Journal of Medicinal Herbs

journal homepage:**www.jhd.iaushk.ac.ir**

Effect of incorporation *Ocimum basilicum* L. powder on some physicochemical properties and oxidative stability of chocolate during the storage period

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ARTICLE INFO

Type: *Original Research*
Topic: *Medicinal Plants*
Received *September 26th2023*
Accepted *January 22th2024*

Key words:
- ✓ *Basil seeds*
- ✓ *Chocolate*
- ✓ *Poly unsaturated fatty acids*
- ✓ *Fiber*
- ✓ *Total phenol*
- ✓ *Hardness*

ABSTRACT

Background & Aim: For over a hundred years, chocolate has been considered to be one of the most popular foods in world due to its high nutritional value and pleasant taste. Foods that are widely accepted by consumers can be enriched with compounds that potentially improve the health of the consumer. In this study, an attempt was made to investigate the enrichment of chocolate with basil seeds powder to improve the nutritional value of the product.

Experimental: Basil seeds powder was added to chocolate formulation at levels of 0, 5, 7.5 and 10%. Then, physicochemical characteristics including protein, fiber and lipid content after production and also the changes in total phenol content, peroxide value and free fatty acids, hardness, fatty acids profile and fat blooming during 4 months of storage were evaluated.

Results: The results showed that by increasing the percentage of basil seeds powder in the formulation, the protein and fiber content, phenolic compounds and essential fatty acids (omega 3 and 6) increased compared to the control sample. On the other hand, the lipid percentage, free fatty acids, peroxide value and the hardness of the chocolate decreased. All samples did not show any signs of bloom until the 80th day. According to sensory evaluation, addition of basil seeds powder up to 7.5% did not cause a negative effect on the sensory attributes.

Recommended applications/industries: Based on the present findings, adding basil seeds powder to the chocolate formulation to a certain extent does not only have a negative effect on the sensory characteristics, but also improves the nutritional value of chocolate by increasing the amount of PUFAs, antioxidant compounds and fiber, as well as oxidative stability during the storage period.

1. Introduction

The global tendency to use medicinal plants and natural compounds in the pharmaceutical and food industries, and the subsequent attention of the public and domestic industries to the use of medicinal and aromatic plants, reveals the urgent need for extensive basic and applied research in this field (Nieto, 2020; Chaachouay and Zidane, 2024; Yu *et al*., 2021). Today, the harm caused by the use of additives and preservatives, essential oils and artificial flavors in the food industry is not secret to anyone (Jha and Jha, 2024; Adhikari, 2021). So, the need for comprehensive research and proper utilization of natural resources and medicinal herbs is very necessary, especially at a time when the world's use of medicinal plants in pharmaceutical and food industries has accelerated so much (Lebelo *et al.*, 2021; Cedillo-Cortezano *et al*., 2024; Amiri *et al*., 2021). Basil is an

important medicinal plant from the mint family (Lamiaceae). This plant is an annual herbaceous plant that has a great diversity in morphology and secondary compounds, especially essential oil. This genus has 50 to 150 herbaceous and shrub species, with some sources even mentioning more than 150 species, for this reason, it is considered one of the largest genera in the mint family and among which *Ocimum basilicum* L. is the most important economic species (Uritu *et al.*, 2018; Azizah *et al.*, 2023; Ramos da Silva *et al*., 2021; Sajjadi, 2006). This plant produces a valuable essential oil that varies depending on the variety of genotype, leaf, flower color, and plant origin. The main compounds inessential oil are citral, eugenol, linalool, methyl chavicol, and methyl cinnamate; which are of particular importance in international trade. The outer shell of basil seeds is covered with a layer of mucilage and they swell rapidly when placed in water. Basil seeds mucilage is emollient, diuretic, laxative, and diaphoretic. Basil extract also showed potential antioxidant properties and important role in improving health and preventing diseases (Tangpao *et al.*, 2022; Kamelnia *et al.*, 2023).

Chocolate is the most popular food in the world (due to its high nutritional value and pleasant taste) with high-calorie and fast metabolism. Its unique taste, texture and flavor stimulate pleasure and this has caused the consumption of all types of chocolate by all level of society. .cocoa and cocoa products are rich in polyphenols which have shown beneficial cardiovascular, metabolic, antiradical and anti-dermatological effects as well as eliciting effects on mood (Shin *et al.*, 2022; Sim *et al.*, 2016). One of the best methods to enhance flavor and phenolic content of chocolate is to add polyphenolic compounds derived from natural sources. Although this enrichment should not affect physical properties such as its rapid melting in the mouth and its hardness and brittleness at room temperature (Samanta *et al.*, 2022; Konar *et al.*, 2016). Therefore, any enrichment requires a comprehensive and sufficient investigation on the characteristics of the obtained product and its acceptance by the consumer. Considering the high content of omega-3 and omega-6 fatty acids, dietary fibers and antioxidant compounds in basil seeds, in this study, an attempt was made to investigate the enrichment of chocolate with basil seed powder at different levels and to evaluate some of physico- chemical properties, oxidative stability, fatty acids profile and consumer acceptance of products during 4 month of storage.

2. Materials and Methods

2.1. Preparation of the basil seeds powder

Basil seeds (*Ocimum basilicum* L.) were purchased from a local market in Shahrekord, Iran. After manual cleaning, the seeds were ground in a laboratory-grinder and finally sieved using a 80 mesh size sieve. Basil powder was stored in a dark plastic bags in the refrigerator until use. The proximate analysis of basil seeds including moisture, protein, lipid, ash, and fiber was carried out according to the method approved by AOAC (2002).

2.2. Determination of total phenol content

The total phenol content of basil seeds was determined spectrophotometrically (Lambda 20, Perkinelemer) at 750 nm according to Arabbi *et al.* (2004) method using the Folin-Ciocalteu·s reagent. Gallic acid was used as a standard to plot the calibration curve. The total phenol content in all chocolate samples was measured during the storage period and at time intervals 1, 40, 80, and 120 days.

2.3. Preparing of the chocolate samples

The chocolates were prepared in Kamvar Chocolate Company, Isfahan, Iran. The main ingredients used in the formulation included isomalt (39.2%), cocoa butter (40.6%), cocoa powder (11%), lecithin (0.2%), vanilla (0.2%), and nonfat milk powder (8.7%). After preparation, the chocolates were stored at ambient temperature (20°C). It is worth noting that 100 ppm of orange flavor was added to all samples. The formulation of the chocolates produced was as follows:

Control (chocolate prepared without basil seed powder)

Chocolate sample containing 5% basil seed powder in 100 g of chocolate

Chocolate sample containing 7.5% basil seed powder in 100 g of chocolate

Chocolate sample containing 10% basil seed powder in 100 g of chocolate

2.4. Determination of fatty acids profile

Fatty acids methyl ester analysis was performed using using an Agilent 6890N gas chromatograph equipped with a Cpsill 88 column and nitrogen was

used as carrier gas at flow rate of 0.7 mL/min. The oven temperature program was set as follows: initial hold at 150°C for 1 min, followed by a ramp to 190 °C at a rate of 5 °C/min, then held at 190 °C for 2 min and finally raised to 240 °C at 5 °C/min and maintained for an extra 8 min. Throughout the process, the FID detector and injector were kept at 250 °C and 150 °C, respectively (Jafari *et al.*, 2020).

2.5. Measurement of peroxide value

AOCS official methods (2004) were used for the determination of the peroxide value (method Cd 8b-90) and free fatty acids content (method Ca 5a-40) at time intervals of 1, 40, 80 and 120 days.

2.6. Texture analysis (hardness)

To measure the hardness of chocolate a texture analyzer (Brokfield CT3, USA) was used. The hardness of the chocolate samples was tested using a flat-bottomed probe and a penetration speed of 1.5 mm/s. The maximum penetration through different chocolates to a depth of 4 mm was investigated and recorded (Meclis, 2019).

2.7. Investigation of fat blooming

All formulations were investigated visually (once a week) on both sides for the appearance of bloom using the method of Ransom-Painter *et al.* (1997). The following visual evaluation code was used in the study: 5 = very glossy; 4 = slightly dull, no gloss; 3 = dull, traces of bloom; 2 = partly bloomed (appearance not acceptable); and 1 = full bloom.

2.8. Sensory evaluation

Sensory evaluation conducted by 10 semi-trained panelists and the 5 point hedonic scale was used to measure chocolate overall acceptance. Sensory evaluation was done on days 1 and at the end of the storage time.

2.9. Statistical analysis

All experiments were conducted in a completely randomized design with three replications. Experimental data were analyzed using SPSS (Version 21) software. The means were compared using Duncan's Multiple Range test at a probability level of 5% ($P<0.05$).

3. Results and discussion

3.1. Proximate analysis and fatty acids profile of basil seeds

The chemical composition of basil seeds, including moisture, ash, protein, lipid, fiber and carbohydrate content as well as total phenol content and fatty acids profile, was determined and the results are presented in Table 1. Basil seeds contain 36.12% fiber, 21.01% lipid, 21.53% carbohydrate, 10.93% protein and 5.42% ash. Therefore, basil seeds are rich sources of oil, protein and fiber. The obtained results are in agreement with the previous reports on physicochemical properties of basil seeds (Erwa *et al.*, 2020) although the moisture, protein, lipid and carbohydrate content were higher in some other studies (Khursheed *et al.*, 2023; Naji-Tabasiand Razavi, 2017; Hosseini-Parvar *et al.*, 2010).

Total phenol content of basil seeds was measured to be 500 mg/g dry weight. In general, the literature review shows that basil seeds have good antioxidant potential, even better than other seeds, such as sesame or red seeds, and could be used to develop new natural antioxidants or be included as ingredients to prevent oxidative deterioration in foods. Aburigal *et al*. (2017) investigated the antioxidant activity and total phenol content of basil (*Ocimum basilicum*) varieties collected from different regions of the world (Sudan, Iraq, Germany, Thiland, Russia, Maldives). Although the amount of total phenol in the seeds collected from Maldives was the highest, however, its amount was lower compared to the present research (Aburigal *et al.*, 2017). According to Bravo *et al.* (2021) study, the seeds from Pakistan presented lower values of total phenol content while seeds from Iran demonstrated higher values.

According to the results of the Table 1, palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$) linoleic acid ($C_{18:2}$), and linolenic acid ($C_{18:3}$) with approximate values of 6.81%, 3.85%, 7.45%, 20.99% and 59.92% , respectively, are the dominant fatty acids of basil seeds oil. Given that the amount of linoleic and linolenic acids in basil seeds oil are predominant, this oil can be consider as a valuable source of unsaturated fatty acids, especially omega 3 and omega 6 fatty acids. According to scientific evidences, linolenic acid showed anti-

cancer, anti-inflammatory, anti-diabetic effects and potential to reduction of blood cholesterol concentration (Avato and Tava, 2022; Momeni and Asadi-Gharneh, 2021; Takic *et al.*, 2022). The present results are in agreement with the results of Erwa *et al.* (2020) and Angers *et al.* (1999) that showed high amount of linoleic and linolenicacid in basil seeds (Angers *et al.*, 1996; Erwa *et al.*, 2020).

Table1. Proximate analysis and fatty acids profile of basil seeds.

Chemical parameters	Quantity
Moisture (%)	4.99±0.057
Ash (%)	5.42±0.010
Protein (%)	10.93±0.057
Lipid (%)	21.01±0.057
Fiber (%)	36.12±0.015
Carbohydrate (%)	21.53±0.075
Total phenol (Gallic acid equivalents, mg/g)	500±0.057
Fatty acids compositions (%)	
Lauiric acid ($C_{12:0}$)	0.05±0.04
Myristic acid($C_{14:0}$)	0.17±0.06
Myristoleic acid ($C_{14:1}$)	0.06±0.05
Palmitic acid ($C_{16:0}$)	6.81±0.05
Palmitoleic acid ($C_{16:1}$)	0.06±0.05
Heptadecanoic acid ($C_{17:0}$)	0.03±0.05
Heptadecenoic acid ($C_{17:1}$)	0.02±0.01
Stearic acid ($C_{18:0}$)	3.85±0.02
Oleic acid($C_{18:1\ Cis}$)	7.45±0.08
$C_{18:1\ Trans}$	0.033±0.01
Linoleic acid($C_{18:2}$)	20.99±0.04
Linolenic acid($C_{18:3}$)	59.92±0.05
Arachidic acid ($C_{20:0}$)	0.21±0.04
cis-13-Eicosenoic acid ($C_{20:1}$)	0.14±0.06
SFAΣ	11.12
USFAΣ	88.85

3.2. Fiber, protein and Lipid content in chocolate samples

Table 2 shows the fiber content of chocolate samples after production. As can be seen, there is a statistically significant difference between the chocolate samples in terms of fiber content ($P<0.05$). The highest fiber content was found in chocolate containing 10% basil seeds powder and by reducing the percentage of basil seeds in formulation, the fiber content has also reduced. As mentioned before, basil seeds contained 36.12% fiber (Table 1), therefore, adding it to chocolate enriches the product with fiber. A direct link has been proven between consuming of high-fiber diets and reducing the risk of some chronic diseases, including colon cancer, constipation, obesity, diabetes, and heart disease (Nunez-Gomez *et al.*, 2023; Alahmari, 2024).

The present results are in agreement with the results of Hassan Nezhad *et al.* (2017) who reported an increase in the fiber content in chocolate samples with the addition of flaxseed powder (Hassan Nezhad *et al.*, 2017). Peighambardoust *et al.* (2017) also reported an increase in fiber content by adding date pit and sesame seed powder mixture to milk chocolate (Peighambardoust *et al.*, 2017).

As can be seen in the Table 2, there is a statistically significant difference between the chocolate samples in terms of protein and lipid content ($P<0.05$). The highest protein content was found in chocolate containing 10% basil seeds powder, and as the percentage of basil seeds in the chocolate formulation decreased, the protein content also decreased. Basil seeds used in formulation contained 10.93% protein (Table 1), and its addition to the chocolate increased the protein content.

The highest lipid content was found in the control chocolate, and as the percentage of basil seeds in the formulation increased, the lipid content significantly decreased ($P<0.05$) which could be due to the reduction in cocoa butter percentage with the addition of basil seeds. The present results are in agreement with the results of Hassan Nezhad *et al.* (2017), who reported a decrease in lipid content in chocolate samples by adding flaxseed powder (Hassan Nezhad *et al.*, 2017).

Table 2. Comparison of average fiber, protein and lipid content (%) in chocolate samples containing different proportions of basil seeds powder after production.

Treatments	Fiber	Protein	Lipid
Control	0.80±0.015^{d}	4.78±0.035^{d}	36.16±0.056^{a}
Chocolate containing 5% basil seeds powder	2.28±0.025^{c}	5.19±0.52^{c}	35.57±0.055^{b}
Chocolate containing 7.5% basil seeds powder	3.41±0.026^{b}	5.83±0.035^{b}	35.15±0.045^{c}
Chocolate containing 10% basil seeds powder	4.41±0.120^{a}	6.72±0.036^{a}	34.68±0.055^{d}

*Small letters in each column indicate a significant difference between treatments ($P<0.05$).

3.3. Total phenolic compounds, peroxide values and free fatty acids content in chocolate samples during storage time

According to results presented in Table 3, it was observed that adding basil seeds powder to the chocolate formulation had a significant effect (P<5%) on the total phenol content of the chocolates and as the percentage of basil seeds powder increased, the average total phenol content of the samples increased, too. A comparison between samples showed that the lowest and highest total phenol content on the first day of production belonged to the control and the sample containing 10% basil seeds powder, respectively. The total phenol content decreased over time in the treatments, however, at the end of the storage period, with a significant difference (P<0.05) compared to others, the highest phenol content was observed in the sample containing 10% seeds powder.

Polyphenols are natural antioxidants found in plants, and their role in improving the oxidative stability and sensory and nutritional properties of foods has been repeatedly confirmed (Zhu *et al.*, 2024; Niu *et al.*, 2024; Caponio *et al*., 2024; Fotiadou *et al.*, 2024). The basil plant is generally composed of specific phenolic compounds, including phenylpropanoids, monoterpenes, and sesquiterpenes, as well as methyl chavicol, citral, and methyl eugenol (Bozyel *et al*., 2024; Spence, 2024; Azizah *et al.*, 2023). According to previous researches, there is a direct relationship between the concentration of phenolic compounds and antioxidant activities. In chocolate samples with a higher percentage of phenolic compounds, due to the increased number of hydroxyl groups present in the reaction medium, the probability of donating hydrogen to free radicals increases, and consequently the oxidative stability of the products increase (Ouamnina *et al.*, 2024; Indiarto *et al.*, 2024; Sun *et al*., 2023; Hatami *et al*., 2014).

The present results are in agreement with the results of Belscak-Cvitanovic *et al*. (2015) who reported an increase in phenolic compounds in chocolate samples containing nettle (*Urtica dioica* L.) seed extract (Belscak-Cvitanović1 *et al.*, 2015). Belscak-Cvitanovic and Komes (2012) had also reported an increase in phenolic compounds in chocolate samples containing raspberry (*Rubus idaeus* L.) extract (Belscak-Cvitanović and Komes, 2012).

According to Table 3, there were significant differences (P<0.05) between the different samples in terms of the peroxide values during the storage period. By adding basil seeds powder, the peroxide value was significantly affected. The peroxide value decreased with increasing in basil seeds powder in the chocolate formulation, so that the lowest and highest peroxide values were found for the sample containing 10% seeds powder and control sample, respectively. In all samples, the peroxide value showed an increasing trend over time, although this increase was much more evident in the control sample than the other treatments maybe due to the high content of total phenol in the seeds, which was also confirmed in the previous section. The anti-radical and antioxidant activity of basil can be attributed to the presence of oxygenated monoterpenes such as linalool and linalyl acetate (Hanachi *et al.*, 2018).

A noticeable increase in the peroxide value was observed from day 40 to 80, although this increase was more for the control sample than the others. The maximum permissible peroxide value for chocolate is 2%, and the values obtained up to the 40th day have been within the standard range. In Rossini *et al*. (2011) study the peroxide value in chocolate increased over time for all samples and the highest value was observed for the sample without antioxidant at 28°C after 10 months of storage (Rossini *et al.*, 2011)

The amount of peroxide is directly related to the amount of free fatty acids (FFA). Because FFA are highly susceptible to oxidation, the amount of FFA must be considered when it comes to product stability and shelf life. Table 3 shows the FFA content of chocolates containing different amount of basil seeds powder at 1, 40, 80, and 120th days of storage. As can be seen, there is a statistically significant difference (P<0.05) in FFA content, and this factor was significantly affected by the addition of basil seeds powder. According to the Table 3, FFA decreased with increasing percentage of basil seeds powder in formulation, so that the lowest acidity was found in the sample containing 10% basil seed powder and the highest value was for the control sample. Also, in all samples, FFA content increased over time and during storage, although this increase was much more evident in the control sample than the other treatments. The maximum FFA content allowed in chocolate according to the Iranian national standard is 1.5%, and all samples showed the values within the standard range.

Addition of basil seeds powder to the chocolate formulation had a significant effect (P<0.05) on the hardness of chocolates. By increasing the percentage of basil seeds powder in the chocolate formulation, the average hardness of the samples decreased. The highest hardness was found for the control sample on the first day of production and the lowest hardness was observed for the sample containing 10% basil seeds powder. Also, the hardness decreased over time in all treatments, regardless of the type of formulation. A very important characteristic of chocolate is its hardness and the sound it makes when broken. A decrease in hardness with the addition of seeds powder can be attributed to various factors. The composition of the formulation and particle size are main factors affecting the hardness of the chocolate samples, but the solid fat content is the most important factor in this case (Zaric *et al*., 2011). At high percentages of basil seed powder, the texture cohesion decreased due to high moisture and fiber content, resulting in less force to break the chocolate. On the other hand, due to the changes in fatty acids profile and increase in unsaturated fatty acids content, the consistency and firmness of the resulting chocolate have decreased. Any changes in the composition of the fat phase in chocolate can lead to textural changes (Beckett, 2009). Particle size is also an important parameter that influences the hardness of chocolate. As the size of the chocolate particles decreases, the chocolate's resistance to breakage increases and the texture becomes harder (Afoakwa *et al*., 2009).

The present results were in agreement with the Shah *et al.* (2010) findings who reported a decrease in the hardness of milk chocolate samples due to the addition of stevia extract as a sweetener and inulin and polydextrose (Shah *et al*., 2010). Bitaraf *et al.* (2013) also reported a decrease in hardness of prebiotic dark chocolate samples due to the replacement of sucrose with inulin and sucralose (Bitaraf *et al.*, 2013).

Table 3. Comparison of average changes in total phenol content, peroxide value, free fatty acids and hardness of chocolate samples during 4 months of storage.

Parameters	Storage time (days)	Control	5% basil seeds powder	7.5% basil seeds powder	10% basil seeds powder
Total phenol (Gallic acid equivalents, mg/g)	0	253.33 ± 0.05^{Ac}	261.00 ± 0.01^{Ac}	294.00 ± 0.02^{Ab}	312.66 ± 0.02^{Aa}
	40	250.00 ± 0.00^{Ac}	260.33 ± 0.05^{Ac}	290.00 ± 0.02^{Ab}	300.00 ± 0.02^{Ba}
	80	233.00 ± 0.01^{Bd}	240.00 ± 0.01^{Bc}	270.00 ± 0.00	290.00 ± 0.00^{Ca}
	120	220.00 ± 0.05^{Cc}	240.00 ± 0.0^{Bc}	256.66 ± 0.05^{Cb}	280.33 ± 0.00^{Da}
Peroxide values (meq O_2/kg oil)	0	1.82 ± 0.25^{Ba}	1.73 ± 0.25^{Db}	1.65 ± 0.15^{Dc}	1.65 ± 0.15^{Dc}
	40	1.97 ± 0.57^{Ca}	1.91 ± 0.57^{Cb}	1.85 ± 0.25^{Cc}	1.85 ± 0.25^{Cc}
	80	6.67 ± 0.36^{Ba}	5.83 ± 0.23^{Bb}	5.27 ± 0.37^{Bc}	5.27 ± 0.37^{Bc}
	120	6.84 ± 0.26^{Aa}	5.93 ± 0.15^{Ab}	5.52 ± 0.10^{Ac}	5.52 ± 0.10^{Ac}
Free fatty acids (%)	0	1.29 ± 0.01^{Ca}	1.25 ± 0.05^{Db}	1.16 ± 0.01^{Dc}	1.14 ± 0.05^{Cd}
	40	1.34 ± 0.01^{Ba}	1.27 ± 0.05^{Cb}	1.20 ± 0.05^{Cc}	1.18 ± 0.01^{Bd}
	80	1.36 ± 0.01^{Ba}	1.30 ± 0.05^{Bb}	1.25 ± 0.01^{Bc}	1.22 ± 0.01^{Ad}
	120	1.39 ± 0.01^{Aa}	1.34 ± 0.01^{Ab}	1.27 ± 0.05^{Ac}	1.24 ± 0.01^{Ad}
Hardness (N)	0	1846.16 ± 25.79^{Aa}	1739.16 ± 16.17^{Ab}	1558.66 ± 15.62^{Ac}	1352.00 ± 20.79^{Ad}
	40	1765.00 ± 33.28^{Ba}	1623.86 ± 36.51^{Bb}	1429.00 ± 18.52^{Bc}	1286.00 ± 14.10^{Bd}
	80	1630.00 ± 27.01^{Ca}	1555.66 ± 11.23^{Cb}	1352.66 ± 19.69^{Cc}	1244.66 ± 13.72^{Cd}
	120	1540.16 ± 44.75^{Da}	1474.66 ± 18.0^{Db}	1279.00 ± 28.04^{Dc}	1148.33 ± 12.89^{Dd}

*Lowercase and uppercase letters in each column and row indicate significant differences between treatments at same day and each treatment at different days, respectively (P<0.05).

3.4. Fatty acids profile analysis

Table 4 shows the fatty acids of the chocolate samples at the beginning (first day) and end (120th day) of the storage period. Chocolate enrichment with basil seed powder had a significant effect on the amounts of fatty acids. The predominant fatty acids in the control sample were lauric acid (19.65%), myristic acid (7.56%), palmitic acid (19.63%), stearic acid (27.31%), and linoleic acid (20.7%). By adding basil seeds powder to chocolate, these values changed and it was observed that the total saturated fatty acids were reduced while the amount of polyunsaturated fatty acids (PUFAs) was increased. In general, the amount of polyunsaturated fatty acids increased from 2.61% in the control sample to 6.71% in chocolate containing 10% basil seeds powder, and saturated fatty acids decreased from 76.39% to 71.07%.

Over time, sum of PUFA showed slight reduction probably due to destruction of fatty acids by oxidation process. The present findings show that incorporation of basil seeds powder in chocolate formulation improves its nutritional value regarding to essential fatty acids.

Table 4. Fatty acid composition (%) of enriched chocolates containing basil seed powder (mean ± SD) at 1st and 120th days of storage.

Storage time (days)	Fatty acids composition	Control	5% basil seeds powder	7.5% basil seeds powder	10% basil seeds powder
1	C8:0	0.74±0.07	0.63±0.05	0.71±0.03	0.70±0.02
	C10:0	0.91±0.05	0.90±0.03	0.99±0.04	0.86±0.02
	C12:0	19.65±0.75	18.38±0.15	18.17±0.25	18.1±0.45
	C14:0	7.56±0.55	7.48±0.15	7.31±0.75	6.02±0.25
	C16:0	19.63±0.45	19.19±0.75	18.95±0.75	18.92±0.15
	C16:1	0.09±0.05	0.09±0.02	0.01±0.04	0.1±0.05
	C18:0	27.31±0.15	27.28±0.45	26.06±0.45	25.86±0/75
	C18:1 Cis	20.70±0.25	21.11±0.75	21.55±0.25	21.95±0.25
	C18:1 Trans	0.21±0.05	0.18±0.05	0.17±0.04	0.17±0.07
	C18:2	2.44±0.75	2.84±0.15	3.04±0.09	3.41±0.08
	C18:3	0.17±0.03	1.31±0.15	2.38±0.05	3.3±0.03
	C20:0	0.59±0.02	0.61±0.02	0.57±0.02	0.61±0.01
	SFAΣ	76.39	74.47	72.76	71.07
	ΣPUFA	2.61	4.15	5.42	6.71
120	C8:0	0.69±0.01	0.67±0.02	0.69±0.01	0.70±0.01
	C10:0	0.95±0.01	0.90±0.03	0.93±0.02	0.85±0.01
	C12:0	19.46±0.45	18.45±0.15	19.18±0.25	17.89±0.15
	C14:0	7.61±0.15	7.29±0.15	7.28±0.25	7.14±0.15
	C16:0	19.43±0.75	19.38±0.50	18.92±0.50	18.9261±0.75
	C16:1	0.14±0.02	0.09±0.02	0.1±0.01	0.09±0.01
	C18:0	27.41±0.25	27.28±0.35	26.11±0.75	25.21±0.15
	C18:1 Cis	20.68±0.15	21.11±0.15	22.41±0.25	23.51±0.15
	C18:1 Trans	0.41±0.05	0.22±0.02	0.21±0.01	0.20±0.01
	C18:2	2.42±0.15	2.75±0.10	3.01±0.15	3.0±0.05
	C18:3	0.11±0.02	1.26±0.01	2.25±0.02	3.01±0.01
	C20:0	0.69±0.01	0.6±0.01	0.51±0.01	0.69±0.01
	ΣSFA	76.47	74.67	72.82	72.09
	PUFAΣ	2.53	4.01	5.26	6.11

3.5. Fat blooming observation

Table 5 shows the results of bloom observation in chocolate samples over a period of 4 months at ambient temperature. As shown in the Table 5, up to the 80th days, there was no difference in fat blooming between the control sample and the samples containing basil seeds powder, and all samples had a completely shiny surface without bloom, but at the end of the storage time (4 months), the fat blooming was observed on the surface of the chocolates. However, there was still no difference in bloom formation between the control sample and chocolates containing basil seeds powder up to 7.5%. With the increase in the basil seed powder to 10%, the fat blooming also increased maybe because of larger particle size and consequently the presence of pores and intermolecular distances which accelerate the movement of fat to the surface

In the chocolate industry, fat blooming appears with white bloom-like or dark-colored spots on the surface of the chocolate. Generally, this phenomenon occurs when the moisture content of the product is high or other causes involved including improper heating of the chocolate during the manufacturing process, improper cooling method, the presence of liquid fat in the chocolate, improper storage, adding fats incompatible with cocoa butter to the chocolate, hand contact with the chocolate, etc. (Lonchampt and Hartel, 2006; Trapp *et al.*, 2024).

Table 5. Comparison of fat blooming in chocolate samples during 4 months storage.

Treatment	Storage time (day)			
	1	40	80	120
Control	5	5	5	4
Chocolate containing 5% basil seeds powder	5	5	5	4
Chocolate containing 7.5% basil seeds powder	5	5	5	4
Chocolate containing 10% basil seeds powder	5	5	5	3

5 = very glossy; 4 = slightly dull, no gloss; 3 = dull, traces of bloom; 2 = partly bloomed (appearance not acceptable); and 1 = full bloom.

3.6. Overall acceptance of chocolate based on sensory evaluation

Table 6 shows the overall acceptance of chocolates after production and at the end of storage time at ambient temperature. The results showed that after production, the samples had significant differences ($P<0.05$) in overall acceptance, and the highest score was recorded for the control sample. Sensory attributes showed a lower score in all samples at the end of storage period, but the control sample still had a higher score. Adding basil seeds powder, up to 7.5%, did not make a significant difference in the acceptance of the chocolate, but by increasing the percentage of basil seeds powder to 10%, due to the perception of the taste and smell of the plant seeds, as well as the feeling of particle size in the mouth, a lower score was given to the sample.

Table 6. Evaluation of sensory characteristics of chocolate at 0 and 120 moments.

Chocolate samples	Storage time	
	0	120
Control	4.80 ± 0.87^{Aa}	4.30 ± 0.51^{Aa}
Chocolate containing 5% basil seeds powder Chocolate containing 7.5% basil seeds powder Chocolate containing 10% basil seeds powder	3.80 ± 1.08^{Ab}	3.80 ± 0.52^{Ab}
	3.50 ± 0.91^{Ab}	3.80 ± 0.48^{Ab}
	2.80 ± 0.91^{Ac}	2.70 ± 0.94^{Ac}

*Lowercase and uppercase letters in each column and row respectively indicate significant differences between treatments at same day and each treatment at different days ($P<0.05$).

4. Conclusion

Today, due to the low intake of essential fatty acids, especially omega-3, antioxidants and fiber in the diet, consumption of foods enriched with such health-promoting compounds is considered essential. Our findings showed that incorporation of basil seeds powder in chocolate, increased its fiber, protein, total phenol content, omega-3 polyunsaturated fatty acids and oxidative stability during storage. The dominant saturated fatty acids in the control chocolate sample were lauric acid, myristic acid, palmitic acid, stearic acid, but in formulation containing basil seeds powder total saturated fatty acids were reduced and the amount of polyunsaturated fatty acids (PUFAs) was increased. Adding basil seed powder to chocolate (in the amounts used in this research) increased its omega-3 content up to 6%. Also, according to the sensory evaluations, chocolates containing up to 7.5% of basil seeds powder, in addition to improving some nutritional properties, were comparable to the control sample. In this way, it was possible to produce functional chocolate with improved nutritional properties and according to consumer acceptance.

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