THERMAL AND LIGHT STABILITY OF ANTHOCYANINS FROM STRAWBERRY BY-PRODUCTS NON-ENCAPSULATED AND ENCAPSULATED WITH INULIN

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ABSTRACT

Background. Strawberry by-products were explored as sources of anthocyanins for the extraction of natural colorants in the development of new value-added products for the food industry. For this purpose, the stability of strawberry anthocyanin extracts was evaluated for color and total anthocyanin content. The anthocyanins were encapsulated with inulin to protect them from processing at high temperatures and exposure to light. Microcapsules were obtained by two drying processes (spray and freeze drying) in order to study their use as coloring ingredients for their use in the food industry.

Materials and methods. Thermal (using the response surface methodology – RSM) and light stability tests were performed, simulating long-term processing and food storage. Antioxidant activity, total anthocyanin content and color analysis were quantified using several methods, and the microcapsules were characterized using scanning electron microscopy. Anthocyanins and their derivatives were identified by high resolution mass spectrometry.

Results. The strawberry extracts showed high antioxidant capacity and total anthocyanin content. The RSM of the thermal stability test showed that temperature is the variable with the most significant effect on color stability and total anthocyanin content. The anthocyanins showed more stability at 50°C/60 min, 57°C/102 min, 93°C/18 min and with up to 8 days of light. Microencapsulation of the strawberry extracts with inulin obtained by spray and freeze drying improved the stability of anthocyanins. The spray drying process can offer better applications for the food industry due to the more regular shape of the microcapsules, which supports the potential use of strawberry by-products as coloring ingredients for application in the food industry.

Conclusion. This study can serve as a technical reference for the development of anthocyanin microcapsules with inulin from strawberry by-products obtained by spray drying, resulting in stable natural colorants to be used as ingredients in the food industry.

Keywords: anthocyanins, encapsulation, inulin, thermal and light stability, spray and freeze drying

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INTRODUCTION

The agro-food industry produces a large quantity of by-products that are often rejected along the production chain due to climatic issues, disease, harvesting damage and economic factors (regulations and public or private standards for quality and appearance), as well as fluctuations in product demand (Otles et al., 2005). However, the use of some by-products is a good opportunity for the development of new value-added products, like natural ingredients, that can have a great impact on the economy of the food industry (Pintado and Teixeira, 2015).

The strawberry is a much-appreciated berry because of its good taste and nutritional value, but it is highly susceptible to microbial spoilage and hence tends to be difficult to commercialize (Huang et al., 2009). The disposal of this fruit usually represents a problem that is further exacerbated by legal restrictions. Thus, new ideas concerning the use of this waste as a by-product for further exploitation in the production of food additives with high nutritional value have gained increasing interest because this is a high-value product and its recovery may be economically attractive (Djilas et al., 2009). Strawberry by-products are a reliable source of anthocyanins that can be used as a raw material for the extraction of natural colorants for food purposes. However, many limitations exist for their commercial application due to their high raw material cost and poor stability during processing and storage. The development of suitable technologies that facilitate an increase in anthocyanin stability will enhance the application of fruit rejected from the production chain and will promote the economic growth of small fruit. Anthocyanins are a group of natural water-soluble pigments that confer a large color spectrum to flowers and fruits, especially purple, dark blue and red colors, and are classified as a subgroup of flavonoids (Cavalcanti et al., 2011). Structurally, anthocyanins are heterosides whose aglycone or anthocyanidins, derived from the flavylium or 2-phenylbenzopyrillium cation, are glycosylated with various sugar substitutes. Among the 21 anthocyanidins described in the literature, six are widespread in nature (Nicoué et al., 2007): pelargonidin (Pg), cyanidin (Cy), peonidin (Pn), delphinidin (Dp), petunidin (Pt), and malvidin (Mv). The strawberry has a simple profile with Pg-3-glucoside as the major anthocyanin pigment (Rein, 2005). Nowadays, the interest in anthocyanins as natural colorants is related to their antioxidant capacity, which leads to a broad range of beneficial effects in human health and disease prevention. They can prevent neuronal and cardiovascular illnesses, cancer, diabetes, and infections, among other things (Verbeyst et al., 2010). Therefore, due to the health problems of certain certified and banned colorants, and consumer preferences, there is an increasing interest in the use of food colorants from natural sources to substitute synthetic dyes (Castañeda-Ovando et al., 2009). In this context, although anthocyanins represent an enormous potential application for food, their use has been limited because of their instability, which is affected by several factors during food processing and storage, such as temperature and light (Cavalcanti et al., 2011). Anthocyanin stability decreases during processing and storage as increases in temperature lead to the formation of brown products (Rein, 2005). Many authors have studied the influence of temperature on anthocyanin stability from various sources proving that heating has a damaging effect on color and anthocyanin content (Cesa et al., 2017; Jiménez et al., 2010; Kirca et al., 2007; Méndez-Lagunas et al., 2017; Wang et al., 2010). The presence of light also accelerates the degradation of anthocyanins. These pigments preserve their color much better when kept in the dark; the difference can already be seen after 24 h when anthocyanins are stored in light and dark conditions at room temperature (Kearsley and Rodriguez, 1981). Encapsulation is a process that has been used to protect colorants from processing and environmental conditions, such as the undesirable effects of light, temperature, oxygen, etc. (Ersus and Yurdagel, 2007; Mahdavi et al., 2016). Inulin, a storage carbohydrate found in many plants that has been used as a food ingredient offering interesting nutritional properties such as soluble fiber and prebiotic effects and with some important technological benefits such as the stabilization of foams and emulsions (Frank, 2006), can be used as a wall material for encapsulation. Since there are no reports on the encapsulation of anthocyanin extract using inulin as a wall material, this study intends to contribute to a better understanding of the color stability of anthocyanin microcapsules using inulin as a wall material for application as a natural colorant in food products.
The aim of this work was to study the potential of the use of non-marketable strawberries for anthocyanin encapsulation, using inulin as a wall material.

MATERIALS AND METHODS

Chemicals and reagents

Ethanol p.a, hydrochloric acid (370 g·L⁻¹), iron(II) sulfate heptahydrate, iron(III) chloride hexahydrate, and sodium acetate trihydrate were purchased from Merck (Darmstadt, Germany). Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and potassium hexacyanoferrate (III), gallic acid (990 g·L⁻¹) were purchased from Sigma (Sternheim, Germany). Anhydrous sodium carbonate was obtained from BDH (Poole, UK), while 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ, 990 g·L⁻¹) and ferric chloride were acquired from Fluka (Buchs, Germany), and anhydrous sodium sulfate from Panreac (Barcelona, Spain). Ethanol absolute anhydrous was purchased from Carlo Erba (Marseille, France). Peonidin-3-glucoside and malvidin-3-glucoside were acquired from Extrasynthése (Genay, France). Inulin was purchased from Alfa Aesar (Zeppelinstraße, Germany). All other unlabeled chemicals and reagents were analytical or HPLC-MS Optima grade.

Plant material

Low caliber strawberries (Fragaria × ananassa) and those with defects were used in this study and were purchased during June/July in local markets in Lisbon, Portugal. The fruits were stored at –18°C until further analytical measurements were carried out.

Preparation of strawberry aqueous extracts (SAE)

Approximately 500 g of fruit per replicate was homogenized from a batch of 5 kg of fruit and used in the preparation of the SAE. Anthocyanins were extracted from the fruit using deionized water at pH = 4.6 (250 g·L⁻¹) by grinding in an Ultra-Turrax (IKA T-25, Janke and Kunkel) for 3 minutes at room temperature (20°C). The extracts were placed in a magnetic stirrer (Are, VelpScientifica) and stirred for 15 minutes at room temperature (20°C), and centrifuged (Sigma and Laborzentrifugen, 1k15) at 3000 rpm for 10 min at 5°C. Finally, the supernatant was collected and filtered through a Whatman no 41 filter (Whatman, Maidstone, UK) and brought to a known volume with deionized water. The extracts were stored at –18°C until analysis.

Preparation of strawberry microcapsules by encapsulation (SME)

A volume of 200 mL of inulin 30% aqueous solution was added to 400 mL of strawberry aqueous extract. The solution was dehydrated by Laboratory Scale Spray Dryer (Lab-Plant SD-05, Keison International Ltd, UK) according to Beirão-da-Costa et al. (2013) and freeze dried (LyoQuest, Azbil Telstar Technologies). The microencapsulates were stored in petri dishes in desiccators in the dark until analysis. To measure the total anthocyanin content and total phenolic content, and to evaluate the antioxidant capacity, the SME were prepared by weighing 1.5 g per replicate into 20 mL falcon tubes, then adding 10 mL of water and keeping them hydrated for 24 hours. The solution was stirred (Ultraturrax T 25) for 5 min and centrifuged (Sigma and Laborzentrifugen, 2k15) at 5000 rpm for 10 minutes. Finally, the supernatant was collected and filtered through a Whatman no 41 filter (Whatman, Maidstone, UK) and brought to a known volume with deionized water. The extracts were stored at –18°C until analysis.

Characterization of strawberry aqueous extracts and microcapsules

Color analysis. The colors of SAE and SME were measured in a Minolta Chroma Meter CT-310 and a CR-310 colorimeter, respectively, using an illuminant D65 and a 2° observation angle. The SAE and SME tristimulus color parameters were expressed as \( L^* \) – lightness, \( a^* \) – red/green index, \( b^* \) – yellow/blue index. The chroma (\( C^* \)), indicating color intensity, was calculated using the formula: \( C^* = (a^2 + b^2)^{1/2} \), and the hue angle (\( H^* \)), indicating tonality, using the formula: \( H^* = \tan^{-1}(b/a) \).

Total anthocyanins content (TAC). The total anthocyanin content was determined using the pH differential method, described by Wroslstad (1976) using a UV-visible spectrophotometer (double-beam; Hitachi U-2010). Approximately 1 mL per replicate of SAE and SME were diluted to 5 mL with deionized water and the absorbance was measured at 515 and
700 nm at pH 1.0 (0.025 M potassium chloride) and pH 4.5 (0.4 M sodium acetate) buffers, respectively. The difference in absorbance between the two samples was measured as follows (equation 1):

\[
\text{Absorbance} = \left[ A = (A_{510 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH 1.0}} - (A_{510 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH 4.5}} \right]
\]

TAC was calculated using the following equation 2:

\[
\text{TAC} = \left( A \times \text{MW} \times \text{DF} \times 100 \right) / \left( \varepsilon \times 1 \right)
\]

where:
- \( A \) – total absorbance,
- \( \text{MW} \) – molecular weight of pelargonidin-3-glucoside (433.2 g·mol\(^{-1}\)),
- \( \text{DF} \) – dilution factor,
- \( \varepsilon \) – molar absorptivity (22 400 L·mol\(^{-1}\)·cm\(^{-1}\) for pelargonidin-3-glucoside), and 1 is for a standard 1 cm path length.

Total anthocyanin content was expressed as mg pelargonidin-3-glucoside per g of dry weight for all samples.

**Antioxidant activity of strawberry aqueous extracts and microcapsules**

**Ferric ion reducing antioxidant power (FRAP).** The FRAP assay was carried out using a method described by Serrano et al. (2011). FRAP values are presented as \( \mu \text{mol Fe}^{2+} \cdot \text{g}^{-1} \) of dry weight for all samples. All determinations were performed in triplicate.

**Free radical scavenging (DPPH).** The scavenging effect of the DPPH free radical was performed according to Serrano et al. (2011). The percentage inhibition was calculated using equation 3 and the concentration that caused 50% inhibition (EC\(_{50}\)) was estimated from a plot of percentage inhibition versus concentration of extract. All determinations were performed in triplicate.

\[
\text{Inhibition, \%} = \left( A_{\text{control}} - A_{\text{sample}} \right) / A_{\text{control}} \times 100
\]

where:
- \( A_{\text{control}} \) – the absorbance of the control reaction (blank with 0.1 mL ethanol and DPPH),
- \( A_{\text{sample}} \) – the absorbance of the sample reaction (0.1 mL sample diluted in ethanol and DPPH).

To standardize the DPPH results, the antioxidant activity index (AAI), proposed by Scherer and Godoy (2009), was calculated using equation 4:

\[
\text{AAI} = \frac{C_{\text{DPPH}}}{\text{EC}_{50}}
\]

where:
- \( C_{\text{DPPH}} \) – the DPPH concentration in the reaction mixture, \( \mu \text{g} \cdot \text{mL}^{-1} \).

The samples were classed as showing poor antioxidant activity when AAI < 0.5, moderate antioxidant activity when 0.5 < AAI < 1.0, strong antioxidant activity when 1.0 < AAI < 2.0, and very strong when AAI > 2.0.

**Total phenolic content (TPC).** The TPC in SAE and SME were estimated using the Folin-Ciocalteu colorimetric method described by Serrano et al. (2011). The concentrations of total phenolic compounds were determined as mg GAE·g\(^{-1}\) of dry weight for all the samples. All determinations were performed four times.

**Thermal stability of strawberry aqueous extracts**

In this study, a central composite rotatable design (CCRD) was employed to systematically study the effects of two variables and their interactive effects on the characteristics of the SAE. The investigated ranges for temperature (\( \times 1 \)) and time (\( \times 2 \)) were 50–100°C and 0–120 min, respectively. A total of 15 experiments were carried out: 4 factorial points (experiments 1–4); 4 star points (experiments 5–8) and 7 central points (experiments 9–11). A repetition of central points is used to determine the experimental error, which is assumed to be constant along the experimental domain. The experiments were performed randomly to avoid systematic errors. Approximately 10 mL per replicate of SAE were measured into 20 mL tubes which were hermetically sealed. The tubes containing SAE were placed in a thermostat bath (Unitronic-OR, Selecta, Barcelona) according to the conditions established in Table 1.

The response variable results of total anthocyanin content and color \( (C^*, h^\circ, L) \) of the 15 experiments of the CCRD were fitted to the second order polynomial model equation (equation 5) using software “StatisticaTM” version 7 (Statsoft, USA).

\[
Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_{11}x_1^2 + \beta_{22}x_2^2 + \beta_{12}x_1x_2
\]
Light stability of strawberry aqueous extracts and microcapsules

Approximately 10 mL per replicate of SAE was introduced into 20 mL transparent tubes, hermetically sealed, and approximately 0.1 g of SME per replicate was weighed into 1 mL transparent tubes. The tubes containing SAE and SME were placed in a climatic chamber (Fitoclima 750 E, Aralab) at 25°C with constant illumination. The luminous intensity (0.014 W·m⁻²) was measured with a light meter (LI-COR model LI-250A). The tubes containing SAE were removed after 3, 5, 8, 12, 22 and 29 days and those containing SME were removed after 5, 12, 22 and 29 days. All the samples were evaluated for color.

**Anthocyanin profile**

The anthocyanin profile in SAE was determined using liquid chromatography tandem mass spectrometry (LC-DAD-MSⁿ) performed on a HPLC Dionex Ultimate 3000 system equipped with a diode array detector (DAD), scanning from 220 to 600 nm, coupled online to a LCQ Fleet™ ion trap mass spectrometer equipped with an electrospray ionization (ESI) source (Thermo Scientific). Separations were carried out with a Kinetex C18 column (150 mm × 4.6 mm, 5 µm, Phenomenex) at a controlled temperature (35°C) using (A) acidulated water with 1% (v/v) formic acid and (B) methanol as mobile phases. The following linear gradient was used: 0 min 7% B, 6 min 15% B, 17 min 75% B, 20 min 95% B, 28 min 75% B. The column was equilibrated for 7 min prior to analysis. The flow was set to 300 μL·min⁻¹, and the injection volume was 10 μL. ESI was performed in the positive mode, and the instrumental parameters were optimized as follows: ionization spray voltage, +4.5 kV, transfer capillary voltage 18 V, capillary temperature 270°C and focus lens voltage –58 V. Nitrogen was used in both nebulized form and as a dry gas at 80 and 10 arbitrary units, respectively. The tandem mass spectra (collision induced dissociation experiments) were obtained with an isolation window of 2 Da, a 20–30% relative collision energy and with an activation energy of 30 msec. The spectra corresponded to the average of 20–35 scans and were acquired in a range of 100–1000 Da. The data were processed using Xcalibur 2.2 SP1.48 software.

**Morphology of microcapsules**

The morphology of the microcapsules was evaluated through Scanning Electron Microscopy according to Beirão-da-Costa et al. (2013).

Table 1. CCRD: Coded and decoded levels of the experimental factors used

| Points       | Coded levels | Decoded levels |
|--------------|--------------|----------------|
| Factorial    | Temperature  | Time           | Temperature | Time |
| 1            | –1           | –1             | 57          | 18   |
| 2            | –1           | +1             | 57          | 102  |
| 3            | +1           | –1             | 93          | 18   |
| 4            | +1           | +1             | 93          | 102  |
| Star points  |              |                |             |      |
| 5            | 0            | –0             | 75          | 0    |
| 6            | –0           | 0              | 50          | 60   |
| 7            | 0            | +0             | 75          | 120  |
| 8            | +0           | 0              | 100         | 60   |
| Central points |             |                |             |      |
| 9            | 0            | 0              | 75          | 60   |
| 10           | 0            | 0              | 75          | 60   |
| 11           | 0            | 0              | 75          | 60   |
| 12           | 0            | 0              | 75          | 60   |
| 13           | 0            | 0              | 75          | 60   |
| 14           | 0            | 0              | 75          | 60   |
| 15           | 0            | 0              | 75          | 60   |

where:

\[
Y \quad \text{– defined as the response variable,} \\
\beta_0 \quad \text{– the constant,} \\
x_1, x_2 \quad \text{– the independent variables,} \\
\beta_{11}, \beta_{22} \quad \text{– the quadratic coefficients,} \\
\beta_{12} \quad \text{– the cross-product coefficient.}
\]

The linear, quadratic and interaction effects were calculated, and their significance was evaluated by analysis of variance. The response surfaces described by a first or second-order polynomial equation were fitted to the experimental data points. First and second order coefficients were generated by regression analysis. The fit of the models was evaluated using the determination coefficients (\(r^2\)) and adjusted \(r^2\) (\(r^2_{adj}\), Montgomery, 1991).

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Statistical analysis
The experimental design for the thermal stability study and all statistical analyses were performed using Statistica™ v 8.0 software (Statsoft, Inc., Tulsa, OK, USA). The differences between the extracts were tested using analysis of variance (ANOVA). To satisfy the ANOVA assumptions, the data were transformed, followed by multiple comparison tests (Tukey HSD) to identify differences between the groups. Statistical analyses were tested at a 0.05 level of probability.

RESULTS AND DISCUSSION

Total anthocyanins content (TAC)
The TAC values of SAE and SME produced by the spray drying (SD) and freeze drying (FD) processes were 6.81, 3.61 and 3.75 mg·g⁻¹ pelargonidin-3-glucoside, respectively (Fig. 1). In this study, SAE had a higher value than that observed by Henríquez et al. (2011) (5 mg·g⁻¹). Such differences might be attributed to the maturity stage at harvest, the genotype and the growing season, and the extraction methods (Frederes et al., 2014; Hosseinian and Beta, 2007; Josuttis et al., 2012; Wang et al., 2012; Zhao et al., 2013). Lacerda et al. (2016) obtained similar TAC in jussara pulp microparticles produced with inulin. The differences in the TAC of SAE and SME might be attributed to the efficacy of the microencapsulation process, which provides good protection to the anthocyanin colorants and avoids its solubilization in water. No significant differences (p < 0.05) were observed in TAC between SD and FD processes.

Antioxidant capacity of strawberry aqueous extracts and microcapsules
The FRAP values of SAE and SME produced by SD and FD were 172.50, 122.50 and 126.59 μmol Fe²⁺·g⁻¹, respectively (Fig. 2A). The results obtained for the antioxidant activity of SAE were higher than those observed by Henriquez et al. (2011) (132 μmol Fe²⁺·g⁻¹). The highest antioxidant activity found in the strawberry extracts used in this study can be explained due to the use of different strawberry varieties, causing a synergistic effect owing to the diverse composition in anthocyanins (Kondo et al., 2009).

The EC₅₀ values for SAE and SME produced by SD and FD were 133, 700, 697 and the AAI values were 0.51, 0.54 and 0.55, respectively (Fig. 2B). The results obtained for the EC₅₀ of SAE were lower than those observed by Huang et al. (2012) for strawberries in methanol extracts (810 µg·mL⁻¹). Such differences might be attributed to the use of low caliber fruit and to the higher extraction of anthocyanins in methanol solvents compared to water (Zhao et al., 2013). The SAE and SME produced by SD and FD revealed a moderate antioxidant activity according to the AAI index and antioxidant categories defined by Scherer and Godoy (2009).

The TPC values for SAE and SME produced by SD and FD were 17.01, 12.40, 13.82 mg GAE·g⁻¹, respectively (Fig. 2C). Phenolic compounds, including anthocyanins, contribute significantly to their antioxidant activity so it was expected to observe the same trend between FRAP, TPC and TAC since anthocyanins are the most abundant group of phenolic compounds present in strawberries. No significant differences (p < 0.05) were observed between spray-drying and freeze-drying processes, showing that both provide a good level of protection.

Thermal stability of strawberry aqueous extracts
The results of all fifteen experiments of the CCRD set for total anthocyanin content (TAC), chroma (C*), hue (h°) and luminosity (L*) of SAE are shown in Table 2.

The linear and quadratic effects of these factors, as well as their effects on the results are presented in Table 3. The results show that temperature was the most
significant variable in color stability and TAC, in comparison to time, having linear and quadratic effects for most of the dependent variables studied. Four-dimensional surfaces, described by second-order polynomial equations, such as factors, functioning with significant effects on the results, or those with important enough effects not to be neglected, were fitted to the data points used in the experiments. High values for $r^2$ and $r^2_{adj}$ indicated a good fit for the results of the experimental models.

SAE showed anthocyanin degradation with an increase in temperature and time (Fig. 3A) leading to a decrease in concentration in the TAC. The same effect was also reported by Wang et al. (2010). The color of SAE also showed fading with an increase in temperature and time. The $C^*$ of SAE (Fig. 3B) decreased with an increase in temperature and time resulting in a less intense color. Similar changes could also be observed in the hue angle ($h^\circ$; Fig. 3C). It was not possible to represent the variation of lightness ($L^*$) of SAE because the model did not present adequate adjustments.

**Light stability of strawberry aqueous extracts and microencapsulated extracts**

The presence of light in SAE leads to a significant degradation of TAC (Fig. 4). The results of TAC for SAE showed a gradual variation over time with a significant
decrease ($p < 0.05$) until day 22 (from 5.01 to 2.08 mg·g⁻¹). After 29 days of exposure to light there was a loss of 59% of TAC. These results agree with Wang et al. (2010) who found the same effect of light on TAC for blueberry extracts.

SAE tristimulus color (Fig. 5) showed an increase in $L^*$ and a decrease in $C^*$, resulting in discoloration and a less intense color. The $h^\circ$ of SAE exposed to light showed a slight variation over time.

The light stability was evaluated only in SME produced by freeze-drying since we didn’t obtain enough microcapsules during spray-drying to perform all tests. The results of the light stability tests with SME produced by FD showed no changes in tristimulus color parameters ($L^*$, $C^*$, $h^\circ$) over time (Fig. 6). These results show that encapsulation provides a good level of protection and color stability to light. The same results were found by Jiménez-Aguilar et al. (2011) for

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**Table 2.** Experimental results of the CCRD set for total anthocyanin content (TAC) and color ($C^*$, $h^\circ$ and $L^*$) of SAE

| Temperature, °C | Time, min | TAC   | $C^*$ | $h^\circ$ | $L^*$ |
|----------------|-----------|-------|-------|----------|-------|
| 57             | 18        | 5.07  | 57.09 | 59.79    | 70.07 |
| 57             | 102       | 4.66  | 56.70 | 59.80    | 67.43 |
| 93             | 18        | 4.78  | 56.60 | 59.81    | 67.61 |
| 93             | 102       | 4.54  | 56.79 | 58.27    | 66.50 |
| 75             | 0         | 3.66  | 52.77 | 57.24    | 64.15 |
| 50             | 60        | 3.57  | 53.87 | 57.26    | 66.68 |
| 75             | 120       | 3.71  | 52.82 | 56.87    | 66.29 |
| 100            | 60        | 3.92  | 53.58 | 58.03    | 67.13 |
| 75             | 60        | 3.79  | 51.52 | 58.16    | 68.60 |
| 75             | 60        | 3.80  | 52.72 | 58.00    | 68.24 |
| 75             | 60        | 3.74  | 52.94 | 57.73    | 66.60 |
| 75             | 60        | 3.70  | 51.13 | 57.05    | 67.85 |
| 75             | 60        | 3.45  | 52.91 | 57.90    | 67.53 |
| 75             | 60        | 1.73  | 44.51 | 55.13    | 68.78 |
| 75             | 1.55      | 42.20 | 58.16 | 70.17    |

**Table 3.** Fitted models equations for total anthocyanin content (TAC) and color ($C^*$, $h^\circ$, $L^*$) of SAE

| Second-order polynomial equations               | $r^2$ | $r^2_{adj}$ |
|-----------------------------------------------|-------|-------------|
| TAC $27.30 – 8.28T - 3.81T^2 - 4.35t - 3.59Tt$ | 0.93  | 0.89        |
| $C^*$ $58.89 – 9.19T - 3.03T^2 - 4.14t + 1.67t^2 - 4.30Tt$ | 0.97  | 0.95        |
| $h^\circ$ $57.62 – 1.87T - 2.03t$            | 0.81  | 0.70        |
Anthocyanin profile

Anthocyanins present in SAE exposed to thermal and light stability tests are summarized in Table 4, and tentatively identified based on their characteristic absorption wavelengths of approximately 500–530 nm, together with their MS² profiles. The major anthocyanin identified in SAE was Pg-3-O-glucoside (m/z 433), as previously reported by Seeram et al. (2006) and Lopes da Silva et al. (2007). Minor di- and mono-glucose compounds such as Cy-3-O-glucoside (m/z 449) and Pg-3-O-malonyl-glucoside (m/z 519) were assigned based on the fragmentation patterns observed on the MS² spectra. A decrease in anthocyanin stability with an increase in temperature was observed in SAE, as shown in Figure 7. The identified anthocyanins showed better stability under...
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the following thermal conditions: 50°C/60 min, 57°C/102 min and 93°C/18 min. A gradual decrease in anthocyanin stability was also observed with longer exposure to light, and these compounds showed more stability under light conditions up to 8 days (Fig. 8).

**Morphology of microcapsules**

The morphologies of the microcapsules produced by SD and FD are presented in Figure 9 and 10. The microcapsules obtained by SD had a spherical shape with a smooth and continuous wall surface without cracks. Some wrinkled and concave microcapsules were also observed. Figure 9B shows the inside of a microcapsule and it can be observed that the microcapsules produced by SD are hollow and have a thick and consistent wall. Beirão-da-Costa et al. (2013) observed similar morphologies in inulin microcapsules with oregano oil obtained by spray-drying. The microcapsules obtained by FD have a roughly spherical shape with a wrinkled, spongy and cracked surface and tend to form agglomerates.

The variation of particle sizes of the microcapsules produced by SD and FD are shown in Figure 11 and 12, respectively. The particle size distribution of

| $t_p$, min | $\lambda_{max}$, nm | [M]+, m/z | MS2, m/z | Proposed compound       | TD sample | PD sample |
|-----------|------------------|----------|--------|------------------------|----------|---------|
| 14.1      | 282, 502         | 595      | 271, 433 | Pg-3-O-diglu           | x        |         |
| 15.8      | 280, 518         | 595      | 287    | Cy-3-O-rut             | x        | x       |
| 16.1      | 518              | 449      | 287    | Cy-3-O-gluc            | x        | x       |
| 16.6      | 500, 428, 278    | 433      | 271    | Pg-3-O-gluc            | x        | x       |
| 17.3      | 502              | 579      | 271, 433 | Pg-3-O-rut             |         | x       |
| 17.8      | 331, 502         | 519      | 271    | Pg-3-O-malonyl-gluc    | x        | x       |

Pg – pelargonidin, Cy – cyanidin, diglu – diglucoside, glu – glucoside, rut – rutinoside.

TD sample – thermodegradated sample, PD sample – photodegradated sample.

**Fig. 7.** Anthocyanins identified in strawberry aqueous extract and area variation during temperature stability tests: T0 – initial time, T1 – 50°C/60 min, T3 – 57°C/102 min, T4 – 75°C/60 min, T6 – 93°C/18 min, T8 – 100°C/60 min

![Graph showing peak area variation](image-url)
microcapsules formed by SD was very wide, ranging from 1.4 to 12.9 μm and 80% of the microcapsules had a diameter of less than 7.7 μm. The average diameter of these microcapsules was 5.5 μm. The microcapsules formed by FD showed a narrow particle size distribution, ranging from 5.5 to 8.8 μm and 80% of the microcapsules had a diameter of less than 7.2 μm. These microcapsules presented an average diameter of 6.6 μm.

Fig. 8. Anthocyanins identified in strawberry aqueous extract and area variation during light stability tests: T0 – initial time, T1 – 8 days, T2 – 57°C/102 min, T3 – 22 days, T4 – 29 days

Fig. 9. Scanning electron microscopy microphotographs of strawberry microcapsules produced by spray-drying. Magnifications: A – 500 ×, B – 2000 ×
CONCLUSIONS

It was demonstrated that SAE showed a strong antioxidant capacity, and a high TPC and TAC, allowing it to be used as a food colorant. Additionally, the strawberry extracts maintained the anthocyanin structure for a long time at about 50°C and were less affected by light exposure until 8 days. The spray drying process can improve anthocyanin stability and offer better applications for the food industry due to the more regular shape of the microcapsules. Micro-encapsulation supports the potential use of strawberry by-products, for example as a natural red colorant to be used as an ingredient in the food industry that requires thermal processing and has a relatively long shelf life. However, the evaluation of the pH variation, thermal stability, and safety issues of the released microcapsule colorants needs to be assessed further.

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