Cellular and Molecular Biology

Journal homepage: www.cellmolbiol.org

Physicochemical properties, nutrient profile, microbial stability, and sensory evaluation of cupcakes enriched with pomegranate seed oil

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ARTICLE INFO

Original paper

Article history:
Received: July 25, 2021
Accepted: October 30, 2021
Published: December 01, 2021

Keywords:
Cupcakes; GC-MS; Physicochemical traits; Pomegranate seed oil; Sensory analysis

ABSTRACT

Excessive use of refined flour, solid fats, and sugar in preparing baked products are considered to be unhealthy and is intricately linked with the development of lifestyle diseases. Replacing refined flour with whole wheat flour and solid fats with cold-pressed oil serves as an alternate option. The study was aimed at evaluating the physicochemical properties, nutrient composition, sensory attributes, and shelf life of cupcakes enriched using pomegranate seed oil (PSO). Vanilla and chocolate cupcake variants were prepared using 25 and 50% of PSO. A sensory panel consisting of 30 semi-trained participants was selected for evaluating the formulated products using a five-point hedonic scale. Nutrient content was estimated using standard techniques. The stability of the formulated product was determined by evaluating the physicochemical traits and microbial growth on the 0th, 4th, and 7th day. Mean scores of the sensorial analysis showed that the incorporation of PSO in cupcakes was highly accepted by the panel members. Chocolate cupcake containing 50% of PSO was found to be the most preferred product (3.53±0.94), followed by vanilla cupcake containing 25% of PSO (3.4±0.62). The moisture, protein, and fat content of chocolate cupcakes containing 25% of PSO were high. Cupcakes prepared with PSO can be stored for four days at room temperature. GC-MS analysis showed the presence of punicic acid, oleic acid, tocopherols, campesterol, sitosterols, stigmasterol, and α-tocopheryl acetate as pre-dominant fatty acid in unheated and heated PSO. In conclusion, cupcakes prepared using PSO showed acceptable physicochemical qualities and sensory properties which indicated its successful consumption by people affected with metabolic disorders.

DOI: http://dx.doi.org/10.14715/cmb/2021.67.4.5

Introduction

The bakery unit occupies an important segment in the food industry. Biscuits, bread, bun, cakes, cookies, doughnuts, pies, rolls, and tarts are consumed by individuals of all age groups. Cakes are commonly preferred by consumers due to their organoleptic properties. The cake is a complex foam and fat emulsion system. Cakes are broadly categorized into individual cakes and whole cakes. Individual cakes are further sub-categorized into cupcakes, cake slices, and pies (1). Cupcakes are small-sized individual sweet products baked in muffin tins that can be nutritionally enriched by adding unique ingredients (2). The principal ingredients used in preparing any type of cake include flour, sugar, and fat. Each of these ingredients contributes to the overall appearance and organoleptic properties of the final product (3). A considerable amount of fat is added to the cake batter

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because fat inhibits contact between flour protein and water, aids in the entrapment of air bubbles, imparts moisture, contributes flavour enhancement, and tenderness (4, 5). In order to obtain the above-mentioned textural, structural, and sensory characteristics, a sizeable amount of fat (15-25%) on a batter weight basis is used while making cakes.

Fruits form an important part of a balanced diet. Pomegranate (Punica granatum) belonging to the Punicaceae family contains a diverse range of bioactive components that renders a wide array of physiological health benefits. The edible portion of the fruit is 50% (40% arils and 10% seeds) (6). Pomegranate seeds contain unsaturated fatty acids, micronutrients, and essential non-nutritive compounds (7). The oil obtained from pomegranate seeds possess several active compounds. Studies on the fatty acid profile of pomegranate seed oil (PSO) confirm the presence of punicic, elagic, gallic, linoleic, and catalpic acid. Phytosterols such as β-sitosterol, campesterol, and stigmasterol along with α and γ-tocopherol are also present in PSO (8-11). Medicinal properties of PSO include antioxidant, anticancer, anti-estrogen, anti-inflammatory, hypoglycaemic, and hypolipidemic effects (12-15).

Fats used in baking units include solid fats and processed oils obtained from plant sources. Baked foods are rich in fat. Excess intake of foods rich in dietary fat contributes to weight gain and increases the risk of developing lifestyle diseases. Taking into consideration the consumers burgeoning need for availability and consumption of healthy foods, more attention is given towards the use of healthier fats. Oil needs to be selected based on certain factors such as physicochemical properties, shelf life, ability to withstand oxidative rancidity, and most importantly, its fatty acid profile. Oils contain both saturated and unsaturated fatty acids. Selecting oils rich in unsaturated fats and low in saturated fats is necessary. Considering the therapeutic and nutritional properties of PSO, the study was intended to determine the nutrient composition, sensory analysis, and storage condition of cakes made using PSO.

Materials and methods

Pomegranate seed oil collection

Cold-pressed PSO was purchased from TVK Beverages Private Limited, Solapur, Maharashtra, India.

Determination of quality index

Refractive index, iodine value, peroxide value, free fatty acids (%), saponification, and unsaponification values were estimated using AOAC methods by heating PSO at 60ºC, 100ºC, and 160ºC (16). Results were compared with the untreated PSO.

Preparation of cupcakes

The dry and wet ingredients required for making cupcakes were procured from a local supermarket in Chennai, Tamil Nadu, India. Cold-pressed PSO was purchased from TVK Beverages Private Limited, Solapur, Maharashtra, India. Two different cupcakes namely vanilla and chocolate cupcakes were prepared. The vanilla cupcake was made using whole wheat flour, maida, sugar, curd, water, vanilla essence, baking powder, baking soda, and refined oil. The chocolate cupcake was made using the above-mentioned ingredients along with the inclusion of cocoa powder (Table 1). In the interest of improving the nutritional value of the final baked product, whole wheat flour was replaced with maida at a fixed proportion. Initially, all the dry ingredients such as flour, baking soda, and baking powder were shifted separately and mixed in a bowl. Oil, sugar, water, and curd were whipped using an electric beater and were gently mixed with the dry ingredients under constant mixing to avoid lump formation. Paper cup liners were placed in a 12 slot aluminium cupcake mould tray before the cake mixture was transferred to the aluminium cupcake mould tray. The oven was pre-heated at 160ºC for 20 min, the cupcakes were baked for 30 min at 160ºC, and cooled at room temperature. After cooling, the cupcakes were wrapped individually with PVC transparent food film and stored at room temperature. In the present study, two variants were formulated under each cupcake category with the incorporation of 25 and 50% of PSO, respectively.

Sensory analysis

A panel of 30 semi-trained participants aged between 21 and 23 years was chosen for examining and evaluating the cupcakes enriched with PSO. The panel members were asked to express their degree of likeness using a 5 point hedonic rating scale where ‘5’ indicates excellent, ‘4’ indicates very good, ‘3’ indicates good, ‘2’ indicates fair, and ‘1’ indicates poor. The participants were provided water in between

sample evaluations to cleanse their palates. The organoleptic attributes evaluated were - appearance, colour, flavour, moistness, softness, taste, tenderness, and overall acceptability.

Proximate composition

Moisture, ash, protein, and fat content were quantified using AOAC standard protocols. The moisture content was determined by drying 5 g of the sample in an oven at 130ºC for about 2 h and then weighed. The ash content was estimated by incinerating the samples in a muffle furnace. The temperature used for determining the ash content ranged between 550ºC and 600ºC. Protein content was analyzed using the Kjeldahl method. Estimation of fat was done by hydrolyzing each of the samples with diluted acid and extracting them with petroleum ether using a Soxhlet apparatus. The carbohydrate content was estimated using the Anthrone method. This method is based on the formation of anthrone – a green colour complex. The absorbance was measured spectrophotometrically at 630 nm (16).

Shelf life analysis

Storage quality was analyzed by wrapping the cakes individually with PVC transparent food film and storing them under aesthetic conditions at room temperature for 7 days. The stability of the product was evaluated by determining the moisture content. The moisture test was carried out by drying 5 g of the test sample in an oven at 130ºC for about 2 h and then weighed again. Moisture content was analyzed on the 0th, 4th, and 7th day. pH was determined using a pH meter. Prior to the pH determination, the samples were homogenized with distilled water in the ratio of 1:10. The pH meter was standardized using buffer solutions of known pH. Samples were transferred to a 25 mL beaker and the pH was measured by placing the pH meter electrodes into the samples directly. Total acidity was determined by diluting 5 g of each sample with 50 mL of distilled water. From this solution, 20 mL of the solution was transferred to another 50 mL conical flask and 3-4 drops of phenolphthalein were added. This solution was titrated against 0.1N NaOH solution until a pale pink colour was obtained. The growth of microorganisms in the samples was tested using the total plate count (TPC) and visible mould growth test (17-18).

Determination of fatty acid profile

The fatty acid composition of unheated and heated (160°C) PSO was determined using Gas chromatography-mass spectrometry (GC-MS) along with a flame ionization detector and an HP-5MS column (0.25 mm in diameter with 0.25 µm film thickness) (Agilent technologies 6890 N JEOL GC Mate II GC-MS model). Briefly, 0.2 µL of the test sample was injected into the capillary column. Helium was used as the carrier gas. The injection was performed in split mode (100:1) at 200ºC with a constant flow of 0.6 mL/min. At first, the column temperature was maintained at 50°C for 2.5 min after which the temperature was steadily increased to 280°C at a rate of 10°C/min. MS temperature was set at 150°C and was run in scan mode using an electron multiplier offset of 200eV. The identification of the peaks in the chromatogram was carried out using the NIST database.

Statistical analysis

Results of the sensory analysis are presented as mean±standard deviation (mean±SD) of individual organoleptic attributes. In order to get concordant values for physico-chemical parameters, proximate analysis, physico-chemical, and microbial tests, the experimental assays were carried out in triplicates. The data were analyzed using analysis of variance (ANOVA) and t-test ($P \leq 0.05$).

Results and discussion

Table 2 indicates the effect of heating PSO at different temperatures on physicochemical parameters. Heating PSO at different temperatures did not have any impact on refractive index, free fatty acids, peroxide value, and unsaponification value. However, the iodine value decreased from 423.16 ± 2.46 to 204.53 ± 12.23 g (I2/100g) and the highest decrement was observed at 160ºC [204.53 ± 12.23 g (I2/100g)]. Decrease in iodine number of fats and oils occur due to diverse irreversible changes that take place in the fatty acid composition during heating.

Table 1. Proportions used for the developmentof the cupcake

Vanilla cupcake										
	Whole Wheat Flour	All-Purpose Flour	Sugar	Curd	Water	Vanilla essence	Baking soda	Baking powder	Refined oil	PSO
Control	35 g	35 g	50 g	82 g	10 g	1.5 g	0.8 g	1.7 g	30 g	-
25% PSO	35 g	35 g	50 g	82 g	10 g	1.5 g	0.8 g	1.7 g	25 mL	5 mL
50% PSO	35 g	35 g	50 g	82 g	10 g	1.5 g	0.8 g	1.7 g	15 mL	15 mL
Chocolate cupcake										
	Whole Wheat Flour	All-Purpose Flour	Sugar	Curd	Water	Cocoa powder	Baking soda	Baking powder	Refined oil	PSO
Control	20 g	20 g	50 g	82 g	10 g	10 g	0.8 g	1.7 g	30 mL	-
25% PSO	20 g	20 g	50 g	82 g	10 g	10 g	0.8 g	1.7 g	25 mL	5 mL
50% PSO	20 g	20 g	50 g	82 g	10 g	10 g	0.8 g	1.7 g	15 mL	15 mL

Table 2. Effect of heating conditions on the physicochemical properties and degradation of vitamin E of PSO

Physico-chemical parameters	Unheated	Heat treatment for 15 min		
		60ºC	100ºC	160ºC
Refractive index	1.48 ± 1.3	1.48 ± 1.3	1.48 ± 1.3	1.48 ± 1.6
Iodine value g (I_2/100g)	423.16 ± 2.46	373.79± 12.21	324.43 ± 12.23	204.53 ± 12.23
Peroxide value (milliequivalent/kg)	10 ± 0.00	30 ± 0.00	30 ± 0.00	30 ± 0.00
Free fatty acids (%)	1.33 ± 0.33	2 ± 0.00	2 ± 0.00	2 ± 0.00
Saponification value (mg KOH/g)	203.84 ± 4.37	275 ± 0.00	253.29 ± 26.63	235.55 ± 31.98
Unsaponification value (%)	1 ± 0.00	1 ± 0.00	1 ± 0.00	1.5 ± 0.36
Vitamin E (µg tocopherol/g)	0.085	0.068	0.134	0.066

GC-MS analysis showed the presence of punicic acid, oleic acid, tocopherols, campesterol, sitosterols, stigmasterol, and α-tocopheryl acetate as pre-dominant fatty acids in unheated and heated PSO (Fig. 1 and 2).

The mean scores of sensory and organoleptic attributes of cupcakes are given in Table 3. In the present study, two variants were formulated under each cupcake category (vanilla and chocolate) with the incorporation of 25 and 50% of PSO. With regard to the overall acceptance of cupcake variants prepared using PSO, chocolate cupcake containing 50% of PSO was found to be the most preferred one (3.53±0.94), followed by vanilla cupcake containing 25% of PSO (3.40±0.62). Nevertheless, the overall acceptability scores of control cupcakes (without PSO) were slightly higher.

Two cupcake variants were selected for further examination based on the scores given by the panel members for the overall acceptability attribute. On analyzing the overall acceptance scores of each cupcake variant, it is evident that chocolate cupcakes containing 50% of PSO and vanilla cupcakes containing 25% of PSO received the highest scores than their counter parts (Table 3). Results of the

proximate analysis indicate that the moisture, protein, and fat content of chocolate cupcakes containing 25% of PSO were high. Differences in nutrient composition observed between the two cupcake varieties could be due to the inclusion of cocoa powder in the chocolate cupcake.

Shelf-life testing is focused on the chemical and microbial attributes of the test samples. Data about shelf life analysis are presented in Tables 4 and 5. A gradual increase in the moisture content was observed for all the samples, while the pH content decreased. Results of TPC highlighted the presence of mild bacterial growth from the 4th day onwards. The presence of mold growth in the test samples was detected only after 7 days (Table 6).

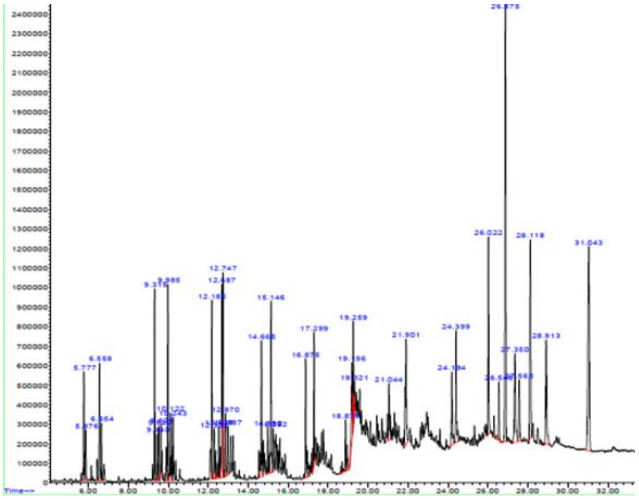

Figure 1. GC-MS analysis of unheated PSO.

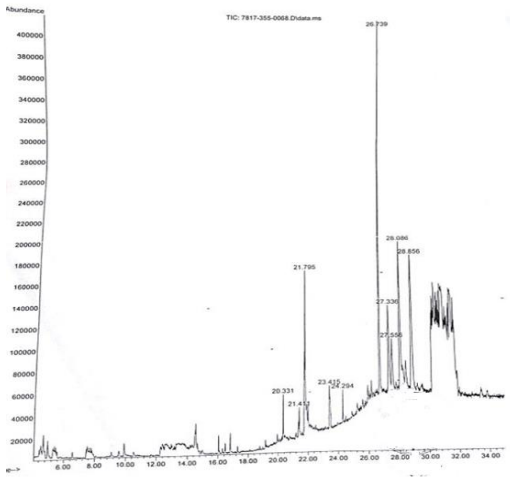

Figure 2. GC-MS analysis of PSO heated at 160ºC.

Fats and oils are considered to be the multi-functional ingredients used in cooking. Commonly used solid fats and cooking oils include butter, ghee, margarine, beef fat, corn, groundnut, sunflower, safflower, rice bran, palm, and coconut oil. The quantity and type of fats or oils used in cooking will have a direct impact on the structural, textural, and rheological aspects of the final product. A plethora of scientific research studies denotes the relationship between dietary fats (saturated fats), blood lipoproteins and the risk of developing cardiovascular diseases (19). Recent studies indicate the use of cold-pressed oil obtained from biological waste by-products such as peels and seeds. Since organic solvents are not utilized in the extraction process of cold-pressed oils, compounds present in these types of oil retain their health-promoting benefits, antioxidant, and physical properties of the oils. PSO extracted from pomegranate seeds contains 6.04% of SFA, 6.22% of MUFA, and 87.73% of PUFA. PUFA present in PSO includes punicic acid (65%-80%) and α eleostearic acid (30%-50%) (20, 22). Polyunsaturated fatty acids present in PSO, especially punicic acid provide multiple health benefits. Cold-pressed seed oils with excellent nutritional properties and unique fatty acid profiles will have immense potential applications in the food industry**.**

Refractive index (RI) refers to the degree of unsaturation and conjugation of fatty acids. From this study, it is evident that heating did not affect RI. Kaseske et al. (21) proved that blanching PSO at different temperatures (80, 90, and 100 ºC) had no significant impact on RI. The authors reported the RI value of cold-pressed PSO to be 1.5215-1.5218**.**

The present study accentuates the sensory attributes, proximate composition, and storage capacity of cupcakes enriched with PSO. Cupcakes prepared with PSO were well accepted by the panel members in terms of appearance, colour, taste, texture, flavour, and overall acceptability. The panel members also commented on the moistness, tenderness, and softness of cakes made using PSO. However, the textural aspects of the control cupcake (without PSO) obtained marginally better scores. With regard to the overall acceptability of cupcake variants, chocolate cupcake containing 50% of PSO was found to be the most highly preferred one (3.53±0.94), followed by vanilla cupcake containing 25% of PSO

(3.40±0.62). Bourekoua et al. (22) studied the sensory attributes, nutrient profile, and antioxidant activity of gluten-free cake formulated using pomegranate seed powder (PSP) in different proportions. The authors of the study stated that the formulated food product had higher antioxidant activity along with high ash, fibre, moisture, and protein content. However, the addition of large quantities of PSP affected the textural and sensorial characteristics. Dib et al. (23) concluded that the addition of 7.5% of PSP to gluten-free cake did not have an impact on the rheological and textural aspects. In this study, cupcakes were prepared with the incorporation of 25 and 50% of PSO. Results of sensorial evaluation point out that the addition of PSO to cupcakes did not affect the organoleptic and textural characteristics.

Phytosterols, tocopherols, and carotenoids provide multiple health benefits. PSO is rich in Vitamin E. Vitamin E has remarkable antioxidant properties and plays a vital role in preventing lipid peroxidation (24). The total tocopherol content of PSO ranges between 68 and 263 mg/100 mL of oil with γ-tocopherol representing nearly 88% to 95% of the total tocopherols content (25). Melo et al. (26) confirmed that PSO had good oxidative stability, despite the high degree of unsaturation of fatty acids**.**

Proximate composition and shelf-life testing were done for the two best cupcake variants. Results of the proximate analysis indicated that the moisture, protein, and fat content of chocolate cupcakes containing 25% of PSO were high. Evaluation of moisture content on three different days highlighted the impact of external environmental factors on the stability of the product. As the relative humidity and temperature of the external environment increased, the moisture content also increased simultaneously. Food spoilage refers to the undesirable chemical and biological changes that occur in food. The two main fundamental factors that contribute to food spoilage are an increase in moisture and water activity. Water activity refers to the free water available for microbial growth. Padmashree et al. (27) explained a strong positive correlation between water activity, TPC, and spoilage of food. As moisture content increased gradually, microbial growth was observed. TPC test provides data on the total number of viable bacteria present in the test sample. This value was obtained by multiplying cfu with the dilution number. Results of TPC highlighted the presence of mild bacterial growth on 4th day. Cupcakes stored at room temperature for a long period will deteriorate quickly as environmental extrinsic factors permit the growth of microorganisms, hence, they can be stored in refrigerators.

Conclusion

In summary, chocolate cupcake containing 50% of PSO was preferred (3.53±0.94) to vanilla cupcake containing 25% of PSO (3.4±0.62). The moisture, protein, and fat content of chocolate cupcakes containing 25% of PSO were estimated high. In addition, cupcakes prepared using PSO were suggested to be stored well for 4 days under room temperature. Findings suggested that cupcakes prepared using PSO had promising organoleptic properties.

Conflict of interest

The authors declare no conflict of interest.

Funding

M.G. was supported by the János Bolyai Research Scholarship (BO/00144/20/5) of the Hungarian Academy of Sciences. The research was supported by the ÚNKP-21-5-540-SZTE New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund. M.G. would also like to acknowledge the support of ESCMID's "30 under 30" Award.

Acknowledgments

The authors like to thank Taif University, Taif, Saudi Arabia, for their support (Taif University Researchers Supporting Project number: TURSP-2020/80).

Table 3. Sensory evaluation of cupcakes enriched with PSO

Attributes		Cupcake flavour	Control	Cupcake with 25% of PSO	Cupcake with 50% of PSO
Appearance	Crust	Vanilla	3.97 ± 0.85	3.47 ± 0.94	3.57 ± 0.82
		Chocolate	3.73 ± 0.79	3.77 ± 0.68	3.57 ± 0.63
	Crumb	Vanilla	3.80 ± 0.93	3.57 ± 0.90	3.47 ± 0.86
		Chocolate	3.50 ± 0.90	3.57 ± 0.73	3.53 ± 0.68
Colour	Crust	Vanilla	4.13 ± 0.73	3.47 ± 0.86	3.60 ± 0.89
		Chocolate	3.87 ± 0.68	3.80 ± 0.66	3.63 ± 0.72
	Crumb	Vanilla	3.90 ± 0.89	3.47 ± 0.86	3.63 ± 0.81
		Chocolate	3.80 ± 0.71	3.70 ± 0.60	3.43 ± 0.86
Crumb cells	Uniformity	Vanilla	4.03 ± 0.67	3.53 ± 0.82	3.47 ± 1.01
		Chocolate	3.63 ± 0.77	3.53 ± 0.86	3.17 ± 0.79
	Size	Vanilla	4.07 ± 0.64	3.40 ± 0.93	3.47 ± 0.86
		Chocolate	3.67 ± 0.81	3.63 ± 0.67	3.33 ± 0.76
	Thickness	Vanilla	3.80 ± 0.85	3.37 ± 0.85	3.60 ± 0.81
		Chocolate	3.57 ± 0.82	3.40 ± 0.68	3.17 ± 0.79
Texture	Moistness	Vanilla	3.80 ± 0.81	3.40 ± 0.93	3.53 ± 0.90
		Chocolate	3.60 ± 0.86	3.47 ± 0.92	3.40 ± 0.81
	Tenderness	Vanilla	3.93 ± 0.79	3.33 ± 0.92	3.63 ± 0.85
		Chocolate	3.70 ± 0.84	3.63 ± 0.81	3.57 ± 0.77
	Softness	Vanilla	4.03 ± 0.77	3.47 ± 1.01	3.83 ± 0.75
		Chocolate	3.93 ± 0.69	3.93 ± 0.69	3.57 ± 0.94
Taste		Vanilla	4.27 ± 0.74	3.13 ± 0.73	3.53 ± 0.82
		Chocolate	3.70 ± 0.84	3.40 ± 0.62	3.23 ± 0.86
Flavour		Vanilla	3.93 ± 0.79	3.33 ± 0.96	3.43 ± 0.86
		Chocolate	3.57 ± 0.94	3.20 ± 0.81	3.37 ± 0.81
Overall acceptability		Vanilla	4.07 ± 0.64	3.30 ± 0.92	3.53 ± 0.94
		Chocolate	3.83 ± 0.75	3.40 ± 0.62	3.43 ± 0.63

Table 4. Proximate composition of cupcakes prepared using PSO

Cake flavour		Ash	Moisture (%)	Carbohydrate (g)	Protein (g)	Fat (g)
Vanilla	Control	1.20 ± 0.07	26.40 ± 0.10	39.45	8.60 ± 0.10	24.19 ± 0.20
	50% PSO	1.67 ± 0.03	27.20 ± 0.20	40.16	6.89 ± 0.20	24.25 ± 0.10
Chocolate	Control	1.69 ± 0.04	26.78 ± 0.08	40.66	6.43 ± 0.10	24.36 ± 0.20
	25% PSO	1.28 ± 0.03	27.41 ± 0.31	38.50	7.15 ± 0.10	24.93 ± 0.10

Table 5. Microbiological aspects of cupcakes prepared using PSO

Cupcake flavour		Total plate count (cfu/mL)			Visible mold growth				
		0^{th} day	4^{th} day	7^{th} day	0^{th} day	4^{th} day	7^{th} day	10^{th} day	12^{th} day
Vanilla	Control	Nil	1	1	-	-	-	-	+
	50% PSO	Nil	1	3	-	-	-	-	+
Chocolate	Control	Nil	1	1	-	-	-	+	+
	25% PSO	Nil	3	9	-	-	-	+	+

Table 6. Physicochemical aspects of cupcakes prepared using PSO

Moisture (%)					
Cupcake flavour	Vanilla	Control	0th day	4th day	7th day
			26.40 ± 0.10	31.72 ± 0.23	32.44 ± 0.01
		50% PSO	27.20 ± 0.20	29.16 ± 0.03	33.25 ± 0.06
	Chocolate	Control	26.78 ± 0.08	28.79 ± 0.11	32.49 ± 0.49
		25% PSO	27.41 ± 0.31	29.32 ± 0.06	33.51 ± 0.01
pH					
Cupcake flavour	Vanilla	Control	0th day	4th day	7th day
			8.71 ± 0.02	8.02 ± 0.01	7.86 ± 0.05
		50% PSO	8.77 ± 0.04	7.79 ± 0.01	7.50 ± 0.01
	Chocolate	Control	8.26 ± 0.01	7.71 ± 0.01	7.52 ± 0.01
		25% PSO	8.10 ± 0.07	7.83 ± 0.01	7.29 ± 0.02
Acidity (%)					
Cupcake flavour	Vanilla	Control	0th day	4th day	7th day
			0.90 ± 0.01	1.80 ± 0.01	1.80 ± 0.01
		50% PSO	0.90 ± 0.01	1.80 ± 0.01	1.80 ± 0.01
	Chocolate	Control	0.90 ± 0.01	0.90 ± 0.01	2.40 ± 0.01
		25% PSO	0.90 ± 0.01	1.80 ± 0.01	1.80 ± 0.01

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