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FORMULATION OF PROTEIN & IRON-RICH CRACKERS USING BIOWASTE AND THE STUDY OF IRON BIO-ACCESSIBITLITY IN THE SNACK

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ABSTRACT

Protein-energy malnutrition (PEM) and anemia are the major health and nutritional problems in India. Not only they are an important cause of childhood morbidity and mortality but also leads to permanent impairment of the physical and mental growth of those who survive. A considerable amount of biowaste is generated while processing foods in the form of peel, seed and inedible pulp material, which can be re-used for edible purposes after appropriate processing because of the nutrient density. Two variations of crackers were developed using sattu flour & soy flour along with other ingredients (seeds, vegetable peels). Nutritional composition was analysed, Moisture sorption isotherm, iron bioaccesibility, organoleptic evaluation (9 point hedonic scale) was studied. The results of the present study demonstrated that the protein content of the crackers ranged from 12.65 to 27.02%, the total dietary fibre ranged from 32.56 to 46.93%, the iron content was high among the developed crackers 7.08±0.56 and 6.06±0.66mg/100g compare to the control crackers. Overall, both the products developed (Sattu & Soy crackers) had better acceptability compared to control crackers. The in-vitro iron bioaccessibility of sattu crackers and soy crackers was 6.27% and 6.35% respectively. The products developed were good sources of protein and iron. Therefore the waste part of fruits and vegetables, having high iron content, can be used as a natural source to improve the iron bioaccessibility by the nutraceutical industries, so this will not only act as value-added but also helps in reducing environmental problems.

Keywords: Anemia, PEM, Bioaccessibility, Crackers, Soy flour, Sattu flour

INTRODUCTION

Protein-energy malnutrition (PEM) is possibly a fatal body depletion disorder, particularly among children due to their high protein and energy needs related to their body weight and their particular susceptibility to infection **(Ubesie 2012, Dulger *et al*., 2002)**. PEM leads to chronic short- and long-term mental, physical retardation and worse resistance to the infection **(Pelletier 1995)**. Iron deficiency is the most common micronutrient shortfall associated with PEM **(Kotecha, 2011, Simbauranga *et al*., 2015; Gautam *et al* l., 2019)**. Iron deficiency also occurs to be a prevalent cause of morbidity and mortality in PEM individuals **(Saka AO et al., 2019)**. Malnutrition affects over one in five children under five in developing countries, and it continues to be a leading cause of child death and poor health. According to the World Health Organization, the single thing posing a threat to the global health issue is hunger and its associated malnutrition (United Nations Development programme).
Good health is achieved by eating proper food rich in nutrients. A considerable amount of biowaste is generated while processing foods in the form of peel, seed, and inedible pulp material, which can be re-used for edible purposes after appropriate processing and may be utilized in the formulation of new products to increase the nutritive value of the food. Ridge gourd peel has a high nutrient value and is often called a nutrition powerhouse because of its affluent and varied nutrient contents. It is also rich in vitamin C, flavonoids, calcium, potassium, sodium, iron, and essential amino acids. The peel contains glycerides of palmitic, stearic, and myristic acids **(Kandoliya *et al*., 2016, Vassilios *et al*., 2019)**. Ridge gourd peel powders, as well as their various solvent fractions, were evaluated for anti-oxygenic activity using different methods **(Swetha and Muthukumar 2016)**. Pumpkin seeds have 290 parts per million of iron **(Habib, 2015)**. As per Braide **(2012)**, watermelon seeds are recognized as having great nutritional value; they include ample amounts of protein, fat, vitamin B, minerals like magnesium, potassium, phosphorus, sodium, iron, zinc, manganese, copper, and phytochemicals. According to **Tabiri *et al.* (2016)**, the iron concentration of three different types of watermelon seeds—Carolina gray, crimson sweet, and black diamond—was determined to be 3.71, 2.72, and 4.60mg, respectively. In affluent nations, plant proteins are regarded more as biologically active components or adaptable functional ingredients than as necessary nutrients **(Marcello, 1997).**
Legumes are said to enhance overall nutritional status when they substitute for animal diets **(Guillon, 1996)**. Glycine max, or soybeans, have gained prominence recently due to their high protein content. It is a leguminous crop that is grown annually to supply raw materials for industry, animal feed, and human consumption **(Abbey, 2001)**. It is the highest food value of all plant foods produced worldwide, with 35–40% of its protein coming from it. It is used as composite flour to make bread **(Kure, 1998)**. Leading baby food producers in the nation employ soybeans due to their high nutritional content. In addition, soya beans are processed into flour, and the local paint, cosmetic, and soap-making businesses use their oil **(International Institute of agriculture, 1990)**. The most extensively grown pulse crop globally is chickpea (*Cicer arietinum* L). It provides several health benefits and is a rich source of macro and micronutrients, including dietary fiber and protein **(Ghadge *et al*., 2008)**. The most affordable ways of preventing and treating anemia and PEM are food-based techniques such as dietary supplementation and food fortification with micro and macronutrients. It is commonly known that eating a diet high in iron can help prevent and treat iron-deficiency anemia. Skin and peel, which can be used for human consumption after appropriate processing, generate an excess of waste together with the complete spectrum of the food manufacturing industries. In light of this, two items that are high in protein and iron were prepared and investigated for the acceptability of the product as well as the nutrient composition and bioaccessibility of iron.
The objective of our study was to develop iron and protein rich crackers using waste seeds, vegetable peels, sattu flour (chickpea), and soy flour & check the bio-accessibility of iron in developed products.

MATERIAL AND METHODS

Ingredients required

Moringa leaves, curry leaves, spinach leaves, and ridge gourd (peel) were procured from “More Megastore”, Mysore. Whole wheat flour, sattu flour, onions, green chilies, watermelon seeds, pumpkin seeds, ajwain seeds, cumin seeds, sesame

seeds, black pepper powder, red chili flakes, garam masala powder, baking soda, oil, and salt were procured from local market of Mysore, Karnataka, India.
The standardization of crackers is mentioned in table 1.

Table 1 Standardization of Crackers

Ingredients (%)	Control	Soy Crackers	Sattu crackers
Wheat flour	79	8.67	8.67
Soy flour	-	34.69	34.69
Sattu flour	-	34.69	34.69
Curry leaves	4.33	5.33	5.33
Onions, Green chilies, Ajwain seeds, Cumin seeds, Sesame seeds, Black pepper powder, Red chili flakes	11.23	11.23	11.23
Baking soda	0.21	0.21	0.21
Oil	4.33	4.33	4.33
Salt	0.86	0.86	0.86
Moringa leaves, Spinach leaves, Ridge gourd peel, Watermelon seeds, Pumpkin seeds	-	34.69	34.69

Packing and storage of crackers

Developed crackers were packed in silver foil packets and stored at room temperature for 30 days, it was made sure during packing that there was no leakage of air from inside the packets

Organoleptic evaluation

The developed crackers were served to semi-trained panel members (n=30) for organoleptic evaluation on a nine-point hedonic scale, with a score of 9 as excellent and a score of 1 as disliking. The sensory properties such as color, texture, aroma, taste, and overall acceptability of the finished product were evaluated on the basis of 9-point hedonic scale.

Analysis

Proximate composition of crackers viz., carbohydrate, protein, fat, total fiber, soluble fiber, insoluble fiber and ash values in crackers were carried out by **(AOAC, 2005)**, iron by **(AOAC, 2016)**. Free fatty acids estimation was determined by the standard method of **(Horwitz, 2000)** and the Peroxide value of stored products was determined by the method of **(Horwitz, 2000).**

Moisture sorption isotherm

The crackers were analyzed using **Lazou and Krokida, (2011**) with slight modification, through sorption isotherm to evaluate the keeping quality. Different salts having different Relative Humidities (RH) were chosen and the trends of moisture uptake were studied. The crackers were pre-dried over for 2 hours. 5g of each dried cracker was weighed into standardized Petri dishes and were kept in pre saturated desiccator overnight and weights were recorded the following day. This procedure was followed for 6 days with all the selected salts. Salt used for moisture sorption isotherm and their relative humidity- Potassium acetate- RH 22, and Sodium nitrate- RH 64.

Iron bioaccessibility

In vitro iron availability was determined by **Luten *et al.* (1996)** method. Dialyzable iron: Free form of iron in the dialysate reacts with α α bipyridyl to yield a colored complex which was determined by AOAC method (2005).

Statistical Analysis

The data was entered in excel and the mean was obtained for the triplicate values and reported as a percentage (%) and mean±SD

RESULTS AND DISCUSSION

Characteristics of crackers

The crackers were evaluated for basic characteristics, with the results presented in Table 2. Measurements included weight, width, height, thickness, and moisture content. For the control sample, these parameters were 3.18, 4.72, 4.66, 0.84, and 2.89, respectively. The soy crackers exhibited values of 3.08, 4.63, 4.56, 0.78, and 2.48, while the sattu crackers showed values of 3.06, 4.55, 4.45, 0.76, and 2.68, respectively.

Macro and Micro Nutrients Composition of Crackers

The crackers were evaluated for their nutritional composition, with the results summarized in Table 3. The energy content, total carbohydrates, total dietary fiber, soluble fiber, insoluble fiber, protein, fat, and ash in the control sample were 490.48 Kcal, 68.03%, 32.56%, 20.15%, 12.41%, 12.65%, 18.64%, and 1.65%, respectively. In soy crackers, these values were 444.55 Kcal, 54.17%, 46.47%, <0.50%, 46.47%, 27.02%, 13.31%, and 2.6%, respectively. The corresponding values for sattu crackers were 449.21 Kcal, 62.61%, 46.93%, <0.50%, 46.93%, 18.53%, 13.85%, and 2.3%. Additionally, the iron content was measured at 3.61 mg, 7.08 mg, and 6.06 mg per 100 g for the control, soy, and sattu crackers, respectively.

Table 2 Characteristics of crackers of the control, soy and sattu crackers

Particulars	Control (mean±SD)	Soy crackers (mean±SD)	Sattu Crackers (mean±SD)
Weight (g)	3.18±0.23	3.08±0.53	3.06±0.22
Width (cm)	4.72± 0.89	4.63±1.17	4.55±1.05
Height (cm)	4.66±0.56	4.56±0.21	4.45±0.31
Thickness (cm)	0.84±0.01	0.78±0.03	0.76±0.05
Moisture (%)	2.89±0.08	2.48±0.02	2.68±0.03

Table 3 Macro and Micro Nutrients Composition of Crackers per 100g

Nutrients (%)	Control	Soy crackers	Sattu Crackers
Energy (Kcal) (mean±SD)	490.48±12.65	444.55±15.51	449.21±18.21
Total Carbohydrates	68.03	54.17	62.61
Total Dietary fiber	32.56	46.47	46.93
Soluble dietary fiber	20.15	<0.50	<0.50
Insoluble dietary fiber	12.41	46.47	46.93
Protein	12.65	27.02	18.53
Fat	18.64	13.31	13.85
Ash	1.65	2.6	2.3
Iron (mg/100g)	3.61±0.12	7.08±0.56	6.06±0.66

Moisture Sorption Isotherm Analysis

Moisture content plays a key role in many food-processing operations. Moisture sorption isotherms are critical for predicting the shelf life of dried foods due to their sensitivity to changes in moisture levels. Understanding sorption isotherms is essential for forecasting stability and quality changes during packaging and storage. This data helps in selecting appropriate packaging materials to prevent quality deterioration. Additionally, sorption data provides insights into deteriorative reactions in food, serving as a useful tool for evaluating the physical, chemical, and microbiological stability of dehydrated foods. Recent research has increasingly focused on the impact of sorption isotherms on storage stability and the diffusion of water vapor, especially in processes aimed at removing residual moisture from foods.

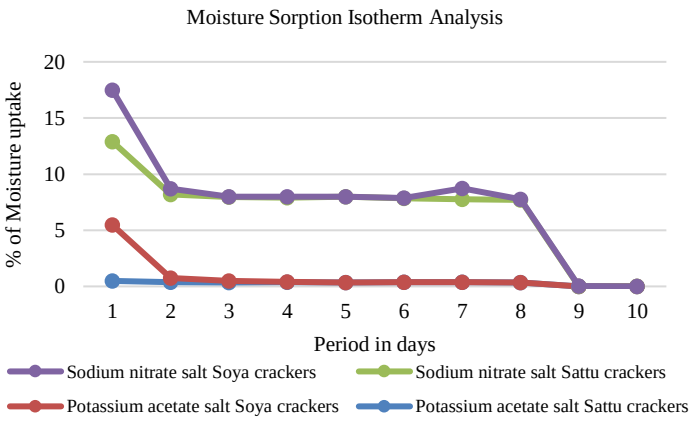

Figure 1 Moisture Sorption Isotherm Analysis

Bio accessibility of iron

Figure 2 presents the data on the bioaccessibility of iron in sattu and soy crackers. Following gastrointestinal digestion simulation, the iron bioaccessibility was 6.35% for soy crackers and 6.27% for sattu crackers. Although both values are similar, sattu crackers exhibited slightly higher iron bioaccessibility than soy crackers.

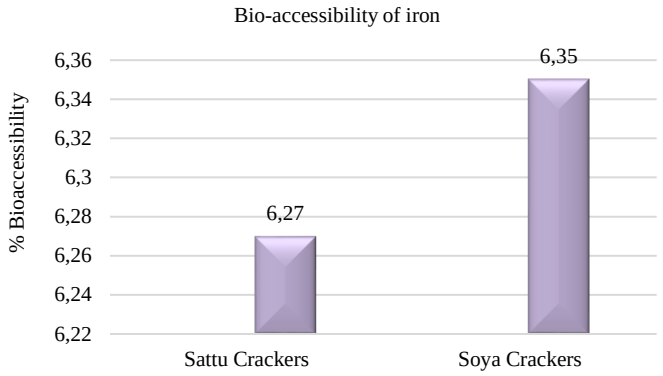

Figure 2 Bio accessibility of iron in sattu and soy based crackers

Organoleptic evaluation of Crackers

Acceptability, based on sensory attributes, is a key criterion for evaluating foods. In this study, 30 semi-trained panelists evaluated the products. Samples were randomly selected from each experimental batch, coded, and presented to the panelists, who assessed the crackers for appearance, color, aroma, taste, texture, and overall acceptability using a 9-point hedonic scale. As illustrated in Figure 3, sattu crackers achieved higher overall acceptability compared to both the control and soy-based crackers.

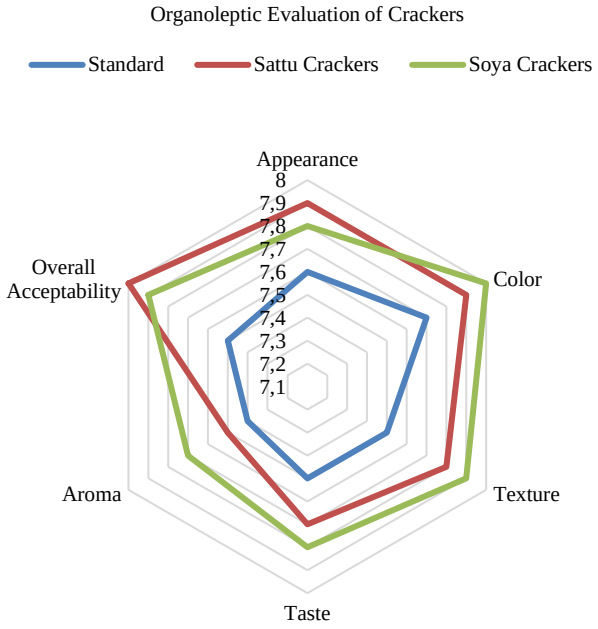

Figure 3 Organoleptic evaluation of Crackers

Shelf Life Studies of Crackers

The shelf life of any product shows its potential for being stored for a definite period without any deteriorating effects on its quality parameters. Storage life indirectly shows the market life of the product. Crackers were evaluated periodically for quality parameters viz., free fatty acids & peroxide value & organoleptic evaluation for overall acceptability was studied in the stored samples. The acceptability scores have helped to establish the shelf life of the products.

Quality Parameters

The changes in free fatty acid content (FFA) and peroxide values of the cracker samples during storage are shown in Figures 4 and 5 respectively. The FFA content of the cracker samples was increased with time. However, during the entire storage period, none of these samples exceeded the 1% limit prescribed by the standardization agencies (SLS 251: 2010). The FFA content of the control cracker showed the highest value and the FFA values of the soy crackers showed the lowest FFA values among all the crackers. A slight increase was seen in peroxide values of cracker samples with time. Up to the level, control cracker samples showed higher peroxide values than those of soy and sattu-based crackers. However, the higher peroxide values did not exceed the maximum peroxide value of 10 mEq/kg during the 3 months of storage.

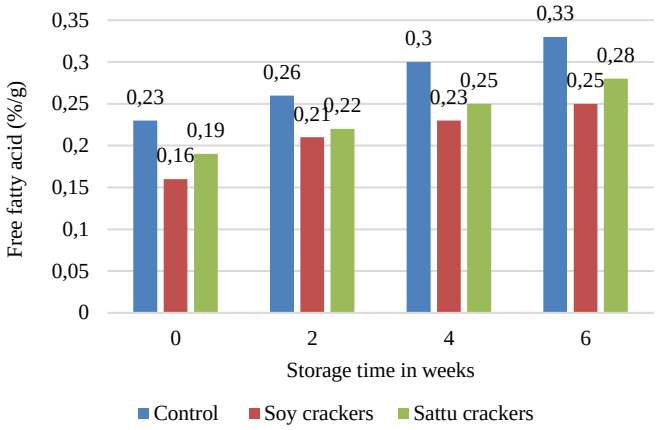

Figure 4 Free Fatty Acid Content of Crackers

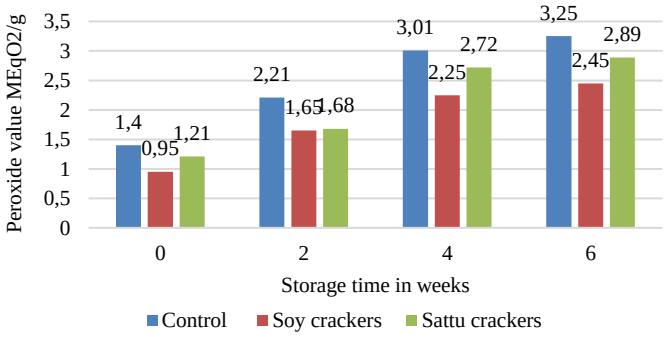

Figure 5 Peroxide Value of Crackers

Organoleptic Evaluation of stored product

The crackers developed were stored at both room and refrigerated temperatures. The products were stored in silver foil packets for 45 days at room temperature, it was observed that the crackers remained stable without any microbial activity, on further observation it was noticed that absorption of moisture in sattu variation was quite faster compared to soy crackers due to this the crispiness and grittiness of sattu based crackers was weak compared to the soy crackers (table 4). At refrigerated temperature the products were kept in the fridge for 4 weeks (30 days), it was observed that the texture, and grittiness and crunchiness remained the same but the taste was weak and no signs of microbial activity was observed (table 5).

Table 4 Organoleptic evaluation of stored product- Room temperature

Crackers	Period in weeks	Taste (mean±SD)	Color (mean±SD)	Appearance (mean±SD)	Texture (mean±SD)	Aroma (mean±SD)	Overall acceptability (mean±SD)
Control	0	7.4± 0.8	7.4± 0.8	7.6± 0.9	7.5 ±0.9	7.4± 0.8	7.4± 0.9
	2	7.2±0.7	7.3±0.7	7.5±0.6	7.4±0.8	7.3±0.7	7.31±0.7
	4	7.1±0.6	7.2±0.5	7.4±0.5	7.3±1.2	7.2±0.66	7.25±0.6
	6	7.0±0.1	7.1±0.9	7.2±0.4	7.1±1.11	7.0±0.9	7.14±0.8
Soy crackers	0	7.8±1.2	8.0±1.1	7.8±1.1	7.4±1.2	7.7±1.2	7.9 ±1.0
	2	7.7±0.8	7.9±1.0	7.7±1.21	7.3±1.23	7.6±1.11	7.82±0.9
	4	7.6±0.9	7.8±1.3	7.6±1.32	7.2±1.32	7.5±1.31	7.66±0.8
	6	7.4±1.0	7.6±1.2	7.5±1.25	7.0±1.06	7.4±1.01	7.59±1.0
Sattu Crackers	0	7.7±1.12	7.7±1.05	7.8±1.02	7.7±1.12	7.7±1.18	8.0±1.19
	2	7.6±1.21	7.6±1.1	7.7±1.23	7.6±1.22	7.6±1.21	7.9±1.21
	4	7.5±1.11	7.5±1.09	7.6±1.28	7.5±1.32	7.5±1.25	7.8±1.33
	6	7.4±1.22	7.4±1.07	7.5±1.01	7.4±1.11	7.4±1.12	7.7±1.01

SD- Standard Deviation

Table 5 Organoleptic evaluation of stored product- Referigerated temperature

Crackers	Period in weeks	Taste (mean±SD)	Color (mean±SD)	Appearance (mean±SD)	Texture (mean±SD)	Aroma (mean±SD)	Overall acceptability (mean±SD)
Control	0	7.4± 0.7	7.4± 0.8	7.6± 0.9	7.5 ±0.9	7.4± 0.8	7.4± 0.9
	2	7.2±0.6	7.2±0.5	7.4±0.8	7.3±0.6	7.33±0.6	7.33±0.6
	4	7.1±0.2	7.1±0.4	7.45±0.6	7.28±1.1	7.29±0.5	7.32±0.4
	6	7.0±0.3	7.0±0.8	7.25±0.2	7.0±1.2	7.0±1.2	7.21±0.5
Soy crackers	0	7.8±1.1	8.0±1.1	7.8±1.1	7.4±1.2	7.7±1.2	7.91 ±1.0
	2	7.6±0.6	7.8±1.1	7.75±1.12	7.35±1.3	7.61±1.21	7.88±0.7
	4	7.2±0.2	7.7±1.2	7.64±1.21	7.22±1.32	7.15±1.31	7.76±0.6
	6	7.0±0.5	7.5±1.5	7.52±1.34	7.1±1.1	7.54±1.1	7.65±1.1
Sattu Crackers	0	7.7±1.15	7.7±1.05	7.8±1.02	7.7±1.12	7.7±1.18	8.0±1.19
	2	7.4±1.1	7.7±1.32	7.68±1.12	7.65±1.05	7.65±1.1	7.89±1.1
	4	7.1±1.5	7.6±1.02	7.65±1.15	7.45±1.21	7.45±1.5	7.87±1.03
	6	6.9±0.9	7.3±1.01	7.6±1.2	7.48±1.01	7.34±1.2	7.78±1.0

SD- Standard Deviation

DISCUSSION

The main factors that consumers consider while buying any product are the sensory attributes. The sensory characteristics such as color, taste, texture, appearance, aroma, and overall acceptability showed that soy and sattu incorporated crackers were in the acceptable range for up to 45 days. **Broyart *et al*. (1998)** reported that the initial acceptance of baked products is influenced by color, which can also be an indicator for the completion of baking. The control, soy, and sattu incorporated crackers were rated for the highest scores by the panelists for the color.

The texture is perceived when food is chewed between molars and is usually expressed in terms of hardness and fracturability. Substitution of soy and sattu flour affected the crispiness of the crackers, which is an important textural characteristic of crackers. **Sudha *et al*. (2007)** have also reported similar findings in crackers, prepared from different cereal fiber. The crackers were found to be crispy at the incorporation level of 40% oat bran, with a very small increase in hardness. However, the texture and taste of the crackers would be dependent on the type of flour used for substitution. The second significant factor that customers take into account when purchasing snack foods is the dietary composition of the crackers. The proximate composition of soy and sattu-incorporated crackers showed a good nutritional profile when compared to the control. The increase in ash content may be due to the high mineral content in the soy and sattu crackers. It's probable that the control and sattu crackers' superior water retention capacity accounts for their comparatively higher moisture content. It is going to be more prone to deterioration and mold growth than the crackers with soy in them. **(Fennema and Tannenbaum, 1996)**. Furthermore, the results indicated that crackers made from soy and sattu had a higher total mineral content. Ash content is indicative of the overall mineral content of the products. Because soy and sattu crackers have a high fiber content, they can help reduce constipation and colon cancer in people **(Anderson *et al*. 2009)**. Consequently, the incorporation of soy and sattu to the crackers rendered them healthier for adults and patients with noncommunicable diseases. The nutritional data suggests that the cracker samples had a modest amount of fat in them. Patients who are health-conscious or anorexic can eat crackers because fat makes up a big portion of a diet's total calories.

In comparison to soy, sattu and control crackers, the control had high total carbohydrate content. These findings showed that the soy and sattu-incorporated crackers' low calorie intake was caused by their high fiber and low carbohydrate content. According to the examination of the nutrient composition, the crackers containing soy and sattu had a better nutrient profile than the control crackers, making them appropriate for patients of all ages.

Given the crackers' sensory, physical, and nutritional qualities, they ought to retain their quality well over storage. The current investigation indicates that within a 45-day period, chemical alterations were within an acceptable range and had no negative impact on the crackers' quality. One way to maintain quality is to use appropriate packaging materials. All of the cracker samples in this investigation were stored in triple-laminated aluminum foil, which has high moisture-barrier qualities. Nevertheless, for the course of the 45-day storage period, none of these samples exceeded the 4% limit set by the standardizing authorities **(SLS 251: 2010)**. Free fatty acid and peroxide value is another measurement of rancidity development from the autoxidation. Triple laminated Aluminum foil as a good packaging material can be affected to lower values which have light, moisture, gas barrier properties. In addition, control cracker had the higher free fatty acid and peroxide value than the soy and sattu incorporated crackers.

CONCLUSION

The data presented in this study demonstrate the potential for industrial exploitation of waste seeds, soy and sattu through processing into snack food items such as crackers. It can be concluded that soy and sattu-incorporated crackers were nutritionally rich with better sensory attributes than control crackers. In addition, the results of this study indicate that, waste seeds, vegetable peels, soy, and sattu can be considered as potential ingredients for food products, increasing their content of total dietary fiber, iron, and protein and improving their technological properties. Hence, the development and utilization of such ingredients can be used to improve the nutritional status of the population as well as to overcome non-communicable diseases. The findings of this experiment may help to generate technology to diversify the use of waste seeds by food processing enterprises. More studies should be conducted to investigate the possibility of using waste seeds and vegetable peels as an ingredient in other food products to increase applications of such value-added food ingredients. In addition to that soy and sattu can be considered as a good protein source for food processing.

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