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IMPLICATION OF DIFFERENT HEATING PERIODS ON SOME CHEMICAL COMPONENTS AND NUTRITIONAL VALUE OF *Nigella sativa* L.

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ABSTRACT

The objective of the present work was to follow up the effect of different heating periods i.e. 10, 20, 30 and 40 min. at 100°C on some chemical components and nutritional value of *Nigella sativa* L. *Nigella sativa* L. seeds are rich in crude fat content 35.22%, crude protein content (26.73%) and carbohydrate content (26.95%). The levels of antinutritional factors (ANFs) were found to be 1.70%, 1.12%, 4.3 mg/g and 51.40 (1 ml 0.1 N NaOH/100 g meal) for total phenolic compounds, phytic acid, trypsin inhibitor and lipase activity, respectively.

It was found that heating of *N. sativa* L. seeds generally and gradually reduced activity of trypsin inhibitor, phenolic compounds and phytic acid. Moreover lipase activity was totally destroyed after 30 min of heating. Concerning fatty acids present in *N. sativa* L. seed oil palmitic, oleic and linoleic acids seemed to be dominant ones.

Also, chemical properties and fatty acids composition of extracted oil were affected by the heat treatment of the seeds. The most dominant amino acids in *N. sativa* L. seeds were glutamic and aspartic acids, whereas the cysteine and methionine were of the least dominance. Heating seemed to reduce slightly contents of most the amino acids, whereas it increased protein digestibility.

Key word: *Nigella sativa* L. – Heating - Antinutritional factors – Fatty acids – Amino acids.

INTRODUCTION

Black cumin (*Nigella sativa* L.) is cultivated in Egypt, and known under different Arabic names: Habbah soeda, kamun Aswan and Habbet El-Baraka. The seeds possess aromatic odour and taste (Salama, 1973). The seeds are also used in the cooking and on bread products as a flavouring agent in Egypt, India and Turkey (El-Komey, 1996). Black cumin seeds have many medical uses such as digestive stimulants, carminative aromatic, diuretic, diphonetic stomachic and antheimitec agents (Agarwal. *et al.*, 1979 and Ibrahim, 1997). Compounds which have antimicrobial activities were found in the volatile oil of *N. sativa* L. seeds by Egyptian workers (El-Alfy *et al.*, 1975; Aqel and Shaheen, 1996).

Many reports and articles have been written (Datta *et al.*, 1987; Al-Jassir, 1992; Abdel-Aal and Attia, 1993a, b; Hailat *et al.*, 1995; and Ibrahim, 1997) indicating the importance of *N. sativa* L. seed oil in increasing immunity and maintaining good health.

Proteins and amino acids compositions of *N. sativa* L. seeds have been reported by Al-Jassir (1992); Abdel Aal and Attia (1993a) and Naroz (1997). On the other hand, antioxidative effects of black seeds were also discussed by Sosulski (1979), Nergiz (1991) and Nergiz and Otles (1993). Antioxidant substances are present in *N. sativa* L. seeds such as α , β and γ -tocopherols (about 340 $\mu\text{g/g}$) and polyphenols (about 1750 $\mu\text{g/g}$) in the seed oil. These compounds may be considered as native medicine and flavouring agents in several foods.

Oil of undamaged seeds is relatively stable due to physical separation between oil and seed lipases. When this physical separation is disrupted by grinding, inherently high lipase activities cause hydrolysis of natural fat to free fatty acids and glycerol. Because free fatty acids accumulate to unacceptable levels, the lipase must be inactivated. The only known practical method, which has commercial potential, is heat treatment (Desikachar, 1974).

The seeds and their oil may increase the human immunity (Toppazada *et al.*, 1965). They help in delaying menses, lactation, flatulence respiratory depression, asthma, cough, as a diuretic, antioxidants and reduce the microbial growth El-Mofty *et al.*, 1997; Atta and Imaizumi, 1998 and Ibrahim, 1999).

Phenolic compounds are known to adversely affect the utilization of protein in animal and human diet due to their ability to bind and

precipitate proteins and inhibit some digestive enzymes such as trypsin and α -amylase (Davis and Hoseny, 1979). On the other hand, several epidemiological studies have shown that an increased dietary intake of natural phenolic antioxidants correlates with reduced coronary heart disease (Hertog *et al.*, 1993). The phenolic compounds have also, been found to exhibit many health-related properties include anticancer, antiviral anti-inflammatory and an ability to inhibit human platelet aggregation (Benavente-Gracisa *et al.*, 1997).

Phytic acid and phytate are natural plant inositol hexaphosphate usually present in seeds. In the meantime, phytic acid and phytate have an adverse effect on minerals bioavailability in nutrition. They reduce the solubility of starch and proteins and may decrease their digestibility. (Thompson, 1993). Trypsin inhibitors are proteins with molecular weight ranging from 8,000 to 21,000 dalton (Liener, 1986). They hinder the digestibility of food proteins (El-Bagoury *et al.*, 1999).

Salem *et al.* (2001) found that *Nigella* flours defatted by pressing or by petroleum ether contained 643 and 620 mg/100 g phenolic compounds, 852.72 and 832.01 mg/100 g phytic acid and 7.45 and 10.21 trypsin inhibitor units. Heat treatments decreased the phenolic compounds by about 25-30%, phytic acid by 10-15% and trypsin inhibitors by 29-100%. In respect to amino acids composition, both defatted *Nigella* flours were rich in most of essential and non-essential amino acids compared to the FAO/WHO standards (1985).

Ghazi *et al.* (1992), El-Kady (2000) and Moussa (2001) used protein isolated from sugar-beet leaves flaxseed meal and *Nigella* meal for preparing a good sausage, pan bread and spaghetti macaroni.

Nigella seed oil is produced in Egypt by pressing or by organic solvents, but the pressed cake or defatted flour (by-product) has not efficiently been utilized. The objective of the present work was to determine the levels of antinutritive factors in *Nigella sativa* L., the effect of dry heating on antinutritive factors and evaluation of their nutritional value.

MATERIALS AND METHODS

Nigella sativa L. seeds were obtained from Agricultural Research Center, Giza, Egypt and were roasted by heating at 100°C for 10, 20, 30 and 40 min and then left to cool at room temperature, cleared and

finely ground. Hexane (B.P. 40-60°C) was used for the extraction of oil from the ground seeds.

Chemical analysis:

Moisture, crude fat, total protein and ash content were determined in *Nigella sativa* L. seeds according to the standard methods described in A.O.A.C. (1995).

Acid value, peroxide value and iodine value were determined according to the standard method described by A.O.A.C. (1995).

The total fatty acids after a saponification of the oil samples were methylated by diazomethane which was prepared from methyl amine and urea as reported by Vogel (1975). The fatty acids methyl esters were analyzed using a Pye Unicam Series 304 Gas-liquid Chromatography, with Flame Ionization Detector.

The trypsin inhibitor activity (TIA) was measured by the method described by Hamerstrand *et al.* (1981) and modified with respect to the initiation of the TIA assay, *i.e.* trypsin was added last to the inhibitor-substrate mixture (Stauffer, 1993), using N-benzoyl-DL-arginine-*p*-nitroanilide hydrochloride (BAPA) as synthetic substrate for trypsin. The assay was run in triplicate and average values were used in expressing the results.

Phytic acid content in raw and processed *Nigella* samples was estimated colorimetrically using Wade reagent (Latta and Eskin, 1980). The free bound or conjugated and total phenols were calorimetrically determined in the ethanolic extracts by using the Folin Denis reagent as described by Gutfinger (1981). Lipase activity in ground *nigella* seeds were determined according to the method of Ranakrishnan and Nevgi (1951). The activity was expressed as the difference between sample and blank titers (1 ml 0.1N NaOH/g seeds).

Molecular weights of subunits of protein extracted using (0.02 N NaOH) from different flours were determined using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method described by Laemmli (1970). The digestibility of protein *in vitro* was carried out as described by Singh and Jumbunathan (1981). The amino acids in *Nigella* seeds were determined using the amino acid analyzer (Model 121) as described by Moore *et al.* (1958).

RESULTS AND DISCUSSION

Results of chemical analysis of *N. sativa* L. seeds are presented in Table (1). These results reveal that *N. sativa* L. seeds are rich in crude fat (35.22%), crude protein (26.73%) and carbohydrate (26.95%). The crude protein of *N. sativa* L. was relatively higher than that reported by El-Malt *et al.* (1998) but, on the other hand, it was in a good agreement with that reported by Salem *et al.* (2001) and Atta (2003). Such variation in crude protein content might be attributed to the variations in both physical and chemical properties on which the plants were grown. Also, the different fertilization treatments may account for these variations.

Table (1): Chemical composition of *Nigella sativa* L. seeds.

Components	
Moisture (%)	6.30
Ash (%)	4.80
Fat (%)	35.22
Crude protein (%)	26.73
Carbohydrate (%)	26.95
Polyphenols (%)	1.70
Phytic acid (%)	1.12
Trypsin inhibitor (mg/g)	4.30
Lipase activity (1 ml 0.1 N NaOH/100 g)	51.40

The levels of antinutritional factors (ANFs) in *N. sativa* meal were found to be 1.70%, 1.12%, 4.3 mg/g and 51.40 (1 ml 0.1 N NaOH/100 g meals) for total phenolic compounds, phytic acid, trypsin inhibitor activity and lipase activity, respectively.

The polyphenolic compounds content of *N. sativa* seeds (1.7%) is different from that reported by El-Malt *et al.* (1998), but is in agreement with that reported by Nergiz and Otles (1993) which was (1.744%). The contents of the other ANFs in *N. sativa* seeds are in agreement with those reported by Shaker *et al.* (1997), Moussa (2001) and Ramdan *et al.* (2003).

Chemical properties of oils extracted from *N. sativa* seeds heated on different periods are presented in Table (2). There were slightly and gradually increases in acid value and peroxide value whereas a decrease occurred in iodine value with increasing time of heating. The

increase in acid value may be attributed to a slight random hydrolysis of triacylglycerols by heating that produces free fatty acids and diacylglycerols (Yoshida *et al.*, 1992). The increase in peroxide value is probably due to the oxidation of unsaturated fatty acid occurred as a result of heating of the *N. sativa* seeds (Yoshida *et al.*, 1990). On the other hand, the decrease occurred in iodine value in oil extracted from *N. sativa* seeds might be attributed to the effect of heating on oxidation of the unsaturated fatty acids which results in a decrease in the relative percentage of total unsaturated fatty acids and a corresponding increase in the relative percentage of total saturated fatty acids (Yoshida *et al.*, 1990).

Table (2): Effect of dry heating on chemical properties of *N. sativa* L. seeds oil.

Parameters	Time of heating (min)				
	0	10	20	30	40
Acide value	11.50	11.30	12.70	13.90	14.98
Peroxide value	6.18	6.90	7.12	7.90	8.97
Iodine value	125.60	124.13	123.13	122.10	121.21

The fatty acids composition of oils extracted from *N. sativa* seeds heated for different periods are presented in Table (3). The obtained results show that the major constituents of these fatty acids were palmitic, oleic and linoleic acids whereas low the other fatty acids i.e. myristic, stearic, arachidic and linolenic acids were detected in lower percentages. These results are in good agreement with those reported by Ramadan and Morsel (2002), Bahman *et al.* (2003) and Talaat and El-Din (2005). The percentage of total saturated fatty acids increased whereas the percentage of total unsaturated fatty acids decreased due to heating at 100°C for different periods, yet the effect seemed more pronounced by increasing period of heating. These results are in good agreement with those reported by Moussa (2001).

Table (3): Effect of dry heating on fatty acid composition of *N. sativa* L. seeds oil.

Fatty acids	Time of heating (min)				
	0	10	20	30	40
C _{12:0}	0.45	0.60	0.67	0.74	1.15
C _{14:0}	0.97	1.60	1.11	1.80	1.99
C _{16:0}	17.70	17.85	18.11	18.55	19.80
C _{18:0}	2.69	2.70	2.95	3.11	3.27
C _{20:0}	0.20	0.25	0.27	0.30	0.41
C _{18:1}	21.37	20.17	20.40	20.86	19.90
C _{18:2}	56.88	56.13	55.88	54.20	54.18
C _{18:3}	0.74	0.70	0.61	0.44	0.30
Total saturated fatty acids	22.01	23.00	23.11	24.50	25.62
Total unsaturated fatty acids	78.99	77.00	76.89	75.50	74.38

Effects of dry heating on antinutritional factors contents of lipase activity, trypsin inhibitor activity, polyphenolic compound and phytic acid in *N. sativa* L. seeds were examined and the results are presented in Table (4) and illustrated graphically in Fig. (1). Dry heating of *N. sativa* L. seeds for 40 min reduced activity of trypsin inhibitor by 78%. Polyphenolic and phytic acid were more resistant to heat treatments compared to trypsin inhibitor activity where heating for 40 min reduced their contents by 55 and 59%, respectively.

Lipase activity was totally destroyed after 30 min of heating of *N. sativa* seeds. These results are, to some extent, in agreement with those reported by El-Kady (2000) and Salem *et al.* (2001).

Table (4): Effect of heating on antritional factos (ANFs) of *N. sativa* L. seeds:

Time of dry heating (min)	Lipase activity (%)	Trypsin inhibitor activity (%)	Phytic acid (%)	Polyphenols (%)
0	100	100	100	100
10	42	75	71	70
20	21	45	68	65
30	0.0	29	45	55
40	0.0	22	41	45

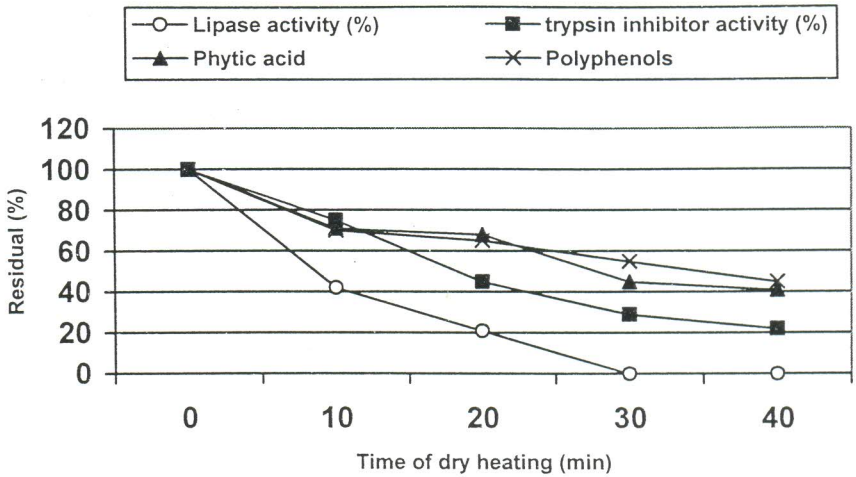


Fig. (1): Effect of dry heating on antinutritional factors (ANFs) of *N. sativa* L. seeds.

The amino acids profile of *N. sativa* seeds as affected by dry heating for 30 and 40 min is shown in Table (5). The most dominant amino acids in *N. sativa* seeds were glutamic acid (17.3 g/100 g protein) followed by aspartic acid (8.3 g/100 g protein) whereas the cysteine and methionine were the least dominant amino acids. The defatted *N. sativa* seeds (meal) were rich in both essential and non-essential amino acids. Threonine, isoleucine and aromatic amino acids were present in *Nigella* flours in amounts higher than those reported by FAO/WHO (1985). Heating seemed to reduce slightly contents of most of the amino acids. Such results are in agreement with those found by Salem *et al.* (2001) who found that heat treatment had a little effect on amino acid of *Nigella* protein isolate.

The solubility curve Fig. (2) showed that about 91% of the protein content was soluble between pH 8 and 10. Minimum solubility about (18.5%) occurred at pH 4.2 which is represents the isoelectric point. These results are in agreement with the results of Abd El-Galil and Latif (2003) who reported that maximum value of protein solubility was found at pH range 9-12 and minimum solubility was at pH range 3-5. The results obtained herein referrers that all heat treatments increased the extractability of protein from defatted *Nigella* flour.

The effect of heating treatments on protein subunits of *N. sativa* seeds are shown in SDS-PAGE patterns (Table, 6 and Fig., 3). The unheated seed showed 14 subunits with molecular weights ranging from 90,000 to 16,000 KD. On the other hand, seeds heated for 20 min showed only 9 subunits with molecular weight ranging from 55,000 to 16,000 KD. Increasing heating time for 30 and 40 min showed a reduction in the number of subunits to 7 and 6 subunits, ranging from 39,000 to 16,000 and 33,000 to 16,000, respectively. In general, it could be concluded that heat treatment caused subunits of the high molecular weights to disappear probably due to their dissociation to lower molecular weight subunits.

Table (5): Effect of dry heating on the amino acids percentage (g/100 g protein) of *N. sativa* L. seeds.

Amino acids	Heating time at 100°C (min)			FAO/WHO (1985) pattern (g/100 g protein)
	0	30	40	
Essential amino acids (E.A.A.):				
Lys.	3.81	3.55	3.35	5.50
Leu.	6.97	6.75	6.63	7.00
Isoleu.	3.70	3.70	3.64	3.50
Cys.	2.15	2.11	1.89	3.50
Met.				
Phe.	3.85	3.84	3.69	6.00
Tyr.	4.31	4.29	4.25	
Thr.	4.56	4.56	4.53	4.00
Val.	4.91	4.90	4.82	5.00
Try.	1.98	1.85	1.64	1.00
Total E.A.A.	36.24	35.55	34.44	
Non essential amino acids (N.E.A.A.)				
His.	3.95	3.89	3.89	
Arg.	6.54	6.51	6.11	
Asp.	8.30	8.12	8.00	
Glu.	17.30	17.32	17.35	
Ser.	4.40	4.38	4.34	
Pro.	4.52	4.42	4.28	
Gly.	6.41	6.41	6.22	
Ala.	4.52	4.49	4.33	
Total N.E.A.A.	55.94	55.54	54.52	
Total A.A.	92.18	91.09	88.96	

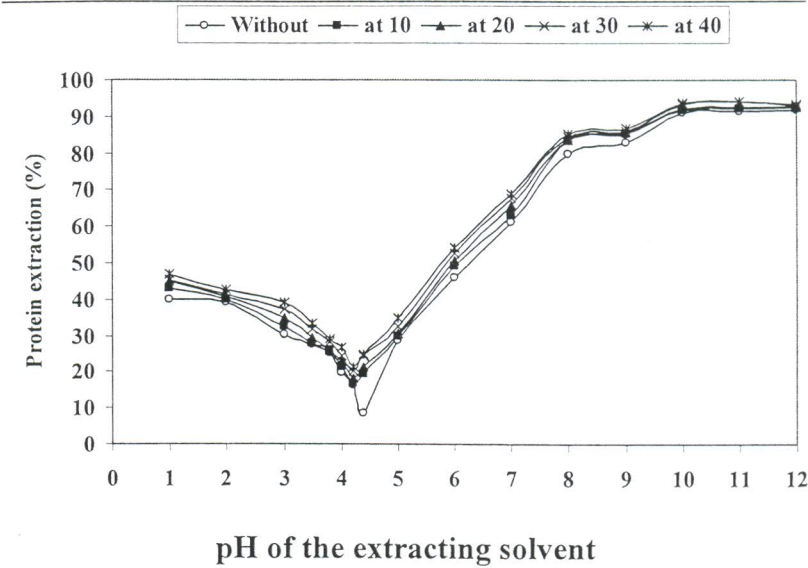


Fig. (2): Effect of dry heating on the stability of *N. sativa* L. seed protein.

Table (6): Molecular weights of *N. sativa* L. protein subunits extracted at different times of heating (as determined by SDS-PAGE, KD).

Band	Heating time (min)				
	0	10	20	30	40
1	90000	---	---	---	---
2	82000	82000	---	---	---
3	77000	77000	---	---	---
4	72000	72000	---	---	---
5	68000	68000	---	---	---
6	55000	55000	55000	---	---
7	42000	42000	42000	---	---
8	39000	39000	39000	39000	---
9	33000	33000	33000	33000	33000
10	29000	29000	29000	29000	29000
11	25000	25000	25000	25000	25000
12	23000	23000	23000	23000	23000
13	21000	21000	21000	21000	21000
14	16000	16000	16000	16000	16000

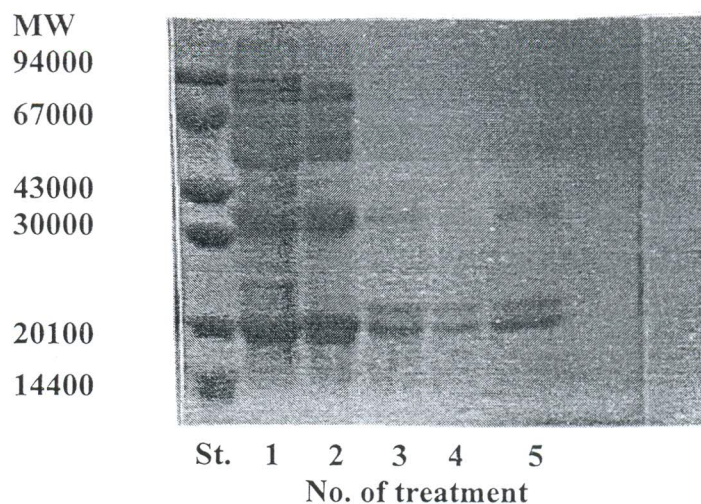


Fig. (3): SDS-PAGE pattern of *N. sativa* L. protein extraction after heating treatments.

- 1- Without heating.
 2- Heating at 100°C for 10 min. 3-Heating at 100°C for 20 min.
 4- Heating at 100°C for 30 min. 5- Heating at 100°C for 40 min.

The results in Fig. (4) show that the *in vitro* protein digestibility of unprocessed N-sativa seed was 81.30%. Heat treatment increased protein digestibility to 87.6% at 40 min. The increase in protein digestibility after heating could be partially attributed to the protein denaturation which improves protein susceptibility to attach with enzyme. Furthermore, elimination or reduction of antinutritional factors would certainly improve protein digestibility (Salem *et al.*, 2001).

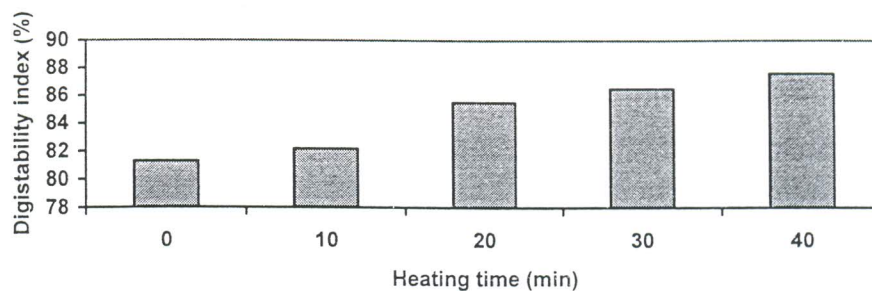


Fig. (4): Effect of heating *N. sativa* L. seeds on *in-vitro* protein digestibility.

The aforementioned results revealed that dry heating at 100°C for periods extending to 40 min reduced or even eliminated the antinutritional factor (ANFs) and hence improved the protein digestibility.

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تداعيات فترات تسخين مختلفة على بعض المكونات الكيميائية والقيمة الغذائية لحبة البركة

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يهدف البحث إلى دراسة تأثير التسخين على درجة ١٠٠°م لفترات زمنية مختلفة (١٠، ٢٠، ٣٠، ٤٠ دقيقة) على بعض المكونات الكيميائية والقيمة الغذائية للحبة السوداء. ومن النتائج المتحصل عليها وجد أن الحبة السوداء غنية في محتواها من الزيت والبروتين والكربوهيدرات. حيث احتوت على هذه المكونات بنسب ٣٥،٢٢%، ٢٦،٧٣%، ٢٦،٩٥% على الترتيب. كما احتوت على مضادات التغذية مثل المركبات الفينولية وحمض الفيتيك ومضاد الترسين ونشاط أنزيم الليبيز. كما وجد أن تسخين الحبة السوداء لفترات زمنية مختلفة أدى إلى انخفاض تدريجي في محتواها من مضاد الترسين والمركبات الفينولية وحمض الفيتيك كما أدى التسخين لمدة ٣٠ دقيقة إلى تحطم كامل لنشاط أنزيم الليبيز.

وقد وجد أن الأحماض الدهنية السائدة في زيت حبة البركة هي حمض البالميتيك، والأوليك، واللينوليك. كما أدى التسخين إلى حدوث تغير في الصفات الكيميائية وكذلك في نسب الأحماض الدهنية في الزيت المستخلص من حبة البركة. كما وجد أن الأحماض الأمينية السائدة في بروتين حبة البركة هي الجلوتاميك والأسبارتيك وأقل الأحماض الأمينية تواجدا هي السستين والميثيونين. كما أدى التسخين لفترات مختلفة إلى انخفاض بسيط وتدرجي في معظم الأحماض الأمينية.

ولقد أوضحت الدراسة أن التسخين على درجة ١٠٠°م لفترات امتدت إلى ٤٠ دقيقة أدى إلى خفض أو التخلص من بعض مضادات التغذية مما أدى إلى تحسين في معامل هضم البروتين.