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FORMULATION OF VALUE ADDED LOW-CALORIE, HIGH FIBRE BISCUITS USING FLAX SEEDS AND *STEVIA REBAUDIANA*

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ABSTRACT: Worldwide scientists are focusing on developing functional food products which are healthy and low in calories. The possibility of incorporating roasted flax seeds flour (RFF) and substituting sugar with *Stevia rebaudiana* leaves powder (SLP) for developing nutritious, healthier and high quality biscuits has been investigated. Overall six biscuit formulations were designed besides control. SLP was used as a natural sweetener and RFF was used as a source of dietary fibre. Standardisation was done by evaluating the sensory quality of the formulated biscuits prepared from a mixture of blends. As soon as standardisation of blends was completed, the standardised flour mix was used to formulate biscuits by substituting sucrose with SLP at levels of 5.5%, 6%, 6.5%, 7%, 7.5% and 8%. The treatment (T_4) was found to be the best among different variations on the basis of sensory evaluation with incorporation of 8% (RFF) and 7% (SLP) resulted in increased moisture (7.57%), ash (3.69%), protein (18.88 g/100g), fat (10.97 g/100g), fibre (4.52 g/100g), calcium (408.23 mg/100g), phosphorus (445.03 mg/100g) and iron (10.01 mg/100g) content in comparison to control (T_0) which was high in calories (384 Kcal/100g) and carbohydrate (62.34g/100g) content. Thus, results signify that incorporation of RFF at 8% and SLF at 7% enhance the functional properties of developed biscuits by reducing the calorie density and improving the health benefits.

INTRODUCTION: In recent era, the people are more concerned for their health and lifestyle; there is a demand on the production of low calorie, high fibre ready to eat food products. Numerous studies have been conducted for appropriate replacement of sucrose (sugar) with artificial/natural sweeteners.

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Consumption of high sucrose content is associated with common conditions that show negative effects on human body. It can lead to life threatening complications like high blood pressure, atherosclerosis, coronary heart disease, obesity, cancer, diabetes mellitus type 2, high blood cholesterol, insomnia, bone and joint diseases.

The increased prevalence of these non-communicable diseases is due to decreased physical activity and sedentary life style, high level of mental stress and increased consumption of unhealthy diet rich in starch, sugars, saturated fat and excessive calories.

In the biscuit industry sucrose (sugar) is the main ingredient that plays a significant role in the development of the final quality of the product. It's a very big challenge to replace sugar from the biscuit industry as it improves the taste and boost the flavour of the product. Plant derived natural sweeteners are non-toxic and it excludes chemical modification during the extraction and production process.

Worldwide, for the past few years the demand for bakery products is increasing and biscuits are one such ready-to-eat, inexpensive and convenient processed food product which is widely consumed in India. The major ingredients required for the preparation of biscuits are wheat flour, butter/oil and sugar, which overall imparts flavour, taste, texture and overall acceptability along with providing dense calories, saturated fat, low fibre and mineral contents.

Now a day, Flaxseeds (*Linum usitatissimum*) is being identified as functional foods and being used as nutraceuticals due to its health promoting and therapeutic properties. It possesses significant quantities of linolenic acids (omega-3), dietary fibres and lignins. These compounds help in lowering blood cholesterol and reduce the risk of heart disease, stroke, immunological and inflammatory disorders [1]. Still the consumption of flax seeds is very low in India but due to growing interest of consumers to adopt healthy lifestyle and eating habits they demand for nutritionally adequate and sensory quality food.

Stevia rebaudiana (Bertoni) is one such herb possessing medicinal and commercial importance contributing low calorie sweetness and bioactive properties [2]. The non-toxic sweet tasting, natural constituents present in the plant are ent-kaurene diterpene glycosides – stevioside, rebaudiosides A–G, dulcoside A, rubusoside and steviolbioside. These glycosides are 250-300 times sweeter than sucrose on a weight basis [3]. In addition to sweetness, various therapeutic activities of these glycosides have been identified such as anti-cancer [4], anticariogenic [5], anti-hyperglycaemic [6], anti-inflammatory [7] and antioxidant activity [8]. This is one of a most desirable option for consumers looking after healthy alternatives to sucrose. In 2007, the specifications were laid down by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) suggesting that at least 95% of the known steviol glycosides should be present in the steviol glycoside sweeteners [9]. Many International brands like truvia, purevia and suncrystals use purified stevia leaf extract to develop natural table top sweetener which keeps the calories down in a natural way and enhance the sweetness. Therefore there is an urgent need to substitute sugar with natural low calorie sweeteners like *Stevia rebaudiana* leaves powder which will increase the nutritional profile of the products. Therefore, it is important to develop new products which include nutritional and functional characterization, with special emphasis on consumer acceptance.

Alpaslan and Hayta [10] reported that consumption of bakery products like biscuits are the best possible way for delivering bioactive compounds in human diet. There is an increased demand for health oriented functional food products such as low calorie, sugar free and high fibre products.

Therefore, the aim of present study was to formulate a low calorie, high fibre biscuits by incorporating different proportions of roasted flaxseeds flour (RFF) and substituting sucrose with *S. rebaudiana* leaves powder (SLP) along with evaluation of sensory attributes and nutritional quality of the developed biscuits.

Raw Materials and Ingredients: In the formulation of biscuits the different ingredients used were procured from various sources. Fresh leaves of *S. rebaudiana* were purchased from Bioved Research Institute, Allahabad. All the required basic ingredients such as whole wheat flour, oats meal, flaxseeds, sugar, refined oil, cinnamon powder, salt, skimmed milk powder, vanilla essence, baking powder and carom seeds (ajwain) were purchased from the specialised and certified food markets of Allahabad city.

Preparation of Stevia Leaves Powder: Fresh leaves of *S. rebaudiana* were cleaned; stems and unwanted parts were removed and washed with lukewarm water to remove dirt particles, excess water was drained out and leaves were dried under shade for about 5 days and then powdered by using a high-speed blender (25000/min) then sieved through 52 mesh sieve to obtain a particle size of 355 µm or less and stored in airtight polythene bags at 4 °C for analysis of further study [11].

Preparation of Biscuits: Overall 7 biscuit formulations were prepared which includes control and different treatments as described in **Table 1**. First of all oat meal and flax seeds was roasted and then allowed to cool which was then coarsely grinded. For the preparation of dough different ingredients are mixed together in specified proportions like skimmed milk powder, whole wheat flour, roasted oat meal flour, roasted flax seeds flour, baking soda, baking powder, cinnamon powder, carom seeds and salt. In a large bowl, vegetable oil and granulated sugar was blended together for 4-5 minutes in case of control biscuits whereas SLP was used in case of treatment biscuits, with the addition of vanilla essence. In to this the above flour mixture was added and homogenously mixed to a crumbly texture. For dough preparation required amount of water was added for kneading until just combined which was allowed to rest for 30 min.

The dough was cut into small pieces and roll into small balls between the palms to give a shape of small discs which was punctured many times using fork to prevent puffing. The prepared biscuits were baked in a pre-heated oven at 150 °F for 10-15 min. which was followed by cooling for 3 minutes on baking sheet, then transfer in metalized LDPE pouches (20.87 µm thick) and stored at 37 °C for further analysis.

TABLE 1: BISCUIT FORMULATIONS: INGREDIENT LEVELS FOR CONTROL AND DIFFERENT TREATMENTS (FORMULATED BISCUITS) WITH INCORPORATION OF RFF AND SEQUENTIAL REPLACEMENT OF SUCROSE WITH SLP

Ingredients (g)	Biscuit Formulations						
	Control (T_0)	T_1	T_2	T_3	T_4	T_5	T_6
Roasted oats meal flour	25 g	25 g	25 g	25 g	25 g	25 g	25 g
Roasted flaxseeds flour	-	9.5 g	9 g	8.5 g	8 g	7.5 g	7 g
Whole wheat flour	25 g	25 g	25 g	25 g	25 g	25 g	25 g
Refined oil	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
Skimmed milk powder	25 g	25 g	25 g	25 g	25 g	25 g	25 g
Vanilla essence	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Cinnamon powder	1 g	1 g	1 g	1 g	1 g	1 g	1 g
Salt	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g
Carom seeds	1 g	1 g	1 g	1 g	1 g	1 g	1 g
Baking soda	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g
Baking powder	1 g	1 g	1 g	1 g	1 g	1 g	1 g
Sucrose	15 g	-	-	-	-	-	-
Stevia leaves powder	-	5.5 g	6 g	6.5 g	7 g	7.5 g	8 g
Total	100	100	100	100	100	100	100

Organoleptic Evaluation: The developed freshly baked low calorie and high fibre biscuits (six treated samples at a time, incorporated with RFF and replacement of sucrose with SLP) were served to 30 semi trained panellists for the evaluation of sensory attributes (colour, texture, flavour, taste and overall acceptability) on a nine point hedonic scale with a scores ranging from 9 to1 (1 - dislike extremely, 2- dislike very much, 3- dislike moderately, 4- dislike slightly, 5- neither like nor dislike, 6- like slightly, 7- like moderately, 8- like very much, and 9- like extremely) [12]. The parameters for quality were quantified and the mean scores were calculated.

Nutritive Value of Developed Biscuits: The developed biscuits were subjected to chemical analysis as detailed under this section.

Moisture Estimation: The proximate composition of the samples was determined using the methods of the AOAC [13]. In washed, dried and weighed crucible, 1.0g of dried sample was placed. This was transferred in an oven and dried at 105 °C for three hours. The sample was allowed to cool in desiccators and then reweighed. The content of moisture in percentage was calculated using the formula.

$$MC\ (\%) = \frac{W_o}{W_i} \times 100$$

Where, W_o = loss in weight (g) on drying and W_i = initial weight of sample (g).

Total Ash Estimation: Ash content measures the total amount of minerals or inorganic content present in the sample which can be estimated by igniting a known amount of dried material in a muffle furnace. A known amount of dry sample (2 to 5 g) was placed in a crucible of constant weight, which is then transferred in a muffle furnace and heated at 550 ± 5 °C for 12 hours, allowed to cool and then transferred to a dryer and again carefully weigh the crucible with the ash.

Calculation: Ash content (%) = 100XA-B/C

Where, A=weight of crucible with sample (g)
B=weight of crucible with ash (g)
C=weight of sample (g).

Protein Estimation: Determination of crude protein was done by determining the total organic nitrogen using the Macro-Kjeldhal Method [14]. This involved 3 steps: digestion, distillation and titration. About 1.0 g of the sample was weighed in triplicate and placed in digestion flasks. The total organic nitrogen (TON) was then calculated using the formula:

$$\% \text{ TON} = \frac{\text{TV x NE x TV}_d}{\text{M}_s \text{ x V}_d} \text{ x } 100$$

Where, TV = titre value, NE = mg nitrogen equivalent to molarity of acid, TV_d = total volume to which digest was diluted, M_s = mass of sample (g) and V_d = volume of digest distilled.

% crude protein = % TON x 6.25

(6.25 is a general factor suitable for products in which the proportions of specific proteins are not well defined).

Fat Estimation: Determination of crude lipid content of the samples was done using direct solvent extraction method [14]. The solvent used was petroleum ether (boiling range 40 °C – 60 °C). 3.0 g of the dried sample was weighed in triplicate and secured in soxhlet extraction thimble. The percentage crude lipid was calculated using the formula:

$$\text{CL } (\%) = \frac{\text{M}_{ex}}{\text{M}_s} \text{ x } 100$$

Where M_{ex} = mass of extract (g) and Ms = Mass of sample used (g).

Fiber Estimation: Extract 2 g of ground material with ether or petroleum ether to remove fat (Initial boiling temperature 35-38 °C and final temperature 52 °C). After extraction with ether boil 2 g of dried material with 200 mL of sulphuric acid for 30 min with bumping chips. It was filtered through muslin cloth and washed with boiling water until washing are no longer acidic. 200 mL of sodium hydroxide solution was boiled for 30 min. Filter through muslin cloth again and wash with 25 mL of boiling 1.25 % H_2SO_4, three 50 mL portions of water and 25 mL alcohol. The residue was removed and transferred to ashing dish (pre-weighed dish W_1). Dry the residue for 2hr at 130 ± 2 °C. Cool the dish in a desiccator and weigh (W_2). Ignite for 30 min at 600±15 °C. Cool in a desiccator and reweigh (W_3).

$$\% \text{ crude fiber} = \frac{\text{Loss in weight on ignition } (W2-W_1) - (W_3 - W_1) \text{ x100}}{\text{Weight of the sample}}$$

Carbohydrate Estimation: Total carbohydrate content of each sample was estimated by 'difference' method. The sum of the percentages of all the other proximate components was subtracted from 100.

Total CHO (%) = 100 – (% moisture + % crude protein + % crude fat + % ash)

Calcium Estimation: 5 ml of concentrated hydrochloric acid was added in a 10 g of ash sample; the upper part of dish is rinsed and evaporated to dryness. 2 ml of hydrochloric acid was added in the residue and covered with watch glass and then ash solution was filtered into 100 ml volumetric flask and diluted to 100 ml volume. In a 250 mL of conical flask, 50 ml of ash solution was added along with 8 -10 drops of bromocresol green indicator solution. In the above sample 20 % of sodium acetate was added gradually until the solution turns blue (pH at this point was 4.8-5.0) and then covered with watch glass and heated to boiling.

Drop wise added 3 % oxalic acid solution until the solution colour turns green (pH 4.4 to 4.6). The boiling was continued for one minute and allowed it to stand until clear or overnight.

The obtained supernatant solution was filtered and discarded through Whatman no. 42 filter paper. The precipitate in the conical flask was washed and filtered with 50 ml of dilute ammonium hydroxide solution; it was again washed with hot distilled water. Finally the filter paper was washed with a solution containing 125 ml distilled water and 5 ml concentrated sulphuric acid, the tip of filter paper was broken and the washing was collected in original empty conical flask. The solution was heated to 90 °C and titrated with 0.1N potassium per magnate solution until solution turns pink.

Calculation: 1ml of 0.1N $KMnO_4$ = 2 mg Calcium

$$\text{mg calcium in 100g of sample} = 2 \times (A - B) \times \frac{V_1}{V} \times \frac{100}{W}$$

Weight of the sample (W) = 5 g
Volume of ash solution made (V) = 100 ml
Volume of ash solution used (V_1) = 50 ml
Volume of 0.1N $KMnO_4$ used for sample = A
Volume of 0.1N $KMnO_4$ used for blank = B

Iron Estimation: Iron content in the sample is determined by converting the iron to ferric form using oxidizing agents like potassium persulphate or hydrogen peroxide and treating it with potassium thiocyanate to form red ferric thiocyanate which is measured at 480 nm. For the experiment, saturated potassium persulphate ($K_2S_2O_8$) solution was prepared by dissolving 7-8g of potassium persulphate in distilled water and then filtered. The 3N potassium thiocyanate was also prepared by dissolving 146 g KCN in 500mL distilled water and filtered. 20ml of pure acetone was added in the filtrate to improve the keeping quality.

TABLE 2: THE SOLUTIONS ARE PIPETTED INTO THE THREE SEPARATE STOPPERED MEASURING CYLINDERS

Reagents	Blank(ml)	Standard(ml)	Sample (ml)
Standard iron solution	0.0	1.0	0.0
Sample ash solution	0.0	0.0	5.0
Distilled water	5.0	4.0	0.0
Conc.H_2SO_4	0.5	0.5	0.5
Potassium persulphate	1.0	1.0	1.0
Potassium thiocyanate	2.0	2.0	2.0

For the preparation of standard iron solution, 0.351g of ferrous ammonium sulphate was dissolved in 50ml water and 2.5ml concentrated sulphuric acid was added, the solution was warmed slightly and potassium permanganate solution was added drop by drop until a drop produces a permanent colour. The volume was making up with 500 ml distilled water. Finally the ash solution of the sample prepared by dry ashing was used for the colour development. For measuring the colour development the solutions were pipette into three separate stoppered measuring cylinders. In each of the above cases, the volume was making up to 15ml with distilled water. The colour developed was measured spectrophotometrically at 480 nm setting the blank at 100% transmission.

Calculation:

$$\text{Iron (mg/100g)} = \frac{\text{OD of sample} \times 0.1 \times \text{total volume of ash solution} \times 100}{\text{OD of standard} \times 5 \text{weight of sample taken for estimation}}$$

Phosphorous Estimation: Phosphorus reacts with molybdic acid to form phosphorus molybdate complex. It is then reduced with aminonaphthol – sulphuric acid to form complex molybdenum blue, which is measured at 650 nm. 5 ml of ash solution was taken which was obtained by dry ashing and 5 ml of molybdate reagent was added along with addition of 2ml of aminonaphthol – sulphonic acid solution and then volume was raised up to 50 ml. The blank was similarly prepared using distilled water in place of the sample. After 10 min. the OD was taken at 650 nm setting blank at 100% transmission.

Calculation:

$$\text{Phosphorus (mg/100 g)} = \frac{\text{O.D. of sample} \times \text{Total vol. of ash solution} \times 0.01}{\text{O.D. of std. solution} \times \text{Vol. of ash taken for estimation} \times \text{Weight of sample taken for estimation}}$$

Statistical Analysis: The obtained results was compiled, tabulated and analyzed by using one-way analysis of variance (ANOVA) and t-test was computed to evaluate the statistical significance of the data by using statistical software SPSS version 12.0.

RESULTS AND DISCUSSION: The entire experiment was under taken to develop low calorie, high fibre biscuits and its evaluation was done by incorporating different percentage of RFF and SLP. Results related to the development and standardization of biscuits *i.e.* sensory evaluation and nutritional analysis were discussed under this section.

Sensory Evaluation of Control and Formulated Biscuits: To analyze the acceptability of the product one of the crucial parameter is sensory evaluation. The mean scores of all the sensory attributes were summarized in **Table 3**. It was observed that control biscuits (T_0) had the highest sensory scores for colour (8.03), flavour (8.10), taste (8.46) and overall acceptability (8.10) than other treatments or variations. The other levels of incorporation were also liked at various degrees, although a little less than treatment T_4. It was observed that there was no significant difference ($p \leq 0.05$) in the sensory scores for flavour and overall acceptability. However, control biscuit (T_0) showed higher values than the formulated biscuits. In general, addition of RFF and replacement of sucrose with SLP alters the colour, texture and taste of the developed biscuits where as flavour and overall acceptability did not vary significantly ($p \leq 0.05$) in different treatments. According to Abdel–Shafi [15] substitution of sucrose with SLF inhibits the formation of acrylamide and also alters dough rheology along with baking attributes of biscuits. The quality attributes (viz. moisture content, colour development, breaking force, spread ratio) and acceptance of the biscuits gets affected if sucrose is substituted by more than 30% with Stevia. Ganorkar and Jain [16] studied that incorporating RFF from 5 to 30% with substitution of refined wheat flour decrease the colour and sensory score along with spread ratio. Incorporation of RFF above 15% adversely affects the cooking quality. Therefore, incorporating 15% of RFF in cookies results in product acceptability.

TABLE 3: AVERAGE SENSORY SCORE (9-POINT HEDONIC SCALE) OF DIFFERENT PARAMETERS IN CONTROL AND TREATED BISCUIT SAMPLES

Sensory Characteristics / Treatments	Scores on 9 point hedonic scale				
	Colour	Texture	Flavour	Taste	Overall acceptability
T_0 (Control)	8.03±0.48	7.60±0.45	8.10±0.21	8.40±0.39	8.10±0.35
T_1	7.45±0.43	7.65±0.41	7.80±0.91	7.45±0.28	7.58±0.17
T_2	7.85±0.24	7.70±0.25	7.45± 0.49	7.50±0.04	7.62±0.18
T_3	7.75±0.425	8.35±0.33	7.45±0.68	7.25±0.54	7.70±0.47
T_4	7.90±0.39	8.60±0.21	8.05±0.83	7.65±0.53	7.87±0.16
T_5	7.55±0.43	8.35±0.33	7.50± 0.52	6.75±1.11	7.53±0.65
T_6	7.50±0.47	8.25±0.35	7.40±0.51	6.70±1.08	7.46±0.63
F value	4.98*	13.88*	2.22	7.272*	1.066
CD (P=0.05)	0.373	0.307	0.568	0.605	0.379

9-point hedonic scale is as follows: 1-dislike extremely, 2-dislike very much, 3-dislike moderately, 4-dislike slightly, 5-neither like or dislike, 6-like slightly, 7-like moderately, 8-like very much, 9-like extremely.
* -Significant difference is at 0.05 levels; Values are means (± SEM).

Standardization of Recipe: The data illustrated in the above pertaining to the average sensory scores clearly indicate that the most acceptable variation was found to be treatment T_4 (**Table 3**). In obtaining the highly acceptable scores the biscuits were constantly prepared and standardized by three consecutive trials and through sensory evaluation as shown in **Table 3**. The overall mean scores of trials ranged from 6.70 to 8.60.

Therefore, from the mean values of sensory attributes it can be discerned that treatment T_0 (control) was found to be the best but substitution of sucrose with SLP and fortification of flaxseeds were very well acceptable. Among all the treatments, the treatment T_4 (incorporation of 8% RFF and substitution of sucrose with 7% SLP) had highest overall acceptability after control (T_0) and were highly acceptable by the panel members.

Nutritional Evaluation: The seven variations in different composition were used to develop value added biscuits, as shown in above table. RFF and SLP was the prime ingredient used in different proportions. On the basis of sensory evaluation, the treatment (T_4) received maximum mean score after control (T_0) which was identified and analyzed in the laboratory for nutritional properties such as moisture, ash, protein, fat, carbohydrate, energy, fibre, calcium, phosphorous and iron by AOAC [13] methods, the results are shown in (**Table 4**). The moisture content was increased in developed biscuit T_4 (7.57 %) as compared with control biscuits T_0 (7.42%).

This might be due to the presence of high fibre (gum mucilage) in flaxseeds which retain maximum moisture content [17]. According to Garcia–Serna [18] substitution of 100% sucrose with

Stevia significantly ($p<0.05$) increased the percentage moisture of the biscuits.

Ash content in treatment T_4 (3.69%) was increased than in control T_0 (2.61%). The reason for this could be attributed to higher mineral content of RFF and SLP [19]. Additionally, it provides high fat (10.97 g/100 g), protein (18.88 g/100 g) and fibre (4.52 g/100 g) content in the treatment T_4 than control (T_0). This accounts that flax seeds is a good source of fat as ground flaxseeds enhances the bioavailability of α-linolenic acid which helps in lowering low-density-lipoprotein-cholesterol by 18% and total serum cholesterol by 9%.

It also decreases postprandial glucose by enhancing the level of n-3 fatty acids in plasma and erythrocytes [20]. Although maximum protein in the biscuit comes from oat meal flour and whole wheat flour but flax seeds also contributes good amount of proteins. High amount of aspartic acid, glutamic acid and arginine was found in flaxseed proteins which play an effective role in lowering plasma cholesterol and triglycerides (TAG) [21]. No deleterious effect on the sensory attributes of biscuits was seen when flax seeds flour was supplemented upto 15 percent [22]. Flaxseeds are rich in both soluble and insoluble fibres and the presence of these fibres add bulk to the waste products present in the gut and increase the bile movement in the gastrointestinal tract.

TABLE 4: NUTRITIONAL COMPOSITION (PER 100 g) IN CONTROL (T_0) AND BEST TREATMENT (T_4) OF DEVELOPED BISCUITS

Nutrients	Control (T_0)	Best Treatment (T_4)
Moisture (%)	7.42 ± 2.10	7.57 ± 1.83
Ash (%)	2.61 ± 1.72	3.79 ± 1.80
Protein (g)	16.56 ± 2.10	18.88 ± 1.98
Fat (g)	7.47 ± 1.01	10.97 ± 1.91
Carbohydrate (g)	62.34 ± 3.21	54.67 ± 2.98
Energy (kcal)	384 ± 1.57	349 ± 2.01*
Fiber (g)	1.55 ± 1.76	4.52 ± 2.10*
Calcium (mg)	367.87 ± 2.37	408.23 ± 1.53
Phosphorus (mg)	436.01 ± 1.89	445.03 ± 1.32
Iron (mg)	2.41 ± 2.69	10.01 ± 1.72*

*Significant difference is at 0.05 levels

These fibres have potential effect in increasing insulin secretion and in maintaining plasma glucose homeostasis. Ganorkar and Jain [23] observed that 15% flaxseed flour incorporated cookies contains terms of quality attributes. The treatment (T_4) incorporated with 8 % of RFF and 7 % SLP was higher amounts of fat, protein, dietary fibre, macro- and micronutrients in comparison to standard one (control).

Totally 100 g of value added developed biscuit (T_4) provide less calories (349 Kcal) and carbohydrate (54.67 g) content. Leaves of *S. rebaudiana* contain steviol glycosides which provide natural sweetness and fewer calories. Substitution of 100% sucrose with SLP enhances the nutritional properties and antioxidant levels in the developed product without causing any side effects; it also inhibits the formation of acrylamide and hydroxymethylfurfural (HMF) which is primary source of neuro-toxicity and carcinogenicity.

Jenkins *et al.*, [24] reported that incorporating 25% of flaxseed in the preparation of bread lowers the glycemic response by 28% than control (no flaxseed).

The developed biscuit (treatment T_4) contain maximum mineral (calcium, phosphorus and iron) content (408.23 mg/100g, 445.03 mg/100g and 10.01 mg/100g) in comparison to control (T_0). It was observed that energy, fibre and iron content of the optimized product differ significantly ($p < 0.05$) from the control. The high mineral content was due to incorporation of RFF and substitution of 100% sucrose with SLP. The major minerals calcium and phosphorus are needed for maintaining electrolyte and fluid balance within the cells of the body; they together mineralize the bone tissue and increase the bone mineral density.

Body requires iron to synthesise oxygen transporting proteins particularly haemoglobin and myoglobin. One of the major functions of iron is in the formation of heme enzymes and other iron-containing enzymes involved in oxidation-reductions and electron transfer. Gambus *et al.*, [25] reported that incorporation of 10 to 13% of flaxseeds in the preparation of bread; enhance the macro and micro-nutrients in comparison to standard one.

CONCLUSION: The results signify that the incorporation of RFF and SLP in formulation of value added biscuits can improve the nutritional value of the product, which is highly acceptable in best considered in sensory attributes and nutritional qualities. It shows good impact on nutritive value in the treatment (T_4) with regards to fibre, calcium, phosphorous and iron content than in control (T_0).

Thus, this new formulated functional biscuit had lower calorie count (349 Kcal/100g) than control product (384 Kcal/100g) and can be included in the diet of every age group which will increase the sensory characteristics and nutrient intake by maintaining the good health and promoting immunity against infections.

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