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Utilization of *Nigella sativa* L. Essential Oil to Improve the Nutritive Quality and Thymoquinone Contents of Baked Products

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Abstract: *Nigella sativa* L. (Black cumin) is important medicinal plant and hold religious importance too. The core objective of present research study was to utilize Black Cumin Essential Oil (BCEO) as functional ingredients to improve the nutritive quality and thymoquinone contents of cereal-based bakery products. The results indicated that addition of essential oil influenced the physical and chemical characteristics of cookies non-significantly. Thymoquinone contents of cookies were highest in cookies containing 0.3% BCEO (7.25±0.482 mg/100 g) as compared to control (0.0 mg/100 g). Additionally, BCEO improved the oxidative stability of the cookies as indicated from decreased Peroxide Value (POV) and TBA value. Moreover, progressive increase in essential oil in cookies formulation didn't confer any deleterious impact on overall acceptability and judged by the trained taste panel during sensory appraisal. Present research investigation brightened the prospects of using black cumin essential oil in different food products that may produce healthy impact on end consumers.

Key words: Black cumin, essential oil, phytochemicals, thymoquinone, sensory appraisal

INTRODUCTION

Functional and nutraceutical foods are gaining immense attention from the researchers owing to increased demands. However, most of the healthy foods are having centuries old applications in the herbal medicines. The herbs and medicinal plants were in use since immemorial to prevent and cure various maladies (Butt *et al.*, 2009). The nutritionists of modern era found it quite plausible to include such foods in daily diet through different modules of diet based regimens (Ali *et al.*, 2008; Butt and Sultan, 2011). However, the scenario is not the same for all the functional foods as color tone of some of them is unlikely to attract the consumers. In such conditions, the incorporation of these healthy foods in dietary staples is one option to improve the intake of foods rich in bioactive molecules. Globally, efforts are directed to utilize phytochemical rich foods including garlic, ginger, guar gum, mulberry, oat, fenugreek, amla, bitter gourd, Echinacea, gingko biloba, etc. (Butt and Sultan, 2012; Sultan *et al.*, 2012).

Black cumin (*Nigella sativa* L.) is once such example that is known as "kalonji" in South Asian countries and considered as a good source of nutritionally essential components (Sultan *et al.*, 2011). Black cumin or black seed contains various functional ingredients e.g. thymoquinone, Polyunsaturated Fatty Acids (PUFA), phytosterols, tocopherols, etc. (Butt and Sultan, 2010). Black cumin seeds have been used as herbal medicine by various civilizations to treat and prevent various maladies to ensure good health. Its health enhancing potential has been attributed to the active ingredients that are mainly concentrated in fixed or essential oil (Ramadan, 2007). Essential oil extracted from black cumin is also of functional importance and naturally bestowed with antioxidant rich volatiles (0.40-1.50%); contain 18.4-24% thymoquinone and 46% monoterpenes (Al-Jassir 1992; El-Tahir *et al.*, 1993; Ashraf *et al.*, 2006; Ali *et al.*, 2008). Black cumin essential oil is also a rich source of antioxidants including ?-cymene, carvacrol, t-anethole and 4-terpineol (Nickavar *et al.*, 2003). Likewise, Burits and Bucar (2000) analyzed essential oil using GC-MS and characterized many components like thymoquinone (27.8-57.0%), ?-cymene (7.1-15.5%), carvacrol (5.8-11.6%), t-anethole (0.25-2.3%), 4-terpineol (2.0-6.6%) and 1.0-8.0% of longifoline (Mozzafari *et al.*, 2000; Ashraf *et al.*, 2006). The antioxidant potential of black cumin essential oil and its fractions containing active ingredients is proven. In this regard, Burits and Bucar (2000) observed IC_{50} value in DPPH (2,2-diphenyl-1-picrylhydrazyl) assay for black cumin essential oil, thymoquinone and carvacrol i.e. 460.0, 211.0 and 28.8 mg/mL, respectively.

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Several pharmacological investigations explored that thymoquinone is effective against oxidative stress, cancer, immune dysfunction and diabetic complications (Sultan *et al*., 2011). Furthermore, it also regulates several hematological and serological functions; maintains body homeostasis and bears hypocholesterolemic effect (Gali-Muhtasib *et al*., 2004; Hussein *et al*., 2005). It holds insulinotropic properties and helpful in maintaining β-cells integrity and both these properties are important in mediating diabetes mellitus. Likewise, antioxidants present in black cumin mitigate diabetic complications arising due to free radical production and elevated cholesterol level (Mansi, 2005; Kaleem *et al*., 2006). In the last few decades, scientific advancement in the domain of nutrition have established pivotal link between dietary components and human health (Anton *et al*., 2008). Black cumin essential oil is promising candidate in dietary modifications and can be utilized as a functional ingredient in cereal-based products (Ramadan and Morsel, 2002).

Cookies being higher in fat contents would be a better carrier for provision of desirable quantities of antioxidants to the vulnerable segments by adding up BCEO in various proportions. Keeping in view, present research project was designed to enhance the nutritive value and improve oxidative stability of cookies using BCEO.

MATERIALS AND METHODS

Black cumin seeds of indigenous variety were obtained from Barani Agricultural Research Institute (BARI), Chakwal. Raw materials for cookies preparation were procured from local market while reagents (analytical and HPLC grade) and standards were purchased from Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan) and Merck (Merck KGaA, Darmstadt, Germany). Infection free and healthy Sprague dawley rats (120-130 gm) were procured from National Institute of Health (NIH) Islamabad.

Extraction and analysis of essential oil: The oil from the black cumin seed was extracted through solvent (n-hexane) extraction system as described in AOCS (1998); hexane was recovered by Rotary Evaporator (Eyela, Japan). The results regarding physical and chemical analysis of black cumin oil has been already published (Sultan *et al*., 2009a).

Product development: Cookies containing BCEO were evaluated for their functional importance. After conducting some preliminary trials, cookies with varying proportions of BCEOs (Table 1) were prepared according to the modified method described in AACC (2000).

Table 1: Treatments used in the study plan

Treatments	Black cumin fixed oil (%)
*T_0	0.0
T_1	0.3
T_2	0.6
T_3	0.9
T_4	1.2
T_5	1.5

*T0 = Acts as control

Physical analysis of cookies: The cookies were analyzed for diameter, width and spread factor at 0, 15, 30, 45 and 60 days according to method as described in AACC (2000).

Chemical analysis: Cookies were analyzed for proximate composition; moisture (Method No. 44-15A), ash (Method No. 08-01), protein (Method No. 46-30), fat (Method No. 30-25), fiber (Method 32-10) and nitrogen free extract according to their respective methods (AACC, 2000).

Determination of thymoquinone in cookies samples: Thymoquinone in BCEO sample was estimated by slurring the oil sample in methanol followed by overnight rotamix in mechanical shaker, vortexed for 1 min and centrifuged at 1400 rpm for 25 min. The supernatant was aspirated and aliquot of 20 µL was injected in HPLC with UV detector. Mobile phase was methanol: water (75: 25) with flow rate of 1 mL/min. Standard curve for thymoquinone was drawn with concentrations of 25, 50, 100, 150, 200 and 250 mg/mL. The peaks were compared and quantity of thymoquinone was determined in oil samples (Al-Saleh *et al*., 2006).

Oxidative stability of cookies: Oxidative stability of cookies was estimated by determining peroxide and TBA value as described in AACC (2000).

Sensory evaluation: The cookies were rated using 9-point hedonic score system (9 = like extremely; 1 = dislike extremely) by trained taste panel (Meilgaard *et al*., 2007). They were asked to express their opinion about the end product by giving score to attributes like color, flavor, taste, texture and overall acceptability at 0, 15, 30, 45 and 60 days (Appendix-I). During sensorial evaluation, cookies with different oil compositions were placed in transparent cups, labeled with random codes. Cold water and crackers were supplied to panelists for rinsing their mouths between the samples. In each session, panelists were seated in separate booths equipped with white fluorescent lighting in an isolated room.

Statistical analysis: Data obtained was analyzed statistically using statistical package i.e. Cohort V-6.1

(Co-Stat-2003). Sample for each analysis was run quadruplet; values expressed are means ± standard deviation. Analysis of variance was applied to check level of significance and mean were compared through Tukey's HSD tests (Steel *et al*., 1997).

RESULTS

Black Cumin Essential Oil (BCEO) was extracted through hydro-distillation. Results pertaining to the black cumin and its essential oils with special referenence to phytochemicals density are already published (Sultan *et al*., 2009a). The safety assessment of BCEO was carried out by Sultan *et al*. (2009b) and results claimed the safe status. In the present project, cookies were prepared and analyzed for various traits and results are herein;

Physical and chemical characteristics of cookies: The statistical analysis regarding physical attributes of cookies predicted the non-significant impact of black cumin essential oil. Means presented in Table 2 showed that progressive increase in BCEO influenced the physicals characteristics significantly. Progressive increase in black cumin essential oil showed varying trend and affected cookies diameter non-significantly that ranged from 49.87±0.08 to 51.36±0.063 mm. Likewise, data pertaining to thickness of cookies revealed that varying levels of black cumin essential oil affected this trait non-significantly and thickness remained in the range of 10.08±0.022 mm to 9.88±0.008 mm. Owing to non-significant changes in diameter and thickness, spread factor varied non-significantly. The addition of black cumin essential oil depicted non-momentous impression of treatments on spread factor and values were found to vary between 49.83±0.083 to 51.41±0.062.

Table 2: Effect of treatments on physical parameters of fortified cookies

Treatments	Diameter	Thickness	Spread factor
T_0	50.22±0.029	10.08±0.022	49.83±0.083
T_1	51.36±0.063	9.99±0.023	51.41±0.062
T_2	50.66±0.050	9.96±0.017	50.87±0.041
T_3	49.87±0.080	9.95±0.008	50.12±0.048
T_4	50.48±0.069	9.89±0.020	51.05±0.067
T_5	50.19±0.052	9.88±0.008	50.82±0.023

Means carrying same letters in a column do not differ significantly. T_0 = Normal cookies; T_1 = Cookies with 0.06% BCEO; T_2 = Cookies with 0.12% BCEO; T_3 = Cookies with 0.18% BCEO; T_4 = Cookies with 0.24% BCEO; T_5 = Cookies with 0.30% BCEO

It was evident from means for proximate composition (Table 3) that in cookies containing varying level of essential oil; moisture, protein, fat, fiber, ash contents and NFE values ranged in 3.13±0.046 to 3.20±0.026, 4.56±0.018 to 4.68±0.009, 23.59±0.126 to 23.78±0.122, 0.457±0.001 to 0.461±0.001, 1.523±0.05 to 1.528±0.005 and 6.51±0.100 to 66.51±0.099%, respectively (Table 3).

Oxidative stability of cookies: POV and TBA are indicators of oxidative stability of the produce. In this research, progressive increase in BCEO improved oxidative stability of cookies (Table 4). Mean squares regarding these attributes revealed that cookies with added levels of black cumin essential oil affected the oxidative stability of the products significantly. Essential oil yielded greater stability of cookies and minimum peroxide value (0.192±0.019) was recorded in T5 (0.30% black cumin essential oil) followed by 0.19±0.019 in T4 (0.24% black cumin essential oil). Data pertaining to TBA value indicated that progressive increase in black cumin essential oil decreased the TBA value momentously and minimum TBA value of 0.036±0.002unit recorded in T5 (0.30% black cumin essential oil) and maximum (0.054±0.007unit) was in T0 (control).

Sensory evaluation: Sensorial evaluation reflects the acceptability of product for the public of intended use. It is based on the traits liked or disliked by the end user and is essential in determining best composition/optimum levels before characterization/ commercialization. In this research project, cookies were prepared with varying levels of black cumin essential oil and attributes like color, taste, texture, flavor, crispness and overall acceptability were assigned scores on 9-point hedonic score system. During sensory evaluation, addition of BCEO imparted significant impact on the color, flavor and taste scores, while rests of the parameters remained non-significant. Color is one of the most desirable attributes for any product to be accepted by the consumers. Varying levels of black cumin essential oil added cookies showed non-significant differences for color scores and values were

Table 3: Effect of treatments on proximate composition (%) of cookies

Treatments	Moisture	Protein	Fat	Fiber	Ash	NFE
T_0	3.20±0.026	6.73±0.027	21.64±0.134	0.459±0.001	0.548±0.005	67.42±0.141
T_1	3.14±0.043	6.74±0.037	21.78±0.122	0.457±0.001	0.546±0.005	67.33±0.122
T_2	3.18±0.038	6.81±0.027	21.61±0.137	0.461±0.001	0.546±0.006	67.39±0.133
T_3	3.13±0.046	6.79±0.036	21.62±0.123	0.458±0.001	0.543±0.005	67.46±0.118
T_4	3.16±0.043	6.82±0.014	21.59±0.126	0.459±0.001	0.547±0.005	67.43±0.103
T_5	3.19±0.033	6.87±0.014	21.63±0.117	0.460±0.001	0.544±0.006	67.30±0.105

Means carrying same letters in a column do not differ significantly. T_0 = Normal cookies; T_1 = Cookies with 0.06% BCEO; T_2 = Cookies with 0.12% BCEO; T_3 = Cookies with 0.18% BCEO; T_4 = Cookies with 0.24% BCEO; T_5 = Cookies with 0.30% BCEO

Table 4: Effect of treatments on POV and TBA value

Treatments	POV (meq/kg)	TBA (mg malonaldehyde/kg)
T_0	0.288±0.061a	0.054±0.007a
T_1	0.243±0.038b	0.047±0.002b
T_2	0.234±0.035c	0.046±0.003b
T_3	0.203±0.022d	0.044±0.002b
T_4	0.198±0.019de	0.040±0.002c
T_5	0.192±0.019e	0.036±0.002d

Means carrying same letters in a column do not differ significantly. T_0 = Normal cookies; T_1 = Cookies with 0.06% BCEO; T_2 = Cookies with 0.12% BCEO; T_3 = Cookies with 0.18% BCEO; T_4 = Cookies with 0.24% BCEO; T_5 = Cookies with 0.30% BCEO

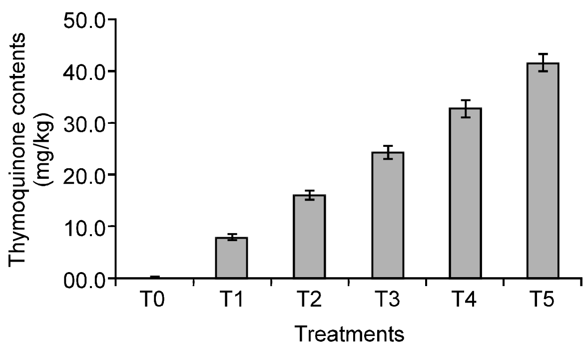

Fig. 1: Effect of treatments on thymoquinone cookies

in the range of 6.93±0.087 to 7.07±0.034 (Table 5). Means squares indicated the significant influence of treatments containing black cumin fixed oil on total taste scores of cookies. Progressive increase in black cumin essential oil and storage intervals imparted non-differential impact on taste sensation of the panelists. Varying levels of black cumin essential oil added cookies showed non-significant differences for taste scores and values were in the range of 6.84±0.036 to 7.02±0.051 (Table 5). Means for treatments and depicted non-significant variation in texture scores and values remained in the range of 6.90±0.086 to 7.13±0.081 in black cumin essential oil cookies. Flavor scores when subjected to statistical analysis revealed the non-significant difference of treatments on flavor scores of cookies containing black cumin fixed oil. Black cumin essential oil addition imparted non-significant flavor perception and all treatments behaved alike. Crispness indicates the crunchy perception of the cookies. Essential oil treatments showed non-momentous impact and values for crispness ranged from 7.28±0.097 to 7.44±0.093 (Table 5). Overall acceptability indicates the end user perception and involves multifarious trials. Trained panelist in this study rated scores for the cookies containing varying levels of black cumin fixed oil and black cumin essential oil. Cookies prepared with added levels of black cumin essential oil were acceptable to the panelists and they rated all treatments alike (Table 5). However, a mild bitter sensation was recorded for the treatments containing higher levels of BCEO and overall acceptability scores were in the range of 6.87±0.085 to 7.11±0.088 (Table 5).

DISCUSSION

Black cumin seeds are important in herbal medicines commonly employed in eastern civilization (Ramadan, 2007) especially Pakistan. In the current era, the centuries old concepts were researched and resulted in coinage of terms like functional and nutraceutical foods. However, most of them are effective in preventing different maladies thus their incorporation in daily diet is essential for improving human health. The diet-based therapies involving functional foods often aimed at maximizing their physiological benefits require meticulous product development (Siro *et al*., 2008). Perspectives of product development include selection of product, keeping in view the segments of population to be addressed (Urala and Lahteenmaki, 2007; Paradiso *et al*., 2008).

The present research project was one step ahead in this direction and black cumin essential oil was incorporated in the cookies formulation. However, care was taken to retain the maximum volatile constituents in final product. According to author knowledge this type of study has not yet been conducted to utilize black cumin essential oils in product development. The results regarding functional ingredient present in Black Cumin Essential Oil (BCEO) have been already published (Sultan *et al*., 2009b). Results regarding the physical traits like diameter, thickness and spread factor indicated the non-significant variations among treatments as a function of varying level of BCEO. Spread potential and size of cookies depends on flour particle size and moisture contents

Table 5: Effect of treatments on sensory traits of cookies

Treatments	Color	Flavor	Taste	Texture	Crispness	OA
T_0	7.07±0.034	6.88±0.070	7.02±0.051	7.13±0.081	7.44±0.086	7.10±0.088
T_1	7.06±0.055	6.87±0.054	7.00±0.058	7.07±0.084	7.44±0.093	7.11±0.073
T_2	7.01±0.056	6.83±0.056	6.92±0.046	7.01±0.076	7.38±0.092	7.10±0.072
T_3	6.96±0.064	6.79±0.058	6.96±0.053	6.99±0.063	7.33±0.062	7.03±0.078
T_4	6.96±0.099	6.77±0.045	6.89±0.044	6.95±0.090	7.30±0.087	6.99±0.085
T_5	6.93±0.087	6.72±0.053	6.84±0.036	6.90±0.086	7.28±0.097	6.87±0.085

Means carrying same letters in a column do not differ significantly. T_0 = Normal cookies; T_1 = Cookies with 0.06% BCEO; T_2 = Cookies with 0.12% BCEO; T_3 = Cookies with 0.18% BCEO; T_4 = Cookies with 0.24% BCEO; T_5 = Cookies with 0.30% BCEO. OA = Overall Acceptability

(Gaines and Donelson, 1985) and spread factor is the ratio that depends on the values of thickness and diameter of the cookies (Jacob and Leelavathi, 2007) that strengthened the results of present exploration.

Chemical parameters were not affected as a function of progressive increase in essential oil and values for moisture, protein, fats, fiber, ash and NFE remained in the ranges recorded in the literature (Bartolozzi *et al.*, 2000). However, peroxide and TBA values were significantly reduced with BCEO. Findings could be correlated with the research investigations of Gouveia *et al.* (2007) and Paradiso *et al.* (2008). They were of the view that polyphenol-rich vegetable materials may improve oxidative stability of the products. Results regarding bioactive molecules have also shown practical significance as thymoquinone contents increased as a function of progressive increase in BCEO addition. The increase in thymoquinone contents possibly attributed to rich phytochemistry of BCEO. Thymoquinone as functional ingredient is present in the amounts of ~50% (Ramadan and Morsel, 2002; Al-Saleh *et al.*, 2006). Thymoquinone is active ingredient of black cumin and it is mainly concentrated in black cumin essential oil (Sultan *et al.*, 2009b). Presence of these important phytochemicals in whole seeds has been highlighted in two earlier studies; initially conducted by Ramadan and Morsel (2002) and later by Al-Saleh *et al.* (2006). They observed the concentration of thymoquinone (3098.5±1519.66 mg/kg) in black cumin seeds.

Cookies containing 0.30% BCEO (T_5) were rated ~6.90/9.0 considering the hedonic response. However, it is evident from the results that cookies with all formulations were quite acceptable. In many investigations, variations in sensory scores as a function of supplementation of oil and antioxidants and storage are supporting the present findings (Pasha *et al.*, 2002; Sharif *et al.*, 2005; Arshad *et al.*, 2007). Flavor and scores for color of cookies showed progressive increase with the addition of essential oil; the remaining sensory traits also followed the similar trend. BCEO is rich in phytochemicals that mainly account for its health promoting perspectives (Ramadan, 2007).

Conclusion: From the present exploration, it is deduced that black cumin essential oil is rich in thymoquinone. The products prepared with 0.30% black cumin essential oil showed better oxidative stability and performed better during sensorial appraisal. Overall, the bakery items like cookies are more useful in incorporation of BCEO as functional ingredient. Further studies are required to assess the therapeutic potential of BCEO, as results from such studies would be important for meticulousness of present findings.

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