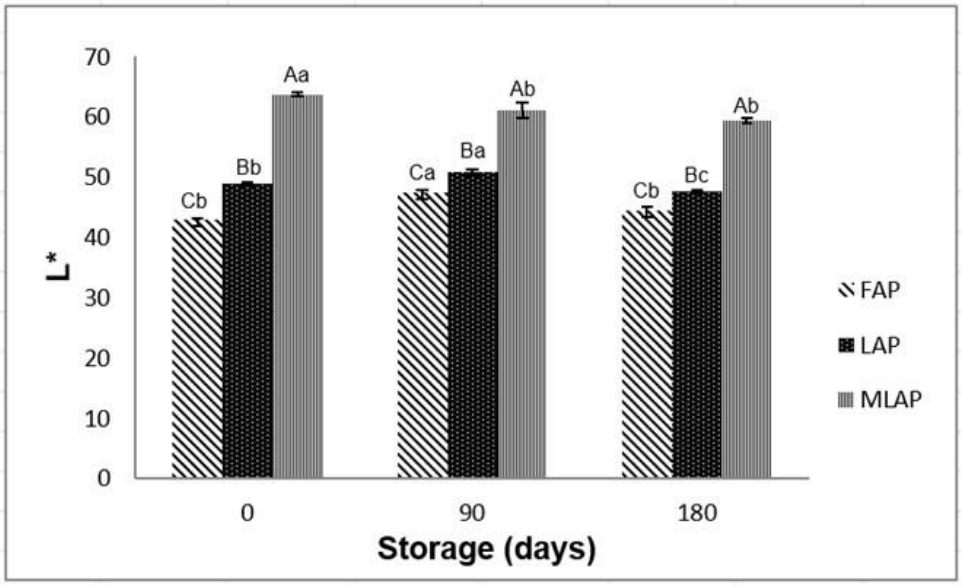
The results of the colorimetric analysis are presented in Figure 3 on the basis of three parameters (L*, a*, and b*), taking into account the angle at which the sample is detected, which may affect the reflectance curve, used as a reference for the CIE (International Commission on Illumination) curve.[29] Figure 3 (A) refers to the lightness (L*) and/or contrast of the sample, wherein at zero days the values were 42.97 ± 0.15 (FAP), 48.92 ± 0.20 (LAP), and 63.71 ± 0.31 (MLAP) differing statistically ($p \leq 0.05$) from each other due to the lyophilization process and maltodextrin addition. Both processes will contribute to increased lightness and, to a greater extent, the maltodextrin addition which is a white powder, it acts as an encapsulant when dissolved in the acerola pulp resulting in a clear sample. After 90 and 180 days of storage, the samples retained their values without significant differences ($p > 0.05$).

Figure 3 (B) concerns chromaticity (a*), i.e., visible light radiation characterized by two chromatic coordinates. The samples showed values ranging from 0.56 to 13.49, with positive values and their color tending towards the red color spectrum. At zero-day storage time, the sample with the highest red trend was FAP (13.49 ± 0.38), followed by MLAP (5.16 ± 0.02) and LAP (0.56 ± 0.04), showing a significant difference ($p \leq 0.05$) among them. Concern 90 days storage period, the FAP (7.82 ± 0.18), and MLAP (4.47 ± 0.18) samples were reduced, while the LAP (2.01 ± 0.16) samples were increased in intensity, differing significantly ($p \leq 0.05$). At 180 days FAP (6.64 ± 0.03) and LAP

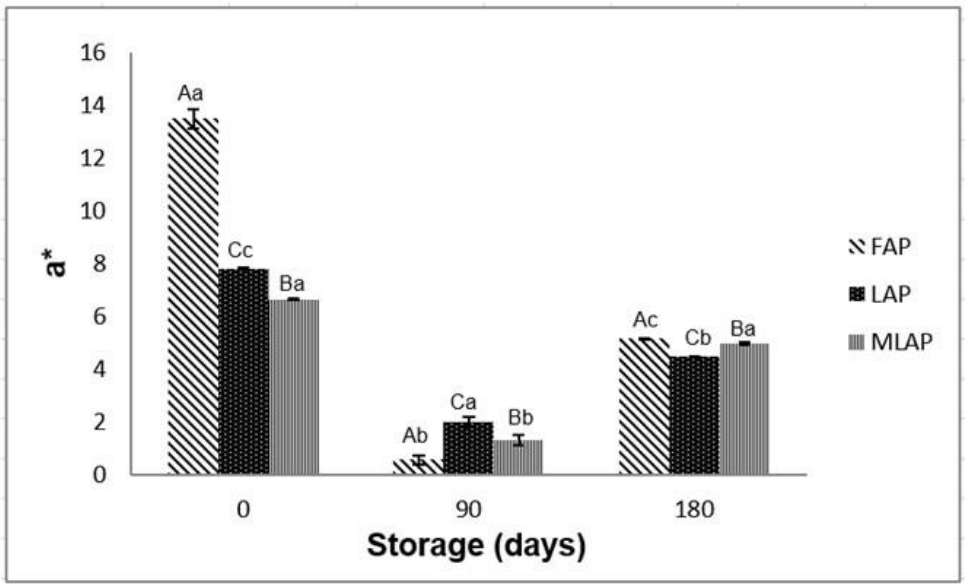
(1.31 ± 0.03) showed a small reduction and MLAP (4.96 ± 0.04) remained stable without significant differences ($p > 0.05$).

Regarding b* (Figure 3 (C)), it refers to the blue/yellow chromatic intensity, and the samples showed values ranging from 22.64 and 31.23, with positive values and their color tending towards the yellow color spectrum. The b* values for the different storage times were not significantly different ($p > 0.05$). The trend toward red and yellow is expected due to the presence of natural pigments such as anthocyanins and carotenoids found in the samples.

(A)


70
60
50
40
30
20
10
0
L*
Aa
Ab
Ab
Bb
Ba
Bc
Cb
Ca
Cb
0
90
180
Storage (days)
FAP
LAP
MLAP

(B)


16
14
12
10
8
6
4
2
0
a*
Aa
Cc
Ba
Ab
Ca
Bb
Ac
Cb
Ba
0
90
180
Storage (days)
FAP
LAP
MLAP

(C)

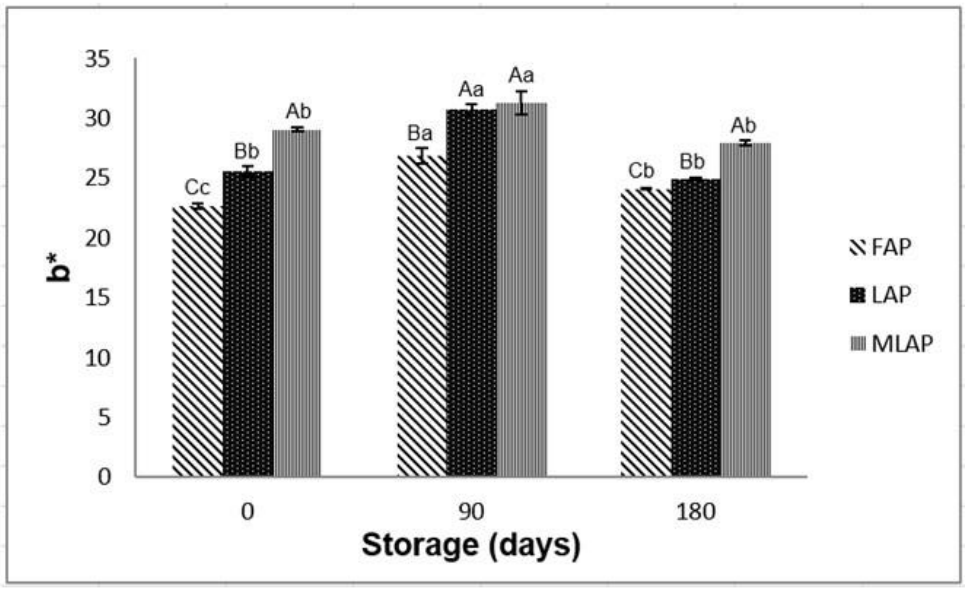

35
30
25
20
15
10
5
0
b*
Cc
Bb
Ab
Ba
Aa
Aa
Cb
Bb
Ab
0
90
180
Storage (days)
FAP
LAP
MLAP

Figure 3. Colorimetric analysis (A) L* Brightness; (B) a* intensity of red when the value is positive/green when the value is negative; (C) b* yellow intensity when the value is positive/blue when the value is negative, at the storage times of 0, 90, and 180 days in different treatments. FAP: Frozen acerola pulp; LAP: Lyophilized acerola pulp; MLAP: Microencapsulated and lyophilized acerola pulp. Data are expressed as mean ± triplicate standard deviation. Equal capital letters mean that treatments do not differ significantly by Tukey's test at 5% probability, equal lower-case letters mean that storage times do not differ significantly by Tukey's test at 5% probability.

Bioactive compounds

Figure 4 shows the results concerning the BC study. For TPC (A) the results demonstrate that there was a significant difference ($p \leq 0.05$) in the concentration of the storage time of zero days, with the highest concentration for the MLAP sample (494, 25 ± 1.79 mg GAE / 100g), followed by LAP (478.36 ± 2.13 mg GAE/100g) and FAP (444.83 ± 2.07 mg GAE/100g). After 90 days of storage, the samples behaved differently, with increase in FAP (481.84 ± 0.65 mg GAE/100g), reductions in LAP (455.17 ± 8.74 mg GAE/100g), and MLAP (493.16 ± 3.31 mg GAE/100g) remained stable and no significant differences ($p > 0.05$). In regard to180 days of storage the FAP samples (501.78 ± 0.31 mg GAE/100g) and MLAP (535.56 ± 0.56 mg GAE/100g) increased in concentration while LAP (460.34 ± 0.57 mg GAE/100g) remained stable. The increase in TPC concentration during freezer storage was observed in the Mallik & Hamilton[38] study evaluating wild blueberries, corroborating the results obtained in this work as a result of chemical degradation and oxidation reactions. The main phenolic compounds reported in acerola are the classes of compounds derived from benzoic acid

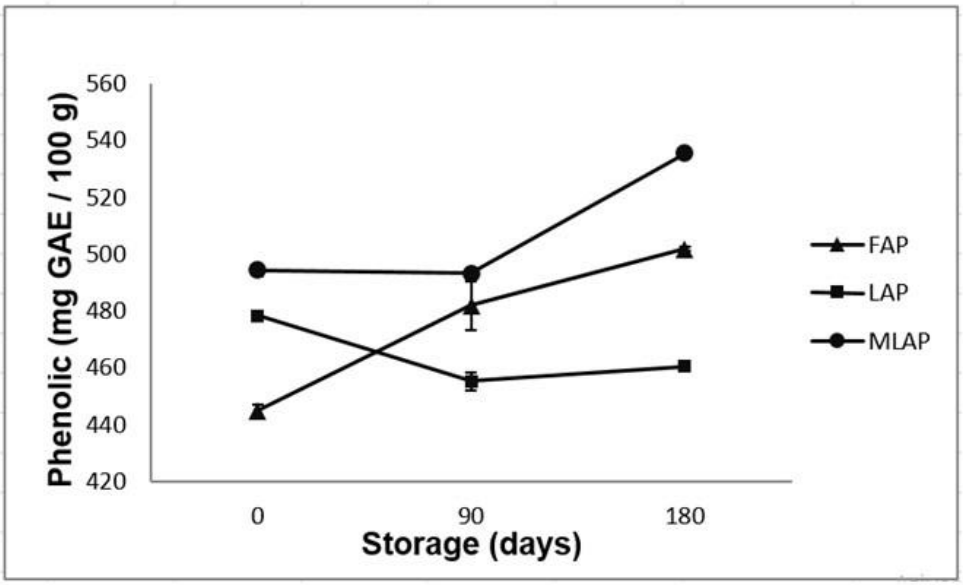
(gallic acid and syringic acid), phenylpropanoid derivatives (*p*-cumaric, ferulic, caffeic, and chlorogenic acids), flavonoids and anthocyanins, compounds responsible for high concentrations of phenolics found in the acerola pulp.[39]

The TF results (Figure 4 (B)) in the storage time of zero days had no significant difference ($p > 0.05$) in the concentrations for the FAP (161.55 ± 2.45 mg QE/100g) and MLAP (160.40 ± 1.99 mg QE/100g), differing only for the LAP sample (155.81 ± 0.84 mg QE/100g). For 90 and 180 days of storage, the samples remained stable with no significant differences ($p > 0.05$) over the storage time. The sample with the highest concentration over the 180-day storage period was MLAP (160.53 ± 1.04 mg QE/100g). The main flavonoids commonly found in acerola are the compounds catechin, epicatechin, epigallocatechin gallate, quercetin, rutin, kaempferol, luteolin, dihydroquercetin, procyanidin B1, and aceronidin.[39] These flavonoid compounds remain protected when microencapsulation is applied, corroborating the work of Sansone *et al.*[40] which evaluated the microencapsulation of flavonoids to increase the useful life, studying the stability during storage.

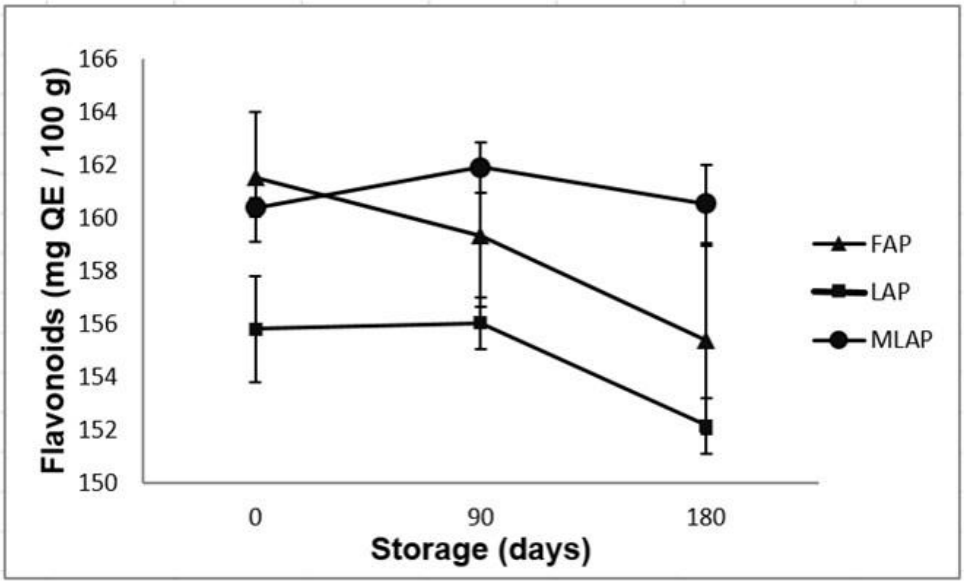
For the results of AT (Figure 4(C)) in the storage time of zero days, the FAP sample (8.46 ± 0.07 mg 100 g^{-1}) showed a higher concentration, differing statistically ($p \leq 0.05$) from PAL (7.61 ± 0.07 mg 100 g^{-1}) and MLAP (7.57 ± 0.18 mg 100 g^{-1}), that is, lyophilization and microencapsulation affected the initial TA concentrations in the samples. After 90 days of storage, the FAP (6.47 ± 0.23 mg 100 g^{-1}) and MLAP (5.98 ± 0.02 mg 100 g^{-1}) treatments had a 23.5% reduction in AT concentrations and 21% respectively, while LAP (7.49 ± 0.12 mg 100 g^{-1}) remained stable with no significant difference ($p > 0.05$). During the 180 days storage period, the samples with lower concentrations were FAP (5.08 ± 0.03 mg 100 g^{-1}) and LAP (4.25 ± 0.01 mg 100 g^{-1}), decreasing 21.4% and 43.2% respectively, while MLAP (5.70 ± 0.03 mg 100 g^{-1})

remained stable in TA concentrations, the treatment with a higher concentration at the end of the storage period. According to Mahdavi *et al.*[41] anthocyanins are hydrophilic dyes and specifically compatible with a water-based gel formulation, such as maltodextrin. After drying, the production of spherical particles is observed, coating the pigment in the peripheral region of the microcapsules, therefore the MLAP treatment had the best result compared to the others in the last 180 days of storage time.

(A)


Phenolic (mg GAE / 100 g)
560
540
520
500
480
460
440
420
0
90
180
Storage (days)
FAP
LAP
MLAP

(B)


Flavonoids (mg QE / 100 g)
166
164
162
160
158
156
154
152
150
0
90
180
Storage (days)
FAP
LAP
MLAP

(C)

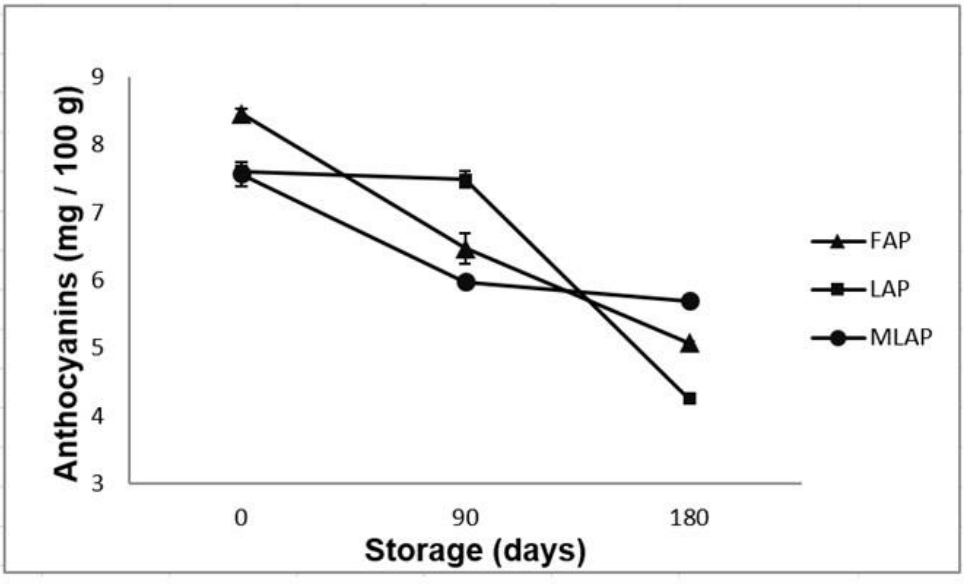

Anthocyanins (mg / 100 g)
9
8
7
6
5
4
3
0
90
180
Storage (days)
FAP
LAP
MLAP

Figure 4. Bioactive compounds content. (A) Total phenolic compounds (TPC); (B) Total flavonoids (TF); (C) Total anthocyanins (TA) at storage times of 0, 90, and 180 days in different treatments. FAP: Frozen acerola pulp; LAP: Lyophilized acerola pulp; MLAP: Microencapsulated and lyophilized acerola pulp. Data are expressed as mean ± triplicate standard deviation.

AA identification by mass spectrometry with direct electrospray infusion (ESI-MS)

To study the best storage method and storage stability of acerola pulp over 180 days, three treatments were evaluated. The ESI-MS analysis was carried out to verify variations in ascorbic acid concentration in treatments as well as in storage, and the results are described in Figure 5. Error bars were not added in the Figure 5 because it would be difficult to see between treatments; standard deviations were less than 5% of all experimental points.

According to Figure 5, there was variation in the concentration of ascorbic acid in the different treatments over the days. Furthermore, in the first 90 days, an increase in AA concentration was observed, with an increase of 39.2% for the FAP, 8.1% for the LAP, and 20.3% for the MLAP. However, it was noticeable that there was a decrease in the AA concentration in FAP after 90 days, in contrast, the other two forms of treatment, LAP and MLAP, significantly increased in concentration, of 39.9% and 60.6% respectively.

This effect is due to the intrinsic characteristics of ascorbic acid, mainly because of its high-water solubility, which directly affects oxidation and reduction reactions. For the frozen sample, after 90 days its concentration was reduced, probably as a result of the interaction of ascorbic acid molecules with water, because hydrogen bonds reduce

the movement of molecules, mainly in solid materials. Moreover, the size and concentrations of the particles directly affect the oxidation reactions, thus reducing the concentration. As previously said, the concentration of LAP and MLAP increased over the days since water was removed during the lyophilization process and therefore ascorbic acid increased. However, over the days the concentration of ascorbic acid is high, this fact occurs due to the lyophilization process, which may release the encapsulated component and therefore produce a degradation product from the incomplete degradation of ascorbic acid into the dehydroascorbic acid.[42]

However, it is important to note that the AA stability is influenced by the intrinsic properties of the product and by the characteristics of the process that are subjected to different handling and storage conditions. These conditions are influenced by aerobic or anaerobic pathways, as well as prolonged storage, pH, humidity, temperature, cold injury, water influence as explained above, the acidity of the medium, the presence of metallic ions, concentration of enzymes, proteins, carbohydrates and amino acids contained in the sample.[43]

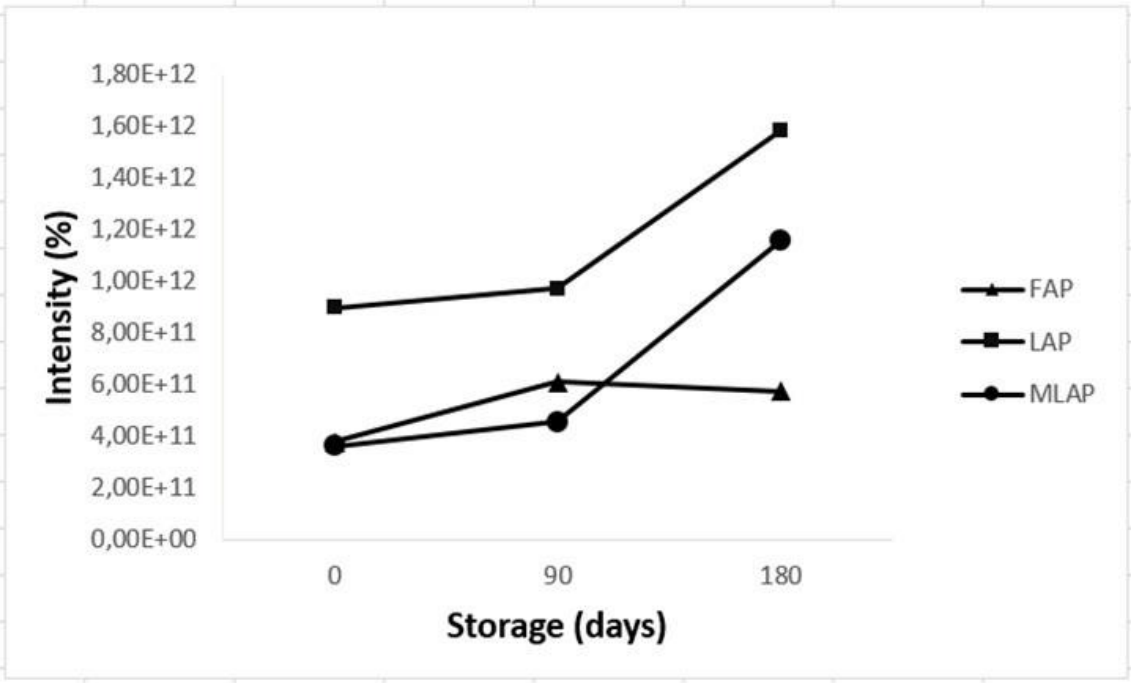


Figure 5. Intensity of ascorbic acid in acerola in the storage time of 0, 90, and 180 days in different treatments. FAP: Frozen acerola pulp; LAP: Lyophilized acerola pulp; MLAP: Microencapsulated and lyophilized acerola pulp.

Conclusion

The microencapsulation technique associated with the lyophilization that was used in the acerola pulp was efficient to obtain a stable product and with superior quality to the other applied treatments. Higher values were found in the concentrations of bioactive compounds and antioxidant activity at the end of the storage period of 180 days. Furthermore, the MLAP treatment achieved a higher pH stability over the assessed storage period. It was observed that the soluble solids had an increase due to the addition of the encapsulating agent (maltodextrin). For color obtained higher values of luminosity (L*), tendency to red color (a*), but with less intensity and tendency to yellow color (b*), with greater intensity in relation to the FAP and LAP treatment. A

significant increase in the AA concentration was observed in the LAP and MLAP treatments, that is, the samples that underwent drying (lyophilization) were able to preserve and concentrate the AA content. The present study showed the feasibility of employ the microencapsulation technique to obtain a powder product with stability of its bioactive compounds, which can be used in the development of new functional products and/or in their reconstitution in water.

Supplementary Information

Supplementary Information is available free of charge at http://jbcs.sbq.org.br".

Declaration of Competing Interest

The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Paraná for their financial support.

CRediT authorship contribution statement

Bruno Henrique Figueiredo Saqueti: Methodology, investigation, formal analysis, and writing - original draft; Eloize da Silva Alves: Investigation and formal analysis; Matheus Campos Castro: Investigation and formal analysis; Patrícia Daniele da Silva Santos: Investigation, formal analysis, and validation; Nayane Braga Mattos Sinosaki: Investigation, formal analysis, and validation; Carlos Eduardo Rubio Senes: Writing - Review & Editing; Jesuí Vergilio Visentainer: Funding acquisition and supervision; Oscar de Oliveira Santos: Conceptualization, visualization and Supervision.

References

1. Rezende, Y. R. R. S.; Nogueira, J. P.; Narain, N. ;*Food Chem.* **2018**, *254*, 281.
2. Silva, P. B.; Duarte, C. R.; Barrozo, M. A. S. ;*Innov. Food Sci. Emerg. Technol.* **2019**, *52*, 350.
3. Barbalho, S. M.; Damasceno, D. C.; Spada, A. P. M. H.; Palhares, M.; Martuchi, K. A.; Oshiiwa, M.; Sazaki, V.; Silva, V. S. Da ;*Exp. Diabetes Res.* **2011**, *2011*.
4. Düsman, E.; Almeida, I. V.; Tonin, L. T. D.; Vicentini, V. E. P. ;*Genet. Mol. Res.* **2016**, *15*.
5. Luveena, A. A. M.; Karthiayini, K.; Sreekumar, K. P. ;*Int. J. Res. Phytochem. Pharmacol. ©JK Welf. Pharmascope Found. | Int. J. Res. Phytochem. Pharmacol.* **2012**, *2*, 45

6. Paz, M.; Gúllon, P.; Barroso, M. F.; Carvalho, A. P.; Domingues, V. F.; Gomes, A. M.; Becker, H.; Longhinotti, E.; Delerue-Matos, C. ;*Food Chem.* **2015**, *172*, 462.
7. Bortolotti, C. T.; Santos, K. G.; Francisquetti, M. C. C.; Duarte, C. R.; Barrozo, M. A. S. ;*Can. J. Chem. Eng.* **2013**, *91*, 1871.
8. Mohammed, M.; *Woodhead Publishing*, **2011**, 27.
9. da Silva, L. M. R.; de Figueiredo, E. A. T.; Ricardo, N. M. P. S.; Vieira, I. G. P.; Figueiredo, R. W.; Brasil, I. M.; Gomes, C. ;*Food Chem.* **2014**, *143*, 398.
10. da Costa, G. A.; Mercadante, A. Z. ;*J. Food Compos. Anal.* **2018**, *68*, 53.
11. Braga, M. B.; Veggi, P. C.; Codolo, M. C.; Giaconia, M. A.; Rodrigues, C. L.; Braga, A. R. C.; *Food Res. Int.* **2019**, *125*, 108557.
12. Pacheco, C.; García-Martínez, E.; Moraga, G.; Piña, J.; Nazareno, M. A.; Martínez-Navarrete, N.; *Powder Technol*. **2020**, *362*, 11.
13. Robert, P.; Torres, V.; García, P.; Vergara, C.; Sáenz, C. ;*LWT - Food Sci. Technol.* **2015**, *60*, 1039.
14. Sampaio, G. L. A.; Pacheco, S.; Ribeiro, A. P. O.; Galdeano, M. C.; Gomes, F. S.; Tonon, R. V. ;*Lwt.* **2019**, *116*, 108589.
15. Santana, A. A.; Cano-Higuita, D. M.; De Oliveira, R. A.; Telis, V. R. N. ;*Food Chem.* **2016**, *212*, 1.
16. Rezende, Y. R. R. S.; Nogueira, J. P.; Narain, N. ;*LWT - Food Sci. Technol.* **2017**, *85*, 158.
17. Tarone, A. G.; Cazarin, C. B. B.; Marostica Junior, M. R. ;*Food Res. Int.* **2020**, *133*.
18. Ribeiro, A. M.; Estevinho, B. N.; Rocha, F. ;*Trends Food Sci. Technol.* **2019**, 1.
19. Carvalho, I. T.; Estevinho, B. N.; Santos, L. ;*Int. J. Cosmet. Sci.* **2016**, *38*, 109.
20. Ye, Q.; Georges, N.; Selomulya, C. ;*Trends Food Sci. Technol.* **2018**, *78*, 167.
21. Ezhilarasi, P. N.; Karthik, P.; Chhanwal, N.; Anandharamakrishnan, C. ;*Food Bioprocess Technol.* **2013**, *6*, 628.
22. De Rosso, V. V.; Mercadante, A. Z. ;*Food Chem.* **2007**, *103*, 935.
23. Jaeschke, D. P.; Marczak, L. D. F.; Mercali, G. D. ;*Food Chem.* **2016**, *199*, 128.
24. Manela-Azulay, M.; Lacerda, C. A. M.; Perez, M. de A.; Filgueira, A. L.; Cuzzi, T. ;*An. Bras. Dermatol.* **2003**, *78*, 265.
25. Ferrari, C. C.; Ribeiro, C. P.; Aguirre, J. M. de ;*Brazilian J. Food Technol.* **2012**,

15, 157.

26. Brand-Williams, W.; Cuvelier, M. E.; Berset, C. ;*LWT - Food Sci. Technol.* **1995**, *28*, 25.
27. Boroski, M.; de Aguiar, A. C.; Boeing, J. S.; Rotta, E. M.; Wibby, C. L.; Bonafé, E. G.; de Souza, N. E.; Visentainer, J. V. ;*Food Chem.* **2011**, *125*, 696.
28. Association of Official Analytical Chemists-AOAC. Official methods of analysis of the Association of Official Analytical Chemists, 2005.
29. McGuire, R. G. ;*HortScience* **1992**, *27*, 1254.
30. Singleton, V. L.; Rossi, J. A.; *American journal of Enology and Viticulture*. **1965,** *16*, 144.
31. Moo-Huchin, V. M.; Moo-Huchin, M. I.; Estrada-León, R. J.; Cuevas-Glory, L.; Estrada-Mota, I. A.; Ortiz-Vázquez, E.; Betancur-Ancona, D.; Sauri-Duch, E. ;*Food Chem.* **2015**, *166*, 17.
32. Rodrigues, C. A.; Nicácio, A. E.; Boeing, J. S.; Garcia, F. P.; Nakamura, C. V.; Visentainer, J. V.; Maldaner, L. ;*Food Chem.* **2020**, *309*, 125694.
33. Silva, F. A. S.; Azevedo, C. A. V. *African Journal of Agricultural Research*, **2016,** *11*, 3733.
34. Michalczyk, M.; MacUra, R.; Matuszak, I. ;*J. Food Process. Preserv.* **2009**, *33*, 11.
35. Di Majo, D.; La Neve, L.; La Guardia, M.; Casuccio, A.; Giammanco, M. ;*J. Food Compos. Anal.* **2011**, *24*, 265.
36. Jeong, H. R.; Cho, H. S.; Cho, Y. S.; Kim, D. O. ;*Food Sci. Biotechnol.* **2020**, 2.
37. Souza, K. O.; Moura, C. F. H.; Lopes, M. M. de A.; Rabelo, M. C.; Miranda, M. R. A. ;*Rev. Bras. Frutic.* **2017**, *39*, 1.
38. Mallik, A. U.; Hamilton, J. ;*J. Food Sci. Technol.* **2017**, *54*, 1545.
39. Belwal, T.; Devkota, H. P.; Hassan, H. A.; Ahluwalia, S.; Ramadan, M. F.; Mocan, A.; Atanasov, A. G. ;*Trends Food Sci. Technol.* **2018**, *74*, 99.
40. Sansone, F.; Picerno, P.; Mencherini, T.; Villecco, F.; D'Ursi, A. M.; Aquino, R. P.; Lauro, M. R. ;*J. Food Eng.* **2011**, *103*, 188.
41. Mahdavi, S. A.; Jafari, S. M.; Assadpoor, E.; Dehnad, D. ;*Int. J. Biol. Macromol.* **2016**, *85*, 379.
42. Yuan, J. P.; Chen, F. ;*J. Agric. Food Chem.* **1998**, *46*, 5078.
43. Mercali, G. D.; Schwartz, S.; Marczak, L. D. F.; Tessaro, I. C.; Sastry, S. ;*J.*

Agric. Food Chem. **2014**, *62*, 5865.

Evaluation of the storage in the conservation of bioactive compounds of the acerola pulp (*Malpighia* spp.) through microencapsulation and lyophilization

Bruno H. F. Saqueti, [a] ***Eloize S. Alves,*** [a] ***Matheus C. Castro,*** [b] ***Patrícia D. S. dos Santos,*** [b] ***Nayane B. M. Sinosaki,*** [b] ***Carlos E. R. Senes,*** [b] ***Jesuí V. Visentainer,*** [a, b] ***and Oscar O. Santos*** [a, b] *******

[a] *Departamento de Ciência de Alimentos, Universidade Estadual de Maringá (UEM), 87020-900 Maringá-PR, Brasil*

[b] *Departamento de Química, Universidade Estadual de Maringá (UEM), 87020-900 Maringá-PR, Brasil*

* corresponding author:

Phone: + 554430113663

e-mail: oliveirasantos.oscardeoliveira@gmail.com

ORCID ID https://orcid.org/0000-0002-9631-8480

Supplementary material

Table 1 DPPH (radical scavenging activity) assay carried out at storage times of every 15 days for 180 days in different treatments.

Sample	FAP	LAP	MLAP
0 day	184.31±2.17Babc	199.39±1.63Aa	200.93±2.42Aa
15th day	183.49±1.22Cabc	188.38±1.22Bbcd	193.68±1.36Ab
30th day	182.27±1.63Cabc	187.97±1.90Bbcd	194.36±0.91Ab
45th day	179.41±0.68Bc	191.85±0.95Aabc	192.78±1.51Ab
60th day	185.12±0.68Babc	191.44±1.22Abc	194.58±1.65Ab
75th day	191.23±1.49Aa	193.48±2.17Aab	191.87±0.76Ab
90th day	188.99±1.22Aab	187.57±1.90Abcd	185.08±1.51Ac
105th day	183.49±1.22Babc	187.97±1.09Abcd	183.04±1.21Bc
120th day	181.86±1.49Abc	186.55±1.63Abcd	174.21±0.61Bd
135th day	182.47±1.77Aabc	184.71±1.22Acd	172.85±1.51Bd
150th day	178.19±0.68Ac	182.27±2.04Ad	171.74±0.27Bd
165th day	156.46±2.22Ad	153.71±2.28Ae	161.66±1.71Ae
180th day	155.63±1.00Ad	152.78±1.11Ae	161.58±1.14Ae

FAP: Frozen acerola pulp; LAP: Lyophilized acerola pulp; MLAP: Microencapsulated and lyophilized acerola pulp. Values are expressed as mean ± triplicate standard deviation. Equal capital letters mean that the treatments do not differ significantly by the Tukey test at 5% probability, equal lower letters mean that the storage time (days) do not differ significantly by the Tukey test at 5% probability

Table 2 pH analysis (hydrogen potential) carried out in the storage times of 0, 90 and 180 days in different treatments.

Sample	FAP	LAP	MLAP
0 day	3.87±0.01Aa	3.66±0.00Ba	3.72±0.04Ba
90th day	3.44±0.04Bb	3.59±0.00Ab	3.58±0.01Ab
180th day	3.36±0.01Bc	3.36±0.02Bc	3.54±0.02Ab

FAP: Frozen acerola pulp; LAP: Lyophilized acerola pulp; MLAP: Microencapsulated and lyophilized acerola pulp. Values are expressed as mean ± triplicate standard deviation. Equal capital letters mean that the treatments do not differ significantly by the Tukey test at 5% probability, equal lower letters mean that the storage time (days) do not differ significantly by the Tukey test at 5% probability

Table 3 Soluble solids analysis carried out in the storage times of 0, 90 and 180 days in different treatments.

Sample	FAP	LAP	MLAP
0 day	5.97±0.04Bc	6.13±0.04Bc	10.93±0.49Aab
90th day	7.97±0.04Bb	8.00±0.07Bb	10.17±0.04Ab
180th day	9.77±0.04Ba	9.70±0.00Ba	11.30±0.07Aa

FAP: Frozen acerola pulp; LAP: Lyophilized acerola pulp; MLAP: Microencapsulated and lyophilized acerola pulp. Values are expressed as mean ± triplicate standard deviation. Equal capital letters mean that the treatments do not differ significantly by the Tukey test at 5% probability, equal lower letters mean that the storage time (days) do not differ significantly by the Tukey test at 5% probability

Table 4 Color parameters results carried out in the storage times of 0, 90 and 180 days in different treatments.

Sample	FAP	LAP	MLAP
0 day L*	42.97±0.15Cb	48.92±0.20Bb	63.71±0.31Aa
0 day a*	13.49±0.38Aa	0.56±0.04Cc	5.16±0.02Ba
0 day b*	22.64±0.24Cc	25.55±0.47Bb	29.05±0.13Ab
90th day L*	47.45±0.41Ca	50.82±0.54Ba	61.10±1.25Ab
90th day a*	7.82±0.18Ab	2.01±0.16Ca	4.47±0.18Bb
90th day b*	26.83±0.61Ba	30.71±0.43Aa	31.23±0.97Aa
180th day L*	44.45±0.64Cb	47.62±0.15Bc	59.36±0.34Ab
180th day a*	6.64±0.03Ac	1.31±0.03Cb	4.96±0.04Ba
180th day b*	24.08±0.03Cb	24.89±0.10Bb	27.90±0.22Ab

L * lightness; a * intensity of red when the value is positive/green when the value is negative; b * intensity of yellow when the value is positive/blue when the value is negative. FAP: Frozen acerola pulp; LAP: Lyophilized acerola pulp; MLAP: Microencapsulated and lyophilized acerola pulp. Values are expressed as mean ± triplicate standard deviation. Equal capital letters mean that the treatments do not differ significantly by the Tukey test at 5% probability, equal lower letters mean that the storage time (days) do not differ significantly by the Tukey test at 5% probability

Table 5 Total phenolic compounds results in the storage times of 0, 90 and 180 days in different treatments.

	FAP	LAP	MLAP
0 day	444.83±2.07Cc	478.36±2.13Ba	494.25±1.79Ab
90th day	481.84±0.65Ab	455.17±8.74Bb	493.16±3.31Ab
180th day	501.78±0.31Ba	460.34±0.57Cb	535.56±0.56Aa

FAP: Frozen acerola pulp; LAP: Lyophilized acerola pulp; MLAP: Microencapsulated and lyophilized acerola pulp. Values are expressed as mean ± triplicate standard deviation. Equal capital letters mean that the treatments do not differ significantly by the Tukey test at 5% probability, equal lower letters mean that the storage time (days) do not differ significantly by the Tukey test at 5% probability

Table 6 Total flavonoids results in the storage times of 0, 90 and 180 days in different treatments.

Sample	FAP	LAP	MLAP
0 day	161.55±2.45Aa	155.81±0.84Bab	160.40±1.99Aa
90th day	159.34±2.71ABa	156.03±0.95Ba	161.92±0.98Aa
180th day	155.39±3.57ABa	152.15±1.47Bb	160.53±1.04Aa

FAP: Frozen acerola pulp; LAP: Lyophilized acerola pulp; MLAP: Microencapsulated and lyophilized acerola pulp. Values are expressed as mean ± triplicate standard deviation. Equal capital letters mean that the treatments do not differ significantly by the Tukey test at 5% probability, equal lower letters mean that the storage time (days) do not differ significantly by the Tukey test at 5% probability

Table 7 Total anthocyanins results in the storage times of 0, 90 and 180 days in different treatments.

Sample	FAP	LAP	MLAP
0 day	8.46±0.07Aa	7.61±0.07Ba	7.57±0.18Ba
90th day	6.47±0.23Bb	7.49±0.12Aa	5.98±0.02Bb
180th day	5.08±0.03Bc	4.25±0.01Cb	5.70±0.03Ab

FAP: Frozen acerola pulp; LAP: Lyophilized acerola pulp; MLAP: Microencapsulated and lyophilized acerola pulp. Values are expressed as mean ± triplicate standard deviation. Equal capital letters mean that the treatments do not differ significantly by the Tukey test at 5% probability, equal lower letters mean that the storage time (days) do not differ significantly by the Tukey test at 5% probability

Evaluation of the storage in the conservation of bioactive compounds of the acerola pulp (*Malpighia* spp.) through microencapsulation and lyophilization

Bruno H. F. Saqueti, [a] ***Eloize S. Alves,*** [a] ***Matheus C. Castro,*** [b] ***Patrícia D. S. dos Santos,*** [b] ***Nayane B. M. Sinosaki,*** [b] ***Carlos E. R. Senes,*** [b] ***Jesuí V. Visentainer,*** [a, b] ***and Oscar O. Santos*** [a, b] *******

[a] *Departamento de Ciência de Alimentos, Universidade Estadual de Maringá (UEM), 87020-900 Maringá-PR, Brasil*

[b] *Departamento de Química, Universidade Estadual de Maringá (UEM), 87020-900 Maringá-PR, Brasil*

* corresponding author:

Phone: + 554430113663

e-mail: oliveirasantos.oscardeoliveira@gmail.com

ORCID ID https://orcid.org/0000-0002-9631-8480

Supplementary material

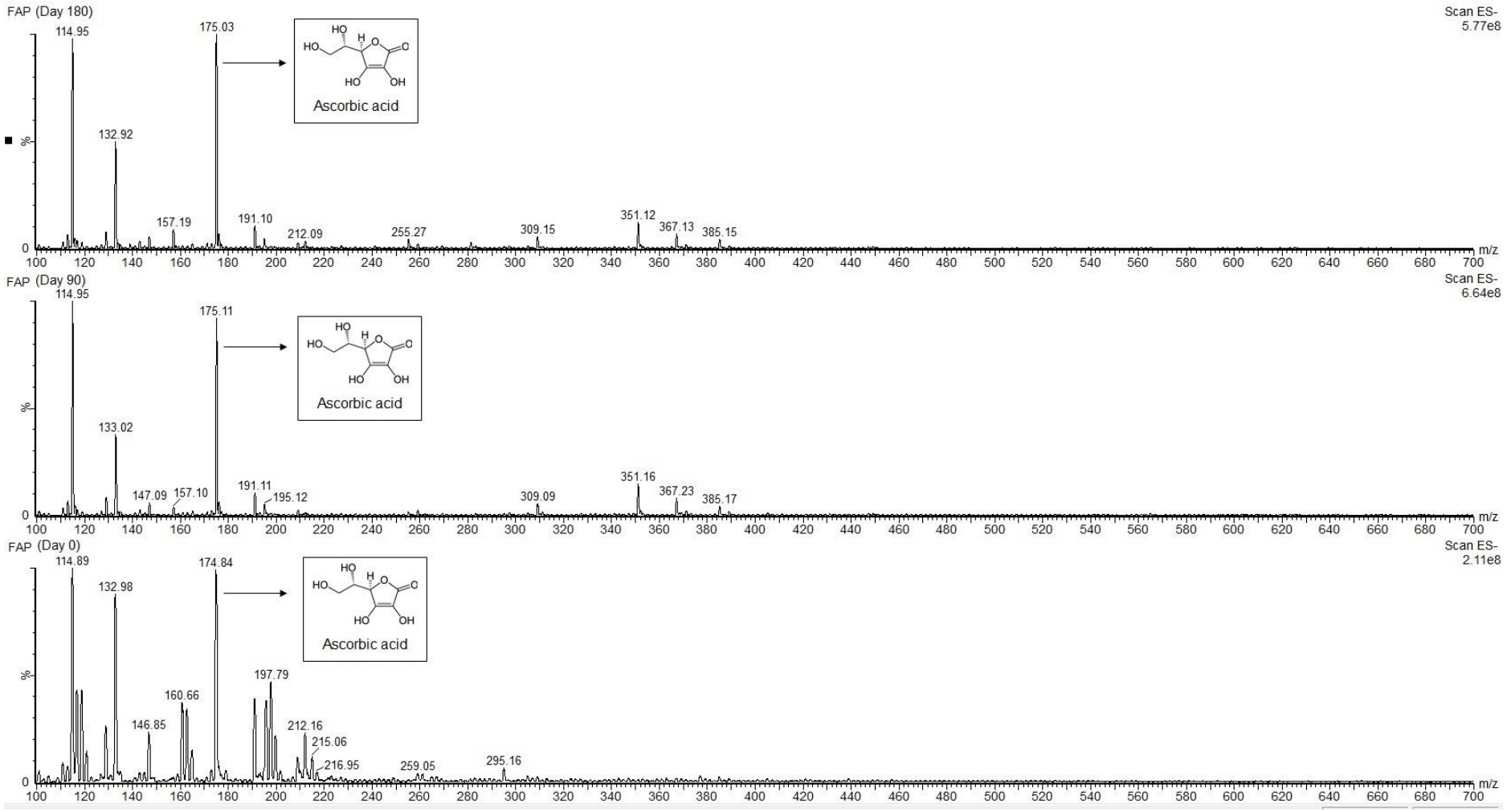


Figure S1. Frozen acerola pulp extract (FAP) spectrum at different storage times.

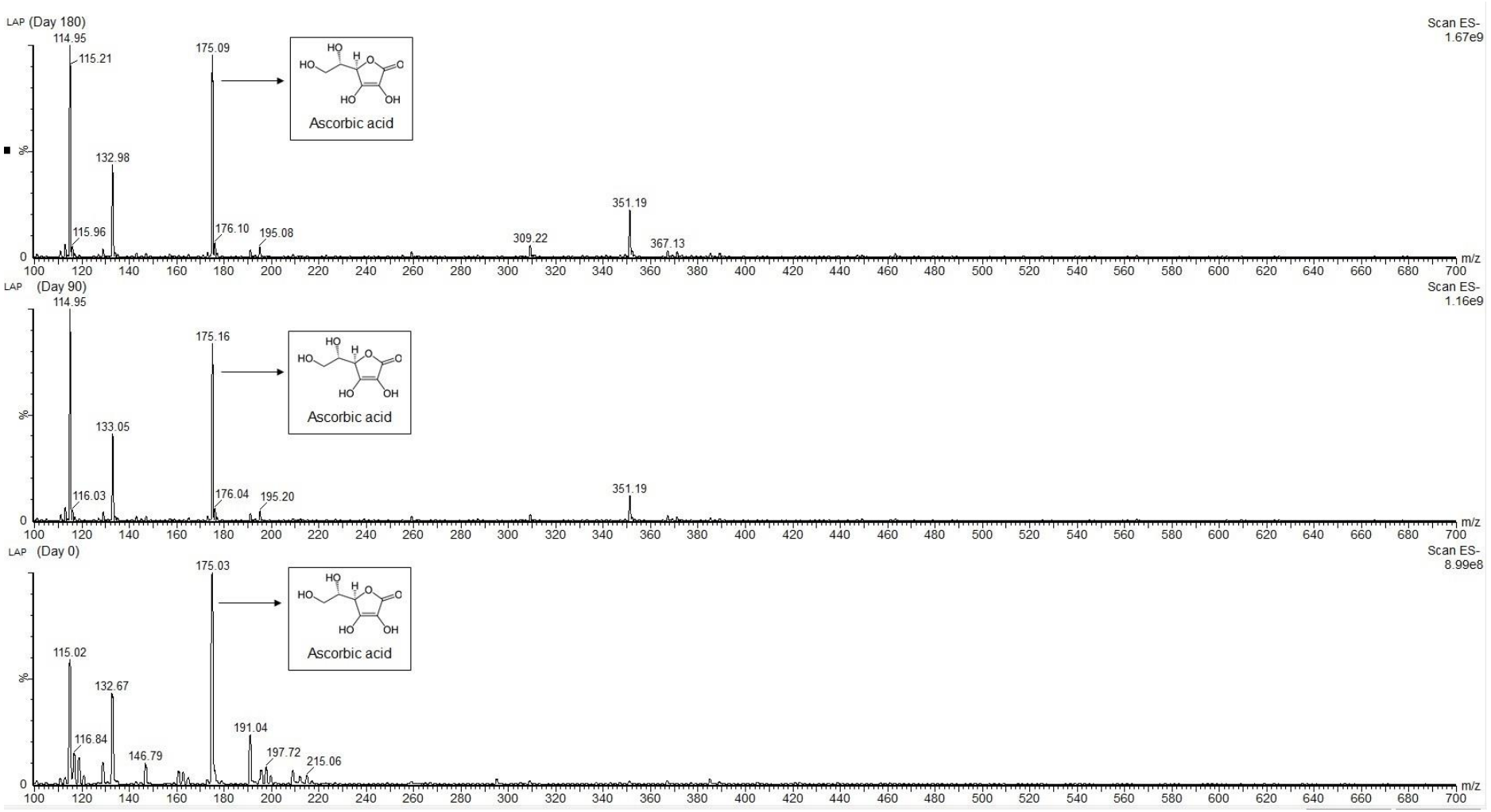


Figure S2. Lyophilized acerola pulp (LAP) spectrum at different storage times.

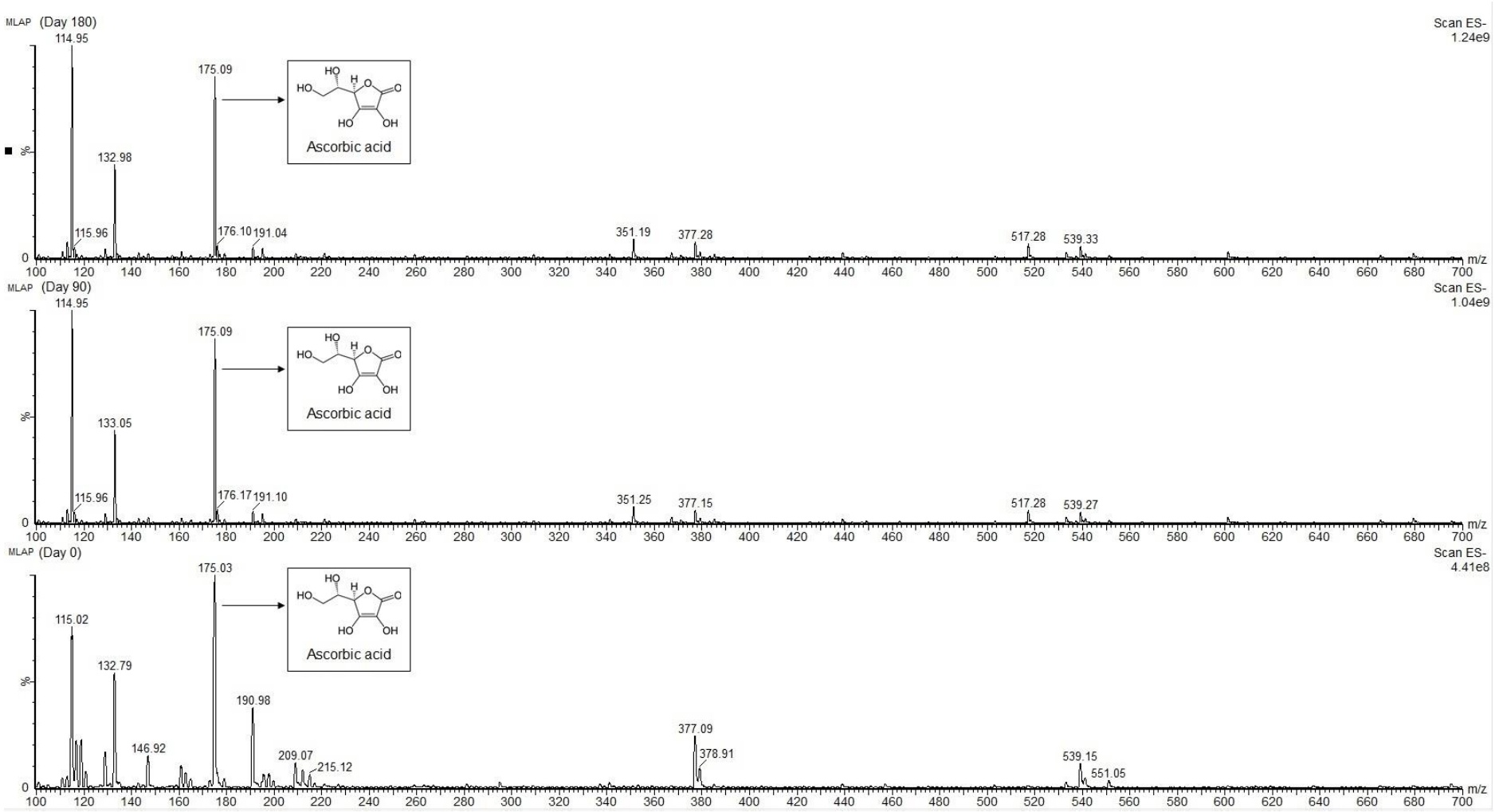


Figure S3. Microencapsulated and lyophilized acerola pulp (MLAP) spectrum at different storage times.