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Formulation of Reduced Calorie and Trans-free Fat Biscuits Using Palm Oil and Sucralose: Study of Their Hypoglycemic Activity on Albino Rats

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Abstract Biscuits are one of the most popular bakery items consumed all over the world. Up to now, the most frequently formulated biscuits in the market suffered from high caloric value with unhealthy saturated fats and in some cases trans-fatty acids. Based on the consumers demand, food scientists are now focusing on developing low-calorie/high-fiber functional foods with healthy fats. In the current work, the effect of use palm oil (as fat replacer) and sucralose (as sweetener) individually or simultaneously in the formulation of wheat biscuits has been investigated. Furthermore, the use of highly nutritious germinated sweet white lupin flour(as partial replacer of wheat flour) along with incorporation of palm oil and sucralose has been studied. The formulated biscuits have been evaluated for their physical properties, proximate analysis and calorie as well as their fatty acids composition and sensory acceptability. Results indicated that the complete replacement of butter and sucrose by palm oil and sucralose did not significantly influence the spread factor of wheat and lupin biscuits. The microstructural analyses of biscuits using SEM showed an open structure consisting of gaps in between the material layers after using palm oil in the formulation. The formulated lupin biscuits with palm oil and sucralose had 15.2% lower energy and 46.7% less fat content than that of control. The saturated fatty acids content showed a significant decrease of 17% in wheat biscuits and 30.3% in lupin biscuits made with palm oil compared to the control biscuits. Furthermore, trans-fatty acids have not been detected in these biscuits. As well, the effect of treatment by formulated biscuits on the food intake, body weight and serum glucose of hyperglycemic rats has been studied. It was found that the treatment by lupin biscuits containing sucralose reduced the serum glucose of hyperglycemic rats by about 55% at the end of 4 weeks experiment. Moreover, cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-c), creatinine, aspartate amino transferase (AST) and alanine amino transferase (ALT) was decreased by 37.9%, 25.0%, 74.1%, 49.7%, 41.8% and 54.5%, respectively, while high density lipoprotein (HDL-c) was increased by 59.7% when diabetic rats treated with new formulations of biscuits. Therefore, the current study demonstrated that sensory acceptable reduced-calorie biscuits with healthy fats and hypoglycemic activity can be produced by using wheat-lupin composite flour incorporation with palm oil and sucralose. It is expected that this kind of biscuits could be recommended for the diet regimen of diabetic persons and also as nutritive foods with low calorie and healthy fats for the public.

Keywords: *low calorie biscuits, lupin, palm oil, sucralose, glucose, sensory*

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1. Introduction

Biscuits are one of the most popular bakery items consumed all over the world [1], and they can be defined as small products from a dough or batter whose mainly ingredients are flour, fat and sugar.There are numerous trials to improve the nutritive value of flour in biscuits by partial or complete replacement of wheat giving attractive results with high fiber content [20,21,32]. Still, a few of studies has been concerned with the improvement of healthy quality of fat and sugar in biscuits and their effect on the physical and sensory attributes. The most frequently used plastic fats in biscuits are margarine and butter, all of which contain high levels of saturated fatty acids (SFAs) and in some cases trans-fatty acids (TFAs) [13]. Fats act as alubricant during mixing of the biscuit ingredients; they also prevent the formation of agluten network in dough [29] where fats surrounded the proteins and the starch granules, isolating them from the water, thereby breaking the continuity of protein and starch structure. According to a study conducted in 2013, 29.5% of biscuits available in the market contained hydrogenated fat [24]. During hydrogenation, oil changes its consistency from liquid to solid. Unhealthy forms of TFA isomers may be formed as a result of this process [33]. Therefore, using of TFA free vegetable oils, which have concurrently lower SFA content, would be an interesting option for obtaining healthier biscuits.

Palm oil is an edible vegetable oil derived from the mesocarp of the fruit of the oil palms. Palm oil has a balanced fatty acid composition in which the level of saturated fatty acids is almost equal to that of the unsaturated fatty acids as well as trans-free fatty acids. Palm oil renders its solid character at room temperature in temperate regions, making it a cheap substitute for butter or trans fats in biscuits. One may argue that trans-free solid fats could be made by interesterifing blends of oils with fully hydrogenated fats. However, such processes are more expensive than just using the natural palm fats. There are also many potential hazards of hydrogenation plants and environmental pollution of catalyst waste especially in a technologically back ward nation. Moreover, Zhang *et al.* [34] showed that palm oil has the effect of decreasing total blood cholesterol and 'bad' LDL-cholesterol and increasing the level of 'good' HDL-cholesterol among hypercholesterolemic subjects. Furthermore, Sundram*et al.* [27] indicated that palm oil diet did not raise serum total cholesterol and caused a significant increase in the 'good' HDL-cholesterol and a significant reduction in 'bad' LDL-cholesterol.

In recent years, a number of artificial sweeteners such as sucralose, which are sweeter than sucrose and nontoxic, have been developed and identified to replace cane sugar or sucrose in baking products. Sucralose has gained the approval of the Food and Agriculture Organization/World Health Organization [7] after 20 years of study. Sucralose, the only non-calorie sweetener created from sucrose by the replacement of three hydrogen atoms with chlorine is about 320 to 1,000times sweeter than sucrose. It is stable under heat and over a broad range of pH conditions. Therefore, it can be used in baking or in products that require a longer shelf life. Sucralose with its sugar like taste and stability offers a significant opportunity for the baking industry to provide consumers with a new generation of great tasting, healthy and reduced-caloriebiscuits [8]. Till now, there are many challenges to improve the nutritional profile of biscuits, including a reduction in sugar content or complete replacement of cane sugar [26]. Furthermore, Literature reports support the choice of sucralose in the formulation of food that may be directed to people with diabetes. Sucralose can be beneficial for controlling glucose uptake in clinical trials in people with diabetes [14,19]. Argyri *et al.* [4] also found that the consumption of sucralose in combination with fiber source with meal by people with type 2 diabetes either did not increase postprandial levels of glucose, insulin, or C-peptide. As well, sucralose ingestion did not affect the secretion of gut hormones GLP-1, PYY, or ghrelin [10].

Fervent changes inthe quality, quantity, and source of food consumed along with a high level of mental stress and physical inactivity have led to an increase in the prevalence of hyperglycemic diseases. The recent published IDF Diabetes Atlas (Sixth edition, 2013) reports that there are382 million people living with diabetes in the world and it is expected to rise by 55% to 592 million in 2035 [1]. In order to limit the prevalence of hyperglycemic diseases, it is recommended to reduce the calorie intake through sugars and fats. The food industry has focused for the last couple of decades on the production of low-fat/low-calorie, high-fiber foods in response to public interest for these functional products. Therefore, the main objective of the current work was to formulate a functional lupin biscuits containing high fiber content with reduced calorie by a simultaneous complete replacement of butter and cane sugar. Lupin biscuits(25% wheat flour replacer), as they are a rich source of dietary fiber and phenolic compounds, have been prepared from germinated sweet white lupin (SWL) flour (Mousa, [21,22]. Sprouting of lupin grains has many nutritional benefits. The content of bioactive antinutritional factors such as alkaloids, oligosaccharides and phytate is reduced while that of beneficial isoflavones and phytosterols and some vitamins is increased. In order to reduce the calorie content of the lupin biscuit, palm oil and sucralose were tried singly and/or in combination to replace all of the butter and sucrose. The proximate chemical composition, caloric value and mineral compositions as well as physical characteristics and sensory quality attributes of biscuits formulae have been measured. As well, the hypoglycemic activity of lupin biscuits on hyperglycemic albino ratshas been investigated.

2. Experimental

2.1. Materials

Sweet white lupin grains (Lupinusalbus, composition data provided by the supplier: 36.0% protein, 30.0% dietary fiber and6.0% fat), refined wheat flour (composition data provided by the supplier: 9.8% protein, 74.0% carbohydrates, 0.8% fat,0.4% minerals, and 15.0% moisture), palm oil (composition data provided by the supplier: saponification value 198, iodine value 52, melting point 36°C, palmitic acid 42.0%, stearic acid 5.0%, oleic acid 43.0% and Linoleic acid 10.0%), butter (Cow's milk ≥ 85.0% saturated fats), cane sugar (Nile Sugar Company, Egypt, pure and melting point 186 °C), sucralose (Innova Comapny, Egypt, pure and melting point 125 °C), sodium bicarbonate, ammonium bicarbonate, skimmed milk powder (Defatted Cow's milk powder), orange (*Citrus sinensis*(L.)) peel powder, corn starch and corn oil have been procured from local market. Casein, vitamins, minerals, cellulose, alloxan and choline chloride have been purchased from El-Gomhoreya Company, Cairo Egypt. Nitric acid, perchloric acid, petroleum ether, hexane, sodium methoxide have been obtained from Fluka company Co. (Germany).

2.2. Preparation of Biscuits

In the beginning, germination of lupin grains has been performed as previously described by Mousa [21,22]. Germination has been used to improve the quality of SWL seeds by diminishing anti-nutritional factors such as phytate, trypsin inhibition, α-galactoside and alkaloids. At the end of germination, the grains have been dried under the sun. Then, the germinated SWL grains have been ground and then sieved thrice through 10 mesh to flour. Grinding has been done in Ushamixer Grinder. The obtained SWL flour has been stored in the dark at 4°C prior to use.

For the preparation of wheat biscuits, 10.0 g of butter (F1) or 7.0 g of palm oil (F2) were creamed with 20.0

gcane sugar for 3 min in a mixer at 60 rpm. The same step has been repeated by mixing butter with 5.0 g sucralose (F3) or by mixing palm oil with 5.0 g sucralose (F4). Then, 7.0 g of skim milk powder, 1.0 g of salt, 0.4 g of sodium bicarbonate, 1.5 g of ammonium bicarbonate and 5.0 g of orange peel powder have been added in water (11-27.6mL based on the components of fat and sugar) and mixed together for 8 min at 125 rpm. The wheat flour (45.0 g) has been added to the above mixture and mixed again for 3 min at 60 rpm. The dough has been sheeted to a 3.5-mm thickness and cut with a biscuit cutter (50-mmdiameter). The biscuits have been baked on an aluminium tray in an electric oven at 200°C for 5 min. They have been cooled for 30 min at room temperature and stored in low density polyethylene bags until further use.

In the case of SWL biscuits, four formulations have been prepared by the replacement of wheat flour with 25% (g/g) germinated SWL flour (11.25 g) in the presence of butter + cane sugar (F5)or palm oil+ cane sugar (F6) or butter + sucralose (F7) or palm oil+ sucralose (F8).This amount percent of SWL flour has been found to be the optimal one as described in the previous reports of Mousa [21,22].

2.3. Evaluation of Biscuits

All biscuits formulations have been evaluated for the thickness (cm), width (cm), spread ratio and spread factor. The spread ratio and spread factor were calculated according to Manohar and Rao [18]. Five biscuits have been used for the evaluations from each of the eight studied biscuits and averages have been recorded. The spread ratio and spread factor have been calculated using the following equations:

$$\text{Spread ratio} = \text{width} / \text{thickness}$$

$$\text{Spread factor} = \begin{pmatrix}\text{spread ratio} \\ \text{of sample}\end{pmatrix} / \begin{pmatrix}\text{spread ratio} \\ \text{of control sample}\end{pmatrix}.$$

Scanning electron microscopy (SEM) has been used to investigate the microstructure of baked biscuits. The samples have been mounted on individual metallic stubs and sputtered with a Balzers SCD 030 conductive coating of gold (Balzers Union LTD. Liechtenstein). Samples have been imaged using a JEOL6060LV variable pressure SEM instrument (Jeol (UK) Ltd). Electron micrographs have been produced for cross-section of each biscuit type at several different magnifications.

The standard method of AOAC [2] has been employed for proximate analysis including moisture, crude protein, crude fat, crude fiber and ash contents of the baked biscuits. Total carbohydrates have been calculated from the sum of moisture, crude protein, crude fat, ash and crude fiber, and lastly subtracting it from 100 [2]. The caloric value has been calculated using values of 4.0k.cal/g of protein, 4.0k.cal/g of carbohydrate and 9.0k.cal/g of fat according to Livesy [17]. Details of each determination in the baked biscuits have been recently published elsewhere [21].

In order to determine fatty acid composition in the studied biscuits, oil extraction from finely ground samples of biscuits has been carried out by soxhlet extraction method using petroleum ether (40-60°C) [3]. The extracted oils have been kept in an oven at 60°C for 1 h to expel solvent before storing at -20°C [29]. Before analysis, the oil samples have been removed from frozen storage, and left static at room temperature for 1 h and then warmed at 60°C until they became completely molten. Then, fatty acid methyl esters (FAME) have been prepared by dissolving 50.0 mg portion of oil in 0.8 mL of hexane and adding 0.2 mL portion of 1M solution of sodium methoxide, then analyzed on a gas chromatograph (Agillent Technologies, Germany) fitted with a FID detector. All the instrumental conditions were as described in the previous report of Yanty et al. [30]. The identification of the peaks of the samples has been done with reference to a chromatographic profile containing FAME standards (Supelco, Bellefonte, PA). The percentage of fatty acid has been calculated as the ratio of the partial area to the total peaks area.

In order to investigate the mineral content of biscuits, the main elements including sodium, potassium, magnesium, calcium, iron, copper, zinc and phosphorus have been determined based on the methods published in AOAC [2]. The samples have been firstly digested using a mixture of nitric acid and perchloric acid (HNO_3, $HClO_4$, 2:1 v/v). After the complete digestion of samples, the amounts of iron, copper, calcium, magnesium, manganese and phosphorous have been determined using atomic absorption spectrometry (AAS) (Agilent Technologies, California, U.S.A). Sodium and potassium have been determined by flame photometer (Jenway, U.K.).

2.4. Rats Experimental Design

Sixty adult male white albino rats (Sprague) weighing between 200 and 210grams have been housed as groups in wire cages under standard laboratory conditions (20-25 ºC, 50-60% humidity, 10-12 hours light/dark cycle) in Animals Department, Assiut University, Egypt. The animals have been fed one week on basal diet for adaptation in *ad-libitum*. The basal diet has been prepared according to Reeves *et al.*, [25]. It consists of casein 14.0% (protein 80%); soybean oil 4.0%, cholinechloride 0.2%, vitamin mixture 1.0%, mineral mixture 3.5%, fibers 5.0%, L-cystine0.18%, sucrose 10.0% and the reminder was corn starch.

After a period of adaptation on basal diet, the rats have been divided into two main groups. The first main group (6 rats) fed on basal diet as a negative control group (Gp1). The second main group (54 rats) injected intraperitoneally with alloxan (120.0 mg/kg/day) in sterile saline for 5 days to induce hyperglycemia in rats. Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rats. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes"), with characteristics similar to type 1 diabetes in humans [16]. After 5 days of alloxan injection, the hyperglycemic rats (glucose level > 200 mg/dL) were divided into different groups comprising of 6 rats each. Group 2 (Gp2) fed on basal diet as a positive control group. Groups 3 (Gp3), 4 (Gp4), 5 (Gp5) and 6 (Gp6) fed on basal diet containing 10%(w/w) wheat biscuits formulae F1, F2, F3 and F4, respectively. Groups 7 (Gp7), 8 (Gp7), 9 (Gp9) and 10 (Gp10) fed on basal diet containing 10%(w/w) lupin biscuits formulae F5, F6, F7 and F8, respectively. The aforementioned treatments have been started from the same day for a period of 4 weeks.

During this period, animals in all groups had free access to water.

2.5. Determination of Food Consumed, Body Weight Gain, Serum Glucose and Other Biochemical Parameters

During the experimental period (4 weeks), the diets consumed and bodyweights have been recorded twice weekly. At the end of the experiment, the animals have been fasted overnight, then the rats have been anaesthetized and sacrificed, and blood samples have been collected from the aorta. The blood samples have been centrifuged and serum has been separated to estimate serum glucose. Serum glucose level has been analyzed by colorimetric procedures kits developed by Diamond Diagnostics Kits Cairo, Egypt using 550 nm according to Tietz [28]. Serum glucose has been estimated by the enzymatic colorimetric treatment with GDD-DAP method as follows: To a 10 serum, 10 ml of the enzymatic solution at 37°C for 10 minutes has been added and the obtained color has been read at 550 nm using Beckman DU-6µ aspects photometer. Standard glucose and blank samples have been read and the value of glucose has been obtained using the following equation:

$$\text{Serum glucose}\left(\text{mg}/100\text{ml}\right)=\frac{100\text{x }\Delta\text{A}\left(\text{sample}\right)}{\Delta\text{A}\left(\text{standard}\right)}$$

where 100 = The concentration of the standard solution and ΔA = The absorbance.

Moreover, other biochemical parameters such as serum cholesterol [9], triglycerids [11], HDL-c [31], LDL-c [12], creatinine [23], aspartate amino transferase (AST) and alanine amino transferase (ALT) [31] have been quantified in the serum samples.

2.6. Sensory Analysis

All biscuits formulae have been checked for their sensory acceptability by fifty judges [21]. All of them are free from cold or sinus problem during the period of evaluation. Ten panelists have been called for sensory valuation at a time under "daylight" illumination and in isolated booths. Samples (2 biscuits of each type) have been served in paper plates identified by random three digits codes. Care has been taken to maintain the sensory environment at 25°C. Panelists have been asked to assess their degree of liking of the samples on paper ballot with a nine-points hedonic rating scale (9—like extremely, 8—like very much, 7—like moderately, 6—like slightly, 5—neither like nor dislike, 4—dislike slightly, 3—dislike moderately, 2—dislike very much, 1—dislike extremely. The panelists evaluated the samples in terms of color, appearance, aroma, texture, taste and overall acceptability. They allowed swallowing samples and they instructed to clean their palate with cold and filtered tap water before tasting each sample.

2.7. Statistical Analysis

Values have been expressed as means ± SD. Analysis of variance has been carried out using statistical software version-7 (State Soft Corporation, Tulsa, USA). The multiple response regression analysis has been carried out using Minitab statistical software.

3. Results and Discussion

3.1. Physical Characteristics of Biscuits

The mean values of physical characteristics of wheat biscuit and wheat-SWL formulated biscuits in the presence/absence of palm oil and sucralose are presented in Table 1. It is obvious that there is a gradual increment in the spread ratio values of the biscuits formulae (F5, F6, F7 and F8) compared to the reference sample (F1). On the contrary, there is no any observed difference in the spread ratio between F1 control sample and biscuits formulae (F2, F3 and F4). These observations confirmed that the partial substitution of wheat flour by 25% (w/w) germinated SWL flour enhanced the spread ratio of biscuits. In parallel, the substitution of butter by palm oil and the substitution of sugar by sucralose did not influence the spread ratio of wheat and lupin biscuits. Considering the spread factor of reference biscuit (F1, 100% wheat flour in the presence of butter and can sugar) as 100, results indicated that the values have been significantly increased from 115.7 to 129.4% within F5 to F8 by the substitution with 25% germinated SWL flour. On the other hand, the substitution of butter and sugar by palm oil and sucralose gave results of spread factor very close to the reference biscuits (F1).Moreover, the simultaneous substitution with palm oil and sucralose (F8) recorded a slight change in the spread factor compared to lupin biscuits with butter and sucrose (F5). In general, these replaced amounts of palm oil and sucralose could be used for the preparation of commercially wheat or lupin biscuits with acceptable physical characteristics.

Table 1. Physical characteristics of the studied biscuits

Sample	Width (D, mm)±SD[a]	Thickness (T, mm)±SD[a]	Spread ratio (D/T)±SD[a]	Spread factor[b]
F1 (reference)	56.0±1.5[a]	11.0±1.7[a]	5.1±0.9[a]	100.0
F2	53.0±0.9[a]	10.8±0.8[a]	4.9±1.1[a]	96.1
F3	53.3±1.2[a]	10.7±1.5[a]	5.0±0.8[a]	98.0
F4	53.4±2.0[a]	10.8±1.3[a]	5.0±1.5[a]	98.0
F5	67.7±1.2[b]	10.4±1.3[b]	6.6±0.9[b]	129.4
F6	63.4±1.7[b]	10.2±1.1[b]	6.2±1.5[b]	121.6
F7	64.2±1.6[b]	10.1±1.6[b]	6.4±1.0[b]	125.5
F8	62.9±1.1[b]	10.6±1.2[a]	5.9±0.9[b]	115.7

[a]SD: the standard deviation of 5 measurements.

[b]Spread factor = spread ratio of sample/spread ratio of reference.
Values which don’t share the same letter in each column are significantly different (p<0.05).

Scanning electron microscopy (SEM) is often employed to reveal the internal geometrical structure of a product indetail in food structure analysis. In this study, microstructural analyses using SEM have been carried out to examine and characterize the microstructure formed using palm oil in the formulation. Representative SEM images of the internal cross-sectional area of biscuits are shown in Figure 1. The biscuits showed an open structure consisting of gaps in between the material layers. The expansion of bubbles of gases as a result of increasing temperature, which also increases the water vapor pressure within them, may result in an increased tensile stress in the membrane initiating rupture and the formation of holes and tunnels in the product through which the gas finds a way to the outside [5]. No major conclusion can be drawn in relation to the resultant cross-sectional openness because the size of the sample used during measurement using SEM might contribute to the results due to only a small portion of the biscuit has been used. However, there are similarity in the porosity observed between biscuits made of palm oil, and butter, where the air cells in the structure seem to be more homogenous, uniform, and well-dispersed.

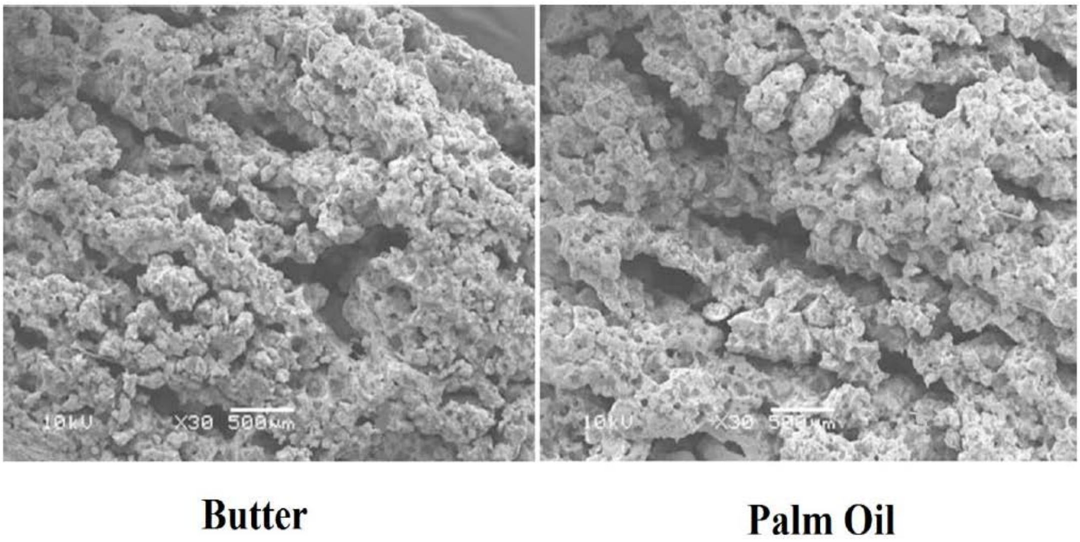

Figure 1. Scanning electron microscopy photographs showing internal structure of biscuits: Bars= 500 µm

3.2. Proximate Chemical Composition and Caloric Value of Biscuits

The mean values of proximate chemical composition and caloric values of wheat and SWL biscuits are given in Table 2. The data ofcrude protein and fiber contents revealed that the substitution of butter and sucrose simultaneously or individually by palm oil and sucralose showed a little difference in measurements among biscuits. However, the substitution of wheat flour by 25% germinated SWL flour (F5) significantly increased protein and fiber more than 2.5 and 35.5 fold increments compared to the control biscuits (F1), respectively. This result confirmed that germinated lupin flour is a rich source of protein and fiber. The reason could be attributed to the fact that during germination step the micro-organisms utilized the carbohydrate content in the sample to synthesis amino acid needed for their growth and development. These observations are in good agreement with previous reports by Mousa [21,22].

Table 2. Proximate chemical composition and caloric value (mean value± SD, n=5) of the studied biscuits

Sample	Moisture content (g/100g)	Crude protein (g/100 g)	Crude fat (g/ 100g)	Ash content (g/100 g)	Crude fiber (g/100g)	Carbohydrates (g/ 100g)	Caloric value (kcal/100g)
F1	5.3±0.1^{a}	7.0±0.1^{a}	11.9±0.1^{a}	1.5±0.2^{a}	0.2±0.1^{a}	74.1±0.1^{a}	431.5±0.1^{a}
F2	5.9±0.3^{b}	7.1±0.3a	7.3±0.2^{b}	1.6±0.2^{a}	0.2±0.1^{a}	77.9±0.2^{b}	405.7±0.1^{b}
F3	6.8±0.1^{c}	6.9 ±0.2^{a}	11.4±0.1^{a}	1.1±0.1^{b}	0.2±0.1^{a}	73.6±0.1^{a}	424.6±0.1^{a}
F4	5.9±0.2^{b}	7.1±0.4^{a}	7.4±0.1^{b}	1.0±0.1^{b}	0.3±0.2^{a}	78.3±0.2^{b}	408.2±0.2^{b}
F5	5.6±0.1^{b}	17.1±0.3^{b}	9.2±0.4^{c}	1.9±0.2^{c}	7.1±0.1^{b}	59.1±0.3^{c}	387.6±0.3^{c}
F6	5.9±0.1^{b}	17.3±0.1^{b}	5.0±0.3^{d}	1.7±0.1^{a}	7.0±0.3^{b}	63.1±0.2d	366.6±0.2^{d}
F7	7.1±0.2^{c}	17.0±0.1^{b}	9.0±0.2^{c}	1.1±0.1^{b}	7.4±0.1^{b}	58.4±0.2c	382.6±0.3^{c}
F8	6.4±0.2^{c}	17.5±0.4^{b}	4.9±0.1d	1.2±0.2^{b}	7.1±0.3^{b}	62.9±0.2^{d}	365.7±0.2^{d}

Values which don’t share the same letter in each column are significantly different (p<0.05).

In the case of moisture and ash content, the replacement of sucrose by sucralose significantly (P<0.05) increased the moisture and decreased the ash among wheat and lupin biscuits. On the other hand, the replacement of butter by palm oil increased a little bit of moisture content but did not have any effect on the ash content among wheat and lupin biscuits as indicated in Table 2. The simultaneous replacement by palm oil and sucralose in wheat biscuits (F4) increased the moisture value by 11.3% and reduced the ash value by 50.0% compared to the control wheat biscuits (F1). The same results have been recorded with lupin biscuits that the replacement by palm oil and sucralose in combination (F8) increased the moisture value by 14.3% and reduced the ash value by 58.3% compared to the lupin biscuits with butter and sucrose (F5).

The fat content has been also measured for all formulations. It was found that the fat content of F2and F6 samples has been markedly reduced by 38.7% and 45.7% by complete replacing of butter with palm oil in wheat and lupin biscuits, respectively. On the other hand, the simultaneous replacement of butter and sucrose by palm oil and sucralose in wheat biscuits (F4) and in lupin biscuits (F8) reduced the fat content down to 37.8% and 46.7% compared to control wheat biscuits (F1) and lupin biscuits (F5), respectively. These results reflected the successful achievements by formulating low fat lupin biscuits (F8). It is well known that the use of saturated fats is therefore central to biscuit technology. Fat gives biscuits their characteristic ‘melt in the mouth’ crumbly texture. The mechanism by which fat contributes this property to biscuits is by coating flour particles during the mixing of the biscuit dough. This fat coating prevents gluten from absorbing water during mixing and becoming elastic and extensible. A secondary role of fat is to coat any air bubbles present in order to enhance their stability during baking. The ability to coat bubbles to retain integrity during dough handling and baking is also best found in a fat with a developed crystalline structure such as palm oil rather than in liquid oil. Palm oilhas a melting point of about 36°C and a balanced constituents between saturated and unsaturated fats.

Fatty acid composition of the studied biscuits is presented in Table 3. The total saturated fatty acids (SFA) content showed a significant differences (p<0.05) between different types of biscuits. Comparison of the SFA content of biscuits made with palm oil (F2 and F6) showed a significant decrease of 17.0% in the case of wheat biscuits (F2) and 30.3% in the case of lupin biscuits (F6) compared to the control biscuits (F1). The lowest percent of total SFA (40.1%) has been obtained in the lupin biscuits contained palm oil (F6 and F8) in the absence/presence of sucralose. Palmitic acid (C16:0) constituted the main component of the SFA, ranging from 25.5±0.5 to 42.1±0.1%. On the other hand, total monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) recoded the highest amounts (47.5 and 12.4%, respectively) in the lupin biscuits contained palm oil (F6 and F8) as a source of fat. These biscuits have been found to contain oleic (C18:1), and linoleic (C18:2) as major MUFA and PUFA fatty acids. As well, the wheat and lupin biscuits formulated by palm oil (F2, F4, F6 and F8) have been cancelled from the trans fatty acids (TFA). All these observations indicated that palm oil can be used as an animal fat replacer in dough biscuits, enabling significant reductions in both total and also SFA content to be made. As well, the formulation of lupin biscuits using palm oil gave the lowest SFA content and free TFA compared with the related wheat biscuits.

Table 3. Overall fatty acid composition of the studied biscuits (%, mean ± standard deviation of 5 replicates)

Sample	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	Others	SFA	MUSFA	PUSFA	TFA
F1	0.1 ± 0.1^{a}	9.2 ± 0.1^{a}	28.4 ± 0.5^{a}	1.4 ± 0.1^{a}	18.7 ± 0.9^{a}	21.2 ± 0.1^{a}	7.4 ± 0.1^{a}	13.6 ± 0.1^{a}	57.5	29.9	10.7	1.9
F2	0.3 ± 0.1^{b}	1.1±0.1b	41.9 ± 0.1^{b}	0.2 ± 0.1^{b}	4.4 ± 0.1^{b}	39.1 ± 0.2^{b}	12.2 ± 0.1^{b}	0.8 ± 0.1^{b}	47.7	40.1	12.2	nd
F3	0.1 ± 0.1^{a}	9.0 ± 0.1^{a}	28.1 ± 0.5^{a}	1.4 ± 0.1^{a}	18.8 ± 0.9^{a}	21.2 ± 0.1^{a}	7.6±0.1a	13.8 ± 0.1^{a}	57.0	30.0	10.9	2.1
F4	0.3 ± 0.1^{b}	1.1 ± 0.0^{b}	42.1 ± 0.1^{b}	0.1 ± 0.1^{b}	4.5 ± 0.1^{b}	39.0 ± 0.2^{b}	12.2 ± 0.1^{b}	0.7 ± 0.1^{b}	48.0	39.9	12.1	nd
F5	0.1 ± 0.1^{a}	7.1 ± 0.1^{a}	25.7 ± 0.5^{a}	1.4 ± 0.1^{a}	16.5 ± 0.9^{a}	30.0 ± 0.1^{b}	11.8 ± 0.1^{b}	7.4 ± 0.1^{c}	49.9	37.5	12.1	0.5
F6	0.2 ± 0.1^{b}	1.0 ± 0.1^{b}	34.7 ± 0.1^{b}	0.5 ± 0.1^{b}	4.2 ± 0.1^{b}	41.5 ± 0.2^{b}	12.3 ± 0.1^{b}	5.6 ± 0.1^{d}	40.1	47.5	12.4	nd
F7	0.1 ± 0.1^{a}	7.1 ± 0.1^{a}	25.5 ± 0.5^{a}	1.6 ± 0.1^{a}	16.5 ± 0.9^{a}	29.0 ± 0.1^{b}	11.8 ± 0.1^{b}	8.4 ± 0.1^{c}	49.7	37.7	12.2	0.4
F8	0.2 ± 0.1^{b}	1.0 ± 0.1^{b}	34.5 ± 0.1^{b}	0.4 ± 0.1^{b}	4.4 ± 0.1^{b}	41.6 ± 0.2^{b}	12.4 ± 0.1^{b}	5.5 ± 0.1^{d}	40.1	47.5	12.4	nd

nd: not detected; Values which don’t share the same letter in each column are significantly different (p<0.05).

The carbohydrate and caloric values have been calculated as indicated in Table 2. The carbohydrate content varies from a high value of 78.3g/100 g in wheat formulation (F4) to a low value of 58.4g/100 g in the formulated lupin biscuit (F7). Refined wheat flour is a result of refining and it contains a higher proportion of starch as compared to the coarse grains like lupins and this might explain the significant (P<0.05) difference in carbohydrate content. The simultaneous substitution with palm oil and sucralose reduced significantly (P<0.05) the caloric values of wheat biscuits (F4) down to 408.2±0.2 kcal/100g compared with control wheat biscuits (F1). Further reduction of caloric values down to 365.7±0.2 kcal/100g has been perceived by the replacement with 25% germinated SWL flours (F8). Therefore, the formulation of biscuits with germinated lupin flour, palm oil and sucralose reduced the energy by 15.2% compared to the control wheat biscuits. The above findings confirmed that the use of lupin biscuits in the presence of palm oil and sucralose as a complete replacement of butter and sucrose improved the nutritional profile of control biscuits which is subsequently used for the formulation of low calorie, high fiber, high protein, low SFA and free TFA biscuits.

3.3. Mineral Composition of Biscuits

The mean values of minerals composition of wheat biscuits and lupin formulated biscuits are outlined in Table 4. The data revealed that the individual or in combination replacement of butter and sucrose with palm oil and sucralose among wheat biscuits did not show any marked changes in the content of all studied minerals compared to control biscuit (F1). Meanwhile, the substitution of wheat flour with 25% germinated SWL flour (F5-F8) increased the content of all minerals in the order of K >P>Fe>Na>Ca>Zn>Mg>Cu. Therefore, it was observed in this study that the substitution with germinated SWL flour improved the mineral composition of the samples except in Cu more than that of 100% wheat biscuits (p<0.05). This observation could be due to biosynthesis and activities of micro-organisms during germination process [15]. Nutritionally, the ratio of Na/K ratio of 0.41has been found in the sample F8 compared to 2.2 Na/K ratio in the case of control wheat sample (F1). This finding indicates that the consumption of this formulated biscuits (F8) is suitable for hypertensive patients. Potassium has a beneficial effect on sodium balance. A high intake of potassium has been reported to protect against increasing blood pressure and other cardiovascular risks. A Na/K ratio less than one is recommended in the diets of people who are prone to high blood pressure [6].

Table 4. Determination of Ca, Mg, K, Na and P (g/ 100 g ± SD, n=5) as well as Fe, Cu and Zn (mg/kg ± SD, n=5) in the studied biscuits

Sample	Ca	Mg	K	Na	P	Fe	Cu	Zn
F1	0.02±0.11^{a}	0.02±0.13^{a}	0.10±0.18^{a}	0.22±0.20^{a}	0.16±0.05^{a}	33.13±0.12^{a}	3.28±0.18^{a}	10.26±0.11^{a}
F2	0.03±0.22^{a}	0.02±0.18^{a}	0.14±0.10^{a}	0.22±0.2^{a}	0.18±0.06^{a}	32.30±0.13^{a}	3.26±0.05^{a}	10.34±0.15^{a}
F3	0.02±0.20^{a}	0.02±0.11^{a}	0.13±0.10^{a}	0.21±0.11^{a}	0.17±0.08^{a}	32.25±0.12^{a}	3.20±0.09^{a}	10.28±0.13^{a}
F4	0.03±0.12^{a}	0.02±0.14^{a}	0.14±0.08^{a}	0.20±0.11^{a}	0.15±0.08^{a}	33.50±0.13^{a}	3.20±0.08^{a}	10.24±0.18^{a}
F5	6.51±0.11^{b}	3.10±0.04^{b}	23.02±0.20^{b}	9.12±0.20^{b}	14.05±0.16^{b}	45.90±0.26^{b}	3.41±0.11^{b}	16.56±0.19^{b}
F6	6.41±0.27^{b}	3.30±0.16^{b}	22.06±0.24^{b}	9.11±0.10^{b}	15.85±0.06^{b}	46.10±0.21^{b}	3.32±0.03^{b}	15.95±0.18^{b}
F7	6.32±0.18^{b}	3.20±0.03^{b}	23.12±0.30^{b}	9.10±0.30^{b}	14.01±0.08^{b}	46.50±0.20^{b}	3.21±0.10^{b}	15.66±0.13^{b}
F8	6.31±0.20^{b}	3.60±0.14^{b}	22.32±0.20^{b}	9.14±0.15^{b}	15.25±0.16^{b}	46.08±0.30^{b}	3.32±0.06^{b}	15.45±0.19^{b}

Values which don't share the same letter in each column are significantly different ($p<0.05$).

3.4. Effect of the Studied Biscuits on Food Intake and Body Weight Gain (%) of Hyperglycemic Rats

Data presented in Table 5 showed the mean value of food intake(g/day) and body weight gain % (BWG%) of normal rats, hyperglycemic rats and hyperglycemic rats treated with different formulations. The mean values of food intake in control group fed on basal diet only (healthy rats) was 15.018 g/day for each rat, while the mean values of food intake of injected group with alloxan (hyperglycemic group) "positive control group" was 14.622 g / day for each rat. Concerning the wheat biscuits groups, the hyperglycemic group which treated with wheat biscuits formulation F3 (group 5, sucrose replaced by sucralose) had the highest food intake followed by the hyperglycemic group which treated with wheat biscuits formulation F4 (group 6, simultaneous replacement of sucrose and butter by sucralose and palm oil). On the other hand, the mean value of food intake of the groups(3 and 4) which treated with wheat biscuits formulations (F1 and F2) decreased more than that of the groups which treated with lupin biscuits. This could be due tothat germination of the lupin grains could achieve desirable changes in their nutritional characteristics [21]. Furthermore, germination decreased the levels of anti-nutrients to improve the quality of lupin seeds. Feeding hyperglycemic group on basal diet and treated them with lupin biscuits F8 (group 10, simultaneous replacement of sucrose and butter by sucralose and palm oil) showed an increase in the value of food intake, as compared to the controls (+ve and –ve groups) and other treated lupin biscuits groups. From these results, it could be concluded that, the hyperglycemic groups which have been treated with wheat or lupin biscuits containing sucralose and palm oil in combination achieved the highest food intake, as compared to the control groups (+ve and –ve groups) and other treated groups except group 5.

Regarding the results of body weight gain % (BWG %), the mean value of (negative control group) fed on basal diet had higher in BWG% compared with positive control. The data in Table 5showed that, treatments of hyperglycemic groups with different lupin biscuits led to significant changes in BWG% compared to wheat biscuits. Data also showed that, feeding hyperglycemic rats with diets containing individual sucralose (group 9) or in combination with palm oil (group 10) showed the best results in BWG%, because these treatments led to non-significant changes (p>0.05), as compared to the negative control group. All these findings reflected the benefits of the simultaneous substitution of wheat control biscuits by 11.25g/100ggerminated SWL flour, 5.0g/100g sucralose and 7.0g/100g palm oil to significantly improve the food intake and BWG% of treated diabetic rats (group 10).

Table 5. Effect of the studied biscuits on food intake and body weight gain% (BWG%) of rats suffering from hyperglycemia

Groups \ Parameter	Food intake (g/day) for each rat	BWG%
Group 1 (negative control)	15.018	34.230±4.221^{a}
Group 2 (positive control)	14.622	7.987±3.510^{d}
Group 3	14.482	8.127±4.138^{d}
Group 4	14.833	11.748±4.138^{cd}
Group 5	17.504	18.125±2.015^{c}
Group 6	16.754	17.523±4.135^{c}
Group 7	15.136	27.140±3.061^{b}
Group 8	15.136	27.140±3.061^{b}
Group 9	15.471	34.182±4.011^{a}
Group 10	16.276	34.343±4.121^{a}

Values are expressed as mean ± SD, n=5.
Values which don't share the same letter in each column are significantly different ($p<0.05$).

3.5. Effect of the Studied Biscuit formulations on Serum Glucose of Hyperglycemic Rats

Results in Table 6 showed the effect of different formulations of biscuits on the concentration of serum glucose of diabetic rats. Initial serum glucose levels showed significant decrease P<0.05 in the control negative group (healthy rats), as compared to other groups. Final serum glucose level of control positive group recorded significant increase (P<0.05), as compared to initial serum glucose level. This means that the injection of rats with Alloxan (120.0 mg/kg/day) for 5 days increased significantly the concentration of serum glucose. While the concentrations of serum glucose of all treated groups at the end of the experimental period showed significant decrease, as compared to initial, except groups of rats fed on basal diet containing 10% wheat biscuits with sucrose (groups 3 and 4). This means that sucrose (disaccharide from fructose and glucose) in biscuits is consider a main source to increase glucose in the blood. Treated groups with wheat biscuits containing sucralose instead of sucrose (groups 5 and 6) led to significant decrease in the serum glucose, as compared to the positive control group. This is due to the reduction of caloric value in the formulated wheat biscuits (F3 and F4). Further reduction in serum glucose at the end of experiment has been observed in groups 9 and 10 compared to the positive control group and very close to the negative control group. In these groups, rats have been treated with lupin biscuits containing sucralose individually or in combination with palm oil. These results are interesting and confirmed also the role of sucralose in lupin biscuits to reduce serum glucose very close to the normal level. Our results cleared that, using the combination of germinated lupin flour, sucralose and palm oil in treated rats (group 10), which suffer from hyperglycemia, decreased serum glucose by about 57%, comparing with the positive control group.

Table 6. Effect of the studied biscuits on serum glucose of hyperglycemic rats

Parameter / Groups	mg/dL	
	Initial glucose	**Final glucose**
Group 1 (negative control)	85.235±3.356^{a}	87.254±4.631^{a}
Group 2 (positive control)	200.865±3.549^{b}	234.632±4.418^{c}
Group 3	207.945±4.732^{b}	214.456±3.354^{cb}
Group 4	203.378±4.331^{b}	206.150±4.247^{b}
Group 5	206.843±2.186^{b}	188.540±4.113^{bd}
Group 6	205.265±4.633^{b}	182.345±2.543^{bd}
Group 7	207.532±4.254^{b}	191.287±3.321^{bd}
Group 8	204.822±2.554^{b}	195.543±5.234^{bd}
Group 9	203.396±4.183^{b}	102.365±2.865^{d}
Group 10	206.322±2.836^{b}	100.926±2.110^{d}

Values are expressed as mean ± SD, n=5
Values which don't share the same letter in each column are significantly different (p<0.05).

As mentioned before, sucralose is made by replacing three hydrogen-oxygen groups on a sucrose molecule with three chlorine atoms. These chlorine atoms help to create a structure that is very stableat high temperatures and gave more than 600 times sweeter than regular sugar. All these characteristics gave an opportunity to reduce the amount of sucralose in biscuits formulations compared to sucrose and subsequently reduced the sugar dose in the blood of treated rats. Further reduction has been achieved in lupin biscuits by decreasing the carbohydrate content due to the replacing of 100% wheat biscuits by 25% germinated lupin flour. As shown in Table 6, the replacement by sucralose in lupin biscuits (group 9) reduced serum glucose by about 55% more than 20% in case of refined wheat biscuits (group 5), comparing with the positive control group. Another effect, the use of germinated lupin flour may assist the hypoglycemic action of sucralose through stimulation of β-cells of islets of langerhans to release more insulin. This action may be due to the presence of phenolic compounds and vitamins in the germinated lupin grains [21].

Furthermore, the functions of liver and kidney have been checked in the alloxan-hyperglycemic rats (positive control) by measuring serum cholesterol, triglycerides, HDL-c, LDL-c, creatinine, AST and ALT as shown in Table 7 and Table 8. Injected rats with alloxan led to significant increase (p<0.05) in serum cholesterol, triglycerides, LDL-c, creatinine, AST and ALT, while HDL-c decreased, in comparison with non-injected rats. Data in Table 7 and Table 8 revealed also that, treating diabetic groups with wheat biscuits containing palm oil RWG% instead of butter led to significant decrease in the mean value of serum cholesterol, triglycerides, LDL-c, creatinine, AST and ALT, while HDL-c increased, as compared to the negative control group. Further reduction in these parameters has been achieved by using wheat-lupin composite flour. In group 10 which composed of treated diabetic rats for 4 weeks with biscuits containing wheat-lupin composite flour, palm oil and sucralose, reduced cholesterol, triglycerides, LDL-c, creatinine, AST and ALT by 37.9%, 25.0%, 74.1%, 49.7%, 41.8% and 54.5% while HDL-c increased by 59.7%, as compared to the negative control group. These observations confirmed also the role of phenolic compounds and vitamins in the germinated lupin grains as well as the complete replacement by palm oil and sucralose to improve the functions of liver and kidney in diabetic rats as well. Therefore, in the current study, lupin (a rich source of fiber and phenolics) biscuits formulated with sucralose individually or simultaneously with palm oil could have a favorable effect on the serum glucose level in diabetic rats. These achievements are in good agreement with Grotz*et al.*[14], Mezitis*et al.*, [19] and Argyri *et al.*, [4].

Table 7. Effect of the studied biscuits on serum cholesterol, triglycerides, HDL-c, LDL-c and creatinine of hyperglycemic rats

Parameter / Groups	mg/dL				
	Cholesterol	**Triglycerides**	**HDL-c**	**LDL-c**	**Creatinine**
Group 1 (negative control)	103.345±1.254^{a}	60.562±2.661^{a}	63.211±2.601^{a}	28.022	0.611±0.211^{a}
Group 2 (positive control)	171.526±2.769^{b}	84.312±2.438^{b}	24.220±3.811^{b}	130.444	1.367±0.431^{b}
Group 3	160.432±1.252^{c}	76.136±2.524^{c}	34.220±3.811^{c}	110.985	1.171±0.301^{c}
Group 4	140.854±2.331d	69.110±3.417^{d}	42.110±2.101^{d}	84.922	0.931±0.450^{d}
Group 5	155.311±3.166^{c}	73.500±2.823c	38.210±2.471^{c}	102.401	1.112±0.398^{c}
Group 6	131.225±2.313^{e}	67.311±2.312^{d}	46.017±2.831^{d}	71.746	0.852±0.510^{d}
Group 7	141.214±2.554^{d}	69.056±1.007^{d}	49.712±2.302^{d}	77.691	0.811±0.421^{d}
Group 8	118.113±3.132^{a}	66.101±2.102^{d}	55.017±2.831^{e}	49.876	0.732±0.128^{e}
Group 9	128.585±2.483^{e}	69.114±2.714^{d}	49.215±2.162^{d}	65.547	0.800±0.200^{d}
Group 10	106.549±1.472^{a}	63.211±2.601^{a}	60.100±3.051^{ae}	33.807	0.687±0.542^{ae}

Values are expressed as mean ± SD, n=5
Values which don't share the same letter in each column are significantly different (p<0.05)
LDL-c = Cholesterol – (triglycerides/5) – HDL-c.

Table 8. Effect of the studied biscuits on AST and ALT of hyperglycemic rats

Parameter / Groups	U/L AST	ALT
Group 1 (negative control)	94.152±1.511[a]	29.254±2.631[a]
Group 2 (positive control)	182.512±1.412[b]	74.632±2.418[b]
Group 3	177.082±1.103[b]	71.922±2.400[b]
Group 4	157.421±2.632[c]	66.150±1.247[c]
Group 5	176.672±2.321[b]	70.540±1.113[b]
Group 6	151.200±1.311[c]	62.345±2.543[c]
Group 7	140.022±1.142[d]	67.287±3.321[c]
Group 8	121.200±1.311[e]	34.601±2.000[a]
Group 9	139.219±1.112[d]	62.365±2.865[c]
Group 10	106.216±1.312[ae]	33.926±2.110[a]

Values are expressed as mean ± SD, n=5
Values which don't share the same letter in each column are significantly different (p<0.05).

3.6. Sensory Evaluation of Biscuits

Table 9 shows the mean sensory scores of overall sensory evaluation of the 8 formulae of biscuits have been carried out among 10 panelists, using 9-points hedonic scale method. All biscuits exhibited the acceptable scores for all the sensory attributes appreciably higher than the minimum acceptability score, i.e. 5.00. It has been observed that the sensory attributes of wheat biscuits formulated by the simultaneous replacement with palm oil and sucralose are significantly different (P<0.05), but still the overall acceptability of them are higher than 5.00. The same observation has been obtained among lupin biscuits. The overall acceptability of lupin biscuits containing palm oil and sucralose in combination was 6.5 ± 1.3. Therefore, the use of palm oil and sucralose in the formulation of biscuits is sensory acceptable.

Table 9. Sensory characteristics of the studied biscuits

Sample	Color	Appearance	Aroma	Texture	Taste	Overall Acceptability
F1	8.4 ± 1.0[a]	8.6 ± 0.9[a]	7.7 ± 1.6[a]	7.9 ± 1.3[a]	8.0 ± 1.0[a]	8.4 ± 1.1[a]
F2	7.5 ± 1.4[b]	7.5 ± 0.4[b]	6.7±1.3[b]	6.8 ± 1.8[b]	7.0 ± 1.3[b]	7.3 ± 1.0[b]
F3	7.6 ± 1.2[b]	7.5 ± 1.3[b]	6.5±1.1[b]	7.0 ± 1.0[ab]	7.6 ± 1.3[b]	7.6 ± 1.7[b]
F4	7.1 ± 1.1[b]	7.6 ± 1.0[b]	7.0±1.0[b]	7.1 ± 0.9[ab]	7.7 ± 1.8[b]	7.7 ± 1.3[b]
F5	6.0 ± 1.0[c]	6.6 ± 0.9[c]	6.4±0.9[c]	6.5 ± 1.7[c]	6.6 ± 1.0[c]	6.9 ± 1.0[c]
F6	6.1 ± 1.6[c]	6.7 ± 1.7[c]	6.3±1.5[c]	6.3 ± 1.1[c]	6.5 ± 1.5[c]	6.4 ± 1.3[c]
F7	6.4 ± 1.8[c]	6.8 ± 1.3[c]	6.4±1.3[c]	6.3 ± 1.2[c]	6.6 ± 1.0[c]	6.6 ± 1.8[c]
F8	6.3 ± 1.2[c]	6.7 ± 1.3[c]	6.4±1.0[c]	6.3 ± 1.2[c]	6.7 ± 1.0[c]	6.5 ± 1.3[c]

Values which don't share the same letter in each column are significantly different (p<0.05).

4. Conclusion

It can be concluded from the above study that germinated lupin flour, palm oil and sucralose can be used successfully in combination to replace 25% of the refined wheat flour, 100% butter and 100% sucrose to formulate healthy reduced caloriebiscuits having the additional benefit of trans-free fats. The formulated functional biscuits had 15.2% lower energy content than the control wheat product. The study demonstrated that highly acceptable reduced-calorie biscuits can be produced by using wheat-lupin composite flour with palm oil (as fat replacer) and sucralose (as sweetener).The replacement by sucralose in lupin biscuits reduced serum glucose by about 55% comparing with the positive hyperglycemic group of rats. Moreover, the proposed lupin biscuits improved cholesterol, triglycerides, LDL-c, HDL-c, creatinine, AST and ALT as compared to the untreated diabetic rats. Therefore, lupin (as rich source of fiber, phenolics and vitamins) biscuits formulated with sucralose individually or simultaneously with palm oil could be recommended for the diet regimen of diabetic persons and also as a nutritive food with low calorie and healthy fats for the public.

Statement of Competing Interests

Author has no competing interests.

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