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EXTRAÇÃO E ESTABILIDADE DE COMPOSTOS BIOATIVOS DE SUBPRODUTOS DE UVA BRS Violeta

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Maringá
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Tese apresentada ao programa de Pós-Graduação em Ciência de Alimentos da Universidade Estadual de Maringá, como parte dos requisitos para obtenção do título de doutor em Ciência de Alimentos

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BIOGRAFIA

EDILSON BRUNO ROMANINI, nasceu em onze de julho de mil novecentos e oitenta e quatro, NA CIDADE DE Maringá do Paraná-PR. Possui graduação em TECNOLOGIA DE ALIMENTOS pela Universidade Tecnológica Federal do Paraná - UTFPR e mestrado em Ciência e Tecnologia de leite e derivados pela UNOPAR. Tem experiência nas áreas acadêmica e industrial atuando principalmente nos seguintes temas: controle de qualidade, pesquisa e desenvolvimento e extração de compostos bioativos.

Dedico

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APRESENTAÇÃO

Esta tese de doutorado está apresentada na forma de dois artigos científicos:

1. Edilson Bruno Romanini; Leticia Misturini Rodrigues; Aline Finger, Talita Perez Cantuaria Chierrito; Monica Regina da Silva Scapim; Grasiele Scaramal Madrona. Ultrasound assisted extraction of bioactive compounds from BRS Violet grape pomace followed by alginate-Ca^{2+} encapsulation. Food Chemistry, v.338, 2021. Qualis Capes: A1. Artigo publicado.

2. Edilson Bruno Romanini; Leticia Misturini Rodrigues; Talita Perez Cantuaria Chierrito; Ana Paula Stafussa; Aline Finger; Grasiele Scaramal Madrona. Bioactive compounds from BRS grape pomace: New insights into extraction and microencapsulation, stability protection and food application. Food Packaging and Shelf Life. Artigo a ser submetido. https://www.elsevier.com/journals/food-packaging-and-shelf-life/2214-2894/guide-for-authors

GENERAL ABSTRACT

INTRODUCTION

The hybrid cultivar BRS Violet is well adapted to southern Brazil. It is recommended for the preparation of juice, containing high levels of anthocyanins and other polyphenols, found mainly in the skin. Grape pomace is an important source of polyphenols such as phenolic acids, flavonoids, anthocyanins, proanthocyanidins and resveratrol, which are recognized for their antioxidant properties, including free radical scavenging ability, prevention of cancer, inflammation, cardiovascular disease and disorders related to aging. Therefore, the bioactive phenolic compounds recovered from grape pomace can potentially be used in the food, cosmetic and pharmaceutical areas, promoting health benefits and reducing the environmental impact. Among the current extraction technologies, ultrasound-assisted extraction (UAE) has been widely recognized as environmentally friendly, cheap, fast and efficient for phenolic extraction due to acoustic cavitation caused by the passage of the ultrasonic wave. Acoustic cavitation consists of the formation, growth and collapse of microbubbles on the solid surface, leading to corrosion and erosion, resulting in the breakdown of cell walls, allowing solvent penetration and improving mass transfer, which leads to an increase in the yield and to a decrease in the extraction time. Now, the great challenge is to apply these phenolic compounds in order to guarantee their properties after extraction from the grape, since they are unstable when exposed in the long term as they easily undergo degradation, oxidation, epimerization and polymerization. The chemical structure of polyphenols responsible for the antioxidant activity is itself targeted by those instabilities by the action of radiation or enzymes and variations in temperature, pH and oxygen. In this context, the use of substances that can transport these bioactive compounds in order to provide protection until their final destination has been described in the literature, for example, the encapsulation techniques by lyophilization, ionic gelation and spray drying are the most common. In this case, the core (phenolic compounds) is physically protected by an encapsulating material such as alginate-Ca^{2+}, maltodextrin and xanthan gum.

AIMS

The objective of this research was to investigate the effect of ultrasound on the extraction of total phenolics and anthocyanins from grape pomace, followed by encapsulation in an alginate-Ca^{2+} matrix and in a mixture of maltodextrin and xanthan gum to increase the shelf life and stability of phenolics and antioxidants, aiming their application in a food matrix (gelatin).

MATERIAL AND METHODS

Initially, grape juice was extracted by steam distillation using an artisanal juice extractor. The pomace generated during the juice production was subjected to a drying process and the skin were manually separated from the rest of the residue and ground in a food processor. A high intensity ultrasonic processor was used for the experimental design of ultrasound-assisted extraction (UAE). The extraction was carried out using water as a solvent at a ratio of 1:200 (w/v, g/mL). After preliminary tests, the factors were defined. The independent variables were sonication time (X1 = 2.5, 5 and 10 min), ultrasound frequency (X2 = 20, 30 and 40%) and temperature (X3 = 25, 40 and 55 °C), and the responses were the concentration of total anthocyanins (TA) and total phenolic compounds (TP). Conventional extraction (CE) was performed for 6 min, at room temperature (25 ºC) and without ultrasound, as a control sample. After determining the best extraction conditions, samples were characterized regarding the contents of bioactive compounds (TA, TP and TF-Total Flavonoids), antioxidant activities

(DPPH, FRAP, ABTS) and quantification of individual phenolic compounds by HPLC (rutin, gallic acid, p-coumaric acid, quercetin, myricetin and cyanidin chloride). The extract was encapsulated with alginate-Ca^{2+} at a concentration of 2% w/v (2 g/100 mL) with stirring and heating (70 ºC ± 4°C) for complete dispersion. For capsule formation, the dispersion was dripped using the Caviar Box® kit in an aqueous solution of calcium chloride (1 g/100 mL) kept for 10 min and washed with deionized water. The capsules containing the extracts were lyophilized. The stability of the microcapsules was evaluated through the degradation of the bioactive compounds (TP and TA) during 28 days of storage in the presence or absence of light. Their concentrations were assessed in intervals of 7 days, until the end of the 28 days. In the second step of the study, encapsulation was carried out with maltodextrin and xanthan gum (99.5% and 0.5%, respectively). The encapsulating agents were added at a 1:1 (w/w) agent:extract ratio. The capsules containing the extracts were lyophilized and stored in different conditions: a chamber (BOD) at a temperature of 25 ºC, in the presence and absence of light; and at 4 ºC for 120 days. In intervals of 30 days, they were monitored for the parameter of total monomeric anthocyanins, total phenolics and color. The lyophilized extract was used as a control sample. The microcapsules, the extract and an artificial dye were applied in a colorless gelatin powder, aiming to evaluate the instrumental color. Thus, the gelatin samples were refrigerated (4 °C) for 30 days, and the color parameters were evaluated at 0, 15 and 30 days. Analyzes were performed in triplicate and submitted to analysis of variance and Tukey's test for the minimum significant difference ($p < 0.05$) between means. Correlation and contour surface were also calculated using the statistical software Excel and Statistic 7.0.

RESULTS AND DISCUSSION

The effect of the three independent variables in ultrasonication (frequency, temperature and time) on extraction results (total phenolic compounds and anthocyanins) was revealed by generating a contour surface. TA values ranged from 3.11 to 3.90 mg of cyanidin-3-O-glycoside/g and TP from 22.30 to 25.03 mg GAE/g. The best extraction conditions for total phenolics and anthocyanins were at 55 °C, with 40% of ultrasound amplitude and for 6 min of treatment. Regarding the color of the extracts, the conventional extraction resulted on a brighter sample. On the other hand, the ultrasound method produced a red extract, indicating a better obtention of the compounds, which correlates with the extraction of anthocyanins with UAE 1.35 higher than with the CE. The concentration and antioxidant capacity results (DPPH, ABTS and FRAP) for UAE were statistically higher when compared to the CE. The results of the three analyzes of total bioactive compound content performed (TP, TA and TF) were different comparing UAE with CE, demonstrating that the use of the ultrasound method was significantly better, on average, 11% for TP, 25% for TA and 34% for TF. Positive correlations were observed between these contents (TP, TA and TF) and DPPH ($r = 0.963$, 0.967 and 0.940 respectively), ABTS ($r = 0.989$, 0.986 and 0.997, respectively) and FRAP ($r = 0.976$, 0.972 and 0.990, respectively). The CE and UAE methods also resulted in significantly different concentrations of anthocyanins and of other compounds identified, demonstrating that the use of ultrasound was significantly better, on average, 29.41% for rutin and quercetin, 50% for acid p-coumaric, 23.93% for cyanidin chloride, 16.67% for myricetin and 15.79% for gallic acid. An encapsulation efficiency (EE) of 62.52% was observed. The stability assay of the extracts with alginate-Ca^{2+} showed that the degradation of anthocyanins and total phenolics (32.80 and 60.26%, respectively) was higher in the presence than in the absence of light (18.51 and 43.59%, respectively). When evaluating the effect of the three parameters (presence, absence of light, and temperature 4 ºC) using maltodextrin + xanthan gum as encapsulating matrix, the degradation was higher in the unprocessed extract (TP = 24.49, 21.38 and 23.07% TA = 20.61, 18.53 and 19.47%, respectively) than in the encapsulated extract (TP = 17.92, 12.06 and 9.69%; TA = 14.71, 12.78 and 12.78%, respectively). Thus, one can observe the protective effect of the capsule comparing to the unprocessed extract. There was a significant difference in the TP

content during the stability test of the encapsulated extract, with higher degradation in the presence of light. On the other hand, the TA content showed practically similar degradation under the conditions studied. Regarding the color at 120 days of storage, the encapsulated extract Cap4 (exposed to a temperature of 4 °C) presented the lowest color change ($\Delta E < 5$), while this parameter was high ($\Delta E > 5$) for the extracts that are independent of the storage conditions and the encapsulated extracts Cap25 (absence of light) and CapLight (presence of light). A first order degradation curve was obtained for all encapsulated samples. The half-life values of the encapsulated content (alginate-Ca^{2+}) of total monomeric and phenolic anthocyanins decreased from 104 to 59 and from 40 to 25 days, respectively, in the presence of light, indicating a longer half-life in the absence of light. When evaluating the encapsulated product (maltodextrin + xanthan gum) versus the extract at both temperatures (25 and 4 ºC), there was a more accentuated loss in the TP content present in the unprocessed extract (t(1/2) of 321 days) compared to the capsules (984 days) 4 ºC. When evaluating the effect of light on the capsules and the extract, TA and TP content losses were similar (t(1/2)). In a general evaluation of the conditions studied, both total anthocyanin and phenolic compounds showed higher degradation in the extract, inferring that the capsule provides protection to bioactive substances. The application of the capsules and the coloring in gelatin showed little difference in total color in 30 days ($\Delta E = 3.35$ and 3.58). During storage, on days 15 and 30, the total color of the extract changed significantly: $\Delta E = 8.37$ and 8.85, respectively. Comparing to the dye and the capsules, the color change when the extract is used is 2.47 and 2.65 times higher, respectively. This demonstrates that the encapsulation of colored bioactive substances can be used as an alternative to artificial coloring in food products, for example, when stored for up to 15 days under refrigeration.

CONCLUSIONS

The aqueous extracts of grape skins (industry by-product) obtained by ultrasound showed higher levels of total anthocyanins and phenolics compared to conventional extraction. The increase in some specific compounds is also evident (29% for rutin and quercetin and 24% for cyanidin chloride). The extract encapsulated in alginate-Ca^{2+} showed less degradation when stored in the absence of light than in its presence. The use of an encapsulating matrix of maltodextrin and xanthan gum proved to be effective in maintaining the stability and color of antioxidant compounds, increasing their half-life. Therefore, it is recommended for protecting antioxidant substances that also provide color in food applications. Finally, grape pomace (BRS Violet) showed a high concentration of phenolic compounds, better extracted by ultrasound, and that can be reused, mainly by encapsulation, for future applications in food matrices.

Keywords: Ultrasound, phenolic compounds, antioxidant, stability, encapsulation.

RESUMO GERAL

INTRODUÇÃO

A cultivar híbrida BRS Violet é bem adaptada ao sul do Brasil, sendo recomendada para o preparo de suco e contém altos níveis de antocianinas e outros polifenóis, encontrados principalmente na casca. O bagaço de uva é uma fonte importante de polifenóis, como ácidos fenólicos, flavonóides, antocianinas, proantocianidinas e resveratrol, que são reconhecidos por suas propriedades antioxidantes, incluindo capacidade de eliminação de radicais livres, prevenção de câncer, inflamação, doenças cardiovasculares e transtornos relacionados ao envelhecimento. Portanto, os compostos fenólicos bioativos recuperados do bagaço da uva podem ser potencialmente utilizados nas áreas alimentícia, cosmética e farmacêutica, promovendo benefícios à saúde e reduzindo o impacto ambiental. Dentre as tecnologias de extração atuais, a extração assistida por ultrassom (EAU) tem sido amplamente reconhecida como ecologicamente correta, barata, rápida e eficiente para a extração fenólica devido à cavitação acústica causada pela passagem da onda ultrassônica. A cavitação acústica consiste na formação, crescimento e colapso de microbolhas na superfície sólida, levando à corrosão e erosão, resultando na ruptura das paredes celulares, permitindo a penetração do solvente e melhorando a transferência de massa, levando a um aumento no rendimento e encurtando o tempo de extração. O grande desafio no momento está em aplicar o uso desses compostos fenólicos de modo a garantir suas propriedades depois de extraídos da uva, já que não apresentam estabilidade quando expostos a longo prazo por sofrerem facilmente degradação, oxidação, epimerização e polimerização. A própria estrutura química dos polifenóis responsável pela atividade antioxidante é alvo dessas instabilidades por meio da ação de radiação ou enzimas e variações de temperatura, pH e oxigênio. Neste contexto, a utilização de substâncias que possam veicular esses compostos bioativos de maneira a conferir proteção até o seu destino final, vem sendo descrita na literatura, como por exemplo, as técnicas de encapsulação por liofilização, gelificação ionica e secagem por pulverização são as mais comuns, neste caso o núcleo (compostos fenólicos) é protegido fisicamente por um material encapsulante como, alginato-Ca^{2+}, maltodextrina e goma xantana.

OBJETIVOS

O objetivo desta pesquisa foi investigar o efeito do ultrassom na extração de fenólicos totais e antocianinas do bagaço de uva, seguido de encapsulamento em matriz de alginato-Ca^{2+} e em uma mistura de maltodextrina e goma xantana para aumentar a vida de prateleira e estabilidade de fenólicos e antioxidantes visando sua aplicação em matriz alimentícia (gelatina).

MATERIAL E METODOS

Inicialmente, o suco de uva foi extraído por destilação a vapor usando um extrator de suco artesanal. O bagaço gerado durante a produção do suco foi submetido a um processo de secagem e as cascas foram separadas manualmente do restante do resíduo e trituradas em processador de alimentos. Para o desenho experimental da extração assistida por ultrassom (EAU), foi utilizado um processador ultrassônico de alta intensidade. A extração foi realizada utilizando água como solvente na proporção de 1:200 (p/v, g/mL). Após testes preliminares, os fatores foram definidos. As variáveis utilizadas foram tempo de sonicação (X1 = 2,5, 5 e 10 min), frequência do ultrassom (X2 = 20, 30 e 40%) e temperatura (X3 = 25, 40 e 55 °C) e como variável resposta a concentração de antocianinas totais (TA) e compostos fenólicos totais (TP), como controle foi realizado a extração convencional (EC) por 6 min a temperatura ambiente (25 ºC), sem ultrassom. Após determinar as melhores condições de extração, a amostra foi caracterizada quanto aos teores de compostos bioativos (TA, TP e TF - Flavonoides totais), atividades

antioxidantes (DPPH, FRAP, ABTS), quantificação de compostos fenólicos individuais por HPLC (rutina, ácido gálico, ácido p-cumárico, quercetina, miricetina e cloreto de cianidina). Realizou-se o encapsulamento do extrato com alginato-Ca^{2+} a uma concentração de 2% p/v (2g/100 mL) com agitação e aquecimento (70 ºC ± 4°C) para dispersão completa. Para a formação da microcápsula, a dispersão foi gotejada utilizando o kit Caviar Box® em solução aquosa de cloreto de cálcio a (1g/100 mL) mantidas por 10 min e lavadas com água deionizada, as cápsulas mais extratos foram liofilizados. A estabilidade das microcápsulas foi avaliada através da degradação dos compostos bioativos (TP e TA) durante 28 dias de armazenamento na presença ou ausência de luz. Suas concentrações foram avaliadas em intervalos de 7 dias, até o final dos 28 dias. Na segunda parte da pesquisa, realizou-se encapsulamento com maltodextrina e goma xantana (99,5% e 0,5% respectivamente). Os agentes encapsulantes foram adicionados a uma relação agente:extrato de 1:1 (p/p). As cápsulas contendo os extratos foram liofilizados e armazenados em diferentes condições: uma câmara (BOD) à temperatura de 25 ºC na presença e ausência de luz; e a 4 ºC por 120 dias. Em intervalos de 30 dias, elas foram monitoradas quanto ao parâmetro de antocianinas totais monoméricas, fenólicos totais e cor. Como controle foi utilizado o extrato liofilizado. As microcápsulas, o extrato e um corante artificial foram aplicados em uma gelatina em pó incolor, visando avaliar a cor instrumental. Assim, as amostras em gelatina foram refrigeradas (4 °C) por 30 dias, e os parâmetros de cor foram avaliados em 0, 15 e 30 dias. As análises foram realizadas em triplicata e submetidas à análise de variância e teste de Tukey para a diferença mínima significativa ($p < 0,05$) entre as médias. A correlação e a superfície de contorno também foram calculadas usando os programas estatísticos Excel e Statistic 7.0.

RESULTADOS E DISCUSSÃO

Para relacionar os efeitos das três variáveis na ultrassonificação (frequência, temperatura e tempo) independentes na extração de compostos fenólicos e antocianinas totais, obteve-se a superfície de resposta, sendo que os valores de TA variaram de 3,11 a 3,90 mg de cianidina-3-O-glicosídeo/g e TP de 22,30 a 25,03 mg de GAE/g. A melhor extração para fenólicos e antocianinas totais, foi a 55 °C, amplitude de 40% e 6 min de tratamento. Em relação à cor dos extratos, a extração convencional apresentou uma amostra mais clara, por outro lado o método por ultrassom produziu um extrato avermelhado indicando melhor obtenção de compostos, o qual se correlaciona com os valores de antocianinas EAU 1,35 maiores que EC. Os resultados de dosagem e capacidade antioxidante (DPPH, ABTS e FRAP) obtidos para EAU foram estatisticamente superiores quando comparados a EC. Para a dosagem de compostos bioativos houve diferenças entre as três análises realizadas (TP, TA e TF) demonstrando que o uso do ultrassom foi significativamente melhor, em média 11% para TP, 25% para TA e 34% para TF. Correlações positivas foram observadas entre o teor de fenólicos, antocianina e flavonoides totais para DPPH ($r = 0,963$, 0,967 e 0,940 respectivamente), ABTS ($r = 0,989$, 0,986 e 0,997, respectivamente) e FRAP ($r = 0,976$, 0,972 e 0,990, respectivamente). Houve diferença significativa entre os dois métodos de extração EC e EAU para as concentrações de antocianinas e entre os demais compostos identificados, demonstrando que o uso do ultrassom foi significativamente melhor, em média 29,41% para rutina e quercetina, 50% para ácido p-cumárico, 23,93% para cloreto de cianidina, 16,67% para miricetina e 15,79% para ácido gálico. Foi observado uma eficiência de encapsulamento (EE) de 62,52%. O ensaio de estabilidade do encapsulado com alginato-Ca^{2+} demonstrou que na presença de luz, a degradação de antocianinas e fenólicos totais (32,80 e 60,26%, respectivamente) foi maior em relação à ausência de luz (18,51 e 43,59%, respectivamente). Já para o encapsulado de maltodextrina + goma xantana ao avaliar o efeito dos três parâmetros (presença e ausência de luz, e temperatura 4 ºC) houve maior degradação para o extrato (TP = 24,49, 21,38 e 23,07% TA = 20,61, 18,53 e 19,47%, respectivamente) do que para encapsulado (TP = 17,92, 12,06 e 9,69%; TA = 14,71, 12,78 e 12,78%, respectivamente). Dessa forma, pode-se observar o efeito

protetor da cápsula em relação ao extrato. Para o ensaio de estabilidade do encapsulado, foi possível observar que houve diferença significativa para o teor de TP, com degradação maior na presença de luz. Diferentemente do observado para o teor de TA, que apresentou degradação praticamente similar nas condições estudadas. Em relação a cor aos 120 dias de armazenamento, o encapsulado Cap4 (exposto a temperatura de 4 °C) obteve a menor alteração de cor ($\Delta E < 5$). Os extratos independentes das condições de armazenamento e os encapsulados Cap25 (ausência luz) e CapLuz (presença de luz) obtiveram altos valores de ($\Delta E > 5$). A encapsulação apresentou uma curva de degradação de primeira ordem para todas as amostras. Os valores de meia-vida do conteúdo encapsulado (alginato-Ca^{2+}) de antocianinas monoméricas totais e fenólicas diminuíram de 104 para 59 e de 40 para 25 dias, respectivamente, na presença de luz, indicando uma meia-vida mais longa na ausência de luz. Ao avaliar o produto encapsulado (maltodextrina + goma xantana) versus o extrato em ambas as temperaturas (25 e 4 ºC) houve perda mais acentuada no teor de TP presente no extrato, sendo evidenciado pelo t(1/2) de 984 dias para cápsula e para o extrato de 321 dias, a 4 ºC. Quando avaliado o efeito da luz sobre o encapsulado e o extrato, houve perdas próximas entre os teores de TA e TP (t(1/2). Em uma avaliação geral das condições estudadas, ambos os compostos antocianinas e fenólicos totais apresentaram degradação maior no extrato, inferindo que a cápsula proporcione proteção as substâncias bioativas. A aplicação do encapsulado e o corante em gelatina apresentaram pouca diferença de cor total entre eles no período de 30 dias ($\Delta E = 3,35$ e 3,58). O extrato nos dias 15 e 30 teve uma mudança significativa na cor total durante o armazenamento ($\Delta E = 8,37$ e 8,85, respectivamente), sendo 2,47 vezes maior que o corante e 2,65 para o encapsulado. O que demonstra que o encapsulamento de substâncias bioativas com cor pode ser empregado como alternativa aos corantes artificiais em produtos alimentícios por exemplo, quando armazenados em até 15 dias sob refrigeração.

CONCLUSÕES

Os extratos aquosos de casca de uva (subproduto da indústria) obtidos por ultrassom assistido apresentaram maiores níveis de antocianinas e fenólicos totais em comparação a extração convencional. Destaca-se também o aumento dos compostos (29% para rutina e quercetina e 24% para cloreto de cianidina). O extrato encapsulado em alginato-Ca^{2+} quando armazenado na ausência de luz apresentou menor degradação do que na presença. A utilização de uma matriz encapsulante de maltodextrina e goma xantana demonstrou ser eficaz na manutenção da estabilidade e cor de compostos antioxidantes aumentando seu tempo de meia vida sendo, portanto recomendada para futuras aplicações alimentícias ao proteger substâncias antioxidantes que promovem cor ao alimento aplicado. Por fim, o bagaço de uva (BRS Violeta) apresentou alta concentração de compostos fenólicos, melhores quando extraídos por ultrassom que podem ser reaproveitados principalmente na forma de encapsulados visando futura aplicação em matrizes alimentícias.

Palavras-chaves: Ultrassom, compostos fenólicos, antioxidante, estabilidade, encapsulamento.

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Ultrasound assisted extraction of bioactive compounds from BRS Violet grape pomace followed by alginate-Ca^{2+} encapsulation

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ABSTRACT

Objective of this study was to recover bioactive compounds from grape pomace, and to investigate the effect of thermosonication in the rate of aqueous extraction. The best extraction for phenolics and total anthocyanins, was at 55 °C, amplitude of 40% and 6 min of treatment. The ultrasound assisted extraction showed superior results when compared to conventional extraction, extraction averages were: 11% total phenolic compounds, 25% total anthocyanins. The extract obtained by ultrasound showed higher antioxidant capacity when compared to the one obtained by conventional extraction. The alginate-Ca^{2+} capsules were stable when stored in the presence or absence of light, with a reduced t1/2 (absence of light), indicating longer half-life in the absence of light. The use of thermosonication favored greater amounts of bioactive compounds in the grape pomace aqueous extract, and this encapsulated extract in alginate-Ca^{2+} shows good stability and less degradation in the light absence.

1. Introduction

Grape culture is probably as old as civilization itself. Grape pomace, considered as an agro-industrial waste, representing about 25% (w/w) of the weight of grapes processed, and thus amounts to more than 9 million tons annually (Sirohi et al., 2020). Approximately 60–65% of phenolic compounds remain in the grape pomace after juice or red wine production, which could be processed, and used as a source of antioxidants, but it is usually discarded as waste or employed as animal feed (de la Cerda-Carrasco, López-Solís, Nuñez-Kalasic, Peña-Neira, & Obreque-Slier, 2015). The increase in agro-industrial and food production residues, have led to the need to study alternative ways to use by-products (Vital, Santos, Matumoto-Pintro, da Silva Scapim, & Madrona, 2018).

Grape pomace is an important source of polyphenols, like phenolic acids, flavonoids, anthocyanins, proanthocyanidins and resveratrol (Tsali & Goula, 2018) which are recognized for their antioxidant properties, including free radical scavenging abilities, prevention of cancer, inflammation, cardiovascular disease and aging-related disorders (Tao, Zhang & Sun, 2014). Therefore, the bioactive phenolic compounds recovered from grape pomace can be potentially used in the food, cosmetic and pharmaceutical areas promoting health benefits and reducing environmental impact (Tao, Zhang & Sun, 2014b).

In 2006 EMBRAPA Grape and Wine (Brazilian Agricultural Research Corporation, Grape and Wine Unit) developed the hybrid cultivar BRS Violet from a cross between IAC 1398-21 and BRS Rubea. This hybrid cultivar is well adapted to southern Brazil, under temperate and subtropical conditions, as well as in tropical regions, being recommended for the preparation of juice (Camargo, Maia, & Nachtigal, 2005). BRS Violet contains high levels of anthocyanins and other polyphenols, mainly found in the peel (Moser et al., 2017).

Among the current extraction technologies, ultrasound has been widely recognized as environment-friendly, inexpensive, fast and efficient for phenolic extraction due to acoustic cavitation caused by the ultrasound wave passage. Acoustic cavitation consists of the formation, growth and collapse of microbubbles on the solid surface, leading to

Abbreviations: DW, dry weight; UAE, ultrasound assisted extraction; TA, total anthocyanins; TP, total phenolics; CE, Conventional extraction; TF, Total content of flavonoids; GAE, gallic acid equivalent; TE, Trolox equivalent; EQ, quercetin equivalent; EC, catechin equivalent; DPPH, 2,2 diphenyl-1 picrylhydrazine; FRAP, Ferric Reducing Antioxidant Power; ABTS, 2,2′- azinobis (3 ethylbenzothiazoline-6-sulfonic acid); HPLC-DAD UV/vis, high efficiency liquid chromatography; EE, Encapsulation efficiency

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corrosion and erosion, resulting in rupture of cell walls, allowing solvent penetration and improving mass transfer, leading to an increase in extraction yield and shortening extraction time (Tao, Zhang & Sun, 2014). The factors that may influence the extraction process in the ultrasound assisted extraction (UAE) are moisture content, sample, sample size, solvent employed, temperature, frequency and probe time (Azmir et al., 2013).

On the other hand, to maintain bioactivity of polyphenols it is important to protect their stability. The chemical structure of these compounds is rich in unsaturated bonds resulting in an easy target to oxidants, light, heat, enzymes, water, and pH variations (Tsali & Goula, 2018). In this way, encapsulation techniques of polyphenols have been reported (Arriola et al., 2019). In addition, encapsulation is used to mask bitter and adstringent taste of polyphenols (Nedovic, Kalusevic, Manojlovic, Levic, & Bugarski, 2011). The most widely used encapsulating material is alginate, a naturally occurring anionic polymer of (1,4)-linked β-D-mannuronate (M) and α-L-guluronate (G) residues, extracted from brown algae, used in pharmaceutical and food industry, due to its biocompatibility, availability, and ease of gelation by addition of Ca^{2+} (Lee & Mooney, 2012). To innovate, considering an ecofriendly process, it was decided to perform aqueous extraction of bioactive compounds from BRS Violet grape pomace, followed by encapsulation with alginate.

Based on these considerations, the aim of this research was to investigate the effect of ultrasound on the total phenolic and anthocyanins extraction from grape pomace, followed by encapsulation in alginate-Ca^{2+} matrix to enhance shelf life and stability of phenolic and antioxidants. Detection and quantification of some phenolic compounds from obtained extracts was also performed by HPLC-DAD.

2. Material and methods

2.1. BRS Violet grapes and chemicals

BRS Violet (BRS Rubea × IAC 1398-21) grapes were collected in November 2018 from a producer from the Marialva region of the state of Paraná (23° 31′09.7 ″S 51° 49′29.7″ W). The grapes were previously sanitized, and stored at −18 °C until processing. The chemicals and solvents used for analysis in this study were of analytical or HPLC grade purchased from JT Baker or Sigma - Aldrich (as example, standards: Cyanidin Chloride, rutin, quercetin, p-cumaric acid, myricetin and gallic acid).

2.2. Pomace obtaintion and drying

Grape juice was extracted by steam distillation using an artisanal juice extractor consisting of three containers (Rizzon, Manfroi, & Meneguzzo, 1998). The pomace (seed, stalks and peels) generated during juice production was subjected to a drying process in a circulating air oven for 36 h at 50 °C (Vital et al., 2018). After drying, the peels were manually separated from the rest of the residue, and crushed in a food processor, resulting in a powder whose particle size was standardized between 0.325 and 0.420 mm. After that, the peel powder was stored at −18 °C protected from light, until further analysis.

2.3. Experimental design: ultrasound assisted extraction (UAE)

A high intensity ultrasonic processor (750 Watts and 20 kHz model, sonicator with probe - Vibra Cell - VC 750, Cole-Parmer, USA) was used. Its amplitude controller allowed the ultrasonic vibrations through the probe tip (13 mm diameter titanium) to be adjusted to the desired level on a scale of 0–40% (in 114 μm) by the controller and cycles of 5 s on and 5 s off. The extraction was performed using water as a solvent in the proportion of 1:200 (w/v, g/mL) with a total extraction volume of 200 mL, according (Rockenbach et al., 2011).

The extraction temperature was controlled manually using a thermometer and a bath with cold water. After each extraction, the extracts were vacuum filtered on Whatman filter paper No. 4, and the filtrates stored at −18 °C ± 2 °C until analysis.

Table 1
Experimental design: optimization of TA and TP ultrasound assisted extraction, with time, amplitude and temperature variables.

Experiment	Variables			Response	
	Time (min.)	Amplitude (%)	Temperature (°C)	TA (mg/g)	TP (mg/g)
1	2.5	20	25	3.18	22.44
2	10	20	25	3.17	22.51
3	2.5	40	25	3.11	22.60
4	10	40	25	3.59	22.30
5	2.5	20	55	3.59	23.89
6	10	20	55	3.64	23.89
7	2.5	40	55	3.83	25.03
8	10	40	55	3.90	24.99
9	5	30	40	3.65	24.00
10	5	30	40	3.65	23.99
11	5	30	40	3.78	24.55

*DW- dry weight.

After preliminary tests, the factors were defined and an experimental design (Rodrigues et al., 2020) was performed (Table 1). Table 2 shows the results of ANOVA and the F-test in order to evaluate the statistical significance of the model used. The used variables was sonication time (X1 = 2.5, 5 and 10 min), ultrasound frequency (X2 = 20, 30 and 40%) and temperature (X3 = 25, 40 and 55 °C), three repetitions in the central points was used for better estimation of a pure error sum of squares. The experiments run order was randomized in order to prevent systematic errors. As response variable the concentration of total anthocyanins (TA) and total phenolic compounds (TP), in triplicate, was evaluated by means of contour surface by the program STATISTIC 7.0.

2.4. Conventional extraction (CE)

For comparative purposes, the aqueous extraction was also performed by the conventional method (CE) for 6 min at room temperature (25 °C), that is, without thermosonication. Thus, the resulting extract was filtered on Whatman No. 4 filter paper and the filtrate stored at −18 °C for further analysis.

Table 2
ANOVA for surface response model of all independent variables.

Factors	TA (Radj 0.912; Rpred 0.941)		TP (Radj 0.723; Rpred 0.848)	
	Coefficient	p-Value	Coefficient	p-Value
Intercept				
β_0	3.799	< 0.0001 *	24.942	< 0.0001 *
Linear				
X_1	0.059	0.0040 *	0.158	0.3217
X_2	0.101	< 0.0001*	298.51	< 0.0001 *
X_3	0.230	< 0.0001*	982.34	< 0.0001 *
Interaction				
X_1X_2	0.062	0.0091*	97.50	0.7916
X_1X_3	−0.036	0.0043*	100.08	0.1147
X_2X_3	0.030	0.1728	226.97	0.0030 *
Quadratic				
X_1^2	−0.216	0.0040*	−785.18	0.0238 *
			Fvalue	p-value
Lack of fit	8.25	0.0036	5.56	0.0028

*Significant at p < 0.005.

2.5. Color, pH and total soluble solids

The colors of the extracts were evaluated in a portable colorimeter CR-400 (Minolta Ltda., Osaka, Japan) using cuvettes, with D65 illuminant −10° angle. The brightness parameters L * (black to white), a * (green to red) and b * (blue to yellow) were checked. The pH was evaluated with the aid of a digital pHmeter (Hanna Instruments, model pH 21 Ph/mv). Total soluble solids content was obtained by digital refractometer (Hanna Instruments, model HI96801) and expressed in ° Brix.

2.6. Analysis of bioactive compounds

Determination of total anthocyanin (TA) content was carried out by the pH differential absorbance method, at 520 and 700 nm at pH 1.0 and pH 4.5 (Lee, Durst, & Wrolstad, 2005). Total anthocyanin content was expressed in mg of cyanidin-3-glucoside/g of grape peel. To determine total phenolic compounds (TP), the Folin-Ciocalteu method was performed. Total amount of phenolic compounds was calculated from the standard calibration curve using gallic acid and the results were expressed in mg of gallic acid equivalent (GAE)/g of grape peel (Pierpoint, 2004). Total content of flavonoids (TF) was obtained according to a colorimetric assay reported by (Zhishen, Mengcheng, & Jianming, 1999), and its total content expressed in μg of catechin equivalent (EC)/mg of grape peel.

2.6.1. Determination of individual phenolic compounds by HPLC-DAD system and antioxidant capacity

The HPLC system, brand Waters, model Alliance e2695 was used, equipped with a quaternary pump (model Waters 2998) and UV–VIS, DAD detectors, with automatic sample injection. The compounds were quantified according to the methodology previously reported with modifications (Giusti, Caprioli, Ricciutelli, Vittori, & Sagratini, 2017). Chromatographic separation was performed using a C18 column (macherey–nagel, 250 × 4.6 mm) with reverse phase. The column temperature was set at 40 °C. The mobile phase used was 0.1% formic acid in water (v/v) (eluent A) and acetonitrile (eluent B). The injection volume was 10 μL of sample, and the flow of the run was 0.9 mL min^{-1} with a total run time of 30 min. The gradient schedule was: 0 min 90% (A), 0–17 min 40% (A), 17–22 min 40% (A), 22–28 min 90% (A) and maintained at 90% (A) until the end. HPLC/DAD analyzes were performed at 8 different wavelengths: 265 nm for rutin, 275 nm for gallic acid, 310 nm for p-coumaric acid, 350 nm for quercetin, 370 for myricetin and 520 nm for cyanidin chloride. Phenolic compound identification was done by comparing the retention time and UV absorption spectra with the available standards. The extracts were filtered (0.45 μL) and automatically injected into the column and quantification was based on the peak area (r2 > 0.99) with the concentrations expressed in mg/g of peel.

Samples UAE (sample that showed better extraction of bioactive compounds) and CE were also subjected to the antioxidant capacity test using three methods, namely the reduction power of iron ion FRAP, scavenging activity of the free radical DPPH and scavenging activity of the radial ABTS.

Antioxidant capacity by the iron reduction method was performed by mixing the sample extracts with the FRAP reagent (Pulido, Bravo, & Saura-Calixto, 2000). The method of reducing the DPPH radical • in alcoholic solution in the presence of an electron donating antioxidant due to the formation of the non-radical form DPPH-H in the reaction (Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Hawkins Byrne, 2006). The antioxidant capacity in the ABTS method was performed using a colorimetric assay (Nenadis, Wang, Tsimidou, & Zhang, 2004). The results were expressed in μM of Trolox equivalent (TE)/mg of grape peel.

2.7. Extraction encapsulation by ionic gelation

The sodium alginate at a concentration of 2% w/v (2 g/100 mL) was dispersed in the extract with stirring (200 rpm) and heating at 70 °C ± 4 °C for complete dispersion. For the formation of the alginate capsule, the dispersion was dripped using the Caviar Box® kit (with 300 μm of diameter) in an aqueous solution of calcium chloride a (1% w/v, 1 g/100 mL), the drip speed was maintained at 4.54 mL/min and the distance between the caviar box kit and calcium solution was 15 cm. The capsules formed were kept in the calcium chloride solution for 10 min, after which they were sieved and washed with deionized water to remove excess calcium and interrupt the complexation process (da Silva Carvalho et al., 2019). After washing, the capsules were frozen for 48 h at −18 °C and subsequently subjected to lyophilization (freeze L108, Liobras) for 2 days to ensure complete drying.

A micrometer (IP 65 Coolant proof, Mitutoyo) was used to measure the size of the encapsulated particles before (3.678 mm ± 0.16) and after lyophilization (3.065 ± 0.18). The encapsulation efficiency was calculated exactly according to (Selamat, Muhamad, & Sarmidi, 2009), by using water as solvent, as shown in the equation below:

$$Encapsulation\ efficiency\ EE\,(\%) = \frac{(FT - FS) \times 100}{FT}$$

where *FT* are the total phenolic compounds inside the microcapsules and *FS* the total phenolic compounds present on the surface.

2.8. Light stability during storage

After weighing, the lyophilized capsules were stored (in replicates) in transparent plastic packaging in a chamber at 25 °C in the presence or absence of light for 28 days (every 7 days) using two fluorescent lamps of 20 W and a dark chamber (Santos, Rodrigues, Costa, De Cassia Bergamasco, & Madrona, 2017), and monitored for the parameter of total monomeric anthocyanins and total phenolics. The experimental data for anthocyanins and phenolics values were adjusted by the first-order reaction kinetics and the half life (t1/2) (Stamp & Labuza, 1983), according to Eq. (1) and (2):

$$\ln(A_t/A_0) = -\ k \times t$$

$$t_{1/2} = \ln 2/k$$

where *t* is time (day), *A* is the TA or TP level, A_0 is either the initial TA or TP level at time zero, and *k* is the rate constant (day^{-1}).

2.9. Data analysis

The analyses were performed in triplicate and subjected to analysis of variance and Tukey's test for the minimum significant difference ($p < 0.05$) between the means. Correlation and contour surface were also calculated using the statistical programs Excel and Statistic 7.0.

3. Results and discussion

3.1. Experimental design by contour surface for ultrasound assisted extraction (UAE)

In this study, it was possible to evaluate the combined effect of frequency, temperature and extraction time on the content of total phenolics (TP) and total anthocyanins (TA) of grape peel during UAE using water as a solvent according the ANOVA table (Table 2), being that the TA values ranged from 3.11 to 3.90 mg of cyanidin-3-O-glucoside/g of peel and TP ranged from 22.30 to 25.03 mg of GAE/g of peel.

Regarding F-test, the low probability value (p-value = 0.0036 (TA) and 0.0028 (TP)) demonstrates the statistical significance of the regression model to represent the relationship between experimental data

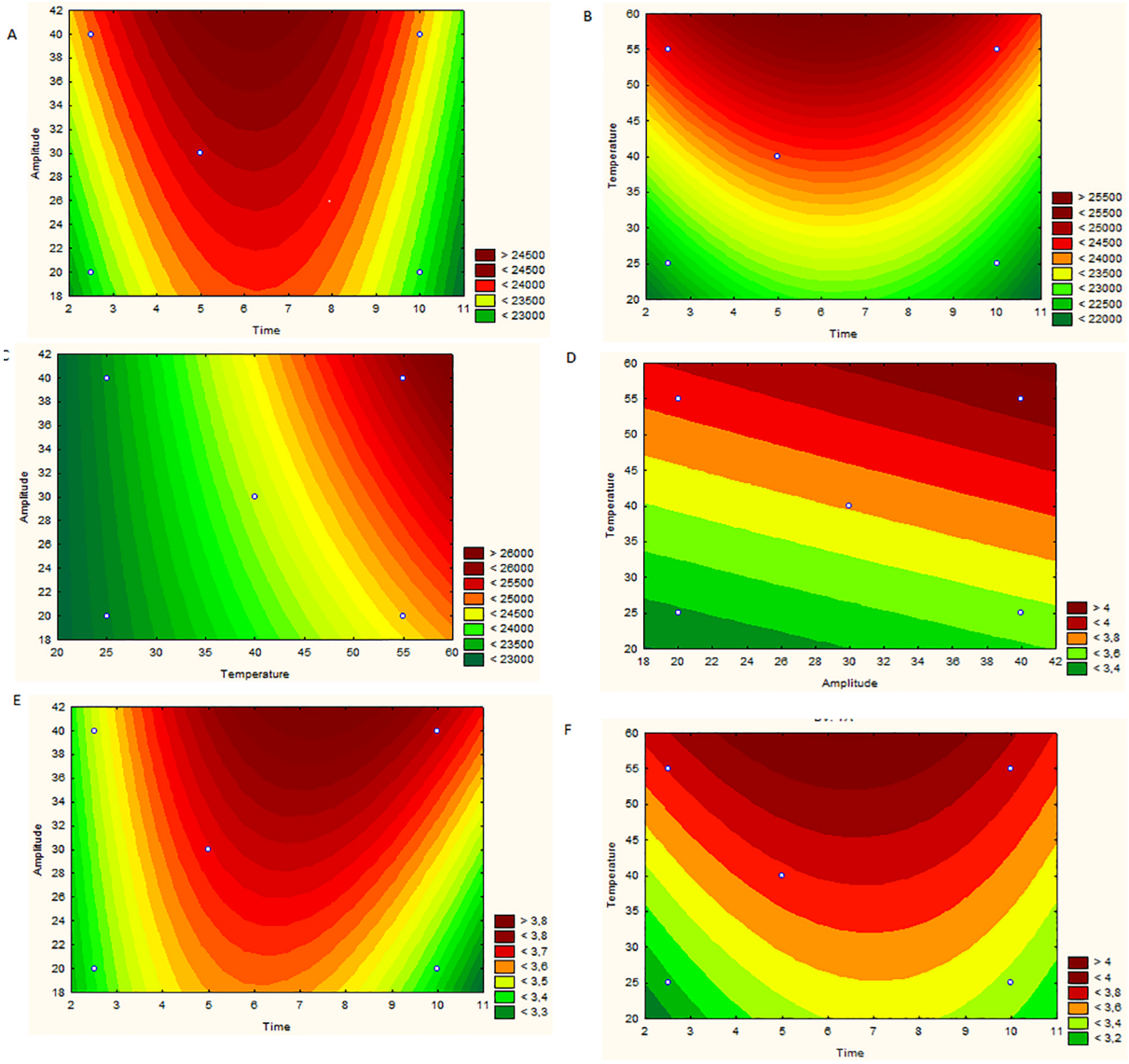

Fig. 1. Contour surface to interactions between three independent variables (time, frequency and temperature) in the extraction of total phenolics (TP, mg of GAE/g, A - C) and total anthocyanins (TA, mg of Cy-3-glu/g, D - F).

and the variables, so the model used was adequate. Furthermore, model quality and its overall predictive capability can be evaluated by the coefficient of determination (R^2) and adjusted R^2, for example, in this study, 91.20% of the variation in TA is connected to the 3 independent variables (even lack of fit was not significant), hence only 8.80% of the total variations are not explained by the model. Also for TP the coefficient of determination demonstrates a good agreement between the experimental and predicted values.

To relate the effects of the three independent variables on the extraction of phenolic compounds and total anthocyanins mediated by ultrasound, the contour surface was obtained. Fig. 1(A, B, C) shows the results for phenolic extraction and (D, E, F) the results of the extraction of total anthocyanins, in both cases the highest values obtained were in the region close to 55 °C, with frequency of 40% and 6 min.

In a study with grape fruit (skin, pulp and seeds) the authors also reported the 6 min time as the best time to recover phenolic compounds and total anthocyanins, in which the efficiency of ultrasound extraction can be explained by the fact that sonication simultaneously improves the hydration and fragmentation process, facilitating mass transfer of solutes to the extraction solvent (Carrera, Ruiz-Rodríguez, Palma, & Barroso, 2012).

Throughout the experiment, the extraction was increased with frequency, mainly from 20 to 40% and with the temperature from 25 to 55 °C. However, high temperatures (greater than 80 °C) can cause degradation reactions due to oxidation of phenolic compounds. Therefore, with high temperatures, it is possible to achieve greater compound recovery, but also higher rates of degradation of phenolic compounds (Carrera et al., 2012).

3.2. Extract characterization, antioxidant capacity and identification by HPLC-DAD for conventional (CE) and ultrasound assisted extraction (UAE)

The extracts had a total soluble solids content of 0.3 ° brix for CE and 0.5 ° brix for UAE. The pH of the extracts ranged from 4.33 (CE) to 4.38 (UAE). Regarding the color of the extracts, the luminosity (L) was 37.89 (UAE) and 45.87 (CE). The tendency towards red (a*) was 24.34 for UAE and 16.93 for CE, this fact can be correlated to the content of anthocyanins (Table 3) in which there was a difference between the extracts, being the highest value found for UAE (25% greater than CE), the tendency for yellow (b*) was 5.77 for UAE and 4.57 for CE. The conventional extraction presented a clearer sample (L = 45.87), on the other hand the ultrasound extraction produced a reddish extract and

Table 3
Content of phenolics, flavonoids, total anthocyanins, antioxidant activity and quantified individual phenolic compounds from extractions (UAE and CE) analyzed by reverse phase HPLC-DAD of BRS Violet grape peel.

	ABS (nm)	TR (min.)	UAE	CE
TP (mg of GAE/g)			$24.63^{a} \pm 0.28$	$21.87^{b} \pm 0.18$
TA (mg of cy-3-O- glc/g)			$3.74^{a} \pm 0.02$	$2.79^{b} \pm 0.01$
TF (μg of EC/mg)			$95.73^{a} \pm 0.46$	$62.93^{b} \pm 2.54$
DPPH (μM ET/mg)			$0.23^{a} \pm 0.02$	$0.12^{b} \pm 0.01$
ABTS (μM ET/mg)			$305.73^{a} \pm 4.00$	$217.73^{b} \pm 6.11$
FRAP (μM ET/mg)			$77.97^{a} \pm 0.71$	$60.97^{b} \pm 0.24$
Rutin (r2 > 0.9987)	265	9.30	$1.7^{a} \pm 0.14$	$1.2^{b} \pm 0.00$
Quercetin (r2 > 0.9999)	350	14.30	$1.7^{a} \pm 0.14$	$1.2^{b} \pm 0.00$
p-cumaric acid (r2 > 0.9999)	310	10.60	$0.4^{a} \pm 0.00$	$0.2^{b} \pm 0.00$
Cyanidin Chloride (r2 > 0.9959)	520	7.99	$11.7^{a} \pm 0.14$	$8.9^{b} \pm 0.14$
Myricetin (r2 > 0.9999)	370	12.26	$1.2^{a} \pm 0.00$	$1.0^{b} \pm 0.00$
Gallic acid (r2 > 0.9998)	275	4.56	$1.9^{a} \pm 0.14$	$1.6^{a} \pm 0.00$

Data are expressed as mean ± standard deviation. Different letters in the same line indicate a significant difference ($p \leq 0.05$). TP = total phenolic compounds; TA = total monomeric anthocyanins; TF = total flavonoids. GAE = gallic acid equivalent, EC = catechin equivalent, ET = Trolox equivalent.

consequently darker indicating better compounds extraction, we can correlated this with the anthocyanins values UAE 1.35 higher than CE (Table 3).

The dosage results and antioxidant capacity obtained for UAE were statistically superior when compared to CE (Table 3), corroborating with data from the literature (Belwal, Dhyani, Bhatt, Rawal, & Pande, 2016; Das, Goud, & Das, 2017). Thus, for the analysis of antioxidant (DPPH, ABTS and FRAP) there was a significant difference between UAE and CE.

For the dosage of bioactive compounds there were differences between the three analyzes performed (TP, TA and TF) demonstrating that the use of ultrasound combined with temperature of 55 °C (thermosonication) was significantly better, presenting higher values when compared to conventional extraction, on average 11% for TP, 25% for TA and 34% for TF (Table 3).

The total phenolic content in the extracts obtained with and without ultrasound was higher than that obtained by another study, that used aqueous extraction and ultrasound bath (5.37 to 31.87 mg of gallic acid/100 g of fresh grape pomace) (González-Centeno et al., 2014). And lower than those obtained by using extraction by mechanical stirring using 80% ethanolic solution (48.6 mg GAE/g of grape peel) (Caldas et al., 2018). Probably this difference is caused by the use of ethanol and agitation in the extraction process, in which this work used a more ecofriendly extraction with water as solvent.

The anthocyanin content extracted by ultrasound (3.74 mg of cyanidin-3-O-glucoside/g of grape peel) was close to that obtained with the same variety BRS Violet (393 mg/kg of peel, as equivalent to malvidin 3,5-diglucoside which correspond to 3.78 mg of cyanidin-3-O-glucoside/g of peel (Rebello et al., 2013). But it showed higher values when compared with other non-wine grape cultivars such as Bordeaux (*V. labrusca)* 1.31 mg of cyanidin-3-O-glucoside/g of peel (Lago-Vanzela, Da-Silva, Gomes, García-Romero, & Hermosín-Gutiérrez, 2011), Fig Leaf (*V. labrusca*) and Niágara Rosada (*V. labrusca*), 0.14 and 3.63 mg/g of cyanidin-3-O-glucoside equivalents respectively (Abe, Da Mota, Lajolo, & Genovese, 2007). Although when compared with other hybrid grape varieties, such as Colobel and Lomanto, they have a lower anthocyanin content ranging from 4.21 to 6.03 mg anthocyanin/g of grape) (Mazza & Francis, 1995).

The antioxidant capacity of grape peel extracts, determined as the capacity to eliminate DPPH radicals, ranged from 0.23 to 0.12 μmol TE/mg dw, (Table 3), with UAE being the process with the highest antioxidant capacity. Positive correlations were observed between the total phenolic content and the ability to eliminate DPPH radical in the samples (r = 0.963). A positive correlation was also observed with the total content of flavonoids and anthocyanin (r = 0.940, 0.967, respectively) between the antioxidant by the DPPH method. The positive correlation between phenolics, flavonoids and antioxidant capacity in grape peel has also been reported by (Rockenbach et al., 2011).

The antioxidant capacity of grape peel extracts, determined as ferric reducing antioxidant power (FRAP), ranged from 77.97 to 60.97 μmol of Fe^{2+}/mg dw, (Table 3). It obtained a high correlation between FRAP antioxidant analysis between phenolic content, anthocyanin and total flavonoids (r = 0.976, 0.972 and 0.990, respectively).

Sequestering activity of the ABTS radical determined in the extracts, varied from 305.73 to 217.73 μM ET/mg dw (Table 3), also demonstrated a good correlation in relation to total phenolics, anthocyanin and total flavonoids (r = 0.989, 0.986 and 0.997, respectively).

The results of antioxidants by the DPPH, FRAP and ABTS methods, demonstrated that the extract obtained by ultrasound showed greater antioxidant capacity when compared to the extract obtained by conventional extraction.

There was a significant difference (Table 3) between the two extraction methods CE and UAE for the concentrations of anthocyanins (cyanidin chloride, responsible for violet color) and between the other identified compounds (rutin, quercetin, p-coumaric acid and myricetin).

There was no significant difference for the gallic acid compound, demonstrating that the use of ultrasound was significantly better, presenting higher values when compared to conventional extraction, on average 29.41% for rutin and quercetin, 50% for p-coumaric acid, 23.93% for cyanidin chloride, 16.67% for myricetin and 15.79% for gallic acid.

To the best of our knowledge, no work was found that detected cyanidin chloride in the BRS Violet grape variety. Another study has identified this compound in cherry and pomegranate extracts ranging from 15.6 to 11.0 μg/g of sample, respectively (Yari & Rashnoo, 2017).

3.3. Microcapsule light stability

The Encapsulation efficiency (EE) was 62.52%. Microcapsule stability was evaluated through the degradation of bioactive compounds (TP and TA) during 28 days of storage (each 7 days) in the presence or absence of light.

For degradation rate constants (k) of microcapsules exposed to light or absence of light, it is observed that there was a reduction of this parameter for both TP and TA, when the samples were stored in the absence of light. The degradation of total anthocyanins in the first 21 days was not significant in the presence of light, however for day 28 there was a difference. In the absence of light, there was no difference between the 28 days analyzed (Fig. 2A). For the degradation of total phenolics, there was a significant difference for the initial and final dosage on the days analyzed with and without light (Fig. 2B).

The sample kept in the dark showed less degradation than the corresponding samples exposed to light. In the presence of light, the

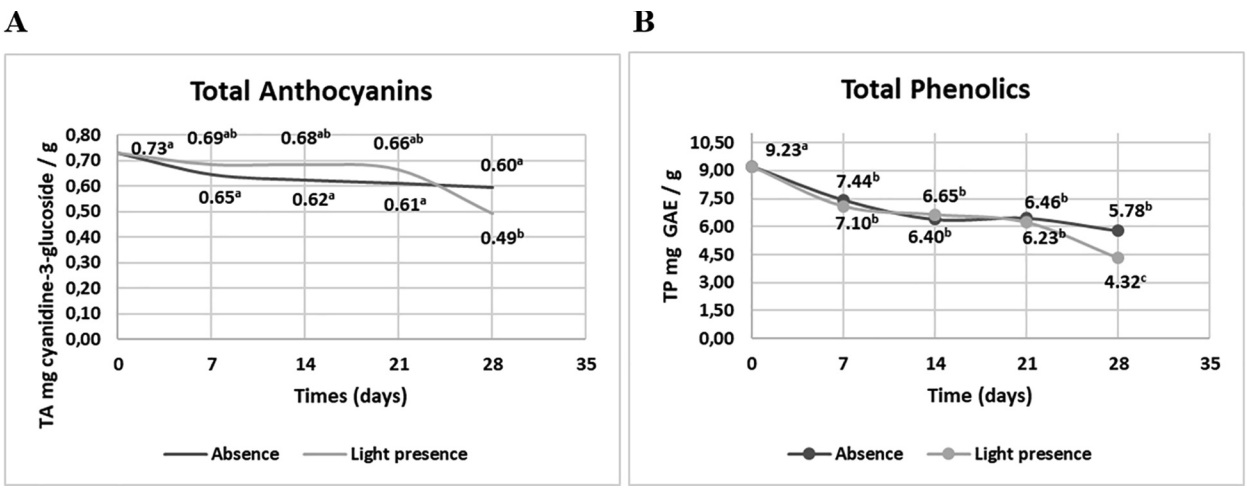

Fig. 2. Degradation of total anthocyanin (A) and total phenolics (B) in the microcapsules during 28 days in the presence or absence of light. Different letters indicate a significant difference in relation to days ($p \leq 0.05$).

Table 4
Kinetic of degradation rate constant (K) and microcapsule half-life period in the presence or absence of light (at 25 °C).

	TA		TP	
	k	$t_{(1/2)}$ (days)	k	$t_{(1/2)}$ (days)
Presence of light	0.0118	59	0.0282	25
Absence of light	0.0066	104	0.0175	40

degradation of anthocyanin and total phenolics (32.80 and 60.26%, respectively) were greater in relation to the absence of light (18.51 and 43.59%, respectively) corroborating with literature that mentions that anthocyanin degrades in the presence of light (Giusti & Wallace, 2009). The effect of phenolic degradation in the absence or presence of light (43.59 and 60.26%, respectively) was greater compared to the same conditions in the analysis of anthocyanins, light exposure and control (32.80 and 18.51%, respectively).

Encapsulation presented a first order degradation curve for all samples (Table 4). First-order kinetics for anthocyanin degradation was reported in jabuticaba peel (Santos, Albarelli, Beppu, & Meireles, 2013) and in concord grape, using a temperature of 30 °C in the presence of light for 15 days (Baublis, Spomer, & Berber-Jiménez, 1994). As light, temperature, pH, oxygen, structure and chemical composition of anthocyanins can influence differently on degradation time (Patras, Brunton, O'Donnell, & Tiwari, 2010).

The half-life values of the encapsulated content of total monomeric and phenolic anthocyanins decreased from 104 to 59 and from 40 to 25 days, respectively when in the presence of light, indicating a longer half-life in the absence of light.

4. Conclusion

The aqueous extracts of grape peel obtained by ultrasound assisted presented higher levels of antioxidant capacity (1.4 times), anthocyanins (1.3 times) and total phenolic (1.2 times) by comparing with the conventional one. The increase in the compounds (29% for rutin and quercetin, and 24% for cyanidin chloride) also stands out, indicating that the ultrasound extraction of bioactive compounds from grape pomace is feasible and ecofriendly.

The extract encapsulated in alginate-Ca^{2+} when stored in the light absence showed less degradation than in the presence being 1.8 and 1.6 times more stable for TA and TP, respectively. Thus, these capsules are recommended for future food applications that need protection against light.

Finally, the studied grape (BRS Violet) pomace showed high concentration of phenolic compounds that could be reused by the food and pharmaceutical industries, both in the form of extract or microcapsules. Thus, it is important to emphasize that the application of clean technologies for the recovery of bioactive compounds shows great promise.

Declaration of Competing Interest

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

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ARTICLE 02

Bioactive compounds from BRS Violet grape pomace: New insights into extraction and microencapsulation, stability protection and food application

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HIGHLIGHTS

The effects of encapsulation on the levels of phenolic compounds from grape pomace extract were investigated.

MD/GX were used as encapsulating agents, ensuring a longer half-life.

The encapsulating matrix was important for the protection of bioactive substances.

Application in gelatin reinforced the importance of the microcapsule for color stability.

Refrigerated MD/GX microcapsules can be used as a natural dye for 30 days.

ABSTRACT

This work aimed to evaluate the encapsulation of grape pomace extract in a combination of maltodextrin and xanthan gum (MD/GX) by lyophilization, and to determine the protective effect of this microcapsule, on the phenolic compounds (TP) and anthocyanins (TA). Thus, the stability of the encapsulated content was determined for a period of 120 days, under different temperature conditions (4 and 25ºC) and in the presence or absence of light. Additionally, a gelatin application test was performed to investigate the effect of the microcapsule in color stability. When comparing the extract versus microcapsules, the microcapsules results were better both for TA (1.69 to 1.54-fold) and TP (3.06 to 1.74-fold), indicating longer half-life after encapsulation. The microcapsule application in gelatin demonstrated that the encapsulating matrix retained the color of the samples better for 30 days. Thus, the encapsulation method can be recommended to preserve the bioactive compounds as well as the coloration in food products.

KEY WORDS: freeze-drying, gelatin, bioactive compounds, maltodextrin, xanthan gum.

1. INTRODUCTION

Since studies have shown a relationship between free radicals and the development of some diseases linked to oxidative stress, compounds that are able to neutralize these particles have gained importance and have been more extensively studied for their application in human health (Karaman, Küskü, & Söylemezoğlu, 2021). In nature, some of these substances are found in vegetables, in which they are responsible for the adaptation in the environment. Among these phytochemicals, phenolic compounds are very important because of their antioxidant action. These compounds can be divided in two groups: flavonoids (anthocyanins, flavano-3-ols, condensed tannins and flavonols) and non-flavonoid compounds (phenolic acids and stilbenes).

A large amount and variety of phenolic compounds are found in grapes, especially the anthocyanins responsible for their color. As stated earlier, these substances determine antioxidant properties related to the prevention of cancer, neurodegenerative and cardiovascular diseases (Xia et al., 2014).

Given the concern related to health and food safety to encourage the use of products of natural origin instead of antioxidants of synthetic origin, the extraction of these substances from natural substrates is increasing. In the case of grapes, it is perfectly possible to extract the phenolic compounds from the pomace generated by the wine industry after pressing (Romanini et al., 2021). After extraction, these substances can be applied in food,

pharmaceutical and cosmetic industries (Jeandet, Clément, Tisserant, Crouzet, & Courot, 2016; Kammerer, Kammerer, Valet, & Carle, 2014).

Nowadays, the great challenge is to apply these phenolic compounds with processes that guarantee their properties for a reasonable amount of time after the extraction from the grape, for example, since these molecules are unstable when exposed in the long term as they easily undergo degradation, oxidation, epimerization and polymerization. The chemical structure of polyphenols responsible for the antioxidant action is the target of radiation, enzymes, variations in temperature, pH and oxygen (Arboleda Mejia et al., 2020; Galmarini et al., 2012).

In this context, the use of substances that can transport these bioactive compounds in order to provide protection until their final destination has been described in the literature, such as the encapsulation techniques by lyophilization and spray drying (Davidov-Pardo, Arozarena, & Marín-Arroyo, 2012; Wilkowska, Ambroziak, Czyzowska, & Adamiec, 2016). In this case, the core (phenolic compounds) is physically protected by an encapsulating material (Aizpurua-Olaizola et al., 2016; Mahdavee Khazaei, Jafari, Ghorbani, & Hemmati Kakhki, 2014).

Among the various microencapsulation agents, maltodextrins (MD) proved to be non-toxic sugar polymers, to have the solubility and to produce the viscosities expected for a good encapsulating agent, in addition to the low cost and the absence of influence in the taste and odor of the final product (Ferrari, Marconi Germer, Alvim, & de Aguirre, 2013; Fredes, Becerra, Parada, & Robert, 2018).

Another natural origin encapsulating agent studied is the high molecular weight anionic biopolymer of xanthan gum, which is colorless, odorless, tasteless and stable at higher temperatures and at pH variations (Jo, Bak, & Yoo, 2018).

In the present study, we obtained the aqueous extract rich in polyphenols from the pomace of grape juice industry of the cultivar BRS Violeta of intense purple color, encapsulated it in a mixture of maltodextrin and xanthan gum (9.5 : 0.5) and observed the stability of the encapsulated content for 120 days. Additionally, the application test in gelatin was carried out to monitor the effect of the microcapsule in maintaining the color of the extract. As far as we know, there are no records in the literature of similar studies, thus indicating the high degree of innovation of the present research.

2. MATERIAL AND METHODS

The residue used from the grape variety (BRS Violeta) came from the city of Marialva (PR, Brazil). The extract (E) was obtained by the ultrasound assisted method using water as solvent in the proportion of 1:200 (w / v, g / mL) with a total extraction volume of 200 mL for 6 min, at 55°C, in a frequency of 40%, performed as described in the study previously published by Romanini et al. (2021). Maltodextrin (DE 10) was supplied by Cargill (SP, Brazil) and xanthan gum was purchased from a local store in Maringá (PR, Brazil). All reagents used in the study were of analytical grade.

2.1. Extract encapsulation and encapsulation efficiency (EE)

The encapsulating agents were added at an agent:extract ratio of 1:1 (w/w), according to (Ferrari, Germer, Alvim, Vissotto, & de Aguirre, 2012). Maltodextrin and xanthan gum were added at concentrations of 99.5% and 0.5%, respectively, as described by (Antigo, Silva, Bergamasco, & Madrona, 2020; Rodrigues da Cruz, Andreotti Dagostin, Perussello, & Masson, 2019). The microcapsules containing the extract and the raw extract were frozen for 48 h at -18 °C and subsequently subjected to lyophilization (freeze L108, Liobras) for 2 days to ensure maximum drying.

The encapsulation efficiency was calculated according to (Selamat, Muhamad, & Sarmidi, 2009), using water as a solvent, according to Eq. (1):

(1) $EE(\%) = ((FT-FS)x100)/FT$

where FT and FS refer to the total phenolic compounds inside the microcapsules and on the surface, respectively.

2.2. Analysis of the bioactive compounds of the extracts

The total anthocyanins content (TA) was determined by the pH differential absorbance method, at 520 and 700 nm, and at pH 1.0 and pH 4.5 (Lee et al., 2005). The total anthocyanin content was expressed in mg of cyanidin-3-glucoside / g of grape pomace. The Folin-Ciocalteu method was used for the determination of total phenolic compounds (TP). The total amount of phenolic compounds was calculated with the calibration standard curve using gallic acid, and the results were expressed in mg of gallic acid equivalent (GAE) / g of grape pomace (Pierpoint, 2004; Singleton & Rossi, 1965).

2.3. Encapsulated content stability during storage

After lyophilization, the microcapsules containing the extract were weighed and then stored in transparent plastic packages in a chamber (BOD Incubator) at 25 ºC in the presence and absence of light, using two 20 W fluorescent lamps (presence of light) and a dark chamber (absence of light), and at 4 ºC, for 120 days. (Santos, Rodrigues, Da Costa, Bergamasco, & Madrona, 2017). The parameters of monomeric total anthocyanins, total phenolics and color were monitored. The lyophilized extract was used as control.

The following samples and storage conditions were obtained and evaluated: Cap4 = encapsulation at 4ºC; Ext4 = extract at 4°C; Cap25 = encapsulation at 25ºC without light; Ext25 = extract at 25°C without light; CapLight = encapsulation in the presence of light; ExtLight = extract in the presence of light

Experimental data for anthocyanin and phenolic values were analyzed by first-order reaction degradation kinetics and half-life (t1 / 2) methods (Stamp & Labuza, 1983), according to Eq. (2) and (3):

(2) $\ln (A_t / A_0) = - k \times t$

(3) $t_{1/2} = \ln 2 / k$

where *t* is time (day), A is the level of TA or TP, A_0 is the initial TA or TP level at time zero, and k is the rate constant (day^{-1}).

The percentage of total loss of anthocyanins and phenolic compounds during the storage period was determined by the ratio between the concentration on the last day of storage (d120) and the initial concentration (d0), as mentioned in (Souza et al., 2014).

2.4. Gelatin application

The microcapsules, the extract and an artificial dye (Gran chef, easy gel – wine colored, Brazil) were applied in a colorless powdered gelatin (Dr. Oetker®). The preparation of pure gelatin followed the manufacturer's recommendations. Then, the powder samples were added directly to the gelatin solutions and manually shaken until complete homogenization. The proportion of microcapsule, extract and dye to dissolved gelatin was 1.8% to 98.2%, as described by (Rodrigues, Januário, dos Santos, Bergamasco, & Madrona, 2018). Subsequently, the samples were poured into Petri dishes and stored randomly at 4 °C until analysis, simulating consumption conditions. The gelatin samples were refrigerated (4 °C) for 30 days, and the color parameters were evaluated at 0, 15 and 30 days.

2.5. Color parameters

The color of the samples was evaluated with a portable colorimeter CR-400 (Minolta Ltd., Osaka, Japan), with illuminant D65 - angle of 10°. The luminosity parameters L* black to white, a* green to red and b* blue to yellow were verified. The color difference (ΔE) was calculated according to Eq. (4), following the method suggested by (da Silva et al., 2017):

$$(4)\ \Delta E = \sqrt{(L^{*}_{dayX} - L^{*}_{day0})^{2} + (a^{*}_{dayX} - a^{*}_{day0})^{2} + (b^{*}_{dayX} - b^{*}_{day0})^{2}}$$

where L, a * e b * - color parameters; day0 - initial; dayX = 15 and 30 days of storage, respectively.

2.6. Data analysis

All readings were performed in triplicate and submitted to analysis of variance and Tukey's test for the minimum significant difference ($p < 0.05$) between the mean values using Excel as statistical software.

3. RESULTS AND DISCUSSION

3.1. Microcapsule stability and encapsulation efficiency (EE)

EE for MD/GX was 68.10%. These results were better than those observed by Antigo et al., (2020), that obtained EE of 43.45% using beet extract and encapsulation by lyophilization with MD/GX. The study of Akhavan Mahdavi, Jafari, Assadpoor, & Dehnad (2016), using barberry extract and encapsulation by spray drying with three different wall materials, that is, combining maltodextrin, gum arabic, maltodextrin and gelatin, obtained higher values (ranging from 89.06 to 96.21%) when compared to the ones observed in the present work. These data demonstrate that EE may be related to the composition of the encapsulating agent, that confers different physicochemical properties for film formation, as well as the drying method (lyophilization or spray drying, for example) (Yinbin et al., 2018).

The stability of total anthocyanins and phenolics in the powdered freeze-dried microcapsules and control samples (extract) were evaluated under different storage conditions (Table 1).

Table 1: Total phenolic compounds (TP), total monomeric anthocyanins (TA) and delta E during 120 days of storage in the presence / absence of light, and at 4 and 25 °C (in the dark)

Stability (days)	Cap4	Ext4	Cap25	Ext25	CapLight	ExtLight
	TP (mg GAE/g)					
0	14.73^{a}±0.21	46.17^{a}±0.04	14.73^{a}±0.21	46.17^{a}±0.04	14.73^{a}±0.21	46.17^{a}±0.04
30	14.52^{a}±0.11	44.33^{b}±0.13	14.68^{a}±0.07	46.51^{a}±0.59	14.7^{a}±0.12	45.36^{a}±0.42
60	14.56^{a}±0.10	43.82^{b}±0.31	14.64^{a}±0.03	42.46^{b}±0.10	14.73^{a}±0.04	43.71^{a}±0.47
90	14.41^{a}±0.16	39.41^{c}±0.22	14.34^{a}±0.18	40.35^{c}±0.50	14.33^{a}±0.17	38.19^{b}±0.24
120	13.30^{b}±0.07	35.42^{d}±0.38	12.95^{b}±0.13	36.30^{d}±0.74	12.09^{b}±0.08	34.86^{c}±0.46
Loss (%)	9.69	23.27	12.06	21.38	17.92	24.49
	TA (mg cyanidine-3-glucoside/g)					
	Cap4	**Ext4**	**Cap25**	**Ext25**	**CapLight**	**ExtLight**
0	2.16^{a}±0.02	5.30^{a}±0.04	2.16^{a}±0.02	5.30^{a}±0.04	2.16^{a}±0.02	5.30^{a}±0.04
30	2.02^{b}±0.04	5.21^{a}±0.11	2.05^{ab}±0.15	5.08^{ab}±0.11	2.04^{ab}±0.04	5.19^{a}±0.11
60	2.00^{b}±0.03	5.15^{a}±0.03	2.01^{ab}±0.09	5.03^{ab}±0.02	2.04^{ab}±0.03	4.91^{b}±0.03
90	1.97^{b}±0.01	4.88^{b}±0.11	2.00^{ab}±0.03	4.82^{c}±0.06	1.98^{ab}±0.12	4.67^{c}±0.10
120	1.88^{c}±0.08	4.27^{c}±0.03	1.88^{b}±0.02	4.32^{d}±0.10	1.85^{b}±0.18	4.21^{d}±0.03
Loss (%)	12.78	19.47	12.78	18.53	14.17	20.61
	Delta E					
	Cap4	**Ext4**	**Cap25**	**Ext25**	**CapLight**	**ExtLight**
120	4	11	6	10	8	12

Means values ± standard deviation (n = 3 repetitions). The time difference was evaluated by % of loss. Different letters in the same column indicate a significant difference in terms of days ($p \leq 0.05$).
d = days; GAE = Gallic Acid Equivalent; Cap4 = encapsulation at 4 ºC; Ext4 = extract at 4 ºC; Cap25 = encapsulation at 25 ºC in the dark; Ext25 = extract at 25ºC in the dark; CapLight = encapsulation in the presence of light; ExtLight = extract in the presence of light.

Overall, the encapsulated content stability assay showed higher degradation for the extract (control) and when exposed to light and temperature, both for TP and TA as expected and also demonstrated by Rodrigues et al., (2018) (Table 1). Some authors also mention that the use of encapsulating agents in adequate proportions can guarantee the protection of the active substance and the preservation of its functionality (Rodrigues da Cruz et al., 2019).

The data indicate that there is a protective effect of the microcapsule on TA and TP comparing to the non-encapsulated extract (control), which showed a considerable loss of TA and TP in 120 days, demonstrating even higher degradation in the presence of light: 20.61% and 24.49% for TA and TP, respectively, while the loss when encapsulation was performed was 14.17% and 17.92% for TA and TP. In addition, the time effect was essential for the degradation of both TA and TP. As shown in Table 1, there was a significant difference between all samples when comparing the beginning (days) and the end of the analysis (120 days).

When verifying the effect of temperature for TA (4 °C x 25 °C), the encapsulated content was not altered in 120 days, apparently. For TP there was a loss of 9.69% at 4ºC and 12.06 at 25ºC. Idham, Muhamad, & Sarmidi (2012), using spray drying and different encapsulating matrices for anthocyanins, observed that the storage time and the encapsulating agent significantly affected the color change, while the storage temperature did not prove to be an influencing factor. Differently from this report, in another research, using maltodextrin and dextrose as encapsulating agents, the anthocyanin content of the encapsulated black carrot extract decreased by 11% and 33% over a period of 64 days of storage at 4 and 25° C, respectively (Ersus & Yurdagel, 2007).

Ravichandran et al., (2014) extracted betalains from beetroot and encapsulated them in different matrices (maltodextrin, guar gum, gum arabic, pectin and xanthan gum) in different concentrations and the samples were dried by lyophilization or spray drying. The lyophilized MD/GX sample showed better stability between the conditions analyzed, corroborating the results obtained in the present study.

When comparing the presence and absence of light during storage at 25 ºC, concentrations decreased in both cases for TA (14.17% with light and 12.78% without light) and TP (17.92% with light and 12.06 without light). TP was more degraded than TA, indicating that the presence of light influenced the degradation of this group of compounds, as already reported elsewhere (Antigo et al., 2020; Ersus & Yurdagel, 2007; Romanini et al., 2021). Burin, Rossa, Ferreira-Lima, Hillmann, & Boirdignon-Luiz (2011) evaluated the influence of temperature and exposure to light on the stability of anthocyanins from Cabernet Sauvignon, and their results also showed that light is an important accelerating factor in the degradation of anthocyanins.

At 120 days of storage, the smallest color change observed was for Cap4 ($\Delta E < 5$). The extracts, regardless of storage conditions (temperature, with and without light) showed the greatest color variations ($\Delta E > 5$). The protective effect of MD/GX was also compared to the extract by Rodrigues et al., (2018), whose jabuticaba extract, stored for 36 days under the same conditions evaluated in the present study, was more degraded than the encapsulated samples. According to Romero-González, Shun Ah-Hen, Lemus-Mondaca, & Muñoz-Fariña (2020) using three polysaccharides (maltodextrin, gum arabic and inulin) as encapsulating agents and lyophilization for drying, a value of $\Delta E < 27$ was obtained, even after 60 days of storage at 25 °C, attributing this result of color stability to low water activity.

Due to the best results presented by Cap4 for color and TA and TP contents, this sample was applied in a food matrix (gelatin) to evaluate color stability during storage for 30 days. Gelatin was chosen because it can be stored in the dark at 4 °C.

Considering that the focus of the present research is to evaluate the encapsulated content, Figure 1 presents the specific data during storage. When evaluating the effect of the three parameters (presence and absence of light at 25 °C and absence of light at 4 ºC) on the stability of the encapsulated content after 120 days, a significant difference was observed in the TP content, with higher degradation in the presence of light. The evaluation of the stability of anthocyanins subjected to a temperature of 25 °C, in the presence and absence of light, and to a temperature of 4 °C showed that the samples were not significantly different. However, TA contents were significantly different after 120 days, approximately from 13 to 14% loss in 120 days. The encapsulation process can improve the stability of anthocyanins against light and temperature (Figure 1).

A B

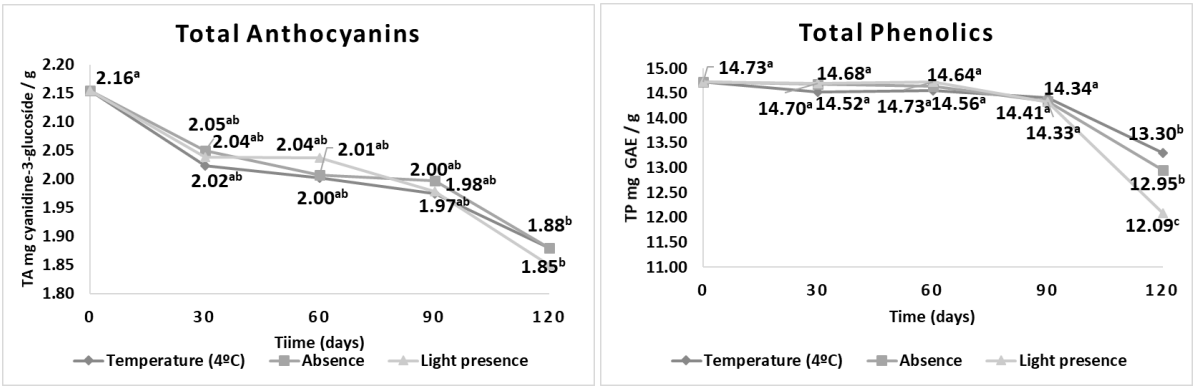

Figure 1: Degradation of total anthocyanin (A) and phenolics (B) of the microcapsules during 120 days stability at low temperature (4 ºC) and at room temperature (25 °C) in absence or presence of light. Different letters indicate a significant difference when different times are compared ($p \leq 0.05$).

Table 2 demonstrates the degradation kinetics and half-life of the encapsulated content evaluated during the storage period (120 days).

Table 2: Kinetics of the degradation rate constant (K) and half-life of the encapsulated content and of the extract during storage in the presence/absence of light, and at 4 and 25°C (in the dark).

	TA		**TP**	
	K	**$t_{(1/2)}$ (days)**	**k**	**$t_{(1/2)}$ (days)**
Cap4	0.0010	698	0.0007	984
Ext4	0.0017	417	0.0022	321
Cap25	0.0010	694	0.0009	741
Ext25	0.0015	450	0.0021	334
CapLight	0.0011	621	0.0014	495
ExtLight	0.0019	367	0.0024	283

Cap4 = microcapsules at 4 ºC in the dark; Ext4 = extract at 4 ºC, in the dark; Cap25 = microcapsules at 25 ºC, in the dark; Ext25 = extract at 25 ºC, in the dark; CapLight = microcapsules at 25 °C, in the presence of light; ExtLight = extract at 25 °C, in the presence of light.

The encapsulated content showed a first-order degradation curve for all samples (Table 2) corroborating previous reports. For example, first-order kinetics for degradation of anthocyanins extracted from blueberries (Righi da Rosa et al., 2019), from hibiscus, with temperatures from 5 ºC t 30 ºC for 50 days (de Moura, Berling, Germer, Alvim, & Hubinger, 2018), and from wine residues with long-term stability of 6 months in the absence and presence of light (Aizpurua-Olaizola et al., 2016).

When evaluating the encapsulated product *versus* the extract (control) at both temperatures, there was a higher loss in the TP content present in the extract. This is evidenced by the t(1/2) of 984 and 221 days at 4ºC for the microcapsules and the extract, respectively. When evaluating the effect of light on the microcapsules and the extract, there were similar losses for TA (t(1/2) of 621 and 367 days) and for TP (495 and 283 days, respectively, for the microcapsules and the extract). In a general evaluation of the analyzed conditions, both anthocyanin and phenolic compounds presented higher degradation in the extract, inferring that the microcapsule provides protection to the bioactive substances.

3.2. Microcapsules application in gelatin

Colorless gelatin was used as the base in which the dye, the extract and the microcapsules were applied (Figure 2) to study the variation in the color of the sample during storage at 4 ºC for 30 days.

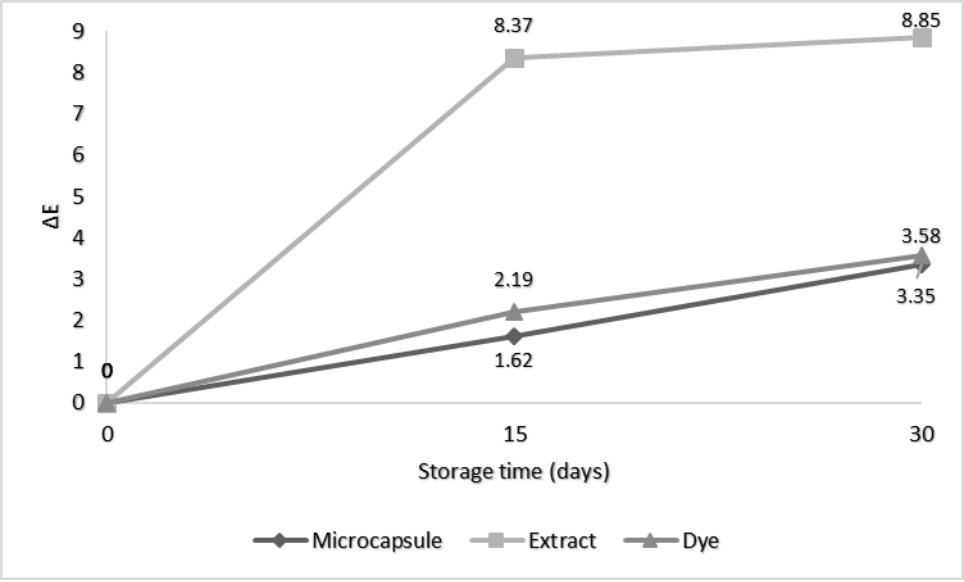

Figure 2: Variation in the color of the gelatin (ΔE) during the 30 days of storage at 4 °C.

The use of MD/GX provided a significant increase in luminosity (L*), from 37,65 (raw extract) to 44,00 (encapsulated extract). That is, the dry microcapsules are brighter than

the dry extract, probably due to the white coloration of maltodextrin, which has already been reported in other studies (Kha, Nguyen, & Roach, 2010; Santos et al., 2017).

Color differences from 0 to 1.5 can be considered small and almost identical for visual observation. They can be distinguished in the range of 1.5 to 5, while color differences are only evident for a ΔE higher than 5 (Obón, Castellar, Alacid, & Fernández-López, 2009).

The total color of the microcapsules and the dye were similar in the period of 30 days (ΔE < 5). The extract showed color change (ΔE > 5) during storage (days 15 and 30), 2.47 times higher than the dye and 2.65 higher than the microcapsules in 30 days, indicating the fragility of this compound, according to the previous analyzes (Tables 1 and 2), even when added to a complex food matrix (such as gelatin). Rodrigues et al., (2018) encapsulated jabuticaba bark extract in maltodextrin and xanthan gum by lyophilization and applied in a gelatin matrix to evaluate the color stability for 60 days at 25 ºC. The extract alone presented the highest color variation (ΔE > 5), while the incorporation of microcapsules into gelatin had the smallest change (ΔE < 5). Moser et al., (2017) evaluated the color stability of BRS Violeta red grape juice powder encapsulated (maltodextrin and proteins) by spray drying during storage for 150 days at 35 ºC and, regardless of the type of carrier agent used, temperature and storage time did not influence the color of the corresponding reconstituted grape juice. Differences (ΔE) were lower than 1.8 for any time/temperature combination. In another research, a powdered food coloring was obtained by spray drying of *Opuntia stricta* fruit juices using glucose syrup as a drying aid. The dye was tested in two food models, yogurt and soft drinks, stored under refrigeration (4 °C) for one month, and there was no evident change in color (ΔE < 5) (Obón et al., 2009).

These results strongly suggest that the combination of MD/GX as an encapsulating agent provides protection to colored substances from natural extracts and that it can be used as an alternative to artificial colorings in food products, such as gelatin, when stored for up to 30 days under refrigeration.

4. CONCLUSION

The use of an encapsulating matrix of maltodextrin and xanthan gum proved to be effective in maintaining the stability of phenolic compounds and total anthocyanins of the encapsulated grape pomace extract during storage time (120 days) and also under different conditions (temperature, absence and presence of light), increasing its half-life comparing to the isolated extract.

To evaluate changes in color, the MD/GX encapsulation was applied to a food matrix (gelatin). After 30 days of refrigerated storage, the initial color of the encapsulated content was preserved ($\Delta E < 5$).

These results are important to expand the use of encapsulated natural dyes, enabling the application of these compounds in the food, pharmaceutical and cosmetic industries.

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