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Research Article
Studies on the Application of Nano-fortified Interesterified Olein: Stearin Vegetable Butter in Biscuits Preparation and Its Nutritional Evaluation

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Abstract

Objective: This study is concerned about using nanotechnology in the preparation and fortification of vegetable butter (margarine) in order to improve the absorption and bioavailability of added nutrients and hence to increase the nutritional value of food products. **Methodology:** Olein: stearin fat blends (70:30 and 60:40 w/w) were chemically interesterified for 45 min using different conditions of temperature (60 and 80°C) and catalysts (0.6 and 0.4%NaOH:glycerol:H_2O (1:2:3 w/w) or 0.2% sodium methoxide). The interesterified blends were subjected to FTIR analysis to ensure the absence of trans acids and evaluated for their triglyceride structure and solid fat content. **Results:** The best results were recorded and the best sample was used to prepare two vegetable butter samples (speed only and 6-cycles), which were fortified with natural sources for omega fatty acids, vitamins, minerals and antioxidants using high speed and high pressure homogenizers, respectively. Transmittance Electron Microscope (TEM) images showed that the particle size of 6-cycles vegetable butter lies in the nano-range. Produced vegetable butter samples and market vegetable butter were applied to prepare biscuits which are known as a common food product. The prepared biscuits were evaluated for their chemical composition, color measurements, physical properties and sensory evaluation, then used in diet in the nutritional experiments. **Conclusion:** Results showed that applying fortified vegetable butter samples in biscuits preparation enhanced somephysical properties, taste and mouth feel score but had no effect on its chemical composition. Also, nutrition results showed that both vegetable butter samples have beneficial effects on lipid profile levels and can be protective against hyperlipedemia and in turn possible atherosclerosis.

Key words: Chemical interesterification, fat blends, nano-fortified vegetable butter, biscuits, nutritional evaluation

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Different attempts have been made in the food sector to improve food products by maximizing their nutritional value and reducing health hazards that may accompanied with different processing. In the field of fats and oils, partial hydrogenation process was an attempt to improve the physical and technological properties of vegetable oils but it was found to be responsible for the formation of trans acids that act as coronary artery disease risk factor[1]. In response, many health organizations have recommended reducing consumption of foods containing trans fatty acids[2]. So, the safe replacement of hydrogenation process is interesterification reaction due to it can manage to produce fat product with zero trans fatty acids without affecting the degree of saturation[3].

On the other hand, food fortification is a promising strategy to overcome micronutrient malnutrition that may be caused by poverty. A world health organization report[4] defined food fortification as the practice of deliberately increasing the content of essential micronutrients, such as vitamins, phytochemicals, nutrients orminerals (including trace elements), in food to improve the nutritional quality of the food supply and to provide a public health benefit with minimal health risk. More advanced techniques in food fortification is the addition of nanoparticles. The general approach is to develop nano-size carriers or nano-sized materials (nanoceuticals) in order to improve the absorption and the bioavailability of added materials[5]:

- To produce fat food product (vegetable butter) through chemical interesterification followed by nano-fortification with different natural nutrientsin order to obtain cheap, healthy and high nutritional value product
- To study the effect of the produced vegetable butter on the manufacture and different properties of biscuits which could be a good candidate for the addition of functional ingredients due to its daily consumed, long shelf-life and contain relevant level of fats[6,7]
- To study the effect of investigated vegetable butter samples on the different biological parameters through feeding rats by the produced biscuits

MATERIALS AND METHODS

Materials: Refined, bleached and deodorized palm olein (57.0 g iodine/100 g) and palm stearin (32.0 g iodine/100 g) were purchased from the Integrated Oil Company, Alexandria, Egypt; sodium methoxide was purchased from Merck (Germany). Lecithin was purchased from Extracted Oils Company, Damanhour Factory. Watercress (seeds and leaves), linseed oil, rosemary, milk, butter flavor, sugar, baking soda (baking powder) and salt were purchased from the local market. Watercress is known as natural source for volatile oil, glycosides, fiber, protein with amino acids (arginine, histidine, isoleucine, leucine, lysine, threonine, phenylalanine, methionine, tryptophan, valine, folic acid, coumarins), vitamins (A, B1, B2, B3, B5, B6, B17, C, D, E, K) and minerals (calcium, phosphorus, potassium, iron, magnesium, copper, manganese, florine, sulphur, chlorine, iodine, germanium silica, zinc[8]. Linseed oil was used as a source of omega fatty acids[9] and rosemary herb as a source of natural antioxidants[10]. Fresh wheat flour *Triticum aestivum* (72% extract) was obtained from North Cairo Flour Mills Company, Egypt. Market vegetable butter (80% palm oil derivatives and 20% palm stearin) as control sample was obtained from IFFCO Egypt Company, Suez, Egypt. All used chemicals and solvents were of highly pure grade.

Methods

Chemical interesterification process: The reaction was carried out according to Affifi[11] under nitrogen atmosphere using different weight ratios of palm olein:stearin and different catalysts stirred and heated under different temperatures for 45 min as shown in Table 1. The reaction was stopped by adding an excess of citric acid to neutralize the catalyst and then washed by warm water to remove both citric acid and catalyst. Residual water was removed using excess of anhydrous sodium sulfate, followed by decantation.

Determination of trans fatty acids: All interesterified samples were subjected to FTIR apparatus (Nexus 670 Fourier Transform Infrared spectrometer, Thermo Nicolet, USA) to detect the presence of trans fatty acids. A fixed volume (5 µL) of each sample was homogeneously and carefully spread between two KBr disks of fixed weights. The samples were measured at wave number ranged 900-1100 cm^{-1} and referenced to their own blank KBr disks[12] and the FTIR spectra was analyzed using "Omnic 5.2a" software.

Determination of triglyceride structure: Triglyceride structure of all interesterified samples and the controls were determined using High Performance Liquid Chromatography (HPLC) according to Gonzalez *et al.*[13] as follows: A 10 µL sample solution in chloroform was injected into the column,

Table 1: Interesterification conditions of olein: stearin fat blends

		Interesterification conditions (45 min)	
Sample	Fat ratio (olein:stearin)	Temperature (°C)	Catalyst
Control 1	70:30	**Non-interesterified**	
A-1	70:30	60	0.6% NaOH : glycerol: H_2O (1:2:3 w/w)
A-2	70:30	80	0.6% NaOH : glycerol: H_2O (1:2:3 w/w)
B-1	70:30	60	0.4% NaOH : glycerol: H_2O (1:2:3 w/w)
B-2	70:30	80	0.4% NaOH : glycerol: H_2O (1:2:3 w/w)
C-1	70:30	60	0.2% sodium methoxide
C-2	70:30	80	0.2% sodium methoxide
Control 2	60:40	**Non-interesterified**	
D-1	60:40	60	0.6% NaOH : glycerol: H_2O (1:2:3 w/w)
D-2	60:40	80	0.6% NaOH : glycerol: H_2O (1:2:3 w/w)
E-1	60:40	60	0.2% sodium methoxide
E-2	60:40	80	0.2% sodium methoxide

NUCLEOSI® 5 C_{18}, (250×4.6 mm ID), column temperature (40°C) using eluents; acetone: acetonitrile (50:50) and the UV detector was set at 220 nm.

Determination of Solid Fat Content (SFC): Solid Fat Content (SFC) of all interesterified and control samples were measured according to AOCS[12] Official Methods Cd 16-93b using pulse NMR (Nuclear Magnetic Resonance), apparatus model Moran SFC, Company Oxford (England). The SFC was measured in temperature range 20-40°C and the calibration and verification by standard tubes (0, 29.65 and 70.3%).

Preparation of vegetable butter using nanotechnology

Preparation of watercress oil and watercress leaves extract: watercress seeds were crushed and its oil was extracted using petroleum ether 40-60°C, the solvent was evaporated and its traces were removed using vacuum oven and stored in glass vial still used. While, watercress leaves were crushed and blended with a little amount of water and filtered to obtain filtrate contains water soluble vitamins and minerals.

Preparation of rosemary water extract: Rosemary herb was blended, soaked in distilled water overnight at room temperature then filtered to obtain natural antioxidants extract. All extracts were added to the selected interesterified sample for preparing two vegetable butter samples.

Speed only vegetable butter: The selected interesterified fat blend was weighed (220 g) and mixed with linseed oil (3 g), watercress oil (1.4 g), lecithin (0.6 g) and butter flavor (1 g). Milk (65 g), water (10 g), rosemary water extract (0.3 g) and watercress leaves water extract (0.3 g) were mixed and added to the previous mixture. The mixture was homogenized using high speed homogenizer (Ingenieurbüro CAT, M. Zipperer GmbH) at rpm 25.000 min^{-1} for 30 min produce speed only vegetable butter.

6-cycles vegetable butter: Previous processing for speed only vegetable butter followed by homogenization for six cycles was done using high pressure homogenizer IKA single piston HPH 2000/4 5SH under 1700 bar pressure.

Determination of solid fat content and particle size: The solid fat content of both vegetable butter samples (A and B) was determined according to AOCS[12] Official Methods Cd 16b-93 and their particle size was measured using the Transmittance Electron Microscope, JEM-1230, Japan.

Both samples (A and B) and market vegetable butter were applied for biscuits preparation.

Biscuits formulation and preparation: Biscuits were prepared at the Unit of Baking, Food Industries and Nutrition Division, National Research Centre, Cairo, Egypt. The dough was prepared according to Maohar and Rao[14] by mixing the vegetable butter (300 g) for 3-4 min using high speed mixer with sodium bicarbonate (4 g), ammonium bicarbonate (10 g) and sodium chloride (10 g). Wheat flour (1 kg) sieved twice and baking powder (3 g), were added and mixed for 3 min. Market vegetable butter as control sample, speed only vegetable butter and 6-cycles vegetable butter were used individually for biscuits preparation. Biscuits dough were sheeted to a thickness of 3.5 mm and baked in a special oven at 160°C for 15 min, allowed to cool at room temperature, packed in air tight plastic bags and analyzed for chemical, physical properties and sensory evaluation.

Chemical composition: All biscuits samples were analyzed for moisture, total protein, total fat, crude fiber and ash contents according to AOAC[15]. The total carbohydrate was calculated by the difference [100-(ash%+fiber%+protein %+fat%)]. Energy values (kcal) were calculated using the factors 4, 9 and 4 for each gram of protein, lipid and carbohydrate, respectively.

Surface color measurement: The surface color of biscuit samples were measured using Hunter Colorimeter model D2s A-2 (Hunter Assoc. Lab Inc., USA). Tristimulus values of the color namely L, a, b and BI were measured using the corresponding button on the colorimeter as follows:

$$X = \frac{a+1.75\ L}{5.645L+a-3.012b}$$

Where:

L = Value represents darkness from black (0) to white (100)
a = Value represents color ranging from red (+) to green (-)
b = Value represents yellow (+) to blue (-)
BI = Browning index = BI = [100 (x-0.31)] 10.72

Physical properties: Physical characteristics (diameter, thickness, spread ratio, weight, volume, specific volume and density) of different biscuits samples were measured according to AACC[16]. All determinations were carried out in triplicate.

Sensory evaluation: Biscuits samples were evaluated (after the nutritional experiment) for their appearance, texture, color, flavor, mouth feel and overall acceptability by 15 staff members of Food Industries and Nutrition Division, National Research Center, Dokki, Egypt.

Nutritional experiment: Experimental diets were prepared according to the method described by Reeves *et al.*[17], vitamin mixture was prepared according to Campbell[18], while, salt mixture was prepared according to Hegseted *et al.*[19]. The diet formula ingredients were prepared in order to make the final ratio of protein12 and 10% fat after adding biscuit samples made by speed only, 6-cycles and market vegetable butter (Table 2).

The nutritional experiments were performed in compliance with the appropriate laws and institutional guidelines of National Research Center (NRC). Eighteen normal male and female (Sprague Dowally strain) rats with an average weight 130±10 g were obtained from animal house, National Research Center (NRC).

Rats were divided into 3 groups (6 each) and housed in galvanized metal cages. Food and water and *ad libtum* were supplied for 6 weeks. All rats were adapted for three days to the control diet before starting the experiment. After 6 weeks, the experimental rats were fasted overnight (12 h) and anesthetized with diethyl ether for blood analysis. Blood samples were collected in clean dry centrifuge tubes from

Table 2: Composition of the experimental diets (g kg^{-1})

Ingredients	g kg^{-1}
Skim milk powder	300
Sucrose	100
Biscuits*	250
Salt miximum	040
Vitamins miximum	010
Cellulose	050
Choline chloride	0.25
L-.cystine	0.18
Corn starch	269.57
Total	1000

*Three different biscuits samples according to the used vegetable butter type (speed only, 6-cycles and market vegetable butter)

hepatic portal vein then centrifuged for 15 min at 3000 rpm to separate the serum, which carefully transferred into dry clean tubes and kept frozen at (-20°C) till analysis[20].

Biochemical analyses: Total lipids of blood samples were determined according to the method described by Knight *et al.*[21], phospholipids according to Trinder[22], triacylglycerides according to Bucolo and David[23], total cholesterol according to Richmond[24]. The HDL-cholesterol according to Lopes-Virella *et al.*[25], LDL-cholesterol according to Friedewald *et al.*[26], lipid peroxide according to Ohkawa *et al.*[27] and Serum VLDL-cholesterol was calculated according to the following equation:

$$\text{VLDL-cholesterol} = \text{Triglycerides}/5$$

Liver and kidney functions were determined according to Henry *et al.*[28], Young[29] and Patton[30], respectively.

Statistical analysis: The experimented data were expressed as the mean values and standard error for three replicates and statistically analyzed by performing analysis of variance technique (ANOVA) using SAS PROC GLM/STAT[31]. Differences among means were identified using Duncan's Multiple Range test.

RESULTS AND DISCUSSION

The FTIR of the controls and different interesterified samples showed that no trans fatty acids were detected in all samples under test, which means fulfilling the main target of interesterification process which became the main method for modifying fats and oils to produce plastic fats with low or even absence of trans acids and hence avoiding their bad nutritional impact on health[2]. Same results were previously found by the authors[32].

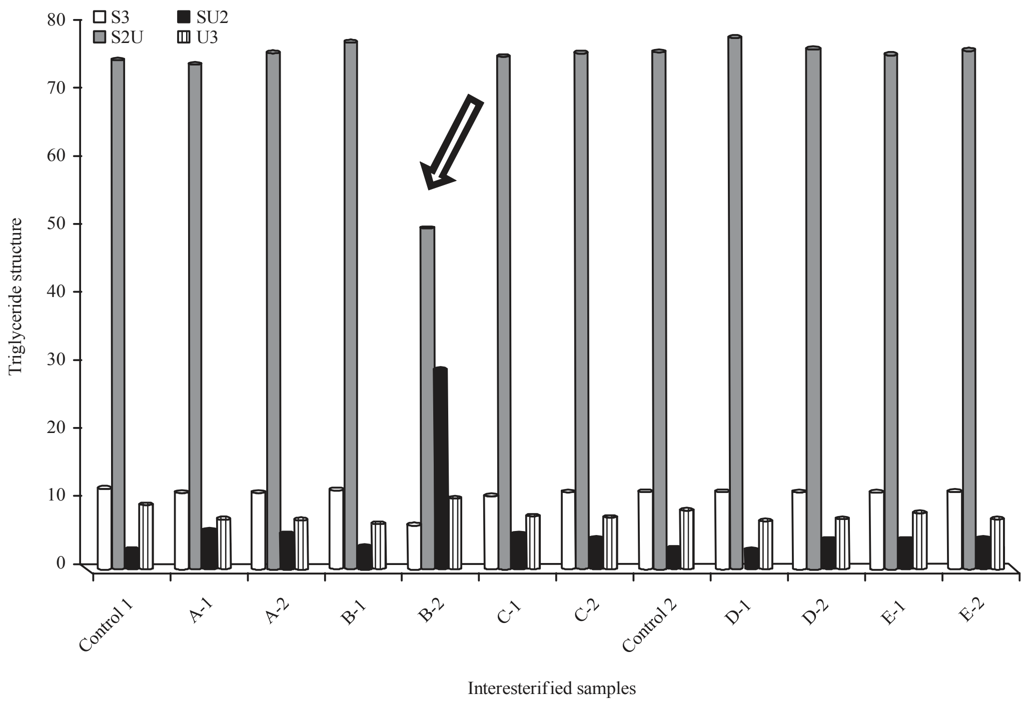

Fig. 1: Triglyceride structure of different interesterified olein: Stearinfat blends; where: S3 trisaturated fatty acid glycerides S2U: Disaturated monounsaturated fatty acids glycerides, SU2: Monosaturated diunsaturated fatty acids glycerides U3: Triunsaturated fatty acid glycerides, Control 1 and samples A-C: Interesterified olein:stearin (70:30 w/w) blends under different conditions (Table 1), Control 2 and samples D-E: Interesterified olein:stearin (60:40 w/w) blends under different conditions (Table 1)

Triglyceride structure: The triglyceride structure of all interesterified and the control samples are represented in Fig. 1. It can be noticed that chemical interesterification sharply affected the triglyceride structure of sample B-2 (interesterified at 80°C using 0.4 mL% NaOH: glycerol: H_2O (1:2:3 w/w) catalyst), which dramatically caused a decrease in the trisaturated (S_3) and disaturated-monounsaturated (S_2U) glycerides, while monosaturated-diunsaturated (SU_2) and triunsaturated (U_3) glycerides were increased compared to the controls and other interesterified samples under test. Such triglyceride structure where SU_2 predominates provides the suitable lubricity to be easily used over temperature range 5-25°C. Also, Ribeiro *et al.*[2] reported that U_2S triacylglycerols type have an important effect on food properties regarding the mouth feel and special functionality at room temperature and U_3 triacylglycerols affects the food softness as their melting points ranging 14-1°C. De Martini Soares *et al.*[33] mentioned that interesterified blends contains more than 50% palm olein shows good relation between the four different glyceride structures (S_3, S_2U, SU_2 and U_3) making these blends available for a wide variety of uses. From a nutritional point of view, fat products containing high level of unsaturated fatty acids (mono and poly) cause a decrease in plasma lipids and hence exhibit an anti-in ammatory effects on the endothelium which lead to improve the vascular function[34].

Solid fat content: Solid Fat Content (SFC) is an important parameter in the field of fat formulations and gives an indication of product characteristics like spread ability and organoleptic properties[35]. Table 3 shows solid fat content of the interesterified samples compared to the non-interesterified samples (the controls) at different temperatures.

It could be noticed from Table 3 that SFC at 20°C of the interesterified samples prepared using NaOH; glycerol: H_2O as a catalyst (A, B and D) tend to be lower than that of control samples, while it was found to be higher or unaltered in almost all samples at the remaining temperatures. On the other hand, SFC of samples prepared using sodium methoxide catalyst (C and E) showed an increase inSFC at all temperatures. Karabulut *et al.*[36] reported that the SFC at 20 and 22°C determines the product's stability and resistance to oil exudation at room temperature: Value higher than 10%

is essential to prevent oiling off. The SFC between 35 and 37°C determines the thickness and flavor release properties of the fats in the mouth. Margarines without a waxy mouthfeel containsolid fat less than 3.5% at 33.3°C and melt completely at body temperature.

The triglyceride structure and solid fat content of the different interesterified samples showed that sample B-2 (70:30 olein stearin, interesterified at 80°C for 45 min. Using 0.4 mL% NaOH: glycerol: H_2O (1:2:3 w/w) catalyst) has the best results, so it was selected to be used to prepare fortified vegetable butter samples using two techniques named speed only vegetable butter and 6-cycles vegetable butter as previously mentioned in the methods section.

Solid fat content of vegetable butter samples: Table 4 represents SFC of the produced vegetable butter made from interesterified sample (B-2) at different temperatures compared to the market vegetable butter. It can be noticed that the SFC of B-2 vegetable butter is lower than that of the market vegetable butter due to its triglyceride structure where U_2S predominates. Also, solid fat content of B-2 vegetable butter was found to be lower than that of B-2 interesterified sample (Table 3) due to the different additives added during vegetable butter processing.

Table 3: Solid fat content (%) of the interesterified blend samples at different temperatures

	SFC at different temperatures (%)				
Sample No.	20°C	25°C	30°C	35°C	40°C
Control 1	34.67	21.27	13.79	8.67	3.35
A-1	32.60	21.70	14.06	9.81	5.11
A-2	32.95	21.63	14.25	9.62	5.18
B-1	34.41	21.75	13.46	8.65	4.14
B-2	34.33	21.03	13.81	9.00	4.10
C-1	34.18	22.63	14.42	8.86	5.83
C-2	35.62	23.68	15.63	9.77	7.17
Control 2	41.56	28.19	19.21	13.01	7.71
D-1	41.75	29.32	19.06	12.98	7.86
D-2	40.53	27.17	18.49	12.45	7.64
E-1	46.26	34.72	23.14	15.86	10.07
E-2	45.83	33.15	23.13	15.14	9.90

Where: Control 1 and samples A-C: Interesterified olein: Stearin (70:30 w/w) blends under different conditions (Table 1), Control 2 and samples D-E: Interesterified olein: Stearin (60:40 w/w) blends under different conditions (Table 1)

Vegetable butter particle size: Transmittance Electron Microscope (TEM) images (Fig. 2 and 3) showed that the particle size of 6-cycles vegetable butter (B-2) sample was found to lie in the nano range which should be less than 100 nm, while the particle size of speed only vegetable butter (B-2) sample was found to be out of the nanorange (>100 nm)[37].

Chemical composition of biscuits: The chemical composition of the biscuits prepared by different types of vegetable butter (speed only and 6-cycles compared with market vegetable butter) is shown in Table 5. Results showed that butter types had no significant effect on protein, fat, crude fiber and total carbohydrate contents in different biscuits samples. Also, there were no significant differences in ash contents between the three types of vegetable butter

Table 4: Solid fat content (%) of vegetable butter samples at different temperatures

	SFC at different temperatures (%)				
Vegetable butter No.	20°C	25°C	30°C	35°C	40°C
B-2	29.00	19.60	11.80	7.00	2.40
Market vegetable butter	36.30	25.22	16.80	11.20	5.70

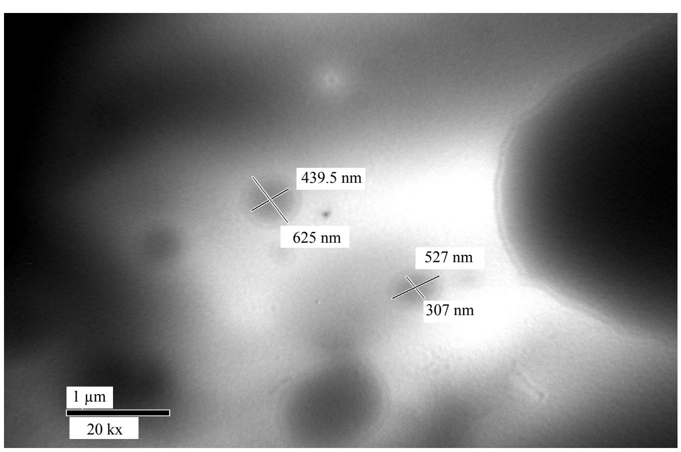

Fig. 2: TEM image of B-2 speed only vegetable butter sample

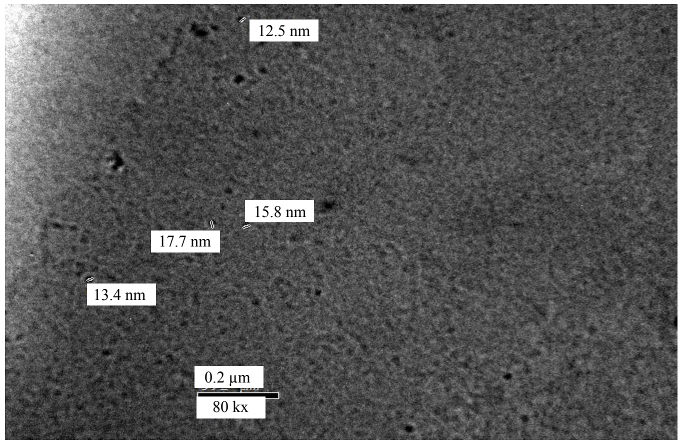

Fig. 3: TEM image of B-2 6-cycles vegetable butter sample

Table 5: Chemical composition of biscuits prepared using different types of vegetable butter

		Chemical composition					
Biscuit samples	Moisture	Protein	Fat	Ash	Crude fiber	Total carbohydrate	Value of energy Cal/100
Market vegetable butter	4.99±0.11[b]	7.39±0.20	19.13±0.44	1.03±0.03[b]	0.18±0.02	72.27±0.02	490.84±1.99
Speed only vegetable butter	5.43± 0.12[a]	7.40±0.12	19.03±0.34	1.11±0.01[a]	0.21±0.01	72.25±0.36	489.87±1.82
6-cycles vegetable butter	4.59± 0.01[c]	7.30±0.12	19.15±0.86	1.09±0.06[ab]	0.18±0.02	72.27±1.00	490.64±4.21

Means±Standard Error, means within the same column followed by the same superscripted letter(s) are not significantly different at $p \leq 0.05$

Table 6: Hunter color values of biscuits prepared from different types of vegetable butter

	Color values*			
Biscuits samples	L	a	b	ΔE
Market vegetable butter	60.24±0.73[a]	4.85±033[b]	16.85±0.24[c]	62.74±0.71[b]
Speed only vegetable butter	56.11±0.66[b]	5.87±0.21[a]	18.20±0.07[a]	59.28±0.61[c]
6-cycle vegetable butter	62.14±0.63[a]	6.21±0.31[a]	19.26±0.20[a]	70.37±0.60[a]

Means±Standard Error, means within the same column followed by the same superscripted letter(s) are not significantly different at $p \leq 0.05$, L: Lightness, a: Redness and b: Yellowness

but the significant decrease was presented between speed only and market vegetable butter. Only moisture content of the biscuit was significantly affected ($p \leq 0.05$) by the different butter types.

These results are in agreement with those obtained by Bashir *et al.*[38], Perego *et al.*[39], Mamat *et al.*[40] and Mamat[41].

Surface color of biscuits: The lightness (L), redness (a) and yellowness (b) values of different biscuit samples are shown in Table 6. Biscuits produced by 6-cycles vegetable butter had the highest significant (L) value with no significant differences ($p \leq 0.05$) with market vegetable butter biscuits and significantly higher than speed only vegetable butter which increased the darkness compared to other biscuits. The 6-cycles vegetable butter increased the redness (a) and yellowness (b) values in produced biscuits followed by speed only vegetable butter with significant differences with Market vegetable butter. These results could be due to the browning reactions in cereal products as a result of protein incorporation with the present sugar or the effects of caramelization or the Millard browning reaction[42,43]. The total color difference (E) in the biscuits was significantly ($p \leq 0.05$) decreased using speed only vegetable butter compared to the market and 6-cycles vegetable butter.

Physical characteristics of biscuits: Physical properties of biscuits prepared by speed only and 6-cycles vegetable butter compared with market vegetable butter are shown in Table 7. There was a significant difference ($p \leq 0.05$) between samples in all parameters under test except biscuits diameter and thickness. The change in width and thickness are reflected in spread ratio which was calculated by dividing the width (diameter) by thickness of the biscuit. The 6-cycles vegetable butter had significant effect on the biscuits spread ratio, weight, volume and density compared with both speed only and market butter. Maohar and Rao[14] reported that density was found to be the best index of sensory texture of biscuits.

Table 7: Physical properties of biscuits prepared by different types of vegetable butter

Biscuit samples	Physical properties Diameter (cm)	Thickness (cm)	Spread ratio	Weight (g)	Volume (cm^3)	Specific volume (cm^3/g)	Density (g cm^{-3})
Market vegetable butter	4.28±0.02	0.66±0.06	6.52±0.26^b	10.30±0.19^b	19.6±0.51^a	1.90±0.02^a	0.53±0.02^b
Speed only vegetable butter	4.48±0.09	0.66±0.06	6.83±0.27^b	11.28±0.25^a	17.8±0.03^b	1.58±0.08^b	0.63±0.09^a
6-cycle vegetable butter	4.56±0.05	0.64±0.03	7.16±0.24^a	11.59±0.43^a	19.0±0.89^a	1.64±0.03^b	0.61±0.05^a

Means±Standard Error, means within the same column followed by the same superscripted letter(s) are not significantly different at $p \leq 0.05$

Table 8: Serum lipid profiles and serum malondialdehyde (MDA) of rats fed diet containing biscuits made by different vegetable butter

Rats group No.	Diet containing biscuits made by	Total lipids (350-500)	Phospholipids (55-90)	Triglycerides* (40-200)	Total cholesterol* (120-140)	HDL-C (35-80)	LDL-C (less than 100)	VLDL-C (according to TG and TC)	(MDA) (4-8.7)
		(mg dL^{-1})							
1 M	Market vegetable butter	457.17±7.05^a	65.33±7.15^a	44.71±2.4^a	93.78±1.33^a	53.34±3.62^b	31.79±1.22	8.94±0.48	4.33±0.13
2 S	Speed only vegetable butter	428.55±11.23^b	60.67±6.19^b	42.65±0.73^b	92.95±1.46^b	54.78±1.97^a	31.2±1.64	8.56±0.14	4.43 ±0.11
3 C	6-cycles vegetable butter	421.07±7.1^b	56.00±1.69^c	40.84±1.63^c	91.07±2.15^c	55.15±3.22^a	30.7±3.43	8.27±0.399	4.35 ±0.12

Table 9: Liver and kidney functions of rats fed on different biscuits samples

Rats group No.	Diet containing biscuits made by	Liver function GOT (U mL^{-1}) (45.7-80.8)	GPT (U mL^{-1}) (0-35)	Bilirubin (mg dL^{-1}) (0.29-1.4)	Kidney function Creatinine (mg dL^{-1}) (1-2)	Urea (mg dL^{-1}) (20-40)
1 M	Market vegetable butter	63.57±0.86^a	35.00± 0.21	0.582±0.06^b	1.49±0.17^a	29.02±0.87^b
2 S	Speed only vegetable butter	60.97±0.68^c	34.67±0.15	0.651±0.78^a	1.38±0.38^b	31.88±1.27^a
3 C	6-cycles vegetable butter	62.13±3.77^b	34.67±2.42	0.646±0.425^a	1.51±0.10^a	29.63±1.78^b

Means±Standard Error, means within the same column followed by the same superscripted letter(s) are not significantly different at $p \leq 0.05$

Effect of diet containing biscuits made by different vegetable butter on serum lipid profile and serum malondialdehyde (MDA) of rats: It could be noticed from Table 8 that the total lipids content of rats fed by diet containing biscuits made by speed only or 6-cycle vegetable butter decreased significantly ($p \leq 0.05$) compared to market vegetable butter (the control) with no significant differences between them. Using 6-cycles vegetable butter to make biscuits decreased the serum phospholipids, triacylglycrides (TG) and Total Cholesterol (TC) significantly ($p \leq 0.05$) followed by speed only vegetable butter compared with the control (market butter). Also, using 6-cycles or Speed only vegetable butter decreased the LDL-cholesterol (LDL-D), VLDL-cholesterol (VLDL-C), values of rats compared to the Market butter. Regarding to serum malondialdehyde (MDA), there is no significant differences between the vegetable butter under test. Lowering effect of produced vegetable butter on serum total lipidscould be due to the presence of phenolic compounds[44,45]. Also, both produced vegetable butter contained α-linolenic acid (ALA, C18:3 n-3) rich diet which reduces hepatic lipid accumulation by stimulating β-oxidation and suppressing fatty acid synthesis[46]. In addition, linseed oil could exerted the protective effect and be a good substrate for mitochondrial and peroxisomal β-oxidation[47].

Pon and Dongmin[48] reported that cardio protective effect of flavonoids could be attributed to its antioxidant, anti-thrombogenic, lipid lowering properties and promoting endothelial function.

Effect of diet containing biscuits made by different vegetable butter on liver and kidney function of rats: Table 9 shows a decrease in the values of serum Glutamic Oxaloacetic Transaminase (GOT) significantly ($p \leq 0.05$) by using both produced vegetable butter compared with the market butter, while, Glutamic Pyruvate Transaminase (GPT) did not affected. Salemi and Pooya[49] foundan increasing (non significantly) in serum levels of GPT and GOT of rats groups fed by local margarine compared with control groups. Serum bilirubin values were changed by speed only or 6-cycles vegetable butter significantly ($p \leq 0.05$) compared with market vegetable butter.

For kidney function, Table 9 shows that biscuits made by speed only vegetable butter decreased the creatinine and increased the serum urea values significantly ($p \leq 0.05$) compared to biscuits made by 6-cycles or market vegetable butter. These results proved that dietary formulas containing speed only or 6-cycles vegetable butter are safe with regard to the liver and kidney functions.

Table 10: Serum minerals of rats fed on different biscuits samples

Rats group No.	Diet containing biscuits made by	Mg	Fe	Mn	Zn
		(µg dL^{-1})			
1 M	Market vegetable butter	1.38±0.146[c]	1.46±0.07[c]	0.15±0.01	79.85±9.09[b]
2 S	Speed only vegetable butter	1.67±0.877[b]	1.62±1.31[b]	0.15±0.02	85.66±0.495[a]
3 C	6-cycles vegetable butter	1.88±0.080[a]	1.74±0.17[a]	0.15±0.01	74.55±1.20[c]

Means±Standard Error, means within the same column followed by the same superscripted letter(s) are not significantlydifferent at $p\leq 0.05$

Table 11: Sensory evaluation of biscuits prepared by different types of vegetable butter

Biscuit samples	Sensory parameters: Appearance	Texture and crispiness	Color	Taste and mouth feel	Overall acceptability
	(10)				
Market vegetable butter	8.27±0.23	7.67±0.29[b]	7.93±0.20	8.40±0.29	7.93±0.25[b]
Speed only vegetable butter	8.73±0.27	8.76±0.25[a]	8.53±0.29	8.33±0.21	8.67±0.15[a]
6-cycles vegetable butter	8.47±0.20	8.67±0.36[ab]	8.27±0.31	8.60±0.19	8.47±0.36[ab]

Means±Standard Error, means within the same column followed by the same superscripted letter(s) are not significantly different at $p\leq 0$

Effect of diet containing biscuits made by different vegetable butter on serum minerals of rats: The values of serum minerals of rats fed diet containing biscuits made by speed only and 6-cycles vegetable butter compared with market vegetable butter are shown in Table 10. Serum levels of (Mg and Fe) for rats fed biscuits made by 6-cycles vegetable butter were increased significantly ($p\leq 0.05$) followed by speed only biscuits compared with market vegetable butter (control group). Serum level of Zn was affected by biscuits made using speed only significantly followed by biscuits market vegetable butter compared with biscuits made by 6-cycles vegetable butter. Serum levels of Mn did not changed by feeding biscuits prepared using the vegetable butter under test.

Sensory evaluation of biscuits: Sensory evaluation of biscuit samples prepared by different types of vegetable butter is presented in Table 11. There was no significant difference ($p\geq 0.05$) in the appearance of biscuit made by the vegetable butter under test. Biscuits made by speed only and 6-cycles vegetable butter obtained higher texture and color scores than biscuits made by market vegetable butter with no significant differences. Zoulias *et al.*[50] reported that texture, flavor and appearance are the main quality attributes of cookies and its fat is very important because it could be contributes to texture, pleasing mouth feel; positively impacts flavor intensity and perception.

Taste and mouth feel score of the biscuits were affected by the types of vegetable butter, 6-cycle vegetable butter obtained the highest score followed by market vegetable butter compared with speed only vegetable butter with no significant difference.

Biscuit made by speed only vegetable butter got the highest overall acceptability scores followed by biscuit made by 6-cycle vegetable butter compared to the biscuit made by market vegetable butter. Ghotra *et al.*[51] mentioned that fat functionality is very important in baked products and it is responsible for tenderness and overall texture of the final product improving mouth feel, structural integrity, incorporation of air, heat transfer and extended shelf life. Also, Rios *et al.*[52] reported that fat generally have numerous functions in bakery products such as, improve structural and sensory properties in cakes and biscuits.

CONCLUSION

This study has confirmed that chemical interesterification is the safe way to modify the physical properties and glyceride structure of a fat blend with no formation of trans acids. Palm olein and palm stearin fat blend was chemically interesterified under different conditions of catalyst, temperature and fat ratio. According to the results of triglyceride structure and solid fat content an interesterified sample was chosen to prepare two fortified vegetable butter samples (speed only and 6-cycles) to be applied in biscuits preparation. The particle size of 6-cycles vegetable butter was found to lie in the nano-range. The two types of vegetable butter had no significant effect on biscuits chemical composition except the moisture content. The 6-cycles butter had a significant effect on biscuits spread ratio, weight, volume and density compared with both speed only and market butter. Also, 6-cycle vegetable butter obtained the highest taste and mouth feel score, while, biscuits prepared by speed only vegetable butter got the highest overall acceptability score followed by 6-cycles vegetable butter compared with market vegetable butter. Nutrition results showed that using biscuits made by fortified 6-cyles and speed only vegetable butter as a diet significantly decreased total lipids profile, serum phospholipids, triglyceride, total cholesterol, LDL-cholesterol and VLDL-cholesterol. On the other hand, both diets significantly increased serum Mg and Fe. Results also proved that dietary formulas containing speed only or 6-cycles vegetable butter are safe with regard to the liver and kidney functions. So, it can be concluded that fortified vegetable butter under test have an increased nutritional value and can be protective against hyperlipedemia and in turn possible atherosclerosis.

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