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Nutritional Assessment of Biscuits Formulated by Simultaneous Substitution with Sweet White Lupinoil and Extracted Flour after Germination

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Abstract Lupin in the recent years was considered as an innovative food ingredient with more health benefits. In the present work, the simultaneous partial substitution of wheat flour and fat in biscuits with sweet white lupin (SWL) flour and SWL oil after germination was firstly investigated. The individual and/or simultaneous substituting with SWL extracted flour and SWL oil was studied in the range of 20-30% (w/w) and 30-40% (v/w) through ten biscuits formulae, respectively. Proximate chemical composition, mineral composition, caloric value and total phenolic compounds (TPCs) were measured. It was found that the simultaneous substitution with germinated SWL flour at 25% and SWL oil at 35% raised significantly the values of protein, fiber and TPCs. However, the proposed substitution showed marked reductions in the values of fat, carbohydrate, calorie and Na/K ratio. The physical and sensory evaluations of the proposed SWL-wheat biscuits were also performed. The proposed substitution increased the spread factor of samples with a breaking strength of 1.7±0.4 kg force. The sensory acceptability of all formulae was checked showing acceptable attributes of taste, appearance, texture and aroma with an observable improvement in the color of SWL-wheat biscuits. All these findings confirmed the role of substitution with SWL extracted flour and oil after germination in order to develop nutritional biscuits with low costs. It is expected that these biscuits could have effective impacts for the improvement of public health including patients suffered from obesity, over-weighted, high blood pressure and hyperlipidemic diseases.

Keywords: *lupins, germination, substitution, nutritional, sensory, biscuits*

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1. Introduction

Lupin (lupine) is a genus of flowering plants in the legume family. The legume seeds of lupins were cultivated all over the world with centers of diversity in Europe, America, Australia and north Africa. Lupin can tolerate frost, drought, and poor soils (Mohamed and Rayas-Duarte, 1995). Therefore, lupin is considered an economically and agriculturally valuable plant with rising of human consumption in recent years. There are four main cultivated species of lupins that are sweet white lupin (SWL, Lupinusalbus, Termis), Australian sweet lupin (Lupinusazngustifolius), yellow sweet lupin (Lupinusluteus) and Andean sweet lupin (Lupinusmutabilis) (Rumiyati and James, 2013). Sweet white lupin seeds are most commonly found in Mediterranean countries especially in Egypt, Portugal, Greece, and Italy (Hassanei, *et al.*, 2011). It is available for all levels of society (limited or high incomes).

The chemical composition of SWL confirmed that it is a good source of nutrients, not only proteins but also lipids, dietary fibers, minerals, vitamins and healthy oil. The amount of SWL oil was found to be in the range of 5-20% (v/w). SWL oil was characterized by the low percent of unhealthy saturated fatty acids (≤ 10%), absence of trans fatty acids, absence of cholesterol and high percent of healthy unsaturated fatty acids (≥90%) including 30-50% oleic acid, 15-45% linoleic acid and 3-11% linolenic acid (Hassanei, *et al.*, 2011). Therefore, it was recently recommended to be used as fat mimetic (healthy oil), improving the fatty acid profile of the food product while maintaining its positive sensory properties (Gravelle, *et al.*, 2012; Stortz, *et al.*, 2012; Tarancón, *et al.*, 2013).This kind of healthy food product is essential for the peoples having the risk of obesity, overweighed, high blood cholesterol and coronary heart diseases (Biguzzi, *et al.*, 2014). These diseases were significantly distributed among peoples all over the world. Hence, there is a great demand for the substitution of fats with healthy oils in food products.

Lupin flour is also widely considered as an excellent raw material for supplementing different food products. It has always been a rich of proteins and dietary fibers with the range between 36-52% and 30-40% (w/w), respectively (Mohamed and Rayas-Duarte, 1995). The lupin protein has two major fractions, albumins and globulins in a ratio of 1:9, respectively. The variations in composition among

SWL species are due to genetic, growing conditions and soil types (Anwar and Harbans, 2004; Sironi, *et al.*, 2005). Lupin flour was incorporated into wheat flour to improve the nutritional value of the final products without detrimental effects on the quality(Pollard, *et al.*, 2002) but may transport a particular allergenic risk (Peeters, *et al.*, 2007). The complete substitution of wheat flour by SWL flour is not recommended because lupin does not also contain gluten and subsequently the gluten structure in the dough could be unable to retain air during proofing and baking. In general, the contribution up to 20% lupin flour in the mixture with wheat flour was found to be the optimal acceptable value (Mohamed and Rayas-Duarte, 1995). As well, the presence of lupin flour influenced the increase of water absorbability. Increase of water quantity produced an increase in fluidity of dough, easy to kneading and time stability (Martinez-Villaluenga, *et al.*, 2006; Scarafoni, *et al.*, 2009). Therefore, the partial substitution by SWL flour can play a valuable role in food products as a rich source of a variety of specific ingredients. Till now, the substitution by 20% lupin flour in bakery foods is the optimal one but still there are other processes could improve this amount of substitution and could develop more the nutritional impact of the food product.

Germination was used to modify the chemical composition of legumes involves a variety of reactions including synthesis, degradation and transformation of biomolecules during transformation of seeds into a plant. To improve the quality of lupin seeds, germination was used to change protein, lipid and carbohydrates levels (Dagnia, *et al.*, 1992; Dueñas, *et al.*, 2009). Analysis of the oil extract after germination revealed that the concentration of phytosterols and total polyphenol content were increased (Rumiyati and James, 2013). Furthermore, the concentration of anti-nutritional factors such as phytate, trypsin inhibition, α-galactoside and alkaloids in lupin were significantly diminished (Cuadra, *et al.*, 1994). Therefore, the previously reported improvement of lupin by germination would be expected to further use of germinated lupin as an innovative food ingredient with more health benefits.

Biscuits are the most popular bakery food product consumed by nearly all levels of society. Flour, fat, water, and sugar are the main components in a biscuit formulation. Fat are needed in biscuit manufacturing because they have suitable plasticity properties, allowing the air to be incorporated during dough formation and enabling the dough to withstand the high temperatures reached during baking and hold its shape for longer (Lee, *et al.*, 2008). The most frequently used plastic fats are margarine and butter, all of them contain high levels of unhealthy saturated fatty acids and in some cases trans-fatty acids. These kinds of fatty acids are the main causes of variant diseases and their replacement by healthy fats is of tremendous importance. Besides, the high cost and shortage of wheat flours encourage researches to substitute with other flours. Limited trials in biscuits were performed to replace butter or wheat flour by lupin oil or flour (Anne, *et al.*, 2012; Jayasena and Nasar-Abbas, 2011). Lupin oil and flour were individually succeeded to replace 30% fat and 20% wheat flour, respectively in biscuits. Still these findings lacked from essential nutritional information and authors did not try to improve the limits of lupin substitution in food products and also to develop their health benefits using technological processes such as germination.

Therefore, the objective of this study is to produce biscuits using simultaneous partial substitution of butter and wheat flour with sweet white lupin (SWL) oil and extracted flour after germination, respectively. This replacement could obviously improve the nutritional value of formulated wheat biscuits that simply prepared with low cost. To the best of my knowledge, this is the first time to demonstrate the role of germinated SWL extracted flour and SWL oil in the manufacturing of healthy and economic biscuits. Hence, this investigation was considered to study the proximate chemical composition, caloric value, total phenolic compounds (TPCs) and mineral compositions as well as physical characteristics and sensory quality attributes of ten biscuits formulae.

2. Materials and Methods

2.1. Materials

Sweet white lupingrains (Lupinusalbus, Termis), refined wheat flour (TriticumAestivum, 72% extraction hard red winter), butter (Cow's milk≥ 80% fat), sugar powder (100% pure cane sugar), sodium bicarbonate, ammonium bicarbonate, skim milk powder (Defatted Cow's milk powder), salt(NaCl), orange (*Citrus sinensis* (L.)) peel powder were procured from local market. Methanol, n-hexane, ether, nitric acid and perchloric acid were obtained from Flukacompany (Germany). Authentic samples of fatty acid methyl esters were purchased from Sigma company (Germany). All other ingredients were obtained from the local market. Double distilled water was used for the chemical analysis.

2.2. Germination of Sweet White Lupin (SWL) Grains

Prior to germination, clean lupin grains (300.0 g) were soaked in 2 L of distilled water at room temperature, in the dark, for 18 h. Following this the soaking water was decanted and the grains were twice rinsed with water. Then, grains were spread on dishes lined and covered with moistened paper towels. These dishes were placed on trays in an incubator set at 30 °C and relative humidity (RH) 90–95 %. The seeds in dishes were germinated for 3 days(Rumiyati and James, 2013).During the germination period, grains were washed twice per day with distilled water. At the end, the grains were dried under the sun. The above process was done in three replications to confirm the precision of the results. Finally, the germinated SWL grains or ungerminated SWL grains were ground and then sieved thrice through 10 mesh to flour (Mousa, 2014). Grinding was done in Ushamixer Grinder. The obtained flours were stored in the dark at 4 °C prior to use.

2.3. Simultaneous Procuration of SWL Oil and Extracted Flour

A portion of the obtained germinated/ungerminated SWL flour (200.0 g) was exposed for oil extraction. The oil was extracted by n-hexane using a Buchi E-816 Soxhlet extraction unit (Switzerland). The weighed samples were placed into a thimble and put on pre-weighed extraction cups that placed on the extraction chamber in the Soxhlet (Rumiyati and James, 2013). Consequently, the oil was extracted by n-hexane for 60 min. The extracted oil samples were kept in well stoppered dark containers under 4°C (to prevent their autoxidation) until use as a fat replacer in the formulated biscuits. Simultaneously, the remained flour amount from oil extraction was dried over anhydrous sodium sulphate and the solvent was removed off by rotatory evaporator under vacuum. The dried extracted flour was cooled, weighed and used for the partial substitution of wheat flour in the formulated biscuits.

2.4. Preparation of Formulated Biscuits

Ten different formulations of biscuits were prepared as described in Table 1. For the preparation of control wheat biscuit (F1), butter (10.0 g) and sugar powder (20.0 g) were creamed together for 3 min in a mixer at 60 rpm. Then, 2.0 g of skim milk powder, 1.0 g of salt, 0.4 g of sodium bicarbonate, 1.5 g of ammonium carbonate and 5.0 g of orange peel powder were added in water (20.0 mL) and mixed together for 8 min at 125 rpm. The wheat flour (100.0 g) was added to the above mixture and mixed again for 3 min at 60 rpm. The dough was sheeted to a 3.5-mm thickness and cut with a biscuit cutter (50-mm diameter). The biscuits were baked on an aluminium tray in an electric oven at 200°C for 5 min. They were cooled for 30 min at room temperature and stored in low density polyethylene bags until further use(Mousa, 2014).

Table 1. Variations in the component of biscuits formulations

Sample	Wheat Flour (g)	SWL Flour (g)	Butter (g)	SWL oil (mL)	Water (mL)
F1 (control-1)	100.0	---	10.0	---	20.0
F2 (ungerminated SWL flour)	80.0	20.0	10.0	---	24.0
F3 (germinated SWL flour)	80.0	20.0	10.0	---	24.0
F4 (control-2)	100.0	---	7.0	3.0	20.0
F5 (Ungerminated)	80.0	20.0	7.0	3.0	24.0
F6 (Germinated)	80.0	20.0	7.0	3.0	24.0
F7	75.0	25.0	7.0	3.0	26.0
F8	70.0	30.0	7.0	3.0	27.0
F9	75.0	25.0	6.5	3.5	26.0
F10	75.0	25.0	6.0	4.0	26.0

For the preparation of wheat-SWL flour biscuits, two formulations (F2 and F3) of biscuits were prepared (Table 1). In F2 sample, the wheat flour was replaced by 20% (w/w) ungerminated SWL extracted flour. In case of F3 samples, germinated SWL extracted flour was used to replace the same percent of wheat flour (20%, w/w).

For the preparation of butter-SWL oil biscuits, three formulations (F4, F5 and F6) were prepared. In these formulations, butter was replaced by 30% (v/w) of SWL oil without germination for the preparation of samples F4 (control-2) and F5 (in the presence of ungerminated SWL extracted flour). At the same percent of fat mimetic (30%), SWL oil after germination was used for the formulation of F6 sample in the presence of germinated SWL extracted flour.

The remained formulations (F7-F10) cited in Table 1 were prepared by the simultaneous variation of SWL oil in the range of 30-40% (v/w) and SWL extracted flour in the range of 20-30% (w/w, including F6) after the process of germination.

2.4. Physical Analysis and Proximate Chemical Composition of Biscuits

All biscuits formulations were 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 (1997). Five biscuits were used for the evaluations from each of the ten studied biscuits and averages were recorded. The spread ratio and spread factor were calculated using the following equations:

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

$$\text{Spread factor} = \text{spread ratio of sample} / \text{spread ratio of control sample}$$

The breaking strength (kg force) was also measured using a Texture Analyser (TA-Hdi, Stable Micro System, UK) by following the triple beam technique described by Gains (1991). A cross head speed of 10 mm/min with a load cell of 50 kg was used in studies and the force required to break five biscuits were recorded and reported as average value.

The standard method of AOAC (1995) was employed for proximate analysis including moisture, crude protein, crude fat, crude fiber, soluble carbohydrates and ash contents.

Moisture content was estimated by weighing 10 g of each sample. Sample was then dried in hot air oven at 130°C in pre-weighed dishes till constant weight. The dish with dried sample was transferred to desiccators and cooled to room temperature. The dish was then weighed and moisture content in percent was calculated from loss in weight.

Crude protein was estimated by weighing 1.0g of each sample. Then, sample was digested with concentrated sulphuric acid (20 mL) and digestion mixture (10.0 g) in Kjeldahl digestion flask. The contents were cooled and transferred to 250 mL volumetric flask. The volume was made up to the mark with distilled water and mixed. Measured aliquot (5 mL) was poured in distillation flask followed by 40% sodium hydroxide and ammonium borate was collected through a condenser in a flask containing 10 mL of 4.0% boric acid solution. The distillate was titrated with 0.1 N sulphuric acid. A blank sample was also run along with the samples. Finally, protein was calculated using the factor 6.25 for converting nitrogen content into crude protein.

Fat content was determined by the ether extract method using Soxhlet extraction apparatus for 6 hr. The ether extract was filtered in pre-weighed beakers, ether was evaporated completely from the beakers and the increase in weight of beaker represented the fat content.

Crude fiber was determined by transferring 2 g sample was transferred to 600 mL beaker and 200 mL of 1.25% H_2SO_4 was added. Beaker was placed on digestion apparatus with readjusted hot plate and boiled for 30 min. Filter the contents through a filter paper. Wash the residue free of acid using hot distilled water and then transferred to the same beaker to which add 200 mL of 1.25% sodium hydroxide. Digest the contents for half an hour, filter and wash free of alkali using hot distilled water. The residue was transferred to crucibles, weighed, dried in an oven overnight at 105°C, and then placed in the muffle-furnace at 550°C for 3 hrs. The loss in weight after ignition represents the crude fiber in the sample.

Ash was estimated by weighing a known quantity of biscuit sample was taken in a pre-weighed silica crucible and charred over the heater to make it smoke free. The crucible along with the sample was ignited in the muffle furnace at 550°C for 3 hrs. When muffle furnace was slightly cooled, the crucible with ash was taken out, kept in desiccators to cool down, and weighed to a constant weight. The difference between the weight of silica crucible as empty and with ash was the amount of total ash and then the percent of ash was calculated.

Total carbohydrates were calculated from the sum of moisture, crude protein, crude fat, ash and crude fiber and lastly subtracting it from 100 (AOAC, 1995). The caloric value was calculated using values of 4 k.cal/g of protein, 4 k.cal/g of carbohydrate and 9 k.cal/g of fat according to Livesy(1995).

2.5. Mineral Analysis of Formulated Biscuits

In order to investigate the mineral content of biscuits, the main elements including sodium, potassium, magnesium, calcium, iron, copper, zinc and phosphorus were determined based on the methods published in AOAC (1995). The samples were 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 were determined using atomic absorption spectrometry (AAS) (Agilent Technologies, California, U.S.A). Sodium and potassium were determined by flame photometer (Jenway, U.K.).

2.6. Analysis of Total Phenolic Compounds

The methanolic extract which contains the majority of the phenolic compounds was prepared and used for total phenolic compounds (TPCs) analysis. TPCs were determined colorimetrically using Folin-Ciocalteu reagent, as described previously (Pasko, *et al.*, 2009). Flour samples were extracted with 80% methanol by shaking in a water bath for 2 h at room temperature. The mixture was then centrifuged at 4000 rpm for 15 min. A sample (0.3 mL) of extracted solutions was mixed with 2.7 mL of deionized water, 0.3 mL of 7 g/100 g Na_2CO_3 and 0.15 mL of Foline-Ciocalteu reagent. Absorbance of mixture was measured at 725 nm using the spectrophotometer Jasco UV-530. The TPCs were expressed as gallic acid equivalent (g of GAE/100 gdried sample).

2.7. Sensory Analysis

All biscuits formulae were checked for their sensory acceptability by fifty judges (Mousa, 2014). All of them are free from cold or sinus problem during the period of evaluation. Ten panelists were called for sensory valuation at a time under "daylight" illumination and in isolated booths. Samples (2 biscuits of each type) were served in paper plates identified by random three digits codes. Care was taken to maintain the sensory environment at 25°C. Panelists were 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.

2.8. Statistical Analysis

Values were expressed as means ± SD. Analysis of variance (Mousa, 2014) was carried out using statistical software version-7 (State Soft Corporation, Tulsa, USA). The multiple response regression analysis was 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 are presented in Table 2. Figure 1 depicts the photos of all formulated biscuits (F1-F10 samples). It is obvious that there is a gradual increment in the spread ratio values of the biscuits formulae (F2, F3, F5 and F6) compared to the control samples (F1 and F4). On the contrary, there is no any observed difference in the spread ratio between F1 control sample and F4 control sample. These observations confirmed that the partial substitution of wheat flour (20%) by SWL extracted flour enhanced the spread ratio of biscuits. In parallel, the substitution of butter (30%) by SWL oil did not influence the spread ratio of biscuits. This is also confirmed by the observation of an increment in the spread ratio from 6.4 (F6) to 7.2 (F8) (i.e. 12.5% increment) due to the increase in the replaced amount of wheat flour from 20% (F6) to 30% (F8) by SWL germinated extracted flour. In contrast, the substitution of butter by 30% (F7) to 40% (F10) SWL oil did not reveal any significant change in the spread ratio as shown in Table 2. Considering the spread factor of control biscuit (F1, 100% refined wheat flour) as 100, results indicated that the values were significantly increased up to 130.6% (F2) and 132.7% (F3) by the substitution with 20% ungerminated SWL extracted flour and germinated SWL extracted flour, respectively. Further increment up to 136.7% (F9) was observed by the replacement with 25% SWL germinated flour in the presence of 35% SWL oil.

Breaking strength of the proposed formulations were also measured. It was observed that the replacement of wheat flour by 20% germinated/ungerminated SWL extracted flour (F2 and F3) reduced slightly the values compared to the control sample (F1). On the other hand, the substitution of butter by 30% SWL oil (F4) increased the breaking strength up to 1.7 relative to 1.5 in the case of the control biscuit (F1). Moreover, the simultaneous substitution with germinated SWL oil and extracted flour (from F6 to F10 samples) recorded a slight change in the breaking strength values. The higher value was achieved in the F9 formulation which was prepared by simultaneous substation with 25% and 35% of germinated SWL extracted flour and oil, respectively. In general, these replaced amounts could be used for the preparation of commercially biscuits with acceptable physical characteristics.

Table 2. Physical characteristics of the studied biscuits

Sample	Width[a] (D, mm)	Thickness[a] (T, mm)	Spread ratio (D/T)	Spread factor[b]	Breaking strength (kg force ± SD[c])
F1	55.0	11.2	4.9	100.0	1.5 ± 0.5
F2	64.5	10.1	6.4	130.6	1.4 ± 0.3
F3	67.7	10.4	6.5	132.7	1.3 ± 0.4
F4	52.2	10.9	4.8	98.0	1.7 ± 0.3
F5	62.1	9.8	6.3	128.6	1.5 ± 0.7
F6	65.0	10.2	6.4	130.6	1.4 ± 0.8
F7	68.2	10.1	6.8	138.8	1.6 ± 0.6
F8	70.6	9.8	7.2	146.9	1.6 ± 0.3
F9	68.0	10.1	6.7	136.7	1.7 ± 0.4
F10	67.3	10.0	6.7	136.7	1.4 ± 0.7

[a] Width and thickness of 5 biscuits in series
[b] Spread factor = spread ratio of sample/spread ratio of control sample.
[c] SD: the standard deviation of 5 measurements.

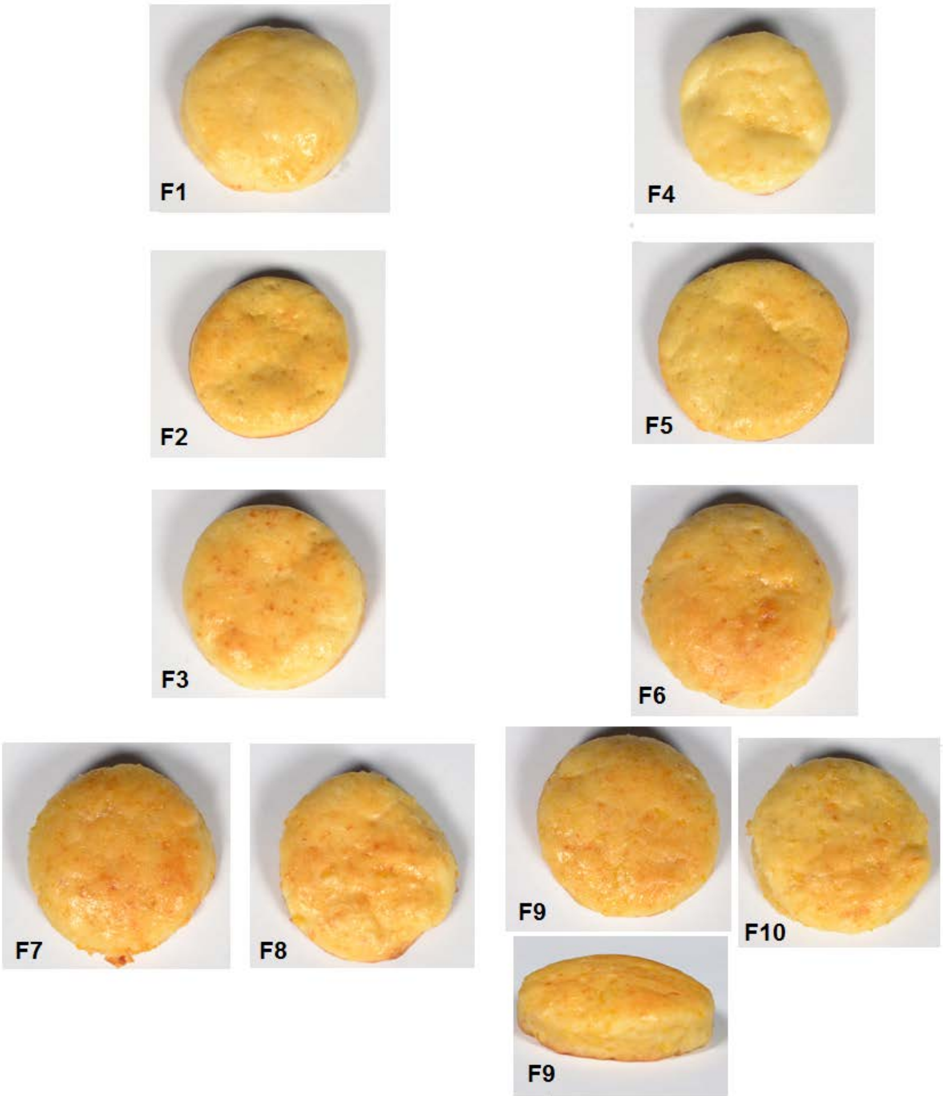

Figure 1. Photos of the formulated biscuits (from F1 to F10)

3.2. Proximate Chemical Composition, Caloric Value and Total Phenolic Compounds of Biscuits

The mean values of proximate chemical composition and caloric values of wheat biscuit and other formulated SWL-wheat biscuits are given in Table 3. The data of moisture and ash contents revealed that the substitution of wheat flour and butter simultaneously or individually by germinated/ungerminated SWL oil and extracted flour showed a little difference in moisture and ash measurements among biscuits. The lowest value of moisture (4.9 g/100g) was achieved by substitution with 25% and 35% germinated SWL flour and oil (F9 sample). It is well known that low moisturecontent of food samples is a desirable phenomenon, since the microbial activity is reduced and the storage period of the product is increased (Alozie, *et al.*, 2009).

Table 3. Proximate chemical composition, caloric value and total phenolic compounds (mean value± SD, n=5) of the studied biscuits

Sample	Moisture (g/100g)	Crude protein (g/100 g)	Crude fat (g/ 100g)	Ash (g/100 g)	Crude fiber (g/100g)	Carbohydrates (g/ 100g)[a]	Caloric value (k.cal/100g)	Total phenols (mg/100g[b])
F1	5.3±0.1	7.0±0.1	11.9± 0.1	1.5±0.2	0.2±0.1	74.1±0.1	431.5±0.1	90.0±0.1
F2	5.2±0.2	14.3±0.2	9.7±0.6	1.9±0.1	7.1±0.2	61.8±0.3	391.7±0.4	120.5±0.2
F3	5.6±0.1	17.1±0.3	9.2±0.4	1.9±0.2	7.1±0.1	59.1±0.3	387.6±0.3	210.0±0.2
F4	5.0±0.3	7.7±0.2	7.5±0.5	1.3±0.2	0.4±0.1	78.1±0.3	410.7±0.3	95.0±0.1
F5	5.1±0.2	14.5±0.3	5.1±0.5	1.8±0.2	7.3±0.1	66.2±0.3	368.7±0.4	122.3±0.1
F6	5.3±0.2	17.6±0.2	4.7±0.3	1.6±0.3	7.5±0.1	63.3±0.2	365.9±0.2	246.1±0.1
F7	5.1±0.1	19.5±0.1	4.0±0.2	1.8±0.1	9.2±0.2	60.4±0.2	355.6±0.2	285.3±0.2
F8	5.6±0.2	20.9±0.2	3.1±0.1	1.9±0.1	10.9±0.2	57.6±0.2	341.9±0.2	332.4±0.1
F9	4.9±0.3	19.8±0.1	2.5±0.3	1.7±0.2	9.5±0.1	61.6±0.2	348.1±0.2	310.4±0.2
F10	5.0±0.3	19.9±0.2	1.1±0.4	1.6±0.1	9.3±0.1	63.1±0.2	341.9±0.2	315.2±0.1

[a] Calculated by difference.
[b]TPCs expressed as gallic acid equivalent (GAE) (mg GAE/100 g dried sample).

The crude protein content of biscuits indicated that the substitution of wheat flour with 20% ungerminated SWL extracted flour increased the value about two times (14.3 g/100g) higher than that of control F1 (7.0 g/100g). This result confirmed that lupin is a rich source of protein and the procedure of oil lupin extraction using n-hexane as solvent does not damage the lupin protein. Further increment of protein (about 2.5 fold compared with F1) was obtained by 20% germinated SWL extracted flour. Moreover, the simultaneous substitution with germinated SWL oil and extracted flour increased markedly the protein content (F6-F10 samples) as shown in Table 3. This observation agreed with other scientific findings that the processing techniques such as germination improved the nutritional quality of the food products, particularly in terms of protein content (Ijarotimi, 2012; Fasasi, 2009). 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.

The fat content was also measured for all formulations. It was found that the fat content of F4 samples was markedly reduced by 37% compared to control wheat biscuit (F1). On the other hand, the substitution of wheat flour by ungerminated SWL flour (F2) and germinated SWL flour (F3) reduced the fat content down to 18.5% and 22.7%, respectively. Further reduction of fat content was perceived by the simultaneous substitution with ungerminated/germinated SWL extracted flour and SWL oil down to 57.1% (F5) and 60.5% (F6) relative to control sample F1. Moreover, the fat content of F9 and F10 biscuits were further significantly reduced down to 79% and 90.8%, respectively. Therefore, F9 and F10 samples recorded the most fat-reduced samples among all studied biscuits due to the substitution with lupin oil. These observations are similar to those previously observed (Drewnowski, *et al.*, 1998).

As well, the crude fiber content of all samples was measured. It was found that fiber content markedly increased up to 7.1 g/100 g by the substitution with 20% SWL extracted ungerminated/germinated flour (F2 and F3) compared to 0.2 g/100 g in the case of control wheat biscuit (F1).On the contrary, the substitution of butter with 30% SWL oil (F4) did not perceive any significant change in the fiber content. The simultaneous substitution with SWL oil and SWL extracted flour (F5-F10) produced biscuits with high fiber contents. The highest values of fiber (9.5-10.9 g/100 g) were obtained by replacement with 30% germinated SWL extracted flour and 30% SWL oil (F8) as well as 25% germinated SWL extracted flour and 35% SWL oil (F9). In addition, the contribution of high fiber germinated lupin products(F8 and F9) in the mixture with wheat flour affects the increase of water amount during the preparation steps in comparison with control dough (F1) as indicated in Table 1. This could certainly improve the rheological properties of dough such as its development, time stability, and index tolerance to kneading (Zlatica, *et al.*, 2011).

The carbohydrate and caloric values were calculated as published elsewhere (AOAC, 1995; Livesy, 1995). The carbohydrate content varies from a high value of 78.1 g/100 g in control formulation (F4) to a low value of 57.6 g/100 g in the formulated biscuit (F8). 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 ($P \leq 0.05$) difference in carbohydrate content. The simultaneous substitution with ungerminated SWL oil and extracted flour reduced significantly the carbohydrate and caloric values of control biscuits. Further reduction of carbohydrate and caloric values was perceived by the replacement with germinated SWL oil and extracted flours. The lowest values of carbohydrate and energy were obtained by simultaneous substitution with 30% of both germinated SWL oil and SWL extracted flour (F8). The same observation was obtained by simultaneous substitution with 25% germinated SWL extracted flour and 35% germinated SWL oil (F9) with very close values of 61.6 g/100 g carbohydrates and 348.1 k.cal/100 g calorie. The carbohydrate content and energy values of germinated samples were lower than those of ungerminated and control wheat samples; this observation could be due to the utilization of carbohydrate for biochemical activities of the germinated grains as well as the soaking in acidic medium prior to germination(Mousa, 2014). The above findings confirmed that the germination of SWL grains improved their nutritional profile which is subsequently used for the manufacturing of low fat, low carbohydrate, low calorie, high protein and high fiber biscuits.

In addition, the effect of germination on the concentration of bioactive compounds in formulated biscuits was examined in the present study (Table 3). The concentration of total phenolic compounds (TPCs) were determined in methanolic extracts and then were measured colorimetrically using Folin-Ciocalteure agent (Pasko, *et al.*, 2009). The concentration of TPCs expressed as gallic acid equivalent(GAE) (mg GAE/100 g dried sample). It was found that the substitution with germinated SWL extracted flour (F3) increased the TPCs value by 42.9% higher than that of ungerminated SWL flour (F2) and raw wheat flour (F1). This finding is in an agreement with previous findings published elsewhere (Stortz, *et al.*, 2012; Dueñas, *et al.*, 2009; Fernandez-Orozco, *et al.*, 2006) but with a little difference in the measured TPCs value. The reason for this could be due to the differences in germination conditions such as temperature. Furthermore, in the present study, the simultaneous substitution with germinated SWL extracted flour and SWL oil enhanced the content of TPCs. The highest range of TPCs (310-332.4 mg/100g) was achieved in the biscuits formulae F8, F9 and F10. Therefore, the substitution with lupin flour and oil after germination improved the antioxidant power of the biscuits since many phenolic compounds are natural antioxidants. Therefore, these findings supported our target to use the germinated SWL extracted flour in parallel with SWL oil as a healthy food ingredient in biscuit.

3.3. Mineral Analysis of Biscuits

The mean values of minerals composition of wheat biscuits and SWL-wheat formulated biscuits are outlined in Table 4. The data revealed that the replacement of butter with 30% SWL oil (F4 sample) did not show any marked changes in the content of all studied minerals compared to control wheat biscuit (F1). Meanwhile, the substitution of wheat flour with 20% ungerminated SWL flour (F2) increased the content of all minerals in the order of K >Fe >P>Zn>Ca>Mg > Na > Cu. By substitution with the germinated SWL extracted flour (F3), the minerals contents were further increased compared with ungerminated one (F2) and control wheat biscuits (F1). The highest increments were observed in the content of K, Na, P, Ca and Fe. In comparison, the simultaneous substitution with germinated SWL flour and oil increased slightly the content of some minerals. The observable changes were happened in the content of K, Na and P. Therefore, it was observed in this study that the simultaneous substitution with germinated SWL extracted flour and oil improved the mineral composition of the samples except in copper more than that of 100% wheat biscuits ($p \leq 0.05$). This observation could be due to bio-synthesis and activities of micro-organisms during germination process (Ijarotimi, 2012).Nutritionally, the ratio of Na/K of the formulated biscuits F6-F10 samples was ranged between 0.38 and 0.57. The Na/K ratio of 0.57 was found in the sample F9 by substation with 25% germinated SWL extracted flour and 35% germinated oil compared to 1.3 Na/K ratio in the case of control wheat sample (F1). This finding indicates that the consumption of this formulated biscuits (F9) is suitable for hypertensive patients. Potassium has a beneficial effect on sodium balance. Ahigh 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 (Cappuccio and McGregor, 1991).

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
	(g/ 100 g)					mg/kg		
F1	0.02±0.15	0.02±0.19	0.11±0.14	0.24±0.20	0.18±0.09	30.33±0.14	3.40±0.20	10.42±0.21
F2	4.01±0.19	3.01±0.24	16.08±0.20	1.15±0.21	10.75±0.25	44.32±0.19	3.72±0.25	16.55±0.16
F3	6.71±0.21	3.21±0.09	24.12±0.24	9.12±0.26	14.05±0.16	45.90±0.26	3.41±0.11	16.56±0.19
F4	0.03±0.24	0.02±0.21	0.12±0.16	0.22±0.25	0.19±0.08	30.37±0.16	3.36±0.09	10.40±0.25
F5	4.05±0.10	3.11±0.11	16.03±0.08	1.11±0.11	10.70±0.19	44.30±0.21	3.76±0.24	16.50±0.14
F6	6.74±0.19	3.18±0.25	24.10±0.08	9.16±0.07	14.03±0.14	45.95±0.07	3.41±0.14	16.55±0.08
F7	7.42±0.25	3.30±0.14	29.05±0.19	15.60±0.19	18.34±0.17	46.35±0.25	3.48±0.19	16.55±0.24
F8	7.95±0.09	3.31±0.26	32.11±0.07	18.06±0.14	20.66±0.10	46.95±0.08	3.49±0.26	16.56±0.11
F9	7.46±0.11	3.32±0.20	29.05±0.14	16.63±0.17	18.34±0.24	46.41±0.14	3.50±0.16	16.56±0.08
F10	7.41±0.27	3.30±0.16	29.06±0.24	15.61±0.10	18.35±0.07	46.40±0.24	3.52±0.09	16.55±0.20

3.4. Sensory Evaluation of Biscuits

Table 5 shows mean sensory scores of overall sensory evaluation of the 10 formulae of biscuits was carried out among 10 panelists, using 9-points hedonic scale method (Youssef and Mousa, 2012).All biscuits exhibited the acceptable scores for all the sensory attributes appreciably higher than the minimum acceptability score, i.e. 5.00. There was low difference in the mean scores of aroma, appearance and texture among the 10 formulae. No serious texture defects were detected by the sensory panel in all formulas but formula 9 had higher score (9.1±0.05). On the other hand, color and taste of the biscuits prepared from simultaneously or individually germinated SWL extracted flour and oil (F3 and F6) were rated significantly ($P \leq 0.05$) higher than the biscuits prepared from ungerminated SWL extracted flour and oil (F2 and F5). Furthermore, the gradual increasing of both germinated SWL extracted flour and SWL oil in the formulated biscuits improved significantly the sensory attributes of biscuits. The highest value of color (9.4±0.13) was obtained in the case of substitution by 25% germinated SWL extracted flour and 35% germinated SWL oil (F9 sample). This observation could be attributed to the substitution of wheat flour by germinated SWL flour decreased the darkness of wheat biscuit color (Pollard, *et al.*, 2002). Finally the overall acceptability (9.1±0.15) of the simultaneous substitution with germinated SWL extracted flour and SWL oil (F9) is very close to the overall acceptability (9.0±0.09) of control wheat biscuits (F1).

Table 5. Sensory characteristics of the studied biscuit samples (mean ± SD, n=50)

Sample	Color	Appearance	Aroma	Texture	Taste	Overall acceptability
F1	9.0±0.14	8.5±0.05	8.5±0.12	9.0±0.22	9.0±0.12	9.0±0.09
F2	8.9±0.10	8.1±0.24	8.4±0.20	8.6±0.04	8.4±0.14	8.7±0.12
F3	9.0±0.19	8.6±0.12	8.4±0.07	8.6±0.19	9.0±0.22	8.7±0.04
F4	8.8±0.04	8.4±0.11	8.5±0.15	9.0±0.02	9.0±0.04	9.0±0.18
F5	8.8±0.02	8.2±0.08	8.4±0.05	8.7±0.05	8.5±0.12	8.7±0.05
F6	9.0±0.22	8.5±0.15	8.4±0.11	8.7±0.08	9.0±0.05	8.9±0.02
F7	9.2±0.05	8.5±0.11	8.4±0.15	8.8±0.18	9.0±0.10	9.0±0.21
F8	9.1±0.15	8.5±0.04	8.4±0.10	8.9±0.04	9.0±0.02	9.0±0.18
F9	9.4±0.13	8.6±0.17	8.5±0.22	9.1±0.05	9.1±0.05	9.1±0.15
F10	9.2±0.12	8.5±0.09	8.5±0.05	9.1±0.12	9.0±0.15	9.0±0.22

4. Conclusion

The need for plant-derived nutrients is expected to grow due to the economic and environmental factors as well as the support of the development of new, safe, and healthy foods. Recently, lupins are considered as economically and agriculturally valuable plants with rising of human consumption. Previously, lupin flour and lupin oil were individually used for the substitution of wheat flour and fat in food products with limited portions. In the current study, for the first time, the simultaneous substitution with sweet white lupin (SWL) flour and SWL oil was studied in biscuits. As well, the substitution levels of 25% germinated SWL extracted flour and 35% SWL oil instead of wheat flour and butter in biscuits increased significantly the content of protein, fiber and total phenolic compounds (TPCs). On the other hand, this proposed substitution decreased markedly the content of fat, carbohydrate and caloric values. Furthermore, the contents of K, Na, P, Ca and Fe in the proposed biscuits were obviously increased compared to commons. Nutritionally, the Na/K ratio of germinated SWL-wheat biscuit formulation achieved value around 0.5 which is admired by peoples prone to high blood pressure. These findings proved that the proposed formulated biscuits could be considered nutritious functional and healthful foods. Moreover, they could be recommended for caloric reduced diets that are substantial for obese and over-weight persons. Likewise, the rather relative low carbohydrate content in the biscuits could be recommended for the diet regimen of diabetic persons. The low fat content of the proposed biscuits is a great advantage especially for the patients of hyperlipidemic diseases. In addition, The sensory evaluation acceptability of the proposed germinated SWL-wheat biscuits showed acceptable attributes of taste, appearance, texture and aroma with an observable improvement in their color. Therefore, in general, the use of lupin flour and oil after germination has to provide adequate nutritional and useful technological functionality to the foods (such as bread, cakes, etc)in which they are incorporated in order to meet the needs of consumers and the food industry. Moreover, the current work can be adapted to larger production scales of obtaining biscuits with lupin oil or germinated lupin flour under the adequate control on the period of grains germination, the storage of extracted oil and the dryness of extracted lupin flour.

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Statement of Competing Interests

Author has no competing interests.

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