

Effects of fat replacing with eggplant puree on characteristics of chocolate spread

Abstract

Nowadays, the interest in searching for natural sources to fat replacer has increased to reduce obesity and the related with health risks. Chocolate spread products are the most important products of candy that are popular and more consumed, especially by children where it is a source of energy also its high nutritional value. This research aims to assess the fat replacement by eggplant puree at 25% and 75 % from the total fat content and its effects on the sensory properties, texture, chemical composition, minerals content, fatty acid composition, polyphenol compound content as tannic acid and storage stability as free fatty acid (FFA), thiobarbituric acid (TBA), total plate count (TPC) and yeasts and molds count and compared by control sample. The obtained results showed that no significant difference in sensory properties between all the products, while Chocolate spread containing 25% eggplant puree substituted were the highest in most Texture properties, the chemical composition was affected by the increasing of eggplant puree, that noted an increasing moisture, ash, dietary fiber and minerals contents while the crude ether extract and energy values were significantly ($p < 0.05$) decreased. The fatty acid profile for the three products was within the permissible limit, tannic acid values were 318.92, 401.28 and 661.46 mg/100g. Finally found that the FFA values which were ranged from 1.73% to 10.75%, TBA values which were ranged from 2.21 to 6.87 mg Malon-aldehyde/kg sample. The use of these fat replacements has improved all the properties of Chocolate spread except the storage stability.

Keywords: spread chocolate; eggplant puree; fat replacer; polyphenol; storage stability

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Introduction

Recently consumer requirements have changed not only to produce food to close the hunger gap but to eat healthy food to improve the physical and mental health of people and prevent the spread of food diseases.¹ Fat is a major dietary component for the human body and is one of the major ingredients in our daily diet.² Although the nutritional benefits are well known, the excessive consumption of fats, especially saturated types, was found to be associated with some health problems like obesity, cardiovascular and coronary heart diseases.³ Fats import many functional and sensory properties in food products which help develop texture, mouth feel, odor, taste and flavor of foods.⁴ Chocolate is one of the most favorite foods in children and adults alike. It is a rich source of energy and characterized by rapid metabolism and suitable digestion. However, it is high in fat. Fat decrease is a major challenge for producers.⁵ Chocolate spread is usually made of milk powder, sugar, fat, cocoa powder and lecithin. The spread usually uses in bread toasts and muffins.^{6,7} Fat replacer play an important role in reducing fat or calories in food and are divided into three main types carbohydrate, lipid, or protein-based fat replacers.⁸⁻¹⁰ Also pawpaw fruit puree,¹¹ avocado puree,¹² cocoa fiber¹³ and apple sauce⁴ used as natural fat replacers. Eggplant (*Solanum melongena L.*) are vegetable belonging to the Solanaceae.¹⁴ Also it considered to be one of the most species vegetables and the best, because of its high content of Antioxidant, which eliminate the harmful effects of free radical.¹⁵

Also, eggplant is an excellent source of phytochemical like phenolic compound which play an important role in diseases prevention and types of cancer and chronic diseases.¹⁶ In addition, it contains minerals such as potassium, calcium, sodium and iron.^{17,18} Also considered as good source of dietary fiber which play important to reduce the risk of cardiovascular diseases, diabetes, obesity and

certain types of cancers.¹⁹ Eggplant was chosen as the fat replacement because of its health benefits. The researchers reported that there is an opposite relationship between the amount of vegetables in products and the dominance of metabolic syndrome.²⁰ Also they knew how eggplant good affects the cholesterol absorption whereas the eggplant have a good ability to bind the salts of bile which using in metabolism of the cholesterol.²¹ The objective of the present study is to evaluate the effects of fat replacing with eggplant puree on characteristics of chocolate spread.

Materials and methods

Materials

Eggplant (*Solanum melongena L.*), skimmed milk liquid, honey with beeswax, butter, cocoa powder, and lecithin were obtained from the local market, Alexandria, Egypt. All chemicals and reagents used in this study were of analytical grade and purchased from Sigma Co.

Methods

Preparation of eggplant puree: The eggplant free of seeds were selected, it was washed with tap water to remove any soil particle and dirt. it was peeled and shredded into rings in a stainless-steel vessel a 100 ml water with 0.1% citric acid was add and boiled for 8-10 minutes at 100°C then left to cool at room temperature, then blended until smooth texture obtained and finally put in plastic bags in freezer.²²

powder was mixed

Preparation of chocolate spread: Chocolate spread was prepared in a straight mixed process according to the methods described by El-Hadad²³ with some modifications. Initially, it is mixed 100g honey,

50ml skimmed liquid milk and 0.25 g Citric acid then, the mixture was heated in water bath. 77 g butter, 3 g lecithin and 20 g cocoa powder was mixed in at low speed for 2 min using a hand blender, finally, the two mixtures were mixed at low speed for 30 min, then packaged in glass jars. The butter was replaced with 25 (17.33 g) and 75% (57.75 g) from eggplant puree with the same mixing steps that were followed in the control sample.

Sensory analysis: The three samples formulated in the present study were served to ten staff members of Special Food and Nutrition Research Department, Food Technology Research Institute, Egypt. The panelists were asked to judge for color, taste, odor, texture, and overall acceptability of chocolate spread according to standard hedonic rating scale from 9 (like extremely) to 1 (dislike extremely) according to Kramer & Twigg.²⁴

Texture profile analysis: Samples were measured by Brookfield Pro CT V1.2 Build 9. A probe TA4/1000 penetrated into each formula using speed 2 mm/s to distance 7.5 mm into each sample at two cycles, the load cell was 1000g, test speed and return speed were 2 mm/s and the force was recorded as gram.²⁵

Chemical components analysis: Proximate Chemical composition including moisture, crude ether extract, crude protein, crude fiber and total ash.²⁶

Minerals contents: Minerals (zinc, iron, magnesium, manganese, calcium, potassium, sodium and phosphorus) contents were determined by dry ash according to produce of the U.S. EPA²⁷ using an inductively coupled plasma atomic emission spectroscopy (ICP-OES).

Fatty acid composition: Preparation of fatty acid methyl esters of oils extracted from chocolate spread was performed according to the procedure of Radwan et al.,²⁸ using 1% sulfuric acid in absolute methanol. The fatty acid methyl esters obtained were separated by Shimodzu gas chromatograph (GC- 4 CM- PFE) under the following conditions: column, 10 % DEGS on 801100 chromosorb Q III; Detector temperature 270°C; flow, N₂ and chart speed, 5 min. Standard fatty acid methyl esters were used for identified action. The area under each peak was measured by the triangulation method as percentage of each fatty acid was regard to the total area.

Tannic acid content and antioxidants activity: HPLC System Agilent using to determine tannic acid in the three chocolate spread samples, in this method 10 mg sample was dissolved in 10 ml of mobile phase (Methanol: Water (50:50)) and the extract was injected in apparatus with pump LC-P-100, detector LC-UV-100 UV/VIS, detector column temperature ambient and wavelength of detection 270. The chromatographic conditions for analysis were flow rate 1mL/min, sample volume 20 µL, run time 12 min and retention time 3.1 min. The quantification of tannic acid was calculate using an absolute calibration curve method using standard solutions of tannic acid.²⁹ Radical scavenging activity of chocolate spread sample was measured using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) according to Brandwilliams et al.³⁰ The determination of DPPH scavenging for chocolate spread sample using 1mM solution of DPPH in ethanol and also 1mg/1 ml extract solution in ethanol was prepared and 1.5ml of this solution was added to 1.5 ml of DPPH. The absorbance was measured at 517 m against the corresponding blank solution which is prepared by taking 3ml ethanol and control O.D. was prepared by taking 3ml of DPPH. The assay was performed in triplicates. Percentage inhibition of free radical DPPH was calculated based on control reading by following equation

$$\text{Scavenging DPPH \%} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

Storage stability determination

FFA and TBA values analysis: According to Poonam et al.,³¹ with modification in extraction method of fat from chocolate spread sample Folish cold extraction method using to extract fat from the samples at zero time and after 3 months of cold storage. Dissolved 5g fat in 50 ml of hot neutralized alcohol in presence of 1.0 ml of phenolphthalein as indicator. Titrate the hot mixture with the standard 0.1 N NaOH solution to end point (pink color) for at least 15 seconds.

Free fatty acids (as Oleic acid), per cent by mass = $VX N \times 28.2 / M$

Where,

V = volume in ml of the standard sodium hydroxide solution used for titration

N = normality of the standard potassium/sodium hydroxide solution,

M = mass in g of the sample taken for the test

Regarding thiobarbituric acid (TBA) value was determined for the samples at zero time and after 3 months of cold storage according to Kirk & Sawyer.³² Ten g sample were distilled with 47.5 ml of distilled water + 2.5 ml of 4N HCl for 10 min, 5 ml of distilled sample was added to 5 ml of TBA solution, and then heated in boiling water bath for 35 min. After cooling, absorbance was measured at 538 min by using Spekol 11 Spectrophotometer. The TBA value was calculated as mg malonaldehyde per kg sample by multiplying the absorbance A (OD) by the factor 7.8.

Microbiological evaluation: Total plate count (TPC) was determined for the samples at zero time and after 3 months of cold storage by using pour plate method and plate count agar as medium according to ISO 8443.³³ For Yeasts & moulds were determined by plating 0.05 ml of diluted sample on potato dextrose agar (Oxoid CM) incubated for 5 days at 25 °C, yeasts & moulds colonies were counted separately according to ICMSF.³⁴

Statistical Analysis: All data of the present study were expressed as mean values \pm SD of variance (ANOVA) followed by using t- test (LSD) at $P \leq 0.05$ being considered statistically significant using (SAS) software program.³⁵

Results and discussion

Sensory analysis

The sensory properties of all formulas were ranged from extremely like to very like acceptance with no significant differences in all sensory properties between the three formulas except the color in formula 25% which was recorded lowest color score between the three samples. Meanwhile the formulas with 75% eggplant puree as a partial fat replacer from the ratio of total fat had the highest score in color, taste, odor, texture and overall acceptability between the three ones as mentioned in Table 1. Nazir & Azad,³⁶ stated that the lipid composition in chocolate product effects in sensory properties such as taste and mouth feel. Structure of fat especially, triglycerides in spread Chocolate which contain saturated fatty acids such stearic and palmetic and also oleic acid as monosaturated fatty acid are reasonable to its mouth feel evaluation. On the other hand, the sensory characteristics affects by the chocolate ingredients such as type of milk and cocoa type and also the manufacturing method which explain that sensory analysis of chocolate spread in this paper may

differ due to the difference in the percentage of fat content, ingredients and the processing method. As well Hussein³⁷ observed that there no differences between the acceptability of cakes made with vegetable puree up to 75% and control cakes. Using of vegetable puree did

not accompany by an unwanted color. Whereas, the high moisture content in vegetable puree cakes were more accepted by the panelists.

Table 1 The sensory properties of chocolate spread formulas

Properties	Formulas			
	Control (100%butter)	25% Substitution	75% Substitution	LSD
Color	8.40±0.70 ^a	7.70±0.95 ^b	8.50±0.53 ^a	0.68
Taste	7.90±0.74 ^a	7.70±0.82 ^a	8.10±1.20 ^a	0.86
Odor	8.20±0.63 ^a	8.20±0.63 ^a	8.30±0.67 ^a	0.59
Texture	7.90±0.88 ^a	7.60±0.84 ^a	8.10±0.99 ^a	0.83
Overall acceptability	8.10±0.88 ^a	7.70±0.82 ^a	8.30±0.82 ^a	0.77

25% eggplant puree and 75% eggplant puree from butter in chocolate spread.

*Each value of each formula is a mean of three replicates ±SD.

Means followed by the same letter, in each row are not significantly different (ANOVA, LSD test $p \leq 0.05$).

Texture profile analysis

Spread ability of chocolate spread is the most texture property affecting its acceptance. The spread ability explained as hardness which is amount of the force using to penetrate the probe of Brookfield apparatus the sample. The spread ability has an opposite correlation with hardness value. Table 2 showed the hardness values (g) of the three experimental samples which recorded the highest hardness in cycle 1 and 2 was the sample which contain 25 % eggplant puree then 75% and finally whole butter sample. The degree of adhesiveness (mJ) was the highest in control sample then 25 % and the lowest one was 75% the degree of decrease was reached to the double between each sample that may explain the distribution of emulsion particles in each sample which affecting by samples composition, mixing technique and processing method. The springiness length (mm) was the highest in 25% followed by control and 75%, respectively. The gumminess (g) and chewiness (mJ) were the highest in substitution of 25% eggplant puree 510 g and 85.4 mJ, these values in 75% sample were 252 g and 14.8 mJ and finally, the control one values were 133g and 17.4 mJ. Figure 1 showed the deformation of samples in cycle 1 and 2 and we noticed that the deformation was the highest in 75% substitution sample and the lowest in the control one that may refer to the degree of adhesiveness in the samples.

Table 2 The texture profile analysis of chocolate spread formulas

Texture profile analysis	Formulas		
	Control (100%butter)	25% Substitution	75% Substitution
Hardness cycle 1 (g)	266	580	339
Hardness cycle 2 (g)	234	463	326
Adhesiveness (mJ)	37.4	18.5	9.1
Springiness length (mm)	7.09	8.29	5.97
Gumminess (g)	133	510	252
Chewiness (mJ)	17.4	85.4	14.8

25% eggplant puree and 75% eggplant puree from butter in chocolate spread.

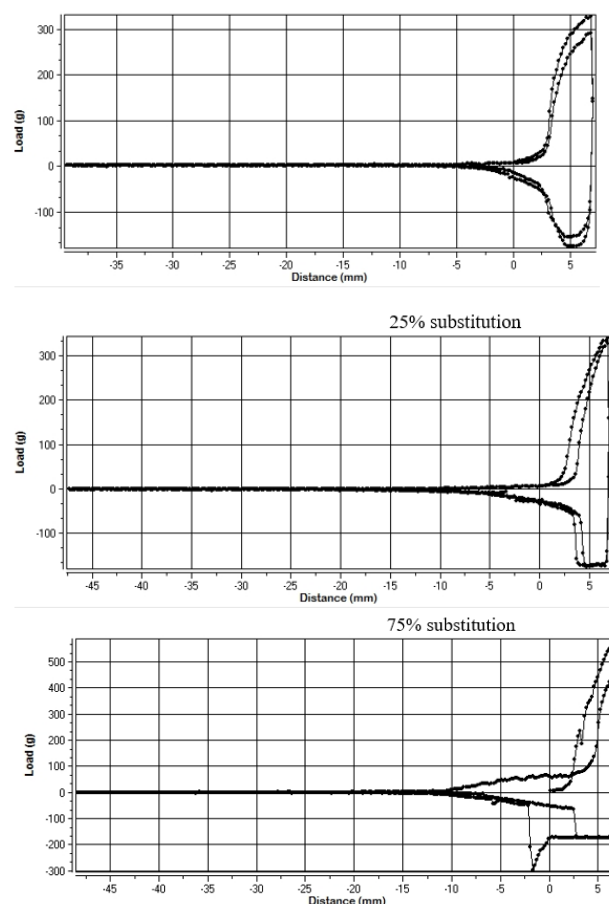


Figure 1 Deformation at hardness of chocolate spread formulas.

Fat content have been important factor that is affecting instrumental texture values in different researches, that fat is coating particles decreases attraction between them and decreased the flow in dark chocolate³⁸ and that in opposite relationship in this study which the full cream sample (control) had the highest adhesiveness that may refer to the method of processing and the degree of mixing. Full et al.,³⁹ found that spread ability and hazelnut oil content were in an

opposite relationship because the solid fat content was changed. In the same opposite relationship effect of solid fat content on hardness values was found in the chocolate spreads. In the current study when the ratio of fat decreased the hardness increased and spread ability decreased is due to the replacement the fat by eggplant puree which contain more fiber and carbohydrate. Hussein³⁷ noticed that the use of vegetable puree as a fat replacer in preparing cakes a significant increase in hardness, springiness and chewiness, but to a limited extent the cohesiveness.

Chemical components analysis and Minerals content

Table 3 illustrated the chemical composition of the samples, and it can note that the moisture contents were 30.35, 33.87 and 42.53% in control, 25% and 75% eggplant puree, respectively. These ratios in

the formulas which were contained the eggplant puree as a partial fat replacer of the animal fat were higher than the control sample and the increasing in moisture ratio was in direct proportion to the increase in the proportion of eggplant puree. This increasing in eggplant puree ratio in formulas was accompanied by decreasing in protein content, where the protein content was decreased to a third in the 75% eggplant puree formula (1.27%) with marked and desirable decreased in crude ether extract reached to 8.87% compared with same content in the control formula was 34.14%. That was decreased about 4 times in crude ether extract accompanied increased in ash, carbohydrate and crude fiber content (3.92%, 40.08% and 3.33%, respectively meanwhile decreased in energy value to 241.40 Kcal/100g. These results agree with Hussein³⁷ who reported the decrease in caloric values and fat in cake prepared with vegetable puree as a fat substitute.

Table 3 Chemical composition of chocolate spread formulas on dry weight basis.

Components (%)	Formulas			
	Control (100% butter)	25% Substitution	75% Substitution	LSD
Moisture	30.35±1.15 ^b	33.87±1.62 ^a	42.53±2.95 ^a	4.1
Crude protein	3.59±1.97 ^a	3.94±0.26 ^a	1.27±0.28 ^b	2.31
Crude ether extract	34.14±2.16 ^a	27.17±0.34 ^b	8.87±0.48 ^c	2.58
Ash	3.55±0.68 ^a	3.68±0.34 ^a	3.92±0.24 ^a	0.91
Carbohydrate*	27.54±2.95 ^b	28.45±1.75 ^b	40.08±2.70 ^a	5.03
Crude fiber	0.84±0.16 ^c	2.87±0.13 ^b	3.33±0.33 ^a	0.44
Energy value (Kcal/100g)	420.99±8.98 ^a	363.31±4.72 ^b	241.40±14.27 ^c	20.2

25% eggplant puree and 75% eggplant puree from butter in chocolate spread. Each value of each formula is a mean of three replicates ±SD. Means followed by the same letter, in each row are not significantly different (ANOVA, LSD test $p \leq 0.05$).

*Calculated by difference

Minerals content

The minerals content was recorded in Table 4 and observed that the third formulas which had 75% eggplant puree recorded the highest content in all investigated minerals (Fe, Ca, K, Na, Zn, Mg, Mn and P) then 25% eggplant puree formula and at the end. The increased in calcium and phosphor contents were little more than the double (from 194.80 to 442.55 mg/100g and from 267.88 to 542.10 mg/100g), also the increased in potassium content was the double (from 1738.70 to 2552.90 mg/100g) and this increase was not equaled with the

increased in sodium content which was 263.41 mg/100g in control formula and it was became 392.65 mg/100g in the 75% eggplant puree one. Beside there was increased in the rest minerals which near to the double. Adubofuor et al.⁴⁰ found a significant increase in magnesium, sodium and potassium contents accompanied by the level of substitution by partially dried ripe pawpaw pulp in rock buns and cakes prepared from the pawpaw pulp incorporated into wheat flour. The increasing in the inorganic minerals have good nutritional effects such as potassium which play important roles in the maintenance of normal glucose-tolerance and in the release of insulin from beta cells.

Table 4 Minerals Content of chocolate spread formulas (mg/100g) on dry weight basis

Formulas	Element							
	Fe	Ca	K	Na	Zn	Mg	Mn	P
Control (100% butter)	16.95	194.8	1738.7	263.41	3.65	133.51	1.54	267.88
25% Substitution	17.25	213.9	1750.25	254.1	3.95	146.33	1.63	251.28
75% Substitution	28.65	442.55	2552.9	392.65	5.77	226.46	2.65	542.1

25% eggplant puree and 75% eggplant puree from butter in chocolate spread.

Fatty acid composition

Table 5 illustrated the fatty acid profile of the three formulas of chocolate spread and this tables showed that the formulas were contained many short chain fatty acids such as caprylic (C8:0), capric (C10:0), undecylic (C 11:0) and tridecylic (C13:0) acids in small quantities on the other side, the control formula was not contained such fatty acids but it was contained other long chain saturated fatty acids in varying amounts starting such as myristic (C14:0), palmitic (C16:0), margaric (C17:0) and stearic (18:0) acids beside large amounts of linoleic (C18:1c, 42.88%) and linolenic (C18:2c, 17.84%) acids in cis form. The same fatty acids were found in the other two

eggplants puree formulas over more some unsaturated fatty acids such as palmitoleic (C16:1) and paullinic (C20:1) acids, the last fatty acid was appeared only in 75% eggplant puree formula (0.146%). The saturated fatty acids to unsaturated fatty acids ratios were calculated for the three formulas, they were 1:1.39, 1:1.37 and 1:1.45 for control formula which was contained 100, 75 and 25% animal butter, respectively. Kara et al.⁴¹ determined the fatty acid composition in 13 samples from 10 different brands of chocolate spread in Turkey. The samples contained 14 fatty acids, these fatty acids were palmitic acid (9.82-27.19 %), oleic acid (26.27-56.05 %) and linoleic acid (10.87-52.40 %). The saturated fatty acids (SFA) of the samples ranged from 17.01 to 32.03 %, monounsaturated fatty acids (MUFA) ranged from

19.74 to 57.00 % and polyunsaturated fatty acids (PUFA) ranged from 11.01 to 52.51 %. These results found the contents of the fatty acids in the samples correlated with legal limits. These results were relatively

agreed with our ratios of saturated and polyunsaturated fatty acids for the experimental samples of chocolate spread.

Table 5 Fatty acids composition (%) of chocolate spread formulas

Fatty acids (%)	Formulas			Fatty acids (%)	Formulas		
	Control (100% butter)	25% Substitution	75% Substitution		Control (100% butter)	25% substitution	75% Substitution
C8:0	ND	0.064	0.433	C16:1	ND	0.146	0.549
C10:0	ND	ND	0.047	C18:1c*	42.88	41.88	39.53
C11:0	ND	0.067	0.092	C18:2c	17.84	15.4	15.97
C13:0	ND	0.071	0.243	C18:3ω3**	ND	ND	0.081
C14:0	1.37	0.908	0.109	C20:1	ND	-ND	0.146
C16:0	38.37	37.45	35.09	SFAs	43.69	41.98	38.90
C17:0	0.122	0.113	0.107	USFAs	60.72	57.43	56.28
C18:0	3.83	3.31	2.78	SFAs: USFAs	1: 1.39	1: 1.37	1: 1.45
***	4.41	0.59	4.82	-	-	-	-

25% eggplant puree and 75% eggplant puree from butter in chocolate spread.

*C is isomerism,

**omega-3 fatty acids, SFAs saturated fatty acids and USFAs unsaturated fatty acids.

*** is unknown compound

Tannic acid content and antioxidants activity

As shown in Figures 2 & 3, the tannic acid content and antioxidant activity of the three samples of chocolate spread. It was found that both values increased with increasing the ratio of eggplant puree which was a good source of polyphenols which act as antioxidants. The tannic acid contents were 318.92, 401.28 and 661.46 mg/100g for control, 25% and 75% samples, respectively. That was paralleled with the antioxidant activities of the three formulas which the 75% eggplant puree formula was also the highest in its antioxidant activity (88.22%) then 25% eggplant puree formula (84.75%) and finally, the lowest one was the control chocolate spread (66.59%). Stommel & Whitaker⁴² showed that eggplant is consider one of among the ten best vegetables by its ability to absorb oxygen radicals because of its high content of phenolic compounds. Flavonoids, phenolic acids and polyphenols are the main classes of dietary phytochemicals, phenolic polymers, commonly known as tannins, are compounds of high molecular weight that are divided into classes, hydrolysable tannins and condensed tannins and they found that eggplant fruit phenolic content provide opportunities to improve eggplant quality and value and they reported that about of 64.3% to 96% of total hydroxycinnamic acids HCA was chlorogenic acid. Also, they illustrated that vegetables have antioxidant quality comparable to that of pure phenol and superior to that of antioxidant vitamin A, C and E.

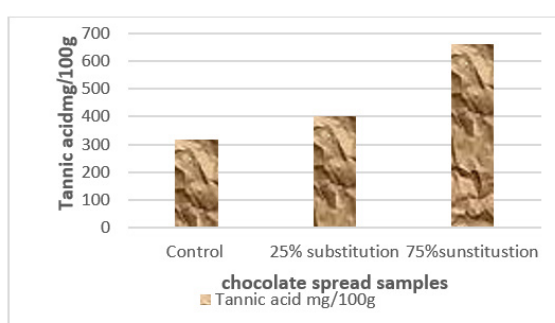


Figure 2 Tannic acid content mg/100g in samples of chocolate spread.

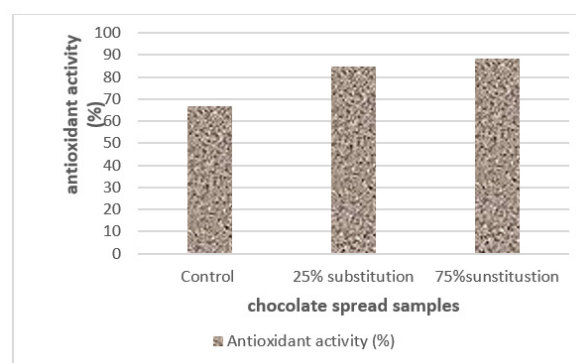


Figure 3 Antioxidant activity (%) in samples of chocolate spread.

Storage stability

Results in Table 6 showed that the different parameters for estimating the quality of prepared chocolate spread formulas in comparison between the formula which was made with animal butter and the other two formulas with 25% and 75% eggplant puree as partial fat replacer. Firstly, free fatty acids were 4.31, 5.74 and 10.57% in the control, 25% eggplant puree and 75% eggplant puree formulas, respectively after three months of storage. And these values were increased with the increasing of the percent of eggplant puree in the formula 25% and 75% to near to the double referring to increasing the amount of moisture and increasing the water activity in formula 75% eggplant puree and occurring hydrolysis of the fat and liberation of some fatty acids. Secondly, the values of thiobarbituric acid were 6.0, 6.33 and 6.87 mg Malonaldehyde/ kg in control, 25% and 75% samples, respectively. Finally, the total count and yeasts and molds was not detected in the control formula but they were detected in the two eggplant puree and there were significant differences between the both. The count of total bacteria was 14.33 and 97.00 log cfu/g for 25% and 75% formulas, respectively. On the other hand, molds and yeasts were less than 10 cfu/g after 3 months of cold storage in 25%

formulas while were more than 30 cfu/g in 75% formulas. Patange et al.⁴³ stated that FFA increased in low fat ghee-based spreads with and without potassium sorbate as preservative during storage at 5 °C

and packaged in 200 g polystyrene tubs and the rate of increase was higher in the product without the preservative where in free fatty acids increased from initial 24.0 to 32.8 μ equiv/g after 5 weeks of storage.

Table 6 Storage stability as free fatty acids (%), thiobarbituric acid (TBA as mg malon aldehyde / kg), Microbiological evaluation as total count and yeasts and molds (cfu/g) of chocolate spread formulas.

Components	Formulas						
	Control (100%butter)		25% substitution		75% substitution		LSD
	Zero time	After 3 months	Zero time	After 3 months	Zero time	After 3 months	
Free Fatty acids (%as oleic acid)	1.73 \pm 0.22 ^c	4.31 \pm 0.42 ^b	2.58 \pm 0.24 ^b	5.74 \pm 0.63 ^b	4.51 \pm 0.15 ^a	10.57 \pm 0.42 ^a	1.59
TBA (mg Malon-aldehyde/kg)	2.21 \pm 0.18 ^b	6.00 \pm 2.50 ^c	2.81 \pm 0.13 ^a	6.33 \pm 0.00 ^b	2.88 \pm 0.11 ^a	6.87 \pm 0.01 ^a	0.04
Microbiological evaluation							
Total bacterial count (cfu/g)	ND*	ND*	ND*	14.33 \pm 3.06 ^b	ND*	97.00 \pm 22.00 ^a	25.62
Yeasts & moulds (cfu/g)	ND*	ND*	ND*	< 10	ND*	> 30	-

25% eggplant puree and 75% eggplant puree from butter in chocolate spread.

*ND not detected

Each value of each formula is a mean of three replicates \pm SD. Means followed by the same letter, in each row are not significantly different (ANOVA, LSD test $p \leq 0.05$.)

The rate of increase in FFA was much lower in product with potassium sorbate than the product without it, the value increasing from initial 23.6 to 29.4 μ equivalent/g after 11 weeks of storage. On the other hand, the initial TBA numbers of the chocolate spread without and with added 0.05 % (w/w) preservative potassium sorbate at 5°C products had 1.25 and 1.27 mg Malon aldehyde/kg, respectively, which increased to 1.55 and 1.62 mg Malon aldehyde/kg at the end of the respective storage periods of 5 and 11 weeks at 5 °C. Further, the TBA value remained almost unchanged during the first 2 weeks of storage in both the spreads, but tended to increase later. We noticed the FFA and TBA values in experimental samples in this paper were relatively high due to the high moisture content in the ingredient especially the eggplant puree.

The count of total bacterial and yeast & moulds was not detected at zero time for all samples, and it was increased after 3 months for 25% and 75% substitution samples to 14.33 and 97.00 cfu/g for TVC and < 10 and < 30cfu/g for yeasts & moulds, respectively. This corresponds to the microbiological acceptable standards for chocolate confectionary according to FDA (2013) was 1000 cfu/g and 10 cfu/g for yeast and molds. In ghee-based low-fat spread, there was a significant increase in the total viable count (TVC) of control spread (from 2.1 to 3.0 to 48 log cfu/g) as well as in the preservative added spread (from 2.1 to 3.2 log cfu/g) between 0 to 5 weeks of storage. Between 6 and 9 weeks of storage, the increase in TVC continued up to 4.4 log cfu/g in the spread with preservative. However, during the last two weeks of storage the count increased only slightly (from 4.6 to 4.7 log cfu/g). It was thus apparent that the preservative had little effect on TVC. The increase in the TVC with progressive storage indicated that the product with a substantial initial bacterial load provided a fairly good medium for the growth of the organisms.⁴³

These results of the current study are in agreement with those found by Amevor et al.,⁴⁴ who stated that the microbial load of the chocolate spread samples was within acceptable standards for chocolate candy according to FDA.⁴⁵ As well as TPC were ranged from 250 to 350 cfu/g which was lower than 1000 cfu/g reported in the FDA standards. Yeast and mold for the Samples were also less than 10 cfu/g. Microorganisms such as bacteria, mould and yeast multiply at a high water activity and since most confectionery products have a very low water activity (< 0.75), they are stable against microbial spoilage

and could be said to be ambient-stable products.⁴⁶ The considerably low levels of TPC and yeast and moulds of samples are indicative of proper handling of raw materials used and satisfactory processing conditions which yielded high hygienic quality of samples.⁴⁷

Conclusion

The present study was made three formulas of chocolate spread which target to minimize the ratio of fat in such product and compare two low fat formulas with third one which had a full fat ratio, the minimize of fat was by partial replacing with 25% and 75% eggplant puree from the total ratio of fat. These replacements were good for healthy food to improve the physical and mental health of people and prevent food diseases.

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Conflicts of interest

The authors declare that there was no conflict of interest.

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