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Nutritional studies on Nano-Fortified Zero Trans Vegetable Butter from Palm Olein and Stearin Interesterified Fat Blend

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Abstract: This work aims to apply nanotechnology in the preparation and fortification of zero trans vegetable butter and to nutritionally evaluate the product to ensure its safety and health beneficiary. Zero trans vegetable butter (margarine) was prepared by chemical interesterification reaction of palm olein and palm stearin in a weight ratio 70:30 using 0.6% and 0.4% NaOH : glycerol: H_2O (1:2:3 w/w) or 0.2% sodium methoxide catalysts at 80°C and 100°C for 45 minutes. The produced interesterified blends were evaluated for their basic physical and chemical properties. Three proper samples were selected and analysed for their solid fat content (SFC) and triglyceride structure (TG.S) and were subjected to FTIR analyses to make sure of the absence of trans acids. These samples were used in the preparation of vegetable butter fortified with essential fatty acids (omega fatty acids), minerals and vitamins like zinc, iron, vitamin c obtained from plant sources and natural antioxidants to preserve the product from oxidation and rancidity using nanotechnology. Transmittence Electron Microscope (TEM) images showed that vegetable butter particle size of all samples has reached the nano-range. According to the results of SFC and TG.S, interesterified sample prepared at 80°C for 45 minutes using 0.4% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst showed the best results among the other samples which means better chemical and physical properties, This sample was selected to prepare fortified vegetable butter using two different methods of preparation named speed only and 6-cycles (nano-technique). These prepared samples in addition to a market vegetable butter sample were nutritionally investigated through a nutritional experiment followed by biochemical analyses of blood samples and histopathological evaluation of rat's liver and kidney. Nutrition results showed that the tested vegetable butter samples are safe and have no health hazard effects.

Key words: Chemical Interesterification, Palm Olein, Palm Stearin, vegetable butter, nanoparticle, nano-nutrition.

Introduction

As a challenge towards the food crises, the world is seeking for a food product that can fulfill all health, safety, low price and satisfaction demands of consumers. fats and oils are one of the most important food constituents as it is the main source of energy and at the same time it has an important role in improving the palatability of foods especially the highly spread fast foods. On the other hand, high fat intake can cause obesity beside other cardiovascular disease, which may be caused by the high saturation content or trans acids content in the consumed fat.

Vegetable butter (margarine), one of the fat food products, is a vegetable oil based butter substitute formulated to overcome the high saturation content. Recently interesterification of edible fats and oils has been considered as an alternative method for hydrogenation to improve the physical properties of fats with zero or reduced trans acids which are associated with coronary heart disease, inflammation and cancer[1,2].

The production of edible fats requires fat blends that are able to impart plasticity to products such as vegetable butter and shortening. To achieve these properties, fat blends may be modified. Chemical interesterification is less expensive than enzymatic interesterification and leads to a random distribution of fatty acids on the triacylglycerols [3].

Palm oil product and their fractions (palm olein and palm stearin) are becoming important raw material and an alternative choice for food manufacturers in producing plastic fats such as vegetable butter, shortenings and fat spreads[4]. The trend nowadays is to incorporate a much liquid oil as possible into the solid fraction in order to claim the highest possible amount of polyunsaturated or monounsaturated fatty acids following current nutritional recommendations[4].

Also, in order to increase the nutritional value of a product, fortification was done to incorborate some external value added materials like vitamins, minerals, antioxidants …etc. It was reported by Fulekar[5] that developing nano-size carrier or nano-sized materials (nanoceuticals and food supplements) improve the absorption and hence, potentially the bioavailability of added materials such as vitamins, phytochemicals, nutrients or minerals.

So, this work aims to fulfill the following points :

a- Using chemical intersesterification with different conditions for the preparation of a fat blend based on palm olein and palm stearin (70: 30 w/w) suitable for vegetable butter formulation.

b- According to the evaluation results, the proper interesterifeid samples were selected and used in the preparation of vegetable butter fortified with different neutaceuticals.

c-Using nanotechnology in the preparation and fortification process to utilize its benefits in increasing the absorbitivity and bioavailabity.

d- Nutritionally examine the produced nano-vegetable butter on rats to determine the effect of nanoparticles on rat's health through biochemical evaluation

Materials and Methods

A. Preparation of fat blend

- Materials

- Refined, bleached and deodorized palm olein (57.0 g iodine /100g) and palm stearin (32.0 g iodine /100g) were purchased from the Integrated Oil Company.

- Sodium methoxide was purchased from Merck.

- Sodium hydroxide and glycerol were purchased from El-Nasr Pharmaceutical Chemicals Company.

- All used chemicals and solvents were of highly pure grade.

- Methods

1. Interesterification process

Interesterification process was carried out under nitrogen atmosphere in a closed system where100g of the fat blend (Olein : Stearin (70: 30 w/w)) were stirred and heated under the selected temperatures (80 and 100°C). When the reaction temperature was reached, the catalyst (0.6 ml% or 0.4 ml% of NaOH : glycerol: H_2O (1:2:3 w/w) or 0.2% sodium methoxide) was added while the blend was vigorously stirred. After the required period (45 minutes) the reaction was stopped by adding an excess of citric acid to neutralize the catalyst. The excess of citric acid and catalyst was removed by thrice warm water washings. Residual water was removed with an excess of anhydrous sodium sulfate, followed by decantation.

The regular and principal chemical and physical characteristics of interesterified blends and the produced vegetable butter were done.

2. Chemical and physical characteristics of the interesterified blend.

- Peroxide value (AV), iodine value (IV), acid value (PV) and melting point determination.

Determination of peroxide value (AV), iodine value (IV), and acid value (PV) were carried out according to AOCS Official Methods Cd 3d-63, Cc 18-80 and Cd 8-53 respectively[6]. Also, melting point was determined for the non- and interesterified blends by using electric melting point apparatus (Electrothermal 9100) according to AOCS Official Method Cc3-25[6].

3. Determination of trans acids by Fourier transform infra red (FTIR) instrument

Some selected interesterified samples were subjected to FTIR analysis on a Nexus 670 Fourier Transform Infra Red spectrometer, Thermo Nicolet, USA. The FTIR spectra were analyzed using "Omnic 5.2a" software. A fixed sample volume (5µl) of each sample was carefully and homogeneously spread between two KBr disks of fixed weights. The samples were referenced to their own blank KBr disks. For collection of the data, a DTGS detector and KBr beam – splitter were used. The measuring at wave number range 900-1100 cm^{-1} according to AOCS Official Methods Cd 14-61[6].

4. Determination of solid fat content (SFC)

Solid fat content was measured using pulse NMR (Nuclear Magnetic Resonance), apparatus model Moran SFC. Company Oxford (England). SFC was measured in temperature range 10°C - 40°C, calibration and verification by standard tubes (0, 29.65, 70.3%) according to AOCS Official Methods Cd 16b-93[6].

5. Determination of triglyceride structure by high performance liquid chromatography (HPLC).

The selected interesterified samples in addition to the control sample were analyzed for the determination of their triacylglycerols structure. High performance liquid chromatography (HPLC) instrument was employed for this estimation of triglyceride (TG) profiles of the samples. A 10µl solution of the sample in chloroform was injected into the column, NUCLEOSIL® 5 C_{18} ,(250 X4.6 mm ID); eluents: Acetone : Acetonitrile (50:50), column temperature(40°C), Detector: UV set at 220 nm. This method was carried out according to González et al[7].

The selected interesterified samples were used for vegetable butter preparation:

B-Vegetable butter preparation

- Materials

- Interesterified olein: stearin fat blend.
- Natural antioxidant extract was obtained by extracting rosemary herbs with water
- Plant extract was obtained by

  * Watercress seeds were crushed and the oil was extracted using petroleum ether 40- 60ºC, the solvent was then evaporated to obtain the oil. The traces of the solvent were removed by using vacuum oven.

  * Watercress leaves were also crushed and blended with a little amount of water and then filtered to obtain a filtrate with water soluble vitamins and minerals.

Watercress constituents are: 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).

- Lecithin was purchased from extracted oils company, Damanhour Factory.
- Linseed oil rich in omega fatty acids was purchased from Serga.
- Milk was purchased from the local market.
- Butter flavor was purchased from the local market
- Filtered tap water
- Market vegetable butter (control) was kindly obtained from EFCO Company. This vegetable butter was prepared from 80% palm oil derivatives and 20% palm stearin which indicates that control sample

compose 10% unsaturated fatty acids more than the other two samples as well as 10% saturated fatty acids less than than the other samples.

- Method of preparation using nanotechnology

For the preparation of vegetable butter using nanotechnology one may have to undergo two steps:

Step I: Using high speed homogenizer

The selected interesterified fat blend was weighed (220gm) and linseed oil (3gm), watercress oil (1.4gm), lecithin (0.6gm) and butter flavor (1gm) were added to the fat part. Milk (65gm) and water (10gm) with the addition of rosemary extract (0.3 gm) and watercress leaves water extract (0.3 gm) will comprise the 25% aqueous part. The aqueous part was then added to the fat part while homogenizing using high speed homogenizer at rpm 25.000 min^{-1} for 30 minutes.

Step II: Using high pressure homogenizer

The mixture was then homogenized under 1700 bar pressure for six cycles using high pressure homogenizer IKA single piston HPH 2000/4 5SH .

- Evaluation of the prepared vegetable butter samples

The prepared vegetable butter samples were evaluated by determining Peroxide, iodine, acid values and solid fat content (SFC) according to the previously mentioned reference[6].

- Particle size determination using Transmittance Electron Microscope (TEM)

The prepared vegetable butter samples were analyzed using Jeol transmittance electron microscope (TEM) (Japan) JEM-1230 for the microscopic study to determine the exact particle size.

According to the evaluation results one interesterified sample was selected and prepared as follows in addition to a market vegetable butter in order to carry out nutritional experiments on the following three samples

1- Market vegetable butter **(Named Market vegetable butter)(control).**

2- Fortified Vegetable butter prepared by using only high Speed homogenizer (step 1) **(Named Speed only vegetable butter).**

3- Nano-fortified vegetable butter prepared by using high Speed homogenizer (step 1) followed by high pressure homogenizer for 6 cycles (step 2) **(Named 6- cycles vegetable butter).**

These three samples were subjected to a nutritional experiment as follows:

C- Nutritional Experiment

1. Biological evaluation

- Diet preparation

Diets were prepared according to the method described by Reevese et al[8]. The vitamin mixture was prepared according to Campbell[9]. The salt mixture was prepared according to Hegsted et al[10]. The composition of the experimental diets are shown in the following table

Composition of different tested diets (g/Kg).

Ingredients	Formula(1)*Control	Formula(2)**	Formula(3)***
Skimmed Milk	300	300	300
Sucrose	100	100	100
Vegetable butter	130	130	130
Salt mix.	040	040	040
Vit. mix.	010	010	010

Cellulose	**050**	**050**	**050**
Choline chloride	**0.25**	**0.25**	**0.25**
L.cystine	**0.18**	**0.18**	**0.18**
Corn starch	**369.55**	**369.55**	**369.55**
Total	**1000**	**1000**	**1000**

These ingredients were prepared in order to make the final ratio of protein 12% and 10% fat in all diets

*Containing vegetable butter from Local Market (margarine)
**Containing vegetable butter prepared by speed only technique
***Containing vegetable butter prepared by 6-cycles technique

- Experimental animal Design

Eighteen normal male and female Sprauge_Dawely albino rats were obtained from our own colony (National Research Center) divided into 3groups (6 each) as following:

Group1 (G1): Rats fed on Formula (1) as normal control,
Group2 (G2): Rats fed on Formula (2),
Group3 (G3): Rats fed on Formula (3).

- Blood sampling

At the end of the experiment rats were fasted overnight (about 12 hrs) and anesthetized with diethyl ether. Blood samples were collected in clean dry centrifuge tubes from hepatic portal vein. All blood samples were centrifuged for 15 minutes at 3000 rpm to separate the serum. Serum was carefully separated and transferred into dry clean a bandore of tubes and kept frozen at (-20°C) till analysis[11].

- Biochemical analyses

Blood samples were used for the determination of the following parameters. Total lipids by Knight et al[12], Phospholipids by Trinder[13], Triacylglycride by Bucolo & David[14], Total cholesterol by Richmond[15], HDL-cholesterol by Lopes-Virella, et. Al[16], LDL-cholesterol by Freidwald et al[17], Lipid peroxide by Ohkawa et al[18] and Serum VLDL-cholesterol was calculated according to the following equation: VLDL-cholesterol = Triglycerides / 5[17]**.**

Liver functions and Kidney Functions were determined according to Henry et al[19], Yound[20] and Patton[21] respectively.

- Statistical Analysis:

Biochemical data were analyzed statistically using software packages namely (SPSS, version 16) according to the method of Black[22] and Snedecor & Cochran[23]

2. Histopathological Examination

Histopathological examination of organs: the rats were killed by decapitation at the end of experiment. Liver and kidney were separated from each rat and kept in glass gars with 10% formalin until histopathological analysis was performed.

Results and discussion

After interesterification using different conditions of temperature and catalysts, chemical and physical properties were determined as they are the fundamental factors in determining the application of fats and oils[1].

A. Evaluation of the interesterified fatty blends

- Determination of peroxide, iodine, acid values and melting point

It is clear from Table (1) that interesterification conditions caused a degree of oxidation and hydrolysis reflected in an increase in both peroxide and acid values respectively in almost all samples. This effect was found to be high and clear in case of using sodium methoxide as the catalyst. In spite of this increase, all peroxide and acid values remain in the allowed range of the standard specifications.

Table (1): Chemical and physical characteristics of interesterified olein : stearin fat blend (70: 30 w/w) using different conditions.

Interesterification conditions	Peroxide value	Acid Value	Iodine Value	Melting Point (°C)
*Control	0.35	0.1	51.18	49.1
at 80°C for 45 minutes 0.6 ml% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst	0.70	0.55	49.85	44.0
at 100°C for 45 minutes 0.6 ml% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst	1.61	0.42	49.36	48.3
at 80°C for 45 minutes 0.4 ml% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst	4.04	0.64	48.76	44.0
at 100°C for 45 minutes 0.4 ml% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst	3.30	0.42	48.5	44.50
at 80°C for 45 minutes 0.2 gm% sodium methoxide catalyst	7.38	0.35	49.21	49.3
at 100°C for 45 minutes 0.2 gm% sodium methoxide catalyst	7.47	0.61	50.70	51.10

*** Noninteresterified olein : stearin fatty blend (70:30 w/w) (control)**

Iodine value is a valuable characteristic in fat analyses, which measures unsaturation but does not define the specific fatty acids. Iodine values of the interesterified blends are changed by means of the proportion of hard stock in the blends. If the proportion of liquid oil in blends increases, the iodine value of blends increases. Iodine values of the fat blends were not affected by interesterification[24].

A slight decrease in iodine value was noticed after interesterification as shown in Table (1) and this may be attributed to the effect of interesterification conditions of which affected the unsaturation content.

Melting point is a parameter of significant importance for characterizing and developing interesterified fats of interesterified samples[25]. Melting point was found to decrease after interesterification process as shown in Table (1). This decrease in melting point was found to be clear in samples prepared by using NaOH : glycerol:H_2O (1:2:3 w/w) catalyst in comparison with those prepared using sodium methoxide catalyst.

From the previous results for evaluating interesterified fat blends under different conditions of temperature, time and catalyst, interesterified samples prepared at 80°C were selected taking into consideration mild results (i.e saving time and energy). These samples were anlaysed for the determination of trans fatty acids, solid fat content and triglyceride structure.

- Determination of trans fatty acids of the interesterified blends

FTIR charts shown in Figures (1-4) of the selected interesterified fat blends in addition to control olein: stearin (70:30 w/w) fat blend demonstrated that no trans acids were detected in interesterified samples. This ensures that chemical interesterification is a successful process for the production of zero trans acids products.

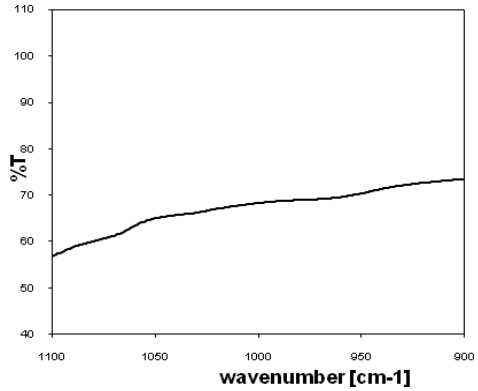

Figure (1): FTIR chart in the range 900-1100 cm^{-1} of control olein: stearin (70: 30 w/w) fat blend

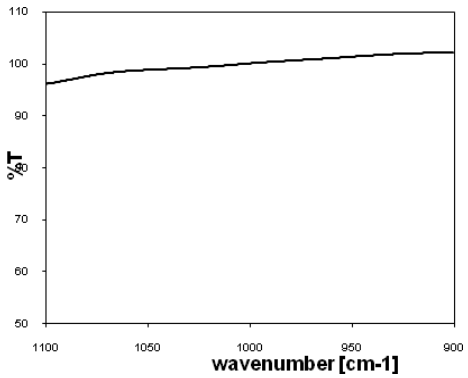

Figure (2): FTIR chart in the range 900-1100 cm^{-1} of sample interesterified at 80˚C for 45 min. using 0.6 ml% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst

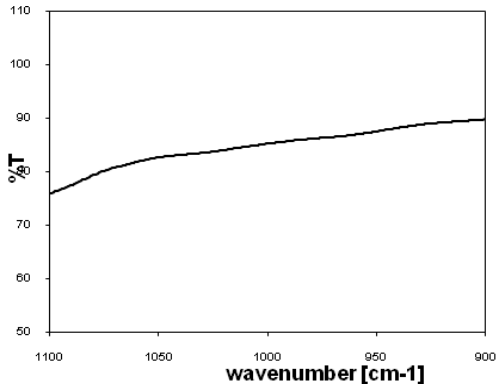

Figure (3): FTIR chart in the range 900-1100 cm^{-1} of sample interesterified at 80˚C for 45 min. using 0.4 ml% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst

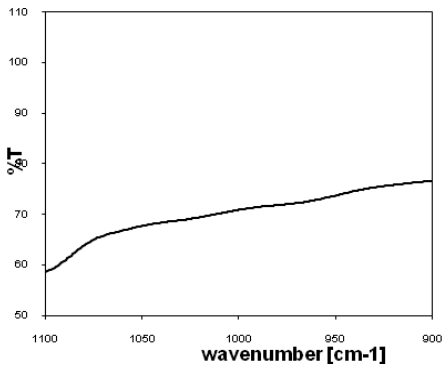

Figure (4): FTIR chart in the range 900-1100 cm^{-1} of sample interesterified at 80˚C for 45 min. using 0.2 gm% sodium methoxide catalyst

- Determination of triglyceride structure by using high performance liquid chromatography (HPLC)

Analysis of triacylglycerol composition represents a true indication of randomization, and is extremely useful for monitoring modification of interesterified fats and outlining specific applications for them[25]. The selected interesterified samples in addition to control samples were subjected to the determination of their triglyceride profile and the results are tabulated in Table (2). For food product formulation, the physical properties of a fat are more easily interpreted when triacylglycerols are designated by their degree of unsaturation: S_3 (trisaturated), S_2U (disaturated–monounsaturated), U_2S (monosaturated–diunsaturated) and U_3 (triunsaturated), instead of by the individual triacylglycerol species[1]. Chemical interesterification distributes fatty acids equally through the three positions of the glycerol backbone[1]. Table (2) shows a reduction in trisaturated (S_3) and triunsaturated (U_3) triacylglycerol contents of all samples, except the sample prepared by using 0.4 ml% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst where there is an increase in its triunsaturates,. On the other hand, disaturated-monounsaturated (S_2U) and monosaturated-diunsaturated (SU_2) triacylglycerols increased also except for the sample prepared by using 0.4 ml% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst

where SU_2 highly increased and S_2U highly decreased. These changes are due to the probabilistic distribution of fatty acids in the triacylglycerols.

Table (2): Triglyceride structure of interesterified olein : stearin fatty blend (70: 30 w/w) at 80°C for 45 minutes using different catalysts.

Catalyst	Triglyceride structure			
	S_3	**S_2U**	**SU_2**	**U_3**
***Control**	11.98	74.91	3.00	9.49
0.6 ml% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst	11.28	75.95	5.35	7.42
0.4 ml% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst	6.62	50.22	29.43	10.63
0.2 gm% sodium methoxide catalyst	11.45	75.95	4.71	7.66

*** Non-interesterified olein : stearin fatty blend (70:30 w/w) (control)**

where:
S_3 = Trisaturated fatty acid glycerides
S_2U = Disaturated monounsaturated fatty acids
SU_2 = Monosaturated Diunsaturated fatty acids
U_3 = Triunsaturated fatty acid glycerides

Soares et al[3] demonstrated that fat blends having more than 50% of palm olein presented a good relation between trisaturated, disaturated, diunsaturated, triunsaturated triacylglycerols and increasing the possibilities of use of these fats.

According to Rodrigues & Gioielli[26], the functional properties of butters and margarines can be related to the triacylglycerol composition of the fat they contain. The S_3 triacylglycerols, with melting points from 54 to 65 ˚C, and some S_2U triacylglycerols, with melting points from 27 to 42 ˚C, are responsible for these products' structure. The U_2S triacylglycerols are important to their mouthfeel and their functionality at room temperature. The U_3 triacylglycerols, with melting points between -14 and 1˚C, contribute to food softness and offers the nutritional benefits of polyunsaturated fatty acids.

Thus, the increased U_2S content in the sample chemically interesterified by using 0.4 ml% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst is associated with enhanced technological functionality, improved sensorial properties and, therefore greater potential for applying this interesterified base in foods.

Generally, the interesterification process improved unsaturated fatty acids content in the triglyceride profile which can lower the risk of heart diseases by reducing the total cholesterol and low density lipoprotein (LDL) cholesterol levels in blood[26].

- Determination of solid fat content (SFC)

Solid fat content curves give good indications of a fat's overall behavior and are useful in formulating and developing new products. The solid fat content is responsible for many characteristics of margarines and shortenings, including their appearance, ease of packaging, spreadability, oiling-out and organoleptic properties[25]. The solid fat content (SFC) of both original and interesterified fat blends is displayed in Table (3). It was mentioned by da Silva et. al.[1] that SFC values at temperatures between 33 and 38˚C influence "mouth feel' or waxy sensations that will be exhibited by the fat. The SFC at 35˚ C is particularly important for table margarines, because this property is related to the extent of melting that takes place in the mouth. Table (3) shows that the samples had similar SFC profile. Also it can be noticed that SFC of all samples was found to slightly increase at almost all temperatures especially at 35 and 40 °C. This could be attributed to the changes happened in the triacyglycerol composition of the fats after interesterification[3]. The SFC profile of the sample prepared using 0.4 ml% NaOH : glycerol: H2O (1:2:3 w/w) catalyst was found to be the most suitable one.

Table (3): Solid Fat Content (SFC) of interesterified Olein : Stearin fatty blend (70: 30 w/w) at 80°C for 45 minutes using different catalysts.

Catalyst	SFC at different temperatures				
	20 °C	**25 °C**	**30°C**	**35°C**	**40°C**
***Control**	34.67%	21.27%	13.79%	8.67%	3.35%
0.6 ml% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst	32.95%	21.63%	14.25%	9.62%	5.18%
0.4 ml% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst	34.33%	21.03%	13.81%	9.00%	4.10%
0.2 gm% sodium methoxide catalyst	35.62%	23.68%	15.63%	9.77%	7.17%

*** Non-interesterified olein : stearin fatty blend (70:30 w/w) (control)**

B. Evaluation of the prepared vegetable butter samples using nanotechnology

- Chemical Characteristics of vegetable butter samples:

Table (4): Chemical characteristics of selected interesterified olein : stearin fat blends at 80°C for 45 minutes using different catalysts in addition to control olein : stearin blends (70:30 w/w) and their vegetable butter samples

Catalysts	PV of Intere. Sample	PV of its vegetable butter	AV of Intere. Sample	AV of its vegetable butter	IV of Intere. Sample	IV of its vegetable butter
***Control**	0.35	1.03	0.1	0.54	51.18	50.63
0.6 ml% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst	0.70	3.03	0.55	1.07	49.85	54.09
0.4 ml% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst	4.04	1.20	0.29	0.51	49.21	49.59
0.2 gm% sodium methoxide catalyst	7.38	3.20	0.35	0.75	49.21	52.21

*** Non-interesterified olein : stearin fatty blend (70:30 w/w) (control)**

Table (4) shows the chemical characteristics of the selected interesterified fat blends in addition to control olein : stearin blend (70:30 w/w) and their vegetable butter samples. It can be noticed that peroxide values of decreased after vegetable butter preparation for all samples may be due to the addition of different constituents causing ratio differences. Acid values of all vegetable butter samples were slightly increased than those of the interesterified fat blend samples. This is may be due to the presence of water in the vegetable butter causing a slight fat hydrolysis. Finally, iodine values of vegetable butter were generally increased than those of the interesterified fat blend samples in accordance to the presence of the added linseed oil in the vegetable butter samples.

In general, all of the previous values lie in the range of the standard specifications.

- Solid Fat Content of the selected vegetable butter samples

The selected vegetable butter samples were analyzed for their solid fat content and results are recorded in Table (5).

Table (5): Solid Fat Content (SFC) of the selected olein : stearin vegetable butter samples in addition to a market vegetable butter sample.

Interesterification Conditions	SFC at different temperatures (%)				
	20 °C	**25 °C**	**30°C**	**35°C**	**40°C**
at 80°C for 45 min. using 0.6 ml% NaOH : glycerol: H_2O (1:2:3) catalyst	24.40%	16.01%	15.30%	6.92%	3.73%
at 80°C for 45 min. using 0.4 ml% NaOH : glycerol: H_2O (1:2:3) catalyst	29.00%	19.60%	11.80%	7.00%	2.40%
at 80°C for 45 min. using 0.2 gm% sodium methoxide catalyst	27.66%	18.50%	12.25%	8.06%	4.50%
Market vegetable butter (80% palm oil derivatives: 20% palm stearin)	36.30%	Not measured	16.80%	11.20%	5.70%

By comparing solid fat content of the selected interesterified fat blends before (Table 3) and after vegetable butter preparation (Table 5) we may report that the solid fat content of vegetable butter in particular at 30 and 35°C were lower than those of the interesterified fat blends. This issue is corresponding to the presence of liquid phase in the produced vegetable butter. This decrease will give a better vegetable butter product without a waxy mouth feel and an enhanced melting at body temperature. On the other hand, market vegetable butter have solid fat content higher than that of the interesterified fat blends and vegetable butter samples as shown from the tables.

- Particle size determination

Transmittence Electron Microscope (TEM) images showed that the particle size of all of the nano-fortified vegetable butter samples lies in the nanometer range as shown in Figures (5-7), This realizes the preparation of vegetable butter in the nanometer range (1-100 nm).

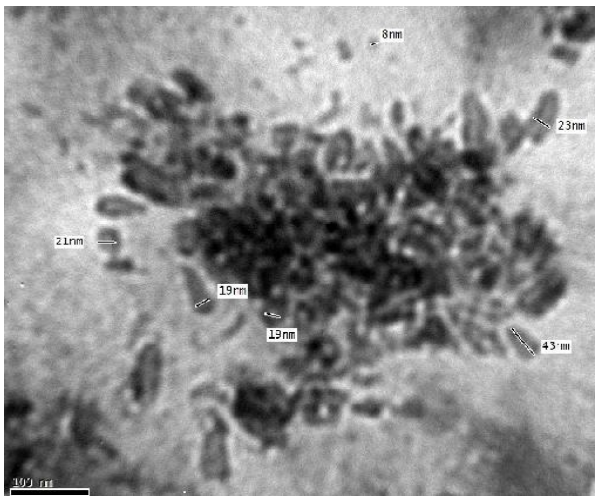

Figure (5): TEM image of vegetable butter sample prepared from the interesterified blend using 0.6 ml% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst at 80˚C for 45 min.

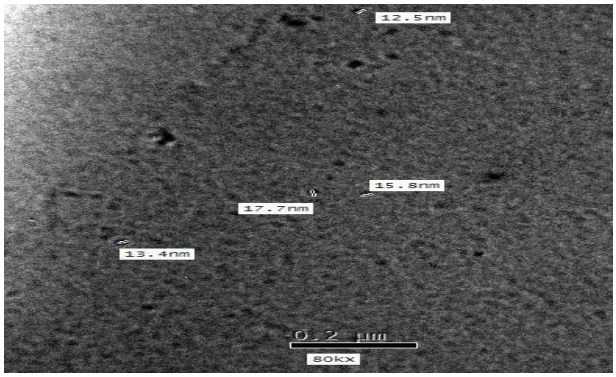

Figure (6): TEM image of vegetable butter sample prepared from the interesterified blend using 0.4 ml% NaOH : glycerol: H_2O (1:2:3 w/w) catalyst at 80˚C for 45 min.

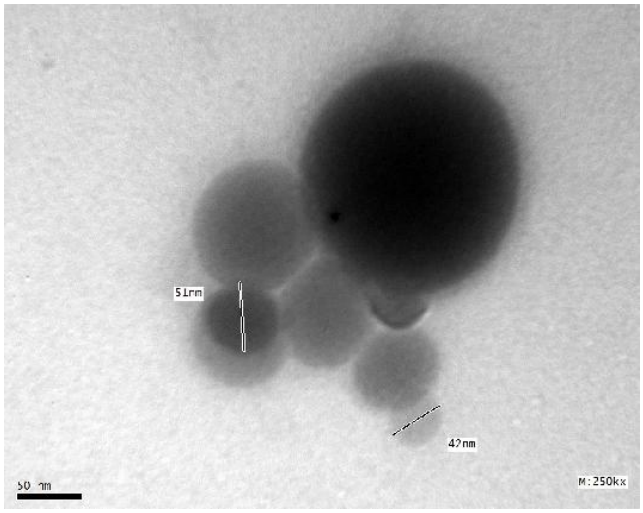

Figure (7): TEM image of vegetable butter sample prepared from the interesterified blend using 0.2% sodium methoxide at 80˚C for 45 min.

From the previous evaluations the sample interesterified using 0.4 ml% NaOH : glycerol: H_2O (1:2:3 w/w) was found to be the most suitable one, regarding its triacylglecerol composition and solid fat content, to be used in vegetable butter preparation which was done by using two ways as follows:

* Using only high Speed homogenizer **(Named Speed only vegetable butter) (Formula 2).**

* High Speed homogenizer (step 1) followed by high pressure homogenizer for 6 cycles (step 2) **(Named 6-cycles vegetable butter) (Formula 3).**

- **In addition to**

* Market margarine **(Named Market vegetable butter)(control) (Formula 1).**

These three samples were subjected to nutritional experiments and blood samples were analysed as follows

C. Nutritional Experiment

1. Biological Evaluation

- Effect of feeding vegetable butter prepared by speed only or 6-cycles technique on serum lipid profile of rats compared with local market margarine

These formulas contain food sources such as (rosemary herbs, watercress and linseed oil) that are rich sources of a variety of nutrients; including vitamins, trace minerals, dietary fiber, omega-3 fatty acids, phenolic compounds and many other classes of biologically active compounds[27-29].

The effects of feeding vegetable butter whether prepared by speed only (formula 2) or 6-cycles technique (formula 3) on serum total lipid (T.L.),triglycerides (T.G.), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), phospholipids and malondialdehyde (MDA) of rats fed tested diets compared with local market margarine as control were studied.

Table (6): Serum lipid profiles and serum Malondialdehyde (MDA) of rats fed different formulas

Rats Group No.	Tested diet	Total lipids mg/dL	Phospholipids mg/dL	Triglycerides mg/dL	Total cholesterol g/dL	HDL-C mg/dL	LDL-C mg/dL	VLDL-C mg/dL	(MDA) mmol/dL
1	Formula(1) as control	414.32	67.83	44.9	94.09	52.04	33.43	9.74	4.47
		26.88	6.37	3.16	3.42	2.03	1.52	0.704	0.18
	Mean								
	SD SE	10.98	2.6	1.29	1.397	0.828	0.62	0.29	0.071
2	Formula (2)	353.13	57.17	42.38	92.29	53.61	32.01	8.68	4.15
	Mean	17.85	9.06	2.83	7.87	10.33	4.69	0.51	0.41
		7.29	3.7	1.57	3.21	4.22	1.92	0.208	0.17
	SD	1.805	0.486	0.3	0.059	0.366	0.29	0.161	0.887
	SE tp	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
3	Formula (3)	366.62	79.17	40.73	91.77	55.61	31.6	8.34	3.53
	Mean	4.02	7.88	2.97	5.59	5.61	4.61	0.44	0.33
		1.64	3.22	1.21	2.28	2.29	1.88	0.18	0.13
	SD	1.099	1.739	1.229	0.493	1.057	0.086	1.176	6.169
	SE tp	P< 0.005	P< 0.01	N.S	N.S	N.S	N.S	N.S	P <0.0005

HDL-C High density lipoprotein-cholesterol VLDL-C Very low density lipoprotein-Cholesterol
LDL-C Low density lipoprotein-cholesterol

The results obtained are shown in Table (6). As shown in the table the total serum lipid content of rats fed on formula (2) was not significantly different from the value obtained for control rats, while rats fed on formula (3) showed significantly lower value than control. The values obtained were 353.13±7.29, 366.62±1.64 mg/dl for rats fed on formula (2 and 3) respectively compared to a value of 414.32± 10.98 mg/dl for control group.

From the same table it can be noticed that no significant difference between the values of serum phospholipids of rats fed on formula (2) and that of control group, while formula (3) showed significantly higher values than control group. The values obtained were 57.17± 3.7, 79.17± 3.22 respectively, compared to a value of 67.83 ± 2.6 mg/dl for control group.

With regard to (T.G.), (TC), (LDL), (VLDL), the values obtained for rats in both formula (2 and 3) were lower than that of control group ,however, that difference was not significant, while the level of serum high density lipoprotein (HDL) showed higher value than control but not significant.

Concerning serum malondialdehyde (MDA) of rats fed on formula (3) showed a significant reduction of MDA, while formula (2) showed no significant difference as compared to control group. The value obtained were 3.53± 0.13.4.15 ± 0.17 respectively compared to a value of 4.47± 0.071/dl for control group.

In this concern, Rashed et al.[30] also reported that, rats fed parsley and pepper showed a significant reduction in plasma total lipids, TC, LDL-c, TG and the ratio of TC /HDLC in different degrees, while HDL-c significantly increased.

The lowering effect of vegetable butter on serum cholesterol can be attributed to the presence of phenolic compounds. In this respect, Pon & Dongmin[31] demonstrated that phenolic compounds possess the bioactivity to beneficially affect the cardiovascular risk factors such as lipoprotein oxidation.

Moreover, α-Linolenic acid (ALA, C18: 3 n-3) rich diet reduces hepatic lipid accumulation both by stimulating β-oxidation and by suppressing fatty acid synthesis[32]. Ide et al.[33] reported that Linseed oil (LO) could have exerted its protective effect probably as a better substrate for mitochondrial and peroxisomal β-oxidation. All these mechanisms may account for the better regulation of hepatic lipid metabolism by LO.

Our results are in agreement with Glen[34] who found that dietary saturated fatty acids (SFAs) are not associated with CAD and other adverse health effects.

On the other hand, Smit LA et al[35] found that Consumption of industrial trans fatty acids (ITFA) increases LDL cholesterol, decreases HDL cholesterol, and is strongly associated with a higher risk of cardiovascular disease (CVD).

- Effect of feeding vegetable butter prepared by speed only or 6-cycles technique on liver and kidney function of rats compared with local market margarine

Table (7): Liver function and Kidney function of rats fed different formulas

Rats Group No	Tested Diet	Liver function			Kidney function	
		GOT U/L	GPT U/L	Bilirubin mg/dL	Creatinine mg/dL	Urea mg/dL
1	Formula(1) as control					
	Mean	64.09	32.17	0.584	1.64	30.94
	SD	5.79	4.07	0.153	0.25	1.74
	SE	2.36	1.66	0.063	0.1	0.71
2	Formula (2)					
	Mean	64.31	27.83	0.663	1.29	30.31
	SD	5.54	2.56	0.052	0.25	2.75
	SE	2.26	1.05	0.021	0.1	1.12
	t	0.068	2.207	1.194	2.384	0.474
	p	N.S	P< 0.05	N.S	P<0.025	N.S
3	Formula (3)					
	Mean	56.58	29.83	0.646	1.53	32.33
	SD	11.04	2.32	0.148	0.24	3.61
	SE	4.51	0.946	0.06	0.099	1.48
	t	1.476	1.22	0.707	0.734	0.849
	p	N.S	N.S	N.S	N.S	N.S

GOT Glutamic Oxaloacetic Transaminase GPT Glutamic Pyruvate Transaminase

The values of serum Transaminases, namely Glutamic Oxaloacetic Transaminase (GOT) and Glutamic Pyruvate Transaminase(GPT) as well as Bilirubin are shown in Table (7) The values obtained for (GOT) of rats fed on both formula whether prepared by speed only, formula (2) or by 6-cycles technique, formula(3) showed no significant difference as compared to control group. As regard to (GPT), rats fed on formula (2) showed significantly lower value than control, while formula (3) was lower than that of control, however that difference was not significant. The values obtained were 27.83± 1.05, 29.83± 0.946respectively, compared to a value of 32.17± 1.66 mg/dl for control group From the same table it can be noticed that no significant changes were observed for serum Bilirubin whether prepared by speed only, formula (2) or by 6-cycles technique, formula (3) as compared with control group.

In this respect Zahra and Samaneh[36] studied the effect of margarine fed to rats on liver enzyme activities. They found that serum levels of AST and ALT in margarine groups were higher than control groups, but it was not significant. This result suggests that consumption of margarine can be safely given to patient suffering from liver disease, but it may be toxic at high ratio of diet for hepatic patients and can cause liver damage.

The kidney functions which indicated by the determination of urea and creatinine were investigated to find out if there is a side effect of tested diets on the experimental rats as a result of using different technology for preparing vegetable butter.

As shown in Table (7) it can be noticed that rats fed on both formula, whether prepared by speed only (formula 2) or by 6-cycles technique (formula 3), showed no significant difference for urea relative to control group.. As regard to creatinine, rats fed on formula (2) showed significantly lower value than control, while formula (3) was lower than that of control, however that difference was not significant. The value obtained were 1.29± 0.1, 1.33± 0.09 respectively compared to a value of 1.64± 0.1 /dl for control. It is worth mentioning that the two formulas recorded the normal range of Creatinine as compared to control group according to Reference range list[37].

The experimental studies proved that these dietary formulas are safe with regard to the liver and kidney

- Effect of feeding vegetable butter prepared by speed only or 6-cycles technique on serum minerals of rats compared with local market margarine

Table (8): serum minerals of rats fed different formulas

Rats Group No.	Tested Diet	Mg µg/dL	Fe µg/dL	Mn µg/dL	Zn µg/dL
1	Formula(1) as control	2.06	1.46	0.14	79.48
	Mean	0.34	0.26	0.019	22.42
	SD SE	0.15	0.12	0.008	10.03
2	Formula (2)	1.84	1.52	0.14	82.78
	Mean	0.51	0.16	0.023	11.61
	SD	0.23	0.074	0.009	5.19
	SE	0.802	0.435		0.119
	tp	N.S	N.S	N.S	N.S
3	Formula (3)	1.74	1.6	0.15	83.44
	Mean	0.42	0.22	0.025	4.35
	SD	0.19	.1	0.01	1.94
	SE	1.34	0.967	0.64	0.388
	tp	N.S	N.S	N.S	N.S

The values of serum minerals of rats fed on both formulas whether prepared by speed only (formula 2) or by 6-cycles technique (formula 3) are shown in Table (8). From data recorded in this table it can be noticed that no significant difference between values obtained for the two groups compared to control group.

2. Histopathological examination

- Histopathological examination of liver and kidney of rat fed vegetable butter prepared by speed only or 6-cycles technique compared with local market Margarine

Histopathological examination of liver as shown in Figure 8 illustrated that feeding animals on local market margarine diet showed massive fatty changes outside the control area, while the central area showed normal hepatocyte. On the other hand feeding animals on 6- cycles vegetable butter diet appeared more or less normal hepatocyte as shown in Figures (9 &10). Meanwhile, histopathological examination of kidney, revealed that feeding rat on local market margarine diet showed normal histology (Figure 11), while (Figure 12) showed cystic dilation of renal tubules.

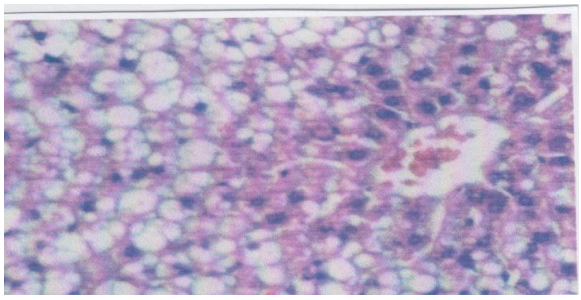

Figure (8): Liver of rat fed on market vegetable butter diet showing massive fatty changes outside the control area, while the central area appears healthy (H and E X300).

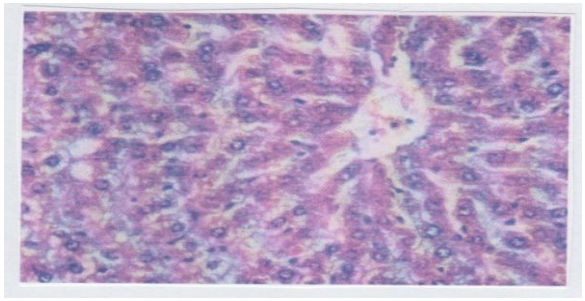

Figure (9): Liver of rat fed on 6- cycles vegetable butter diet showing central vein and hepatocytes that appear more or less like control (H and E X300).

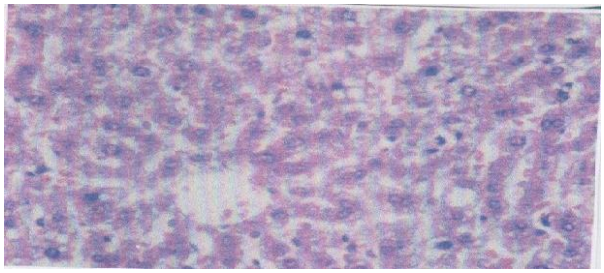

Figure (10): Liver of rat fed on speed only vegetable butter diet showing central vein and hepatocytes that appear more or less like control (H and E X300).

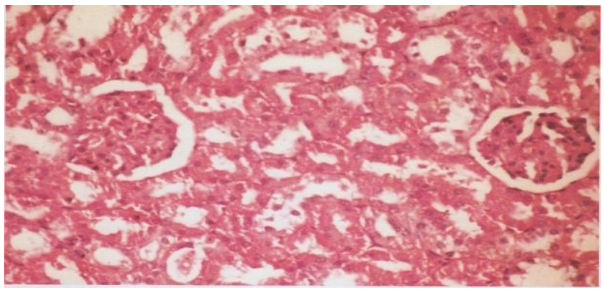

Figure (11): Kidney of rat fed on market vegetable butter diet showing no histopathological changes (H and E X400).

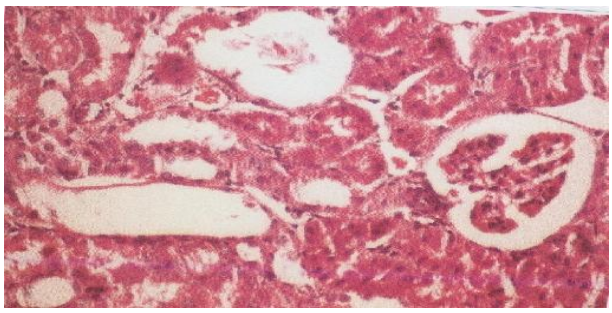

Figure (12): Kidney of rat fed on market vegetable butter diet showing cystic dilation of renal tubules (H and E X400).

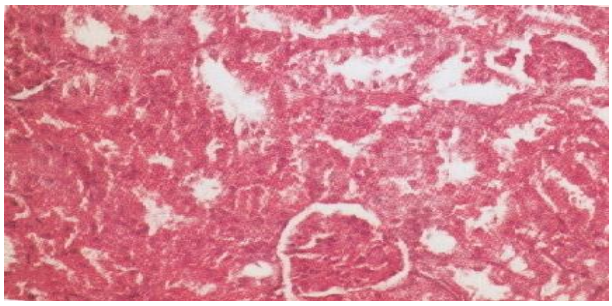

Figure (13): Kidney of rat fed on 6-cycles vegetable butter diet showing no histopathological changes (H and E X400).

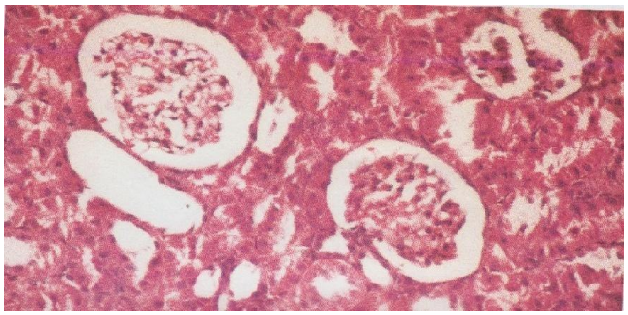

Figure (14): Kidney of rat fed on 6-cycles vegetable butter diet showing vacuolation of endothelial lining glomeruler tuft (H and E X400).

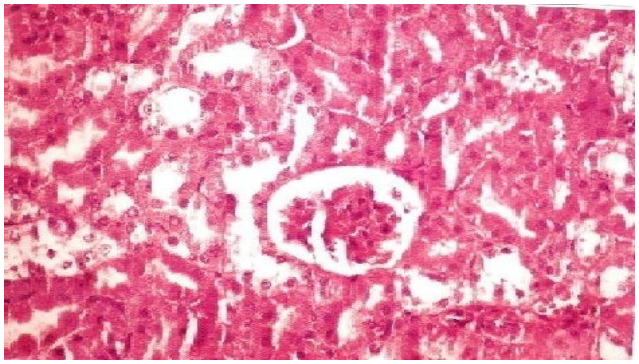

Figure (15): Kidney of rat fed on speed only vegetable butter diet showing no histopathological changes (H and E X400).

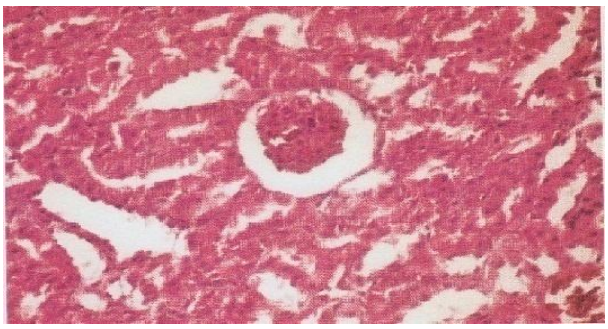

Figure (16): Kidney of rat fed on speed only vegetable butter diet showing slight distension of Bowmen's space (H and E X400).

Figure 13 illustrated that rats fed on 6-cycles vegetable butter showed no histopathological changes, while (Figure 14) showed vacuolation of endothelial lining glomeruler tuft. Also, kidney of rats fed on speed only vegetable butter diet (Figure 15) showed no histopathological changes, while (Figure 16) showed slight distension of Bowmen's space.

The histopathological studies proved that these dietary formulas are safe with regard to the liver and kidney

Conclusion

Chemical interesterification is an effective way to modify the physical properties of palm olein and palm stearin (70:30w/w) fat blend. Interesterification using NaOH: glycerol: H2O (1:2:3 w/w) as a catalyst gave better results than using sodium methoxide catalyst regarding the product stability, melting point, triglyceride structure and solid fat content. Nanotechnology is the recent approach in food fortification to enhance neutraceuticals absorption and to increase bioavailability. Fortified vegetable butter samples were managed to be prepared in the nano-size form. Nutritional experimental studies provide that tested diets containing nano-fortified vegetable butter samples are safe with regard to the liver and kidney functions as compared to control groups. Mean while, our results showed normal values of the lipid profile (T.L, T.G, total cholesterol and LDL) comparable with control group's .It is a good sign for a good health. It is worthy to mention that no histopathological changes are shown. In general, the results of evaluation confirm that these margarines formulas have no health hazard effects

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