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Formulation of Functional Biscuit for Lowering Blood Lipid Level

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Abstract
The purpose of the study is to formulate the functional biscuit for lowering blood lipid level and to elucidate its effect on hyperlipidemic patient. Functional biscuits T_1, T2 & T_3 were prepared by replacing the 10%, 15% and 20% refined wheat flour with soy flour. The prepared biscuits were evaluated for its physical, sensory and chemical properties. Result of sensory (appearance, color, flavor, texture, taste, and overall acceptability) evaluation of biscuits showed that treatment T_1 (10% of soy flour) was best. The other treatments T_2 & T_3 were also found acceptable. The result of chemical analysis of functional biscuit showed moisture, ash, protein, fiber content increases with supplementation whereas fat, carbohydrate and energy value decreases with supplementation. 14 patients suffering from hyperlipidemia were selected for the study and were treated by 60gm of functional biscuit T_1 for 45 days. Blood lipid level – total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides were measured before and after the ingestion of biscuit formula. This study shows that biscuit formulations can be modified significantly to develop functional biscuit which could be useful as a therapy for improving blood lipid profile.

Keywords: Functional biscuit, hyperlipidemia and lipoprotein.

1. Introduction

The CVD is one of the leading causes of death worldwide. The World Health Organization (WHO) estimates 20 million deaths due to coronary vascular disease (CVD) in 2015, which is accounting for 30 percent of deaths worldwide (WHO, 2005). Hyperlipidemia or Dyslipidemia is a major risk factor in the development of atherosclerosis and coronary vascular disease (Steinberg, 1987) [25].Clinical signs of this condition are an increase in the fasting serum cholesterol level (hypercholesterolaemia) or the fasting serum triglyceride level (hypertriglyceridaemia) or both.

Soy flour has a highly digested & high quality soy protein. The isoflavones, a class of phytochemicals found in soybeans have been reported to be responsible for lowering the cholesterol (Potter, 1998) [24]. There are evidences that consumption of soy protein in place of animal protein lowers the blood cholesterol levels and provides other cardiovascular benefits. Anderson (1995) [3, 4] found that every 1% reduction in cholesterol values is associated with an approximate 2–3% reduction in the risk of coronary heart disease. Daily intake of 20–50 grams of isolated soy protein could result in a 20–30% reduction in coronary disease risk (Bakhit *et al*., 1994) [6].

Replacement of saturated fat by MUFA and PUFA in food products decreases the risk factor associated with heart disease. The trend of functional foods is gaining popularity day by day due to increased health awareness among human beings. Due to urbanization people want ready to eat, convenient healthy food which provides health benefits beyond the basics nutrients. Biscuit is most popular convenient snack food with longer self-life. Due to the high consumption of ready to eat, convenient food like biscuit, worldwide attempt has been made to improve the nutritional value of biscuit and functionality by modifying their nutritional composition. Otles and Cagindi (2006) [22] reviewed that wheat, buckwheat, oat, barley soy notified the most common cereal based functional foods. Functional food or medicinal food is a healthy food which have health-promoting or disease-preventing property along with the basic nutrition (Bech-Larsen & Grunert, 2003) [7]. From last few year demand of functional food has being increased as it leads to healthier life without changing the eating habits of the consumer.

.Therefore in present study effort was made to increase the nutritional value of biscuit by composite flour technology and replacing traditional saturated fat by olive oil, which has a beneficial effect in maintaining good lipid profile and blood glucose level.

2. Materials & Methods

The present experimental work "Formulation of Functional Biscuit for Lowering Blood Lipid Level" was carried out in the research laboratory of Department of Food and Dairy Technology, Warner School of Food and Dairy Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences with appropriate methodology.

2.1 Raw Material

Refined wheat flour, soy flour, sugar, olive oil, milk powder, salt and baking powder are purchased from the local market of Allahabad. The equipment & chemical for preparation and analysis were taken from lab of department of Food and Dairy technology, SHIATS, Allahabad.

2.2 Subjects

Fourteen hyperlipidemic people, who had at least one of parameters of blood lipid indices including TC ≥250, HDL ≤30, LDL ≥150 and TAG ≥150 mg/dL with normal blood pressure. Their ages ranged from 45 to 60 years and with equal number of male and female were studied. Their mean body weight was 60.8kg. The protocol and purpose of the study was explained to the subjects. They take 60g biscuit in a day & were advised to take balanced diet as per directed by the dietician for 45days. Patients visit daily in a clinic with their diet dairy and receive

fresh soy biscuits. Venous Blood sample was drawn before and after 45days for testing blood lipid profile.

2.3 Formulating Composite flour

Wheat & soy flours sieved through a metal sieve of 160mm pore size before being mixing into different ratio to obtain different flour blends. Composite flour were prepared by substituting 10%,15%& 20% wheat flour by soy flour in the ratio of 90:10, 85:15, 80:20 as shown below:

2.3.1 Treatments:

T0 - Biscuit made by 100% wheat flour
T1 – 10% Soya flour + 90 % wheat flour.
T2 -15% Soy flour + 85% wheat flour.
T3 -20% Soy flour + 80% wheat flour.

2.4 Preparation of Biscuit

Biscuits were prepared by creaming method. Sugar powder and fat (olive oil) were creamed until the mixture became light and fluffy. Then milk powder was added to the cream while mixing. Salt, ammonium bicarbonate, baking soda were added to the creamed mixture. The flour was slowly introduced into the mixture and mixed.

Table 1: Recipe of Manufacturing of Biscuit

Ingredients	Amount
Composite flour	100g
Grinded sugar	30g
Olive oil	20 ml
Milk powder	10g
Salt	0.5g
Ammonium bicarbonate	2.5g
Baking Powder	1.5g

Then 20 ml of water is added to make dough.Then dough was sheeted to a thickness of 3.5mm with the help of flat rolling board. The sheeted dough was cut into fine circular shape using a wooden cutter. The cut dough was transferred to greased baking tray. The biscuits were baked in an electric oven maintained 175 ^{0}C for 15minutes.The biscuit were cooled for about 30 minutes, packed into LDPE bags for further analysis.

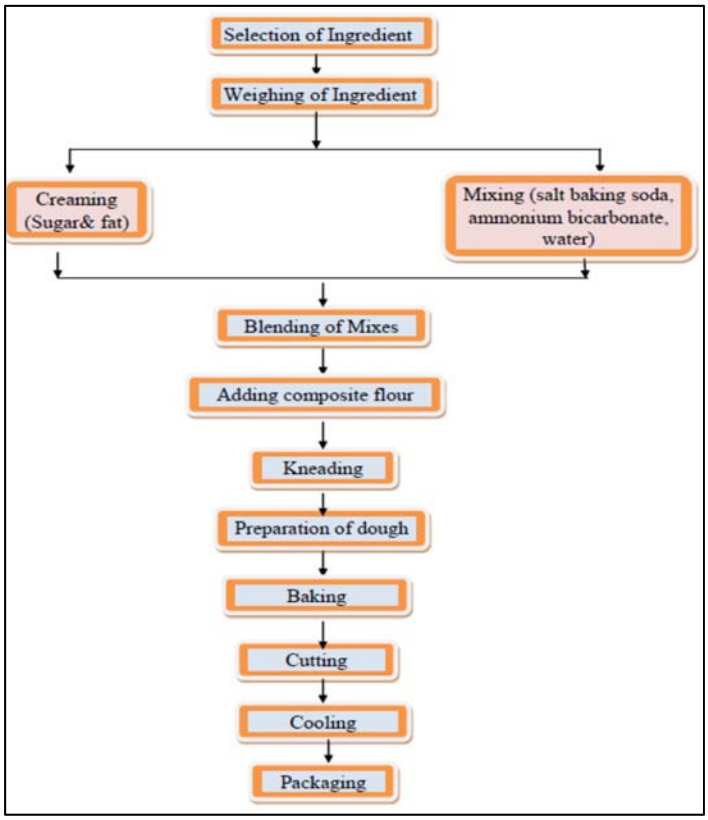

Fig 1: Flow sheet for preparation of functional biscuit

2.5 Sensory Analysis

The sensory analysis of functional biscuit was done by using nine point hedonic scale system as describe by Iwe *et.al;* 2002 [17]. The 20 semi-trained panelist were selected from the staff of department of Food and Dairy Technology, SHIATS, Allahabad for the sensory analysis like color, flavor, texture overall acceptability of prepared functional biscuit. All the products were coded and presented to the panelist to gives score 9-1 to the product, ranging from 'like extremely' to 'disliked extremely' to find out the most suitable composition of biscuit.

2.6 Physical analysis: Width, thickness were measured and spread ratio was calculated as per the method described by AOAC, 2000.

Width (W): Six biscuits were placed horizontally (edge to edge) and rotated at 90° angle for reading.

Thickness (T): Six biscuits were placed one another to compute thickness.

Spread factor (SF): It was calculated according to the following formula:
SF = (W/T x CF) x 10
Where; CF= Correction factor (1.0 in this case).

2.7 Chemical composition
Chemical composition includes moisture content, total ash, crude protein, fat, carbohydrate and crude fibre were determined using AOAC method (2000) [5].

2.8 Measurement of Serum lipid
Blood samples were analyzed for 1) Total serum cholesterol levels were measured using a cholesterol ester/oxidase enzymatic procedure. 2) HDL cholescholesterol concentrations were measured, using cholesterol e ster/oxidase enzymatic procedure, following precipitation of LDL, VLDL and chylomicron fractions by buffered polyethylene glycol 6000. 3) LDL cholesterol levels were measured using a direct enzymatic selective protection method. 4) TAG levels were measured using a glycerol kinase based enzymatic procedure.

2.9 Statistical Analysis
The experiment was conducted by adopting completely randomized design. The mean squares of triplicate scores were determined and subjected to analysis of variance (ANOVA) using SSPP (Statistical package for social Statistics).Difference among means were compared using Duncan's Multiple Range Test at significant level 95% ($p \leq 0.05$).

3. Result and Discussion
3.1 Chemical composition of Flour
The chemical composition of the composite flours affect both physico-chemical properties and nutritional quality of their products (Dhingra and Jood, 2001 [11]. Akhtar *et al.,* 2008; Mashayekh *et al.,* 2008).The obtained result has been presented in Table 2.

Table 2: Chemical composition of refined wheat flour and Soy flour (% on dry weight)

Parameters	Refined wheat flour (%)	Soy Flour (%)
Moisture	12±0.1	7.2±0.5
crude Protein	11.6±0.9	52±1.3
crude fat	1.41±0.2	1±0.1
Total Ash	0.50±0.1	5.7±0.2
Fiber	0.81±0.2	7.2±0.4
Total Carbohydrates	72.5±0.3	23.6±0.2

Data are the means of triplicates ± standard deviation.

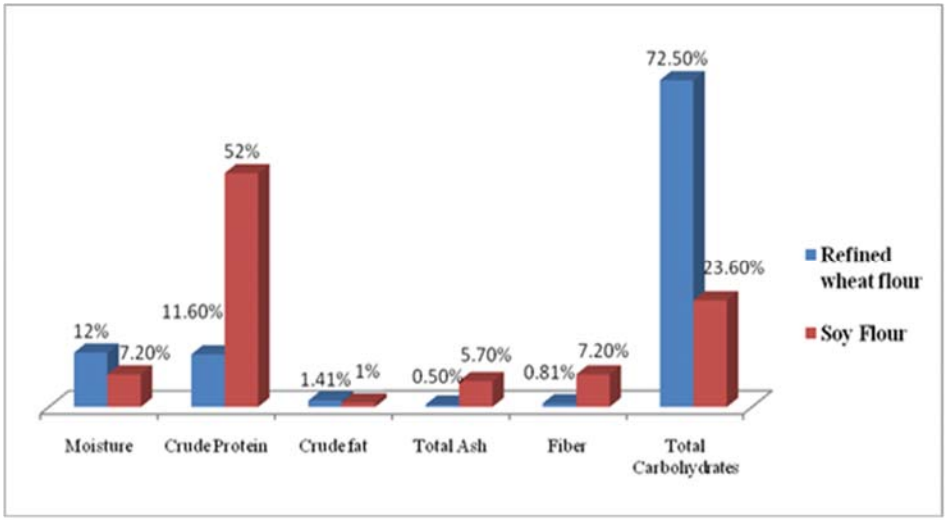

Fig 2: Compare the proximate composition of wheat & soy flour

Figure 2 shows the moisture content of wheat flour (12%) higher than soy flour (7.2%) reveals its shelf life and storage. Whereas the fiber (7.2), protein (52%) and ash content (5.7%) of soy flour was higher than refined wheat flour signifies the importance of soy flour in nutritional enrichment in food preparation.

3.2 Sensory Analysis
For the consumer acceptability of any product sensory analysis is important. The sensory scores obtained from control and different treatments of functional biscuit were presented in Tables 3.

Table 3: Sensory score of biscuits

Treatments	T0	T1	T2	T3
Taste	7.5±0.2a	7.0±0.3b	6.9±0.1b	6.3±0.2bc
Color	7±0.1a	7.2±0.4b	6.9±0.2c	6.8±0.2d
Texture	7.1±0.2a	6.9±0.3a	6.7±0.2ab	6.5±0.3ab
Flavour	7.3±0.1a	6.9±0.2bc	6.8±0.2bc	6.73±0.3bc
Overall acceptability	7.4±0.2a	7.2±0.2b	6.8±0.3c	6.5±0.1d

Means carrying the same letter in superscripts in a column do not differ significantly ($p \leq 0.05$).

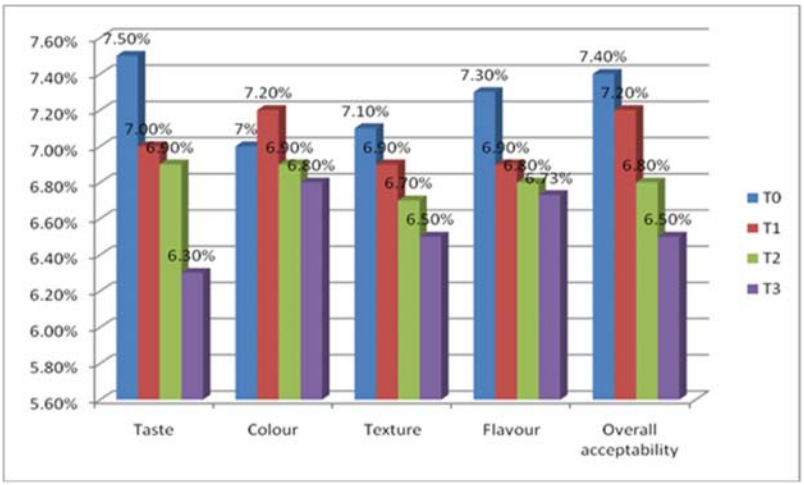

Fig 3: Compare the sensory score of biscuits

3.2.1 Taste: Taste is the important criteria make the product liked or dislike by consumers. Mean score for taste has been shown in Table 3, elucidated decrease in the quality score for taste from 7.5 to 6.3 with the increase in the level of substitution. This difference observed could be as a result of substitution of soy flour and olive oil. Soy flour has a beany flavor which can affect the taste of the biscuit. Drobot & stabikone (1976) [12] found similar finding of decreasing score for taste with soy incorporation.

3.2.2 Color: Mean scores for color declines from 7.2 to 6.8. With increasing level of substitution, the color of biscuit turned from light brown to dark brown, leading to lower acceptance (Latidoyeop and Sobowale, 2011). The darker color of the fortified breads and biscuits have been reported by several authors (Best, 1987 & Gopalan *et.al;* 2001). Browning color of bakery product like bread, biscuit might be due to caramelization, dextrinisation of starch or maillard reaction (Sudha *et.al;* 2007) Soyabean has reported to be rich in lysine protein undergo mailard reaction with reducing sugar molecules,give characteristic brown color (Decker *et.al;* 2002 & Tsuji *et.al;*.2001).

3.2.3 Flavor: Mean scores for flavor decreases 7.3 to 6.7 (Figure 3). In terms of flavor, the functional biscuits were not significantly different ($P \leq 0.05$) from each other. Mean score for flavor decreases due to the beany flavor of soy flour. Similar result was founded by Onweluzo & Iwezu (1998) [20], reported decreasing trend in the flavor score of the biscuit enriched with soy flour. Hussain (1993) also reported a decrease in trend in the biscuit enriched with gram flour.

3.2.4. Texture: The texture of the biscuit reveals the external appearance of the biscuit whether smooth or rough (Banureka and Mahendran, 2009) [27]. Mean score of texture decreases from 7.1 to 6.5 with increasing the level of substitution of soy flour from 10%to 20%(Figure 3).Result reveals the control biscuit T0 (7.1) has smoothest texture. In all treatments, T1(6.9) ranked top and do not differ significantly with control biscuit whereas T3 (6.5) ranked lowest. Similar result was found Mc watter, 1978 [18] in his study. The mean score for the texture decreases may be due to the protein and fiber content of composite flour increases, which affect the texture (i.e. increases hardness).

3.2.5 Overall acceptability: Overall acceptability was determined on the basis of quality scores obtained from the evaluation of color, taste, flavor, texture and crispness of the biscuits. Mean sensory score regarding overall acceptability of biscuits are presented in Table 3. In all treatment biscuit with 10% soy biscuit had highest overall acceptability.

3.3 Physical Analysis: Physical analysis of biscuit is important as an objective judgment of quality. The result of the physical analysis of the functional biscuit is shown in Table 4, which shows that the supplementation of various levels of composite flour has a significant effect on width, thickness and spread ratio of biscuits. The result obtained agreed with result reported by Bunde *et al.,* 2010 [8].

Table 4: Physical score of biscuit

Treatments	T0	T1	T2	T3
Width(mm)	48	46.5	45.2	43.8
Thickness(mm)	9.1	9.3	9.5	9.8
Spread Factor (%)	52.7	48.9	47.5	44.6

*Data are the means of five values of replicates.

The result eluded that weight, width & spread factor of functional biscuit decreases with increasing soy flour whereas thickness increases with incorporation of soy flour.

Width: The mean score of width decreases from 48mm to 43.8mm with increasing in the level of substitution, this may be due to the high fiber content of composite flour than control flour. The fiber has deleterious effects on dough structure & loaf volume, have been suggested to be due to the dilution of gluten network which in turns impairs gas retention rather than gas production **(**Dewettinck *et al.,* 2008; Eiman *et al.,* 2008*;* Elleuch *et al.,* 2011**).** The result shows that control treatment T0 has the maximum width 48mm, followed by T1 (46...5) and T2 (45.2) while minimum width was observed in T3 (43.8).

Thickness: Mean score of thickness increases from 9.1mm to 9.8mm with increasing level of substitution (Table 4). The result shows that T3 has maximum thickness 9.8 followed by T2 (9.5) while minimum thickness was observed in control treatment T0 (9.1). The results of present study are quite close to the observations reported earlier by numerous researchers. Zaker *et*

al.; (2012) [29] elucidated in his study, thickness of biscuits increased with increase in level of supplementation of defatted soy flour may be attributed due to better binding strength of soy protein

Spread factor: Greater spread ratios are desirable and indicate a better biscuit quality **(**Seker *et al*.; 2010**).** The spread ratio was affected by the competition for the available water. The spread factor of biscuit decreases from 47.8 to 34.1 with increasing the supplementation. This reduction in the spread factor of the biscuits attributed to the proteins & fiber present in the composite flour because protein & fiber has more binding power and it binds water and restricts the spread of the biscuits The result shows that control treatment T0 has the maximum spread factor 47.8mm, followed by T1 (44.2) and T2 (41.4) while minimum width was observed in T4 (34.1) (Figure 4).

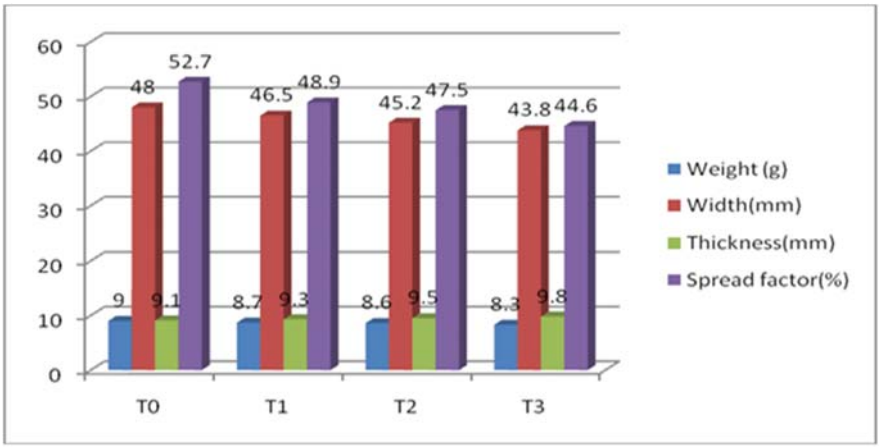

Fig 4: Compare the physical score of biscuits.

3.4 Chemical analysis of Functional Biscuit

The result of the chemical analysis of functional biscuit was shown in Table 5.

Table 5: Chemical analysis of functional biscuit

Treatments	T0	T1	T2	T3
Moisture(%)	2.51±0.4b	2.6±0.3a	2.69±0.2a	2.79±0.2c
Ash(%)	0.7±0.1	1.2±0.3	1.5±0.2	1.85±0.3
Protein(%)	5.5±0.2	9.8±.0.9	11±0.5	12.5±07
Fat(%)	20±0.2b	19.5±0.2b	19.1±0.1bc	18.5±0.3c
Fiber(%)	0.18±0.1a	0.6±0.1bc	0.8±0.2bc	1±0.3c
Carbohydrates(%)	68..69±0.2	64.87±0.1	63.25±0.3	61.6±0.1
Energy	476.76	474.18	468.9	462.9

Data are the means of five values of replicates ±standard error.
Means carrying the same letter in superscripts in a column do not differ significantly ($p \leq 0.05$).

3.4.1 Moisture

The moisture content of biscuit increases with the supplementation of defatted soy flour from 2.51% to 2.79% this could be due to the high water binding capacity of soy flour. The highest moisture content found in T3 (with 20%DFS biscuit) followed by T2 (with15% DFS biscuit) and the lowest moisture was observed in T0 (non supplemented biscuit).This result is similar to the earliar finding of Zaker *et.al*; 2012 [29] but in other study of Awasthi *et.al;* 2012 [16] the moisture content of biscuit decreases with increases the supplementation of soy flour. There is need to further study of moisture content of soy flour supplemented biscuit concerning self-life as the high moistures leads the higher risk of mold growth.

3.4.2 Ash

Mean score for ash content of different treatment (Table 5) of biscuits revealed the significant variation of ash content with supplementation of soy flour. The maximum ash content was observed in T3 (with 20%DSF) followed by T2 (with15% DFS biscuit) and the lowest moisture was observed in T0 (non supplemented biscuit). The linear variation of ash content with DFS may be attributed due the high mineral content of soy flour.

3.4.3 Protein

The result depicts the protein content of biscuit increases with supplementation of soy flour from 5.5% to 12.5%. The maximum protein content was observed in T3(12.5%) followed by T2 & T1 whereas control biscuit has minimum protein content (5.5%).This could be due to high protein value of soy flour. Our result are supported by other study of Awasthi 2012 [16], Banureka and Mahendran 2009 [27]. Soy flour is high quality protein complement to lysine limited cereal protein. Soy incorporation enhances the quantity and quality of protein content of food product which increase its potential for the formation of functional biscuit.

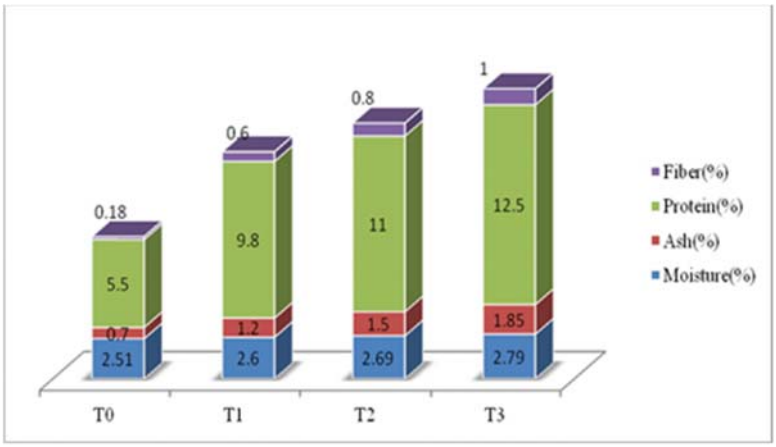

Fig 5: Compare moisture, ash, protein and fiber content of biscuits.

3.4.4 Fiber

The result of fiber content of biscuit (Table 5) depicts the fiber content of biscuit increases from 0.18 to 1.0
Linearly with soy flour supplementation 0% to 20%. The fiber content in biscuit attributed may be due to
The high fiber content of soy flour. Demand of high fiber food product has increased due to its ability
To reduce serum cholesterol level and incidence of cancer. Pereira *et al*; 2004 [23] found in his study that 10gm Increasing in total dietary fiber intake produced 14% reduction in all coronary event and 27% reduction In the risk of coronary death.

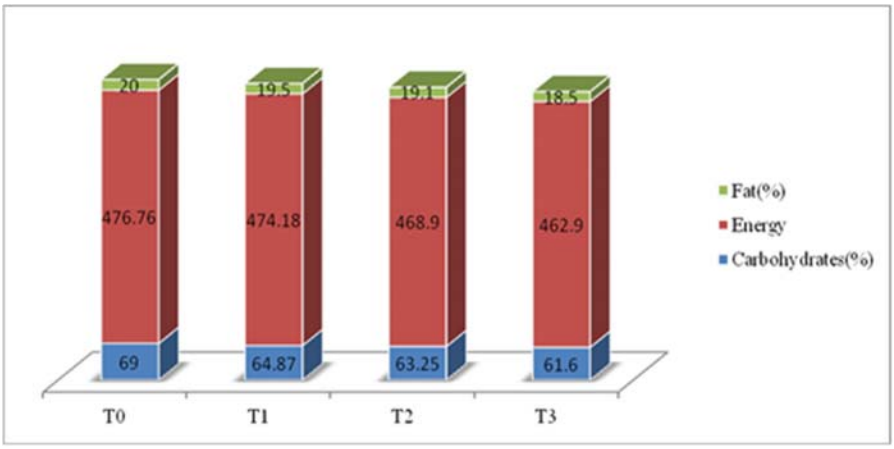

Fig 6: Compare the fat, carbohydrate and energy content of biscuits.

3.4.5 Fat

The fat content of biscuit decreases with increases the supplementation of soy flour from 0% to 20%.The maximum fat content was observed in non-supplemented biscuit T0(20%) which do not differ significantly from fat content of T1(19.5%) while the lowest fat content was observed in T3 (18.5%). The slight reduction of fat content was observed due to the incorporation of soy flour.

3.4.6 Carbohydrate

As shown in Figure 6 the carbohydrate content decreases from 69% to 61.6% with supplementation of soy flour from 0% to 20%. This variation is due to lower carbohydrate content of defatted soy flour than refined wheat flour.

3.4.7 Energy

The calorie content of the biscuits has been decreased from 476.76 to 462.95kcal with supplementation from 0 to 20% (Figure 6). The total calorific value of control samples was observed to be higher 476.76 Kcal compared to 20% supplementation biscuits 462.95kcal; however, it is worthwhile owing that major source of calories in case of treated samples was contributed by protein and fiber which is superior in terms of nutrition point of view.

3.5 Blood Lipid profile

Table 6 elicited that ingestion of biscuit would improve blood lipid profile. This may be prepare functional biscuit were rich in high quality protein, fiber and unsaturated oil-PUFA, MUFA. Traditional biscuit were rich in saturated biscuit and low in protein, fiber which lower its nutritional value whereas addition of soy flour besides increasing protein and fiber also improve fat composition of biscuit. As soy flour rich in PUFA and olive oil rich in MUFA, may improves fatty acid composition of functional biscuit. A recent study conducted in the United States reported that intake of both MUFA and PUFA was associated with reduced CVD risk.

Table 6: Effect of functional biscuit on blood lipid profile

Lipid profile	Before (mg/dl)	After (mg/dl)	% change

TG	209.6	208.5	0.52
LDL	155.6	154.3	0.84
HDL	32	31.8	0.46
TC	242.8	241.5	0.54

4. Conclusion

From present experimental work, it was concluded it is possible to formulate soy biscuit by replacement of wheat flour with 20% defatted soy flour without affecting physical, chemical and sensory characteristic of biscuit. Result of sensory evaluation of biscuits showed that by adding 10% of defatted soy flour had higher overall acceptability. It was found from present study that soy flour biscuit is more nutritious than control as high in protein and fiber. In this investigation to know the effect of experimental biscuit on blood lipid level, 14 hyperlipedimic patient were treated with 60gm most acceptable biscuit for 45days with balanced diet. Result showed this functional biscuit lowers the Serum cholesterol levels by 0.52%, TG level by 0.54%, LDL level by 0.84% whereas increases serum HDL level by 0.46%. There is need to study further to explore the possibility of using soy flour and olive oil for formulation of biscuit to decresing risk of many disease like obesity, CVD, DM. and to know its effct on hyperlipidimic patient large sample group need to be take. In this study we had not studied the shelf life of biscuit, which need to study further.

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