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Betacyanins obtained from alternative novel sources as natural food colorant additives: incorporated in savory and sweet food products†

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The aim of this study was to assess the performance and stability of betacyanin compounds present in enriched extracts of red-fleshed pitaya peels (*Hylocereus costaricensis*) and the flowers of *Amaranthus caudatus*; they were evaluated as natural food colorants in tagliatelle pasta and meringue cookies. The recovered natural extracts showed promising stability, maintaining a deep pink color over a storage time of 14 days, without deeply changing the chemical composition. A number of factors were assessed, including the microbial load, texture, color, nutritional value, and contents of organic acids, fatty acids, and even free sugars of the products. Some significant interactions between the type of colorant and storage time contributed to the changes in some analyzed parameters, as can be observed from the results for organic and fatty acids in the tagliatelle pasta and meringue cookies. Another significant achievement was the reduction in the microbial load during the storage time, which strengthens the antibacterial power of these natural extracts.

1. Introduction

A shift in the consumption habits of the global population during the past few decades has been occurring,[1] and the consumer concern over added ingredients in processed foods has led to dietary changes. Synthetic food additives have been pointed out as having, in some cases, harmful effects on human health.[2] Food additives are essential for preserving and enhancing the organoleptic qualities, while also avoiding degradation and extending the shelf life.[3] These additives are used in the most diverse types of foods, such as bakery,[4] dairy products,[5,6] and candies,[7] among others. Food coloring additives, which are included in many products marketed primarily for children, are currently under high scrutiny since they are possibly the ones who are more likely to experience the harmful effects linked to these substances.[8] Color is an important factor in food, as it can influence consumer decisions to buy a certain product. Color characteristics are seen as a first feature that our senses notice and have a significant impact as an indicator of quality and safety of a product.[9] However, color is not just significant in these situations; in fact, some coloring agents, or more specifically, natural colorants, may offer some advantages to the consumer, mainly due to the bioactive properties they entail.[10,11] Pigments found in plants are not only responsible for the vivid colors of some plants, but also play important functions in their metabolism, attracting pollinators, and chemical defenses.[12] Curcumins,[13] chlorophylls,[14] and anthocyanins[15] are only a few of the families of naturally occurring molecules that have the ability to color. Stability issues with natural pigments can be a challenge when using them in foods, but modern scientific knowledge and methodologies can help overcome these obstacles.[9] Natural colorants have an added cost for procuring and transforming natural ingredients, which is why bio-waste from various industrial processes is a great way to lower the cost of acquiring them while adhering to the principles of a circular economy.[16] For instance, betacyanins, which have not received much attention, may have positive impacts on human health in addition to adding color, since they present various bioactivities. Although this particular class of compounds already exists as a natural substitute on the market (E162), they are preferably recovered from beetroot (*Beta vulgaris* L.), though there are other sources available. Examples of this include fruits and peels from *Hylocereus* spp.[17] and flowers from *Gomphrena*

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globosa L.[18,19] and *Amaranthus caudatus* L.,[20] which have all been investigated as potential sources of betacyanins. In previous works, Roriz *et al.*[17,20] optimised the recovery of these particular compounds by ultrasound assisted extraction (UAE) with the support of a response surface methodology (RSM). This study used betacyanins extracted from *Amaranthus caudatus* L. (Amaranthaceae) and *Hylocereus costaricensis* (F.A.C. Weber) Britton and Rose (Cactaceae). The results were used to evaluate their performance as coloring agents in two types of food products: savory and sweet. The savory product was a tagliatelle pasta, while the sweet product was sweet meringue cookies. The goal was to determine if these compounds could improve color parameters and their effect on nutritional composition, individual sugars and fatty acid profiles.

2. Materials and methods

2.1. Plant samples

Amaranthus caudatus L. plants were acquired from "Cantinho das Aromáticas", a Portuguese company located in Gaia, Portugal. The peel samples of *H. costaricensis* were kindly provided by Ángel Ferrero, from "Pitayas de Galicia", a research project on the acclimatization and cultivation of this fruit in the region. The botanical identification was made by a botanist from the herbarium of Escola Superior Agrária (BRESA), Polytechnic Institute of Bragança (Portugal). The *A. caudatus* flowers were manually separated from the rest of the plant, and frozen. The *H. costaricensis* peels were frozen, and were subsequently lyophilised (FreeZone 4.5, Labconco, Kansas City, MO, USA) along with the flowers, ground to 20 mesh, and kept in a container protected from light and humidity at −20 °C until further analysis.

2.2. Preparation of betacyanin's enriched extracts

A. caudatus extracts enriched in betacyanins were obtained using an UAE (ultrasonic homogeniser, model CY-500, Optic Ivymen Systen, Barcelona, Spain; 20 kHz frequency) equipped with a titanium probe, working at 500 W, for 13.3 min, using water (treated in a Milli-Q water purification system, TGI Pure Water Systems, Greenville, SC, USA) as the preferred extraction solvent and a liquid-to-solid ratio of 5 g L^{-1}, as described previously by Roriz *et al.* (2021). *H. costaricensis* betacyanin-enriched extracts were obtained using an UAE working at 487 W, for 38 min, resorting to the same extraction solvent and solid–liquid ratio, following the procedure previously reported by Roriz *et al.* (2022). The obtained extracts were then dried by lyophilization (FreeZone 4.5, Labconco, Kansas City, MO, USA).

2.3. Preparation of the tagliatelle pasta and meringue cookies with different coloring agents

2.3.1. Tagliatelle pasta preparation. To prepare the tagliatelle pasta, 900 g of flour (self-raising wheat flour; composition per 100 g: 1421 kJ or 333 kcal; 1 g of lipids, of which 0.3 g were saturated; 71 g of carbohydrates, of which 2 g were sugars; 3 g of fibre; 9 g of protein; and 2 g of salt) was mixed with 22.5 g of salt, 450 g of water and 72 g of olive oil (composition per 100 mL: 3378 kJ or 822 kcal; 91 g of lipids, of which 13 g were saturated; 0 g of carbohydrates; 0 g of protein; and 0 g of salt) with a stand mixer (Food Processor SKM 550 A1, SilverCrest, Hamburg, Germany). The tagliatelle pasta dough was divided into three equivalent parts and identified as: (i) control (tagliatelle pasta dough without coloring agents); (ii) tagliatelle pasta dough colored with E162 (commercial natural food colorant – 2 g, *i.e.* ≈4 mg per 100 g pasta dough); and (iii) tagliatelle pasta dough colored with freeze-dried *A. caudatus* extract – 1 g, *i.e.* ≈2 mg per 100 g pasta dough; these colorant doses were chosen to ensure that the concentration of the betacyanins in both colorants were comparable. For each batch, the tagliatelle pasta dough was divided into approximately 133 g, and then stretched and cut into thin strips, using a fresh pasta manual machine. The dough was then wrapped in flour and left to dry for 15 min at room temperature. After the drying process, the tagliatelle pasta was packed in zip lock bags, and stored at room temperature for the subsequent analysis at different storage times. Prior to the analysis, all tagliatelle pasta samples were lyophilized, finely crushed and analyzed (in triplicate) immediately after preparation and at two more sampling times (7 and 14 days of storage).

2.3.2. Meringue cookie preparation. To prepare the meringue cookies, 132 g of pasteurized liquid egg white (pasteurized liquid egg white; composition per 100 g: 208 kJ or 49 kcal; 0.5 g of lipids, of which 0.5 g were saturated; 0.7 g of carbohydrates, of which 0.5 g were sugars; 7 g of protein; and 0.38 g of salt) was mixed with 0.75 g of salt, 140 g of powdered granulated sugar (granulated white sugar; composition per 100 g: 1700 kJ or 400 kcal; 0 g of lipids; 100 g of carbohydrates, of which 100 g were sugars; 0 g of fibre; 0 g of protein; and 0 g of salt) and 0.77 g of cream of tartar (composition per 100 g: 1031 kJ or 238 kcal; 0 g of lipids, of which 0 g are saturated; 79.3 g of carbohydrates; 0 g of protein; and 0 g of salt) with a stand mixer. The meringue cookie mixture was made separately for each of the different batches and identified as: (i) control (meringue cookie dough without coloring agents); (ii) meringue cookie dough colored with E162 (commercial natural food colorant; 2 g, *i.e.* ≈7 mg per 100 g meringue dough); and (iii) meringue cookie dough colored with freeze-dried *H. costaricensis* extract (2 g, *i.e.* ≈7 mg per 100 g meringue dough). For each batch of the meringue cookies, dough was piped into individual meringue cookies on a tray lined with parchment paper and baked for 60 min at ≈90 °C. After baking, the meringue cookies were left to cool inside of the oven for approximately 1–2 hours, and separated to be analysed at three different storage times, using 20 cookies at each time of analysis. Prior to the analysis, all cookie samples were lyophilized, finely crushed and analysed (in triplicate) immediately after preparation and at two more sampling times (7 and 14 days of storage). Meringue cookies were packed in a sealed plastic bag and stored at room temperature.

2.4. Chemical composition of the different foods

2.4.1. Nutritional value. The samples (tagliatelle pasta and meringue cookies) were meshed and the macronutrients

(moisture, proteins, fat, carbohydrates, and ash) were analysed in accordance with the AOAC protocols,[21] while the crude protein content (Nx6.25) was calculated following the macro-Kjeldahl method, the crude fat content was determined by Soxhlet extraction with petroleum ether, the ash content was determined by incineration at 600 ± 15 °C, the total carbohydrate content was determined by difference, and the energetic value was determined using the following equation: energy (kcal) = 4 (g protein + g carbohydrate) + 9 (g fat).

2.4.2. Organic acids. The samples (2 g) were extracted with 25 mL of metaphosphoric acid (25 °C, 150 rpm, 45 min) and subsequently centrifuged (10 000*g* for 5 min) and then filtered through 0.2 μm Whatman nylon filters. The organic acids were determined using a high-performance liquid chromatograph coupled to a photodiode detector (UFLC-PDA) according to a procedure previously described by Barros *et al.* (2013).[22] The detection of organic acids was achieved using a DAD system, applying a wavelength of 215 nm (and 245 nm for ascorbic acid). The quantification of the compounds was carried out by comparing the area of their recorded peaks at the wavelengths mentioned above with the calibration curves obtained from the standards of the respective compound. The results were expressed in g per 100 g (fw).

2.4.3. Soluble sugars. The internal standard technique (IS, melezitose, Sigma-Aldrich, St Louis, MO, USA) as previously reported by Barros *et al.* (2013)[23] was used to determine the soluble sugars using a HPLC connected to a refraction index (RI) detector (Knauer, Smartline System 1000, Berlin, Germany). Acetonitrile : water (70 : 30 v/v, acetonitrile HPLC-grade, Lab-Scan, Lisbon, Portugal) was used as the mobile phase, and a Eurospher 100–5 NH_2 column (4.6 250 mm, 5 m, Knauer) was used to separate the compounds, while Clarity v.2.4 software was used to process the results (DataApex, Prague, Czech Republic). The results were expressed in g per 100 g of dry weight.

2.4.4 Fatty acids. A gas chromatograph (DANI1000, Contone, Switzerland) equipped with a split/splitless injector and a flame ionisation detector (GC-FID at 260 °C) and operating under the parameters mentioned by Barros *et al.* (2013)[23] was used to determine the fatty acids. By comparison of the relative retention times of fatty acid methyl esters (FAME) reference standard mixture, (Sigma-Aldrich, St Louis, MO, USA), it was possible to identify and quantify the fatty acids. CSW 1.7 software was used to record and process the results (Data Apex 1.7, Prague, Czech Republic). The results were expressed in relative percentage.

2.5. Stability of the prepared cookie formulations

2.5.1. Physical parameters

2.5.1.1. Moisture. The moisture content of the tagliatelle pasta and meringue cookie samples was measured using a moisture analyser (Adam Equipment, PMB 163). This apparatus progressively increases the temperature to 105 °C to drive out moisture from the food sample. The sample is weighed again once the weight reaches a constant value. The calculations were done using the formula: moisture % = (iw − fw)/iw × 100, where fw is the weight after obtaining a constant weight and iw is the starting weight.

2.5.1.2. Color analysis. The exterior color as well as the powder of the ground samples at three distinct regions on the tagliatelle pasta and meringue cookies were measured at each storage time. The D65 illuminant, a standard illuminant established by the International Commission on Illumination (CIE), was used to measure the color readings using a portable CR400 colorimeter from Konica Minolta (Chiyoda, Tokyo, Japan). This illuminant simulates the noon light in Europe (daylight illuminant). With a 10° observation angle and an 8 mm aperture, the CIE L^* a^* b^* color space from 1976 was used, where L^* stands for lightness, a^* for redness (red-green), and b^* for yellowness (yellow-blue).

2.5.2. Microbiological analysis. The stability of both prepared foods was examined with regard to microbial growth control over shelf life. Briefly, 1 g of powdered and homogenised tagliatelle pasta or meringue cookie was added to 9 mL of peptone water (PW, Liofilchem, Italy). From this suspension, successive decimal dilutions were made until 10^{-3} mL was reached. Following that, several counts were made:

2.5.2.1. Total viable count (aerobic plate count; ISO 4833-2:2013). Using the pour plate technique, 1 mL of the produced suspensions was inoculated into 15 mL of melted PCA (plate count agar, Liofilchem, Italy), in duplicate; LOQ = 1 log CFU per g. The plates were incubated upside down for 48 to 72 hours at 30 °C. Colonies were counted on plates with 15 to 300 colonies.

2.5.2.2. Coliforms (including E. coli; ISO 4832:2006). The pour plate technique was used to inoculate 1 mL of the produced suspensions in 15 mL of melted VRBLA (violet red bile lactose agar, Liofilchem, Italy), in duplicate; LOQ = 1 log CFU per g. After being homogenised, the plates were allowed to harden and a top layer of 4 mL of VRBLA was added to the medium. The plates were then incubated upside down for 48 hours at 30 °C. Only plates with 10 to 150 colonies were subjected to counting.

2.5.2.3. Mold and yeast (ISO 21527-1:2008). By using the spread plate technique, 0.2 mL of the produced suspensions was pipetted onto a plate containing 15 mL of DRBC (dichloran rose bengal chloramphenicol, Liofilchem, Italy), in duplicate; LOQ = 1.7 log CFU per g. The plates were then incubated for five days at 25 °C in the upright position. Counting was done on plates with less than 150 colonies; after 3 and 5 days of incubation, respectively, yeast and mold colonies were counted separately.

2.5.2.4. Bacillus cereus (ISO 7932:2004). Using the spread plate technique, 0.2 mL of the produced suspensions was pipetted onto a plate containing 15 mL of MYP (mannitol yolk polymyxin, Liofilchem, Italy, in duplicate; LOQ = 1.7 log CFU per g). The plates were placed upside down and incubated at 30 °C for 24–48 hours. The plates with 10 to 150 colonies were used for the counting.

The microbiological loads of the tagliatelle pasta and meringue cookies were evaluated immediately after their production (T0), after seven days (T7) and after fourteen days (T14).

2.6. Statistical analysis

The mean and standard deviation were used to express all the data in the study. Using SPSS Software, version 25, a two-way ANOVA was performed using type III sums of squares for the characterization of both the tagliatelle pasta and the meringue cookie. The two variables, storage time (ST) and colorant type (CT), were treated as independent in this multivariate general linear model. This allows the effects of each variable to be examined separately and offers more insight into how each element contributed to the final result. The estimated marginal means (EMM) plots were used to draw some general conclusions and tendencies if a significant interaction ($p > 0.05$) between the two components (ST × CT) was observed. If a significant interaction was not found ($p > 0.05$), each component was then categorised independently using Tamhane's T2 test for non-homoscedastic samples, and a Tukey's multiple comparison test for homoscedastic means. Levene's test was employed to assess homoscedasticity.

For the microbiological analysis, the results were treated in the same manner, using one-way ANOVA and the same tests for different levels of homoscedasticity. The significance level throughout the study was set at 0.05.

3. Results and discussion

Natural alternatives are being searched to address the harmful effects of artificial food additives, to promote innovation in the food industry. When choosing natural colorants, specifications of each food type must be taken into account. Several studies have been conducted on the replacement of synthetic coloring additives with natural counterparts, for instance, in yogurts,[24] candies,[7] confectionary products,[25] ice creams,[26] and baked products,[27] among others. This research topic focuses not only on the replacement of artificial food colorants, but also on improving the nutritional properties of some of these products due to the fact that natural food colorants have some interesting bioactivities.

3.1. Nutritional profile

For the determination of the chemical profile, as stated in the Statistical analysis section, a 2-way analysis of variance was applied, allowing for an individual assessment of each parameter, and at the same time allowing the assessment of the interaction between them. In this way, Tables 1–3 are divided in two sections, the upper part regarding the results obtained for the tagliatelle pasta, and the lower part – the results obtained for the meringue cookies. Each of these parts is then divided in two more sections: the upper part representing the different STs, and the lower part representing the CT. In this sense, for each tested ST in the upper part of the table, all tested CTs are included, and for each tested CT in the lower part of the table, all STs are also included. This representation allows for the aforementioned individual assessment of each parameter, which means that the standard deviation should not be regarded as the accuracy of the analysis, but rather a range of values for the non-fixed parameter (CT or ST). If between these parameters, a significant interaction is detected, by presenting a p-value of CT × ST lower than 0.05, no multiple comparisons can be extracted, which means that both parameters (CT and ST) significantly contributed for the changes, only allowing tendencies to be obtained from the Estimated Marginal Means (EMM) plots. Conversely, if the p-value of CT × ST is higher than 0.05, each parameter is analysed individually.

3.1.1. Tagliatelle pasta. In Table 1, in the left section, the centesimal profile is presented, in the middle section – the organic acids are shown, and the right section represents the soluble sugars; all expressed in g per 100 of dry weight. The centesimal profile includes fat, protein, ash, carbohydrates and energy values (expressed as 100 g of fw weight). The nutrients detected in a higher amount were carbohydrates, which was expected since the main ingredient in the tagliatelle pasta is flour, followed by protein and fat. A non-significant interaction was detected among the two analysed parameters (ST × CT > 0.05) for ash and energy, meaning that for these nutrients, ST and CT contributed significantly for the obtained results. Only general tendencies could be extracted from the estimated marginal means (EMM) plots for them (Fig. 1). For fat and proteins, there were significant differences over the storage time, showing a decrease in these nutrients, especially from the 7th day onwards. In terms of the colorant type, the commercial and *Amaranthus*-incorporated samples showed lower fat, although the samples revealed a significantly higher amount of proteins. For carbohydrates, a statistically significant increase was detected from day 0 to day 7 and the control samples showed a significantly higher amount when compared to the other two samples in terms of colorant incorporation. Ash and energy (Fig. 1A and B), obtained from the EMM showed a decrease over time, although overall the control sample was the one with the least amount of ash and the highest energy. Two organic acids were detected: malic acid as the major compound and oxalic acid as the minor. A significant interaction among the two analysed parameters (ST × CT < 0.05) was also detected, which hindered any individual assessment of the individual contribution of each parameter. For the soluble sugars, where the highest amount was found for trehalose, followed by sucrose, fructose and finally glucose, the only compounds that allowed for classification was sucrose. Thus, a significant decrease in this sugar was detected over time, probably due to a breakdown into monosaccharides, while also being present in a significantly higher amount in the commercially colored tagliatelle, followed by the control sample, and only trace amounts in the *Amaranthus* colored samples.

3.1.2. Meringue cookies. Similar to what was done for the tagliatelle pasta samples, the same parameters were evaluated for the meringue cookies. From the results shown in Table 1, carbohydrates followed by proteins were the most abundant nutrients. Only oxalic acid was detected in terms of organic acids, and only one soluble sugar was detected, which was sucrose. All but the proteins showed a significant interaction (ST × CT < 0.05), meaning that both factors had an influence on the outcome. No tendencies could be obtained from the

Table 1 Nutritional profile of the different tagliatelle pasta types (upper part of the table) and the different meringue cookies (down part of the table), along the 14-day storage time (left section); expressed in g per 100 g of fresh weight (fw); organic acids (middle section) and soluble sugars (right section) are represented in g per 100 g of dry weight (dw)

							Organic acids			Soluble sugars				
Tagliatelle pasta		Fat	Proteins	Ash	Carbohydrates	Energy, kcal	Oxalic	Malic	Total	Glucose	Fructose	Sucrose	Trehalose	Total sugars
Storage time (ST)	0 day	6.54 ± 0.26[c]	9.71 ± 0.22[b]	1.83 ± 0.49	81.9 ± 0.40[a]	425 ± 3.00	0.07 ± 0.01	0.18 ± 0.03	0.25 ± 0.02	0.09 ± 0.13	0.11 ± 0.16	0.24 ± 0.20[b]	1.38 ± 0.16	1.82 ± 0.26
	7 days	6.27 ± 0.13[b]	9.65 ± 0.19[b]	1.74 ± 0.43	82.3 ± 0.42[b]	427 ± 2.35	0.0669 ± 0.0004	0.12 ± 0.02	0.19 ± 0.02	0.06 ± 0.09	0.07 ± 0.11	0.22 ± 0.17[a,b]	1.32 ± 0.11	1.68 ± 0.16
	14 days	6.08 ± 0.26[a]	9.39 ± 0.29[a]	1.58 ± 0.38	82.9 ± 0.57[c]	424 ± 2.33	0.06 ± 0.01	0.12 ± 0.02	0.18 ± 0.01	0.06 ± 0.09	0.11 ± 0.16	0.21 ± 0.17[a]	1.33 ± 0.23	1.71 ± 0.32
p-Value (n = 9)	ANOVA	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	<0.001	<0.001	<0.001	<0.001	0.006	0.028	<0.001
Colorant type (CT)	Control	6.42 ± 0.35[b]	9.43 ± 0.27[a]	1.16 ± 0.13	82.9 ± 0.52[b]	427 ± 1.68	0.06 ± 0.01	0.15 ± 0.05	0.21 ± 0.06	tr	tr	0.25 ± 0.02[b]	1.24 ± 0.11	1.50 ± 0.13
	Commercial	6.37 ± 0.27[b]	9.60 ± 0.24[a,b]	1.88 ± 0.12	82.1 ± 0.53[a]	424 ± 1.07	0.081 ± 0.003	0.11 ± 0.02	0.20 ± 0.02	tr	tr	0.42 ± 0.04[c]	1.24 ± 0.05	1.66 ± 0.05
	Amaranthus	6.11 ± 0.11[a]	9.72 ± 0.24[b]	2.12 ± 0.17	82.0 ± 0.36[a]	422 ± 0.41	0.062 ± 0.003	0.16 ± 0.02	0.22 ± 0.02	0.21 ± 0.03	0.29 ± 0.05	tr[a]	1.56 ± 0.09	2.05 ± 0.14
p-Value (n = 9)	ANOVA	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ST × CT (n = 27)	*p*-Value	0.052	0.063	0.013	0.120	0.010	<0.001	<0.001	<0.001	<0.001	<0.001	0.110	<0.001	<0.001

		Fat	Proteins	Ash	Carbohydrates	Energy, kcal	Organic acids Oxalic	Soluble sugars Sucrose
Meringue cookies								
Storage time (ST)	0 day	0.07 ± 0.03	6.53 ± 0.28[a]	1.34 ± 0.16	92.0 ± 0.30	395 ± 0.49	0.11 ± 0.06	301 ± 7
	7 days	0.11 ± 0.02	6.63 ± 0.36[a]	1.39 ± 0.34	91.9 ± 0.41	395 ± 1.26	0.11 ± 0.06	331 ± 11
	14 days	0.16 ± 0.08	6.71 ± 0.31[a]	1.33 ± 0.13	91.8 ± 0.25	395 ± 0.64	0.10 ± 0.05	299 ± 21
p-Value (n = 9)	ANOVA	<0.001	0.125	0.078	0.003	0.008	<0.001	<0.001
Colorant type (CT)	Control	0.11 ± 0.02	6.86 ± 0.03[b]	1.27 ± 0.18	91.8 ± 0.24	395 ± 0.72	0.051 ± 0.004	309 ± 21
	Commercial	0.12 ± 0.10	6.39 ± 0.29[a]	1.23 ± 0.10	92.2 ± 0.34	396 ± 0.39	0.082 ± 0.002	300 ± 14
	Pitaya	0.11 ± 0.02	6.61 ± 0.27[a]	1.56 ± 0.22	91.7 ± 0.26	394 ± 0.74	0.183 ± 0.008	323 ± 10
p-Value (n = 9)	ANOVA	0.373	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ST × CT (n = 27)	*p*-Value	<0.001	0.080	<0.001	<0.001	<0.001	<0.001	<0.001

tr – trace amounts. In each row, different letters mean statistically significant differences. The presented standard deviations were calculated from the results obtained under different operational conditions. Thus, they should not be regarded as a measure of precision, rather as a range of values.

Table 2 Individual fatty acids – MUFA, PUFA and SFA of the different fresh pastas (upper part of the table) and the different meringue cookies (down part of the table) along the 14 days of storage time, represented as a relative percentage of themselves

		C16:0	C16:1	C17:0	C17:1	C18:0	C18:1n9c	C18:2n6c	C18:3n3	C24:0	SFA	MUFA	PUFA
Tagliatelle pasta													
Storage time (ST)	0 day	17 ± 1	1.8 ± 0.3	0.18 ± 0.03	0.31 ± 0.07	2.39 ± 0.42	68.8 ± 2.95	8.64 ± 0.77	0.94 ± 0.10	0.10 ± 0.14	19.4 ± 1.83	71.0 ± 2.57	9.57 ± 0.86
	7 days	16.5 ± 0.43	1.69 ± 0.07	0.29 ± 0.11	0.31 ± 0.03	2.26 ± 0.11	70.5 ± 0.89	7.51 ± 0.62	0.88 ± 0.04	0.10 ± 0.15	19.2 ± 0.60	72.4 ± 0.98	8.34 ± 0.68
	14 days	19 ± 2	2.00 ± 0.07	0.53 ± 0.18	0.32 ± 0.03	2.63 ± 0.29	71.9 ± 0.72	3.25 ± 1.08	0.42 ± 0.26	0.06 ± 0.09	22.1 ± 1.83	74.2 ± 0.70	3.70 ± 1.32
p-Value (n = 9)	ANOVA	<0.001	<0.001	<0.001	0.014	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Colorant type (CT)	Control	17 ± 2	1.80 ± 0.17	0.34 ± 0.24	0.29 ± 0.03	2.41 ± 0.41	71.0 ± 0.15	5.97 ± 3.07	0.65 ± 0.37	tr.	20.3 ± 3.10	73.1 ± 0.34	6.62 ± 3.44
	Commercial	16.5 ± 0.4	1.75 ± 0.20	0.42 ± 0.19	0.29 ± 0.02	2.38 ± 0.25	70.9 ± 1.39	6.66 ± 1.95	0.82 ± 0.01	0.26 ± 0.05	19.6 ± 0.77	72.8 ± 1.57	7.49 ± 2.00
	Amaranthus	18 ± 1	1.97 ± 0.23	0.24 ± 0.04	0.36 ± 0.03	2.49 ± 0.34	69.3 ± 3.32	6.78 ± 2.42	0.76 ± 0.31	tr.	20.9 ± 1.30	71.6 ± 3.10	7.51 ± 2.67
p-Value (n = 9)	ANOVA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ST × CT (n = 27)	*p*-Value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

		C16:0	C18:0	C18:1n9c	SFA	MUFA
Meringue cookies						
Storage time (ST)	0 day	41.1 ± 8.6	16.3 ± 6.99	43.3 ± 15.2	57.5 ± 15.8	41.6 ± 16.5
	7 days	40.4 ± 5.41	16.8 ± 6.29	42.5 ± 11.8	57.4 ± 11.7	42.5 ± 11.7
	14 days	40.4 ± 3.9	20.7 ± 3.35	38.9 ± 5.67	61.0 ± 5.54	38.8 ± 5.8
p-Value (n = 9)	ANOVA	0.338	<0.001	<0.001	<0.001	<0.001
Colorant type (CT)	Control	33.6 ± 3.46	10.8 ± 4.46	55.8 ± 7.67	44.3 ± 7.59	55.7 ± 7.49
	Commercial	43.5 ± 5.16	22.9 ± 1.60	34.1 ± 4.99	66.5 ± 5.58	32.7 ± 6.61
	Pitaya	45.0 ± 0.67	20.3 ± 1.52	34.8 ± 2.03	65.2 ± 1.92	34.6 ± 2.06
p-Value (n = 9)	ANOVA	<0.001	<0.001	<0.001	<0.001	<0.001
ST × CT (n = 27)	*p*-Value	<0.001	<0.001	<0.001	<0.001	<0.001

tr. – trace amounts. C16:0 – palmitic acid; C16:1 – palmitoleic acid; C18:0 – stearic acid; C18:1n9 – oleic acid; C18:2n6 – linoleic acid; C18:3n3 – alpha linolenic acid; C24:0 – lignoceric acid. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. In each row, different letters mean statistically significant differences. The presented standard deviations were calculated from the results obtained under different operational conditions. Thus, they should not be regarded as a measure of precision, rather as a range of values.

Table 3 Representation of the external (tagliatelle pasta), and the pasta powder (milled sample) colors (upper part of the table) and the same for meringue cookies (down part of the table) according to the *L**, *a**, *b** color space

		External color			Milled sample powder		
		*L**	*a**	*b**	*L**	*a**	*b**
Tagliatelle pasta							
Storage time (ST)	0 day	72 ± 11^{a}	9 ± 8	16 ± 7^{a}	79 ± 3	6 ± 5	15 ± 6
	7 days	73 ± 9.0^{a}	11 ± 8	16 ± 6^{a}	78 ± 3	7 ± 5	13 ± 4
	14 days	76 ± 8.0^{a}	9 ± 6	15 ± 6^{a}	78 ± 4	7 ± 5	12 ± 3
p-Value ($n = 9$)	ANOVA	0.023	0.181	0.002	0.08	0.317	0.072
Colorant type (CT)	Control	83 ± 1.2^{c}	1.4 ± 0.2^{a}	22 ± 2^{c}	78 ± 1	1.3 ± 0.2^{a}	17 ± 2^{b}
	Commercial	76 ± 2^{b}	9.9 ± 2.8^{b}	18 ± 1^{b}	75 ± 1	6.6 ± 1.5^{b}	$14 \pm 3^{a,b}$
	Amaranthus	62.0 ± 6.5^{a}	17 ± 5^{c}	8 ± 1^{a}	78 ± 2	12.5 ± 3.2^{c}	9 ± 4^{a}
p-Value ($n = 9$)	ANOVA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ST × CT ($n = 27$)	*p*-Value	0.739	0.521	0.364	<0.001	0.527	0.786
Meringue cookies							
Storage time (ST)	0 day	83.0 ± 5.3	5.2 ± 6.2	3.52 ± 2.05	89.5 ± 2.2	6.03 ± 4.74	7.6 ± 5.5
	7 days	82.8 ± 5.9	5.3 ± 6.6	4.1 ± 2.3	89.5 ± 1.7	6.2 ± 4.7	8.3 ± 5.9
	14 days	82.2 ± 5.5	6.13 ± 7.7	4.2 ± 2.2	89.7 ± 2.1	5.7 ± 4.7	7.3 ± 5.2
p-Value ($n = 9$)	ANOVA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Colorant type (CT)	Control	90.2 ± 0.6	-1.28 ± 0.02	4.3 ± 0.8	92.0 ± 0.3	1.1 ± 0.3	12.1 ± 1.1
	Commercial	78.1 ± 0.7	3.4 ± 0.1	6.3 ± 0.3	89.5 ± 0.2	4.6 ± 0.3	10.9 ± 0.3
	Pitaya	79.6 ± 0.6	14.5 ± 1.4	1.24 ± 0.06	87.2 ± 0.2	12.2 ± 0.4	0.2 ± 0.1
p-Value ($n = 9$)	ANOVA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ST × CT ($n = 27$)	*p*-Value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

In each row and within each storage period, different letters mean significant statistical differences between different colorant type ($p < 0.05$). The presented standard deviations were calculated from results obtained under different operational conditions. Thus, they should not be regarded as a measure of precision, rather as a range of values.

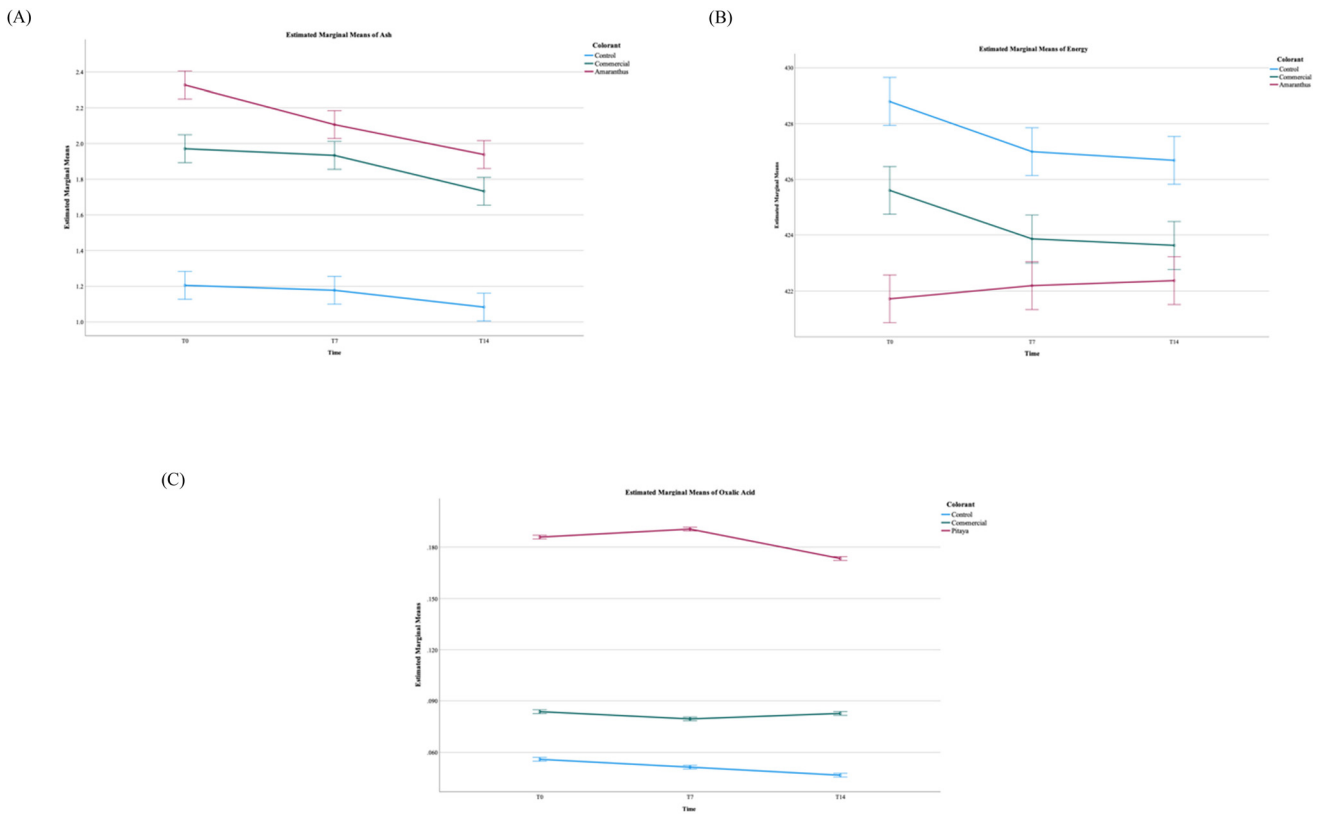

Fig. 1 EMM plots of ash (A) and energy (B) for the tagliatelle pasta and oxalic acid (C) for the meringue cookies.

EMM plots for the nutritional values or sucrose, although from the "partial eta squared" values, the energy variance was seen to be significantly higher for the colorant type, meaning the colorants had a higher influence in the variation of the energy values. For proteins, no significant changes were sought during the storage time, although the control meringue samples were the ones with significantly higher values. The EMM plots showed that the samples colored with pitaya had higher values of oxalic acid than the commercial samples, with little variation in value over time.

3.2. Fatty acids

3.2.1. Tagliatelle pasta. Table 2 shows the individual fatty acids of tagliatelle pasta, expressed in relative percentage, as well as the grouped molecules, namely saturated fatty acids (SFA) – monounsaturated (MUFA) and polyunsaturated (PUFA), detected through GC-FID along the storage time of 14 days. The most abundant individual fatty acid was oleic acid (C18:1), followed by palmitic acid (C16:0) and linoleic acid (C18:2). This presented profile was due to the olive oil ingredient. The MUFA was the prevalent group with values of approximately 70% of the total amount, while SFA reached roughly 20% and PUFA only 10%. Regarding statistical classifications, a significant interaction was sought for all instances (ST × CT < 0.05), meaning that both storage time and colorant type had an influence on the behavior of the fatty acids. Only C17:0 (margaric acid) showed a valid EMM plot (Fig. 2A), where an increase of this fatty acid was sough over time, although the tagliatelle pasta colored with *Amaranthus* showed a smaller amount of this molecule. With these results, it can be concluded that a low influence was observed on the fatty acids by the different types of colorants and storage time.

3.2.2. Meringue cookies. Table 2 shows the individual fatty acids – SFA, MUFA and PUFA of the meringue cookies. Only three fatty acids were detected in these samples, the oleic acid being the major compound (C18:1), followed by palmitic acid (C16:0) and finally stearic acid (C18:0). The presence of these specific compounds was due to the fatty acid profile of the egg whites, which presents the same three fatty acids described above. For all fatty acids, a significant interaction was detected and the profile presented p-values <0.05 for the interaction between the two analysed parameters, meaning that a significant interaction occurs between the two parameters analysed, enabling us to obtain further conclusions.

3.3. Color stability over storage time

3.3.1. Tagliatelle pasta. To ensure the coloring capacity of the used colorants, the color, measured using a portable colorimeter, was analysed on the tagliatelle stripes and in the powder of the milled tagliatelle pasta. This provides better knowledge of the homogeneity of the dispersion capacity of the colorants. The CIELab color space was used to obtain the coordinates of L^* (lightness, variation between −100 and +100, black to white), a^* (greenness and redness, variation between −100 and +100, red to green), and b^* (yellowness/blueness, variation between −100 and +100, yellow to blue). In Table 3, it can be seen that a significant interaction was only sought for L^* of the milled tagliatelle stripes, meaning that only for this coordinate did both factors significantly influence the outcome. Considering the lightness (L^*) of the stripes, there is a significantly lighter hue for the control sample, followed by the commercial sample, and the *Amaranthus* colored sample being the darkest one. Still, over time, no significant differences were detected. The exact same occurred for b^*, in which the control sample showed a yellow hue, while the *Amaranthus* sample showed a tint towards blue, however, over time no significant difference was found. For a^*, the total opposite was found, meaning that statistical differences were found between the different colorants, although the *Amaranthus* colored one showed higher values of a^* – a yellower hue. Considering the storage time for a^* of the pasta stripes, no classifications could be performed. Regarding the milled samples, the same color schemes and differences were recorded, meaning that the colorants showed a satisfactory homogeneous coloring capacity.

Fig. 3 shows the actual color of the tagliatelle stripes and powder obtained from the CIELab coordinates. As can be noticed, the *Amaranthus* colored sample showed a more intense pink coloration when compared to the commercially colored sample.

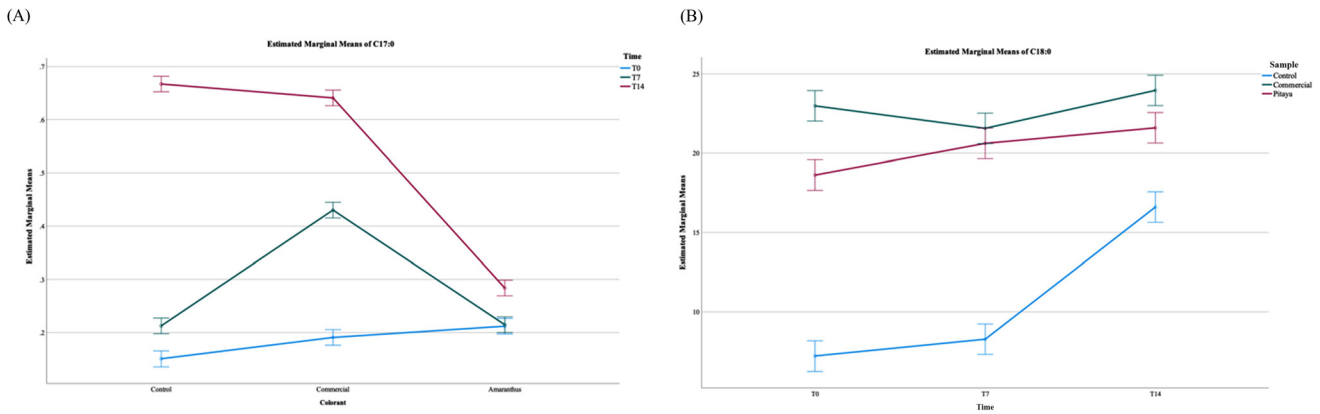

Fig. 2 EMM plots of C17:0 for the tagliatelle pasta (A) and C18:0 for the meringue cookies (B).

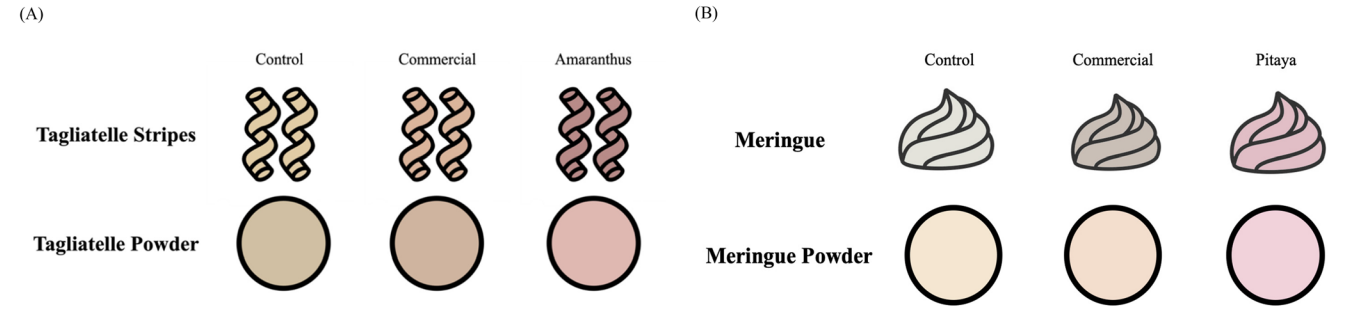

Fig. 3 Representation in RGB colors of the stripes and milled samples of the tagliatelle pasta (A) and the meringue cookies (B) obtained from the conversion of the L^*, a^* and b^* coordinates.

In terms of the homogeneity of the samples, there was barely any difference from the external color and the milled sample. Overall, in terms of coloring, *Amaranthus* proved to be a successful candidate as a natural colorant for tagliatelle pasta.

3.3.2. Meringue cookies. Table 3 shows the external colors of the meringue samples as well as their milled powder. For all parameters, a significant interaction was found, and thus, only general conclusions could be extracted from the EMM plots, as shown in Fig. 1 (ESI†). Regarding L^* of the external color of the meringue samples (Fig. 1A; ESI†), the darkest samples seemed to be those of the pitaya and commercial colored ones (E162), while the control was the lightest. For the redness (Fig. 1B; ESI†), the naturally colored sample showed a higher hue of red, while the control sample was the one with the lowest amount of red. Inversely, for the b^* (Fig. 1C; ESI†), the pitaya colored sample showed the lowest values, indicating a shift towards the blue zone, while the control sample shifted towards the yellow quadrant. The commercially colored meringue sample showed the highest yellow hue with low changes over time. Overall, the meringue colored sample with pitaya flesh showed a vivid pink color, while the commercial colored sample displayed a dark grey tone (as can be confirmed in Fig. 3), obtained from converting the L^*, a^* and b^* coordinates to RGB colors. Furthermore, the milled powder of the meringue samples was also analysed to understand the homogeneity of the colorants throughout the whole sample. In Fig. 1D (ESI†), in terms of lightness (L^*), the lighter samples can be seen as the control ones, followed by the commercial colored, and finally, the pitaya colored meringue samples, which were the darkest. For a^* (Fig. 1E; ESI†), the complete opposite was detected, with the pitaya colored sample being the most red, and the control sample – the one with the least red hue. Finally, for b^* (in Fig. 1F; ESI†), the pitaya sample showed a central value, with no tint towards yellow or blue, while the other two samples showed 10 to 13 units towards a yellow tone. Considering Fig. 3, the powder of the control and commercially colored meringue samples seemed to have an orange hue, clearly different from the external color, while the pitaya colored meringue sample had a pink tint. Pitaya's color showed higher homogeneity than the commercial colorant, matching the color found in the meringue cookie powder with the external color of the cookies.

3.4. Microbiological analysis

To understand the microbial stability over time, the samples were subject to a microbial analysis over the 14 days of storage.

3.4.1. Tagliatelle pasta. The assessment of the microbial load on the tagliatelle pasta showed some growth, namely for the aerobic mesophilic microorganisms, *Enterobacter*, mold and yeast (Fig. 2; ESI†). These contaminants probably originated from the raw materials, in particular, the wheat flour. However, it should be noted that the initial function of these extracts was that of a coloring effect, and their antimicrobial capacity was only an added benefit.

3.4.2. Meringue cookies. Regarding the microbial load for the meringue cookies, growth was only seen for the aerobic mesophilic microorganisms (Fig. 3; ESI†). Nevertheless, the colorant recovered from the pitaya peels exhibited results that were just as good as those of the natural commercial colorant, proving to be a fantastic feasible substitute for this natural colorant.

4. Conclusions

Natural food colourants are not only a healthier alternative because coloured foods are currently very popular, but it also complies with customer demands for the food sector, which helps these new products gain widespread consumer acceptance. When compared to a natural commercial food colorant (E162), the tagliatelle pasta and meringue cookies colored with the natural extracts recovered from Ac (*Amaranthus caudatus*) and Hc (*Hylocereus costaricensis*) displayed a deeper pink coloration.

Additionally, the strong hue was maintained along the stored time (ST), demonstrating the potent coloring potential and stability from betalains. The color of the tagliatelle pasta and meringue cookies and the powder produced after the milling process of the two products indicated how evenly the extracts were distributed – a very significant aspect that contributes to the homogeneous hue of the finished product. The tagliatelle pasta and meringue cookies were colored with the extracts obtained from Ac and Hc, respectively; besides showing no signs of color loss over time, they also showed a general tendency for the microbial load to decline between T0

and T7, which is a bonus for the extracts, given their primary goal to have a strong coloring effect. The high content of betalains in the extracts, a well-known compound with a variety of bioactivities, was responsible for the presence of antibacterial activity. Additionally, the incorporation had little to no effect on the chemical composition of the tagliatelle pasta and meringue cookies, which is a requirement for any food additive to be recognised as such. If the bright pink intensity can be achieved by using less extract, the novel colorants seem to give better results when compared to commercial alternatives. These analogous natural extracts from Ac and Hc could be an interesting alternative to the synthetic colorants being used in various food products. For the reasons mentioned above, as well as the fact that they are recovered using biowaste from different industrial processes, according to the principles of a circular economy, these two sources can and should be considered as viable alternatives for the food industry.

Conflicts of interest

There are no conflicts to declare.

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