EFFECT OF SOY AND RAGI FLOUR INCORPORATION ON THE PHYSICO-CHEMICAL AND ORGANOLEPTIC PROPERTIES OF GLUTEN-FREE MUFFINS

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ABSTRACT:

The present study was conducted to develop and assess nutritional and organoleptic characteristics of millet-based gluten-free muffins incorporated with ragi and soy flour blends. Three standardized recipe of gluten-free muffins T_1– RF: SF: R (80:10:10), T_2 –RF: SF: R (60:20:20), and T_3 – RF: SF: R (40:30:30), were developed and their sensory, physicochemical, and microbiological characteristics were analyzed. The optimal ratio of ragi, rice, and soya flour blends (40:30:30) was found to be more acceptable than other developed muffins. The mean score of overall acceptability control muffins and muffins treatment were lies in the category to like slightly to like very much 6.18 to 8.14 respectively. The physical characteristics indicated height weight, and volume of muffins treatment were significantly ($P \leq 0.05$) increased than control muffins (T_0) except density of muffins treatment. Incorporation of ragi and soy flour in the muffin's recipe increases moisture, ash, and crude protein, while the treatment muffins had carbohydrate and crude fat content was significantly lower than control muffins. The proximate composition indicates that the moisture, ash, crude fiber, protein, crude fat, and carbohydrate content of the most acceptable muffins treatment T_3 were 30.48 gm, 1.50 gm, 0.13 gm, 9.63gm, 0.23 gm and 58.03gm, respectively. The total bacterial count of control and most acceptable muffins treatment was 1.5×10^4 cfu/g, 1.2×10^4 cfu/g respectively. The results showed that the addition of 40 % of ragi and soya flour in the recipe has improved the sensory and physico-chemical characteristics of the treatment muffins, and consequently increased their nutritional value.

KEYWORDS: Gluten, Celiac disease, Ragi, Muffins, Soya.

INTRODUCTION:

Millets are small-grained cereal crops that are tolerant to drought and require low chemical inputs. They are known as Nutri-cereals due to their high nutritional value. Millets are high in fiber, vitamins, and minerals, and have a low glycemic index, which means they release glucose into the bloodstream slowly, providing sustained energy and keeping blood sugar levels stable. Millets are also rich in antioxidants, which help protect the body against inflammation and oxidative stress and may help reduce the risk of chronic diseases such as heart disease and cancer.

In 2018, India celebrated "The Year of Millets" and in 2023, the Food and Agricultural Organization (FAO) declared it as the "International Year of Millets", highlighting the importance of millets. Millets were produced in larger amounts from 164 lakh tons in 2017–18 to 176 lakh tons in 2020–21. From 2017–18 to 2020–21, productivity grew, increasing to 1239 kg/ha. Millets' exports climbed from 21.98 million US dollars in 2017 to 24.73 million US dollars in 2020 (Ministry of Agriculture & Farmers Welfare, 2022).

Gluten is a class of storage proteins, formerly known as prolamins, that are naturally present in several grains, including wheat, barley, and rye. The prolamins found in wheat are gliadins and glutenin's, whereas secalins and hordeins are found in rye and barley, respectively (Theethira *et al.,* 2014). Gluten protein causes the systemic, immune-mediated enteropathy known as celiac disease in people who are genetically predisposed to it (Mohta *et al.,* 2021). A genetically susceptible person who consumes gluten-containing products develops celiac disease, an immune-mediated condition that causes mucosal inflammation and malnutrition of both micro and macronutrients (Sharma *et al.,* 2020). Patients with celiac disease frequently exhibit mucosal atrophy, epithelial lymphocytosis, and crypt and villous atrophy. Consequently, there may be severe vitamin, mineral, protein, carbohydrate, and fat malabsorption (Theethira *et al*., 2014). Nutritional deficiencies related to malabsorption in celiac disease can lead to complications, usually more noticeable in children, such as growth abnormalities and short stature. (Naqash *et al*., 2017). The most effective treatment for celiac disease is a lifelong gluten-free diet which affects between 40 and 60 million individuals worldwide (Valitutti *et al*., 2019).

At least 1% of the world's population is afflicted by celiac disease, which has a widespread distribution. However, there are regional variations in the incidence of disease (Singh *et al.*, 2018) revealed that the prevalence of celiac disease ranged from 0.05% to 2.6% over the world in their systematic review and meta-analysis of the global prevalence of celiac disease. Among children that are genetically predisposed to celiac disease, higher gluten intake during the first five years of life was linked to a statistically significant increase (6 to 7%) in celiac disease prevalence (Aronsson *et al.*, 2019).

The demand for gluten-free products is rising as the prevalence of celiac disease rises. The market for GF products will increase from $4.63 billion in 2015 to $7.59 billion in 2020 at a compound annual growth rate (CAGR) of 10.4% (Naqash *et al.*, 2017). Most GF products on the market are nutritionally unbalanced, having high glycemic indexes (83.3 and 96.1), little protein, and more fat than usual.

Muffins are sweet baked goods highly acceptable by the consumers due to their soft and moist texture and characteristics taste. A pan with cup-sized indentations is used to bake muffins. Replacement of wheat flour in baked products is a complex challenge because gluten is an essential structure-building protein with important physio-elastic properties. (Ureta *et al.,* 2014) Also, gluten-free goods are often not enriched and are generally prepared from refined flour and starch. Therefore, Products with a high nutritional value that are gluten-free are scarce on the market. Ragi flour is naturally gluten-free, making it a great alternative for those with gluten sensitivities or celiac disease. It also has a low glycemic index, meaning it can help regulate blood sugar levels and prevent spikes in blood sugar. For people with diabetes or those trying to control their blood sugar levels, this makes it a perfect alternative. Ragi flour's high fiber content and low glycemic index can aid in weight management by encouraging satiety and lowering cravings.

METHODOLOGY:

Procurement of raw materials:

The present study conducted in the Department of Nutrition and Dietetics, UIAHS, Chandigarh University, Mohali, Punjab. Screening of ingredients was done based on reviews. Raw materials such as Rice, Soyabean flour, Ragi flour and other essential ingredients were purchase from the local market of Kharar, Punjab.

Development of ragi and soya incorporated muffins

Three standardized treatments and controls of muffins were prepare by incorporating Rice flour (RF), Soya flour (SF), and Ragi flour (R) T_1– RF: SF: R (80:10:10), T_2 –RF: SF: R (60:20:20), and T_3 – RF: SF: R (40:30:30) and T_0 -RF: SR: RF (100:0:0) in different ratios to see the critical difference between them. In all three cases, the other ingredients remained the same.

T_1 T_2 T_3

Figure:2 Ragi and soya flour incorporated gluten free muffins

Physico- chemical characteristics:

After the completion of baking process, the muffins were placed on a desiccator to cool for about 20 minutes at room temperature. We measured the muffins' mass and volume right away after they had cooled. Measurements of muffins were made for volume (mL), density (g/cm3), and height (cm) (Ho *et al*., 2013).

Muffins height: The muffin height was measured using an electronic caliper from the top to the bottom of the muffin.

Muffins weight: muffins were weighed in grams (g) using analytical balance.

Muffin volume: Muffins volume was measured by rapeseed displacement method. (Lin *et al.*, 2003)

Muffin density: Muffins density was calculated by following formula after measuring cake volume and weight.

$$\textbf{Density (g/cm}^3\textbf{)} = \frac{\text{Muffin weight}}{\text{Muffin volume}}$$

Proximate composition:

Moisture Content: Moisture content of the muffins were determined by using (AOAC, 2005) method. A five-gram sample was weighed in a petri dish and dried for six hours in an oven at 105°C. After cooling in a desiccator, the sample was reweighed. The following formula was used to calculate moisture content:

$$\text{Moisture \%} = \frac{\text{weight of original sample} - \text{weight of dried sample}}{\text{weight of original sample}} \times 100$$

Ash content: In a muffle furnace, the ash content of muffins was assessed using the procedure outlined in (AOAC 2000). In a weighted silica crucible, 5 g of oven-dried sample was weighed. To make the sample smoke-free, the weighed amount of sample is first burned. The crucible was then placed in a muffle furnace (550°C) for 5-6 hours until white ash was obtained. The crucible was then cooled and weighed in a desiccator. The weight loss represented organic matter, while the residue was the ash content, which was determined using the following formula:

$$\text{Ash content\%} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

Crude Protein:

The protein content of the muffins was assessed using the Kjeltec apparatus in accordance with the procedure described in AOAC (2006). The material (2 g) was first broken down using 1 digestion tablet and 25 ml of concentrated sulphuric acid (H2SO4). When everything was set up and running, the machine was left running for 190 minutes, or until a light green color appeared. The mixture is then removed and diluted to make up the final volume of 250 ml. Then, 10 ml of the diluted material and 10 ml of 40% NaOH were collected and placed in a distillation device. The ammonia produced was collected in a 4% boric acid solution that contained methyl red indicator. The distillate was finally titrated with 0.1N HCl solution till light pink color was produced. The following formula was used to determine the nitrogen concentration of the sample:

$$\text{Nitrogen (\%)} = \frac{\text{volume of H2SO4} \times 0.0014 \times 250}{\text{weight of sample} \times \text{volume of sample taken}} \times 100$$

Crude Fiber:

One gram of a fat-free sample that had been oven dried was added to a litre beaker with 200 ml of 1.25 percent H2SO and a few drops of antifoam. The solution was then heated to boiling in the crude fiber apparatus, where it was maintained at boiling for 30 minutes under a bulb condenser. The contents of the beaker were occasionally mixed by rotating the container. Particles were then removed using a Buchner funnel, and the sample was then put back into the tall beaker with 200 ml of NaOH (1.25%). It was once more brought to a boil and this time it boiled for exactly 30 minutes. Using boiling water until the insoluble material was free of acid, it was all transported to the sintered crucibles. It was washed with acetone and then twice with alcohol before being dried at 100 degrees Celsius to maintain weight. The crucibles were heated to 550°C in a muffle furnace for one hour, cooled in a desiccator, and then reweighed (AOAC, 2000).

$$\text{Crude fiber (\%)} = \frac{\text{weight of insolube matter} - \text{weight of ash}}{\text{weight of sample}} \times 100$$

Crude Fat: The crude fat content of gluten free muffins was analyzed by (A0AC 2000) using the SOSC plus solvent extraction system.

Procedure:

The fat extraction beakers should be properly cleaned before being dried in a hot air oven at 60°C. carried the weight of the empty beakers. A pre-weighed extraction thimble containing 5gm of moisture-free material was filled and left to dry overnight. The material was placed into the fat extraction beaker along with the thimble holder. 100 ml of petroleum ether, with a boiling point of 60 to 80 degrees Celsius, were added to the beaker as needed. Set the temperature in the controller to 90°C (the boiling point of the solvent) after inserting the beakers into the system. At 90°C, the extraction was done for a whole hour. To collect the solvent in the solvent compartment, the temperature was raised to 110°C once the extraction phase was finished. Removed the beaker and the fat and maintained them in a hot air oven set at 60°C until a steady weight was achieved. After cooling in a desiccator, the beaker was weighed.

$$\text{Fat \%} = \frac{Weight\ of\ beaker\ with\ fat - Weight\ of\ emprt\ beaker}{Weight\ of\ sample\ (g)}$$

Carbohydrate: The total amount of carbohydrates was calculated using the formula:

(Jabeen *et al.*, 2022)

Total carbohydrates = {100 - (Moisture + Ash + Crude fiber + Crude protein + Crude fat)}

MICROBIAL ANALYSIS:

The Indian Standard (**IS 5402 :2012**) were applied for estimating the TPC (Total Plate Count) (IS 5403) were applied for estimating TYMC (Total Yeast & Mold), (IS 5887) were applied for estimating E. coli, and (IS 5401 P1) were used for estimation of Coliform content in the sample.

Statistical Analysis

The data were statistically analyzed in complete randomized design for analysis of variance, mean, standards deviation and critical difference according to the standard method (Sheoran and Pannu, 1999).

RESULT AND DISCUSSION

Organoleptic acceptability gluten free muffins

The organoleptic acceptability of the control and three treatments of gluten-free muffins developed by using ragi and soy flour were evaluated organoleptically evaluated by ten semi-trained judges. The organoleptic acceptability of the developed muffins was depicted in Table 1 and Figure 3.

Table.1 Organoleptic Acceptability of gluten free muffins

Treatments	Appearance	Color	Texture	Flavor	Taste	Overall Acceptability
T_0	6.2±0.29	5.8±0.25	6.2±0.20	6±0.21	6.7±0.26	6.18±0.19
T_1	6.8±2.9	6.6±0.30	7.2±0.25	7.2±0.31	7±0.33	6.96±0.25
T_2	7.1±2.33	6.8±0.25	7.4±0.27	7.5±0.17	7.6±0.34	7.28±0.15
T_3	8±0.21	8.4±0.16	8±0.21	7.9±0.1	8.4±0.26	8.14±0.19
CD ($P \leq 0.05$)	0.75	0.71	0.67	0.61	0.82	0.57

Mean±SD of ten independent variables.

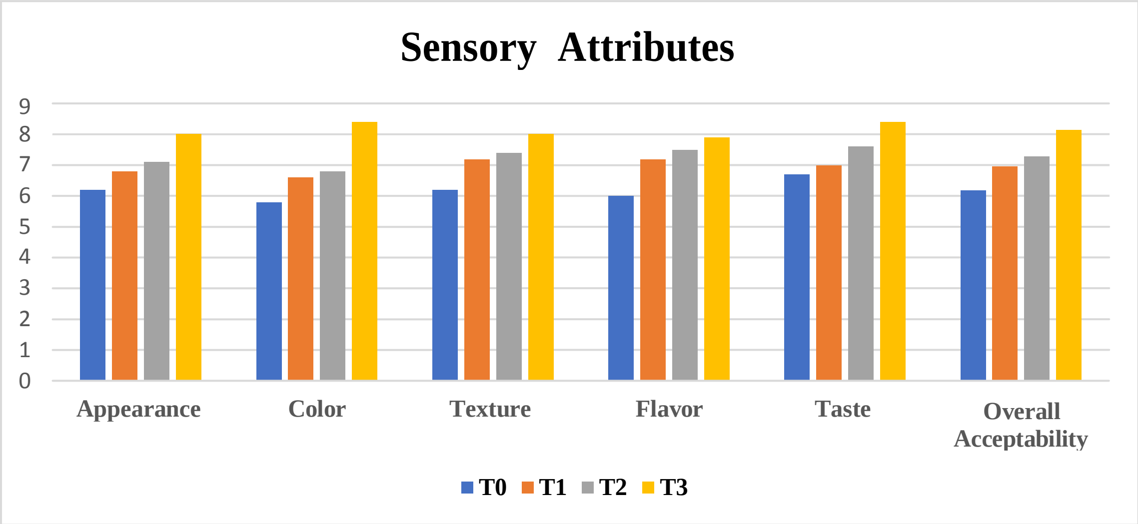

Figure: 3 Organoleptic acceptability of gluten free muffins

The composite of three standardized recipes of muffins and the control (T_0) is subjected to sensory evaluation and the result were given in Fig: 3 and table1. The mean score of overall acceptability significantly increased ($P \leq 0.05$) in ragi and soya incorporated muffins treatments than control T_0. The overall acceptability score of muffins treatment T_1, T_2, and T_3 was 6.96, 7.28 and 8.14 respectively. Based on overall acceptability score T_3 – RF: SF: R (40:30:30) gluten free muffins were most acceptable. Our results agreed with those of other researchers (Sirisha *et al.*, 2019), who made cookies using soy flour, foxtail millet, and refined wheat flour and found that the treatment cookies had higher organoleptic attributes than the control muffins in terms of color, texture, appearance, taste, and general acceptability.

TABLE.2 PHYSICAL CHARACTERISTICS OF GLUTEN FREE MUFFINS:

Treatments	Height (cm)	Weight (gm)	Volume (cm)^3	Density (g/ cm^3)
T_0 Control	2.5±0.29	41.05±0.29	54.61± 0.58	0.75±0.12
T_1	3.4±0.24	39.1±0.29	78.73±0.64	0.50±0.15
T_2	3.3±0.44	39.62±0.2	75.34±0.99	0.53±0.13
T_3	3.7±0.27	40.95±0.29	91.23±0.78	0.46±0.12
CD ($P \leq 0.05$)	N/A	0.893	2.52	N/A

Mean±SD of three independent variables.

The physical characteristics of gluten free muffins was presented in Table 2. The physical characteristics of gluten free muffin's i.e., weight, volume and volume change were significantly increased (P≤0.05) with the increased. The mean height of control (T_0) muffins i.e., 2.5 cm. while the mean height score of muffins treatment were 3.4cm, 3.3 cm and 3.7 for T_1, T_2 and T_3 respectively. The average weight of T_0 (control) muffins 41.05 gm was significantly lower than most acceptable muffins treatment T_3 was 41.95g.

The volume of the muffins treatments was significantly (P≤0.05) increased with addition of ragi and soya flour in recipe. The average volume score of control muffins is 54.61cm^3 while the average volume score of muffins treatment were78.73 cm^3,75.34 cm^3, and 91.23 cm3, for T_1, T_2 and T_3 respectively. The mean density score of control muffins was significantly higher than muffins treatment. The density score of control muffins was 0.75 g/ cm^3. While the mean density score of treatment muffins were 0.5 g/cm^3, 0.53 g/cm^3 and 0.46 g cm^3 for T_1, T_2 and T_3 respectively.

However, the volume (91.23cm^3) and height (3.7cm) of the muffins (T_3) were significantly increased with the increases in the incorporation of ragi and soya flour than the control sample. The density of all the samples gradually decreased as the muffins' volume increased. Similar findings observed by (Ramya, and Anitha, 2020)

Table.3 Proximate composition of developed muffins

TRAEATMENTS	Moisture (g/100gm)	Ash (g/100gm)	Crude fiber (g/100gm)	Crude protein (g/100gm)	Crude fat (g/100gm)	Carbohydrate (g/100gm)
T_0 Control	29.05±0.09	0.7±0.14	0.6±0.12	2.14±0.2	0.12±0.11	65.07±0.18
T_1	31.02±0.47	1.20±0.16	0.10±0.02	8.65±0.72	0.16±0.11	58.64±0.68
T_2	31.50±0.31	1.34±0.14	0.11±0.05	8.54±0.34	0.20±0.1	58.70±0.63
T_3	30.48±0.38	1.50±0.15	0.13±0.06	9.63±0.68	0.23±0.07	58.16±0.45
CD (P≤0.05)	1.133	0.495	0.232	1.760	0.397	1.723

Mean±SD of three independent variables.

The chemical characteristics of muffins are presented in Table 3. The moisture content of T_0 (control) was 29.05 gm which was significantly ($P \leq 0.05$) increased in muffins treatment T_1, T_2, and T_3 were 31.02g, 31.50, and 30.48 g respectively. The highest crude fat content 0.37g was observed in muffins T_0, whereas the muffins treatment had lowest fat content 0. 23gm. Similar result observed by other researchers that increases the amount of ragi and soya flour in the recipe increases the moisture and fat content. The carbohydrate content of control (T_0) muffins was 65.07 gm which was significantly ($P \leq 0.05$) higher than muffins treatments T_1, T_2, and T_3 58.78gm, 58.56 gm, and 58.16gm, respectively. The CHO (carbohydrate) level decreased with the addition of soy and ragi flour. This finding was supported by those of (Adanse *et al.,* 2022) who also found that the addition of soy and rice bran decreases the level of carbohydrate content. The ash content of muffins treatment T_1, T_2, and T_3 were 1.20gm, 1.34gm, and 1.50gm respectively which was significantly ($P \leq 0.05$) higher than control T_0 (0.6gm). The crude fiber content of muffins treatment ranges from 0.10gm to 0.13gm which was significantly higher than the control muffins 0.6gm. The crude protein content of muffins treatment T_1, T_2 and T_3 were 8.65gm, 8.54gm and 9.63gm respectively significantly increased than control muffins T_0 2.14gm. which indicates that supplementation of ragi and soya flour in the muffin's recipe increases the crude protein content. A similar result was observed by (Ali *et al.,* 2013) increasing the ash, crude fiber, and crude protein content in bakery products while incorporating with ragi and soya flour.

Table.4 Microbial analysis of gluten free muffins

Treatments	TPC cfu/gram	TYMC cfu/gram	E. coli	Coliform	Staph. Aures.
T_0	$1.5 \times 10^4 \pm 0.12$	$1.4 \times 10^2 \pm 0.12$	Absent	Absent	Absent
T_3	$1.2 \times 10^4 \pm 0.10$	$1.5 \times 10^2 \pm 0.06$	Absent	Absent	Absent

Mean±SD of three independent variables.

The microbial load of gluten-free muffins was presented in (table 4). The total bacterial count of control and gluten free muffins treatments ranges between 1.5×10^4 to 1.2×10^4 respectively at zero days. Similar results observed by (Pandey, and Sangwan,2016) they found that at the end of storage period total bacterial count was higher in control than that of newly developed Ladoo's. The total yeast and mold count of T_0 and T_3 1.4×10^2 cfu/gm and 1.5×10^2 cfu/gm respectively. The yeast and mold growth are in increasing order due to increase in moisture content, Similar finding observed by (Roshiya *et al.,* 2022). The E. coli, Staph. Aures and coliform were absent in both treatments and control of gluten free muffins. It indicates the absence of gram-negative bacteria during preparation of gluten free muffins which means strict hygienic practice was maintained.

CONCLUSION

The data obtained in this study are sufficient to conclude that ragi and soy flour incorporated muffins were nutritionally superior to control muffins treatments. The gluten free muffins formulated in the ratio of T_3 – RF: SF: R (40:30:30) are the most acceptable in terms of overall consumer acceptability. Hence, it was suggested that gluten-free muffins full of nutrients and organoleptically acceptable be commercialized and promoted, particularly among celiac patients they will like these muffins. It can be considered that the study's investigated recipes can be successfully used on an industrial basis. The use of this approach will significantly improve the population's overall nutritional health and help the environment be preserved, especially for growing children with celiac disease.

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