

BIOACTIVE COMPOUNDS, PHYSICO-CHEMICAL CHARACTERISTICS, AND SENSORY ANALYSIS OF BISCUITS ENRICHED WITH SAFFRON AS NATURAL COLORANT

– Research paper –

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Abstract: Recently, the primary focus of the food industry lies in replacing synthetic additives with natural ones. Saffron (*Crocus sativus*) is an agricultural product used by agri-food industries as naturel colorant. The aim objective of the present work was to explore the application of Saffron (*Crocus sativus*) crocin extracts as natural colorant in biscuits. Furthermore, a comparative study was conducted to evaluate the performance of the cookies during 45 days of refrigerated storage. Physicochemical analysis was carried out on the biscuits prepared during storage. In addition, total phenolics, flavonoids, and crocins content was assessed. The free radical scavenging activity (DPPH) and ferric reducing power were also evaluated. All parameters studied were carried out after 15, 30, and 45 days of storage period. The obtained results indicate that the use of saffron in the manufacture of biscuits gives a product with a good physicochemical quality for a limited duration (45 days of storage). Indeed, the values of moisture content, pH, and acidity are in line with the cookie preparation standard. A variation in these values was recorded during storage. In addition, the saffron biscuit showed a high level of phenolics, flavonoids and condensed tannin contents with a strong antioxidant activity. A difference in phenolic compounds level and in the antioxidant activity during storage period was also recorded for the biscuits prepared with synthetic colorant and those without colorant. A natural additives offer a more convenient alternative for consumers who prefer food products without synthetic additives. Hence, the biscuits enriched with saffron as natural additives can be developed as functional food with healthy benefits.

Keywords: *Crocus sativus*, Biscuit, physicochemical parameters, bioactive compounds, antioxidant activity, sensory analysis.

INTRODUCTION

Crocus sativus L. is the Iridaceae family which characterized as a perennial herbaceous geophyte. Gender *Crocus* includes over 80 species, most of which are disseminated in Southeast Asia and the Mediterranean area (Maleki et al., 2017). In the Mediterranean climate, where the winter is cool and damp, autumn/winter/spring with rainfall, and the summer is dry and warm with small precipitation, the saffron culture is suitable (Lage and Cantrell, 2009). Saffron stigmas considered as one of the most expensive spices named 'red gold'; this is due to the scarcity and nobility of

their functional compounds and also considered as culinary adjuncts that in recent years have attracted greater market interest (Maleki et al., 2017). This aromatic and medicinal plant is endemic to Algeria and serves as a valuable source of functional compounds. These compounds have been used in the treatment of several symptoms and diseases including hypoxia, asthma, cramps, liver disease, menstrual disorders, pain, and cancer pathogenesis (Khorasanchi et al., 2018; Lv et al., 2019).

Crocins are orange-yellow pigments; natural carotenoids found in stigmas of saffron (Rameshrad et al., 2018). These compounds are responsible for both the color and consistency of

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saffron, which is especially used as a food coloring and flavoring ingredients. In the scientific literature, biological and functional attributes of crocins have been assessed and include hepato-, nephro-, cardio- and neuro-protective effects (Lv et al., 2019; Zhu et al., 2019). Moreover, these compounds exhibit notable anti-inflammatory, anti-cancer, anti-hyperlipidemia, and anti-Alzheimer properties, contributing to their wide range of therapeutic effects (Chalatsa et al., 2019; Faridi et al., 2019; Pashirzad et al., 2019). Furthermore, the beneficial effects of these compounds to attenuate the metabolic syndrome-induced osteoporosis, the streptozotocin-induced diabetes, the burning mouth syndrome related to anxiety and/or depression, and the disorders related to oxidative stress are also reported (Pashirzad et al., 2019; Sefidgar et al., 2019). Natural plant extracts have the potential, with their antioxidant properties, to serve as alternatives to synthetic additives. These extracts contribute additional value to the final product (Pasqualone et al., 2015). For the purpose of incorporating

antioxidants, natural extracts from aromatic plants, spices, and fruit powder have already been utilized in the development of bakery, dairy, and meat products (Bajaj et al., 2006).

According to the FAO-WHO committee, a food additive is defined as a substance with or without nutritional value, intentionally added to food for technological, health, organoleptic or nutritional purposes. Its use must improve the qualities of the finished product without presenting any danger to health, at the doses used (Dutau et al., 1996). In addition, the cookies formulated saffron stigma showed acceptable sensory score with enhanced antioxidant properties and good shelf-life (Bhat et al., 2018).

The objective of this work was to assess saffron efficacy in a new food matrix, specifically biscuits with saffron colorant. The study aimed to compare the performance of these biscuits with a control group (biscuits without colorant) and biscuits containing a synthetic colorant SIN102 (SIN: *Système International de Nomenclature*, SIN system) after 15, 30, and 45 days of storage.

MATERIAL AND METHODS

Plant material

In Algeria, saffron (*Crocus sativus* L.), known as Zafren, was harvested from Djebel El-Ouahch in the Constantine region (northeast of Algeria) on October 15, 2018. Their flowers and stigmas were carefully retrieved and then these portions were separated. The stigmas were dried in a dark and dry location at 35° C. Dried stigmas were ground and sieved (<500µm). Then, the sample was conserved at 6°C for further analyses.

Biscuits preparation

For the biscuit preparation, a traditional recipe based on the method developed by Caleja et al. (2017) was followed with minor adjustments. Initially, one egg (60 grams) was thoroughly mixed with 125 g of sugar. Subsequently, 0.1 mg of saffron powder was dissolved in 60 mL of distilled water and added to the mixture. Then, 300 g of durum wheat flour were gradually incorporated into the mixture while mixing vigorously manually. After allowing the dough to rest for 15 minutes, it was rolled out to a thickness of 5 mm and cut into round shapes using a biscuit cutter. Three types of biscuits were prepared: biscuits without any added colorant, biscuits with saffron colorant, and biscuits with synthetic colorant (SIN 102). Then, the biscuits were baked in an electric oven for 10 min at 180 °C. The three

formulation of biscuits were kept at the room temperature. After 0, 15, 30, and 45 days of storage, the biscuits were ground and sieved (<500µm). The powders were packed in a sealed plastic bag covered with aluminum paper and stored at -4°C until further analysis.

Physico-chemical analysis of biscuits

Humidity of biscuits was determined by drying of 4g of each biscuit (Control, SIN 102, and saffron colorants) and place in an oven set at 105° followed by a weight measurement after every 2 hours until weight stability. pH measurements were conducted using a pH meter (Metrohm model 692, Herisau, Switzerland) at a temperature of 20°C. while, acidity was measured by titrating with NaOH (0.1 N) in the presence of a few drops of phenolphthalein (1%) until a color change to pink and pH=8.2. Likewise, The Brix degree was measured using a refractometer, which determines the refractive index of light from a solid or liquid matrix (Ydjedd et al., 2021).

Phytochemical content

Total phenolic contents (TPC)

The total phenolic content (TPC) was assessed using the method described in our previous study (Chaalal et al., 2013). A volume of 150 µL of biscuits extracts was added to 600 mL sodium

carbonate (7.5%) and 750 mL of Folin–Ciocalteu reagent. The absorbance of the mixture was measured at 750 nm. The results were expressed as milligrams of gallic acid equivalent per 100 grams (mg GAE/100g).

Total flavonoid contents (TFC)

The estimation of flavonoid content was carried out using the method reported by Quettier-Deleu et al. (2000).. A volume of 150 L of biscuits extract was mixed with 150 mL of chloride aluminum (2%). After a 15 min incubation, the absorbance of the reaction mixture was measured at 430 nm. The results were expressed as milligrams of quercetin equivalents per 100 grams (mg QE/100g).

Condensed tannin contents (CTC)

The condensed tannin contents were estimated using the butanole HCl assay as described by butanole HCl assay (Maksimović et al., 2005). A volume of 0.5 mL of biscuits extracts was added to 3 mL of butanole HCl (95:5; v/v) and 0.1 mL of iron sulfate (2 %). After 60 min of incubation at 90 °C, the absorbance was measured at 530 nm. The results were calculated using the Beer-Lambert law ($A = \epsilon * L * C$) with molar extinction coefficient of cyanidin $\epsilon = 34,700 \text{ Lmol}^{-1}\text{cm}^{-1}$. The condensed tannin contents were expressed as milligrams of cyanidin equivalent per 100 grams (mg CE/100g).

Total crocin content (TCC)

The total crocin content (TCC) was assessed following the method described by Prieto et al. (2015). The absorbance of the saffron (crocin contents) was measured at 450 nm. The results were calculated using the Beer-Lambert law ($A = \epsilon * L * C$) with molar extinction coefficient of crocin-1 [R-crocin, crocetin-di(-D-gentiobiosyl) ester], $\epsilon = 15.117 \text{ Lmol}^{-1}\text{cm}^{-1}$. The rocin contents were expressed as milligrams of crocin equivalents per 100 grams (mg crocin E/100g).

Antioxidant activities

Free radical scavenging activity (DPPH)

The free radical scavenging capacity (DPPH) was evaluated using the method reported in our previous study (Chaalal et al., 2013). A volume of 200 μL of extract was added to 1000 μL of Methanolic DPPH solution (60 μmol). The absorbance was measured at 517 nm after 30 min of incubation at room temperature. The DPPH radical reduction percentage was determined by applying the formula as follows: $\text{DPPH \%} = [(\text{Abs}_C - \text{Abs}_S) / (\text{Abs}_C)] * 100$. Abs_C represents the absorbance of the DPPH solution without the

sample, and Abs_S represents the absorbance of the DPPH solution with the sample extract.

Ferric reducing power (FRP)

The method described by Chaalal et al. (2013) was used to estimate the free reducing power of the samples. Equal volume of extract, phosphate buffer (0.2 mol/l, pH 6.6), and potassium ferricyanide solution (1%) was mixed. After incubation at 50 °C for 20 min, the same volume of trichloroacetic acid (10%) were added. After centrifugation at 3000 rpm for 10 min, 1.25 mL of supernatant was added to 1.25 ml of distilled water and 0.25 ml of ferric chloride (0.1%). The absorbance was measured at 700 nm. The results were expressed as milligrams of ascorbic acid equivalents per 100 grams (mg AAE/100 g).

Phosphomolybdenum method (PhM)

The phosphomolybdenum method, as described by Ramalakshmi et al. (2008), was used to evaluate the total antioxidant capacities of the extracts. A volume of 100 μL of extracts was added to 1 mL of the phosphomolybdenum solution (sulfuric acid 0.6 mol/l, sodium phosphate 0.028 mol/l and ammonium molybdate 0.004 mol/l). The absorbance of the mixture was measured at 695 nm after 1 hour of incubation at 90 °C. The results were expressed as milligrams of ascorbic acid equivalent per 100 grams (mg AAE/100 g).

Sensory analysis

The sensory analysis was carried out following the method described by Basu et al. (2011). An untrained panel consisting of 30 students, including 12 males and 18 females, with an average age ranging from 21 to 26 years, was used. The biscuit samples were prepared at room temperature and served after a 3 hour after baking. The descriptors evaluated by the panel are color, odor, taste, texture, aspect, and overall acceptability. These descriptors were estimated using a nine-point hedonic scale. The score of 9 indicated "like extremely" and a score of 1 indicated "dislike extremely."

Statistical analysis

All experiments were conducted three times, and the obtained data were expressed as means $\pm$ standard deviation. Statistical analysis of the results was performed using STATISTICA® 5.5 software through analysis of variance (ANOVA). A significance difference was considered at $*P < 0.05$. The letters a, b, c, and d indicating differences between the means.

RESULTS AND DISCUSSION

Physico-chemical parameters

Table 1 presents the physical-chemical of control biscuits, biscuits with SIN 102, and biscuits enriched with saffron colorant during 45 days of storage. The results indicate a decrease in humidity values from 1% (biscuits enriched with SIN102) just after biscuits formulation to 0.72% (biscuits with saffron) over the same 45-day period. These findings suggest that the low water content in the biscuits contributes to their effective preservation. The obtained results were lower than those by Gandhi et al. (2021), who found 4.32% in their study on biscuits enriched with whey protein concentrate-iron sulfate complex (WPC-FeSO₄). The pH parameter is a crucial factor in determining food quality and is one of the parameters that affects the proliferation of microbial flora. The obtained results showed that the pH values ranged from 6.53 for the biscuits with saffron to 7.80 for the biscuit with SIN102 after 45 days of storage. The obtained pH is acceptable compared to the values established by Parveen et al. (2017), who found a pH of 8.50 for the biscuits enriched with fiber.

The acidity analysis allows for the estimation of the rate of hydrolytic, enzymatic, or chemical degradation of triglyceride fatty acids (Türkmen and Ekşi, 2011). Additionally, the results indicate that the acidity values increased from 0.134 (control) to 1.286 (biscuit with SIN102) as the storage period increased from 0 to 45 days. The Brix value indicates the percentage of dry matter soluble in water in a food product (Türkmen and Ekşi, 2011). The results show an increase in values from 2.70% (biscuit with SIN102) at T₀ (immediately after preparation) to 3.70% (biscuit with saffron) after 45 days of storage. Between days 15 and 45 of storage, no significant difference was observed between the Brix values of the three formulation of biscuits. A small

chemical changes (pH, °Brix, and acidity) were observed in the composition of biscuits supplemented with cladode flour (*Opuntia ficus indica*) after 60 days of storage (Nabil et al., 2019). In addition, 2. Ali et al. (2021) found that the brix value of biscuits enriched with date powder was 9.50%. A decrease in humidity values and a controlled pH range might be considered favorable indicators of biscuit quality and stability. An increase in acidity values could signal changes in fatty acid composition, which affected the sensory analysis of biscuits. Likewise, the Brix value changes could be seen as natural variations within acceptable limits or as indicative of changes in soluble solids content.

Phenolics, flavonoids and condensed tannins

The phenolic compounds, known as phytomicro nutrients, are synthesized by plants and which belong to their secondary metabolism. According to Figure 1, the content of total phenolic of the three formulation of biscuits at the first time of storage were 99.705, 107.004, and 88.555 mg GAE/100g for the control, biscuit with INS 102, and for biscuit with saffron, respectively. Therefore, these contents were significantly reduced ($p < 0.05$) to reach the following values: 70.230, 61.175, and 74.780 mg GAE/100g for biscuits control, biscuits with SIN 102, and for biscuits with saffron colorant, respectively. The obtained results were lower than those obtained by Mahloko et al. (2019) and Elhassaneen et al. (2016) for wheat-prickly pear and banana biscuits and biscuit incorporated with prickly pear peel and potato, respectively. The decrease of these compounds (TPC) is attributed to the occurrence of epolymerization of polyphenols and decarboxylation of phenolic acids during the thermal treatment (Krystyan et al., 2015).

Table 1. Physico-chemical analysis of three lots of biscuits studied during storage.

	Time (days)	Humidity (%)	pH	Acidity (g/L)	Brix (%)
Control	0	1.14±0.02 ^a	6.80± 0.02 ^c	0.134±0.003 ^c	2.73±0.06 ^c
	15	0.89±0.02 ^b	6.85± 0.03 ^c	0.286±0.003 ^c	3.10±0.00 ^b
	30	0.80±0.02 ^c	7.08± 0.06 ^b	0.393±0.02 ^b	3.17±0.06 ^{a,b}
	45	0.75±0.02 ^d	7.23± 0.04 ^a	0.430± 0.01 ^a	3.40±0.05 ^a
SIN 102	0	1± 0.02 ^a	6.56 ±0.05 ^c	0.147±0.003 ^d	2.70±0.10 ^b
	15	0.89±0.02 ^b	6.72± 0.02 ^c	0.297±0.01 ^c	3.07±0.06 ^a
	30	0.79± 0.02 ^c	7.19± 0.02 ^b	0.359±0.01 ^b	3.10±0.00 ^a
	45	0.76±0.02 ^d	7.32 ±0.03 ^a	1.286±0.03 ^a	3.20±0.00 ^a
Saffron	0	1.37±0.02 ^a	6.53 ±0.03 ^c	0.169±0.003 ^d	2.80±0.00 ^c
	15	0.85±0.02 ^b	6.70 ±0.06 ^c	0.255±0.003 ^c	3.50±0.00 ^b
	30	0.75±0.02 ^c	7.36 ±0.02 ^b	0.291±0.003 ^b	3.63±0.06 ^a
	45	0.72±0.02 ^d	7.42 ±0.01 ^a	0.614±0.03 ^a	3.70±0.00 ^a

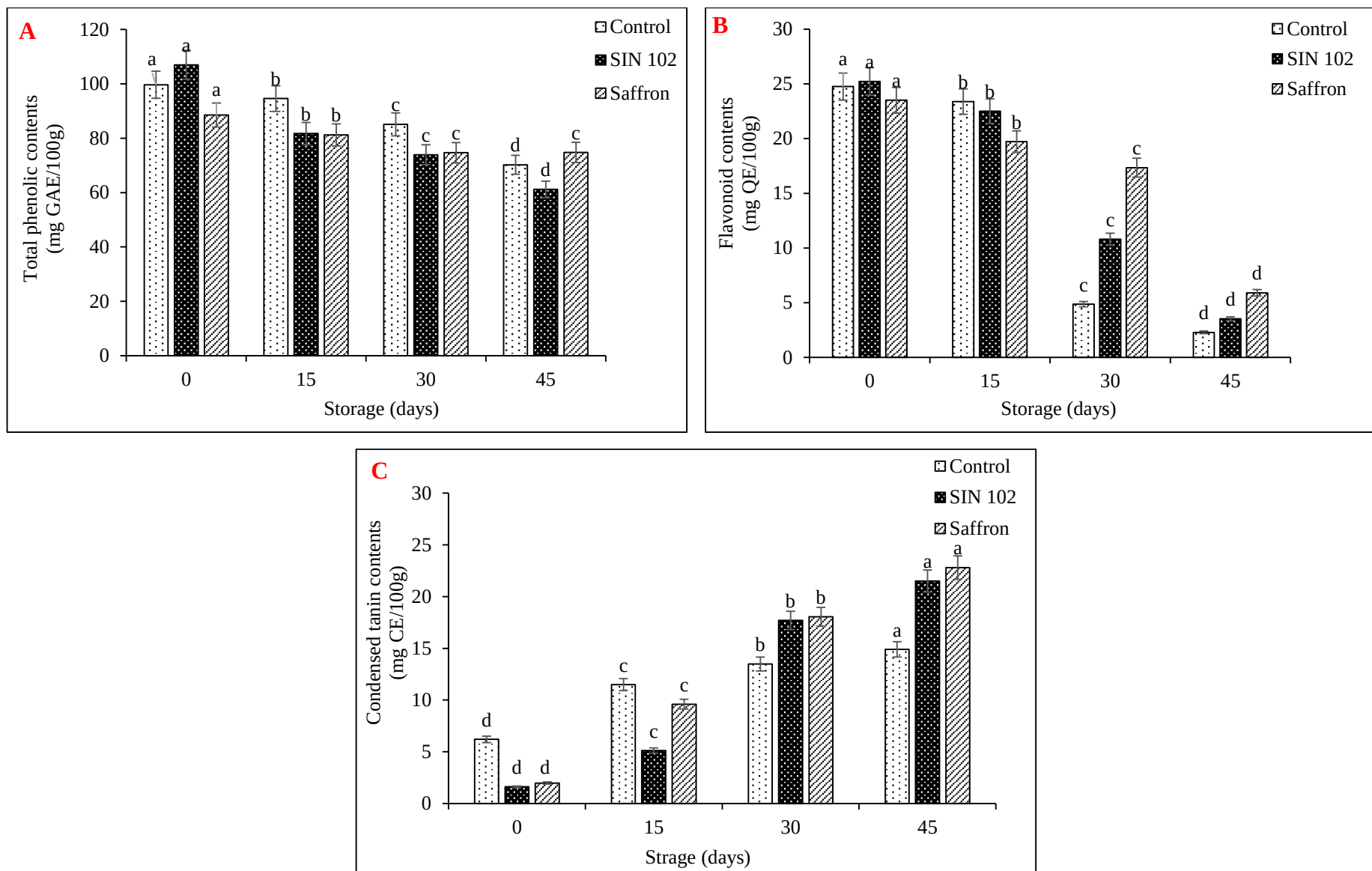


Figure 1. Total phenolic (A), flavonoid (B), and condensed tannin (C) contents of three lots of biscuits during storage.

Regarding the total flavonoids contents (TFC), the results show that the contents of different biscuits formulation were decreased after 45 days of storage. Indeed, the TFC of control biscuits decreased from 24.76 to 2.289 mg QE/100g. Similarly, the biscuits with SIN102 decreased from 25.288 to 3.528 mg QE/100g, while the contents of biscuits with saffron colorant decreased from 23.51 to 5.899 mg QE/100g. Statistical analysis revealed a significant difference ($p < 0.05$) between TFC of three formulation of biscuits. In addition, a significant difference ($p < 0.05$) in TFC was observed during the storage period. The observed differences in the total flavonoid content could be attributed to the formation of meladins. Meladins are brown pigments that are produced as a result of the Maillard reaction, which occurs during the baking process. These pigments can affect the stability and content of flavonoids in the biscuits, leading to a decrease in their levels over time (Manzocco et al., 2000). According to Pasqualone et al. (2015), there was a significant increase ($p < 0.05$) in flavonoids content in biscuits. They attributed this increase to the presence of semolina and shortening as well as incorporation of plant by-products which contribute their volatile compounds. On the other hand, the polyphenols degradation during storage has been reported by Šaponjac et al. (2016). a similar degradation phenomenon could occur in the study conducted by Bhat et al. (2018).

For the condensed tannin contents (CTC), the results showed a significant increase of CTC during the storage period (45 days of storage). Indeed, the values were increased from 6.2 to 14.9 mg CE/100g for biscuits control, from 1.62 to 21.50 mg CE/100g for biscuits with SIN102, and from 1.98 to 22.80 mg CE/100g for biscuits

enriched with saffron colorant. This increase of CTC can be explained by polymerization of polyphenols from condensed tannin during storage.

Total crocin content

Figure 2 illustrates the crocin content of biscuit enriched with saffron powder (crocin) over storage period. At time of T_0 (just after biscuits preparation), the crocin content of biscuit was 69,289 mg crocin eq/100g. However, these contents were significantly ($p < 0.05$) decreased during storage period. indeed, after 15, 30, and 45 days of storage, the TCC values were recorded as 56.289, 50.318, and 35.808 mg crocin eq/100g, respectively. These results suggest that the storage period influence the stability of saffron contents of biscuits. Similar findings were reported by Bhat et al. (2018), who observed a significant decrease in saffron concentrations in cookies during storage. The authors concluded that the increased water activity in the cookies might have accelerated the deterioration of saffron content. In addition, Raina et al. (1996) noted that higher moisture content during storage could contribute to the degradation of saffron metabolites.

Antioxidant activities

Figure 3 presents the results of free radical scavenging activity (DPPH), ferric reducing power (FRP) and Phosphomolybdenum activity (PhM) of three lots of biscuits studied during storage. As showed in the results, the biscuit enriched with saffron colorant showed a higher antioxidant activity (DPPH, FRP, and PhM) throughout the storage period. Likewise, the control biscuits showed the lower antioxidant activities.

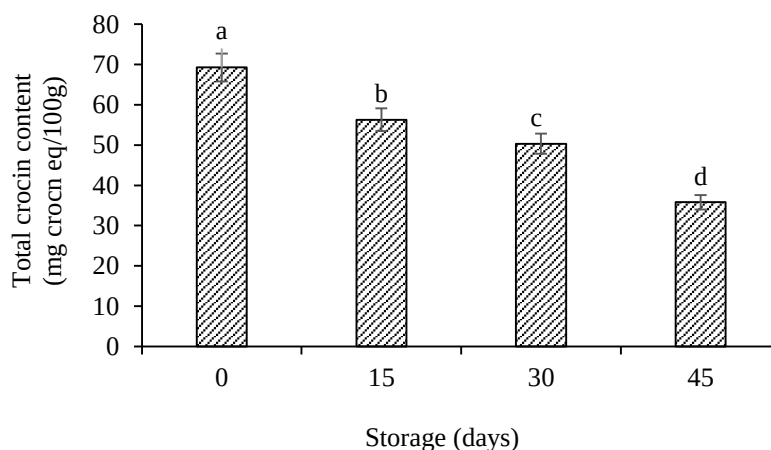


Figure 2. Total crocin contents of biscuits enriched with saffron during storage.

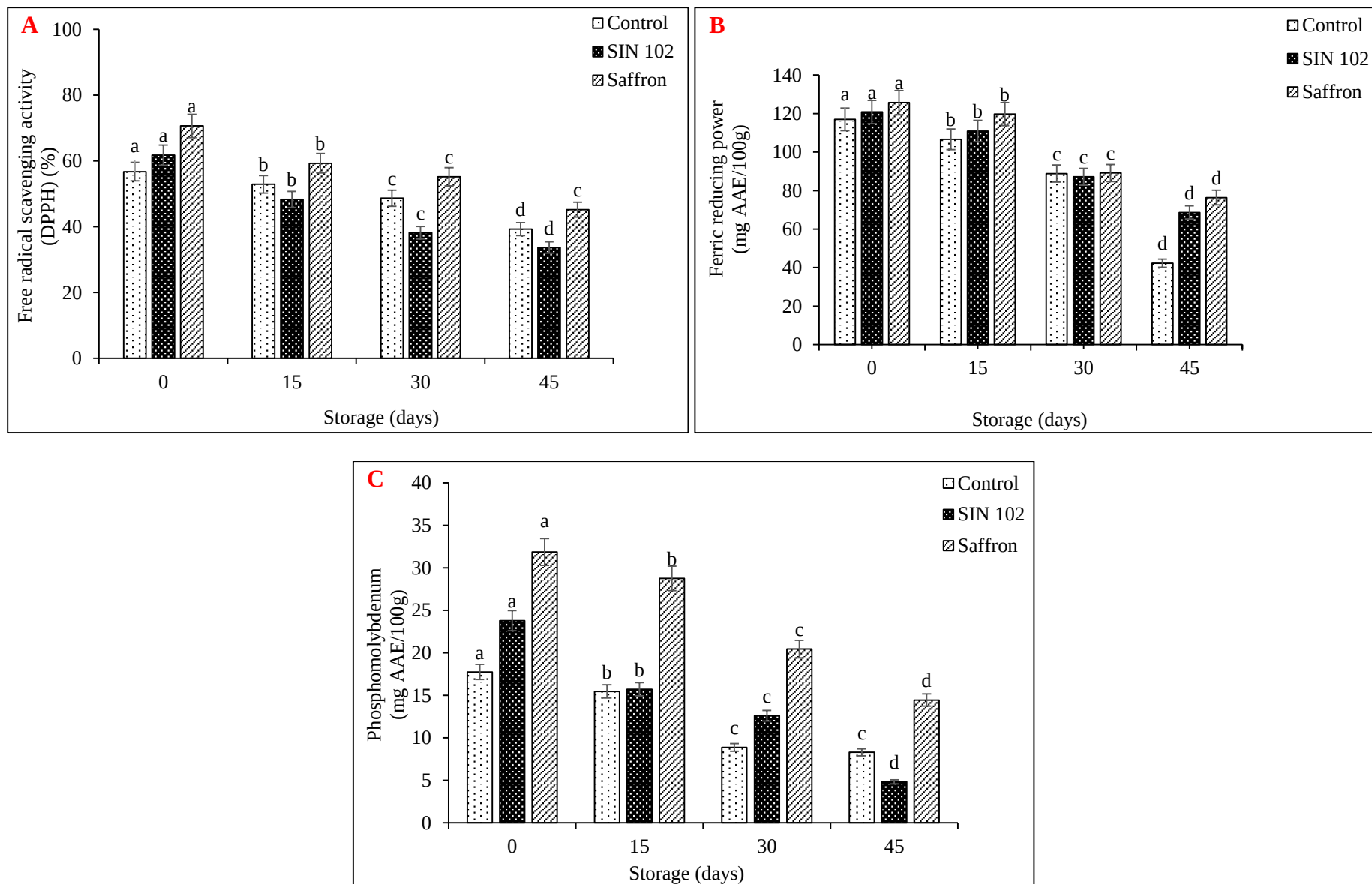


Figure 3. Free radical scavenging activity (DPPH) (A), Ferric reducing power (FRP) (B), and phosphomolybdenum (C) of three lots of biscuits during storage.

Indeed, for free radical scavenging activity (DPPH), the biscuits incorporating with saffron colorant showed a height activity than the biscuits incorporated with SIN102 and the control biscuits. However, this activity was decreased during the storage period from 56.78 to 39.325% for the control biscuits, from 61.79 to 33.707% for the biscuits with SIN102, and from 70.674 to 45.224% for the biscuits with saffron colorant. The values obtained in this study were higher compared to those previously reported by Mahloko et al. (2019) for heat-prickly pear and banana biscuits. The decrease in the free radical scavenging activity (DPPH) of stored cookies can be attributed to the degradation of phenolic compounds during this storage period (Zhou et al., 2014). Similar trend was observed by Bhat et al. (2018) for cookies incorporate by saffron extract. These observations were similarly reflected in the results of ferric reducing power (FRP) and Phosphomolybdenum activity (PhM). Notably, a significant decrease in FRP levels was observed over the storage duration, with values decreasing from 116.963 to 41.304 mg AAE/100g for the control biscuits, from 120.83 to 68.652 mg AAE/100g for biscuits enriched with SIN102, and from 125.709 to 76.385 mg AAE/100g for biscuits containing saffron coloring. These results align with the findings of Mahloko et al. (2019), who reported that FRP values of wheat-prickly pear and banana biscuits decreased during storage. Concerning phosphomolybdenum activity, the highest activity was obtained with the biscuit enriched with saffron with value of 31.867 mg AAE/100g. Likewise, the biscuit incorporated with SIN102 and the control biscuits exhibited a FRP value of 23.783 mg AAE/100g, while and 14.747 mg AAE/100g, respectively. This activity was also decreased during the storage period to allow the following values: 14.44 mg AAE/100g for the biscuit with saffron, 4.82 AAE/100g for biscuit with SIN102, and 8.297 mg AAE/100g for the control biscuits. The decreases of this activity during the storage period can be explained by the decreases of phytochemical compounds during this period of storage. The DPPH inhibition, FRP, and PhM for plant materials of plant materials generally follow a similar pattern as their phytochemicals content. Indeed, an increase in the concentration of phenolic compounds or the degree of hydroxylation of these compounds often leads to an increase in antioxidant activity. In this study, the antioxidant activity was found to be higher when the concentration of the natural additive (saffron) was higher for all evaluated antioxidant activities. Importantly, the presence of the natural extracts can offer additional benefits without deleterious effects.

In a similar study, the incorporation of fresh peel extracts from two different varieties of mango in biscuits demonstrated higher antioxidant properties compared to the biscuits prepared with synthetic antioxidant Butylhydroxyanisole (BHA) (Ajila et al., 2008). Pasqualone et al. (2015) reported that the functional biscuits prepared with wheat purple exhibited high antioxidant activity due to their higher levels of bioactive compounds. Other previous studies have also demonstrated the capacity of fennel and chamomile extracts and prickly pears (*Opuntia ficus indica* L.) betalains extracts to bring antioxidant activity to cottage cheese and yogurt, respectively (Caleja et al., 2017; Ydjedd et al., 2021). These phenolic compounds possess hydrogen atoms that are readily available to scavenge antioxidants. The antioxidant activity of a compound structure depends on the number and position of active groups (OH) incorporated within the compound.

Sensory analysis

Sensory evaluation of three different lots of biscuits were carried out to assess their sensory characteristics including color, odor, taste, texture, aspect, and overall acceptability. The results of this evaluation after 0, 15, 30, and 45 days of storage are presented in Figure 4. Throughout the storage period, the biscuits with added saffron exhibited higher levels of sensory attributes compared to other biscuits. Nevertheless, sensory attributes of three lots of biscuits declined after 45 days of storage. All panelists consistently attributed a higher level of overall acceptability to the biscuits with saffron when compared to the biscuits control and those with SIN 102. Furthermore, the saffron biscuits exhibited a significant differences compared to the control biscuits and those with SIN 102, particularly in terms of color and odor intensity. Overall, these findings indicate that color, odor, and texture are the three most significant factors influencing the overall acceptability of biscuits with saffron. Regarding the texture parameter, the results show that all types biscuits sets exhibited a decrease in values over time of storage. Concerning the taste and aspect of biscuits, all samples showed almost constant levels. As the storage time increased, the sensory characteristics of the cookies gradually decreased. After 45 days of storage, the biscuits enriched with saffron maintained their color, odor, and overall acceptability at a moderate level, indicating that they can be safely stored for up to 45 days without negative effect on their sensory properties. Temporal and projective methods will offer insights into the product's long-term sensory characteristics and market potential. These sensory studies help ensure the product market readiness (Oliveira et al., 2017).

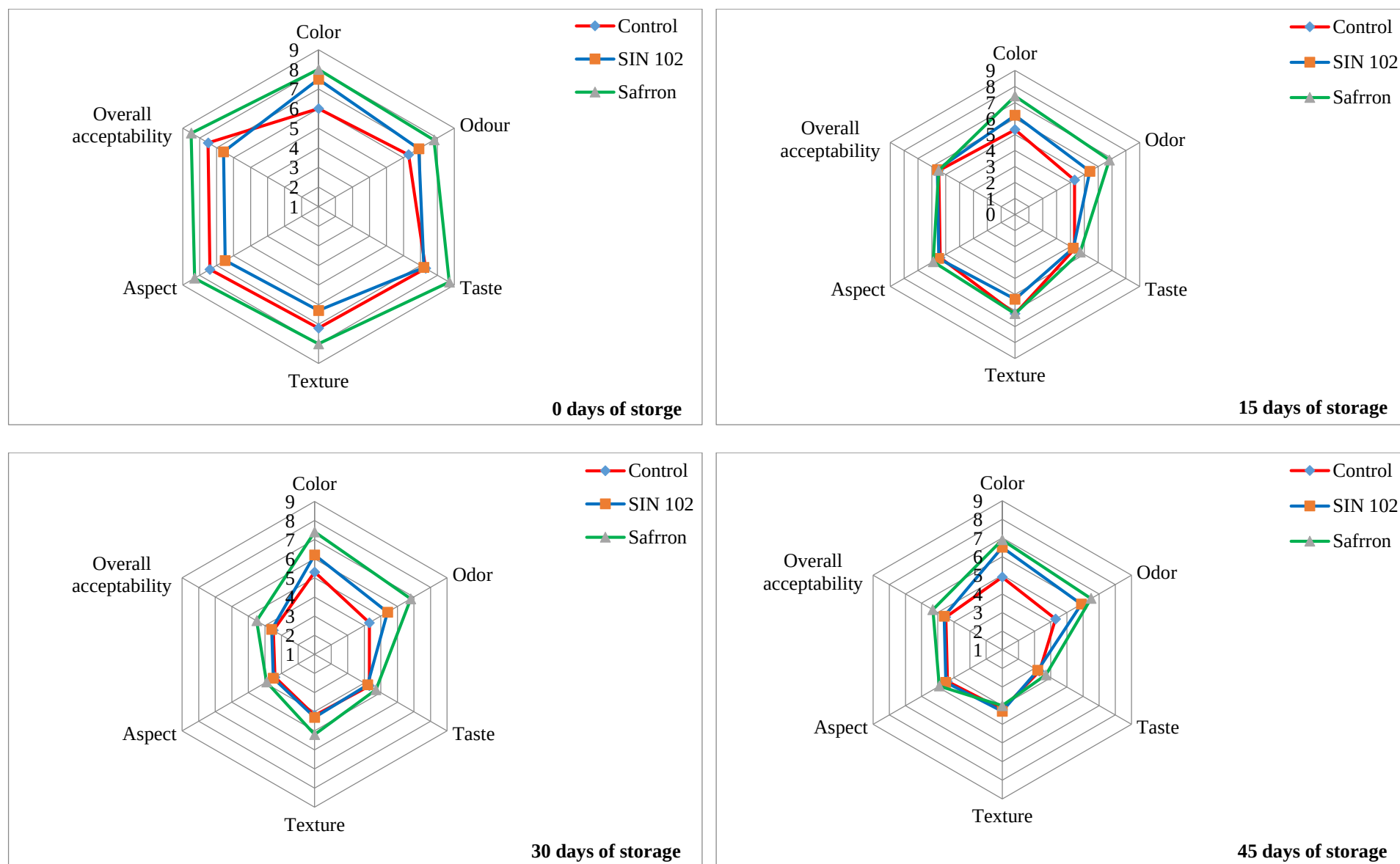


Figure 4. Spider diagrams of descriptive sensory analysis of biscuits (Green line: saffron biscuit; blue line: SIN 102 biscuits; red line: control biscuits) from 0 to 45 days of storage.

Correlation

The correlation coefficient "r" was calculated to determine the relationship between quantified phytochemical compounds and antioxidant activities in three formulation of biscuits during storage. A positive "r" indicates a positive correlation, where both variables increase together, while a negative "r" indicates a negative correlation, with one variable increasing as the other decreases. The correlation between the phytochemical compounds (TPC, TFC, TTC, and CTC) and antioxidant activity (DPPH, FRP, and PhM) of three type of biscuits during storage period was presented in Table 2.

A good correlation was noted between TPC and TFC and the antioxidant activity evaluated (DPPH, FRP, and PhM) with "r" value ranged from 0.75 to 0.996. Likewise, a negative correlation was recorded between CTC and all antioxidant activity tested with "r" value ranged from -0.996 to -0.81. Furthermore, a positive correction was also

observed between TCC and the antioxidant evaluated with "r" values of 0.960, 0.966, and 0.969 between TCC and DPPH, TCC and FRP, and TCC and PhM, respectively. In addition, a positive relationship between the parameters analyzed indicated the good contribution of TPC and TFC as well as the TCC on the antioxidant activity tested. These results indicate that the biscuits enriched with saffron exhibit higher antioxidant activity. Crocins and polyphenols exert a wide range of bioactive effects on antioxidative molecules (Karimi et al., 2010). The bioactivity of these compounds has been strongly associated with their antioxidant activities (Gentile et al., 2015). The antioxidant activity can arise from various mechanisms, and the true antioxidant capacity of a sample cannot be determined by relying on a single antioxidant assessment. (Gaglio et al., 2019). Furthermore, a significant relationship between the antioxidant activity and phenolic compounds of food samples has been reported by Oboh et al. (2008).

Table 2. Correlation matrices between phytochemical compounds and antioxidant activity.

	Control			SIN 102			Saffron		
	DPPH	FRP	PhM	DPPH	FRP	PhM	DPPH	FRP	PhM
TPC	0.82	0.75	0.88	0.996	0.927	0.936	0.960	0.966	0.969
TFC	0.89	0.86	0.99	0.932	0.997	0.936	0.945	0.894	0.936
CTC	- 0.87	- 0.81	- 0.91	- 0.953	- 0.986	- 0.920	- 0.973	- 0.971	- 0.979
TCC	-	-	-	-	-	-	0.960	0.966	0.969

CONCLUSION

Biscuits are widely consumed and appreciated by various consumer groups worldwide. Consequently, the biscuits enriched with antioxidants bio-resources may be attractive for consumers who are increasingly focused on choosing healthy foods. The results of this study revealed that the addition of saffron colorant in biscuits improved their antioxidant activity compared to those prepared by synthetic colorants (SIN 102) and the control biscuits. Similarly, a significant decrease was obtained for both phytochemical compounds and the antioxidant activity of three types of biscuits studied during storage period. Furthermore, a good correlation between the phytochemical compounds and the antioxidant activity evaluated. Importantly,

the addition of saffron on biscuits affected positively the appearance (color) and appreciation of panelists used during the sensory evaluation. Thus, biscuits enriched with saffron extract can be safely stored for a period of 45 days without any adverse changes in their organoleptic properties. The biscuits formulated saffron stigma showed acceptable sensory score with enhanced antioxidant properties and good shelf-life. The incorporation of saffron into the diet can significantly benefit human nutrition by contributing to the intake of dietary antioxidants.

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