Development of functional cookies using saffron extract

Naseer Ahmad Bhat¹ · Afshan Mumtaz Hamdani¹ · F. A. Masoodi¹

Revised: 3 September 2018 / Accepted: 11 September 2018 / Published online: 27 October 2018
© Association of Food Scientists & Technologists (India) 2018

Abstract Saffron extracts of two different concentrations were prepared and used as a source of natural antioxidants in whole wheat flour cookies. The effect on the color, texture and sensory properties of the product was also studied over a storage period of 9 months. Results revealed that spread ratio and hardness of cookies reduced non-significantly with the addition of saffron extract (SE). Color values ‘*L*’ and ‘*b*’ of cookies increased significantly from 50.7 to 53.9 and 36.5 to 47.0, respectively with the addition of SE while ‘*a*’ value decreased non-significantly ($p > 0.05$). DPPH radical scavenging activity, reducing power and inhibition of lipid peroxidation of dough and cookie samples containing SE were enhanced in comparison to control. The concentration of crocins, saffranal and picrocrocin in DS₅₀ and DS₁₀₀ dough samples was found as 28.30, 48.30, 104.6 µg/g and 35.14, 62.38, 118.2 µg/g, respectively. Sensory scores of cookies containing SE were high as compared to control. All the quality parameters of cookies reduced during the storage period (0–9 months). However, the cookies with added SE revealed significantly higher quality attributes up to 6 months of storage without any significant loss in quality.

Keywords Whole wheat flour · Saffron · Color · Antioxidant · Storage

Introduction

Bakery products contain large amount of fats and oils that oxidize slowly with storage and lead to development of rancidity and off-flavor. The development of rancidity due to fats and oils may be reduced by the use of antioxidants. Various synthetic antioxidants such as BHA (butylated hydroxy anisole) and BHT (butylated hydroxy toluene) have been used in processed foods to inhibit oxidation (Reddy et al. 2005). These antioxidants are effective in improving the shelf life of processed foods (Nanditha and Prabhasankar 2009). However, the use of synthetic antioxidants has been restricted due to their toxigenic, mutagenic, and carcinogenic effects. Thus, the demand for use of natural antioxidants derived from plants has increased to a large extent (Dillard and German 2000). The use of extracts obtained from spices, fruits and vegetables as natural antioxidants in bakery products has been reported by Nanditha and Prabhasankar (2009). In this regard, extracts obtained from saffron can be used as a source of natural antioxidants in numerous food formulations especially bakery products.

Saffron (*Crocus sativus* L.), the most expensive spice in the world belongs to the family Iridaceae. The components of saffron i.e. crocin, saffranal and picrocrocin are present in red stigmatic lobes of the flower. The coloring properties of saffron are mainly due to water soluble crocins that are glycosyl esters of crocetin with different sugar moieties (Carmona et al. 2006). Picrocrocin, a colorless glycoside is the major bitter compound of saffron. Furthermore, it acts as a precursor of saffranal, the main compound responsible for aroma (Jan et al. 2014). Several studies have reported that saffron has potent antioxidant activity, mainly due to the presence of crocin. However, the combined effect of other bioactive components of saffron provides it a

✉ Naseer Ahmad Bhat
bnaseerahmad03@gmail.com

Afshan Mumtaz Hamdani
afshanhamdani119@gmail.com

F. A. Masoodi
masoodi_fa@yahoo.co.in

¹ Department of Food Science and Technology, University of Kashmir, Srinagar 190 006, India

significant antioxidant activity. Many other researchers reported that saffron has several health benefits including reduction of coronary artery diseases, hypertension, stomach disorders, dysmenorrhea and learning and, memory impairments (Khazdair et al. 2015). Moreover, saffron has been observed to have anti-inflammatory, anti-atherosclerotic, anti-obesity antigenotoxic and cytotoxic properties (Mashmoul et al. 2013). The aim of the present study was to evaluate the addition of saffron extracts on the color, texture, sensory properties and antioxidant properties of whole wheat flour cookies.

Sample collection and preparation

Soft wheat cultivar HS-240 was obtained from Sheri-Kashmir University of Agricultural Sciences and Technology, Kashmir (SKUAST-K), Srinagar, India. The moisture content of the grains was increased to 14% before milling them in a laboratory mill (Amar Industries, Amritsar, India) to obtain whole wheat flour of uniform particle size.

Extraction and analysis of saffron metabolites

Saffron metabolites were extracted using the method described by Kumpati et al. (2003), with slight modification. 1 g (db) of saffron powder was taken in an amber colored flask and 15 mL of water was added to it. The mixture was homogenized to maximize the extraction of bioactive compounds and kept on magnetic stirrer for 24 h in dark. After extraction, the mixture was filtered and kept under frozen storage (-18 °C) for further analysis.

Quantification of saffron metabolites was done by following the ISO/TS 3632 (2003) procedure. As per this procedure picrocrocin, safranal and crocin are expressed as direct reading of the absorbance of 1% aqueous solution of saffron at 257 nm, 330 nm and 440 nm, respectively.

The results were obtained by taking absorbance, D, at three wave lengths (Hitachi, U-2900, Japan) as follows:

$E_{1\text{cm}}^{\%}$ (257): absorbance at 257 nm (maximum absorbance of picrocrocin)

$E_{1\text{cm}}^{\%}$ (330): absorbance at 330 nm (maximum absorbance of safranal)

$E_{1\text{cm}}^{\%}$ (440): absorbance at 440 nm (maximum absorbance of crocins)

$$E_{1\text{cm}}^{\%} = (D \times 10,000)/(m \times (100 - H))$$

where D is the specific absorbance; m is the mass of the saffron taken in grams; H is volatile and moisture content of the sample.

Crocin, safranal and picrocrocin contents in the saffron extract were observed as 13,917.40 µg/g, 3717.15 µg/g and 7584.48 µg/g, respectively.

Preparation of cookies

Whole wheat flour cookies were prepared by mixing flour with saffron extracts (SE) of two different concentrations. One of the saffron extracts was obtained from 50 mg of saffron powder, while the other extract was obtained from 100 mg of saffron powder. Each of the extracts was added to 100 g of flour. Control cookies (without saffron extract) were also prepared to check the contribution of supplements. Briefly, whole wheat flour, sugar, shortening (hydrogenated fat), skim milk powder, egg white, salt and baking powder were used in different proportions to prepare the cookies. Initially, 100 g of shortening was mixed thoroughly with whole wheat flour (250 g) in a laboratory mixer for 2 min. Subsequently, 112.5 g of sugar, 30 g of skim milk powder, 1.5 g of salt, 1.5 g of baking powder and 25 g of liquid egg white were added. Water was added as per the requirement. A portion of dough was taken for analysis and labeled as DS₅₀ (SE of 50 mg added to 100 g of flour) and DS₁₀₀ (SE of 100 mg added to 100 g of flour). The dough was sheeted to a uniform thickness of 6 mm and cut into round shapes using 5.00 cm diameter cutter. The cookies were baked at 160 °C for 15–20 min and then allowed to cool. After this, cookies were packed in air tight laminated pouches, labeled as CC, CS₅₀ and CS₁₀₀ and stored at ambient temperature for further analysis.

Proximate composition of cookies

Proximate analysis of the cookie samples was performed using standard AOAC (1990) methods.

Sensory evaluation

The evaluation of organoleptic properties of freshly prepared and stored whole wheat flour cookies was done by a total of 20 semi-trained panelists from the department of Food Science and Technology, University of Kashmir, India. Three differently coded samples were presented to panelists and sensory characteristics like appearance, texture, mouth feel, flavor and overall acceptability were recorded on the basis of 9-point hedonic scale.

Physical properties

The physical characteristic of cookies like diameter, weight, thickness and spread ratio were determined using the method of Zouari et al. (2016).

Texture

The hardness of cookies was determined using TA XT Plus, Texture analyzer (Stable Micro Systems, Vienna

Court, UK). The probe was set with pre-test speed = 1 mm/s, test speed = 3 mm/s, post-test speed = 10 mm/s and trigger force = auto. Each cookie was centered on the base plate and the travel distance of the blade was 5 mm. The peak force used to break the cookies represented the hardness of cookies.

Color

The Hunter color 'L' (lightness), 'a' (redness) and 'b' (yellowness) values of cookies were measured using Color Flex Spectrocolorimeter (Hunter Lab Colorimeter D-25, Hunter Associates Laboratory, Ruston, USA).

Total phenolic content (TPC)

Total phenolics of dough and cookie samples were estimated using the method described by Gao et al. (2002), with minor modifications. Each sample (200 mg, db) was extracted with 4 mL of acidified methanol (HCl/methanol/water, 1:80:10, v/v/v) on magnetic at room temperature stirrer for 2 h. Folin-Ciocalteu phenol reagent (1.5 mL) was added to centrifuge tubes containing 200 μ L of the sample extract. Contents were mixed and allowed to equilibrate for 5 min. The mixture was neutralized by adding 1.5 mL of sodium carbonate solution (60 g/L), followed by incubation at room temperature (25 °C) for 90 min. Absorbance of the samples was measured at 725 nm (UV-Spectrophotometer, Model U-2900 2JI-0003, Hitachi, Japan). TPC of samples was calculated using the standard curve of gallic acid and was expressed as mg of gallic acid equivalents (GAE) per gram of sample.

Antioxidant properties

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay

The modified procedure of Brand-Williams et al. (1995) was used to assess the DPPH radical scavenging activity of the whole wheat flour dough and the product. 100 mg (db) of sample was extracted by using methanol (1 mL) for 2 h and centrifuged at 3000 $\times g$ for 10 min. 100 μ L of supernatant was then reacted with 3.9 mL of a 6×10^{-5} mol/L of DPPH solution. Absorbance (A) (UV-vis spectrophotometer, Model, UV-2450, Shimadzu, Japan) of the samples was taken at 515 nm at 0 and 30 min using a methanol blank. Antioxidant activity was measured as % inhibition of DPPH radical calculated as below:

$$\text{DPPH radical scavenging activity (\%)} \\ = [1 - (A \text{ of sample } t = 30 / A \text{ of control } t = 0)] \times 100$$

Reducing power

The reducing power of the samples was determined by the method of Zhao et al. (2008). Samples were extracted in polypropylene tubes using methanol (1 mg/mL, db), vortexing, centrifuging at 3000 $\times g$ for 10 min and collecting the supernatant as extract. Then 1 mL of the sample extract was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 7.4) followed by addition of 2.5 mL potassium ferricyanide (1%). After this, the mixture was incubated at 50 °C for 20 min and 2.5 mL of Trichloro-acetic acid solution (10 g/100 mL) was added, followed by centrifugation (5810R, Eppendorf, Hamburg, Germany) at 10,000 $\times g$ for 10 min. The supernatant of the solution (2.5 mL) was mixed with 2.5 mL deionised water and 0.5 mL ferric chloride (0.1%). The absorbance (UV-Spectrophotometer, Model U-2900 2JI-0003, Hitachi, Japan) of the mixture was read at 700 nm. A standard curve was prepared using different concentrations of ascorbic acid and results were expressed as ascorbic acid equivalents (AAE) per gram of sample.

Lipid peroxidation

Lipid peroxidation of samples was estimated by using the procedure of Wright et al. (1981) with slight modification. The sample extract (250 μ L) was mixed with 1 mL of Linoleic acid (0.1% in ethanol), 0.2 mL of ferric nitrate (20 mM), 0.2 mL of ascorbic acid (200 mM) and 0.2 mL of hydrogen peroxide (30 mM). The reaction mixture was incubated at 37 °C in water bath for 1 h. After incubation, the reaction was stopped by the addition of 1 mL TCA (10%, w/v), followed by addition of TBA (1 mL, 1% w/v). The mixture was then placed in boiling water bath for 20 min and centrifuged at 5000 rpm for 10 min. Absorbance (A) of the mixture was taken at 535 nm against a blank. Lipid peroxidation was measured as % inhibition using the following formula:

$$\% \text{ Inhibition} = [1 - (A \text{ of sample} / A \text{ of control})] \times 100$$

Statistical analysis

All analyses were performed in triplicates and data were subjected to one-way analysis of variance (ANOVA) with a significance level of 5%. Duncan's test was applied to determine the differences between the means using the commercial statistical packages (SPSS, Inc, Chicago, IL, USA).

Result and discussion

Proximate composition

The proximate composition of cookies is presented in Table 1. Moisture content of the cookies varied significantly ($p < 0.05$) and was observed as 2.43, 2.29 and 2.37% for CC, CS₅₀ and CS₁₀₀, respectively. However, protein, fat and ash content of cookies containing saffron extract did not vary significantly ($p > 0.05$) from control and was found in the range of 6.11–6.14%, 27.13–27.41% and 1.83–1.96%, respectively. Kaur et al. (2017) reported the protein and ash content of wheat flour cookies as 7.40 and 1.24%, respectively, which is in line with the results obtained in the present study. Crude fat content of cookies was reported as 21.71% by Bhat and Ahsan (2015), slightly lower than the values obtained in the present study.

Physical characteristics of cookies

Physical properties of cookies prepared from whole wheat flour with added saffron extract are presented in Table 2. There were no significant ($p > 0.05$) differences in the diameter, thickness and spread ratio of cookies containing saffron extract when compared to control and were found in the range of 5.74–6.11 cm, 0.57–0.61 cm and 9.42–9.98 respectively. Škrbic and Cvejnov (2011) reported the diameter and spread ratio of whole wheat flour cookies as 5.16 cm and 3.93, respectively. The results obtained for the former are in agreement with that found in present study. Diameter, thickness and spread ratio of wheat flour cookies were reported as 5.08 cm, 0.60 cm and 8.47, respectively, by Cheng and Bhat (2016) which are close to the results observed in our study. Kaur et al. (2014) reported the spread factor of different wheat cultivars in the range of 6.15–7.25 which is slightly lower than that observed in the current study.

Texture

Hardness of the freshly prepared cookies containing saffron extract i.e. CS₅₀ (50 mg/100 g) and CS₁₀₀ (100 mg/100 g)

varied non-significantly ($p > 0.05$) when compared to control (Table 2). It was observed in the range of 45.98–47.16 N. Aziah et al. (2012) reported the hardness of cookies as 41.50 N, which is slightly lower than that observed in the current study. The hardness of cookies was reported as 49.69 N by Zouari et al. (2016) which in line with the results obtained in the present study. The hardness of cookies prepared from different wheat cultivars was reported in between 30.25 and 78.87 N by Kaur et al. (2014) which is similar to that observed in our study. Storage period of 0–9 months resulted in a decrease in hardness of cookies. However, the decrease was non-significant ($p > 0.05$) for all cookies except those stored for a period of 3 months. The decrease in hardness of cookies might be due to increase in moisture content during storage (Dar et al. 2014).

Color

Color values 'L' (lightness), 'a' (redness) and 'b' (yellowness) of all cookies are presented in Table 3. The surface lightness and yellowness of freshly prepared cookies increased significantly ($p < 0.05$) from 50.7 to 53.9 and 36.5 to 47.0, respectively, with addition of saffron extract when compared to control. However, 'a' value decreased non-significantly from 5.18 to 4.08 with the addition of saffron extract. The increase in 'L' and 'b' values of cookies might be due to the presence of saffron extract which contains crocins that contributed golden yellow color (Bolhassani et al. 2014). Storage period of 0–9 months significantly ($p < 0.05$) affected the surface color of cookies. 'L' value of CC, CS₅₀ and CS₁₀₀ cookies was observed to increase significantly ($p < 0.05$) from 50.7 to 55.6, 50.9 to 55.5 and 53.9 to 56.1, respectively upon storage. In contrast, 'a' and 'b' values of the cookies decreased significantly ($p > 0.05$) with storage. Degradation in the amount of carotenoids (α -carotene) after 3 months of storage has been reported by Chen et al. (1996), which might be a reason for decrease in 'a' and 'b' values of cookies in the present study. Raina et al. (1996) reported that hydrolysis of crocin into crocetin takes place in saffron during storage at ambient temperature, thereby

Table 1 Proximate composition of cookies supplemented with saffron extracts (n = 3)

Sample	Moisture (g/100 g)	Crude protein (g/100 g)	Crude fat (g/100 g)	Ash (g/100 g)
CC	2.43 ± 0.14 ^c	6.13 ± 0.04 ^a	27.18 ± 0.16 ^a	1.83 ± 0.10 ^a
CS ₅₀	2.29 ± 0.10 ^a	6.14 ± 0.14 ^a	27.13 ± 0.10 ^a	1.91 ± 0.07 ^a
CS ₁₀₀	2.37 ± 0.06 ^b	6.11 ± 0.12 ^a	27.41 ± 0.31 ^b	1.92 ± 0.05 ^a

Results are expressed as means (n = 3) ± SD

Values followed by same letter in a column do not differ significantly ($p < 0.05$)

CC control cookie without any supplement, CS₅₀ cookie with SE 50 mg/100 g of flour, CS₁₀₀ cookie with SE 100 mg/100 g of flour

Table 2 Physical characteristics and hardness of cookies supplemented with saffron extracts (n = 3)

Parameters	Sample	Weight (g)	Diameter (cm)	Thickness (cm)	Spread ratio	Hardness (N) Storage (months)			
						0	3	6	9
	CC	13.42 ± 0.03 ^c	6.11 ± 0.02 ^b	0.57 ± 0.00 ^a	9.98 ± 0.17 ^b	47.16 ± 1.00 ^{cA}	44.65 ± 0.43 ^{bb}	43.72 ± 0.23 ^{bA}	39.33 ± 0.41 ^{aA}
	CS ₅₀	13.00 ± 0.70 ^{ab}	5.74 ± 0.03 ^a	0.61 ± 0.01 ^a	9.42 ± 0.34 ^a	45.98 ± 2.29 ^{bA}	44.51 ± 1.8 ^{aA}	44.05 ± 0.23 ^{aAB}	43.4 ± 0.90 ^{ab}
	CS ₁₀₀	12.16 ± 0.11 ^a	5.82 ± 0.02 ^b	0.60 ± 0.32 ^a	9.71 ± 1.30 ^b	46.01 ± 0.58 ^{cA}	45.09 ± 1.4 ^{bb}	44.73 ± 0.49 ^{abb}	43.51 ± 0.22 ^{ab}

Results are expressed as means (n = 3) ± SD

Values followed by same letter in a row and column do not differ significantly ($p \leq 0.05$)

CC control cookie without any supplement, CS₅₀ cookie with SE 50 mg/100 g of flour, CS₁₀₀ cookie with SE 100 mg/100 g of flour

reducing the coloring strength of saffron. This also might be a reason for reduced 'b' value of the CS₅₀ and CS₁₀₀ cookies during storage.

Total phenolic content (TPC)

Total phenolic content of samples is shown in Table 4. It is evident from the data that TPC of dough samples containing saffron extracts showed non-significant ($p > 0.05$) variation when compared to control and was observed in the range of 0.39–0.42 mg GAE/g. Non-significant difference in the TPC of CC, CS₅₀ and CS₁₀₀ might be due to less amount of same in the saffron extract. Makhoul et al. (2011) reported the TPC of aqueous extract of saffron as low as 0.016 mg/mL. The TPC of whole wheat flour was reported as 0.74 mg GAE/g by Li et al. (2015) which is higher than that observed in the current study.

Baking of cookies significantly ($p < 0.05$) increased their TPC content and was found in the range of 0.86–0.94 mg GAE/g. Phenolics are generally bound with cell wall structural proteins and carbohydrates (Parmar et al. 2017) and might get released by rupture of cell wall upon heating resulting in the increase in TPC of cookies. TPC of cookies might also be enhanced due to generation of Malanoidins produced in Maillard reaction at high temperature.

TPC of cookies containing saffron extract decreased non-significantly ($p > 0.05$) after 3 months of storage except control where the decrease was significant ($p < 0.05$). Degradation of polyphenols during storage has been reported by Šaponjac et al. (2016) which might also be the case in our study. In general the TPC of cookies was observed to decrease significantly ($p < 0.05$) after 9 months of storage. However, the TPC of CS₅₀ and CS₁₀₀ cookies was slightly better retained during storage as compared to CC. This indicates better antioxidant activity of the former cookies due to the presence of saffron extract.

Antioxidant properties

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay

DPPH scavenging activity of dough was observed to increase significantly ($p < 0.05$) with the addition of saffron extract (Table 4). It was found in the range of 16.30–24.67%, the highest for DS₁₀₀ and the lowest for CC. The increase in DPPH scavenging activity of DS₅₀ and DS₁₀₀ may be attributed to saffron extract that has been reported to have potent antioxidant activity (Mashmoul et al. 2013).

Table 3 Hunter color values of cookies with added saffron extract ($n = 3$)

<i>L</i>	<i>a</i>									<i>b</i>								
	Storage (months)				Storage (months)					Storage (months)				Storage (months)				
	0	3	6	9	0	3	6	9	0	3	6	9	0	3	6	9		
CC	50.7 ± 0.1 ^{aA}	51.0 ± 0.7 ^{aA}	54.3 ± 0.3 ^{bA}	55.6 ± 0.3 ^{bA}	5.18 ± 1.4 ^{bA}	4.50 ± 0.8 ^{abB}	4.14 ± 0.1 ^{abB}	3.10 ± 0.2 ^{aB}	36.5 ± 0.4 ^{cA}	33.5 ± 0.1 ^{bA}	32.5 ± 0.8 ^{abA}	32.1 ± 0.1 ^{aA}						
CS ₅₀	50.9 ± 0.4 ^{aB}	54.8 ± 1.1 ^{bB}	55.3 ± 0.1 ^{bB}	55.5 ± 0.6 ^{bA}	4.10 ± 0.1 ^{bA}	2.12 ± 0.1 ^{aA}	2.04 ± 0.2 ^{aA}	1.98 ± 0.3 ^{aA}	39.4 ± 0.2 ^{aB}	39.5 ± 0.9 ^{aB}	41.1 ± 0.0 ^{bB}	41.8 ± 0.9 ^{bB}						
CS ₁₀₀	53.9 ± 1.4 ^{aC}	54.4 ± 1.7 ^{aB}	54.1 ± 0.1 ^{aA}	56.1 ± 0.1 ^{aB}	4.08 ± 0.2 ^{cA}	2.86 ± 0.8 ^{bA}	2.48 ± 0.4 ^{abA}	1.82 ± 0.0 ^{aB}	47.0 ± 0.1 ^{cC}	46.8 ± 0.6 ^{cC}	45.9 ± 0.1 ^{bC}	44.0 ± 0.6 ^{aC}						

Results are expressed as means ($n = 3$) ± SDValues followed by same letter in a row and column do not differ significantly ($p \leq 0.05$)CC control cookie without any supplement, CS₅₀ cookie with SE 50 mg/100 g of flour, CS₁₀₀ cookie with SE 100 mg/100 g of flour

DPPH scavenging activity of freshly prepared cookies significantly ($p < 0.05$) increased after baking. Previous studies have reported production of dark brown colored pigments during thermal processing of foods due to Maillard reaction (Sharma et al. 2012). The increase in DPPH scavenging activity may therefore be attributed to Maillard reaction products (mellanoindins) which improved the antioxidant activity of cookies. Increase in antioxidant activity upon thermal processing has also been reported by Sharma and Gujral (2011). Gregory et al. (2005) reported that saffron metabolites were better retained at elevated temperature which might also be a reason of higher DPPH scavenging activity of CS₅₀ and CS₁₀₀ cookies when compared to control.

DPPH scavenging activity of cookies significantly ($p < 0.05$) reduced during storage except CS₅₀ and CS₁₀₀ cookies stored for 3 months which showed a non-significant ($p > 0.05$) decrease when compared to control. The decrease in DPPH scavenging activity of stored cookies may be due to degradation of phenolic compounds (Zhou et al. 2013). In general, DPPH scavenging activity of CS₅₀ and CS₁₀₀ cookies remained high compared to CC throughout the storage which might be due to the presence of saffron metabolites in the former.

Reducing power

Reducing ability of dough and cookies is presented in Table 4. It is clear from the data that reducing power of dough increased significantly ($p < 0.05$) after addition of saffron extract. DS₁₀₀ had the highest (0.42 mg AAE/g) reducing power while DC had the lowest (0.26 mg AAE/g). Reducing power of freshly prepared cookies increased significantly ($p < 0.05$) upon baking and ranged from 0.34 to 0.50 mg AAE/g. Increase in reducing power may be attributed to formation of Malanoidins in Maillard reaction during baking at high temperature. The trend observed in reducing power of cookies was similar to that found in DPPH scavenging activity which indicates a strong relation between phenolic compounds and antioxidant properties. Oboh et al. (2008) have reported a strong correlation between antioxidant activity and phenolic compounds of food samples.

Storage period of 0–9 months adversely affected the reducing power of cookies and it was observed to decrease significantly ($p < 0.05$) with the increase in storage time. Reducing power of CS₅₀ and CS₁₀₀ cookies was found to remain high during storage as compared to CC cookies. This implies that rate of degradation of antioxidant compounds in CS₅₀ and CS₁₀₀ cookies might have been reduced to some extent by saffron metabolites that have potent antioxidant activity (Mashmoul et al. 2013).

Lipid peroxidation

The percent inhibition of lipid peroxidation of samples is presented in Table 4. Inhibition of lipid peroxidation (ILP) by dough was found to increase significantly ($p < 0.05$) from 11.74 to 34.02% with the addition of saffron extract. Inhibition of lipid peroxidation by saffron metabolites in rat liver homogenate has been reported by Hamid et al. (2009) which might be the reason for enhanced ILP by CS₅₀ and CS₁₀₀ as compared to CC. ILP of freshly prepared cookies reduced significantly ($p < 0.05$) upon baking from 11.74 to 7.09%, 27.34 to 11.45% and 34.02 to 13.09%, respectively for CC, CS₅₀ and CS₁₀₀. The decrease in ILP by cookies may be attributed to increase in oxidation of lipids on baking.

A significant ($p < 0.05$) reduction in ILP of all cookies was observed during storage period of 9 months. The main reason for decreased ILP by cookie samples may be because of the increased oxidation of lipids during storage. However, in case of CS₅₀ and CS₁₀₀ cookies ILP was higher

than that of CC cookies throughout the storage. Butt et al. (2004) observed increase in oxidation of lipids in wheat flour cookies with storage which is consistent with the results of present study. Similar results were reported by Singh et al. (2000) regarding oxidation of lipids for soy fortified biscuits.

Quantification of saffron metabolites, crocin, safranal and picrocrocin

The concentration of saffron metabolites in dough and cookie is shown in Table 5. Average values of crocins, safranal and picrocrocin in DS₅₀ and DS₁₀₀ containing saffron extract were observed as 28.30, 48.30, 104.6 µg/g and 35.14, 62.38, 118.2 µg/g, respectively. The three main active compounds identified in saffron i.e. crocins, safranal and picrocrocin (Nassiri-Asl and Hosseinzadeh 2014) of cookies lowered significantly ($p < 0.05$) upon baking. Degradation of saffron metabolites was reported to be temperature dependent by Tsimidou (1997) which might be

Table 4 Antioxidant properties of cookies supplemented with saffron extract (n = 3)

Storage (months)	Sample					
	DC	CC	DS ₅₀	DS ₁₀₀	CS ₅₀	CS ₁₀₀
<i>TPC (mg GAE/g)</i>						
0	0.39 ± 0.014 ^A	0.90 ± 0.03 ^{cB}	0.40 ± 0.01 ^A	0.42 ± 0.14 ^A	0.86 ± 0.04 ^{bB}	0.94 ± 0.14 ^{bB}
3	–	0.79 ± 0.09 ^{bA}	–	–	0.84 ± 0.02 ^{abA}	0.92 ± 0.02 ^{bB}
6	–	0.73 ± 0.05 ^{bA}	–	–	0.82 ± 0.04 ^{abB}	0.85 ± 0.04 ^{aB}
9	–	0.60 ± 0.02 ^{aA}	–	–	0.80 ± 0.01 ^{aB}	0.81 ± 0.02 ^{aB}
<i>DPPH scavenging assay (%)</i>						
0	16.30 ± 0.21 ^A	22.09 ± 0.58 ^{cB}	21.67 ± 0.4 ^B	24.67 ± 2.15 ^C	25.50 ± 0.76 ^{cC}	28.96 ± 0.92 ^{cD}
3	–	17.28 ± 0.27 ^{bA}	–	–	23.33 ± 0.51 ^{cB}	26.87 ± 0.90 ^{cB}
6	–	16.8 ± 0.2 ^{bA}	–	–	16.93 ± 0.46 ^{bA}	19.07 ± 0.72 ^{bB}
9	–	06.20 ± 0.47 ^{aA}	–	–	11.39 ± 0.34 ^{aB}	12.84 ± 1.01 ^{aB}
<i>Reducing power (mg AAE/g)</i>						
0	0.26 ± 0.06 ^P	0.34 ± 0.04 ^{cBC}	0.33 ± 0.03 ^B	0.42 ± 0.02 ^D	0.40 ± 0.05 ^{cCD}	0.50 ± 0.01 ^{cE}
3	–	0.17 ± 0.04 ^{bA}	–	–	0.35 ± 0.01 ^{cB}	0.45 ± 0.01 ^{bC}
6	–	0.12 ± 0.03 ^{abA}	–	–	0.24 ± 0.01 ^{bB}	0.28 ± 0.04 ^{abB}
9	–	0.07 ± 0.02 ^{aA}	–	–	0.16 ± 0.0 ^{aA}	0.20 ± 0.01 ^{aB}
<i>Lipid peroxidation (% inhibition)</i>						
0	11.74 ± 0.59 ^B	7.09 ± 0.43 ^{cA}	27.34 ± 1.57 ^C	34.02 ± 0.90 ^D	11.45 ± 1.62 ^{cB}	13.09 ± 1.43 ^{cB}
3	–	5.11 ± 1.01 ^{cA}	–	–	8.32 ± 0.8 ^{bcBC}	10.36 ± 0.53 ^{bA}
6	–	2.52 ± 0.62 ^{bA}	–	–	4.16 ± 1.2 ^{abAB}	5.86 ± 1.03 ^{apB}
9	–	1.02 ± 0.89 ^{aA}	–	–	3.52 ± 0.47 ^{aB}	4.09 ± 1.78 ^{aAB}

Results are expressed as means (n = 3) ± SD

Values followed by same letter in a row and column do not differ significantly ($p \leq 0.05$)

DC control dough without any supplement, DS₅₀ dough with SE 50 mg/100 g of flour, DS₁₀₀ dough with SE 100 mg/100 g of flour; CC control cookie without any supplement, CS₅₀ cookie with SE 50 mg/100 g of flour, CS₁₀₀ cookie with SE 100 mg/100 g of flour; CC control cookie without any supplement, CS₅₀ Cookie with SE 50 mg/100 g of flour, CS₁₀₀ cookie with SE 100 mg/100 g of flour

	Storage (months)				Storage (months)				Storage (months)			
	Crocin (µg/g)				Safranal (µg/g)				Picrocrocin (µg/g)			
	0	3	6	9	0	3	6	9	0	3	6	9
DS ₅₀	28.30 ± 0.2 ^C	–	–	–	48.30 ± 0.2 ^C	–	–	–	104.6 ± 0.2 ^C	–	–	–
DS ₁₀₀	35.14 ± 0.1 ^D	–	–	–	62.38 ± 0.1 ^D	–	–	–	118.2 ± 0.1 ^D	–	–	–
CS ₅₀	6.22 ± 0.2 ^{bA}	5.97 ± 0.1 ^a	5.86 ± 0.0 ^a	5.76 ± 0.1 ^a	21.69 ± 0.2 ^{dA}	18.12 ± 0.3 ^c	16.34 ± 0.4 ^b	14.5 ± 0.4 ^a	76.22 ± 0.1 ^{dA}	58.86 ± 0.8 ^c	49.8 ± 0.9 ^b	40.77 ± 0.6 ^a
CS ₁₀₀	10.02 ± 0.5 ^{cB}	9.27 ± 0.3 ^b	8.87 ± 0.2 ^{ab}	8.47 ± 0.4 ^a	39.45 ± 0.2 ^{dB}	26.52 ± 0.5 ^c	25.03 ± 0.8 ^b	23.8 ± 0.3 ^a	82.28 ± 0.6 ^{dB}	66.91 ± 0.3 ^c	61.3 ± 0.2 ^b	55.27 ± 0.4 ^a

DC control dough without any supplement, *DS₅₀* dough with SE 50 mg/100 g of flour, *DS₁₀₀* dough with SE 100 mg/100 g of flour, *CC* control cookie without any supplement, *CS₅₀* cookie with SE 50 mg/100 g of flour, *CS₁₀₀* cookie with SE 100 mg/100 g of flour

Storage (months)	CC	CS ₅₀	CS ₁₀₀
<i>Texture</i>			
0	8.00 ± 0.61 ^{cD}	7.50 ± 0.51 ^{aC}	7.70 ± 0.33 ^{bD}
3	7.50 ± 0.12 ^{bC}	7.40 ± 0.60 ^{aC}	7.50 ± 0.21 ^{bC}
6	7.16 ± 0.10 ^{bB}	7.00 ± 0.23 ^{aB}	7.11 ± 0.01 ^{abB}
9	6.17 ± 0.11 ^{bA}	6.07 ± 0.23 ^{aA}	6.10 ± 0.38 ^{abA}
<i>Appearance</i>			
0	8.80 ± 0.68 ^{aD}	9.00 ± 0.42 ^{bC}	9.10 ± 0.22 ^{bC}
	8.52 ± 0.11 ^{aC}	8.70 ± 0.13 ^{bB}	8.80 ± 0.30 ^{cB}
6	8.20 ± 0.60 ^{aB}	8.63 ± 0.27 ^{bB}	8.78 ± 0.34 ^{cBC}
9	6.87 ± 0.16 ^{aA}	7.03 ± 0.67 ^{bA}	7.00 ± 0.44 ^{bA}
<i>Mouth feel</i>			
0	7.15 ± 0.31 ^{aC}	7.78 ± 0.12 ^{cD}	7.60 ± 0.32 ^{bC}
3	7.12 ± 0.11 ^{aC}	7.70 ± 0.29 ^{cC}	7.55 ± 0.50 ^{bC}
6	7.00 ± 0.31 ^{aB}	7.53 ± 0.51 ^{bB}	7.42 ± 0.65 ^{bB}
9	6.00 ± 0.20 ^{aA}	6.90 ± 0.22 ^{cA}	6.27 ± 0.35 ^{bA}
<i>Flavour</i>			
0	7.40 ± .71 ^{aD}	7.71 ± 0.12 ^{bC}	7.50 ± 0.17 ^{aC}
3	7.23 ± 0.42 ^{aC}	7.65 ± 0.21 ^{cC}	7.44 ± 0.36 ^{bC}
6	7.01 ± 0.23 ^{aB}	7.21 ± 0.57 ^{bB}	7.05 ± 0.62 ^{aB}
9	6.16 ± 0.11 ^{aA}	6.20 ± 0.30 ^{aA}	6.25 ± 0.78 ^{aA}
<i>Overall acceptability</i>			
0	7.12 ± 0.31 ^{aC}	7.29 ± 0.23 ^{bC}	7.10 ± 0.09 ^{aB}
3	7.09 ± 0.18 ^{bC}	7.24 ± 0.25 ^{cC}	7.00 ± 0.21 ^{aB}
6	6.87 ± 0.13 ^{aB}	7.13 ± 0.10 ^{cB}	7.00 ± 0.28 ^{bB}
9	5.13 ± 0.32 ^{aA}	5.68 ± 0.32 ^{cA}	5.22 ± 0.99 ^{bA}

DC control dough without any supplement, *DS*₅₀ dough with SE 50 mg/100 g of flour, *DS*₁₀₀ dough with SE 100 mg/100 g of flour, *CC* control cookie without any supplement, *CS*₅₀ cookie with SE 50 mg/100 g of flour, *CS*₁₀₀ cookie with SE 100 mg/100 g of flour

The concentration of saffron metabolites in cookies was observed to decrease significantly ($p < 0.05$) with storage. This may be due to increase in the water activity of cookies that might have accelerated the deterioration of saffron metabolites. Raina et al. (1996) observed that increase in moisture content enhanced the degradation of saffron metabolites with storage.

Sensory properties

The organoleptic properties of cookies with added saffron extracts are shown in Table 6. It was found that CC cookies were rated lowest by sensory panelists for all

organoleptic properties evaluated except for texture when compared to CS₅₀ and CS₁₀₀ cookies. Sample CS₅₀ gained the maximum score for appearance, mouthfeel, flavour and overall acceptability while CC had the lowest score. The sensory panelists liked the flavour of CS₅₀ and CS₁₀₀ cookies. This infers that cookies with added saffron extract that are rich in bioactive components will be preferred by people because of their sensory attributes.

Sensory characteristics of all cookies decreased significantly ($p < 0.05$) with storage. However, flavour, mouthfeel and overall acceptability were still in the category of 'like moderately' even after 6 months of storage. Thus cookies containing saffron extract can be safely stored for a period of 6 months without any adverse changes in their organoleptic properties.

Conclusion

The addition of saffron extracts to cookies significantly changed their quality attributes. Cookies showed excellent antioxidant properties after the addition of saffron extracts. This was attributed to potent antioxidant activity of saffron and stability of its extracts during baking. Cookies with added saffron extract were rated highest for all sensory characteristics except texture when compared to control. CS₅₀ cookies gained maximum scores among all the cookies. This implies that the most optimum concentration which should be added to the cookies for higher consumer acceptability is saffron extract of 50 mg/added to 100 g of flour. It can be concluded that health promoting cookies can be made with the addition of saffron extract as a source of natural antioxidants. In addition, natural antioxidants are safe and can be used to increase the shelf life of bakery products that contain high concentration of fats/oils.

References

- AOAC (1990) AOAC official methods of analysis, 15th edn. Association of Official Analytical Chemists, Arlington
- Aziah AAN, Noor AYM, Ho LH (2012) Physicochemical and organoleptic properties of cookies incorporated with legume flour. *Int Food Res J* 19(4):1539–1543
- Bhat MA, Ahsan H (2015) Physico-chemical characteristics of cookies prepared with tomato pomace powder. *Food Process Technol* 7:1
- Bolhassani A, Khavari A, Bathaie SZ (2014) Saffron and natural carotenoids: Biochemical activities and anti-tumor effects. *Biochim Biophys Acta* 1845:20–30
- Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci Technol* 28:245–251
- Butt MS, Nasir M, Akhtar S, Sharif K (2004) Effect of moisture and packaging on the shelf life of wheat flour. *Int J Food Saf* 4:1–6
- Carmona M, Zalacain A, Sanchez AM, Novella JL, Alonso GL (2006) Crocetin esters, picrocrocin and its related compounds present in *Crocus sativus* stigmas and *Gardenia jasminoides* fruits. Tentative identification of seven new compounds by LC-ESI-MS. *J Agric Food Chem* 54:973–979
- Chen HE, Peng HY, Chen BH (1996) Stability of carotenoids and vitamin A during storage of carrot juice. *Food Chem* 57(4):497–503
- Cheng YF, Bhat R (2016) Functional, physicochemical and sensory properties of novel cookies produced by utilizing underutilized jering (*Pithecellobium jiringa* Jack.) legume flour. *Food Biosci* 14:54–61
- Dar AH, Sharma HK, Kumar N (2014) Effect of extrusion temperature on the microstructure, textural and functional attributes of carrot pomace-based extrudates. *J Food Process Preserv* 38:212–222
- Dillard CJ, German JB (2000) Phytochemicals: nutraceuticals and human health. *J Sci Food Agric* 80:1744–1756
- Gao L, Wang S, Oomah BD and Mazza G (2002) Wheat quality: antioxidant activity of wheat millstreams. In: Ng P, Wrigley CW (eds) *Wheat quality elucidation*. AACC International, St Paul, MN, pp 219–233
- Gregory MJ, Menary RC, Davies NW (2005) Effect of drying temperature and air flow on the production and retention of secondary metabolites in saffron. *J Agric Food Chem* 53(15):5969–5975
- Hamid B, Sam S, Islam T, Singh P, Sharma M (2009) The free radical scavenging and the lipid peroxidation inhibition of crocin isolated from Kashmiri saffron (*Crocus sativus*) occurring in Northern part of India. *Int J PharmTech Res* 1(4):1317–1321
- Jan S, Wani AA, Kamili AN, Khastwari M (2014) Distribution, chemical composition and medicinal importance of saffron (*Crocus sativus* L.). *African. J Plant Sci* 8(12):537–545
- Kaur A, Singh N, Kaur S, Ahlawat AK, Singh AM (2014) Relationships of flour solvent retention capacity, secondary structure and rheological properties with the cookie making characteristics of wheat cultivars. *Food Chem* 158:48–55
- Kaur M, Singh V, Kaur R (2017) Effect of partial replacement of wheat flour with varying levels of flaxseed flour on physicochemical, antioxidant and sensory characteristics of cookies. *Bioact Carbohydr Diet Fibre* 9:14–20
- Khazdair MR, Boskabady MH, Hosseini M, Rezaee R, Tsatsakis AM (2015) The effects of *Crocus sativus* (saffron) and its constituents on nervous system: a review. *Avicenna J. Phytomed* 5(5):376–391
- Kumpati P, Abraham SK, Santhiya ST, Ramesh A (2003) Inhibitory effects of aqueous crude extract of Saffron (*Crocus sativus* L.) on chemical-induced genotoxicity in mice. *Asia Pac J Clin Nutr* 12(4):474–476
- Li Y, Ma D, Sun D, Wang C, Zhang J, Xie Y, Guo T (2015) Total phenolic, flavonoid content, and antioxidant activity of flour, noodles, and steamed bread made from different colored wheat grains by three milling methods. *The Crop J* 3:328–334
- Makhlouf H, Saksouk M, Habib J, Chahine R (2011) Determination of antioxidant activity of saffron taken from the flower of *Crocus sativus* grown in Lebanon. *Afr J Biotechnol* 10(41):8093–8100
- Mashmoul M, Azlan A, Khazaai H, Yusof BNM, Noor SM (2013) Saffron: a natural potent antioxidant as a promising anti-obesity drug. *Antioxidants* 2:293–308. <https://doi.org/10.3390/antiox2040293>
- Nanditha B, Prabhasankar P (2009) Antioxidants in bakery products: a review. *Crit Rev Food Sci Nutr* 49(1):1–27. <https://doi.org/10.1080/10408390701764104>
- Nassiri-Asl M, Hosseinzadeh H (2014) Neuropharmacology effects of saffron (*Crocus sativus*) and its active constituents. In: Watson RR, Preedy VR (eds) *Bioactive nutraceuticals and dietary*

- supplements in neurological and brain disease: prevention and therapy. Academic Press, Cambridge, pp 29–39
- Oboh G, Raddatz H, Henle T (2008) Antioxidant properties of polar and non-polar extracts of some tropical green leafy vegetables. *J Sci Food Agric* 88(14):2486–2492
- Parmar N, Singh N, Kaur A, Thakur S (2017) Comparison of color, anti-nutritional factors, minerals, phenolic profile and protein digestibility between hard-to-cook and easy-to-cook grains from different kidney bean (*Phaseolus vulgaris*) accessions. *J Food Sci Technol* 54(4):1023–1034
- Raina BL, Agarwal SG, Bhatia AK, Gaur GS (1996) Changes in pigments and volatiles of saffron (*Crocus sativus* L.) during processing and storage. *J Sci Food Agric* 71:27–32
- Reddy V, Urooj A, Kumar A (2005) Evaluation of antioxidant activity of some plant extracts and their application in biscuits. *Food Chem* 90:317–321
- Šaponjac VT, Cetkovic G, Canadanovic-Brunet J, Pajin B, Djilas S, Petrovic J, Loncarevic I, Stajcic S, Vulic J (2016) Sour cherry pomace extract encapsulated in whey and soy proteins: incorporation in cookies. *Food Chem* 207:27–33
- Sharma P, Gujral HS (2011) Effect of sand roasting and microwave cooking on antioxidant activity of barley. *Food Res Int* 44:235–240
- Sharma P, Gujral HS, Singh B (2012) Antioxidant activity of barley as affected by extrusion cooking. *Food Chem* 131:1406–1413
- Singh R, Singh G, Chauhan GS (2000) Development of soy-fortified biscuits and shelf-life studies. *J Sci Food Agric* 37:300–303
- Škrbic B, Cvejanov J (2011) The enrichment of wheat cookies with high-oleic sunflower seed and hull-less barley flour: Impact on nutritional composition, content of heavy elements and physical properties. *Food Chem* 124:1416–1422
- Tsimidou M (1997) Kinetic studies of saffron (*Crocus sativus* L.) quality deterioration. *J Agric Food Chem* 45:2890–2898
- Wright JR, Colby HD, Miles PR (1981) Cytosolic factors which affect microsomal lipid peroxidation in lung and liver. *Arch Biochem Biophys* 206(2):296–304
- Zhao H, Fan W, Dong J, Lu J, Chen J, Shan L (2008) Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. *Food Chem* 107:296–304
- Zhou CL, Liu W, Zhao J, Yuan C, Song Y, Chen D, Li QH (2013) The effect of high hydrostatic pressure on the microbiological quality and physical–chemical characteristics of pumpkin (*Cucurbita maxima* Duch.). *Innov Food Sci Emerg Technol* 21:24–34
- Zouari R, Besbes S, Ellouze-Chaabouni S, Ghribi-Aydi D (2016) Cookies from composite wheat–sesame peels flours: dough quality and effect of *Bacillus subtilis* SPB1 biosurfactant addition. *Food Chem* 194:758–769