LWT - Food Science and Technology 73 (2016) 551–556

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Effects of encapsulating agents on anthocyanin retention in pomegranate powder obtained by the spray drying process

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ARTICLE INFO

Article history:
Received 14 April 2016
Received in revised form
27 June 2016
Accepted 28 June 2016
Available online 30 June 2016

Keywords:
Punica granatum L.
Microencapsulating
Gum arabic
Modified starch
Maltodextrin

ABSTRACT

The present work evaluates the effect of different encapsulating agents (gum Arabic, modified starch Capsul™ and maltodextrin DE 5) on anthocyanin retention in microcapsules produced by spray drying of raw pomegranate juice. A high concentration of anthocyanins is required in order to obtain a product that can be used as a functional ingredient. An accurate quantitative and qualitative analysis of the anthocyanins in the pomegranate juice and in the microcapsules was carried out by High Performance Liquid Chromatography (HPLC). Using the simplex-centroid experimental design employed here, the gum Arabic and Capsul™ (1:1) mixture, obtained a high retention (up to 70%) of total monomeric anthocyanins (delphinidin, cyanidin and pelargonidin 3-*O*-glucosides and 3,5-*O*-diglucosides). Pomegranate powder was stored at 25 °C for 3 months in laminated packaging and about 90% of the monomeric anthocyanins were preserved. In order to evaluate this product as a natural colorant for food, the anthocyanins in the microcapsules were also evaluated for color analysis. The results indicated a color hue with a predominance of red.

1. Introduction

Pomegranate fruit contains high levels of bioactive components, such as hydrolysable tannins, and its juice is a source of anthocyanins (delphinidin, cyanidin and pelargonidin 3-*O*-glucosides and 3,5-*O*-diglucosides) (Lansky & Newmann, 2007; Robert et al., 2010). All pomegranate flavonoids, including anthocyanins, show antioxidant activity with indirect inhibition of inflammatory markers (Lansky & Newmann, 2007). Studies in human and murine models have also shown the antiatherogenic, antihypertensive and anti-inflammatory effects of pomegranate juice (Gil et al., 2000).

The anthocyanins in this fruit are responsible for the intense and attractive red color of the juice and other pomegranate products (Jaiswal, DerMarderosian, & Porter, 2010). There is an increased demand for food colorants from natural sources to substitute synthetic dyes (Nayak & Rastogi, 2010). Consequently, the use of pomegranate juice and, in particular, its derivatives as food colorants has promoted the cultivation of this fruit (Al-Maiman & Ahmad, 2002).

Although the phenolic compounds present in pomegranate fruit, such as anthocyanins, have been used as active ingredients in the food industry, they are known to degrade when exposed to adverse environmental conditions, such as oxygen or light. Therefore, stability is a crucial factor to consider if natural pigments are to be used as food colorants, especially when anthocyanin based colorants are compared with synthetic ones (Robert & Fredes, 2015). The stabilization of such compounds for industrial purposes can be improved by microencapsulation (Favaro-Trindade, Pinho, & Rocha, 2008; Nayak & Rastogi, 2010). Robert et al. (2010) observed that the degradation of polyphenols and anthocyanins

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http://dx.doi.org/10.1016/j.lwt.2016.06.059
0023-6438/

in fresh pomegranate juice was faster than in microencapsulated powder, showing the importance of the encapsulating material in preserving the bioactive compounds of this fruit.

The wall material for a microcapsule must have good emulsifying properties, low viscosity at high-solids concentrations, low hygroscopicity and low cost. Most often, the same encapsulating material does not have all of these properties, and so different drying agents are used to promote the desired properties (Shahidi & Han, 1993).

The most common carrier agents used in fruit juices are maltodextrins and gum arabic (Cano-Chauca, Stringheta, Ramos, & Cal-Vidal, 2005; Gabas, Telis, Sobral, Telis-Romero, & Charve, 2007). Maltodextrin is a hydrolyzed starch widely used in the food industry due to its properties of aiding the dispersion of a product and preventing its agglomeration in pipelines (Alexander, 1992; Jafari, Assadpoor, He, & Bhandari, 2008). Furthermore, it offers advantages such as low cost, aroma and mild flavor and low viscosity at high-solids concentrations; however it has a low emulsifying capacity. Gum arabic is a well-known encapsulating agent that increases the stability of emulsions with good volatile retention (Trubiani & Lacourse, 1988). However, there are limitations to its use in Brazil due to the high cost and limited supply. In some cases, modified starches are used in formulations to partially or completely replace the natural gums. Modified starch is a food additive prepared by treating starch or starch granules with the incorporation of lipophilic groups making it easier to dissolve powder products (Miyazaki, Hung, Maeda, & Morita, 2006). The purpose of this modification is to enhance its properties, such as: to improve its water holding capacity, to improve its heat resistant behavior, to reinforce its binding, to minimize syneresis of starch and improve thickening. The modified starch named Capsul ™ was developed specifically for encapsulation processes due to its excellent film-forming properties and low viscosity in high soluble solids concentrations.

The objective of this study was evaluate the influence of carriers like gum Arabic, modified starch Capsul™ and maltodextrin DE 5 to maximize the anthocyanin retention in the powder produced from pomegranate juice by spray drying, in order to find a formulation that would provide the best protection of these functional compounds.

2. Material and methods

2.1. Chemicals

HPLC grade acetonitrile, formic acid 96% and methanol were purchased from Tedia (Ohio, USA). Ultrapure water from Milli-Q™ Gradient 10A System from Millipore (Milford, MA, USA) was used for all analyses. Gum Arabic was purchased from Vetec (Rio de Janeiro, Brazil), modified starch Capsul™ aky-0800 from National Starch (São Paulo, Brazil), and maltodextrin DE5 Globe™ 1805 from Corn Products (São Paulo, Brazil).

2.2. Fruits

Pomegranate fruits (Wonderful cultivar) were supplied by Boa Fruta Farm, located in a semiarid region in Brazil. After washing, the arils of the selected fruits were manually separated.

2.3. Fruit processing

The arils were depulped in horizontal equipment from Itametal, model Bonina df 0.25, in order to separate the juice from the seeds. The juice was stored at −20 °C until the spray drying process.

2.4. Formulations

The interaction of encapsulating agents was evaluated according to a mixture design (Table 1). The concentrations of the encapsulating agent were equal to the solids content in the raw pomegranate juice (16.5°Brix). The total amount of the encapsulating agents in each mixture was always 16.5 g per 100 g. For the formulations containing more than one encapsulating agent, the same proportion of each agent was used.

2.5. Spray drying process

A laboratory scale spray-dryer Buchii brand model B190, atomized with a 1 mm diameter nozzle was fed with pomegranate juice and encapsulating agents at a mass flow rate of 1 kg h^{-1}, the inlet-air temperature ranged from 162 °C to 170 °C and the air flow rate was 500 m^3 h^{-1}. Under these conditions the outlet-air temperatures ranged from 89 °C to 93 °C. The dry product was put into vacuum sealed packages, which remained stored in a desiccator at 25 °C until analysis of their anthocyanin contents and color. Processes were performed in duplicate.

2.6. Anthocyanin analyses

The analyses of the anthocyanins were conducted with 1 g of sample. The anthocyanins were extracted with methanol and formic acid solution in the ultrasonic bath with subsequent centrifugation until discoloration of the solution (Brito et al., 2007). Then, an aliquot of the extract was dried under filtered compressed air, after which it was diluted in methanol and formic acid for chromatographic analysis. All analyses were performed in triplicate. Chromatography was performed on a Waters™ Alliance 2695 system, with a Waters™ 2996 photodiode array detector, a Thermo™ Scientific C_{18} BDS (100 mm × 4.6 mm; 2.4 μm) column, with a flow rate of 1.0 mL min^{-1}, column temperature of 40 °C, injection volume of 20 μL and a gradient elution method with acetonitrile and formic acid. The quantification of the anthocyanins was performed by external standardization, based on the calibration curves made with analytical standards isolated and confirmed by high resolution mass spectrometer Synapt™ Waters ESI-qTOF according to the methodology described by Santiago et al. (2014).

2.7. Anthocyanin stability evaluation

The anthocyanin stability of the selected microcapsules was evaluated over a 120 day period. Time zero was considered to be immediately after the drying process. The subsequent evaluations were performed after 30, 90 and 120 days of storage at 25 °C. The samples were kept at room temperature, in individual aluminum and polyethylene packs and without exposure to light until analysis by HPLC.

Table 1
Experimental mixture design proposed to evaluate the effect of carrier agents on the retention of anthocyanins.

	Emulsions
sample 1	pj + Capsul™
sample 2	pj + maltodextrin
sample 3	pj + gum Arabic
sample 4	pj + Capsul™ + maltodextrin
sample 5	pj + gum Arabic + Capsul™
sample 6	pj + gum + maltodextrin
sample 7	central point (pj + Capsul™ + maltodextrin + gum Arabic)

pj: pomegranate juice; central point: assay in triplicate.