Fruits, 2016, vol. 71(1), p. 3-8

DOI: 10.1051/fruits/2015041

FRUITS

Available online at:
www.fruits-journal.org

ORIGINAL ARTICLE

Characterization of jamelão (*Syzygium cumini* (L.) Skeels) fruit peel powder for use as natural colorant

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Received 14 January 2015 – Accepted 7 September 2015

Abstract – Introduction. Tropical fruit have a good commercial potential as natural colorants; however, they are underexploited. Anthocyanins from jamelão (*Syzygium cumini*) fruit peel have been shown to be an alternative source of natural pigments for the food industry. Due to the toxicity of some synthetic colorants to the environment and human health, these artificial colorants are being replaced by natural pigments. This study was carried out to evaluate the properties and the technological feasibility of using the peel powder of the jamelão fruit as a colorant. **Materials and methods.** Over a 5 month period the anthocyanins from the jamelão fruit peel powder were tested for stability by HPLC every 30 days. **Results and discussion.** A loss of 36% of the total monomeric anthocyanins was noted at the end of the study. Despite this loss, the final product maintained its quality as a colorant, due to the initial high concentration of anthocyanin. **Conclusion.** Besides its intense and attractive color, the peel powder of jamelão also proved to be rich in dietary fibers and thus a good ingredient for low-calorie diets as well as having low lipid content.

Keywords: Brazil / jambolão / jamun / *Syzygium cumini* / antioxidant / natural colorant

Résumé – Caractérisation de la poudre de la peau des fruits du jamelonier (*Syzygium cumini* L. Skeels) pour une utilisation comme colorant naturel. Introduction. Les fruits tropicaux présentent en général un bon potentiel commercial comme colorants naturels ; et pourtant, peu d'entre eux sont exploités. Les anthocyanes de la peau du fruit du jamelonier (*Syzygium cumini*) passent pour être une source alternative de pigments naturels pour l'industrie alimentaire. En raison de la toxicité de certains colorants synthétiques pour l'environnement et la santé humaine, ceux-ci sont remplacés par des pigments naturels. La présente étude évalue les propriétés et la faisabilité technique de l'utilisation de la poudre de zeste du fruit du jamelonier comme colorant. **Matériels et méthodes.** Sur une période de 5 mois, la stabilité des anthocyanes de pelure en poudre des fruits du jamelonier a été testée par HPLC tous les 30 jours. **Résultats et discussion.** Une perte de 36 % du total des anthocyanes monomères a été notée à la fin de l'étude. Malgré cette perte, le produit final a maintenu sa qualité de colorant, en raison de la forte concentration initiale en anthocyanines. **Conclusion.** Outre sa couleur intense et attrayante, la poudre de pelure de fruit du jamelonier s'est également avérée être riche en fibres alimentaires, et donc un bon ingrédient pour les régimes hypocaloriques, d'autant plus qu'elle présente une faible teneur en lipides.

Mots clés : Brésil / jamelonier / *Syzygium cumini* / anti-oxydant / colorant naturel

1 Introduction

Consumption of tropical fruits is increasing due to the growing recognition of their nutritional value and therapeutic properties. A wide variety of native and/or exotic fruits are consumed in Brazil, but there are few studies on the feasibility of introducing the same in domestic markets. These fruits have been consumed in Brazil for many years, are adapted to the different Brazilian climatic conditions and are potential sources of natural antioxidants. They have good commercial potential, but are still underexploited. They represent a future source of opportunity and income for local people to access specialized markets where consumers give importance to the presence of nutrients capable of reducing the risk of degenerative diseases [1, 2].

Jamelão (*Syzygium cumini* (L.) Skeels), also known as jambolão, jambolan, black plum, java plum or jamun, is a native

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species from India, but had been adapted to the Brazilian tropical climate. It belongs to the family *Myrtaceae* [3]. The Java plum is an exotic fruit bearer, widely cultivated in Brazil, as an ornamental tree for shade, mainly along the littoral. It is an evergreen tree, with a frondose, dense crown, 15−20 m tall, and the trunk is usually crooked. The leaves are simple, coriaceous, glabrous on both surfaces, aromatic, lustrous above, 8−14 cm long, with a prominent main nerve and a petiole of 1−3 cm. Flowers, dominant by the white stamens, are arranged on branched, axillary racemes that form from September to November. Fruit is oblong, with a succulent pulp, a slightly sweet and astringent flavour, they contain a single seed and mature in January and February [4]. Since there is no comercialization or even broad consumption by the population, most of the fruit is wasted, which calls attention to the potential use of the same to obtain natural colorant.

The jamelão fruit is small and ovoid in shape, with purple-red to black color peel and white pulp [5]. This dark color is attributed to the presence of anthocyanins. They belong to the most important group of water-soluble pigments in nature and occur as glycosides or akyl glycosides of their respective anthocyanidins. It is possible to find many anthocyanidins in nature, whereas only 6, cyanidin (Cy), delphinidin (Dp), petunidin (Pt), malvidin (Mv), pelargonidin (Pg) and peonidin (Pn), are normally found in food.

The peel of the jamelão fruit has been revealed to contain a high amount of anthocyanins, and therefore is a potential source of natural colorants for the food industry [6]. These anthocyanins have been identified as 3,5-diglucoside derivatives of delphinidin, petunidin, malvidin, cyanidin, and peonidin [7, 8]. The artificial synthesis of anthocyanins still represents a challenge and is expensive. However, these compounds can be extracted from vegetal species, especially fruits, where there is high concentration of anthocyanins.

The commercial cost of isolating chemically pure anthocyanins is very high. Furthermore, due to the toxicity of some synthetic colorants to the environment and human health, artificial colorants are being replaced by natural pigments.

The most difficult challenge when using anthocyanins as colorants is due to their stability. During storage, the structure of anthocyanins, and consequently their color, may change once these compounds are sensitivity to high temperatures, oxygen, light and enzymatic action [9, 10]. However, drying process can be a convenient way to store and handle anthocyanins. Drying prevents the degradation of anthocyanins, limits the growth of microorganisms, and thus limiting the activity of damaging enzymes as well as preventing biochemical deterioration of the products [11].

The use of anthocyanins as food colorants not only confers improvements in product appearance but also prevents autoxidation and lipid peroxidation in biological systems [12]. However, studies about their bioavailability are necessary as well as studies defining their potential health benefits. The incorporation of alternative fruits such as jamelão in the preparation of processed products could contribute to increase the availability of bioactive compounds in such food products. Therefore, the study of this fruit is a great opportunity to promote the use of biodiversity for the benefit of food security and nutrition.

However, the aim of this study was to characterize the jamelão fruit peel powder obtained by a simple drying process, and to evaluate this powder as a natural colorant and source of bioactive compounds.

2 Materials and methods

2.1 Sampling

Jamelão fruit (10 kg) was collected in February (summer season) 2014 in the Guaratiba district, Rio de Janeiro city, Brazil. The ripe fruit was immediately selected and separated into 3 samples with a mean of 500 fruits each sample. The fruit was washed and the peel was manually separated from the pulp. The peel was submitted to a drying process on the same day.

Jamelão fruits were individually weighted in order to establish the mean weight per fruit. Peel, pulp and seeds of jamelão fruit were separated, and each part was individually weighted to define the content percentages (%). This procedure was repeated with 6 fruits.

2.2 Chemicals

Acetonitrile HPLC grade, formic acid 98%, methanol HPLC grade and ethanol ACS grade were purchased from Tedia™ (Ohio, USA). The ultrapure water was obtained from Milli-Q™ Gradient 10A System (Merck Millipore Corporation, Massachusetts, USA). A standard of delphinidin-3,5-diglucoside chloride was purchased from ChromaDex™ (California, USA). ABTS (2,2-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) and Trolox (6-Hidroxi-2,5,7,8-tetrametilchroman-2-carboxylic acid) from Sigma-Aldrich™ (Missouri, USA).

2.3 Convective drying process

The drying process was conducted on a convective layer dryer developed by Embrapa Food Technology. The fruits peels were placed on trays in a single layer and subjected to dehydration at a temperature of 60 °C and air velocity of 1 m s^{-1} for 22 h. The dried product was ground using a blender and stored in aluminum and polyethylene packs at room temperature until analysis.

2.4 Jamelão peel powder centesimal composition

The analyses of moisture, ash, lipid, fiber and proteins followed the standard methods of the Association of Official Analytical Chemists [13]. All analyses were performed in duplicate. The total carbohydrate content was determined by the difference of the other analyzed components.

2.5 Color analysis

The color analysis was carried out in triplicate with a colorimeter, Color Quest XE using the CIELAB and CIELch scale with a 0.375 mm opening diameter, and D65/10 illumination. The angle of observation was 10 mm quartz cuvette. From the L * (lightness), a * and b * (color coordinates) values of the parameters, C * (chroma, color saturation) and h° (hue angle Chromatic) were calculated [14].

2.6 Antioxidant capacity ($ABTS^{\cdot+}$ assay)

The extraction of the antioxidant was conducted using 1 g of sample and solutions of methanol/water (50:50 v/v) and acetone/water (70:30 v/v) [15]. Analyses were performed in triplicate.

The ABTS radical species was prepared by mixing 38.4 mg of ABTS and 6.6 mg of potassium persulfate and dissolved in ultrapure water and left in the dark at room temperature for 16 h before use. The ABTS solution was diluted with ethanol PA to an absorbance of 0.70 ± 0.02 at 734 nm. After the addition of 30 μL of the sample extract or trolox standard to 3 mL of diluted ABTS solution, the absorbance was recorded at 6 min after mixing at 734 nm. The blank assay used ethanol and the antiradical activity was expressed as μM trolox equivalents, determined using a Trolox calibration curve [15].

2.7 Anthocyanin analysis

The anthocyanins were extracted using 1 g of sample and 10 mL of methanol/formic acid solution (90:10 v/v) in an ultrasonic bath with subsequent centrifugation until discoloration of the solution [7]. Then, an aliquot of the extract was dried and diluted with 200 μL of 5 % formic acid solution in water: methanol (90:10 v/v). Analyses were performed in triplicate and were carried out on a Waters™ Alliance 2695 system, using a Waters™ 2996 photodiode array detector, with a Thermo™ Scientific C_{18} BDS (100 mm × 4.6 mm; 2.4 μm) column, a flow of 1.0 mL min^{-1}, column temperature of 40 °C, injection volume of 20 μL and using a gradient elution method with acetonitrile and formic acid. Analyses were performed in triplicate.

The quantification of the main anthocyanins was performed by external standardization, based on calibration curves made with analytical standards isolated in the laboratory, with purities greater than 99% and confirmed by mass spectrometer of high resolution Synapt™ Waters ESI-qTOF [16].

2.8 Mass spectrometry analysis

In order to identify all the anthocyanins present in jamelão, the unidentified ones were collected manually by elution from HPLC system and then injected directly into the high resolution mass spectrometer Synapt™ Waters ESI-qTOF. The mass spectrometry (MS) source was positive electrospray ionization (ESI+) with a temperature of 120 °C, N_2 as the desolvation gas delivered at 12.5 L min^{-1} at 500 °C. The capillary exit was set at 3.0 kV, the sampling cone energy at 25.0 V and the extraction cone energy at 4.0 V.

Table I. Jamelão fruit parts in percentage of the whole fruit fresh weight. Results reported are mean values ± SD (n = 6).

Parts	Values (%)
Peel	10.9 ± 1.5
Pulp	68.9 ± 3.3
Seed	20.4 ± 4.5

2.9 Anthocyanin stability evaluation

The anthocyanin stability of the powder was evaluated every 30 days, over a 5 month period. Time zero was the analysis immediately after the drying process. The samples were kept at room temperature in individual laminated packaging until analysis by High Performance Liquid Chromatography.

2.10 Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey's multiple range test was used to determine significant differences amongst means ($P < 0.05$) of anthocyanin content during the storage. All data were calculated with XLSTAT software (version 2014).

3 Results and discussion

3.1 Fruit parts

The mean weight per fruit was 6.0 ± 0.9 g. The jamelão fruit parts, as percentage (in weight) of peel, pulp and seed, are presented in *table I*. The values indicate that although jamelão fruit peel is the object of the present work, the other parts of the fruit are more abundant and could be used, *e.g.*, for desert (pulp) and oil extraction (seed).

3.2 Convective drying process

The drying process of the Jamelão peel showed a yield ratio of 20%, related to the amount of powder obtained from the peels. The powder had an intense and attractive color (*figure 1*). Although the yield ratio is not high, any use of this fruit is worthwhile since there is an abundant amount of jamelão fruit in some regions. Many of the fruit fall off the trees during the harvest period, and are wasted on the ground.

Usually, food colorants are produced by sophisticated drying methods, such as spray drying and freeze drying, which are considered high cost technologies. Methods like convective drying are also low cost and simpler; and consequently a good alternative for farmers. Besides, oven drying method

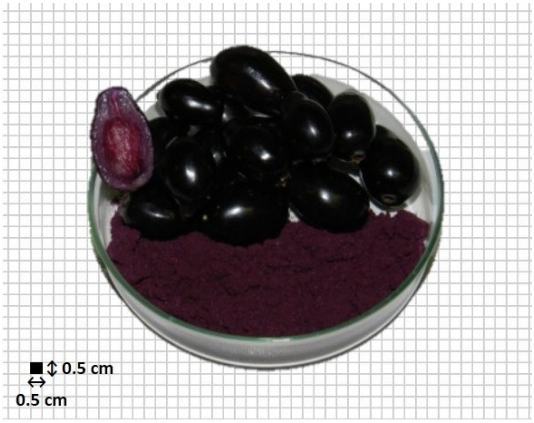

Figure 1. Jamelão fruit and the powder obtained from its peel.

Table II. Centesimal composition of Jamelão fruit peel powder. Results reported are mean values ± SD (n = 6).

	Composition in g 100 g^{-1} fresh weight
Moisture	9.98 ± 0.08
Lipids	0.64 ± 0.01
Ash	3.33 ± 0.03
Protein	3.97 ± 0.00
Dietary fiber	22.51 ± 0.00
Carbohydrate	59.57 ± 0.12

has an advantage over the natural drying by reducing the drying time and is one of the most commonly used methods for drying raw fruit and vegetables [17]. Moreover, under suitable temperatures, this method maintains the quality of the plant compounds [18].

3.3 Jamelão fruit peel powder centesimal composition

The composition values obtained for the jamelão peel powder show that it contains high amounts of dietary fibers. Freeze-dried jabuticaba peel powder, which is also recognized as a food rich in dietary fibers, presents 25% of total fibers [19], a value similar to the one found for the jamelão peel powder in the present work (*table II*). Jamelão peel powder also presented low lipid content, which is important for low-calorie diets.

3.4 Color analysis

The CIELAB color space was represented as Cartesian polar coordinates. The L* axis covers from the top to the bottom and the maximum value is represented by 100 (indicative of color), while the minimum is 0, what indicates black color. The a* parameter indicates a variation of the green/red color intensity (from −80 to zero = green, from zero to +100 = red), while the parameter b * indicates the variation of blue/yellow intensity (from −100 to 0 = blue, from 0 to +70 = yellow) [20, 21]. The jamelão fruit peel powder showed an a* value within the range of red. The C* and h° parameters belong to the CIELCH color system which uses cylindrical coordinates instead of Cartesian coordinates. In this system, C* (chroma, saturation index or brightness) represents the intensity of the color deviation from neutral gray, while h° (hue angle) is referred directly to the color of the object. For the parameter h°, the closer the value is from 360, the redder the color [20, 21]. The evaluation of h° for the powder showed a predominance of the red color (351.47°) (*table III*). These results confirm that the powder product could be used as colorant to substitute for red synthetic dyes, which are considered toxic and are banned in some countries.

Table III. Color analysis of the jamelão fruit peel powder. Results reported are mean values ± SD (n = 9).

Parameters	Fruit peel powder values
L* (lightness)	40.67 ± 0.58
a* (a axis)	6.72 ± 0.53
b* (b axis)	–1.00 ± 0.09
C* (chroma)	6.80 ± 0.53
h* (matriz angle)	351.47 ± 1.07

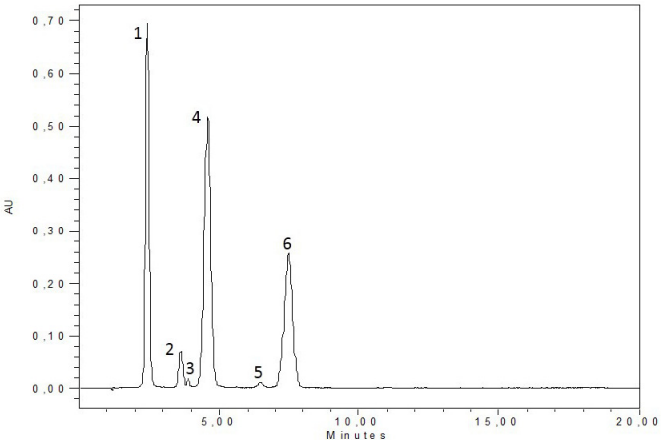

Figure 2. Chromatogram of the anthocyanins from jamelão peel powder extract. Peak identification: Peak 1: delphinidin-3,5-diglucoside; Peak 2: cyanidin-3,5-diglucoside; Peak 3: delphinidin-3-glucoside; Peak 4: petunidin-3,5-diglucoside; Peak 5: peonidin-3,5-diglucoside; Peak 6: malvidin-3,5-diglucoside. AU: absorbance unit.

3.5 Antioxidant capacity (ABTS$^{\cdot+}$ assay)

The measurement of the jamelão fruit peel powder antioxidant capacity indicated a value of 150 μM trolox g^{-1}, which is greater than that reported by Rufino *et al.* [15], when they evaluated jamelão fruit peel powder obtained by the freeze drying process (125 μM trolox g^{-1}).

3.6 Anthocyanin identification

The jamelão anthocyanins profile (*figure 2*) was previously identified by Brito *et al.* [7], except the third peak, which was

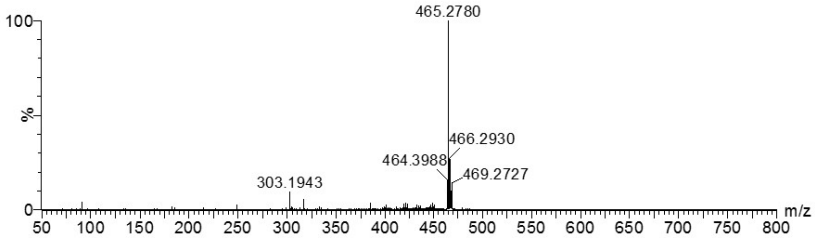

Figure 3. Electrospray ionization coupled to tandem mass spectrometry (ESI-MS/MS) spectrum of the anthocyanin delphinidin-3-glucoside isolated from jamelão fruit peel powder extract. M/Z: mass-to-charge ratio.

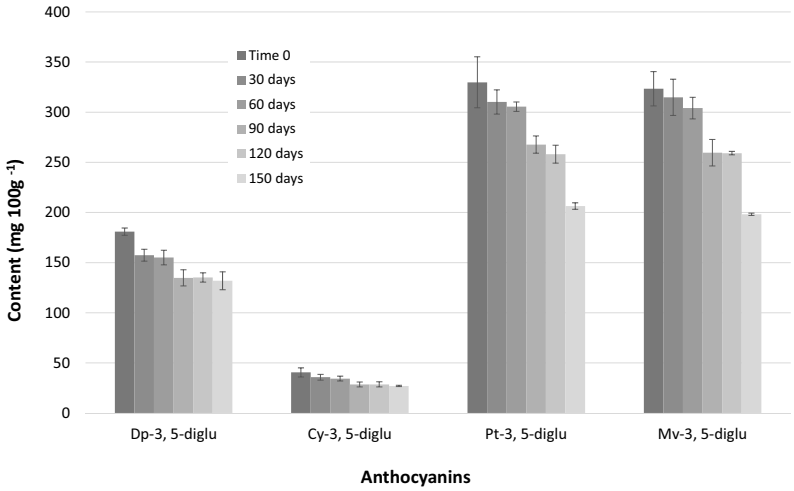

Figure 4. Anthocyanin concentrations over 150 days of the stability test of jamelão fruit peel powder (Dp-3,5-diglu: delphinidin-3,5-diglucoside; Cy-3,5-diglu: cyanidin-3,5-diglucoside; Pt-3,5-diglu: petunidin-3,5-diglucoside; Mv-3,5-diglu: malvidin-3,5-diglucoside). Values are means of 3 sample analyses (n = 500 fruits per sample).

not observed by these authors. In the present work this anthocyanin was identified by mass spectrometry as delphinidin-3-glucoside. This anthocyanin identification was based on data obtained by mass spectrometry for the molecular ion and on its fragments. A molecular ion at m/z = 465.2780, which is the molecular weight of the anthocyanin delphinidin-3-glucoside, and at m/z = 303.1943 by MS/MS, was obtained. This value equals the molecular weight of the aglycone delphinidin (*figure 3*).

3.7 Anthocyanin stability evaluation

The initial anthocyanin content of the powder was 879 mg 100 g^{-1}, which was greater than described previously with freeze dried jamelão fruit peel powder (771 mg 100 g^{-1}) [7]. Until the second month, there was no statistically important loss at the 95% confidence level in the total monomeric anthocyanin content. After the second month of storage, there was a 10% loss and at the end of 5 month storage there was a 36% loss (*table IV* and *figure 4*).

These losses may have occurred due to oxidation of anthocyanins. Tonon *et al.* [22] linked the degradation of anthocyanin in açaí powder to the porosity and heterogeneity of the structure of the dry material, which allow the oxygen diffusion and thus the degradation of anthocyanin pigments present in it. This may explain the occurrence of chemical reactions even in materials with low water content.

Besides this, the possible occurrence of water absorption can promote some undesirable reactions such as anthocyanin polymerization, which causes the reduction of the initial monomeric anthocyanin content.

Despite these losses, the product maintained its quality as a colorant due to the high concentration of anthocyanins.

Table IV. Anthocyanin stability evaluation of the jamelão fruit peel powder. Results reported are mean values ± SD (n = 9).

Storage time (day)	Total monomeric anthocyanin content (mg 100 g^{-1})[y]
0	878.56 ± 31.43 a
30	818.17 ± 30.97 a
60	798.91 ± 19.36 a
90	690.50 ± 31.74 b
120	674.47 ± 16.72 b
150	563.56 ± 14.00 c

[y] Different letters in the last column indicate significant difference (Tukey, $P < 0.05$).

The anthocyanin profile (*figure 2*) remained the same along the five months of the study. The magnitude of the peaks expressed a variation, what is proportional to the concentration of each one. It is important to consider that the variability of the plant material can interfere in the fruit anthocyanin content and consequently in the anthocyanin concentration of fruit peel powders.

4 Conclusion

Jamelão fruit peel powder obtained in the present study maintained its quality as a colorant during the stability evaluation, due to the high initial concentration of these pigments, despite a reduction of anthocyanin contents. This product could also be used as a functional ingredient in the development of food products due its antioxidant properties. Moreover, the powder also proved to be rich in dietary fibers and is thus a good ingredient to be used in low-calorie diets.

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Cite this article as: Manuela C.P.A. Santiago, Ana C.M.S. Gouvêa, Fernanda M. Peixoto, Renata G. Borguini, Ronoel L.O Godoy, Sidney Pacheco, Luzimar S.M. Nascimento, Regina I. Nogueira. Characterization of jamelão (*Syzygium cumini* (L.) Skeels) fruit peel powder for use as natural colorant. *Fruits* 71 (2016) 3–8.