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Valorisation and sensory evaluation of natural colorants for a sustainable food supply

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ABSTRACT

The study explored the potential of several typical Tunisian food plants as sources of natural colorings, focusing on sustainability and ecology. Five common fruits and vegetables were studied: spinach, beetroot, red cabbage, carrot, and turmeric. Their deep pigments, affected by pH, were examined. Red cabbage was found to be the richest in condensed tannins, while turmeric contained the highest levels of anthocyanins. These natural colorings were then incorporated into the formulation of two bakery products, one sweet and the other savory, to give an attractive appearance and stimulate the appetite, as the food industry commonly does with synthetic colorings. Additionally, orange and lemon peels were upcycled by extracting essential oils for analysis. The primary findings indicate that these essential oils are complicated blends of many substances, with monoterpenes and sesquiterpenes being the most prevalent. The last part of this project required using gluten-free flour to make two food products (zouza and ravioli dough), substituting fructose for sucrose, and adding other colorings that had been produced and tested. We also improved the flavor by adding essential oils. Sensory research with sixty unsuspecting panelists revealed that our items were well-liked by all. The result of a very successful initiative is the packaging for these goods and the market research.

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1. Introduction

Throughout history, nations have produced colors, first from natural resources, to create dyes (1). Natural dyes, such as saffron, woad, and madder, have been used to decorate and dye (1). In the food industry, food colors have been used to enhance the visual appeal of products, influencing their perception and palatability (2).

However, natural dyes were dethroned at the beginning of the 20th century with the advent of petrochemicals, which now supply the synthetic pigments widely used today (3).

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They are also responsible for allergies to human skin and generate non-biodegradable waste that pollutes the environment (4). As a result, synthetic dyes are steadily declining due to the many accusations of their harmful effects on human health (3). Several recent studies (5,6) have highlighted the risks of intolerance, allergies, and toxicity associated with these non-biodegradable chemical additives.

As consumer demand for clean, sustainable ingredients has grown, so too has interest in natural colorants derived from natural resources in recent years. Natural plant-based dyes have emerged as a promising alternative to address these worries (7,8). There are many different sources of natural colorants, such as plants (9,10). As a result, plant-based products have emerged as a promising solution to these problems (11). They provide an ideal solution for meeting these expectations, as they are not only natural but also renewable and biodegradable. They also bring benefits in terms of the sensory quality and nutritional value of foods. They can add subtle flavors and pleasant aromas to food products while preserving their nutritional value through the presence of bioactive compounds (12,13). Plants get their natural colors from pigments found in many of them. These pigments include flavonoids, anthocyanins, carotenoids, and chlorophyll (9). Moreover, this richness in secondary metabolites contributes to the preservation of consumer health (12). They are responsible for important biological activities such as antioxidant activity (2,14,15).

For the first time in our knowledge, Tunisian plant dyes are being used in traditional food products. It paves the way for natural, local alternatives to controversial synthetic additives. Recovering little-used plant residues is also an ecologically responsible and sustainable approach.

This study aims to extract, characterize, and integrate plant dyes from dye plants frequently used in Tunisia in food products, and to assess the organoleptic qualities of the finished products with a panel of naive consumers to understand their acceptability and gustatory, olfactory, and visual appeal. This study could open the way for using natural and local alternatives to controversial synthetic dyes, encouraging healthier and environmentally friendly food production in Tunisia.

2. Materials and Methods

2.1. Plant material

The most popular species in Tunisian markets were: beetroot (*Beta vulgaris*); turmeric roots (*Curcuma longa*); red cabbage (*Brassica oleracea*); carrot (*Daucus carota*); and spinach leaves (*Spinacia oleracea*). Lemon (*Citrus limon*) and orange (*Citrus sinensis*) peels were used to add value to the waste.

2.2. Dye extraction

Twenty grams of each species was extracted in 200 mL of distilled water. They were cut into small pieces and then mixed with an electronic blender. The extraction was assisted by ultrasound for 1 h. Büchner filtration (vacuum filtration) was used to separate the solid phase from the liquid phase of the heterogeneous mixture. After vacuum filtration, the filtrates were stored at -80 °C. All extracts were freeze-dried to obtain a dry dye.

2.3. Characterization of dyes

2.3.1. UV-visible spectroscopy

The UV-visible spectrum is obtained using spectrophotometry. Wavelengths from 200 to 800 nm are measured.

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2.3.2. The pH effect

The pH test is used to examine their impact on the plant food colors studied. To do this, three groups were prepared for each sample. In each watch glass, we put the quantity (20 g) of fresh plant material to which we added acid or a basic solution

2.4. Phenolic compound quantification

2.4.1. Determination of condensed tannins

Sun et al. (16) provided the technique used to determine the condensed tannin concentration. 50 µL was added as an aliquot to 3 mL of 1% vanillin and 0.5 mL of concentrated sulfuric acid. The mixture was homogenized and then allowed to sit at room temperature for 15 min. The absorbance was measured at 500 nm. Condensed tannin concentrations were calculated using a typical tannic acid range of 0 to 400 µg/mL. Condensed tannin levels are reported in mg tannic acid equivalent per gram of dry matter (mg TAE/g DW).

2.4.2. Determination of proanthocyanidins

A butanol-HCl test (17) was used to evaluate the proanthocyanidin content, which is given as µg (+)-catechin equivalent per gram dry weight (µg CE/g DW). 500 µL of extracts are combined with 3 mL of butanol-HCl (95:5) and 0.1 mL of iron sulfate. The mixture was homogenized and then incubated for 60 min at 90 °C. At 530 nm, absorbance was measured.

2.4.3. Determination of anthocyanins

Anthocyanins are measured using the Murray & Hackett, (18) method. The anthocyanin content is expressed in µg/g FW. The anthocyanin-rich fraction was extracted by maceration in an acidulated methanolic solvent (HCl 3M/H_2O/MeOH) in the following proportions: 1/3/6, with continuous stirring for 24 h. The mixture was incubated for 4 days at 4°C. The absorbance of the extract was measured at 530 nm and 653 nm. The anthocyanin content was calculated using the following formula:

[Anthocyanins] (µg/g FW) = $(OD_{530}) - 0.24\ (OD_{653})$

2.5. Essential oil extraction

The essential oils were extracted from citrus fruit waste (lemon-orange) using the hydro distillation method.

2.6. Gas chromatography-mass spectrometry (GC-MS) of essential oils

The chemical composition of the essential oils in lemon and orange peels was determined using gas chromatography-mass spectrometry (GC-MS). GC-MS analysis was performed using an HP 7890 gas chromatograph (A), linked to an HP 5972 mass spectrometer (Agilent Technologies, Palo Alto, California, USA) with electron impact ionization (70 eV). An HP-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 mm) was utilized. Helium was the carrier gas at a flow rate of 0.9 mL/min. The column temperature was programmed to rise from 60 to 260 °C at a rate of 5°C/min. The scan time and mass range were 1 s and 50 to 550 m/z, respectively. The injected volume was 1 µL, and the total runtime was approximately 42 min. Identification was achieved by comparing the recorded spectra with those stored in the Wiley NIST 2011 computerized mass spectra library of the GC-MS data system.

2.7. Application of natural dyes and essential oils in food products

2.7.1. Preparation of food products

Savory product: The prepared colorings are incorporated into the dough of the ravioli, which are small squares of stuffed dough.

Sweet product: The dyes and oils are used to color and flavor the "ZOUZA", a biscuit of Tunisian origin in the shape of a nut, formed by the combination of two oval shells with a hollow interior.

2.7.2. Food product analysis

2.7.2.1. Nutritional composition

Determination of humidity content: A 3 to 4 g sample of the biscuit or ravioli dough to be analyzed is dried at 100°C for 6 h. The measured humidity content corresponds to the difference in weight between the initial test sample and the constantly dried sample.

2.7.2.2. Determination of protein content

To determine the protein content, the Kjeldahl method was used (19). Two grams of each sample was mixed with 10 mL of concentrated H_2SO_4 in a heating tube. One gram of selenium catalyst was added to the tube, and the mixture was heated in a carboy. The digest was transferred to a 100 mL volumetric flask and made up to volume with distilled water. A 10 mL portion of the digest was mixed with an equal volume of 45% NaOH solution and poured into a Kjeldahl distillation apparatus. The mixture was distilled, and the distillate was collected in a 4% boric acid solution containing 3 drops of zuazaga indicator. A total of 50 mL of distillate was collected and titrated. The nitrogen content was calculated and multiplied by 5.25 to give the crude protein content.

2.7.2.3. Determination of lipid content

Two grams of the sample were placed in the thimble, which fits into a clean, dried, and weighed round-bottomed flask containing 120 mL of the solvent (ethanol). The sample was heated and left to reflux for 6 h. At the end of the extraction, the solvent was evaporated using a rotary evaporator. The percentage oil content was calculated as follows:

$$\% \text{ Lipid} = (W_2 - W_1) / W_3 \times 100$$

Where W_1 = weight of empty extraction flask, W_2: weight of flask and extracted oil, and W_3: weight of the sample.

2.7.2.4. Determination of carbohydrate content

Total carbohydrate was calculated using the AOAC method Horwitz (19). It is equal to the product of subtracting the sum of moisture, protein, fat, and ash from 100 (expressed as a percentage).

2.7.3. Sensorial analysis

The sensory analysis involved testing these five senses (sight, hearing, smell, taste, and touch). A panel of 60 individuals was formed to assess the organoleptic characteristics of the samples. The test was carried out using a two-part questionnaire: the first part aimed to identify the consumers in terms of age, place of birth, gender, and tobacco consumption, while the second part focused on the responses relating to the organoleptic qualities of the samples tasted. Participants were asked to rinse their mouths between samples during the tasting, and the sensory analysis focused on the overall assessment of consumers' evaluations of the different biscuit samples.

2.8. Statistical analysis

Three replicates were used for each activity in the different analyses described above. Significant differences ($p \leq 0.05$) between treatments were calculated using ANOVA and Duncan's multiple comparison test using XLSTAT version 2013 software.

3. Results

3.1. Characterization of dyes

The extraction of the dyes enabled us to determine the yield of the five species studied (beetroot, turmeric, carrot, red cabbage, and spinach). Table 1 shows that the highest yields were obtained with turmeric roots. For fresh material (beetroot, carrot, red cabbage, and spinach), we note that the best yield is recorded for fresh beetroot, followed by carrot. In addition, the main dyes are various purplish red, blue green, orange to yellow (Table 1).

3.2. UV-visible

We performed a scan from 200 nm to 800 nm. Fig. 1 shows the profiles of the five extracts. The results show that the different extracts are rich in photosynthetic pigments and have different relative absorbances.

3.3. Effect of pH

The results show that in an acidic environment (in the presence of lemon) (Fig. 2), the various samples changed color. The color of the spinach leaves lightened, the beetroot red became darker, and the blue of the cabbage turned purplish red. No color changes were observed in carrots or turmeric. In a basic medium (plus NaOH) (Fig. 2), spinach leaves became darker in color, while beetroot and red cabbage lost their color and became transparent. Turmeric roots turned brick red, while carrots showed no change in color.

3.4. Phenolic compound content

3.4.1. Proanthocyanidin content

The proanthocyanidin content of the species studied ranged from 160 to 100 mg of catechin equivalent per gram of dry matter (mg CE/g DW) (Table 2). We note that this content varies according to the species and that the highest value is recorded with red cabbage extract.

3.4.2. Condensed tannin content

The condensed tannin content of the species studied ranged from 23.9 to 41.4 mg tannic acid equivalent per gram of dry matter (mg TAE/g DW) (Table 2). This content varied according to the species, with the highest value recorded for turmeric extract.

3.4.3. Anthocyanin content

The anthocyanins, or anthocyanosides, are natural pigments found in leaves, petals, fruit, etc., located in the water-soluble vacuoles of cells. These compounds exist in nature in the form of heterosides, or oses. The anthocyanin content of the species studied varies from 5 to 49 mg equivalent per gram of fresh matter (mg/g FW) (Table 2). We note that this content varies according to the species, with the highest content recorded in turmeric extract, followed by red cabbage.

3.5. Study of essential oils

The extraction of the essential oils by the hydro-distillation method enabled us to determine the oil yield from the peels of the two citrus fruits: *Citrus limon* and *Citrus sinensis*.

The yield of oil from the zests of two citrus fruits: Citrus limon and Citrus sinensis. The highest yields of essential oil were found in orange (3.47%). For lemon oil, the yield is only 2.2%.

The essential oil obtained from lemon peel is yellow, while that obtained from orange is orange in color. Orange has a light orange color.

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Table 1. The principal dyes, the yields, and the organoleptic characteristics obtained

Simples	Colors	Yields *(%)*	Apparence	Smell
Beetroot	Red violet	9.36	Powder very wet	Beetroot smell
Red cabbage	Light blue	4.77	Powder wet	No smell
Spinach	Light green	7.29	Glue gel	No smell
Carrot	Light orange	9.01	Powder wet	Spinach smell
Curcuma	Orange to yellow	37.17	Wet paste	Cabbage smell

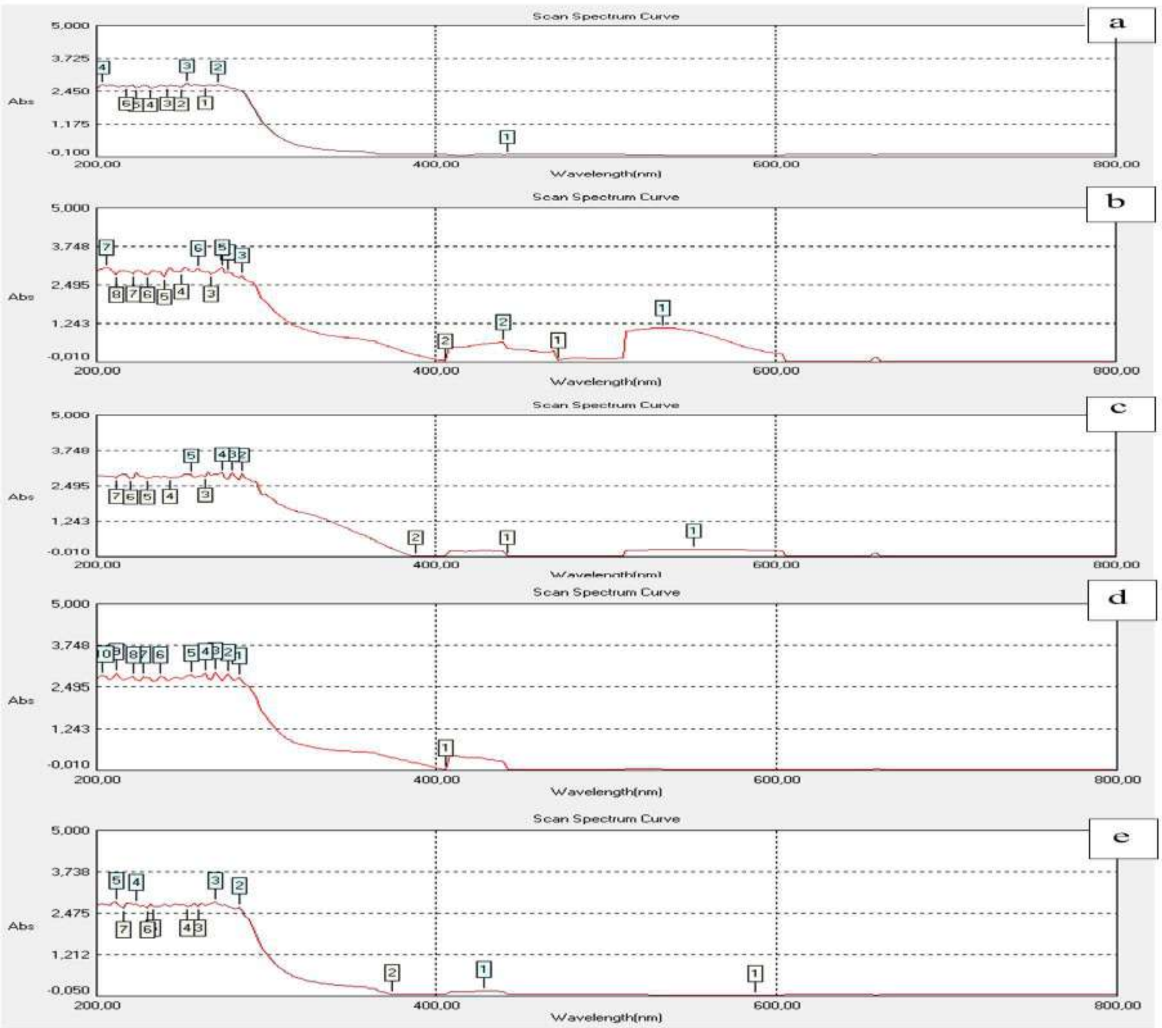

Figure 1. UV-visible spectra of red cabbage (a), carrot (b), beetroot (c), turmeric (d), and spinach (e)

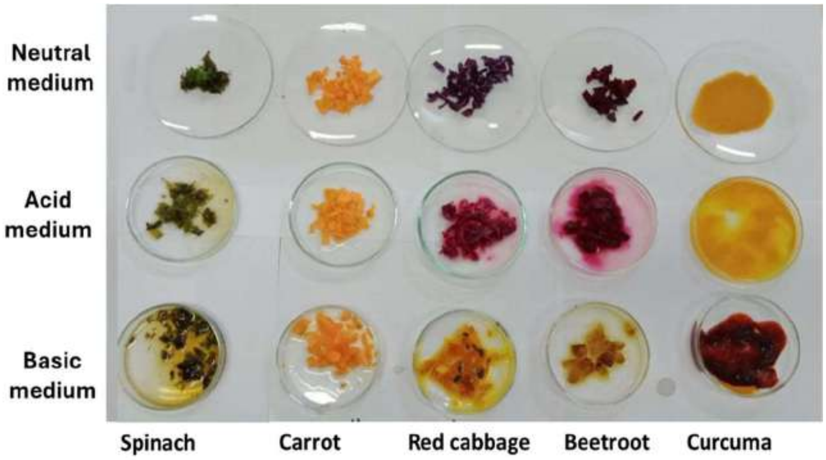

Figure 2. Effect of pH on the five species studied

Table 2. Proanthocyanidin content, condensed tannin content, and anthocyanin content of aqueous extracts of the five species studied (averages of three replicates and 95% confidence intervals)

Simples	Proanthocyanidins content (mg CE/g DW)	Condensed tannin content (mg TAE/g DW)	Anthocyanin content (mg/g FW)
Beetroot	104.17±0.2	23.93±0.05	5.05±0.02
Red cabbage	99.68±0.5	27.70±0.15	4.52±0.1
Spinach	106.02±1.5	41.40±0.5	48.93±0.75
Carrot	119.73±0.77	39.55±0.8	27.07±0.5
Curcuma	163.36±0.5	31.62±0.5	39.96±0.05

3.6. Chemical composition of essential oils

The chemical composition of the essential oils in the dried peel of two citrus fruits was determined by gas chromatography-mass spectrometry.

The results obtained show that essential oils are complex mixtures of several compounds, the most dominant of which are monoterpenes and sesquiterpenes. Table 3 shows the results of the chromatographic analysis of the two samples of orange and lemon essential oils. The compounds are classified according to their retention time (RT). These values are represented in order of elution from the capillary column TRB-5MS. This composition includes a total of 20 compounds for orange peel essential oil. The main orange compounds are limonene (68.63%), β-pinene (11.01%), and δ-terpinene (9.73%). For lemon peel, the main compounds are limonene (89.811%) and β-pinene (4.150%). Both oils have the limonene chemotype (Table 3).

3.7. Incorporation of colors in food products

3.7.1. Savory product

Our savory product is colored ravioli dough (Fig. 3a). The pasta is prepared using special gluten-free flours and colorings extracted and produced by our team.

3.7.2. Sweet product

Our sweet product consists of colored "ZOUZA" filled biscuits (Figure 3b), prepared with gluten-free flours, using fructose as the sugar, our extracted natural colorings, and some fillings flavored with lemon and orange essential oils.

3.7.3. Nutritional composition analysis

Analysis of the nutritional energy composition revealed that the biscuits contain 55 g of carbohydrates, 22 g of fat, and 5.5 g of protein for each 100 g portion. The energy value is therefore 200 kcal per 100 g. Although these biscuits are high in calories, they are lower in energy than other Tunisian pastries. What's more, they offer other interesting nutritional benefits. For example, the gluten-free flour used makes them suitable for people with gluten intolerance or coeliac disease. Replacing sucrose with fructose in the recipe helps to reduce calories. Fructose is also used for its properties in various food preparations. Our sweet and savory products are made without synthetic colorings, additives, or flavor enhancers, which is good for health and the environment (Table 4).

3.8. Sensory analysis

The sensory profile is a summary of all the information obtained from a rigorous analysis of the food product (Fig. 4). For each type of biscuit produced, the descriptors (smell, sight, taste, etc.) were analyzed, and the intensity of each descriptor was assessed on a graduated scale. The results showed that all the biscuits seemed to be appreciated by the tasters, although those colored with red cabbage were slightly better.

Table 3. Chemical composition of the essential oils of orange and lemon peel

TR	Compounds	Orange (%)	Lemon (%)
5.758	α-Thujene	-	0.14
6.007	α-Pinene	1.375	0.71
7.811	Sabinene	-	0.60
7.920	β-Phellandrene	0.518	-
7.942	β-Pinene	4.150	11.01
8.850	β-Myrcene	-	1.54
11.906	α- Terpinene	0.899	0.08
11.979	β-Cymene	-	0.31
12.569	Limonene	89.811	68.63
13.180	δ-Terpinene	0.377	9.73
13.946	Terpinolene	0.189	0.45
21.985	α-Terpineol	-	0.14
25.605	Citral	-	0.75
34.063	Neryl acetate	-	0.76
35.316	Geranyl acetate	0.568	0.37
36.895	Caryophyllene	0.201	0.56
38.206	α-Bergamotene	-	0.82
41.696	α-Elemene	-	0.10
42.400	Bicyclogermacrene	-	0.08
42.426	Humulene	-	0.09
42.735	β-Bisabolene	-	1.16
43.125	Eremophilene	1.765	-
43.748	Cadinene	0.147	-

Figure 3. a: Pasta ravioli, dyed with natural color, b: Zouza dyed with natural color

Table 4. Analysis of the nutritional composition of pasta products (100 g)

Pasta	Carbohydrates	Fats	Protein	Kcal
Pasta with spinach	35.4	1.4	4.2	171
Pasta with beetroot	34.6	1.3	4.4	167.7
Pasta with carrots	35.2	1.4	4.3	170.6
Pasta with Curcuma	35.6	1.5	3.7	170.7
Pasta with red cabbage	37.8	1.3	3.9	177.3

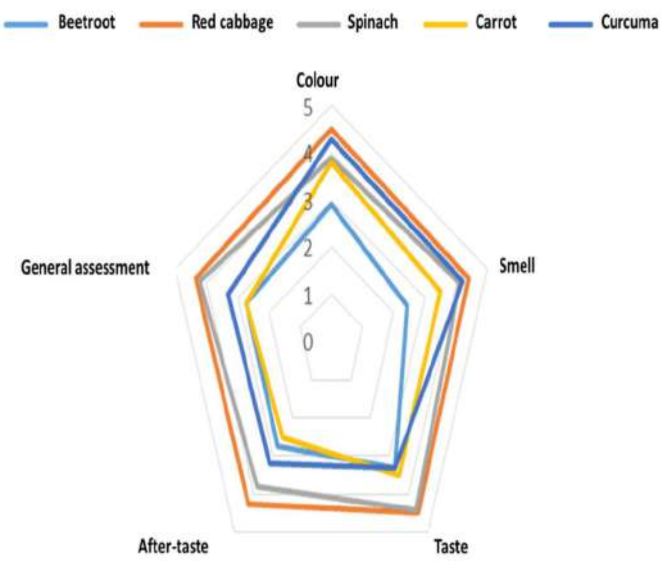

Figure 4. Sensory analysis

4. Discussion

As natural foods become increasingly popular, interest in plant dyes has been rekindled as an alternative to potentially harmful synthetic dyes (15). Widely used synthetic colors are often associated with health problems such as hyperactivity in children, food allergies, intolerances, and even certain types of cancer (20). In this context, the use of natural plant-based dyes is becoming increasingly attractive (11). This study looked at the use of natural colorings extracted from common Tunisian plants: beetroot, turmeric, red cabbage, carrot, and spinach. Each plant offers health benefits in addition to its coloring function, such as antioxidant properties and essential nutrients (21–25).

As an alternative, the dyes in our study were extracted by sonication with water, thus avoiding the use of the organic solvents normally employed. This method has been widely used, mainly to extract bioactive compounds, due to its many advantages (26). This environmentally friendly approach produces extracts rich in natural colorants. The results are consistent with previous research exploring environmentally friendly dye extraction methods. Because of their durability and low impact on human health, these alternative methods are increasingly sought after (27,28). The characterization of the dye extracts enabled the various compounds present to be identified qualitatively and quantitatively, condensed tannin, proanthocyanidins, and anthocyanins. Other studies have identified compounds such as condensed tannins, proanthocyanidins, and anthocyanidins as the main pigments in dye plant extracts (29). These plants are rich in bioactive compounds, as confirmed by the findings of the characterization, which are like those reported in the literature (30). These compounds are known to produce bright, stable colors, making them attractive to the food industry (14,21). The results of Lauria and Silva, (26) suggest that the outer leaves of *Brassica oleracea* L., the barks of *Cucurbita maxima*, *Cucurbita maxima*, *Cucurbita moschata,* and *Beta vulgaris* L. have proved to be excellent resources for obtaining natural pigments. In addition, this approach is original in that it successfully incorporates these coloring extracts into traditional local gluten-free food formulations, in association with essential citrus oils. Chemical analysis of these essential oils revealed a composition rich in monoterpenes and sesquiterpenes, which confer interesting aromatic properties. Our results agree with those of Shaw et al. (30) who suggest that citrus essential oil contains a variety of compounds, both volatile and non-volatile, making it a complex mixture. The extraction of essential oils from citrus peels and the identification of their chemical compounds, including monoterpenes and sesquiterpenes, are in line with established knowledge of the composition of essential oils. They are frequently used to add natural aromas and flavors to foods and can provide biological properties such as antimicrobial activity (32).

In the second part of the study, sweet and savory food products were produced using natural coloring extracts that had been characterized beforehand. Food substitutions were made using ingredients such as gluten-free flour and fructose to replace sucrose. Extracted essential oils were used to flavor the products, masking the original flavors. Nutritional analysis revealed a reduction in calories compared with traditional Tunisian pastries. This approach is in line with current trends in health-focused food choices, using natural food coloring extracts in the manufacture of food products. These results are consistent with previous research, which has shown that similar substitutes can reduce the calorie content and improve the nutritional value of foods (33–35).

The products were subjected to a sensory analysis by a panel of 60 naïve consumers. The results of this evaluation showed a broad appreciation of the products developed, underscoring the acceptability and appeal of natural colorings in food products. The results of the sensory analysis show that consumers are receptive to food products containing natural colors. This is in line with other studies showing that consumers are receptive to food products containing natural colors (35–37). Natural colors are often associated with the idea of healthier, more natural products, which is in line with growing consumer preferences for healthier, environmentally friendly food choices.

5. Conclusion

This study exploited Tunisia's local plant resources as natural colorings and flavorings for innovative food products. The extraction and characterization of pigments from various plants revealed their richness in bioactive compounds with antioxidant properties. The incorporation of these natural extracts into food formulations has enabled the creation of visually appealing sweet and savory products with an improved flavor profile. The sensory evaluation confirmed the acceptability of these products, combining organoleptic, natural, and dietary qualities. These results open new prospects for the use of local plant resources in the agri-food industry, meeting the growing demand for natural, healthy, and sustainable products. A thorough analysis of the life cycle and production costs is needed to assess the viability of the industrial deployment of these innovations.

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Author contributions

R. J. Methodology, Analyses, and Writing-Original draft preparation. M. M. Methodology, Reviewing, Editing, and Writing. A. K. Methodology, Analyses, Reviewing, and Supervision. R. S. Reviewing and Supervision.

Declaration of competing interest

The authors declare no competing financial or personal interests.

Data availability

The data generated and analyzed in this study are available from the corresponding author upon reasonable request.

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