Research Article

Bioactive Characteristics and Storage of Salt Mixtures Seasoned with Powdered Cereal Sprouts

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Sprouting is a process that can improve nutritional and beneficial properties of seeds. This work studies the design of the new healthier product salt seasoned with freeze-dried powdered cereal sprouts. The cereal sprouts mixture (SM), including barley sprouts (BS), oat sprouts (OS), and wheat sprouts (WS), was optimized in terms of total phenolic content (TPh) and antioxidant capacity (AC). The sprouts mixture with optimal features (OSM), composed of 92.9% BS, 0% OS, and 7.1% WS, had 482.82 mg GAE/100 g of polyphenols and 797.97 µmol TE/100 g antioxidant capacity. HPLC analysis showed that the most abundant phenolic compound in OSM was gallic acid (94.27 mg/100 g). OSM was mixed with salt in different ratios (1:1, 1:2, and 1:3) and stored in transparent and amber bottles for six months. Colour, TPh, and AC retention of seasoned salts and OSM was significantly better (p < 0.05) preserved in amber bottles during storage, protected from light. The sprout content was in correlation with TPh and AC retention and colour change. These results suggest that cereal sprouts can be used as a safe ingredient for food products such as seasoned salt, adding value to the basic daily diet with no changes in dietary habits.

1. Introduction

Oxidative stress and damage are involved in the etiology of many chronic and degenerative diseases and in the aging process. Current research suggests that antioxidants, both endogenous and exogenous, are necessary to protect cellular components from damage caused by oxidative stress [1]. Additionally, numerous scientific studies support the observation that increased dietary intake of total antioxidant capacity is associated with reduced risk for diseases such as cancer, coronary heart disease, and diabetes [2, 3].

Cereals are considered as an important part of human diet across the world, apart from the fact that they are also rich in various health-promoting components. Sprouting of cereals is an effective processing method for improving their nutritional and health value in a natural way [4]. The main feature of this process is the change in biochemical, nutritional, and sensory properties due to the activation of dormant enzymes [5]. As a result, the germinated seeds or sprouts are superior to their original seeds with higher levels of nutrients, lower amounts of antinutrients, and increased protein and starch digestibility [6, 7]. Nowdays, the sprouting is gaining more and more attention due to consumers' demand for minimally processed, additive-free, more natural, nutritional, and healthy foods [8].

The most important groups of bioactive compounds in cereals are phenolics [9]. Phenolics and other antioxidants found in cereals may act as free radical scavengers and/or reducing agents, chelating prooxidant metals, and as singlet oxygen quenchers [10, 11].

One of the ancient cereal crops that is currently receiving increasing demands as a functional food ingredient is barley (Hordeum vulgare). This is due to its high content of bioactive compounds such as β-glucan, tocols, and...
phenolic compounds [12–15]. According to Sharma and Gujral [16], sprouts of different barley cultivars showed a significant increase in total phenolic content and antioxidant activity compared to raw seeds. Wheat (Triticum aestivum) is the main cereal used in the human diet. Amici et al. [17] have reported that wheat sprouts are a powerful mixture of molecules such as enzymes, reducing glycosides, and polyphenols. Antioxidant compounds from wheat sprouts are capable of protecting the DNA from the oxidative damage caused by free radicals in vitro. Oats are ranked as the fifth cereal crop in the world by its importance in food and feed. Oats have attracted research and commercial attention mainly due to their high content of β-glucan and phenolic compounds with high antioxidant activities as well as high-quality protein and fatty acids [18].

Today’s consumers have an access to a wide range of products whose consumption provides the recommended daily intake of substances that provide health benefits. Also, the nutritional quality of food products during storage has become one of the major concerns in the evaluation of food shelf life [19]. Modern society and lifestyle impose processed food with longer shelf life. On the contrary, microbiological safety issues and perishableness are the main factors discouraging widespread consumption of sprouts. Drying is one of the traditional methods to prepare foods for longer storage, and freeze-drying is a contemporary technique that is suitable for drying of heat-sensitive materials. Storage can strongly influence the sensory, nutritional, and bioactive characteristics of the food product, affecting its various physical, chemical, and biological properties, which may lead to vitamin and pigment loss and the product degradation. Thus, the preservation method and conditions, such as light, temperature, and humidity, are critical, especially for foods expected to be sources of bioactive compounds [20].

To the best of our knowledge, there are no examples of studies using dried cereal sprouts in food matrices. Some authors have used fresh sprouts in creating novel food products. For example, Simsek et al. [21] tested the antidiabetic, cholesterol-lowering, angiotensin-converting enzyme (ACE) inhibitory, hemagglutinating, and total antioxidant activity of fermented vegetable juices with freshly blended germinated seeds and sprouts of lentils and cowpeas. Frias et al. [22] tested the inositol phosphate content and trypsin inhibitory activity in ready-to-eat (canned and freeze-dried) fresh 4-day germinated rape seeds and radish seeds. Mridula and Sharma [23] developed nondairy probiotic drink utilizing sprouted wheat, barley, pearl millet, and green gram separately with oat meal, stabilizer, and sugar using Lactobacillus acidophilus NCDC14 with soymilk with very good sensory acceptability. The present study was aimed to determine the total phenolic content, antioxidant activity, and colour capacity of potential sprout-based products that contain the optimized mixture of cereal sprouts (barley, oat, and wheat), with highest antioxidant properties, and salt in different ratios (1:1, 1:2, and 1:3). Additionally, these properties were assessed throughout the 6-month storage period, simultaneously exposed to the light and protected from the light.

2. Materials and Methods

2.1. Chemicals and Instruments. Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazine (DPPH), and Trolox were from Sigma Chemical Co. (St. Louis, MO, USA). Methanol was from Lach-Ner (Brno, Czech Republic). All other chemicals and solvents were of the highest commercial grade. For spectrophotometrical measurements, the Multiskan GO microplate reader (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used.

2.2. Plant Materials and Sprouting Conditions. Winter wheat cv. “Simonida” (Triticum aestivum L., ssp. vulgare), oat (Avena sativa L. “Golozrni”), and barley (Hordeum vulgare L. “NS565”) were donated by the Institute of Field and Vegetable Crops (NS Seme), Novi Sad, Serbia, all officially approved varieties by the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, registration numbers 320-04-574/2014-11, 320-04-183/2/2008-08, and 320-04-7228/2/2008-08, respectively. Sprouting of the cereal seeds was performed according to the method by Aborus et al. [24]. Germination lasted for 7 days, and then sprouts were freeze-dried (Alpha 2-4 LSC; Martin Christ, Osterode, Germany) and ground. Powdered wheat sprouts (WS), oat sprouts (OS), and barley sprouts (BS) were packed in vacuumed plastic bags and stored at −20°C.

2.3. Sprouts Mixture Design. The composition of the sprouts mixture with optimal properties (OSM) was defined by the response surface methodology (RSM), adopting a simplex-centroid experimental design for three variables at four levels. Independent variables were the share of oat sprouts (OS), barley sprouts (BS), and wheat sprouts (WS) in the sprouts mixture (SM), where their values were 0, 0.33, 0.50, and 1. The complete design is presented in Table 1.

2.4. Seasoned Salt Preparation. The optimal sprouts mixture (OSM) was used to formulate seasoned salt with sprouts (SS). OSM was mixed with table salt (iodine-enriched NaCl) in three ratios, 1:1 (SS1), 1:2 (SS2), and 1:3 (SS3), and homogenized. OSM, SS1, SS2, and SS3 were placed in transparent (OSM1, SS1t, SS2t, and SS3t) and amber (OSMα, SS1α, SS2α, and SS3α) glass bottles. Transparent bottles were exposed to daylight, while amber bottles were kept in dark. Storage tests included measuring the total polyphenolic content and antioxidant capacity for 6 months, once a month, as well as colour measurements at the beginning and at the end of storage.

2.5. Extraction of Sprouts Mixtures and Seasoned Salts. A sample of SS or SM (50 mg) was extracted with 500 μl of methanol (70%, v/v) in an ultrasonic bath for 20 min,
followed by agitation in a laboratory shaker at 200 rpm (Unimax 1010; Heidolph Instruments GmbH, Kelheim, Germany) under light protection for 2 h, at room temperature, and then filtered (Whatman paper No. 1) [25].

2.6. Total Polyphenolic Content (TPh). Total polyphenols were determined spectrophotometrically, following the Folin–Ciocalteu method [26] adapted to microplates. The content of total polyphenols was expressed as mg of gallic acid equivalents (GAE)/100 g of sample dry weight. Retention of polyphenols after storage was determined using the following formula: TPh retention (%) = (TPh of the sample during storage/TPh of the fresh sample) × 100.

2.7. HPLC Analysis of Polyphenolic Compounds. We followed the methods of Šaponjac et al. [27], where chromatograms were recorded using different wavelengths for individual phenolic compounds: 280 nm for hydroxybenzoic acids (gallic, protocatechuic, and vanillic acids), catechins (catechin and epicatechin), 320 nm for hydroxycinnamic acids (gallic, protocatechuic, and vanillic acids), catechins and sinapic acids, and 360 nm for flavonoids (quercetin). Separation was performed on a Luna 5 μm C-18 RP column, 250 × 4.6 mm (Phenomenex; Torrance, CA, USA), with a C18 guard column, 4 × 30 mm (Phenomenex; Torrance, CA, USA). Two mobile phases, A (acetonitrile) and B (1% formic acid), were used at flow rates of 1 ml/min with the following gradient profile: 0–10 min from 10% to 25% A, 10–20 min linear rise up to 60% A, and 20–30 min linear rise up to 70% A, followed by 10 min reversal to initial 10% A with additional 5 min of equilibration time. Reference substances were dissolved in 50% methanol.

2.8. Antioxidant Capacity (AC). Antioxidant capacity of SS and SM was determined by the extract’s ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, by a spectrophotometrical assay in a 96-well microplate reader, according to Mena et al. [28]. In brief, 250 μl of DPPH solution in methanol (0.89 mM) was mixed with 10 μl of the sample in a microplate well and left in dark at room temperature. Absorbances were read at 515 nm after 50 min. Methanol was used as a blank. AC was calculated using the following formula: AC (%) = ((ACsample - ACcontrol) / ACsample) × 100, where ACsample is the absorbance of the control and ACsample is the absorbance of the sample. Results were also expressed as μmol Trolox equivalents (TE)/100 g sample dry weight. Retention of antioxidant capacity after storage was determined using the following formula: AC retention (%) = (AC of the sample during storage/AC of the fresh sample) × 100.

2.9. Colour Measurements. The colour measurements were made with a Minolta reflectance colorimeter (Chroma Meter CR-300; Minolta, Osaka, Japan) considering the CIELab colour system [29]. The samples were placed in a 15 mm thick and transparent plastic cell without cover. A black plate was used as the background for standardization and a standard white plate for calibration. Chroma or saturation (C*) and total colour difference ΔE* were calculated using the following formulas: C* = √(a*2 + b*2) and ΔE* = √((L0* − L*)2 + (a0* − a*)2 + (b0* − b*)2) [29], where subscripts “0” and “*” refer to the colour value of the samples at the beginning of storage and after 6 months.

2.10. Statistical Analysis. Data were reported as mean ± standard deviation (SD) of three independent experiments. Data were analyzed by one-way analysis of variance (ANOVA) and t-test, where applicable. The level of significance was 95% (p < 0.05), by using OriginPro 8 SR2 software (OriginLab Corporation, MA, USA). Optimizations were carried out using Design-Expert version 10 (Stat-Ease, Inc., MN, USA).

3. Results and Discussion

3.1. Powdered Sprouts Mixture Optimization. Powders of sprouts were mixed in predesigned ratios and tested in terms of their total polyphenolic contents and antioxidant capacities (Table 1). Obtained results have shown that SM5 had the highest TPh (477.61 mg GAE/100 g) and AC (799.36 μmol TE/100 g) pointing to the superior antioxidant characteristics of barley sprouts. Previous reports [24] have reported that barley sprouts from the same cultivar (“NS565”) contained several classes of phytochemicals (phenolics, chlorophylls, xanthophylls, and carotenoids),

### Table 1: Experimental design and results for total polyphenolic contents and antioxidant capacities of cereal sprouts mixtures.

| SM formulation | Independent variables | Dependent variables |
|----------------|-----------------------|---------------------|
|                | BS: Share in SM       | TPh (mg GAE/100 g)  | AC (μmol TE/100 g) |
| SM1            | 0.5                   | 458.63 ± 1.42a      | 670.36 ± 16.25a    |
| SM2            | 0.5                   | 374.17 ± 12.35b,c   | 711.51 ± 0.34cd    |
| SM3            | 0                   | 324.43 ± 15.07b     | 200.20 ± 2.61b     |
| SM4            | 0                   | 381.70 ± 7.43c      | 668.36 ± 1.82b     |
| SM5            | 1                   | 477.61 ± 14.42d     | 799.36 ± 8.42d     |
| SM6            | 0.33                 | 364.10 ± 7.03b,c    | 432.07 ± 30.23c    |
| SM7            | 0.5                  | 328.96 ± 15.80b,c   | 343.58 ± 10.80c    |
| SM8            | 0.5                  | 482.36 ± 3.29cd     | 786.68 ± 9.26ab,d  |

SM: sprouts mixture; TPh: total polyphenolic content; AC: antioxidant capacity. * Values sharing the same letter in the same column are not significantly different at the 0.05 level.
significant antioxidant capacity, and antihyperglycaemic and anti-inflammatory activity, that could be beneficial to health, in addition to their nutritional benefits. On the contrary, the weakest antioxidant potential (324.43 mg GAE/100 g and 200.20 µmol TE/100 g) has been registered for SM3 and SM7, consisting of oat sprouts alone and in mixture with wheat sprouts, respectively.

The response surface methodology was employed to process the results presented in Table 1 and obtain the composition of the most potent mixture, considering both responses, TPh and AC. Multiresponse optimization results are presented in Table 2 and Figure 1. Due to low TPh and AC of OS, they were not considered for OSM formulation consisting of 92.9% BS and 7.1% WS. To validate the accuracy of the mixture optimization model, the observed (actual) values of TPh and AC and of prepared OSM with predicted formulations were compared. The observed TPh (484.63 mg GAE/100 g) and AC (801.06 µmol TE/100 g) were not significantly different from the predicted values at $p < 0.05$ (Table 2).

Contents of individual polyphenolics in the optimal sprouts mixture were quantified by HPLC analysis and presented in Table 3. It could be observed that the most abundant phenolic compounds were gallic (94.27 mg/100 g) and p-hydroxybenzoic (69.56 mg/100 g) acids. The most dominant sprouts in OSM were BS (92.9%). In the study by Aborus et al. [24], contents of gallic acid in barley sprouts in “BSNS” and “BSG” cultivars were 43.25 and 67.47 mg/100 g, respectively, while p-hydroxybenzoic acid was present in a smaller amount in “BSG” (2.18 mg/100 g).

### 3.2. Stability of Seasoned Salts with OSM

Storage and photodegradation studies are useful tools for a better understanding of an active substance behaviour and should be considered in product formulation and packaging studies.

The optimized mixture of cereal sprouts consisting of 92.9% barley and 7.1% wheat sprouts was mixed with salt in three ratios (1:1, 1:2, and 1:3). The mixtures were left at room temperature for six months and exposed to the light in transparent bottles and simultaneously to the dark place in amber bottles. The results for polyphenol retention, presented in Figure 2, showed that the light, as well as OSM, influenced the preservation of polyphenols throughout the storage period. The decreasing order of polyphenol retention after 6 months was OSMa > OSMt > SS1a > SS1t > SS3a > SS2a > SS2t > SS3t. The most significant decrease in the polyphenolic content after 6 months was in the SS3t sample (75.72%) which had the lowest amount of sprouts in the formulation (25%). On the contrary, the highest retention of polyphenols was in the OSMa mixture (71.69%). Furthermore, the sprout concentration and final polyphenol retention were highly correlated, for samples in transparent and amber bottles ($r = 0.95$ and 0.97, respectively). These results could suggest that polyphenols are better preserved when their concentration is higher and that light accelerated polyphenol degradation [30]. The results are in accordance with the work by Teixeira et al. [31] reporting that the rate of artepillin C (3,5-diprenyl-4-hydroxycinnamic acid) loss under the light and in the dark during the first week of storage is similar, but after the seventh day, the artepillin C loss is more pronounced under light. Polyphenols are very unstable compounds and may be degraded when exposed to high temperatures, oxygen, light, enzymes, and other elements [32]. These conditions must be taken into account when processing polyphenol-rich food matrices for the development of functional matrices, as well as upon their storage [33]. Esposto et al. [34] found that, during storage and light exposure of extra-virgin olive oils with higher olearoquin derivatives, the content had higher polyphenol and α-tocopherol loses, thus resulting in superior oxidative stability, suggesting the role of polyphenols as antioxidants and their consumption during oxidative processes.

Figure 3 represents the influence of storage on antioxidant capacities of OSM and seasoned salts. Generally, the rate of final retention of antioxidant capacity, after 6 months of storage, was higher than that for polyphenols. However, the trend was similar, following the decreasing order: OSMa > OSMt > SS1a > SS1t > SS2a > SS3a > SS2t > SS3t. Again, the most significant loss of AC was in the SS3t sample (49.76%), while the highest retention was in OSMa (81.44%). Similar to polyphenols, there was a high correlation between AC and sprout concentration, for samples stored in transparent and amber bottles ($r = 0.90$ and 0.91, respectively). Also, a strong linear relationship ($r = 0.89$) between antioxidant capacity (mg TE/100 g sample) and polyphenolic content (mg gallic acid/100 g sample) throughout the storage period has been determined.

Besides polyphenols, cereal sprouts are confirmed to be also a good source of other bioactive compounds, such as carotenoids and chlorophylls [24]. Carotenoids and chlorophylls are especially light sensitive and tend to degrade during time [35, 36]. Many authors have demonstrated first-order degradation kinetics of phytochemicals during heating or storage [37–40]. Hoffmann et al. [20] reported reduction of the flavonoid content (by 51%) and antioxidant potential against DPPH and ABTS⁺ in butía (Butia odorata) nectar during 90 days of room temperature storage, while there were no changes in polyphenolic compounds content. However, there are numerous reports demonstrating various bioactive compounds acting in a synergistic manner [41]. This may elucidate good retention of AC (>50%) in all mixtures after 6 months.

| Variables | Predicted values | Observed values |
|-----------|-----------------|-----------------|
| BS (%)    | 92.9            |                 |
| OS (%)    | 0               |                 |
| WS (%)    | 7.1             |                 |
| TPh (mg GAE/100 g) | $482.81 \pm 7.21$ | $484.63 \pm 4.68$ |
| AC (µmol TE/100 g) | $797.97 \pm 4.28$ | $801.06 \pm 5.30$ |

BS, OS, and WS: barley, oat, and wheat sprouts; TPh: total polyphenolic content; AC: antioxidant capacity.
Storage and light exposure has adversely affected the colour characteristics of OSM and seasoned salts (Table 4). CIELab coordinates describe the lightness of the colour ($L^*$; equals 0 yields black and $L^*$ equals 100 indicates diffuse white), red/magenta to green colour ($a^*$; negative values indicate green while positive values indicate magenta), and yellow to blue colour ($b^*$; negative values indicate blue and positive values indicate yellow). Lightness and intensity of green and yellow colours have decreased significantly in all samples after 6 months. Samples stored in transparent bottles were lighter and less green and yellow, as demonstrated by higher $L^*$ and $a^*$ values and lower $b^*$ values, probably due to light degradation of pigments. The trend was similar with decreasing sprout concentration. The decrease of chroma (C) values found after 6 months of storage for all samples was in accordance with other reports [42]. There was an inverse correlation between $L^*$ values, in transparent and amber bottles, and sprout concentration ($r = -0.69$ and $r = -0.73$, respectively) and $a^*$ values for samples in transparent bottles ($r = -0.97$). On the contrary, $a^*$ and $C^*$ values for amber bottles and $b^*$ for transparent bottles were found to be highly correlated with sprout concentration ($r = 0.97$, 0.98, and 0.96, respectively). To define the colour differences, $\Delta E^*$, according to CIE76, was calculated between samples in transparent and amber bottles, as well as between samples at the beginning and at the end of storage time ($\Delta E^*_{t/a}$). Since the value 2.3 represents “just a noticeable difference,” it could be observed that the difference in colour characteristics between samples exposed to light and protected from light, $\Delta E^*_{t/a}$ ranging from 5.13 to 9.19, could be noticeably distinguished after the 6-month storage period. This colour change was moderately and inversely correlated with the concentration of sprouts in the mixture ($r = -0.62$). Basically, each sample, regardless of its exposure to light, had severe change in its colour after 6 months, compared to the fresh sample ($\Delta E^*_{0/6}$ in the range of 13.45 to 18.08).

4. Conclusion

Cereal sprouts, due to their enhanced nutritional and bioactive features, could be considered as a valuable ingredient of a healthy diet. Their short shelf life and issues with microbiological safety have limited their use. Freeze-drying was presented as an excellent method for extension and facilitation of their utilization as ingredients of food. In this study, seasoned salt was used as an example of the
powdered cereal sprouts-derived product with the aim to incorporate them in diet in a microbiologically safe and practical way. The optimal mixture of barley, oat, and wheat sprouts, in terms of bioactive potential, was combined and used for formulation of seasoned salt. Despite bioactive compound degradation upon light exposure and storage, seasoned salts remained rich in phenolics and preserved antioxidant activity and colour characteristics. The results of this study provide the bioactive potential of cereal sprouts in a powdered form used for seasoning salt as an example for fortified products. Due to the bioactive potential of cereal sprouts, seasoned salt could be a good candidate for a new healthier product lower in the NaCl content.
| Colour characteristics | OSMt  | OSMa  | SS1t  | SS1a  | SS2t  | SS2a  | SS3t  | SS3a  |
|------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| \( L^* \)              | 63.52±0.69A | 63.76±0.40A | 64.57±0.28A | 63.95±0.28A | 63.69±1.01A | 64.29±0.71A | 64.07±0.47A | 63.45±1.28A |
| \( L^* \)              | 52.09±0.12A | 47.08±0.01B | 51.54±0.01A | 49.35±0.01C,F | 55.30±0.01D | 49.88±0.02C | 56.67±0.01E | 48.79±0.01F |
| \( a^* \)              | −3.17±0.20A | −2.43±0.17B | −3.22±0.00A | −3.22±0.00A | −3.20±0.00A | −3.25±0.09A | −3.52±0.15A | −3.29±0.16A |
| \( a^* \)              | −0.38±0.02A | −0.55±0.02A | 0.15±0.02A | −0.85±0.04C | 0.62±0.01B | −1.12±0.01E | 0.84±0.01F | −1.09±0.03E,F |
| \( b^* \)              | 26.61±0.29A | 26.22±0.03A,B | 26.27±0.29A,B | 25.54±0.03A,C | 25.98±0.24A,B | 25.14±0.48C | 28.7±0.31A,R,C |
| \( b^* \)              | 16.49±0.07A | 19.50±0.01B | 15.02±0.01C | 19.42±0.02D | 14.81±0.01E | 19.84±0.01F | 17.9±0.06E | 19.11±0.01G |
| \( C^* \)              | 26.80±0.28A,C | 26.34±0.05A,R,C | 26.46±0.29A,R,C | 26.41±0.19A,R,C | 25.74±0.03B,C,D | 26.18±0.25C | 25.39±0.49D | 26.07±0.32A,R,C,D |
| \( C^* \)              | 16.50±0.07A | 19.51±0.01B | 15.02±0.01C | 19.44±0.02D | 14.82±0.01E | 19.87±0.01F | 18.1±0.01E | 19.14±0.01G |
| \((\Delta E^*_{ab})_{t/a}\) | 6.06±0.44A | 5.13±0.20B | 5.13±0.20B | 7.59±0.01C | 9.19±0.01D |
| \((\Delta E^*_{ab})_{0/6}\) | 15.31±0.63A,D,E | 17.37±0.42B | 14.17±0.64B,C | 13.45±0.67A,B | 18.08±0.38A,E | 16.27±0.31A,C,D | 15.80±0.73E | 16.29±1.28A,B |

Values are presented as mean ± SD of three measurements. Values marked with different A–E capital letters in the same row are significantly different at the \( p < 0.05 \) level. Subscripts "0" and "6" refer to the colour value of samples at the beginning of storage and after 6 months. \((\Delta E^*_{ab})_{t/a}\): total colour differences between samples in transparent and amber bottles. \((\Delta E^*_{ab})_{0/6}\): total colour differences between samples at the beginning of storage and after 6 months.
Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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