JRIFST
Original Paper

www.journals.rifst.ac.ir
Journal of Research and Innovation
in Food Science and Technology

https://dx.doi.org/10.22101/JRIFST.2022.342704.1360

The Effect of Nigella Sativa Oil on Physicochemical, Antioxidant Properties, and Shelf Life of Savory Cake

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Abstract
This work was undertaken to develop a novelly formulated cake substituted with the cold-pressed Nigella Sativa oil and evaluate their nutritional properties namely Carbohydrate, Protein, Fat, Ash, Moisture, Energy, physicochemical properties, antioxidant activity, and shelf life of the savory cake. The formulated savory cakes in different proportions in ranges from 2.5-10% along with butter (SC1-2.5%, SC2-5%, SC3-7.5%, and SC5 -10 %) as best suited for the recipe and without adding Nigella Sativa oil as taken control (SC0-0%). (Results revealed that nutritional properties of carbohydrate, protein, fat, ash, moisture and energy of various concentrations of savory cake were reported significant differences ($p<0.001$) as compared to the control. The hardness of savory cakes () increases even as the texture profile decreases with the continuing increase of Nigella Sativa oil. Color parameters, L*and a* values of the savory cake were decreased with the increase in the Nigella Sativa oil level. Scanning electronic microscopy reported the spongy structure of savory cake which dominance to increase in expansion ratio. The increased amount of Nigella Sativa oil in the dough formulation resulted in higher antioxidant properties in the final product and reduced lipid peroxidation. Sensory values were reported significantly increased in their acceptance among the savory cakes as compared to the control. The SC3 sample was observed to be most acceptable to sensory panelists towards the formulated cake. Based on the present work, Nigella Sativa oil-based baked products have good potential for consumer receipt, and health-promoting snacks, especially for diabetes patients.

Received: 2022.05.16
Accepted: 2022.07.21

Keywords
Microbial analysis
Microstructure
Nutritional properties
Sensory properties
Wheat flour

Introduction
Fat is one of the major bio-molecules substances endowed with a source of metabolic energy, besides acting as fat-soluble vitamins (e.g. A, D, E, and K), and essential fatty acids (e.g., arachidonic, linoleic, and linolenic). It also plays a vital role in food products particularly bakery products in the form of nutritional and consumer acceptance. Fats substances such as butter in baked products are obtained supplementary exogenously to provide a heat transmit medium for processing and

are functional to get pleasing texture and flavor (Shahidi & Zhong, 2010). Many findings, reported that the various fat (Butter, palm oil, soybean oil, olive oil, and sunflower oil) substances play a major role in the preparation and increase the aroma compounds in the baked products mostly muffins, savory cakes respectively. Savory cakes are flexible and usually consumed in several countries in the world, conventionally made with wheat flour, yeast, sugar, spices, and butter. However, savory cakes are high in butter (10%–15%), and documented also that contains high-fat food items are increased flat to lipid oxidation, retreating the worth of the food product when treated to high thermal processes (Al-Bandak & Oreopoulou, 2011).

Lipid oxidation leads to unfavorable changes in the nutritive value and sensory and physic-chemical properties of the food products. Besides, numerous findings obtained that more consumption of compounds that emerged from oxidation are connected with the growth of certain diseases, such as tumorigenesis or atherosclerosis (Giarnetti et al., 2015; Kanazawa, Sawa, Akaike, & Maeda, 2002) The oxidative deterioration of food products can be decreased with the addition of artificial or natural antioxidant sources. Antioxidants are the major weapon to mitigate the free radicals which keep to less oxidative stress. Free radicals are the unbalanced molecule substances and the reason for the growth of numerous retrogressive diseases (Prathapan et al. 2011). On other hand, antioxidants can play a vital role in inhibiting the dissemination of free radical reactions. However, the use of artificial antioxidants such as TBHQ (Tert-butylhydroquinone), PG (Propyl gallate), BHT (Butylated hydroxytoluene), and BHA (Butylated hydroxyanisole) has been constrained because of potential toxicity and health risks. Thus, there is improved consumer attentiveness to use as natural antioxidants in food products from plant s that carry high phenolic and other phytochemical compounds to preserve and improve food products and also prevent potential toxicological effects of synthetic ones (Hashemi et al., 2017). Nigella sativa L. is a yearly aromatic plant of the Ranunculaceae family, with the seeds habitually called black seed and black cumin seed. Nigella sativa L. (Black cumin) is fine recognized for its peppery taste, is strong, hot, and has various pharmaceutical, nutritional, and traditional therapeutic uses (Ahmad et al., 2013). Nigella sativa L seed and its oil are significantly active compounds and are regarded to have a wide range of bioactive properties. In the food industry, selected Mediterranean plants including Nigella sativa L. oil and olive leaf are used as food additives in the formulation of yogurt, bread, marinades, cheese, and sauces (Ahmad et al., 2013) and replacing of the fat amount to reduce lipid oxidation.

Use in the place of fat substances with option replacers is unquestionably challenging as fat plays a vital role in the organoleptic properties and textural characteristics of food products (Psimouli & Oreopoulou, 2013). In the baking method, fat supports air absorption and embarrassment of gas-bubble coalescence, important to the making of a leavened, tender cake with superior crumbs (Colla et al., 2018; Felisberto et al., 2015). Fat substances also detain starch gelatinization in baked food products by set backing water to move into starch granules during initiating complexes between amylase and polar lipids during baking time (Felisberto et al., 2015). Hence, decreased-fat substances are frequently linked with mouthfeel, less sensory qualities, and consumer acceptance (Colla et al., 2018). Fat products substitute are generally classified as fat replacers and relate to other major molecules such as protein, carbohydrates, and fat sources. It is naturally used as a fractional substitution of fats in foodstuff. To our awareness, no findings have been passed out about to utilization of Nigella sativa L oil with the intent to decrease the lipid oxidation of bakery products. Therefore, the aim of the present study was to develop the savory cake substituted with using Nigella sativa L oil which is good potential for consumer receipt, more proficient restraint of lipid oxidation and high nutritional functional food, and health-promoting snacks, especially for diabetes patients .and investigated the physicochemical, antioxidant properties, microstructure, and shelf life of the savory cake.

Materials and Methods

Materials and sample preparation

Selected ingredients (Black seeds, wheat flour, Chickpea flour, and Vegetable oil and spice items) were procured from M.K. retail (local supermarket) and Big Basket (online supermarket). Nigella sativa oils were washed, dried, and put through a cold-pressing machine to the oil at a temperature of 37ºC and the press speed was maintained at 610 rpm. The cold pressing service was extended by a local food industry situated on Kanakapura road, namely, Safa Honey Co. Analytical grade reagents were purchased from Merck, Mumbai, India. Solvents and antioxidant assays reagents were purchased from Himedia, India.

Formulation of savory cakes

The formulation of savory cakes involved several trials and errors which further resulted in the incorporation of the oil s which is commonly consumed in RTE snacks. The recipes were chosen to keep in mind the preferences and requirements of a diverse population based on the age group. After several trials, the butter was replaced Nigella sativa oil in the savory cakes in different proportions in ranges from 2.5-10% (SC1-2.5%, SC2-5%, SC3-7.5%, and SC5 -10 %) as best suited for the recipe and without adding black seed as taken control (SC0-0%) the amount mentioned in table 1Preparation of savory cake was initially weighing all the ingredients and keeping them aside then to transfer in a bowl and adding lukewarm water, sugar, and yeast and keeping it aside for 15 minutes. Then once the yeast is well proofed, add wheat flour and the yeast mixture to a bowl and knead the dough by adding lukewarm water little by little and knead well for 10 minutes. Add fat to the dough for control and replace black seed for samples in various percentages and knead until it leaves the sides then place the dough in a plate, cover it with a cloth and keep in a warm dark place for 1 hour and prepare the masala to be added on a pan by bringing all the spices together then after 1 hour, remove the excess air from the dough, add the masala and knead until it has mixed evenly and let it rest for another 45 minutes until well proofed then preheat the oven at 200ºC for 10 minutes and line the tray with a parchment paper and grease it and place the dough into the tray and bake at 200 ºC for 25 minutes and finally remove the prepared slices of bread from the baking trays and place on an open plate until completely cooled and then store in an airtight container and kept at room temperature for further investigation.

Table 1. Formulation (g) control (SC0) and *Nigella sativa* L. oil substitution level of savory cakes (SC1, SC2, SC3and SC4)

Ingredient (g/100g)	Variation of Savory cake				
	SC0	**SC1**	**SC2**	**SC3**	**SC4**
Wheat flour	67.5	67.5	67.5	67.5	67.5
Yeast	2.5	2.5	2.5	2.5	2.5
Sugar	5	5	5	5	5
Cinnamon	10	10	10	10	10
All purpose Flour to dust	5	5	5	5	5
Butter	10	7.5	5.0	2.5	0
Nigella sativa L oil	0	2.5	5.0	7.5	10
Total	100	100	100	100	100

SC0: Control; SC1: 2.5 % *Nigella sativa* L. oil; SC2: 5 % *Nigella sativa* L. oil; SC3: 7.5 % *Nigella sativa* L. oil; SC4: 10 % *Nigella sativa* L. oil
substitution level of savory cakes

Proximate composition

The proximate composition including moisture, protein, fat, and ash content of savory cakes was analyzed using standard methods [AOAC 2005]. Moisture content was done by the gravimetric method in a hot air oven at 105ºC until a constant weight was obtained, and the quantity of ash was analyzed using a muffle furnace at 550ºC until a constant weight was attained. The lipid content was analyzed using a Soxhlet ion system and the crude protein content of fish was measured by the standard Kjeldahl method using 6.25×N as the conversion factor. Minerals such as iron, potassium, and calcium were estimated using AOAC methods.(2005).

Physicochemical properties of savory cakes

pH

The pH value of the products was determined by a digital pH meter (Cyber Scan 5105 pH/mV/Temperature meter, EutechInc, Singapore) calibrated at room temperature. 1 gm of the sample was taken and dissolved in 10 ml distilled water and a pH electrode was inserted directly into each sample solution.

Water activity

The water activity of savory cake was evaluated using an electronic dew site water activity meter (Aqualab Series 4TE, Decagon Devices, Inc., USA) at room temperature (25±2°C). A adequate amount of cake was taken in the sample holder and precaution was taken so that sample does not touch the sensor. Measurement of water activity was carried out until the value was concurrent.

Color analysis

The color of products was read as L*, a*, and b* using Hunter Lab Colorimeter (D-25, Hunter Associated Laboratory, USA). The Hunter lab colorimeter was calibrated using Hunter color standards prior to the reading of samples. The color intensity in terms of Chroma (C*) was calculated by the formula, C* = (a*2+ b*2)1/2, whereas hue angle (H◦) was calculated by the formula H◦= tan−1(b*/a*).

Hardness

The hardness of the cake was measured using a Texture Analyzer, TA (TA – HD plus, Stable Micro Systems, Surrey, U.K) according to the method of Shabir Ahmad Mir et al. (2017).

Oxidative stability (Measurement of TBARs)

To measure thiobarbituric acid reactive substance (TBARS), the lipid oxidation of savory cake substituted with Nigella sativa L. oil was performed using the method of Porntip Pasukamonset et al (2017). TBARS values were represent as number of nmol of malondialdehyde (MDA) per g of savory cake.

Antioxidant Properties

Antioxidant properties were carried out with the methanolic of savory cake (5g in 25ml methanol). Total flavonoid content was determined using the method of (Singh et al. 2015) with slight modifications. Total phenolic content was determined using the Folin-Ciocalteau spectrophotometric method (Singh et al. 2015). The method of (Mir, Bosco, Shah & Mir, 2016) was used for the DPPH radical scavenging activity with slight modifications.

Microstructure

The appearance of SC0, SC1, SC3, and SC4 was evaluated using the technique described by Shabir Ahmad Mir et .al (2017) with scanning electronic microscopy (Hitachi, S-3400N, Tokyo, Japan). By using a Freeze drier, the formulated cake was dried and cut into small pieces and congregation of the samples was performed on aluminum stubs with double side adhesive tape to which the samples were fixed and covered with a thin gold layer. An acceleration potential of 15 kV was used during micrography.

Shelf life and microbial analysis

The savory cake was microbiologically analyzed for 6 days to obtain the storage stability of the product. The freshly prepared sample of the savory cake was sealed and stored in an airtight pouch for different days (Day 0, Day1, Day2, Day3, Day4, and Day 5) and container respectively, and was opened only during the time of microbial testing. Consequent serial dilution was performed for both the flour and product. The Petri plates and test tubes were autoclaved on the same day of the analysis to ensure sterilization and prevent contamination. Shelf life study was done using the standard spread plate technique which was conducted every day for a period of 6 days

Sensory evaluation

Developed cakes were evaluated for sensory properties namely appearance, odor, texture, flavor and overall acceptability on different days (Day 0, Day1, Day2, Day3, Day4, and Day 5) using a nine-point hedonic scale rating from dislike extremely to like extremely. A sensory panel of fifteen semi-trained members was involved from the public. Mean ± standard deviation is out of overall acceptability scores by fifteen-panel members

Statistical analysis

All observations and sensory properties were carried out in triplicates. Data collected were analyzed and computed by using SPSS 22 software (SPSS Institute Inc., Cary, USA). Values were manifested as mean ± standard deviation. Single-factor ANOVA was employed to compute the significance of differences between mean values at $p<0.05$.

Result and Discussion

Nutrient properties of Savory Cake

The Carbohydrates, protein, fat, ash, moisture, protein, total fiber, and trace elements (Calcium, Iron, and Potassium) values are presented in Table 2. Nutritional quality is ultimately significant in considering the substituted level of Nigella sativa L. oil as a fat replacer and its successful performance depends principally on functional uniqueness imparted to the final products. The moisture content was slightly increased from 10.7 ± 0.09 % in 10% Nigella sativa L. oil to 9.5 ± 0.18% in 2.5% composite of savory cake. This could be due to the damaged seeds as well as seeds with higher moisture content presented. Wilderjans et al., (2010) reported that the final value of moisture of cake normally ranges from 18 to 28%, which is lesser than bread but higher than cookies. The moisture value of food products is generally used as an indicator and vital role in food quality. It is a dependent factor to measure the other characteristic of products because of its prospective influence on the sensory and microbial properties. The carbohydrate content of savory cake from the various components of oil was observed nonsignificant in the range of 45.5 ± 1.30 g/100g to 44.8 ± 1.04 g/100g. Substitution of oil baked product is mostly processed based on the lipids and wheat flour composition which contain mostly fat and some protein. The previous work reported a similar carbohydrate amount of spongy cake (Jae Hwan Kim et.al. (2012). Protein content was decreased of increase the concentration of oil in the savory cake range between 10.15 ± 0.01 g/100g to 10.0 ± 0.05 g/100g and noted significant ($p<0.05$) of control (SC0) sample respectively. Manuel Gómez & Mario M. Martinez (2017) suggested that the formulation of cakes comprises two major groups that are low lipid content, where the emulsion is well (high quantity of fine bubbles) and someplace egg white proteins play a major role. In the other group, higher lipid content (oil or shortening) results in a coarse emulsion (lower number of coarse bubbles) that is stabilized by the presence of lipids and where the gas expansion is attained by the use of baking powder. Generally, crude protein content was present in ranged between 22.6 to 23.3 % of *Nigella sativa* seeds (S. Cheikh-Rouhou et al.(2007). The Fat content was the most certainly influence parameter of baked products on other hand akes possess more lipids that can mask the unfavorable taste. The fat contents were increased of increase the concentration of oil in the savory cake as range between 26.3 ± 1.19 g/100g to 37.4 ± 0.96 and noted significant ($p < 0.05$) of SC0 (11.7 ± 0.26 g/100g) sample respectively. The high-fat content may be due to its impressive fatty acid profile. Black seed oil contains a high amount of unsaturated fatty acids. Consumption of black seed oil substituted product successfully reduces diastolic and systolic blood pressure (Fallah Huseini, H et al. 2013). Thymoquinone and thymohydroquinone are the major bioactive compounds in the black seed and play multi-purpose health aspects. The fiber content reported a non-significant difference between the control (SC0 7.5 g/100g) and variation (SC1 7.6 g/100g, SC2 7.5 g/100g, SC3 7.4 g/100g, and SC4 7.6 g/100g). The ash content in savory cake composed of black seed oil could be significant in the range between 9.6 ± 0.12 g/100g to 12.7 ± 0.17 g/100g compared with the control. Due to the presence of high levels of trace elements obtained in Nigella sativa seeds (S. Cheikh-Rouhou et al.(2007).). The minerals such as calcium, iron, and potassium determined the savory cake substituted with various concentrations of Nigella sativa oil results obtained in Table 2. Calcium (420.3 - 325.0 mg/100 g) followed by iron (12.13 - 7.85 mg/100 g) and potassium (844.9-447.1 mg/100 g) in savory cakes. S. Cheikh-Rouhou et al.(2007) suggested that Nigella sativa oil has been represented to be rich in essential micronutrients and which be capable of raw material for the formulation of functional foods.

Table 2. Nutritional properties of savory cakes

Nutritional properties (g/100 gm)	Variation of Savory cake				
	SC0	**SC1**	**SC2**	**SC3**	**SC4**
Moisture	8.4 ± 0.59 ^{d}	9.5 ± 0.18^{c}	10.2 ± 0.09 ^{b}	10.5 ± 0.12^{b}	10.7 ± 0.09 ^{a}
Carbohydrates	43.8 ± 1.04^{b}	44.5 ± 1.20 ^{b}	44.7 ± 1.39 ^{b}	44.83 ± 1.25 ^{a}	45.5 ± 1.30 ^{a}
Protein	10.21 ± 0.02 ^{a}	10.15 ± 0.01 ^{b}	10.13 ± 0.01 ^{b}	10.01± 0.01 ^{c}	10.01 ± 0.05 ^{c}
Fat	11.7 ± 0.26 ^{e}	26.3 ± 1.19 ^{d}	28.4 ± 1.05 ^{c}	33.2 ± 1.34 ^{b}	37.4 ± 0.96 ^{a}
Ash	7.72 ± 0.17 ^{e}	9.6 ± 0.12 ^{d}	10.3 ± 0.42 ^{c}	11.6 ± 0.05 ^{b}	12.7 ± 0.17 ^{a}
Fiber	7.5 ± 0.16 ^{a}	7.6 ± 0.01 ^{a}	7.5 ± 0.02 ^{a}	7.4 ± 0.08 ^{a}	7.6 ± 0.20 ^{a}
Minerals (mg/100 gm)					
Calcium	290.4 ± 4.70 ^{e}	325.0 ± 5.01 ^{d}	340.1 ± 5.02 ^{c}	387.7 ± 7.72 ^{b}	420.3 ± 1.33 ^{a}
Iron	5.68 ± 0.14 ^{e}	7.85 ± 0.04 ^{d}	9.80 ± 0.05 ^{c}	11.64 ± 0.16 ^{b}	12.13 ± 0.15 ^{a}
Potassium	261.6 ± 5.69 ^{e}	447.1 ± 5.62 ^{d}	546.6 ± 4.25 ^{c}	701.5 ± 3.02 ^{b}	844.9 ± 2.52 ^{a}

Data expressed as Mean ± Standard deviation of Triplicates. Means within the same row have no common superscripts are significantly different (p<0.05). SC0: Control; SC1: 2.5 % *Nigella sativa* L. oil; SC2: 5 % *Nigella sativa* L. oil; SC3: 7.5 % *Nigella sativa* L. oil; SC4: 10 % *Nigella sativa* L. oil substitution level of savory cakes

Physicochemical properties of savory cake

Hardness

The hardness value of savory cake measured by the texture analyzer was observed that increased gradually with the substitution concentration of Nigella sativa oil (Table 3). The baking process modifies the physicochemical characteristics of baked products which direct to the textural changes. The Nigella sativa oil substitution increased the hardness value from 1.52 ± 0.01 N to 1.68 ± 0.01 N was significantly increased when compared to the SC0 (control) cake respectively. Increase the hardness value due to the communication of hydrogen bonding and hydrophobic relations between protein elements and phenolic compounds, which causes conformational and structural changes of the protein in food (Yuksel et al. 2010; Jakobek 2015)

On other hand, the increase in the hardness of baked products can also be caused by the high level of insoluble fiber and bioactive compounds present in the composition of products (Laguna et al., 2014). The increase in hardness with Nigella sativa oil incorporation of the savory cake may be due to the presence of bioactive compounds such as thymoquinone and thymohydroquinone content of the which may influence the baking process and leads to the texture changes. The hardness results can classify the quality of the complete product and may contribute to selecting the most excellent functional ingredients (Choi et al., 2009).

Table 3. Physicochemical properties of savory cakes

Physical properties	**Variation of Savory cake**				
	SC0	**SC1**	**SC2**	**SC3**	**SC4**
Hardness (N)	1.82 ± 0.06[a]	1.52 ± 0.01[e]	1.58 ± 0.01[d]	1.61 ± 0.01[c]	1.68 ± 0.01[b]
pH	5.52±0.01 [d]	5.62±0.01[c]	5.66±0.01[b]	5.68±0.01[a]	5.68±0.01 [a]
Water activity	0.85 ±0.02 [a]	0.82 ± 0.02 [a]	0.81 ± 0.02 [a]	0.81 ± 0.02 [a]	0.81 ± 0.03[a]
Color parameters					
*L**	54.38 ± 0.06[a]	48.26 ± 0.12 [b]	46.38 ± 0.08 [c]	45.40 ± 0.12 [d]	43.38 ± 0.10 [e]
*a**	12.42 ± 0.03 [a]	10.38 ± 0.07 [b]	9.83 ± 0.04 [c]	9 .28 ± 0.02 [d]	8.98 ± 0.04 [e]
*b**	29.38 ± 0.08 [e]	32.30 ± 0.06 [d]	36.30 ± 0.07 [c]	38.65 ± 0.08 [b]	39.67 ± 0.06 [a]
Chromaticity	31.74± 0.05 [e]	34.06 ± 0.02 [d]	37.24 ± 0.04 [c]	39.35 ± 0.05 [b]	40.28 ± 0.02 [a]
Hue Angle (^{o})	67.07 ± 0.18 [e]	71.59 ± 0.15 [d]	77.25 ± 0.30 [c]	79.32 ± 0.40 [b]	80.23 ± 0.10 [a]
Oxidation stability					
TBARS (mmol/g of cake)	35.03±0.05 [a]	26.12±0.02 [b]	21.84±0.02 [c]	18.06±0.02 [d]	15.04±0.02 [e]

Data expressed as Mean ± Standard deviation of Triplicates. Means within the same row have no common superscripts are significantly different ($p<0.05$). SC0: Control ; SC1 : 2.5 % *Nigella sativa* L. oil ; SC2 : 5 % *Nigella sativa* L. oil ; SC3 : 7.5 % *Nigella sativa* L. oil ; SC4 : 10 % *Nigella sativa* L. oil substitution level of savory cakes

pH and Water activity

The pH and water activity are one of the important attributes in the shelf life and storage of food products. Significant variation ($p < 0.05$) was observed in pH value of control and variation (SC1, SC2, SC3, and SC4) in the range between from 5.52 to 5.68 (Table 3). The pH value can be related to the peroxide value of oil. The higher the peroxide value, the lower will be the pH due to an increase in the acid content of the oil which leads to rancidity. The water activity of savory cake showed no significant difference between variation (SC1, SC2, SC3, and SC4) and lower in control respectively. Results indicating that Nigella sativa oil addition lesser the amount of free/unbound or available water molecules in the savory cakes. These water activity value were less than those founded by others researchers for spongy cake prepared from with Clitoria ternatea , which had a range from (0.94 to 0.95) (Porntip Pasukamonset et. al (2017) observed water activity The results In general, the shelf life of a precise food system is calculated based on its water activity, which is an intrinsic property that denotes the availability of free water in the food system. In the food system, lower water activity provides protection from microbial growth and contamination and delays deterioration through biochemical reactions (Primo-martin et al., 2010).

Color

Color is unique of the important properties in food products mainly judged by the buyer. Maillard reaction and caramelization are the direct substances to affect the color of the baked products during the processing time (Stefani et al., 2019). In addition, the color of the raw materials can also have an impact (Paesani, Bravo-Nú˜nez, & G), mainly replacing the major materials. Therefore, color parameters are of dominant importance for the baked product. The color parameters L*, a*, and b* of the savory cake were observed significant differences in value based on the substituted Nigella sativa oil concentration present in the cake shown in Table 3. Luminosity (L*) was founded lower in Nigella sativa oil substituted sample (SC1, SC2, SC3, and SC4) then compare to control and ranged from 53.2 to 35.8. For all formulated cakes, chroma a* of the coating tended to be red and chroma b* to yellow. It is indicated that the luminosity (L*) decreased in the fragment of the cakes with the incorporation of oil , following the small piece. Further that in these savory cakes, chroma a* transformed the color development from red to green. These researchers also observed color changes, with a decrease in luminosity as the substitution for Nigella sativa oil occurred. The composition of raw materials, baking time and temperature, oven structure, the humidity of savory cakes, and airspeed inside the baking oven (Pertuzatti, Esteves, Alves, Lima, & Borges, 2015), as well as the color of the Nigella sativa oil , are factors that impact the final color of the savory cakes. A significant difference ($p \leq 0.05$) was observed for Chroma (C*) and Hue angle (H°) of control and Nigella sativa oil substituted samples. Hue angle (h◦) is measured as a qualitative aspect of color with the colors that are usually defined as, for instance, reddish and greenish. The Hue angle of 0° is appropriate for the color red and the angle of 90°, for the color yellow. Consequently, as the Hue angle varied from 67.07 ± 0.18 to 80.23 ± 0.10 °, the savory cakes approached the yellowish color. Chromaticity (C*) that represents the saturation of color for color and samples ranged form was 31.74± 0.05 and 40.28 ± 0.02. respectively.

Oxidative stability

High lipid content is a major factor in lipid oxidation in the cake. Kamkaen and Wilkinson (2009) reported that during high baking temperature lipid molecules degraded and noted oxidative instability in bakery products. The previous study reported in a spongy cake that many lipid peroxidation compounds such as 1-octanol and aliphatic aldehydes were detected during the baking process (Zamora and Hidalgo 2016). The range of lipid oxidation products expressed as TBARS in Nigella sativa oil substituted savory cakes are shown in Table. 3. The replacement of Nigella sativa oil (2.5–10%) significantly reduced the TBARS level in savory cakes (15.04–35.03 mmol/g of cake) when compared with the control savory cake ($P < 0.05$). These results showed that Nigella sativa oil at a low substitution of 2.5% caused the reduction of lipid oxidation in the savory cake. Many studies have documented that Nigella sativa oil has antioxidant activity including the TEAC, FRAP, and DPPH assays (Abdol-Samad Abedi et al. 2017; Deepak Kadam and S. S. Lele 2017). A previous study has also reported that Nigella sativa oil containing the phenolic compounds imparts characteristic inhibition of lipid oxidation in meat products (Kiralan, M.; et al. 2014). Findings proved that an increased level of Nigella sativa oil may present an enhanced inhibition impact of TBARs than a less substitution of Nigella sativa oil. In our assessment of the findings shown above, it can be concluded that the antioxidant activity of the Nigella sativa oil may be varied depending on the level of substitution. According to these results, the decreased TBARS of savory cakes capacity is due to the increased content of phenolic compounds in Nigella sativa oil, which perform as a free radical scavenger.

Total phenols, total flavonoids and antioxidant properties of savory cake

The results of the antioxidant activities including total phenol, total flavonoid, and DPPH radical scavenging activity of control and Nigella sativa oil substitution samples are presented in Table 4. Total phenolic compounds (TPC) are the most significant factor for an estimate of the quality of a product because TPCs have been correlated to the shelf life, sensorial attributed, and mainly prevent the oxidation in the product (Asdadi, A et al., 2014). The results noted a significant ($P < 0.05$) increase in total phenolic content increased with the increase of Nigella sativa oil level in savory cakes. The savory cakes formulated from 0% Nigella sativa oil noted 1.37 mg GAE/g of phenolic content for SC0 and 4.54 mg GAE/g and 5.08 for SC1, and SC2 whereas, savory cake prepared from 7.5g Nigella sativa oil showed 5.85 and 6.13 mg GAE/g phenolic content for SC4 (10 g Nigella sativa oil) in savory cake, respectively. The previous study reported the total phenolic compounds of Nigella sativa oil were reported in a range of 4.258 mg GAE/g (Deepak Kadam & S. S. Lele., 2017). The results indicate that the substitution of Nigella sativa oil influences the total phenolic content of the savory cake. Morita et al. (2017) suggested that TPC plays a significant role in mitigating the oxidative damages to the cellular constituent as a consequence of the creation of mediator and chemotactic factors which show the way to diverse degenerative diseases and health problems.

Flavonoids are the major significant secondary metabolite that has strange physiological potential including bioagents. Total flavonoid contents of Nigella sativa oil substituted cake were expressed as mg catechin equivalent per g. The total flavonoid content of savory cakes increased significantly ($P < 0.05$) with the incorporation of Nigella sativa oil. The savory cakes formulated from 0% Nigella sativa oil noted 1.85 mg catechin eq/g of flavonoid content for SC0. With the increase in Nigella sativa oil level, savory cakes reported improved flavonoid content from 8.43 mg catechin equivalent/g to 9.32 mg catechin equivalent/g from SC1 to SC4 savory cakes, respectively. Nigella sativa oil is rich in flavonoids (Deepak Kadam & S. S. Lele, 2017), so the substitution of may increase the level of flavonoids in savory cakes.

The DPPH is a constant free radical commonly used to estimate the antioxidant properties or free radical scavenging activity. The inclusion of Nigella sativa oil in the dough mixtures resulted in savory cake with significantly increased DPPH radical scavenging activity (Table 4). The highest DPPH scavenging capacity was observed by savory cake substituted with 10 g of Nigella sativa oil, which may be attributed due to their highest phenolic content compared to the control sample (SC0). DPPH radical scavenging capacity of savory cakes significantly increased from 87.8% to 95.4% from 2.5% to 10% Nigella sativa oil for the composition of savory cake dough, respectively. Y. Mazaheri et al., (2019) Nigella sativa oil can have potential antioxidative properties as it is a rich source of antioxidative components such as tocopherols and phenolic compounds. Lindenmeier and Hofmann, (2004), and Sensoy et al., (2006) suggested that the baking method was antioxidant parameters such as Maillard reaction products which significantly increase the DPPH radical scavenging activity. The result indicated the inhibition concentration is due to the incorporation of Nigella sativa seed oil in the savory cake, which is a rich source of thymoquinone, a potent antioxidant. Also, there are phenolic compounds such as carvacrol and kaempferol that work along with thymoquinone to furnish an antioxidant effect. (Topcagic et al., 2017).

Table 4. Antioxidant properties of savory cakes

Antioxidant properties	Variation of Savory cake				
	SC0	**SC1**	**SC2**	**SC3**	**SC4**
Total Phenol content (mg GAC eq/g)	1.37 ± 0.02[e]	4.54 ± 0.01[d]	5.08 ± 0.03[c]	5.85 ± 0.02[b]	6.13± 0.02[a]
Total flavonoid content (mg catechin eq/g)	1.85±0.01 [e]	8.42±0.03 [d]	8.66±0.05[c]	9.08±0.03[b]	9.32±0.06 [a]
DPPH (%)	58.24 ±0.07 [e]	87.82 ± 0.08 [d]	90.08 ± 0.05 [c]	92.13 ± 0.04 [b]	95.42 ± 0.08[a]

Data expressed as Mean ± Standard deviation of Triplicates. Means within the same row have no common superscripts are significantly different (p<0.05). SC0: Control ; SC1 : 2.5 % *Nigella sativa* L. oil ; SC2 : 5 % *Nigella sativa* L. oil ; SC3 : 7.5 % *Nigella sativa* L. oil ; SC4 : 10 % *Nigella sativa* L. oil substitution level of savory cakes

Microstructure of savory cake

A scanning electron microscope (SEM) determined the structural changes of savory cake substituted with Nigella sativa seed oil of different compositions. Figure 1 a is an SEM image of the 0 % (SC0) substitution of Nigella sativa seed oil cake and Fig. 1 b to Fig. 1e shows the micrographs of cake that have been substituted by the different percentage of Nigella sativa seed oil respectively. The SC0 noted the squashed internal structure with cakes was observed in some places. The baking treatment leads to the puffiness and fusion of biomolecules resulting in the healing of savory cake and the alteration of the protein matrix. No significant difference was observed in shape; particles in all Nigella sativa seed oil cake showed a flaked structure. Moreover, the surface of the particles was smooth without wrinkles and folding. However, the particle size showed differences, which may be because of differences in the aggregation of lipid particles. However, the 10 % substitution Nigella sativa seed oil cake showed larger wrinkles and protein matrix in comparison to other substitution cakes. The interaction of Nigella sativa seed oil and protein leads to the physical change in the developed cake and hence affects its expansion ratio. Abdol-Samad Abedi et al., (2017) studied the SEM of ion of Nigella sativa L. essential oil and reported that the large number of cells containing essential oil ruptured, atrophic, and seemed wrinkled shapes. Wheat flour and raw materials ingredients also influence the microstructure of savory cake and baked products (Jongsutjarittam and Charoenrein (2013).

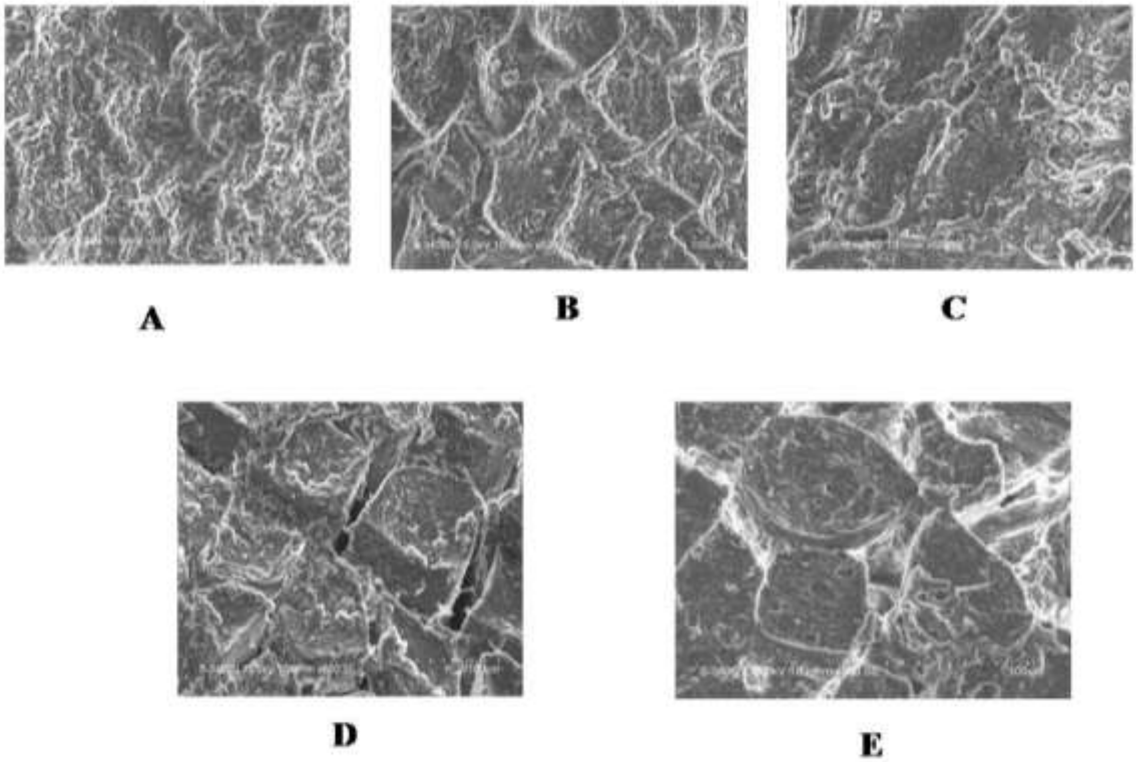

Fig. 1. Scanning electronic micrographs (a) 0 % Nigella sativa L. oil (X500) (b) 2.5% Nigella sativa L. oil (X500) ; (c) 5 % Nigella sativa L. oil (X500) ; (d) 7.5 % Nigella sativa L. oil (X500) ; (e) 10 % Nigella sativa L. oil (X500)

Microbial analysis of savory cakes

The microbial analysis measured of savory cakes estimated by total plate count technique (TPC) from Day 0 to Day 5 storage days is shown in Table 5. The savory cake substituted with Nigella sativa seed oil observed that low colony-forming units (CFU) for bacteria were growth from day 0 to day 5. Although there was a growth of bacterial colonies from on initial in control (SC0), the CFU calculated was extremely negligible and hence can be regarded as 0. However, there was significant growth in all the variations. Generally, Nigella sativa seed oil and essential oil have been observed to acquire antimicrobial activity against several bacteria (Lorina Badger Emeka et al.,2015). The author revealed from microbial analysis results that increasing the concentration of Nigella sativa seed oil in the savory cake prevents microbial growth in the product and oil plays an essential role in the extent of the storage days of the product and shelf life. Microorganism growth usually necessitates at least 0.91 water activity (aw), while below 0.6 aw, microorganisms cannot survive (Pupulawaththa et al., 2014). The author founded that savory cake of water activity was range from 0.81 to 0.82 aw respectively. Generally, the shelf life of a homemade cake is 3-5 days due to no added preservatives and stabilizers. According to the Public Health Legislative Safety Guidelines (PHLSG), the acceptable range for CFU in food products is less than10^{-4} to 10^{-6}. Hence, the formulated product meets the standards of the commercially available products.

Table 5. Microbial analysis (Total Viable Counts of Bacteria) of savory cakes

Storage days	Dilution factor	Variation of Savory cake				
		SC0	**SC1**	**SC2**	**SC3**	**SC4**
0th day	10^{-6}	ND	ND	ND	ND	ND
	10^{-8}	ND	ND	ND	ND	ND
1st day	10^{-6}	0.33×10^{5}	0.11×10^{3}	ND	ND	ND
	10^{-8}	ND	ND	ND	ND	ND
2nd day	10^{-6}	1.66×10^{3}	0.36×10^{3}	0.24×10^{3}	ND	ND
	10^{-8}	0.66×10^{4}	0.20×10^{4}	0.16×10^{4}	ND	ND
3rd day	10^{-6}	3.3×10^{3}	1.2×10^{3}	0.45×10^{3}	0.36×10^{3}	0.13×10^{3}
	10^{-8}	0.93×10^{4}	0.45×10^{4}	0.24×10^{4}	3.33×10^{4}	1.25×10^{4}
4th day	10^{-6}	5.0×10^{3}	4.8×10^{3}	4.4×10^{3}	0.34×10^{3}	0.12×10^{3}
	10^{-8}	4.2×10^{5}	3.7×10^{5}	3.1×10^{5}	2.0×10^{5}	1.3×10^{5}
5th day	10^{-6}	5.88×10^{3}	4.95×10^{3}	3.58×10^{3}	2.2×10^{3}	1.12×10^{3}
	10^{-8}	5.66×10^{5}	5.02×10^{5}	4.86×10^{5}	3.0×10^{5}	2.3×10^{5}

ND=not detected; SC0: Control ; SC1 : 2.5 % *Nigella sativa* L. oil ; SC2 : 5 % *Nigella sativa* L. oil ; SC3 : 7.5 % *Nigella sativa* L. oil ; SC4 : 10 % *Nigella sativa* L. oil substitution level of savory cakes

Sensory evaluation of savory cakes

Sensory characteristics of savory cakes substituted with Nigella sativa seed oil were estimated based on appearance, odor, color, texture, flavor, and overall acceptability (Table 6). Results reported that significant difference among the variations for all the attributes. The mean score for all the attributes was found to be the highest for SC3. The variation SC3 consisted of 7.5 % oil. The panelists had the highest preference for SC3 due to the right balance of flavor, texture, and odor. The spicy flavor of the oil complimented the other spices very well. The previous studies reported that Nigella sativa seed oil was incorporated in bread and found that substitution of 5% of the flour in bread satisfactory consumer acceptability in terms of flavor, texture, aroma, and overall acceptability (Osman MA et al., 2011). Nigella sativa seed oil has been reported to contain more phenolic compounds (Y. Mazaheri et al., 2019) and these constituents play a vital role to enhance the texture of the cake. Moreover, the idea of a savory cake was highly appreciated and very impressive to the panelists. They found it a new desirable addition to the list of commonly consumed foods. The savory cake is a modern concept with traditionally existing ingredients.

Table 6. Sensory evaluation of savory cakes

Sensory parameters	Storage days	Variation of Savory cake				
		SC0	**SC1**	**SC2**	**SC3**	**SC4**
Appearance	0th day	7.2±0.2^{bc}	7.6±0.3^{b}	7.6±0.4^{b}	8.1±0.2^{a}	7.6±0.2^{b}
	1st day	7.1±0.3^{bc}	7.6±0.2^{b}	7.5±0.6^{b}	8.1±0.3^{a}	7.4±0.3^{b}
	2nd day	6.9±0.2^{d}	7.5±0.3^{b}	7.2±0.2^{c}	7.9±0.2^{a}	7.3±0.2 ^{bc}
	3rd day	6.6±0.4^{cd}	7.2±0.2^{b}	6.9±0.4 ^{bc}	7.8±0.5^{a}	7.0±0.3^{bc}
	4th day	6.5±0.1^{d}	6.9±0.4^{b}	6.8±0.4^{b}	7.4±0.3^{a}	6.8±0.2^{,b}
	5th day	6.2±0.2^{c}	6.6±0.2^{b}	6.6±0.4^{b}	7.2±0.2^{a}	6.7±0.2^{b}
Odour	0th day	7.2±0.5^{c}	7.8±0.2^{b}	7.0±0. 2 ^{c}	8.2±0.2^{a}	7.1±0. 2^{c}
	1st day	7.0±0.2^{c}	7.6±0.5^{b}	6.9±0. 4 ^{c}	8.2±0.2^{a}	7.0±0. 4^{c}
	2nd day	6.8±0.2^{c}	7.5±0.3^{b}	6.6±0. 3 ^{c}	8.0±0.2^{a}	6.7±0. 2^{c}
	3rd day	6.7±0.3^{c}	7.2±0.2^{b}	6.5±0. 2 ^{c}	7.5±0.2^{b}	6.6±0. 1^{c}
	4th day	6.3±0.5^{c}	7.0±0.3^{b}	6.3±0. 2 ^{c}	7.2±0.2^{a}	6.4±0. 3^{c}
	5th day	6.2±0.2^{c}	6.8±0.2^{b}	6.2±0. 4 ^{c}	6.8±0.2^{a}	6.2±0. 2^{c}
Texture	0th day	6.4±0.3^{c}	7.8±0.2^{a}	7.4±0.3^{ab}	7.8±0.2^{a}	7.4±0.2^{ab}
	1st day	6.3±0.5^{c}	7.6±0.3^{a}	7.3±0.2^{ab}	7.6±0.2^{a}	7.4±0.4^{ab}
	2nd day	6.2±0.2^{c}	7.5±0.2^{a}	7.2±0.1^{ab}	7.4±0.3^{a}	7.2±0.3^{ab}
	3rd day	6.2±0.4^{c}	7.3±0.4^{a}	7.0±0.3^{ab}	7.3±0.2^{a}	7.0±0.2^{ab}
	4th day	6.0±0.2^{c}	7.2±0.2^{a}	7.0±0.2^{ab}	7.0±0.1^{a}	7.0±0.4^{a}

	5th day	5.9 ± 0.2^{c}	7.0 ± 0.9^{a}	6.8 ± 0.2^{ab}	7.0 ± 0.5^{a}	6.9 ± 0.2^{ab}
Flavour	0th day	6.4 ± 0.4^{a}	6.8 ± 0.3^{b}	7.2 ± 0.2^{b}	7.3 ± 0.2^{b}	7.2 ± 0.1^{b}
	1st day	6.0 ± 0.2^{c}	6.7 ± 0.4^{b}	7.0 ± 0.1^{ab}	7.4 ± 0.2^{a}	7.0 ± 0.3^{ab}
	2nd day	5.9 ± 0.4^{c}	6.5 ± 0.2^{b}	7.0 ± 0.4^{ab}	7.3 ± 0.2^{a}	$6.9\pm0.^{5ab}$
	3rd day	5.8 ± 0.3^{c}	6.3 ± 0.2^{b}	6.8 ± 0.3^{ab}	7.0 ± 0.3^{a}	6.8 ± 0.4^{ab}
	4th day	5.6 ± 0.3^{c}	6.2 ± 0.4^{b}	6.4 ± 0.2^{ab}	7.0 ± 0.2^{a}	$6.6\pm0.^{2ab}$
	5th day	5.4 ± 0.2^{c}	6.0 ± 0.2^{b}	6.2 ± 0.3^{ab}	6.9 ± 0.3^{a}	$6.3\pm0.^{2ab}$
Overall acceptability	0th day	6.8 ± 0.2^{c}	7.6 ± 0.3^{ab}	7.6 ± 0.2^{ab}	8.0 ± 0.3^{a}	7.6 ± 0.1^{ab}
	1st day	6.8 ± 0.3^{c}	7.5 ± 0.2^{ab}	7.4 ± 0.3^{ab}	7.7 ± 0.2^{a}	7.4 ± 0.2^{ab}
	2nd day	6.5 ± 0.2^{c}	7.3 ± 0.3^{ab}	7.2 ± 0.2^{ab}	7.5 ± 0.2^{a}	7.2 ± 0.3^{ab}
	3rd day	6.4 ± 0.4^{c}	7.2 ± 0.2^{ab}	6.9 ± 0.3^{ab}	7.2 ± 0.3^{a}	6.9 ± 0.2^{ab}
	4th day	6.2 ± 0.3^{c}	6.9 ± 0.3^{ab}	6.7 ± 0.2^{ab}	6.9 ± 0.1^{a}	6.7 ± 0.1^{ab}
	5th day	6.0 ± 0.2^{c}	6.6 ± 0.2^{ab}	6.5 ± 0.2^{ab}	6.8 ± 0.1^{a}	6.5 ± 0.3^{ab}

Data expressed as Mean ± Standard deviation of Triplicates. Means within the same row have no common superscripts are significantly different ($p<0.05$). SC0: Control ; SC1 : 2.5 % *Nigella sativa* L. oil ; SC2 : 5 % *Nigella sativa* L. oil ; SC3 : 7.5 % *Nigella sativa* L. oil ; SC4 : 10 % *Nigella sativa* L. oil substitution level of savory cakes

Conclusion

From the findings of the present study, it can be concluded that Nigella sativa seed oil replaced butter concentration in savory cakes was sensory acceptable. Nigella sativa seed oil addition changes the nutritional properties of savory cakes. Savory cakes prepared from 10% Nigella sativa seed oil level have significantly higher antioxidant properties mostly tocopherols and phenolic compounds. The significant variation in physicochemical properties such as saponification value, acid value, FFA, PV, color, and texture was obtained with the incorporation of Nigella sativa seed oil. Savory cakes also showed rich in minerals especially calcium, iron, and potassium. Thus the substitution of Nigella sativa seed oil is proven as a good source of nutritional and functional components of baked products and therefore can be recommended that used in other food products as a functional ingredient.

Acknowledgements

Authors acknowledge the Mount Carmel College, Autonomous, Bengaluru, Karnataka 560052, India for providing financial assistance in the form of Home research project. This project is financially supported by the Department of Nutrition and Dietetics, Mount Carmel College, Autonomous, Bengaluru, Karnataka, India.

Conflict of Interest

The authors declare no conflict of interest.

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