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UTILIZATION OF DEFATTED BLACK CUMIN AND ITS EXTRACT AS NATURAL ANTIOXIDANTS TO KEEPING QUALITY OF CAKE DURING STORAGE

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Abstract

Egyptian black cumin *(Nigella sativa L.,)* (balady) seeds and its defatted meal were analyzed. Crude protein, crude fat, crude fiber, ash and total carbohydrates were 22.37, 35.42 , 8.25, 4.53 and 29.43 % , respectively, in the seeds and 32.30, 4.26, 9.75, 5.82 and 47.87 %, respectively, in the defatted meal. Amino acid profile of defatted meal revealed that the major amino acid is glutamic acid 19.75 g / 100g protein. The essential amino acids content represent 39.15 g/ 100g protein and leucine 7.13 g/ 100g protein is the dominant amino acid.

Defatted black cumin meal was treated by ethanol (70%) followed by acetone and ethyl acetate to extract flavonoid and phenolic compounds as natural antioxidant. Flavonoid (kaempherol, quercetin. myricetin, rutine, cosmosiin and astragelin) and phenolic (ellagic and *p* – cumaric acids) were isolated and identified by paper chromatographic technique. Natural and synthetic (BHT) antioxidants were added separately to cake at 100,200 and 300 ppm levels. Its defatted meal was added at the levels of 5, 10 and 15%. Cakes were stored at room temperature and every two days, peroxide value was determined in the extracted lipids.

The results showed that the natural and synthetic antioxidants at 200 and 300 ppm levels retarded the oxidation of lipids during storage. Also, its defatted meal at level 15% was effective. Whereas, the sensory evaluation showed that cakes containing natural antioxidant had higher panel scores. The defatted meal at 5% level gave the best color and at 10 and 15% levels gave the best cake taste.

INTRODUCTION

Black cumin seeds *(Nigella sativa L.,)* is a herbaceous plant which is a member of the Ranunculacea family. It is known as nigella or black cumin. Black cumin is widely distributed in countries boarding the Mediterranean sea, Middle Europe and Western Asia. Black cumin is a small seed, black and possesses aromatic odor and taste as well as it is cultivated in Egypt (Salama, 1973). Babayan *et al.*, (1978), mentioned that these seeds are used by Egyptians as a diuretic, carminative, flavoring agent by Syrians for cheese flavoring and by the Americans for bakery products and cookies. The seeds are

digestive stimulants as well as carminative, aromatic, diuretic, diaphonetic, stomachic, anthelmintic, ashmatic.

El–Malt *et al.*, (1998) indicated that black cumin seeds are rich in crude fat (38.78 %), crude protein (26.25%) followed by carbohydrate (24.0%). Total soluble sugars, reducing sugars and non – reducing sugars were found to be 6.26 , 1.5 and 4.76 % ,respectively. Amino acid profile of black cumin seed revealed that the major amino acids were glutamic acid (18.41 g/100g protein) followed by aspartic acid (7.95 g/100g protein). The essential amio acids content represents about 38.05 g / 100g protein. The dominant essential amino acids were leucine (7.55%) followed by valline, tyrosine, isolucine and methionine (5.11, 4.57 and 4.16 g / 100 g protein, respectively). Also, El – Malky and Kerolles, (2000) analyzed the Egyptian (local and imported) black cumin seeds and its defatted meal for chemical composition. Protein, fat, carbohydrates, fiber and ash in local seeds were 21.44, 36.22, 22.00, 15.82 and 4.55%, respectively. Whereas, in imported seeds they were 20.55, 35.38, 26.28, 11.67 and 4.11%, respectively. On the other hand, in defatted local seeds the same components were 28.34, 14.5, 40.12, 12.54 and 4.87%, respectively. Also, the defatted imported seeds they were 29.65, 13.86, 42.15, 12.88 and 4.95 %, respectively.

Three new flavonoides glycosides quercetin and kaempferol triglycosidel, quercetin 3- glucoside, kaempferol – 3 glucoside and rutin were isolated and identified from seeds of black cumin (Merfort *et al.*, 1997). Flavonoids are potent antioxidants with free radical scavengers and metal chelators and inhibit lipid peroxidation. The structure requirements for antioxidants and free radical scarvening functions of flavonoids include a hydroxyl group in carbon number four and polyhydroxylation of the A and B aromatic rings (Giese, 1996). Overall antioxidant effect of flavonoids on lipid peroxidation may be related to their hydroxy group and oxygen ion scavenging properties and their reaction with peroxy radicals (Zhishen *et al.*, 1999).

The aim of this investigation was to study the chemical composition and amino acid from defatted black cumin. Also, isolation and identification of flavonoids from defatted black cumin and utilization of the flavonoids and the defatted meal as natural antioxidant to prevent lipids peroxidation in cakes.

MATERIALS AND METHODS

Materials :

Nigella sativa L. seeds (balady) were obtained from local herb grocery. It was finely ground and oil extracted with n-hexan, the residue meal (cake) was dried. Morever, wheat flour, 72% extraction was obtained from South Cairo Mills Co. Also, French butter, vanillia and baking powder were purchased from local market.

Butylated hydroxytoluene (BHT) was obtained from Naarden International Company, Holland. Whereas, the synthetic flavonoid and phenolic compounds were purchased from Sigma Chemical Company, Deisenhofen, Germany.

Methods :

Chemical analysis of black cumin seeds and defatted including crude protein, crude fat, ash , crude fiber and total carbohydrates were determined according to A.O.A.C (1990). While the nitrogen free extract was determined as (% total carbohydrate - % crude fiber). Amino acids of black cumin whole meal cake were determined according to the procedure described by Olson *et al.*, (1978) and amino acids chemical score was calculated according to FAO / WHO Scoring Pattern (1990).

Extraction of flavonoids and phenolic compounds from black cumin seeds.

The seeds were washed, dried and ground in a Willy mill to a fine powder. The powder was immersed in chloroform to remove waxy and resinous materials. The residue was macerated in n–hexane to remove oil and then dried. The dried defatted residue was exhaustively extracted several times with 70% aqueous ethanol till the extract was colorless and the extracts were combined. A brown product was concentrated and the concentrated aqueous ethanolic extract was subjected to fractional extraction using acetone followed by ethyl acetate and kept for flavonoids investigation according to Mabry *et al.*, (1970).

Identification of flavonoid and phenolic compounds.

Acetone and ethyl acetate extracts of nigella sativa were tested by paper chromatographic technique in order to identify the major flavonoid and phenolic compounds

as described by Markham and Mabry (1968). The black cumin extracts and authantic samples were spotled on one dimensional Whatman No.,1 paper chromatography. The eluting solvents were butanal: acetic acid: water (4:1:5) and acetic acid (ACOH) 15%. The different spots (major flavonoid, phenolic compounds and authentic samples) were located by color reaction and the R_f values under U.V.lights with and without the presence of amonia vapours were calculated according to Markham and Mabry, (1968). After that the flavonoid and phenolic acid compounds were U.V. measured according to Mabry *et al.*, (1970) by using Shimadzu U.V. visible Recording Spectrophotometer Model. U.V. 240. Each compound was separately dissovled in pure ethanol and examined for U.V. spectrum with different reagents. A blank was carried out on pure methanol and treated with different reagents.

Preparatoion of butter cake :

The butter cakes were prepared according to Mizukoshi *et al.*, (1979). Flavonoid and phenolic acid compounds as natural antioxidant extracted from black cumin and synthetic antioxidant (BHT) were added separately to butter cakes at 100,200 and 300 ppm. Black cumin defatted meal was added to butter cake at levels of 5 , 10 and 15% , respectively, to test their antioxidant effectiveness.

The ingredient cake with natural, synthetic antioxidants and black cumin meal contained Frensh butter, sugar, whole egg, vanillin, baking powder and water were mixed for 5 min. Wheat flour extract 72% was added and mixed for 10 min. in a mixer. Propunic acid was added at 1% as antimicrobial effect in manufactories. The product was baked at 191C for 25 min. in an electric oven and it was stored at room temperature. The products were tested by ten panelists after 2 hours according to AACC. (1985). The results were statistically analyzed using the method reported by Steel and .Torri, (1980).

Extraction of lipid from cakes :

Fats were extracted from cakes every 2 days by soaking in n – hexan at room temperature for 48 hr. The extract was filtrated and evaporated to dryness. Butter was kept in the deep freezer for further investigation.

Peroxide value as physico – chemical characterietics was determined as /Mequ. O_2 / kg. oil according to A.O.A.C (1990).

RESULTS AND DISUSSION

Chemical composition of black cumin and its defatted meal:

Proximate chemical composition in Table (1) show that the percentages of crude protein, crude fat, crude fiber, ash and total carbohydrates in balady type of black cumin seeds were 22.37, 35.42 , 8.25, 4.53 and 29.43%, respectively. These percentages increased in defatted seeds as shown in Table (1). Protein as an example increased in seeds by 45% after defatting. Moreover, black cumin seeds were found to be a good source of fat, protein and carbohydrates which mean that the seeds possessed medium concentration between cereals and legumes, and utilizing of these components for food supplementation and industry. (El Malt *et al.*, 1998).

The increasing use of black cumin seeds increased the interest in the composition of seed protein. Besides, black cumin seeds could be generally considered a good source of protein especially in the defatted form. The amino acids profile of black cumin is shown in Table (2) listing the concentrations of 17 amino acids. Among these amino acids, the major amino acids in black cumin were glutamic acid (19.75 g / 100g. protein) followed by arginine, aspartic and glycine (7.51, 6.93 and 6.32 g/ 100g protein), respectively. The essential amino acids content represent about 39.15 g / 100g protein and eight essential amino acids were found. The major essential amino acids were leucine (7.83 g/ 100g protein) followed by valine (5.00 g / 100 g protein). The first limiting amino acid was lysine and second limiting amino acid was sulphur containing amino acid. These results were in agreement with those reported by El- Malt *et al.*, (1998). Babayan *et al.*, (1978) regarding the amino acids profile of black cumin seeds, which may be due to the genotypes and or environmental variations. The essential amino acids content of black cumin protein compared with FAO / WHO professional pattern (1990), is shown in Table (2). Results from the Table (2) indicated that their levels, with the exception of lysine exceed the recommendation. Relative to the FAO / WHO (1990) requirements for 2 to 5 years old child, the essential were higher except for lysine and sulphur amino acids were equal the recommended level in FAO/ WHO

(1990) pattern. Whereas, the lysine was found to be the first limiting amino acid in black cumin protein with a score value of 71.27 . These results were reported by El – Malt *et al.*, (1998).

Paper chromatographic anatysis of black cumin extract :

The defatted black cumin was finely ground and extracted with 70% ethanol. The concentrated aqueous ethanolic extract was subjected to fractional extraction using acetone followed by ethyl acetate. Two solvent systems were used namely butanol: acetic acid: water (4:1:5) and acetic acid 15% (ACOH) to identify acetone and ethyl acetate extracts with paper chromatographic technique and compared with authentic samples. The color reaction and R_f values of the flavonoid and phenolic compounds are shown in Table (3). Acetone extract was found to contain four flavonid compounds (kaempferol, quercetin, myricetin and rutine). Ethyl acetate extract contained two flavonoid and two phenolic acids (cosmosiin, astragelin, ellagic acid and *p*- cumaric acid). The structure of the isolated compounds were examined through U.V. spectral in methanol and in the presence of diagnostic reagents are shown in Table (4) Antioxidant activity of the flavonoids increased as the number of phenolic hydroxy groups was increased. Antioxidant activity was in order : myricetin > quercetin > catechin > kaempferol. The aglycons rutin and narginin were consistently weaker antioxidant than the aglycones (Pekkarinen, 1996).

Sensory evaluation of the produced butter cake:

Table (5) shows the sensory evaluation of cakes prepared from Frensh butter. Natural amd synthetic antioxidants had the highest scores for the sensory evaluation parameters and also, exhibits the highest acceptability compared to cake made up from black cumin meal. Sensory evaluation of cakes prepared from black cumin meal at different levels recorded higher score at 5% for the color while 10 and 15% resulted higher score of taste.

Peroxide value of lipids extracted from butter cake was determined every two days up to twenty days, and the results are given in Table (6) and Figs : 1, 2 and 3 respectively. From the table and figures it can be observed that 300 ppm of the black cumin extract effectively inhibited the peroxide formation for a period of four days

(p.v. 2.4 to 2.7) then the peroxide value increased to 3.9 , 5.7, 8.4, 11.5 Mequ. O_2/ kg oil after 8^{th} , 12^{th} , 16^{th} and 18^{th} days , respectively. Very close results were observed by the addition of BHT at 300 ppm. This means that the black cumin extract contained active antioxidants. It is worth to mention that 200 ppm extract also decreased the peroxide value. Whereas, addition black cumin meal at 15% was affected and decreased the peroxide value to period 16^{th} days.

From the aforementioned results, it could be suggested that the addition of natural antioxidant at 200 and 300 ppm had given higher shelf life of butter cake. Also , addition of the black cumin meal at 15% gave a decrease in the peroxide value.

Table 1. Chemical composition of black cumin seeds and its defatted meal (on dry weight basis).

Chemical composition	Black cumin	
	Undefatted seed	Defatted meal
Protein	22.37	32.30
Fat	35.42	4.26
Fiber	8.25	9.75
Ash	4.53	5.82
Total carbohydrates	29.43	47.87
Nitrogen free extract	21.18	38.12

Table 2. Amino acids content of black cumin g/ 100g protein and amino acids chemical score

*NEAA	Black cumin	**EAA	Black cumin	FAO/WHO professional pattern (1990)	Chemical score
Aspartic	6.93	Lysine	3.92	5.50	71.27
Serine	4.98	Theronine	4.67	4.00	116.75
Glutamic	19.75	Cystine	2.01	Cystine +	
Proline	4.77	Methionine	1.57	Methionine 3.50	102.28
Glycine	6.32	Valine	5.77	5.00	115.40
Alanine	4.47	Isolucine	4.75	4.00	118.75
Histidine	2.80	Leucine	7.83	7.00	111.86
Arginine	7.51	Tyrosine	4.82	Tyrosine +	
		Phenylalanine	3.81	Phenylalanine 7.00	120.43
Total	57.53		39.15		
*** E/N	0.68				

* NEAA Non essential amino acids ** EAA Essential amino acids

*** E/N Essential / non essential

Chemical score was calculated by using the following equation:

$$\text{Chemical score} = \frac{\text{EAA in crude protein}}{\text{EAA of FAO/WHO}} \times 100$$

According to FAO / WHO scoring pattern (1990).

Table 3. Color reaction and R_f values of flavonoid and phenolic compounds extracted from black cumin

Compounds	Color reaction		R_f. Values x 100	
	U.V. light	U.V. NH_3	BAW	ACOH 15%
Acetone extract				
Kaempferol	Yellow	Bright – yellow	82	1
Quercetin	Yellow	Bright – yellow	65	4
Myricetin	Yellow	Yellow	29	2
Rutin	Deep – purple	Yellow	44	56
Ethyl acetate extract				
Cosmosiin	Dark – purple	Greensh– green	66	14
Astragalin	Brown	Yellow	70	43
Ellagic acid	Dark	Yellow	38	2
p. cumaric acid	Faint	Violets	69	54

BAW Butanol : Acetic acid; water ACOH Acetic acid

U.V. ultra violet light U.V. NH_3 ultra violet light with ammonia fumes

Table 4. U.V. spectral data of the compounds isolated from black cumin.

Compounds	MEOH (a)	(a)+NaOMe (b)	(a) + $AlCl_3$ (c)	(c) + Hcl (d)	(a)+NaOAC (e)	(e)+H_3BO_3 (f)
Kaempferol	245,268, 322,365	275,325,412	262,270,352, 426	260,271, 350,426	275,300,285	269,295,320, 370
Quercetin	256,268, 374	249,424	273,305,335, 434	266,303, 350,414	264,325,390	362,302,386
Myricetin	254,272, 301,374	262,285,322, 423	231,316,450	266,275, 308,360 428	269,335	258,304,392
Rutin	259,266, 299,359	272,327,410	275,303,433	271,300, 364,402	271,325,393	262,298,387
Cosmosiin	267,333	245,269,301, 326	276,298,384, 375	277,299, 341,382	256,267,355, 387	267,340
Astraglain	267,295, 352	273,328,405	274,300,348, 398	274,300, 345,399	273,300,370	267,295, 353
Ellagic acid	255	245,277	247,271		245	
p - cumaric acid	227,310	335				

MEOH : Methyl alcohol $AlCl_3$: Aluminium chloride

NaOMe : Sodium methoxide Hcl : Hydrochric acid

$H3BO_3$: Bouric acid NaOAC : Sodium acetate

Table 5. Effect of black cumin extract and its defatted meal on the sensory evaluation of butter cake

Addition rate (level)	Taste 30	Odor 10	Shape 10	Crust color 15	Grain crumb 10	Texture 15	Grumb color 10	Total score 100
Control	29.0	9.5	9.5	14.75	9.75	14.0	10.0	97.0
Black cumin								
5%	26.0	8.25	8.5	12.25	8.0	12.5	7.5	85.5
10%	26.25	7.75	7.25	10.5	7.5	9.5	6.0	77.25
15%	26.25	7.75	6.25	8.25	7.25	9.5	4.5	72.25
Synthetic antioxidant (BHT)								
100ppm	29.5	9.5	9.5	14.75	9.75	14.5	10.0	97.5
200ppm	28.5	9.5	9.5	14.0	9.5	14.25	9.5	95.0
33ppm	28.5	9.25	9.25	13.75	9.25	14.0	9.0	93.0
Natural antioxidant extract								
100ppm	29.0	9.5	9.5	14.75	9.75	14.25	10.0	96.25
200ppm	28.5	9.5	9.5	14.25	9.25	14.0	9.25	94.25
300ppm	28.25	9.25	9.0	14.0	9.0	13.75	9.0	92.25
L.S.D. at 5%	0.683	0.476	0.58	0.535	0.436	0.692	0.476	

Control prepared from butter without free antioxidant.

Table 6. Peroxide value of Frensh butter after baking cake as affected with black cumin extract and its meal

Time / day	Control	Natural antioxidant extract, ppm			Synthetic antioxidant (BHT) ppm			Defatted black cumin %		
		100	200	300	100	200	300	5	10	15
Zero	3.4	2.4	2.4	2.4	2.4	2.3	2.3	3.0	2.7	2.7
4	5.1	3.5	3.1	2.7	3.2	2.9	2.6	3.8	3.5	3.2
8	8.3	5.7	4.4	3.9	4.9	4.2	3.7	6.1	5.6	4.8
12	11.7	7.2	6.6	5.7	6.5	6.1	5.4	9.7	8.1	7.5
16		10.4	9.2	8.4	9.4	8.5	6.9	13.8	11.6	10.2
18			12.3	11.5	13.2	11.7	9.5			
20							12.1			

Before baking cake peroxide value of Frensh butter was 2.3 Mequ. O_2 / kg. oil.

Fig. 1. Peroxide value during stored period of cake prepared from natural antioxidant.

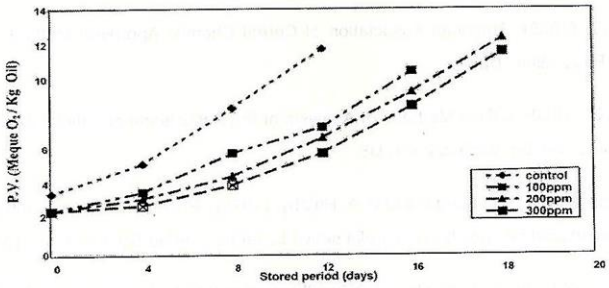


Fig. 2. Peroxide value during stored period of cake prepared from synthetic antioxidant (BHT).

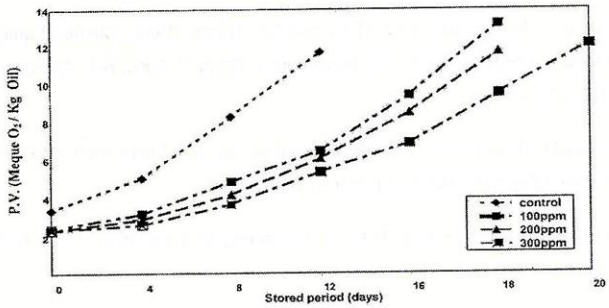
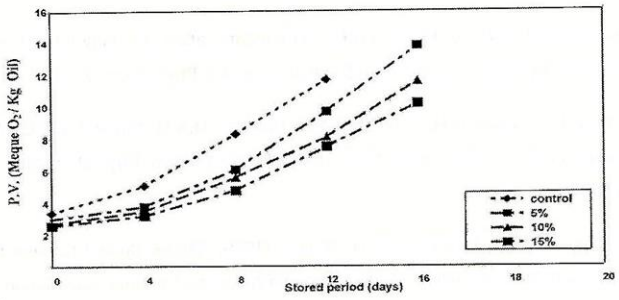


Fig. 3. Peroxide value during stored period of cake prepared from defatted black cumin.



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الاستفادة من حبة البركة المنزوعة الدهن ومستخلصها كمضادات أكسدة طبيعية لحفظ جودة الكيك أثناء التخزين

رأفت نجيب سندق

قسم بحوث تكنولوجيا المحاصيل- معهد بحوث تكنولوجيا الأغذية- مركز البحوث الزراعية -جيزة - مصر.

أوضحت الدراسة أن التركيب الكيماوى لبذور حبة البركة من بروتين - زيت - ألياف - رماد - كربوهيدرات كلية كانت نسبتها على التوالى ٢٢,٣٧ - ٣٥,٤٢ - ٨,٢٥ - ٤,٥٣- ٢٩,٤٣ ٪ أما حبة البركة المنزوعة الدهن كانت النسب على التوالى ٣٢,٣٠- ٤,٢٦ - ٩,٧٥ - ٥,٨٢ - ٤٧,٨٧ ٪. تم تقدير الأحماض الأمينية المتواجدة فى حبة البركة المنزوعة الدهن فوجد أن الحامض الأمينى جلوتاميك هو الحامض الأمينى الرئيسى (١٩,٧٥ ٪ من البروتين) وكان الحامض الأمينى ليوسين (٧,٨٣ ٪ هو السائد فى الأحماض الأمينية الأساسية.

تم استخلاص المركبات الفلافونيدية والأحماض الفينوليه من حبة البركة المنزوعة الدهن بواسطة كحول الإيثايل ٧٠٪ ثم المعاملة بالاسيتون ثم خلات الايثايل وذلك لاستخدامها كمضادات أكسدة طبيعية وتم تفريدها والتعرف على المركبات الفلافونيدية فكانت (كامفيرول - كيروستين - ميرستين - ريوتين - كوزموسين - استراجلين) والأحماض الفيتوليه وكانت (حمض الايلاجيك - البارا-كيوماريك) مضادات الأكسدة الطبيعية والصناعية (BHT) ثم إضافتها على الكيك المصنع من الزبد الفرنسى بنسب ١٠٠- ٢٠٠ - ٣٠٠ جزء فى المليون وقد تم إضافة حبة البركة المنزوعة الدهن بنسب ٥ - ١٠ - ١٥٪ على التوالى وتم تخزين الكيك على درجة حرارة الغرفة.

أوضحت النتائج أن مضادات الأكسدة الطبيعية بنسب ٢٠٠و٣٠٠ جزء فى المليون تعمل على حماية الليبدات من الأكسدة أثناء تخزين الكيك أما حبة البركة المنزوعة الدهن فيتم إضافتها بنسبة ١٥٪ - التقييم الحسى أوضح أن جميع التركيزات من مضادات الأكسدة الطبيعية كانت مفضلة أما حبة البركة المنزوعة الدهن فوجد أن نسبة الإضافة ٥٪ مفضلة من حيث اللون أما نسبة ١٠ ٪ و ١٥ ٪ كانت مفضلة من حيث الطعم.