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Utilization of black seeds meal for bakery products preparations.

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Abstract

Nigella sativa (Nigella sativa) was chosen as one of the natural herbal seeds because of its various medicinal and nutritional applications. Due to its effective medicinal properties. They can be used by replacing 2.5, 5, 7.5 and 10% proportions of wheat flour for preparing healthy bread and flatbread. The sensory evaluation data were statistically analyzed, and the results showed that the fried and flat bread that was replaced with 7.5% low-fat black seed powder had a slight significant difference in the comparison. The aim of this study is to benefit from the addition of black seed in bread products because it contains natural antioxidants and antibacterial that affect the quality of bread. The variables studied were organoleptic assessment (crust colour, crumb colour texture, taste, odour, general acceptability), resulting bread shelf life and flatness. The results indicated that the addition of black seed powder led to a slight increase in the fibre and protein content and had a slight effect on the fat and ash content. It also improved the strength. its durability. and freshness of fried and flat bread, during storage compared to control. Black bean meals also improved the aroma of fried bread and flatbread while affecting other sensory qualities. Fryer and flatbread samples contain more antioxidants than controls. Resulting in inhibition of mould growth and prolonged storage compared to the control. All sensory characteristics recorded the highest addition of 7.5% while the lowest was 10%. Total phenolic compounds increased in healthy flat bread containing 7.5% black seed powder, scoring 0.71 mg/g, followed by potato bread containing 7.5% black seed powder (0.57 mg/g). The shelf life of fried and flat bread increased as the highest values of bacterial and fungal numbers were detected in the control sample compared to the black bean treated samples (5 and 7.5%).

Key words.
Black seed meals, low fat, pan bread, flat bread

Introduction

Nigella sativa (N S) is an annual herbaceous plant in the Ranunculaceae family, called 'Black Seeds' in English and 'Al-Habba Al-Sauda' or '-Habit Al-Barakah' in Arabic. It's a global medicinal plant. It's grown in the Middle East, Mediterranean, Southern Europe, India, Pakistan, Syria, Saudi Arabia, and Turkey. Seeds and oil are used in folk medicine and food. (**Ahmed *et al.*, 2013; Razavi and Hosseinzadeh, 2014).**

Nigella sativa is used as a painkiller, anthelmintic, appetizer, carminative, sudorific, digestive, diuretic, emmenagogue, guaiacol, antifebrile, galactagogue and cathartic. **(Razavi and Hosseinzadeh, 2014)**

The *Nigella sativa* containing active materials known as nogelleone, thymoquinome, and thymohdroquinone that were shown to possess antimicrobial, antitoxic and pharmacological activities via increasing the defense mechanisms against infectious diseases **(Forouzanfar *et al.*, 2014).**

The *Nigella sativa* seeds, depending on the region, contain volatile oils (0.40%–0.45%) and non-volatile (32%–40%) oils, protein (16.00%–20.85%) carbohydrates (31.0%–33.9%), fiber (5.50–7.94%) as reported by **Thilakarathna *et al.*, (2018)**. It was, also, mention that the defatted black seeds meal can be developed further as a value-added product, and it can be used as a rich source of proteins and carbohydrates. Nigella seed cake is a useful by product of the black seed obtained after cold pressing which can be utilized in many fields. Its high content of protein and carbohydrates enhances the importance of the residue of black seed.

Black seeds meal (BSM) is a waste that produced from the oil processing industry of black seeds using the cold pressing method. It contains 8.1% water content, 23.3% crude protein and 9.6% ash **(Ali *et al*., 2012).** While alkaloids, tannins, saponins, minerals such as iron, calcium, potassium, magnesium, zinc and copper (1.79%– 3.44%), vitamin A and C, thiamine, niacin, pyridoxine and folate. **(Al-Mahasneh, *et al*., 2008; Salama, 2010; Sultan *et al.*, 2009).**

Defatted black seeds meal (BSM) contains some residual oils rich in bioactive components even after the oils from the seeds were extracted, in addition to some minerals, like Mg, Fe, Cu, Ca and K. (**Cheikh-Rouhou *et al*.,2007)**

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According to **Al-Okbi *et al*., (2015),** the major amino acids in black seed meal are arginine, histidine, and leucine, while the secondary amino acids are isoleucine and methionine.

The purpose of this study was to determine the effect of substituting a portion of wheat flour with black seeds meal in the preparation of pan and flat breads in order to improve the nutritional value, the antioxidant potential and the shelf life.

Materials and Methods

Materials

Wheat flours (82 % and 72 % extraction rates) were obtained from the South Cairo Mills Company, Cairo, Egypt.

Black seeds meal (BSM)was obtained from a local factory in Hurghada city where the oil was extracted using the cold pressing method from the black seeds. Then it was ground to a fine powder using a high-speed grinder. The other ingredients like sugar, salt, instant active dry yeast were procured from the local market in Giza.

Microorganisms: All strains were obtained from MERCN of Agriculture Department, Ain Shams University

Methods

1-Preparation of the black seed meals extract:

Ground black seeds meal was defatted through extraction with n-hexane in a Soxhlet apparatus at 80°C for 6 h following the procedure described in **Sen *et al.,* (2008).** Then 0.25 g was extracted using 5 ml of 80% ethanol for 1 h at room temperature in a shaker. The homogenate was then centrifuged at 2170 RCF for 30 min. the. The resulting supernatants were tested for total phenolic content and antioxidant activity.

2-Prepation of pan bread and flat bread.

The Pan bread was prepared by Wheat flour 72% according to the method described by **AACC (2010).** Other samples were prepared by replacing flour with different level of black meal (2.5,5,7.5 and 10%). Meanwhile, flat bread sample were prepared by wheat flour 82% according to **Yaseen *et al.,* (2007**) and were cooled at room temperature (25± 2.0 °C) for 60 min, then packed in polyethylene bags and stored for 10 days at room temperature (25± 2.0 °C).

3-Sample extraction of pan bread and flat bread

Sample extraction was carried out based on the method of (**Wahyono *et al*., 2020)** with minor modifications. Pan and flat breads were cut about 1 cm of thickness and then dried in the oven (Jeo-tech OF 21-E) at 40ºC for 24 hours. Then the dry bread then ground to a fine powder using a high-speed grinder. The mashed bread was extracted with 80% methanol with a ratio of 5 grams of bread and 12.5 ml of methanol. Then bread extract was put in a shaker for 2 hours at 37ºC.

4-Determination the chemical composition and bioactive components of raw materials, pan and flat breads

Moisture, ash, crude protein, crude fiber and ether extract were determined in the black seed meal, wheat flour and bread as described by the **AOAC (2012)**. Total carbohydrate was calculated by differences.

4-1 Determination of total phenols and total flavonoid contents:

Total phenolic contents of the extract were determined according to the method described by **Słowianek and Leszczyńska (2016).** Total flavonoid content of the extract was determined according to the method described by **Belwal *et al.,* (2016**).

4-2 Determination of antioxidant activity:

Antioxidant capacity of the samples were determined by the 1, 1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging method as described by **Brand-Williams *et al*., (1995).**

4-3 Identification of phenolic acids and flavonoid compounds using HPLC

The phenolic acid and flavonoid compounds of black seeds meal was extracted according to the method described by **Goupy *et al*., (1999) and Mattila *et al*., (2000).** HPLC analysis was performed using an Agilent 1200 chromatograph (Agilent Technologies Inc., USA) composed of column C18 Zorbax ODS (with particle size 5μm, 4.60mm × 250mm). Elutes were monitored using UV detector set at 280 nm for phenolic acids and 330 nm for flavonoids.

5- Sensory Evaluation:

Pan bread samples were evaluated for crust color, crumb color texture, taste, odor and overall acceptability using a 10-point unstructured hedonic scale by 10 panelists from Bread and Pastry Research Department, Food Technology Research Institute staff according to the method of **Stone and Sidel (1993).**

The flat bread was allowed to cool for about 1h before evaluation. Then loaves of bread, by using suggested blends, were evaluated for sensory characteristics (color, taste, Oder, separation of layers and appearance) by ten panelists from the staff of Bread and Pastry Research Department, Food Technology Research Institute, Agric. Res. Center, Giza. The scoring scheme was established as mentioned by **Yaseen *et al*., (2007)**

6- Texture Properties of Pan and Flat bread

The texture profile analysis for pan bread and flat bread were double compression test to penetrate to 50% depth, at 1 mm/s speed test. the shear force needed to cut the sample (different heights) with a flat knife probe 2,5 mm thickness was registered. all measurements were performed at ambient temperature (20°C). Hardness and chewiness were calculated from the TPA graphic.

7- Physical Properties of Pan and Flat bread

Bread loaf weight (g) was recorded after cooling for 1 h, dimerter (cm^3) was determined by rapeseeds displacement method as described by **AACC (2002).** While physical properties of pan were volume., Hight, weight and Specific volume (cm^3/g) of bread was calculated by dividing the volume by weight,

8- Water Activity (aw) of Pan and Flat bread

Water activity (a_w) of bread samples were tested by using Rotronic Hygrolab 3 instrument (Model CH-8303, Switzerland) as stated by **Shahidi *et al.*, (2008)**

9- Microbiology examination

a-Antimicrobial assay of black seeds meal

The microorganisms, Gram Positive (Bacillus cereus (ATCC 11778), *Staphylococcus aureus* (ATCC 13565), Gram negative (*E. coli* (RCMB010052), *Salmonella typhimurium* (RCMB 006) ATCC 14028, *Klebsiella pneumonia*, *Proteus vulgaricus* and yeast and fungi (*Candida albicans* RCMB 005003 (1), *Aspergilus fumigatus* (RCMB 002008), *Aspergillus niger*. Were uses to test antimicrobial action of black seeds meal extracts via microbial assay agar well diffusion method. At the end of the incubation period, the inhibition zones were measured (**Daoud *et al.*, 2019)**

b- Determined of total counts of bacteria, yeast and mold of pan bread and flat bread

Total mesophilic (total viable count and fungi count (yeast and mold count) was carried out on the bread samples during the storage period (analysis was carried out on a day interval i.e 0, 2, 4, 6 and 8th day) to determine the microbial load of the samples as described by **APHA, (1992).** Bread samples were prepared by mashing and mixing in peptone water. Sub-samples were decimally diluted and spread plated. 0.1 milliliter aliquots were spread on nutrient agar (Oxoid) and incubated at 37° C for 24 hrs. The yeast and mold counts were determined by plating one milliliter of the aliquot on potato dextrose agar (Oxoid) and incubating the plates at 28° C for 48 hrs.

10-Statistical analysis

The statistical analysis was carried out using ANOVA with one factor under a significance level of 0.05. Data were treated as a complete randomization design to **Steel *et al*., (1997).**

Results and Discussion

Chemical composition and bioactive components of raw materials:

The chemical composition of wheat flour. 72%, 82% ext. and black seeds meal on dry weight basis is shown in Table 1. From these data, it could be noticed that black seeds meal had the highest values in crude protein (31.44%), ether extract (10.55%), and ash content (9.53%) compared with wheat flour 72%, 82% ext rate. These results agreed with **Ahmed *et al.*, 2018**. It was found, also, that wheat flour (82% extraction) had higher contents of protein (11.75%); fiber (1.20%) and ash (1.15%) than that of 72% extraction wheat flour which contained 10.82%, 0.66% and 0.54 %, respectively. These results were in agreement with those made by other researchers (**Khorshid *et al*., 2011).**

From the same Table the total phenolic content of the black seed meal was determined as mg gallic acid/ g in dried black seed meal. The total polyphenol content was 4.8 mg/g. This result is in agreement with the results of **Chauhan *et al*. (2017)** who found that the total phenolic contents of black seeds extract using different solvents ranged from 4.4 to 7.4 mg /g as gallic acid of seeds powder. Meanwhile, the total flavonoids content of the black seed meals was 8.92 mg/g as catechin of black seeds meal. The present result is, also, in line with the finding of **Radwan *et al.*, (2017).** Who reported that flavonoids content in black seeds was 9.85 mg/g

The black seed extract's radical-scavenging capacity is indicated in Table. (1). Results show that the black seed meal extract had the high value of antioxidant activity (83.17%) which is in direct proportion with the total amount of phenolic and flavonoid in the black seeds.

Table 1: Chemical Composition and Bioactive component of Raw materials (g/100gm).

Contents	Wheat flour (72% ext.)	Wheat flour (82% ext.)	Black seeds meal (BSM)
Moisture	10.76 ^{b} ±1.21	10.90 ^{b} ±1.03	18.45 ^{a} ±3.75
Protein	10.82 ^{c} ±0.55	11.75 ^{b} ±0.63	31.44 ^{a} ±4.85
Ether extract	1.01 ^{cb} ±0.23	1.17 ^{b} ±0.15	10.55 ^{a} ±1.59
Fiber	0.66 ^{c} ±0.01	1.20 ^{b} ±0.51	14.22 ^{a} ±2.43
Ash	0.54 ^{c} ±0.03	1.15 ^{b} ±0.20	9.53 ^{a} ±1.95
Carbohydrate*	76.21 ^{a} ±2.90	73.83 ^{b} ±2.87	15.81 ^{c} ±3.51
Bioactive component			
Total phenolic (mg/g)	0.65 ^{c} ±0.02	0.71 ^{b} ±0.01	4.8 ^{a} ±0.34
Total Flavonoid(mg/g)	0.076 ^{c} ±0.03	0.092 ^{b} ±0.02	8.92 ^{a} ±3.90
antioxidant activity (%)	48.4 ^{c} ±2.87	52.7 ^{b} ±2.62	83.17 ^{a} ±3.34

*Total carbohydrates were calculated by differences

Each mean value is followed by its ± means within a raw containing the same letters are not substantially different at 0.05level.

Identification of phenolic and flavonoid compounds for black seeds meal.

Phenolic and flavonoid compounds of black seeds meal (mg/100g dried sample) were identified by HPLC. Results in Table (2) show the identification and quantitative analysis of phenolic compounds of the black seeds meal. In particular, the predominant phenolic compounds in the black seed meal are p-hydroxybenzoic, Coumarin and gallic acid with values of 81.553, 31.444 and 29.415 mg/100g in dried sample respectively**.** Meanwhile, Caffeine, Caffeic and 4-Aminobenzoic were found in small amounts. The current results are in agreement with the results of **Mariod *et al*., (2009)** who mention that HPLC–DAD identified the most predominant phenolic compounds in the sample of crude methanolic extract of *Nigella sativa L.* cake and in fractions using water are p-hydroxybenzoic, syringic and p-coumaric acids. In addition, **Mechraoui *et al*., (2018)** found the presence of the important phenolic compound in black seeds meal are gallic acid, kaempferol, rutin, apigenin, naringenin and quercetin, and the absence of other compound(catechin, vanillic acid, epicatechin and coumaric acid).

Results in Table (2), also, demonstrated identification and quantitative analysis of flavonoid of black seeds meal. The highest content of Naringin, hesperdine and Quercetrin were 20.70641 ,23.43962 and 15.37302 mg/100g d.w., respectively.

Table 2: Identification of phenolic and flavonoid compounds for black seeds meal (mg/100g dried sample).

Phenolic compound (mg/100g)	
Pyrogallol	19.12087
Gallic	29.41564
3-OH Tyrosol	4.205932
Catechol	1.288527
4-Aminobenzoic	0.816472
Catechein	3.69891
Chlorogenic	16.77348
Oleuropin	2.036879
Caffeic	0.815177
Vanillic	7.052397
Caffeine	0.270715
Ferulic	5.961348
Coumarin	31.44494
Salycillic	3.229525
P-OH- benzoic	81.55388
Ellagic	27.09383
Flavonoid compound (mg/100g)	
Apiening 6-rhamnose 8-glucose	1.146103
Rutin	2.276149
Naringin	20.70641
Hesperdine	23.43962
Rosmarinic	5.905049
Quercetrin	15.37302
Naringenin	2.201647
Kampferol	2.276831
Apigenin	1.354479

Sensory evaluation of Pan and flat bread:

The results outcomes of the sensory evaluation of pan bread prepared with varying amounts of black seeds meal were illustrated in Table (3). The results indicated that the control pan bread sample recorded the highest score for all attributes in relative to the other pan bread samples. Pan bread samples prepared with different levels (2.5, 5, and 7.5%) of black seeds meal were significantly different (p>0.05) with control bread. Meanwhile, pan bread with 10% black seeds meal recorded the least score for all parameters.

Table (3) Sensory Evaluation of Pan Bread fortified with black seeds meal (BSM)

Pan bread	Crust color	Taste	Oder	Texture	Crumb color
Control	19.7 ± 0.20 [a]	19.7± 0.10[a]	19.8 ±0.10[a]	19.6 ±0.10[a]	19.7 ± 0.10[a]
2.5% (BSM)	18.5 ± 0.20[c]	19.0 ± 0.19[c]	19.4 ±0.19[b]	19.5 ± 0.20[a]	19.5 ± 0.20 [b]
5% (BSM)	18.7 ± 0.20[c]	19.5 ± 0.10[b]	19.5 ± 0.10[b]	19.0 ± 0.10[b]	19.5 ± 0.10 [b]
7.5% (BSM)	18.9 ± 0.41 [b]	19.0 ±0.10[c]	19.5 ± 0.10[b]	19.2 ±0.20[b]	19.5 ± 0.10 [b]
10% (BSM)	14.0 ± 0.50[d]	15.0 ± 0.2[d]	15.0 ±0.19[c]	17.0 ± 0.10[c]	16.0 ± 0.10 [c]

*Means of each value, within the same column, followed by the same letter are not significantly different at 0.05 levels.
*Each value is followed by its standard deviation.

Table (4) shows that increasing the amount of black seeds meal has a significant impact on all of the sensory properties. This can be attributed to the dark color of the black seeds powder. These results agreed with the work of **Pawase and Veer, (2020)** who reported a decrease in overall acceptability of cookies with the increase of black seeds meal levels

Table (4) Sensory Evaluation of flat Bread fortified with black seeds meal (BSM)

Flat bread	Color	Taste	Oder	Separation	Appearance
Control	19.5 ± 0.02 [a]	19.8 ± 0.1 [a]	19.6 ±0.05[a]	19.6 ± 0.19 [a]	19.6 ± 0.1 [a]
2.5% (BSM)	19.2 ±0.25[a]	19.2 ±0.25 [b]	19.5 ± 0.2[a]	19.4 ±0.15 [a]	19.2 ±0.19 c
5% (BSM)	19.4±0.19 [a]	19.3 ±0.10[b]	19.6 ±0.09 [a]	19.4 ± 0.10[a]	19.4 ± 0.09 [b]
7.5% (BSM)	19.2±0.20[a]	19.2 ±0.3[b]	19.6 ± 0.05[a]	19.4 ±0.10 [a]	19.2 ±0.10 [c]
10% (BSM)	16.9 ± 0.10 [b]	17.6 ±0.10[c]	17.5 ±0.10[b]	18.0 ±0.20 [b]	18.0 ± 0.05 [d]

*Means of each value, within the same column, followed by the same letter are not significantly different at 0.05 level.*Each value is followed by its standard deviation.

Table (4) showed the sensory properties of flat bread which were prepared from 82% flour with different levels of black seeds meal. Flat bread were expectable until 7.5% addition level. The data concur with those of Osman et al., (2015), who clarified that the bread was much less acceptable than the control. They attributed this to the dark color of the black seeds powder. From the obtained data (Table 3 and 4), it could be seen that higher level of black seeds meal 10% was not acceptable in both pan and flat bread, so it was eliminated from further coming studies

Chemical Composition of produced pan and flat bread

The chemical composition of pan bread samples with various levels of black seeds meal is shown Table (5). It could be observed that control pan bread content of, protein, ether extract, ash, crude fiber and total carbohydrates were10.64, 1.61, 1.32, 1.42 and 84.69 respecti8vely. Regarding the chemical composition of produced pan bread with 2.5, 5 and 7.5% substitution of wheat flour, upon the addition of 2.5% black seeds protein reached 11.49, 1.73, 1.42, 1.73 and 83.66 Meanwhile, the addition of 7.5% black seeds meal, resulted in that protein, ether extract, ash, crude fiber and total carbohydrates reached 12.34,1.91,1.62, 2.31 and 81.82 respectively. In general, it could be noticed that protein, ether extract, ash, and crud Fiber content of pan bread samples was significantly increased with increase black seeds meal due to the corresponding higher content of chemical composition of black seeds meal, meanwhile the total carbohydrate decreased due to its lower content in black seed meal. These findings are in line with those obtained by **Khalil *et al.*, 2021**

Table 5: Chemical Composition of Pan and flat Bread on dry weight basis (g/100gm)

Sample	Protein	Ether extract	Ash	Fiber	Carbohydrate
Pan Bread					
Control	10.64d ±0.1	1.61d ± 0.15	1.32d ± 0.02	1.42d ±0.09	84.69a ± 0.36
2.5% (BSM)	11.49c ± 0.14	1.73c ± 0.02	1.42c ± 0.01	1.73c ± 0.02	83.66b ± 0.21
5% (BSM)	11.95b ± 0.03	1.84b ± 0.02	1.54b ± 0.02	2.09b ±0.04	82.60c± 0.10
7.5% (BSM)	12.34a ± 0.14	1.91a ± 0.01	1.62a ± 0.04	2.31a ±0.01	81.82d ± 0.11
Flat Bread					
Control	11.65d ± 0.02	1.63d ± 0.02	1.54d ± 0.01	1.70d ± 0.01	83.47a ± 0.06
2.5% (BSM)	12.04c ± 0.01	1.76c ± 0.01	1.81c ± 0.009	2.0c ± 0.01	82.38b ± 0.03
5% (BSM)	12.67b ± 0.02	2.08b ± 0.01	2.21b ± 0.009	2.54b ± 0.01	80.49c ±0.03
7.5% (BSM)	13.09a ± 0.08	2.50a ± 0.01	2.75a ± 0.009	2.94a ± 0.01	78.60d ± 0.01

*Means of each value, within the same column for individual product, followed by the same letter are not significantly different at 0.05 levels.*Each value is followed by its ± standard deviation

From the obtained results in Table (5), It could be observed that the chemical composition of control flat bread contents of was protein, ether extract ash crude fiber and total carbohydrates were 11.65,1.63, 1.54,1.70, and 83.47% respectively. Regarding the chemical composition of flat bread with 2.5, 5 and 7.5% substitution of wheat flour ,upon the addition of 2.5% black seeds protein, ether extract, ash, crude fiber and total carbohydrates reached 12.04, 1.76, 1.81, 2.01 82.38% respectively. Meanwhile, the addition of 7.5% black seeds resulted in that protein reached ether extract, ash, crude fiber and total carbohydrates contents 13.09, 2.50, 2.75, 2.94, 78.60% respectively. In general, it could be noticed that all chemical composition except the total carbohydrate increased with the increase levels of black seeds meal. These findings are in line with those obtained by **Osman *et al.*, (2015).**

Total phenolic, total flavonoid contents and antioxidant activity of black seeds meal fortified bread.

Phenolics compounds have shown a wide scope of cumulative biological impacts including anti-inflammatory, antibacterial, vasodilator actions, anticarcinogenic, antiviral, antithrombotic, anti allergic, and hepatoprotective effects (**Haggag *et al*., 2011 and Mohsen and Ammar 2009).**

Data in Table (6). show the total phenolic content in pan and flat bread prepared from wheat flour with partially substituted by black seeds meal powder at different levels. It could be observed that the phenolic and total flavonoid content and antioxidant activity in all treatments were higher than control and the phenolic content in Flat bread was higher than in pan bread at the same levels of substituted with black seeds meal.

Table (6) Total phenolic, total flavonoid contents and Antioxidant activity of pan and flat bread

Sample	Total phenolic mg/g	Total flavonoid mg/g	Antioxidant activity %
	Pan bread		
Control	0.24d ±0.01	0.75d±0.01	58.94d±0.01
2.5% (BSM)	0.37c ±0.02	0.93c±0.01	63.44c ±0.02
5% (BSM)	0.41b ±0.01	1.21b±0.02	66.49b ±0.00
7.5% (BSM)	0.57a ±0.00	1.44a±0.02	69.42a ±0.01
Flat bread			
Control	0.27d ±0.01	0.82d ±0.01	60.29d±0.01
2.5% (BSM)	0.44c ±0.02	1.06c ±0.01	64.96c ±0.01
5% (BSM)	0.67b ±0.01	1.49b ±0.03	69.23b ±0.01
7.5% (BSM)	0.71a±0.02	1.87a ±0.03	71.90a ±0.01

*Means of each value, within the same column for individual product, followed by the same letter are not significantly different at 0.05 level.*Each value is followed by its ± standard deviation

The value of phenolic compounds was high in flat bread prepared by 7.5% black seeds meal, that recorded 0.71 mg/g followed by pan bread with 7.5% black seeds meal 0.57 mg/g. Meanwhile, the less amount of phenolic compound was in pan bread with 2.5% black seeds meal 0.37 mg/g then flat bread with 2.5% black seeds meal 0.44 mg/g. On the other hand, flavonoid contents of the different prepared bread was slightly significant difference, The highest value of flavonoid was in flat bread with 7.5% black seeds meal that recorded 1.87 mg/g followed by pan bread with 7.5% black seeds meal 1.44 mg/g. While the lowest amount of flavonoid was in pan bread with 2.5% black seeds meal 63.17 mg/g then flat bread with 2.5% black seeds meal 64.78 mg/g.

The substituted In the same context, DPPH test is a frequent assay used to evaluate the radical-scavenging potential of a sample including black seeds meal. Usually, high levels of antioxidant potential 69.42 of DPPH in in pan bread at the 7.5 of substituted with black seeds meal.DPPH test is assay used to evaluate the radical-scavenging capacity. In the same context, high levels of antioxidant capacity fond in flat bread and pan bread prepared with 7.5 of black seeds meal 71.90 and 69.42 respectively, which is in direct proportion with the total phenolic content.

Results obtained confirm the possibility of utilizing the black seed powder in increased antioxidant activities. The recipe for bread with concentrations of black seed meal made bread useful because it is rich in active substances. and this is confirmed with **Różyło *et al* 2021**

Zaky *et al.*, (2021) found that antioxidants play an essential role in reducing oxidative reactions in both dietary models and the human body, especially in systems dependent on fatty foods

Physical Properties of Pan and flat breads prepared addition of black seeds meal

Table (7) Physical Properties of Pan Bread

Sample	Volume (cm^3)	Hight (cm)	Weight (gm)	Specific Volume (cm^3/gm)
Control	450 ^{a} ±0.25	10.0 ^{a} ±0.05	120.0 ^{d} ±0.11	3.75 ^{a} ±0.05
2.5% (BSM)	444 ^{b} ±1.22	9.25 ^{b} ±0.03	124.5 ^{c} ±0.13	3.56 ^{b} ±0.02
5% (BSM)	435 ^{c} ±2.27	8.50 ^{c} ±0.06	130.0 ^{b} ±0.15	3.34 ^{c} ±0.06
7.5% (BSM)	428 ^{d} ±1.22	6.00 ^{d} ±0.07	135.5 ^{a} ±0.17	3.15 ^{d} ±0.04

*Means of each value, within the same column for individual product, followed by the same letter are not significantly different at 0.05 level.*Each value is followed by its ± standard deviation

Data from table 7 showed that the volume and height of pan bread were 450 cm^3 and 10 cm for control and with the addition of black seed the volume decrease reaching 428 and 6 for pan bread with 7.5% black seed. It was also noticed, from data in Table (7), that the height of pan bread with the addition of different levels of black seeds was considerably ($P \leq 0.05$) less than those prepared from wheat flour, while the weight of pan bread was greater in bread made with the addition of varying amounts black seeds. The specific volume of the pan bread made from wheat flour (control) was the highest (3.75 cm^3/gm), whereas the bread made with 7.5% black seeds was the lowest in terms of specific volume (3.15 cm^3/gm). **Afiukwa *et al.*,1997.**

Table (8) Physical Properties of Flat Bread

Sample	Weight (gm)	Dimeter (cm)	Moisture%
Control	125 c ±0.11	21.61b±0.11	30.2 d±0.02
2.5% (BSM)	126 b ±0.15	21.70a ±0.11	32 b ±0.03
5% (BSM)	127 a ±0.10	21.74a±0.11	33 a ±0.07
7.5% (BSM)	125 c ±0.19	21.79a±0.11	31 c ±0.04

*Means of each value, within the same column for individual product, followed by the same letter are not significantly different at 0.05 level.*Each value is followed by its ± standard deviation

Data from table 8 showed that the weight of flat bread was 125 gm for control and with the addition of black seed the weight increase reaching 127 gm for flat bread with 7.5% black seed. The same is true dimeter. The highest moisture content and weight of bread which prepared from black seed was 31%and 125g respectively. The data showed gradually increase in moisture and weight which is associated with the increase of fiber this increase may be due to absorption more water than control these results agreement with that found by **Sozer,2009**.and help to improve functional properties

Texture Analysis of Pan and flat bread

The hardness of pan bread prepared from wheat flour with black seeds meal was significantly similar to control up to 7.5%. Loaves were slightly harder than control, at 7.5%. These results agreed with **Sogi *et al.*, 2002,** who revealed a significant increase with increasing in the levels of defatted seeds meal bread. While, the hardness of flat was gradually increased with increasing of substitution levels of black seeds meal **(Islam *et al.*, 2015)** .Chewiness is one of the texture parameters easily correlated with sensory analyses through trained panels **(Esteller *et al.*, 2004)** The chewiness values had a similar trend of hardness. These results agree with those obtained by **Ibrahim (2011)** who reported that chewiness are parameters dependent on hardness.

Table(9) Texture Analysis of Pan and flat bread

	Hardness (N)				Chewiness (mJ)			
Sample	**Zero**	**24h**	**48h**	**72h**	**Zero**	**24h**	**48h**	**72h**
Pan Bread								
Control	9.90±0.02[a]	10.46±0.14[c]	11.53±0.03[b]	12.92±0.04[a]	3.55±0.01[a]	2.80±0.40[a]	1.50±0.07[a]	0.44±0.04[a]
2.5%(BSM)	9.92±0.02[a]	10.56±0.05[b]	11.67±0.20[b]	12.94±0.01[a]	3.54±0.04[a]	2.53±0.01[a]	1.44±0.04[a]	0.40±0.02[a]
5% (BSM)	9.95±0.03[a]	10.90±0.06[a]	11.90±0.02[a]	12.97±0.03[a]	3.50±0.03[a]	2.50±0.01[b]	1.35±0.04[b]	0.37±0.02[b]
7.5% (BSM)	9.98±0.03[a]	10.32±0.02[c]	11.98±0.21[a]	12.99±0.05[a]	3.47±0.09[a]	2.34±0.03[b]	1.20±0.22[c]	0.33±0.07[c]
Flat bread								
Control	13.58±0.28[d]	15.46±0.29[d]	18.53±0.02[d]	20.92±0.01[d]	27.55±0.05[a]	25.80±0.06[a]	23.50±0.06[a]	18.44±0.05[a]
2.5%(BSM)	13.67±0.01[c]	15.56±0.03[c]	18.67±0.03[c]	20.94±0.00[c]	27.54±0.03[a]	25.53±0.05[a]	23.44±0.08[b]	18.40±0.09[a]
5% (BSM)	13.88±0.02[b]	15.90±0.02[b]	18.90±0.01[b]	20.97±0.02[b]	27.50±0.03[b]	25.50±0.06[a]	23.35±0.05[b]	18.37±0.02[a]
7.5% (BSM)	13.94±0.01[a]	16.32±0.04[a]	18.98±0.05[a]	20.99±0.01[a]	27.47±0.02[b]	25.34±0.04[b]	23.20±0.02[c]	18.33±0.03[b]

Values are means ±SD(n=3),mean number in the same column for individual product bearing different superscript letter are significantly different at p≤5

Water Activity aw of Pan and flat Bread Produced from Wheat Flour and Different Levels of Black Seeds meal

The water activity level in food is of practical importance as it controls the onset and severity of mold spoilage. It is commonly observed that foods most likely to show rapid deterioration, due to biological and chemical changes are usually those with high water content **(Abdullaha et al., 2000).** While **Fellows, (2000)** reported that almost all microbial activity is inhibited below a^w (0.6), most fungi are inhibited below a^w (0.7), most yeasts are inhibited below a^w (0.9).During the storage period of food, the water activity affects the growth of microorganisms. Reduction of aw often affects the microbial growth, therefore, it may increase the shelf life of food as a result of reduced availability of water **(Eskin and Robinson, 2001)**

Water activity of pan and flat bread are reported in Table (9). the degrees of water activity (a^w)during the storage of bread for 72 hours under the effect of different concentrations of black seed meal .The results revealed that the water activity (a^w) decreased by increasing addition with 2.5, 5 and 7.5% respectively during the storage period for 72 hours. The results of this study showed that the water activity (a^w) of pan bread samples tested decreased when the level of black seed meal increased. The lowest value of (a^w) was 0.710 with the pan bread samples 7.5% as compared to 0.989 in the control after 72 hour of storage.

Table (9) Water Activity (aw) of Pan and flat Bread Produced with Black Seeds meal

Treatment	Zero time	24h	48 h	72 h
Pan Bread				
Control	0.947 [c] ±0.02	0.963 [a] ±0.03	0.971 [a] ±0.01	0.989 [a] ±0.01
2.5% (BSM)	0.956 [b] ±0.00	0.859 [c] ±0.02	0.822 [d] ±0.02	0.802 [c] ±0.00
5% (BSM)	0.996 [a] ±0.02	0.935 [b] ±0.02	0.892 [b] ±0.02	0.810 [b] ±0.01
7.5% (BSM)	0.924 [d] ±0.02	0.890 [d] ±0.01	0.838 [c] ±0.01	0.710 [d] ±0.01
Flat Bread				
Control	0.950 [c] ±0.01	0.965 [a] ±0.01	0.972 [a] ±0.02	0.992 [a] ±0.03
2.5% (BSM)	0.967 [a] ±0.02	0.833 [c] ±0.01	0.832 [b] ±0.01	0.800 [b] ±0.01
5% (BSM)	0.953 [b] ±0.02	0.822 [d] ±0.01	0.811 [d] ±0.01	0.788 [c] ±0.01
7.5% (BSM)	0.947 [d] ±0.01	0.851 [b] ±0.02	0.820 [c] ±0.01	0.721 [d] ±0.02

*Means of each value, within the same column for individual product, followed by the same letter are not significantly different at 0.05 levels.*Each value is followed by its ± standard deviation

Microorganisms have an absolute demand of water, for growth can occur. The exact amount of water needed for growth of microorganisms varies. This water requirement is best expressed in terms of available water or water activity (a^w). Each microorganism has maximal, optimal and minimal aw for growth **(Rizk et al., 2000).** After 72 hours of storage

While the water activity , of flat bread samples control at zero time increased from 0.950 to 0.992 . Reduction of water activity may affect the microbial growth in food, in turn; the shelf-life of bread may be increased The lowest value of (a^w) was 0.721 with the pan bread samples 7.5% as compared to 0.992 in the control after 72 hour of storage..

Microbiological examination

A-Antimicrobial assay of black seeds meal:

The antimicrobial properties of black seeds meal aqueous extracts at a concentration of 40% with different volumes (20, 40, 60 and 100 µL) were determined against the selected bacterial and fungi species and shown in Table (10). The results revealed that aqueous extract exhibited a potent inhibitory effect against all the tested bacterial and fungi species and the zone inhibition (mm) increased with raising volume intake. Moreover, aqueous extract of black seeds meal showed stronger antibacterial activity against studied Gram-positive than Gram-negative which recorded the highest inhibition zone against *S. aureus* (21 mm) followed by *B. Ceruse* (20 mm) for 100 µL. Meanwhile, among Gram-negative bacteria, the aqueous extract showed strong activity against *P. vulgaricus* (19 mm) followed by *K. pneumonia and E. coli* for 100 µL. Gram-negative bacteria are more resistant to external agents than Gram-positive bacteria. This is due to the presence of lipopolysaccharides in their outer membranes, which inhibit the penetration of amphiphatic chemicals and multidrug resistance pumps that release toxins over this barrier. antibiotics, detergents and hydrophilic dyes (**Suresh Kumar *et al.*, 2010).**

Table 10: Antimicrobial activity of black seeds meal aqueous extracts.

Concentration (µl/well)	20	40	60	100
	Diameter zone of inhibition in mm			
Bacteria strain				
***Escherichia coli* (RCMB 010052) ATCC 25955**	5	6	9	12
Staphylococcus aureus(ATCC 13565),	2	8	13	21
Klebsiella pneumonia	8	10	11	14
Proteus vulgaricus	3	13	17	19
Salmonella typh (RCMB 006) ATCC 14028	5	7	8	10
***Bacillus ceruse* (ATCC 11778)**	4	9	13	20
Yeast and mold				
Aspergillus niger	2	13	16	20
Aspergillus fumigatus (RCMB 002008)	11	12	14	19
Candida albicanes RCMB 005003 (ATCC 10231)	4	17	22	35

B-Total count of bacteria, yeast and mold of pan bread and flat bread.

In the present work, the microbiological growth in forms of total bacteria (TC) and mold & yeasts (M&Y) counts (log cfu/g) were estimated in both flat and pan bread substituted the wheat flour with black seeds meal powder levels (2.5, 5 and 7.5 %) compared with control during storage periods at room temperatures for eight days as well as the rate of microbial growth at zero time and every two days. The obtained results are listed in Fig (1 &2).

Data reveal that flat and pan bread have no microbial growth in the control sample and other selected ones (2.5, 5 and 7.5%) at zero time. On the other hand, total bacterial and yeast & mold counts increased from 2.95 to 5.90 log cfu/g and 1.48 to 4.86 log cfu/g for flat bread and 1.92 to 5.62 log cfu/g and 1.60 to 4.5 log cfu/g for pan bread by increasing the storage period from two to six and eight days, while no microbial growth was detected in all treatments of flat bread after two days reached maximum to 4.63, 2.85 and 1.48 log cfu/g at 2.5, 5 and 7.5% in the sixth day for the total bacterial count. Meanwhile, total yeast & mold counts reached a maximum (3.56 log cfu/g) at 2.5% while no microbial growth was found during the storage period for 5 and 7.5% of black seeds meal powder after the sixth days.

Similar results were observed in pan bread during the storage period for eight days. The pan bread has no detection of bacteria, yeast and mold growth after two and four days for 5% of black seeds meal and till 6 days for 7.5% of black seeds meal addition. As well as, the total bacterial count arrived maximum after eight days (4.39, 3.41 and 1.95 log cfu/g, respectively) while it reached 2.95, 1.85 and 1.48 log cfu/g at 2.5, 5 and 7.5% addition. These results showed that the shelf life of pan bread increased where the

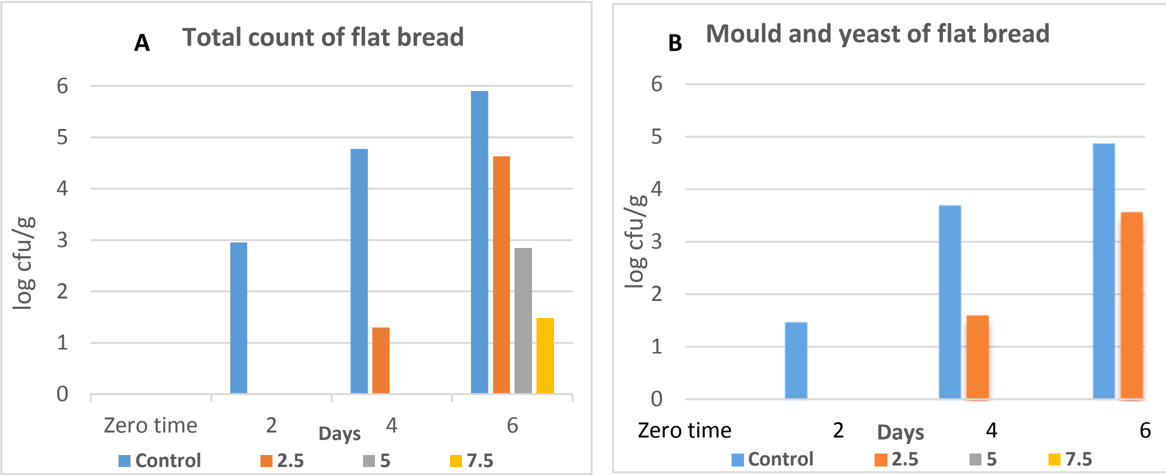

Fig (1) A :Total count of flat bread

B: mold and yeast of flat bread.

Fig (2) A: Total count of pan bread

B: mold and yeast of pan bread

Conclusion

This study aimed to make use of Nigella sativa (Nigella sativa) as a by-product in the preparation of baladi bread and flat bread. loaves. Different levels of significant effects on physical and sensory structures Characteristics of loaves of local bread. Committee members decided The best levels for baking are added from 2.5 to 7.5% of the black bean meal. Fryer and flatbread samples contain more antioxidants than the controls. The shelf life of fried and flat bread increased as the highest values of bacterial and fungal numbers were detected in the comparison sample compared to the black bean treated samples (5 and 7.5%). These loaves of bread are excellent An option for diabetics and hyperlipidemia due to They are rich in fiber, phenols and antioxidants. The black bean meal still needs further research Effect of black seed meal as anti-nutritional agents and how For use in many other functional products such as Such as cakes, drinks, pizza and bakery products

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