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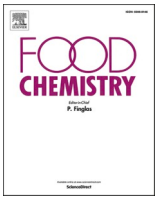

Smart polymers and smartphones for Betalain measurement in cooked beetroots

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ABSTRACT

Betalains in beetroots offer notable colouring properties and health benefits, including antioxidant, anti-inflammatory, hepatoprotective, and antitumorous activities. However, they degrade due to processing and storage conditions like temperature, pH, oxygen, and light-exposure. Traditional betalain determination methods are resource-intensive solid-liquid extractions. This study proposes a novel approach using a smart polymer to rapidly quantify betalains in processed beetroots. The polymer, containing *N,N*-dimethylaminoethyl methacrylate, selectively interacts with compounds like betalains. Characterization shows thermal stability over 250 °C and suitable mechanical properties. The film changes to colour upon interaction with betalains, allowing quantification via smartphone. The sensory polymer's efficacy was validated across 27 beetroot samples, showing no significant differences compared to traditional methods. Combining the smart polymer with a colour analysis app, "Colorimetric Titration," provides a robust and efficient means of quantifying total betalains in beetroot puree, reducing the quantification time from 180 to 90 min, promising implications for routine food industry quality assessments.

1. Introduction

Betalains are water-soluble, nitrogen-containing pigments (Stintzing & Carle, 2004), synthesized from the amino acid tyrosine and classified into two structural groups: betacyanins and betaxanthins (red-purple and yellow-orange, respectively).

The stability of betalains is affected by factors such as temperature, pH value, oxygen, and light (Herbach et al., 2006). Although pH changes cause alteration in their charges, betalains are relatively stable over the broad pH range from 3 to 7 (Azeredo, 2009). Temperature is the most important factor in betalain stability during processing and storage (Herbach et al., 2006) and the effects of high temperatures are increased with light exposure (Lombardelli et al., 2021). However, the light-induced degradation of betalains can be minimized in the absence of oxygen (Azeredo, 2009). Interestingly, betaxanthins show greater stability than betacyanins under the mentioned conditions (Lombardelli et al., 2021). In this sense, previous reports have suggested that the ideal conditions to preserve betacyanins are low temperatures at pH 5 in the dark (Woo et al., 2011). Betalains are restricted to the suborder Chenopodiinae and can be found in roots, fruits and flowers among the Caryophyllales (Stintzing & Carle, 2004). These pigments appear, therefore, in beetroots (*Beta vulgaris*), prickly pear fruits (*Opuntia* spp.), the red pitahaya or red dragon fruit (*Hylocereus polyrhizus*) and amaranth (*Amarathus* sp.) (Gengatharan et al., 2015). Red beetroot (*Beta vulgaris*) has been reported to have more betalain content than other betalain-containing plants (Koss-Mikołajczyk et al., 2019) and it is the major commercially exploited betalain crop (Strack et al., 2003). It is widely grown in Europe, America, and Asia (Akan et al., 2021), and although it is not consumed as much as other vegetables, beetroots have a significant market (Kumar, 2015). Recently, the demand for healthy food has also increased consumer interest in red beetroot (Clifford et al., 2015). A large proportion of commercial production is processed into boiled and sterilized beetroots or canned into pickles (Akan et al., 2021).

Betalains are responsible for the colour of these roots. At the point of purchase, the consumer uses appearance factors as quality indicators (Pathare et al., 2013), and beetroot colour is the most important quality

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indicator for consumer preference (Akan et al., 2021; Esatbeyoglu et al., 2014). Beetroot colour changes are directly related to betalain degradation (Prieto-Santiago et al., 2020). Betalains can suffer degradation during storage or processing, leading to discolouration in beetroot (dos Baião et al., 2020; Herbach et al., 2006). Betalains have also been described as bioactive compounds. In this sense, betanin acts as a free radical scavenger and an inducer of endogenous cellular enzymatic antioxidant defence mechanisms (Esatbeyoglu et al., 2014). Aside from their antioxidant activity, betalains have been described as having immunosuppressive, anti-inflammatory, hepatoprotective, and anti-tumorous biological activity (Clifford et al., 2015).

Therefore, betalains have colouring properties and potential positive effects on human health. Given the dual role of betalains, and their instability upon temperature and storage, the determination of these bioactive pigments could be used as a sensory and functional quality marker for beetroot products. This is why the quantification of these compounds using simple and direct methods could have applications in the industry, both in quality control and in the potential prediction of the time it will take for the colour to change, leading to consumer rejection.

Conventional methods for betalain determination are based on solid−liquid extractions (Ravichandran et al., 2013; Sawicki et al., 2016; Stintzing et al., 2003), which are often unsustainable, due to the amount of organic solvents used, time-consuming, expensive, and require infrastructure and highly specialized equipment. These drawbacks can make implementing this analysis into routine food industry quality assessments challenging.

In this sense, smart polymers, particularly as films, are emerging as a robust solution well-received within the agri-food domain. Recent literature (García Pérez et al., 2022) shows smart polymers can be categorized into two primary groups: reactive polymers and sensory polymers. The former instigates specific actions upon encountering a target (e.g., controlled drug release, alterations in shape), while the latter prompts an alert upon target detection, often manifested through changes in colour or fluorescence.

This study proposes a sensory polymer that selectively interacts with targets exhibiting formal negative charges in their structure, such as betalains. In the case of betalains, the negative charges are provided by the carboxylic acid groups present in their chemical structure (Fig. 1), which have a pKa of approximately 2, and therefore, are deprotonated at the typical pH of beetroots (from 5.5 to 6.6). These light-sensitive coloured compounds become trapped within the structure of the polymeric material, resulting in two relevant outcomes. Firstly, they induce a colour change in the material, enabling quantification through colour analysis. Secondly, the polymeric chains create a protective environment that prevents the degradation of such compounds, as demonstrated in prior studies (Arnaiz et al., 2022; Bustamante et al., 2019), at least for the duration of the analysis. This facilitates their potential utilization in industrial settings, obviating the need for a dark environment. The use of a smartphone as complementary equipment has been significantly enhanced in recent years, which boasts high-quality cameras and processors that complement sensory polymers perfectly, democratizing chemical analysis and making it more efficient and cost-effective (Guembe-García et al., 2022a).

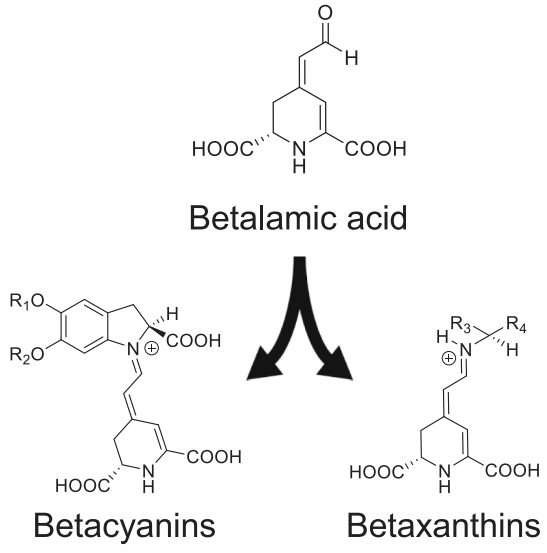

Fig. 1. Chemical structure of the two types of betalains, betacyanins and betaxanthins, as well as the chemical structure from which they derive, betalamic acid.

2. Experimental

2.1. Materials

All materials and solvents were commercially available and used as received unless otherwise indicated. The following materials and solvents were used: methylmethacrylate (**MMA**) (Aldrich, 99%), *N,N*-dimethylaminoethyl metacrylate (**NNDA**) (Aldrich, 98%), 2-hydroxyethyl acrylate (**2HEA**), (Aldrich, 96%), ethylene glycol dimethacrylate (**E**) (Aldrich, 98%), hydrochloric acid (VWR, 37%), acetone (VWR, 99%), KH_2PO_4 (Scharlau, 99%), Na_2HPO_4 (Merck, 99%), ethanol (VWR/BDH Chemicals, 96%). Azo-bis-isobutyronitrile (AIBN, Aldrich, 99%) was recrystallized twice from methanol.

Beetroot samples were both provided by HUERCASA 5ª GAMA S.A. company and purchased in different local markets.

2.2. Instrumentation and general methods

The thermal characterization of the smart polymer was conducted utilizing various analytical techniques. For the adhesives, thermogravimetric analysis (TGA) was performed employing a Q50 TGA analyzer (TA Instruments, New Castle, USA). Samples weighing 3–7 mg were subjected to a nitrogen atmosphere with a heating rate of 10 °C·min^{-1}.

Tensile properties analysis, with 5 × 9.44 × 0.103 mm samples tested at 5 mm min^{-1} (EZ Test Compact Table-Top Universal Tester, Shimadzu Kyoto, Japan).

Infrared spectra (FTIR) were recorded using an infrared spectrometer (FT/IR-4200, Jasco, Tokyo, Japan) equipped with an ATR-PRO410-S single reflection accessory.

The water swelling percentage (WSP), was performed in triplicate, and calculated using the following equation.

$$WSP = \left[\frac{g_{swelled\ material} - g_{dried\ material}}{g_{dried\ material}}\right] \times 100 \quad (1)$$

2.3. Design and synthesis of the film-like smart polymer

For a correct and efficient polymeric material design, it is necessary to consider its final use and the type of users handling it. We aim for this development to be usable by non-specialized personnel and under analysis conditions in industrial environments, or in other words, in environments that are not typical laboratories. That is why we have chosen the film format for our materials, as it can be manipulated by hand without filtration or extraction processes. Additionally, the selected formulation generates a material with gel-like behaviour, meaning it swells in aqueous media, allowing the target species (betalains) to enter the polymeric structure and interact with the material's receptor centres.

The film-like smart polymer (FiLiPo) was prepared by thermally initiated bulk radical polymerization (González-Ceballos et al., 2023; Trigo-López et al., 2016). In this experimental procedure, three commercially available monomers, NNDA, 2HEA, and MMA were

copolymerized in a specific molar ratio 45/45/10, respectively. The function of NNDA is twofold, as it serves both as a monomer that imparts hydrophilicity to the material and as the active monomer that, after a process of protonation of the tertiary amine group, allows the generation of positive charges in the polymer (polycation). Considering that betalains are species with formal positive and negative charges, our detection strategy is based on electrostatic interactions with the polymer through these charges. The 2HEA serves a structural function in the material, providing hydrophilicity and some flexibility. On the other hand, MMA contributes to hydrophobicity and rigidity.

The combination of these monomers generates a material with high hydrophilicity, resulting in a high WSP and, therefore, poor handling. To control swelling, we have incorporated ethyleneglycol dimethacrylate as crosslinking agent. In fact, we have added 15 mol% of crosslinking agent, which is a high amount compared to other materials we have published (González-Ceballos et al., 2021; González-Ceballos et al., 2023; González-Ceballos et al, 2021; Vallejo-García et al., 2023). The proper balance of monomers and crosslinker enables control over the intricate equilibrium between handling, WSP, and the speed of interaction between the target and smart polymer.

Finally, a 1 w% of AIBN (thermal radical initiator), was added to the mixture of monomers and crosslinker, and the resulting solution was injected into a mould (100 μm thickness). The oxygen-free polymerization was carried out at 60 °C overnight. The solid film was demoulded, and dipped into different solutions to both remove nonreacting monomers, and aconditioning for the measurement of betalains. Specifically, it was first immersed in distilled water for 15 min, and then progressively replaced with acetone until reaching 100% acetone. Conversely, acetone was gradually replaced with distilled water until returning to a 100% aqueous medium. This progressive solvent exchange is necessary to avoid abrupt changes in the swelling of the material that could cause film ruptures. Finally, the film was immersed in an aqueous solution of HCl (4%) to protonate the tertiary amine groups contained in the NNDA monomer, thereby generating positive charges in the polymeric chain and enabling electrostatic interaction with betalains. Finally, the material was cut into discs with an 8 mm diameter.

2.4. Preparation of calibration samples (CS)

As previously described in the literature (Prieto-Santiago et al., 2020), the betalain content of beetroots decreases as the temperature increases. In this study, we have utilized this phenomenon to construct a calibration curve that allows the determination of the concentration of betalains using the FiLiPo method. In order to achieve a wide range of betalain concentrations, *Beta vulgaris* cv. Monty was the cultivar selected due to its high betalain content (Carrillo et al., 2017, 2019; Kazimierczak et al., 2019).

Eight kg of fresh beetroots were washed, dried, peeled and chopped into similar-sized slices. The sliced beetroot was vacuum-packed in plastic bags (250 g per bag). Then, samples were boiled, and two bags were taken out at different boiling times (from 0.5 to 7.5 h). Beetroot slices were immediately grinded in a blender to obtain a puree.

2.5. Extraction of betalains from beetroots

A betalain extraction was performed as described elsewhere (Prieto-Santiago et al., 2020). Briefly, 0.5 g of each puree sample were diluted with 5 mL ethanol-water solution (50:50 *v*/v) and immediately shaken (10 min, RT) and centrifuged (10 min, 4 °C, 5500 rpm). Then, the supernatant was collected. Two consecutive extraction cycles were performed by repeating the same procedure. Extracts were stored at −30 °C until analysis. The extraction was performed in triplicate. Samples were carefully handled along the procedure to minimize light exposure.

2.6. Determination of betalain content. Traditional Method

Betalain content was determined by spectrophotometry (UV/Vis) following the method described elsewhere (Nilsson, 1970). The extract was diluted with a phosphate buffer (pH 6.5) until the absorbance at 538 nm was between 0.4 and 0.5. The absorbance of the samples was measured at 538 nm and 476 nm to quantify betacyanins and betaxanthins, respectively. Absorbance at 600 nm was recorded to correct for small amounts of impurities. Betacyanin and betaxanthin concentrations were expressed in mg/kg. Total betalain content was calculated as the sum of betacyanins and betaxanthins.

2.7. Determination of betalain content in beetroots using FiLiPo and the app "colorimetric titration"

Two grams of the beetroots' purees were weighed into 5 mL vials, and an 8 mm diameter FiLiPo disc was introduced for 1 h at room temperature (20 ± 1 °C, 3 replicates). After this time, the discs were removed from the beetroots' purees, the surface was cleaned with a little distilled water, and they were photographed with an iPhone 11 Pro inside a backlit lightbox. The use of a backlight box helps maintain consistent lighting conditions, thus positively contributing to the reproducibility of the method. The colour of the FiLiPo discs was analyzed using the "Colorimetric Titration" app (Vallejos et al., 2021), and the colour parameters were extracted from two different digital colour spaces, the RGB colour space (the most common in electronic devices) and the HSV colour space. Additionally, two different combinations of RGB parameters were analyzed. In total, 8 different digital colour variables were examined (Guembe-García et al., 2022b).

After analyzing the 12 calibration samples (Section 2.4), one specific colour parameter was chosen for the calculations, namely the one that exhibited the best correlation with the betalain content calculated using the traditional method. Subsequently, the 27 beetroot puree samples were analyzed using the FiLiPo method (Fig. 2), and the betalain concentration was calculated using the obtained colour parameters from digital photographs and the calibration curve equation.

2.8. Statistical analysis

Statistical analyses were performed using GraphPad Prism v8 software. To evaluate the statistical significance of the correlation between total betalains derived from the reference method and the different colour parameters obtained with the FiLiPo method, the normality of the data of the calibration samples was analyzed and a Pearson product-moment correlation test was performed. A correlation coefficient (r) value close to 1 or − 1 and a $p < 0.05$ described a positive or negative correlation. To compare the betalain content of the samples obtained by the traditional method and FiLiPo-based method, the normality of the data of the test samples was analyzed and a non-parametric Mann-Whitney test was used ($p < 0.05$).

3. Results and discussion

3.1. Characterization of the film-like smart polymer

Characterizing the handling of such materials is challenging, and often, it is necessary to manipulate and test them under real conditions to determine if they can withstand the process without breaking. There is not a specific technique that indicates this handling, but rather a combination of various techniques, such as the evaluation of their thermal and mechanical properties. Fig. 3 illustrates the proposed material's chemical structure and part of its thermal characterization and infrared analysis. Regarding the thermal behaviour of FiLiPo (Fig. 3b), we can observe that it has a T_5 and a T_{10} of 254 °C and 272 °C, respectively (temperatures at which 5% and 10% of the material weight is lost due to thermal degradation). For this application, it is relevant

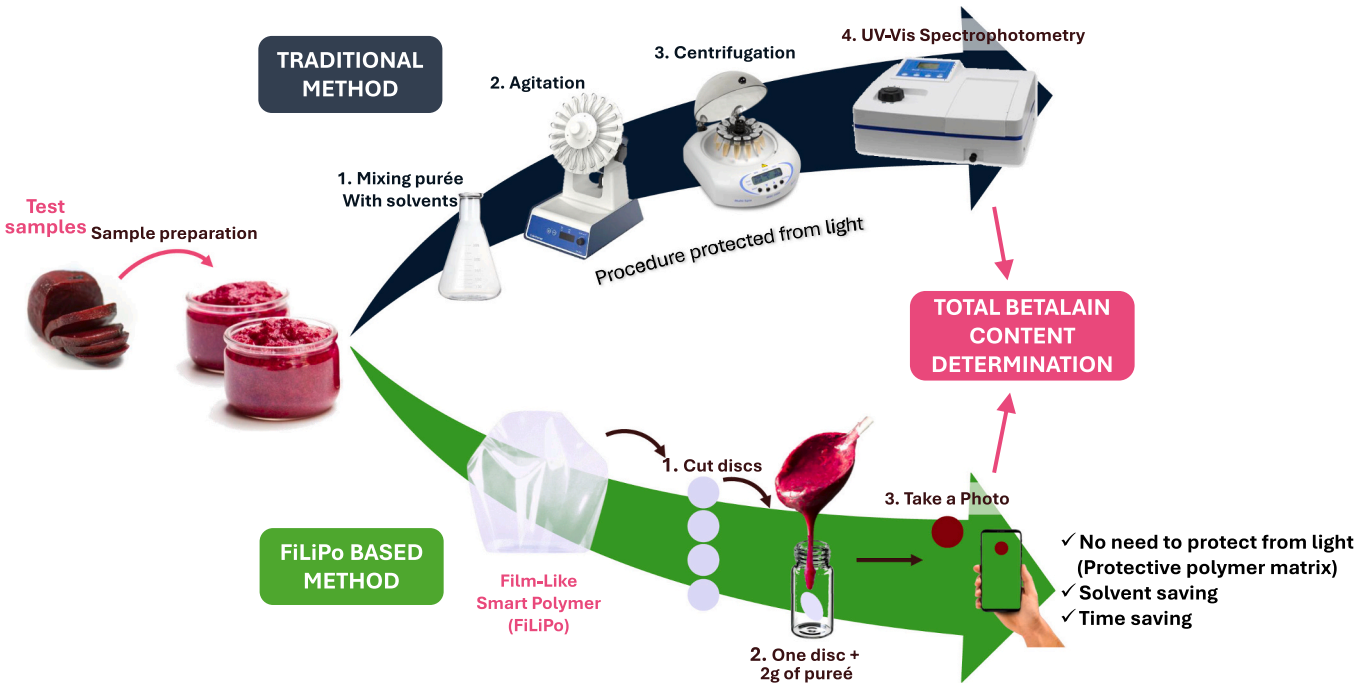

Fig. 2. Table of contents.

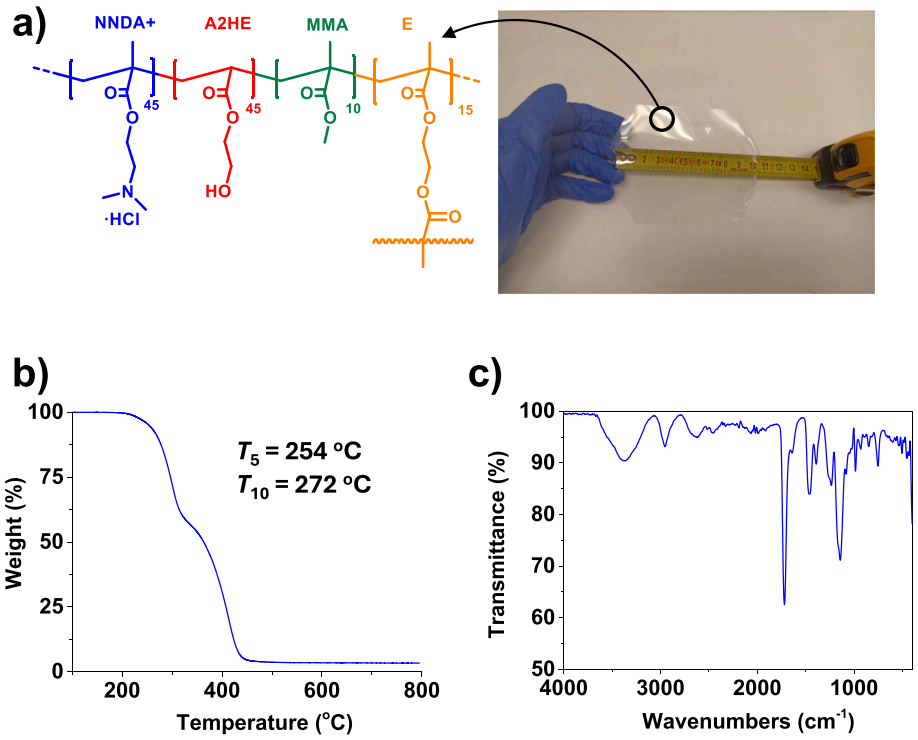

Fig. 3. Structure and properties of the FiLiPo material. a) Chemical structure of the Film-Like Smart Polymer (FiLiPo); b) thermogravimetric curves of FiLiPo at 10 °C/min under a nitrogen atmosphere, displaying T_5 and T_{10} temperatures; c) FT-IR spectrum of FiLiPo.

that we do not have significant weight loss below 100 °C, as the material could potentially come into contact with cooking water, or freshly cooked beetroots, which may reach around that temperature.

The FT-IR analysis confirmed the polymer's structure and revealed characteristic bands of the three different monomers (Fig. 3c). For instance, the broadband observed at 3372 cm^{-1} is indicative of the ν_{O-H} vibration in **2HEA**, the band at around 1233 cm^{-1} corresponds to the ν_{C-N} vibration in **NNDA,** and the bands observed at 2950 cm^{-1} and 1718 cm^{-1} are attributed to the C—H vibration of the sp^3 carbons and the $\nu_{C=O}$ vibration, respectively, present in all the monomers.

The WSP was found to be 55%, a common value in gel-like films used in the detection of targets in food matrices (González-Ceballos et al., 2023; Guirado-Moreno et al., 2023). Typically, with increased swelling, the response speed of the material tends to rise; however, the polymer

matrix absorbs much more water, impacting the material's mechanical properties. Thus, once again, there is an effort to balance swelling and mechanical properties.

The Young's modulus of the material was measured immediately after being demoulded in the FiLiPo preparation process, yielding a value of 291.5 ± 1.4 MPa. This value is not unusual for this type of material (Bustamante et al., 2019; Vallejos et al., 2018). However, it could be improved in future applications, especially by increasing the molar proportion of MMA, albeit sacrificing some of the hydrophilicity provided by 2HEA.

Given that this material is proposed as an option for use in the food industry, it is important to highlight that it has been designed with biocompatible monomers such as [2-(methacryloyloxy)ethyl]trimethylammonium chloride (Ozsoy et al., 2022), 2-hydroxyethyl acrylate (used in the manufacture of contact lenses, (Mutar & Muter, 2015)), and methyl methacrylate (used in the production of dental prosthetics, (Pituru et al., 2020)). Moreover, the risk of compound migration into the food matrix is negligible, as demonstrated in previous studies (Vallejos et al., 2017). These studies have shown that the migration of substances from similar materials into food matrices with which they come into contact is minimal, complying with European Union regulations on food contact (Regulation (EU) No 10/2011 and amendments).

3.2. Results of betalain content and RGB parameters of the calibration samples

Table 1 displays the total betalain content results of the 12 calibration samples, calculated using the traditional method described in Section 2.6. Furthermore, Table 1 presents the different colour parameters obtained through the analysis of the FiLiPo discs using the "Colorimetric Titration" smartphone application. These parameters are extracted from the analysis of the photographs taken of the FiLiPo discs shown on the

Table 1
Results of betalain content in the calibration samples obtained by the traditional method (in blue), and results of the 8 digital colour variables extracted from the photographs taken of the FiLiPo discs (green). Table shows cropped images of the FiLiPo discs (8 mm in diameter) after being immersed for 1 h in 2 g of calibration samples' purees at 20 ± 1 °C. The bottom part of the table shows the Pearson correlation coefficient (r) and the coefficient R^2 between both methods taking into account the different digital colour parameters. Data are means of 3 replicates ± standard error.

Calibration Sample (CS)	Trad. Method	FiLiPo Method										
	Total Betalain (mg/kg)	R	G	B	ΔRGB	RGB	H	S	V	Cropped images of the FiLiPo discs (Replicate 1)	Cropped images of the FiLiPo discs (Replicate 2)	Cropped images of the FiLiPo discs (Replicate 3)
CS#1	751.95 ± 1.70	85.00 ± 3.61	17.00 ± 3.51	51.33 ± 3.48	356.46 ± 4.34	-0.777 ± 0.11	0.915 ± 0.00	0.798 ± 0.04	0.333 ± 0.01			
CS#2	663.59 ± 4.58	81.33 ± 4.63	2.33 ± 0.88	34.00 ± 5.03	377.99 ± 5.51	-1.250 ± 0.17	0.934 ± 0.01	0.972 ± 0.01	0.319 ± 0.02			
CS#3	523.17 ± 1.99	88.00 ± 1.00	2.33 ± 1.86	40.33 ± 1.45	371.24 ± 1.65	-2.008 ± 0.01	0.925 ± 0.00	0.973 ± 0.02	0.345 ± 0.00			
CS#4	484.69 ± 2.87	106.33 ± 4.84	3.00 ± 0.58	66.00 ± 9.50	348.50 ± 7.14	-0.274 ± 0.23	0.899 ± 0.01	0.972 ± 0.01	0.417 ± 0.02			
CS#5	377.06 ± 0.61	115.33 ± 6.67	18.67 ± 12.68	103.33 ± 3.48	314.25 ± 4.86	0.065 ± 0.17	0.853 ± 0.00	0.823 ± 0.13	0.452 ± 0.03			
CS#6	312.04 ± 5.89	112.33 ± 0.33	24.33 ± 0.88	100.00 ± 1.00	312.39 ± 1.28	-0.040 ± 0.04	0.857 ± 0.00	0.783 ± 0.01	0.441 ± 0.00			
CS#7	236.42 ± 3.36	132.67 ± 1.33	4.33 ± 1.76	118.33 ± 3.28	310.62 ± 3.38	0.989 ± 0.09	0.852 ± 0.00	0.968 ± 0.01	0.520 ± 0.01			
CS#8	177.53 ± 1.27	134.67 ± 0.88	32.33 ± 1.20	155.00 ± 1.15	272.15 ± 1.30	0.632 ± 0.03	0.806 ± 0.00	0.791 ± 0.01	0.608 ± 0.00			
CS#9	176.61 ± 1.17	140.33 ± 1.20	16.00 ± 3.79	138.33 ± 4.26	289.64 ± 5.23	0.004 ± 0.13	0.836 ± 0.00	0.887 ± 0.03	0.554 ± 0.01			
CS#10	170.93 ±1.43	137.00 ± 0.00	15.67 ± 1.20	136.67 ± 1.20	291.91 ± 1.47	0.049 ± 0.04	0.833 ± 0.00	0.886 ± 0.01	0.540 ± 0.00			
CS#11	141.65 ± 1.76	135.33 ± 0.67	34.00 ± 0.58	143.00 ± 1.00	275.15 ± 0.38	0.542 ± 0.02	0.822 ± 0.00	0.762 ± 0.01	0.561 ± 0.00			
CS#12	137.86 ± 0.69	131.00 ± 0.58	64.67 ± 3.33	162.33 ± 5.70	245.42 ± 4.79	0.907 ± 0.14	0.781 ± 0.01	0.602 ± 0.01	0.637 ± 0.02			

CORRELATION OF TRADITIONAL METHOD WITH THE DIGITAL COLOUR PARAMETERS								
	R	G	B	ΔRGB	RGB	H	S	V
Pearson Coefficient (r)	-0.960	-0.557	-0.943	0.912	-0.779	0.917	0.418	-0.942
R^2	0.922	0.310	0.890	0.832	0.607	0.841	0.175	0.888

right side of the table. At the bottom, a brief correlation study is displayed between each of the 8 analyzed colour variables and the total betalain concentration data obtained by the traditional method.

The results show that the best correlation is with the parameter "R" of the RGB colour space, thus, we constructed our calibration curve by plotting this parameter against the concentration of total betalains (Fig. 4).

3.3. Results of betalain content of the test samples by both methods

A total of 27 beetroot purees were measured using the traditional and FiLiPo-based methods. For the latter, the parameters "R" obtained from the photographs allowed the calculation of betalain concentration through the calibration curve equation, as shown in Table 2 (the table containing all the colour parameters extracted from the photographs can be found in the Supplementary Material).

The results indicate that there are no statistically significant differences between the total values of betalains obtained with both methods. The statistical test utilized did not find significant differences, although some slightly different data were observed, such as in the case of sample 4 or sample 6.

Upon plotting the betalain content data obtained by both methodologies, we observe a strong visual correlation between the two, as depicted in Fig. 5. This demonstrates that the utilization of the smart polymer FiLiPo, complemented by a colour analysis application such as "Colorimetric Titration," constitutes a robust method for quantifying total betalains in beetroot puree samples. This approach not only saves costs on equipment but also streamlines execution time significantly. The experimental duration per sample measurement is reduced from 180 min to 90 min, and the assay can be conducted by non-specialized personnel.

4. Conclusions

Our study presents a novel advancement in the quantification of betalains in processed beetroot, showcasing the efficacy of an innovative smart sensory polymer film. This cutting-edge approach not only addresses the limitations of traditional methods but also introduces several significant advantages. Firstly, unlike conventional techniques that require working in dark conditions to prevent betalain degradation, our method eliminates this necessity by providing a protective environment within the polymer, thus simplifying the analytical process and enhancing convenience. Additionally, by avoiding the need for extensive solid-liquid extractions, our method significantly reduces the consumption of organic solvents, which contributes to sustainability efforts in analytical chemistry and reduces environmental impact. Moreover,

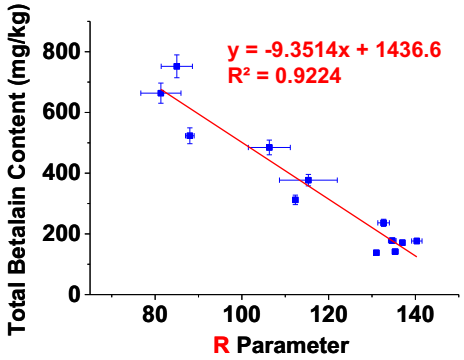

Fig. 4. Calibration curve. The betalain content was calculated according to the method described in Section 2.6. The "R" parameter of digital colour was extracted from photos of 8 mm diameter FiLiPo discs after being immersed for 1 h in beetroots purees at room temperature. The data show the standard error of 3 replicates.

Table 2
Results of betalain content in the test samples obtained by the traditional method (in blue), and FiLiPo-based method (green). Data are means of 3 replicates ± standard error. Statistical analysis: Mann Whitney test ($p < 0.05$); "ns" means no significant differences.

Beetroot purees	Traditional Method (mg/kg)	FiLiPo-based Method (mg/kg)	Statistical analysis	Cropped images of the FiLiPo discs		
				Replicate 1	Replicate 2	Replicate 3
BRP#1	649.33 ± 10.11	604.33 ±10.80	ns			
BRP#2	706.75 ±6.87	729.01 ±8.25	ns			
BRP#3	250.83 ±4.69	220.92 ±28.57	ns			
BRP#4	445.06 ±0.51	336.25 ±3.12	ns			
BRP#5	586.38 ±2.77	566.92 ±42.17	ns			
BRP#6	375.00 ±1.81	270.79 ±27.71	ns			
BRP#7	404.91 ±3.28	389.24 ±10.80	ns			
BRP#8	571.95 ±3.62	492.11 ±65.46	ns			
BRP#9	471.33 ±3.03	367.42 ±3.12	ns			
BRP#10	405.81 ±1.01	345.60 ±11.24	ns			
BRP#11	402.79 ±3.59	420.41 ±16.49	ns			
BRP#12	180.45 ±0.31	183.51 ±5.40	ns			
BRP#13	368.56 ±1.85	389.24 ±16.20	ns			
BRP#14	370.88 ±2.13	370.54 ±5.40	ns			
BRP#15	175.59 ±0.82	180.40 ± 8.25	ns			
BRP#16	846.82 ±4.82	834.99 ±17.36	ns			
BRP#17	286.33 ±1.17	305.08 ±14.28	ns			
BRP#18	453.72 ±2.95	389.24 ±21.60	ns			
BRP#19	776.50 ±2.83	800.70 ±23.53	ns			
BRP#20	405.38 ±2.57	308.20 ±40.52	ns			
BRP#21	492.70 ±1.57	551.33 ±18.96	ns			
BRP#22	448.38 ±1.94	492.11 ±30.06	ns			
BRP#23	223.26 ±0.39	192.86 ±5.40	ns			
BRP#24	474.88 ±1.66	501.46 ±53.99	ns			
BRP#25	727.80 ±1.75	753.95 ±28.05	ns			
BRP#26	453.65 ±1.72	414.18 ±25.51	ns			
BRP#27	387.56 ±2.44	414.18 ±13.59	ns			

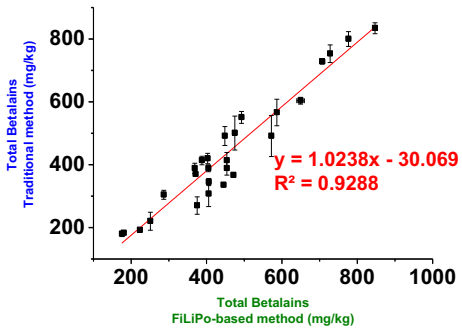

Fig. 5. Total betalains calculated from traditional method and FiLiPo-based method. Data are means of 3 replicates ± standard error of the total betalains for each sample obtained with each method. The trendline illustrates how the results obtained with both methods correlate with each other, with an R^2 value of 0.93.

the smart sensory polymer film exhibits a rapid and selective colour change in response to betalains, enabling swift and precise quantification using ubiquitous devices such as smartphones. This feature eliminates the need for specialized instrumentation and trained personnel, making the process more accessible and cost-effective. The ease of use and reduced dependency on sophisticated equipment offer a practical solution for routine quality assessments in the food industry. Statistical analysis confirms the reliability and accuracy of our method, demonstrating no significant differences in betalain concentrations compared to traditional quantification approaches. This finding underscores the robustness of our methodology and validates its suitability for widespread adoption in quality control processes. In summary, the adoption of this smart polymer-based approach, complemented by colour analysis applications, holds transformative potential for accelerating betalain quantification processes. This innovation facilitates more efficient, sustainable, and cost-effective quality control measures in the production of beetroot-derived products, offering a significant advancement in analytical techniques within the food industry.

Open Data

Open Data is available at https://riubu.ubu.es/handle/10259/5684 under the name "UBU-Polymers Research Group 16042024".

CRediT authorship contribution statement

María Gaona-Ruiz: Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **Jorge Lucas Vallejo-García:** Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **Ana Arnaiz:** Writing – original draft, Validation, Supervision, Formal analysis. **Carlos Sedano-Labrador:** Validation, Methodology, Investigation. **Miriam Trigo-López:** Writing – review & editing, Writing – original draft, Methodology. **Ana Rodríguez:** Writing – review & editing, Conceptualization. **Celia Carrillo:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization. **Saul Vallejos:** Conceptualization, Funding acquisition, Project administration, Methodology, Investigation, Writing – original draft, Writing – review & editing, Supervision.

Declaration of competing interest

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

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Digital colour parameters of Calibration and Test samples. Supplementary data to this article can be found online at [https://doi.org/10.1016/j.foodchem.2024.140358].

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