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Proximate Analysis of Multi-grain Pizza Base

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The ill effects of junk foods are widely known, as it is acknowledged that their regular consumption may cause irreparable damage to the body. The role of millet-rich foods in offering protection against diabetes and heart disease is well established, which has led to an interest in increasing nutrients in developed pizza bases. The present research has been conducted to develop a nutritionally rich "millet-based pizza base "using whole wheat flour, Kodo millet, buckwheat flour, and brown rice flour. The developed pizza base is noted to have advantages over a normal base made from refined wheat flour. Sensory evaluation by a panel of 25 members using a nine-point scale showed that T3 was the most preferred among the samples, suggesting its potential as a standardized pizza base. Nutrient analysis revealed protein content ranging from 7.13% to 9.5%, fat from 1.59% to 1.75%, fiber from 0.798% to 0.913%, moisture from 36.12% to 37.08%, and ash from 0.91% to 0.98%. Overall, the study concluded that these value-added pizza bases offer superior nutritional benefits compared to conventional options.

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Keywords: Kodo millet; buckwheat; brown rice; incorporated; formulation; nutritional composition.

1. INTRODUCTION

In the world where dietary preferences and health-conscious choices reign supreme, the emergence of millet-based pizza bases as a revolutionary product that caters to both taste and dietary preferences is observed. Our composite grains-based pizza base, crafted from nutrient-rich whole wheat flour, kodo millet flour, buckwheat flour, and brown rice flour, is highlighted, which redefining the pizza experience.

In this research, the benefits of millet-based pizza bases are explored. Creating a pizza base using wheat flour, kodo millet, buckwheat, and brown rice flour is considered a fantastic way to enjoy a healthy, nutritious alternative to replacing regular pizza; millet flour is viewed as a much healthier replacement. Research has found that the combination of refined wheat flour with cheese is deemed a very unhealthy combination.

It's interesting to see how pizza has become such a popular food choice in India, reflecting broader cultural shifts influenced by western norms. The statement highlights the growing popularity of fast food in Indian society, with pizza being a significant example. This trend is supported by data indicating substantial growth in the Indian pizza market, which has been expanding rapidly over recent years (Kanaujiya and Singh, 2017).

The mention of INR 1,500 crores as the size of the pizza market in India underscores its economic significance and the increasing consumer demand for this particular type of fast food. The annual growth rate of 26% indicates a robust and sustained increase in consumption, suggesting that pizza has become a staple in the diets of many Indians (Kumar et al., 2016).

This cultural adoption of pizza reflects not just changing dietary preferences but also broader societal changes, such as globalization and the influence of western culture on traditional Indian lifestyles (Biljwanet al., 2019). It's a testament to how food can serve as a marker of cultural exchange and adaptation in today's interconnected world (Bunkar et al., 2021).

Cultural and agricultural significance: wheat is referred to as the "king of cereals" and is one of the most important staple food crops worldwide. It belongs to the grass family (*Triticum)* and is cultivated extensively (Gulia and Kawatra, 2022).

Major wheat producing states in India: Uttar Pradesh, Madhya Pradesh, Punjab, Haryana, Maharashtra, and Gujarat are identified as major wheat-growing states in India. Uttar Pradesh is noted as the largest wheat-producing state in the country (Kaur and Sharma, 2017).

Varieties of wheat: the passage categorizes wheat into three major types: hard, soft, and durum. Each variety differs in its nutritional composition, particularly in protein levels. Hard wheat, for instance, is highlighted for its high protein and gluten content, making it ideal for bread making (Bhavya et al., 2020).

uses of wheat: wheat is primarily milled into whole wheat flour, commonly used to make unleavened flatbread known as "chapati" locally in India. Additionally, wheat flour serves as a major ingredient in a variety of bakery products worldwide, including breads, biscuits, cookies, cakes, pasta, and noodles.

Nutritional and health benefits: wheat offers several nutritional benefits. Whole wheat is recognized for reducing the risk of heart diseases by lowering cholesterol levels, blood pressure, and reducing blood coagulation time. It is rich in magnesium and vitamin e, which are beneficial for asthma management.

Functional properties of gluten: gluten, a protein found in wheat, plays a crucial role in baking. It forms a network of fibres that trap carbon dioxide and steam during baking, creating a light, porous structure in bread and other baked goods.

Kodo millet: Kodo millets or magical millets are the must-have millets in your meals. Kodo millet or *paspalum scrobiculatum* belongs to the family poaceae, and is locally known as rice grass, ditch millet, cow grass in English, *araka* in Telegu and *kodra* in Marathi. Kodo millet grains are annual grains ranging from light red to dark grey. The cultivation of kodo millets started in India about 3000 years ago. Apart from India, it is cultivated in Russia, China, Africa and Japan. In India, it is widely grown in Madhya Pradesh, Tamilnadu, Karnataka, Gujarat and Chhattisgarh (Sharma et al., 2017).

Among all millets available, it is well known for the highest drought resistance and produces high yield in a short duration thus is of great economic value. India is the world leader in the production of kodo millets, and thus its cultivation is of great economic significance. Kodo millets are cultivated in the kharif season (monsoon season) and are available in different varieties, namely indira kodo, jawahar kodo, tnau, etc. Kodo millets are processed into high value foods and drinks. Apart from economic and culinary benefits, kodo millets have numerous health benefits. Let us explore more about this superfood's nutritional value, health benefits, and side effects (Sharma et al., 2017, Swamy, 2013).

Kodo millets are packed with the goodness of carbohydrates, proteins, and dietary fibers. It contains vitamins like niacin and riboflavin and minerals like calcium, iron and phosphorus. The phytochemicals found in kodo millets include antioxidants along with phenolic compounds like vanillic acid, gallic acid, tannins, ferulic acid, etc (Saini et al., 2021).

Kodo millets show numerous scientifically proven properties; some of these properties are it may have antioxidant properties; it may lower blood glucose levels, it, may lower blood pressure.it may have anti-allergic properties, it may be able to halt the abnormal growth of cells, it may have the ability to reduce abnormally high lipid levels and also it may have antibacterial properties.

Buck wheat flour: It is one of the most popular pseudo millets in India and is often used during the fasting period of the Navratri's in India it is a crop suitable for cultivation in marginal lands and yields harvests in a short time. Commonly cultivated in the Himalayan region, it supports the livelihood of the hilly population.

Common buckwheat was domesticated and first cultivated in inland Southeast Asia possibly around 6000 BCE, and from there spread to Central Asia and Tibet, and then to the Middle East and Europe, which it reached by the 15th century. Domestication most likely took place in the western Yunnan region of China (Wang et al., 2022).

The oldest remains found in China so far date to circa 2600 bce, while buckwheat pollen found in Japan dates from as early as 4000 bce. It is the world's highest-elevation domesticate, being cultivated in Yunnan on the edge of Tibetan Plateau or on the plateau itself. Buckwheat was one of the earliest crops introduced by Europeans to North America. Dispersal around the globe was complete by 2006, when a variety developed in Canada was widely planted in China (Wang and Nie, 2022). In India, buckwheat flour is known as *kuttu ka atta* and has long been culturally associated with many festivals like Shivratri, Navratri and Janmastami. On the day of these festivals, food items made only from buckwheat are consumed (Anonymous, 2024a).

Brown rice: Brown rice flour is the lightest of all the whole grain flours that can add texture, nutrition, and subtle flavour to many baked goods. Like any other whole grain flour, brown rice flour contains the exterior bran-rich layer of the grain, making it more nutritious than white rice flour. Brown rice flour has a slightly gritty texture with bone-white colour and has a mild, toasty flavour. However, the taste often ranges from clean and creamy to nutty. It is gluten-free and can enhance lightness in cakes or crispness in cookies. You can use it in place of all-purpose flour to make gluten-free foods (Anonymous, 2024b).

2. MATERIALS AND METHODS

The present study was carried out on the topic entitled "Proximate Analysis of Multi-Grain Pizza Base" the experiment work was conducted in the search of find an replacement of refined wheat flour based pizza base which has better nutritional profile,it was conducted during the period of January to June 2024.

The study aimed to formulate and assess nutritionally enriched pizza base samples labeled as T0 (control) and T_1, T_2, T_3 incorporating varying ratios of whole wheat flour, kodo millet flour, buckwheat flour, and brown rice flour. Treatments T_1 (30% whole wheat, 15.38% kodo millet, 10.25% buckwheat, 7.6% brown rice), T_2 (25.64% whole wheat, 17.94% kodo millet, 12.76% buckwheat, 7.6% brown rice), and T_3 (20.51% whole wheat, 20.51% kodo millet, 15.38% buckwheat, 7.6% brown rice) were developed to enhance the nutritional profile of traditional pizza bases, aiming for improved consumer acceptance (Agrawal and Verma, 2016, Pundir et al., 2024).

Procurement of raw materials: Raw material used in the preparation of the millet-based pizza base is arranged from the local market of Prayagraj. The company Grandma millet provide 100 percent organic millets located in Prayagraj, all the millets used in our experiment were provided from them.

Ingredients: Whole wheat flour, Kodo millet, Buck wheat flour, Brown rice flour, activated yeast, and Salt are key ingredient that are used in the preparation of the pizza base.

Equipment's: These are some major equipment's which is used while formulation of nutritionally rich pizza base Oven, Pizza Stone or Baking Sheet, Pizza Peel, Mixing Bowls, Measuring Cups and Spoons, Rolling Pin, Cutting Board, Sharp Knife or Pizza Cutter, Dough Scraper, Pastry Brush, Stand Mixer with Dough Hook, Dough Proofing Box or Container, Cooling Rack, Perforated Pizza Pan, Thermometer and Pizza Oven.

Preparation of pizza base samples: The nutritious pizza base was developed by mixing of whole wheat flour, kodo millet flour, buckwheat flour and brown rice flour in three different ratios indicated as T_1 (30%:15.38%:10.25%:7.6%), T_2 (25.64%:17.94%:12.82%:7.6%), and the last combination T_3 (20.51%:20.51%:15.38%:7.6%), The pizza base prepared only from refined wheat flour was taken as control (To).

Experimental process: Pizza Base was prepared through the traditional method which is used for the preparation of Pizza Base, dough was prepared by blending all the ingredients in quantity as decided in the above paragraph. Yeast was first activated in luke warm water with salt for 10-15 min at 35-40 °C. Activated yeast was added into the mixed ingredients. Calculated quantity of concentrated flours was added into the mixing bowl for preparation of dough for the experimental samples. Some additional amount of water was added into the dough for maintaining equal water quantity in control samples. Control sample dough was prepared by adding only whole wheat flour. Therefore, control samples were prepared with full replacement of millet flour. All the ingredients, yeast and salt were mixed properly to form uniform dough. Dough was kneaded, covered with wet cloth and kept at room temperature for double volume raise. Thereafter, the dough was portioned to 125 g. Each portion of dough was made into the round ball and flattened with help of wooden roller to diameter of about 7 inches. Docking was done on it by using tip of the knife. The flattened and docked dough was kept at room temperature covered with wet cloth for final proofing for 30 min. The desired proofed dough was baked in electrically operated oven at 200-220°C for 5-10 min. The Pizza Base was then cooled slowly to room temperature and packed in polyethylene pouches. The products were stored in room temperature till the analysis.

Nutritional Composition: The standardized developed pizza base sample T3 and the control sample T0 were subjected to the nutritional analysis for comparison purpose. The AOAC (1990) method was used to determine moisture, crude, ash and crude fiber content. For determination of protein content Micro Kjeldhal method was used, for fat content determination Soxhlet Method is used, for fiber Content determination chemical digestion method is used, for moisture Content determination hot air oven method is used, for the determination of ash content Muffle furnace method is used.

Determination of Protein Content (The AOAC (1990): The micro Kjeldahl method is a widely used technique for determining the nitrogen content in organic compounds, which can then be used to estimate protein content. Here is a step- by-step overview of the process through which protein content was determined:

Equipment and Reagents:

Apparatus: Kjeldahl digestion flask, distillation apparatus, and titration setup.

Reagents: Concentrated sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), and a suitable indicator (like phenolphthalein or methyl red).

Procedure:

Sample preparation: Weighing a small amount of the sample (typically around 0.1 to 0.5 g) accurately.

Digestion: Addition of the sample to a Kjeldahl digestion flask along with sulfuric acid (usually around 5-10 mL) and a catalyst (like copper sulfate and selenium).

then heating of the mixture was done until it becomes clear, indicating that organic material has been converted to ammonium sulfate. This usually takes a couple of hours.

Neutralization: After the cooling, the digested mixture was transfer to a distillation flask and add distilled water.

Neutralization of the solution was done by adding sodium hydroxide until a basic PH is reached (usually around PH 12).

Distillation: Distill the ammonia released by adding a small amount of distilled water and collect it in a receiving flask containing a known volume of dilute acid (typically HCl or H_2SO_4).

Titration: Titrate the excess acid in the receiving flask with a standard solution of sodium hydroxide. Use an appropriate indicator to determine the endpoint.

Calculation: Calculate the nitrogen content using the formula:

$$N\ (\%) = \frac{\text{N of HCL} \times \text{(HCL in sample)}}{\text{weight of sample}} \times 1.4 \qquad (1)$$

Protein % = %N × 6.25

Determination of fat content through Soxhlet method: The Soxhlet method is a well-established technique for extracting fat from a sample, commonly used in food analysis. Here is a step-by-step overview of the process through which fat content was determined:

Equipment and Reagents:

Apparatus: Soxhlet extractor, round-bottom flask, heating mantle or water bath, and a condenser.

Reagents: A suitable solvent (usually petroleum ether or hexane).

Procedure:

Sample preparation: Weighing of the representa -tive sample (typically 2-10 g) and then dry it to remove moisture if necessary. Grind the sample to increase the surface area.

Setup: Placed the dried sample in a thimble (made of porous paper or a suitable material) and inserted it into the Soxhlet extractor.

then extractor is Connect to a round-bottom flask filled with the chosen solvent. The flask is heated to evaporate the solvent.

Extraction: As the solvent vapor rises, it condenses in the condenser and drips into the thimble containing the sample.

The fat dissolves in the solvent, which then fills the extractor chamber. When it reaches a certain level, the solvent will siphon back into the flask, carrying the extracted fat with it.

This cycle continues for several hours (typically 4-8 hours), ensuring thorough extraction.

Collection: After the extraction period, turn off the heat and allow the apparatus to cool.

Solvent Removal: Remove the round-bottom flask and evaporate the solvent using a rotary evaporator or by gentle heating to obtain the extracted fat.

Weight measurement: Once the solvent has completely evaporated, weigh the flask containing the extracted fat.

Calculation:

Determine the fat content using the formula:

$$\text{Fat}\ (\%) = \frac{w^2 - w^1}{w} \times 100 \qquad (2)$$

Determination of the fiber Content through chemical digestion method: The determination of fiber content through chemical digestion is often done using the AOAC (Association of Official Analytical Chemists) methods, specifically for dietary fiber. Here is a step-by-step overview of the process through which fiber content was determined:

Equipment and Reagents:

Apparatus: Analytical balance, beakers, heating mantle, filtration setup, and drying oven.

Reagents: Sulfuric acid (H_2SO_4) and sodium hydroxide (NaOH) for digestion. Ethanol or isopropanol for washing and Acetone for drying.

Procedure:

Sample preparation: Weigh approximately 1-2 g of the sample accurately. Grind the sample if necessary to ensure uniformity.

Neutral Detergent Fiber (NDF) Analysis: Samples were boiled in in neutral detergent solution (usually containing sodium lauryl sulfate) for a specific period (typically 1 hour). This extracts soluble fibers and leaves behind cell walls.

Filtration: Filtration of the mixture through a filter paper or a cloth is done to separate the residue (insoluble fiber) from the filtrate.

Washing: Washing of the residue thoroughly with hot water to remove any remaining soluble material. Then wash with ethanol or isopropanol to remove any residual detergent.

Drying: Dry the residue in an oven at 105°C until constant weight is achieved. Acid and Alkali Digestion:

Subject the dried residue to acid digestion (e.g., boiling in 1.25% H_2SO_4) for about 30 minutes, followed by neutralization with NaOH. This step breaks down the hemicellulose and lignin.

Filtration and Washing: Filter again to collect the remaining insoluble residue (cellulose and lignin). Wash with hot water, followed by ethanol or acetone.

Drying: Dry the final residue at 105°C until the constant weight of the sample was achieved, then

Weigh the dried residue to determine the amount of insoluble fiber.

Calculation: Calculate the fiber content using the formula:

$$\text{Fiber content (\%)} = \frac{\text{mass of fiber}}{\text{mass of sample}} \times 100 \quad (3)$$

Determination of Moisture Content through hot air oven method: Determining moisture content using the hot air oven drying method is a common technique used across various industries to quantify the amount of moisture present in a sample. This method involves drying the sample in a controlled environment to remove moisture without significantly altering other components.

Here is a step-by-step overview of the process through which moisture content was determined Materials Needed:

Sample containing moisture (e.g., food, feed, soil), Hot air oven capable of maintaining temperatures up to 105°C (often specified for moisture determination), Aluminum or stainless-steel drying pans or containers, Analytical balance for weighing, Desiccator for cooling.

Procedure:

Sample Preparation: An appropriate amount of the sample was weighed using an analytical balance, ensuring the amount was sufficient to accurately measure the moisture content without exceeding the capacity of the drying pans or containers.

Preparation of drying containers: Clean and dry aluminum or stainless-steel drying pans or containers, suitable for the sample size and oven, were used.

Initial Weighing: Record the initial weight of the drying pan or container (W1).

Transfer the weighed sample into the drying pan or container and record the combined weight (W2 = W1 + mass of sample).

Drying Process: Placed the drying pan or the container with the sample in it into the hot air oven, set the oven temperature to the specified drying temperature (typically between 100-105°C for most materials) for our sample we did the temperature at 105ºC and allow the sample to dry for a sufficient duration. The drying time can vary and also depends upon the on the moisture content and sample type but generally ranges from 1 to 24 hr.

Cooling and Weighing: After the specified drying period, remove the drying pan or container from the oven and place it in a desiccator to cool to room temperature, once cooled, quickly weigh the dried sample and drying pan or container (W3). Record the final weight.

Calculation of Moisture Content:

$$\text{Moisture \%} = \frac{w^1 - w^2}{W2 - W} \times 100 \quad (4)$$

Determination of ash content through Muffle furnace method: Determining ash content by using a muffle furnace involves heating a sample at very high temperatures to combust the organic materials, leaving behind inorganic residue (ash). This method has been commonly used in various industries, including food, agriculture, and many industries and also in environmental analysis.

Here's a detailed process for determining ash content by muffle furnace.

Materials Needed: Sample containing ash-forming materials (e.g., food, feed, biomass), Muffle furnace capable of reaching temperatures up to 550-1000°C (depending on the sample), Crucibles or ashing dishes made of inert materials (such as porcelain, quartz, or alumina), Analytical balance for weighing, Desiccator for cooling.

Procedure:

Sample Preparation: Weighing the appropriate amount of the sample using an analytical balance. The amount should be sufficient to accurately measure the ash content not exceed the capacity of the crucible, for solid samples, ensure they are finely ground and homogenized to facilitate uniform combustion.

Ashing Process: The weighed sample was placed into the pre-weighed crucible dish or ashing dish made of inert material. The initial weight of the crucible with the sample was recorded.

Heating in the Muffle Furnace: The crucible containing the sample was placed into the muffle furnace. The furnace was gradually heated to the specified temperature (typically between 550-1000°C) and maintained at this temperature for a sufficient duration to ensure complete combustion of organic materials. The heating duration, which depended on the nature of the sample, usually ranged from 2 to 6 hours.

Cooling and Weighing: After the specified heating period, the muffle furnace was turned off, and the crucible on which sample was allowed to cool inside the furnace to avoid contamination. The cooled crucible was then transferred to a desiccator to further cool at the moderate room temperature. Once cooled, the crucible containing the ash residue was weighed using an analytical balance, and the final weight was recorded.

Calculation of Ash Content:

$$\text{Ash content } (\%) = \frac{w^2 - w^1}{w} \times 100 \qquad (5)$$

3. RESULTS AND DISCUSSION

As a result of nutritional and sensory analysis, it is concluded that the treatment T_3 which was millet-based pizza base had better nutritional profile in compare to the regular refined wheat flour pizza base and treatment T_1, T_2, prepared sample T_3 of millet has more protein content, fiber content and ash content and lower fat content, and moisture content among all three treatment T_1, T_2, and T_3. But treatment T_1, T_2, also have higher protein content, fiber and ash content, and lower fat content, and moisture content in compare to the regular refined wheat flour pizza base which is widely used in the markets, and in the sensory analysis containing color, taste, flavor, texture appearance and overall acceptability T_3 have got 8.6 which is the best among all the treatments.

Protein content of treatment T_1, T_2, and T_3 are 7.13%, 8.20%, and 9.5% respectively so on the fiber content of treatment T_1, T_2, and T_3 are 0.798%, 0.875%, and 0.913% respectively, And the fat content of these three treatments is 1.75%, 1.56%, and 1.46% respectively, Moisture of the treatments T_1, T_2, and T_3 are 37.08%, 36.95%, and 36.12%, The ash content of the treatment T1, T_2, and T3 are respectively are 0.91%, 0.93%, and 0.98% respectively. sample T_3 among all the samples T_1, T_2, T_3 was superior on the sensory parameter according to the panelists. So, sample T_3 considered as the standardized sample.

After protein content analysis it is found out that as the amount of composite flour content kodo millet, buck wheat flour and brown rice increase the amount of protein content also increases that is why the content of protein is higher in sample T_3 in comparison of sample T_0 as mentioned in Fig. 1.

Table 1. Score card for organoleptic evaluation of millet-based pizza base Name of the product: Pizza Base

Sample	Organoleptic score					
	Colour	**Taste**	**Flavour**	**Texture**	**Appearance**	**Overallacceptability**
T1	8	6	6	7.4	8.2	7.8
T2	8.6	8.2	8.2	7.8	8.6	8.2
T3	9	8.6	8.6	7.8	8.6	8.6

Table 2. Comparative proximate content of control and T3 pizza base

Nutrients	Control T0	Standarized T3
Protein	6	9.5
Fat	1.75	1.46
Fiber	0.605	0.913
Moisture	38.25	36.12
Ash	0.95	0.98

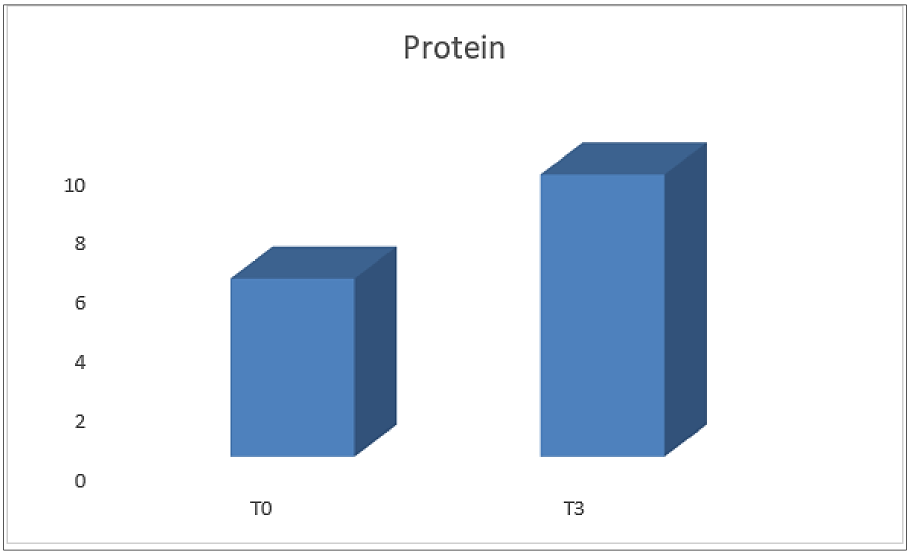

Fig. 1. Protein content comparison of T0 and T3 pizza base samples

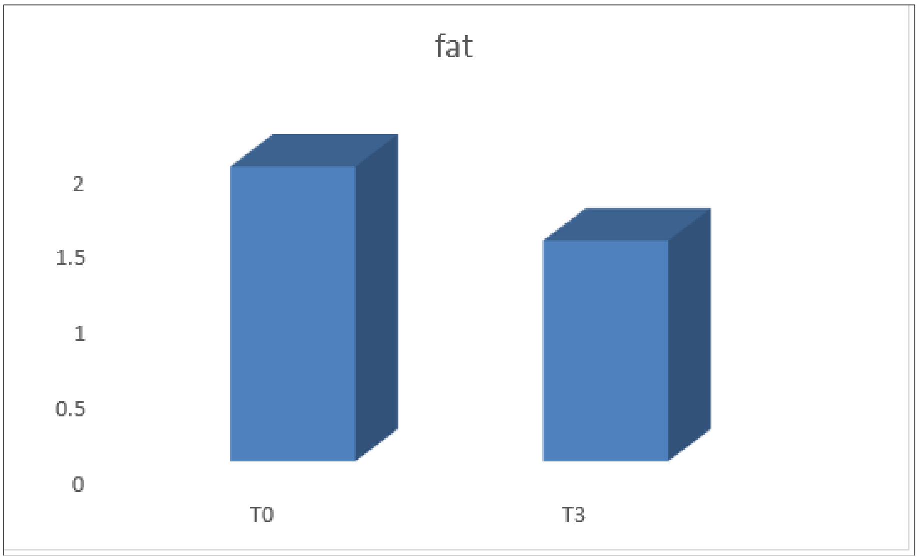

Fig. 2. Fat content comparison of T0 and T3 pizza base samples

During the fat content analysis, it is observed that the content of fat decreases as the amount of composite flour content increases in the sample that why the sample T_3 has the lowest amount of fat in compare to all other samples and also lower in compare to T_0, as shown in Fig. 2.

In the analysis of the fiber content of the sample, it is analyzed that the amount of fiber content increases as the content of composite flour increases that is why sample T_3 has higher fiber content as compare to the control sample T_0, as mentioned in Fig. 3.

After ash content analysis it is found out that as the content of kodo millet, buck wheat flour and brown rice increase the amount of ash content is increases that is why the content of ash is higher in sample T_3 in comparison of sample T_0 as mentioned in Fig. 4.

During the moisture content analysis, we observe that the content of moisture decreases as the amount of composite flour content increases in the sample that why the sample T3 has the lowest amount of moisture content in compare to all other samples and also lower in compare to T0, as shown in Fig. 5.

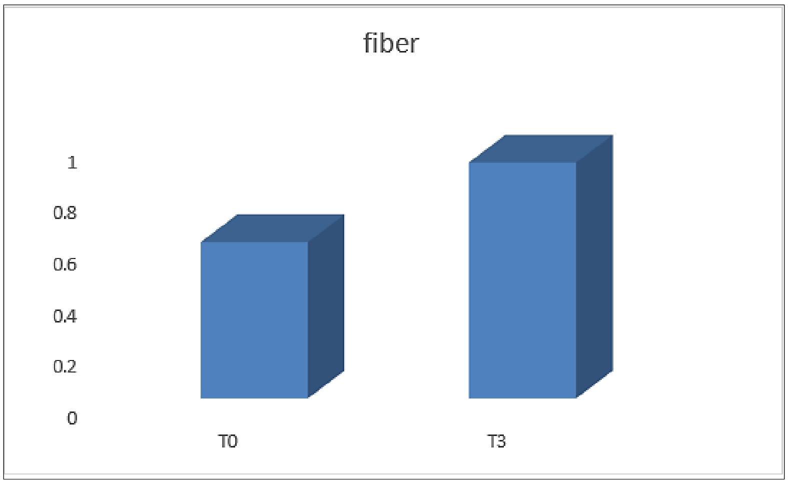

Fig. 3. Fiber content comparison of T0 and T3 pizza base sample

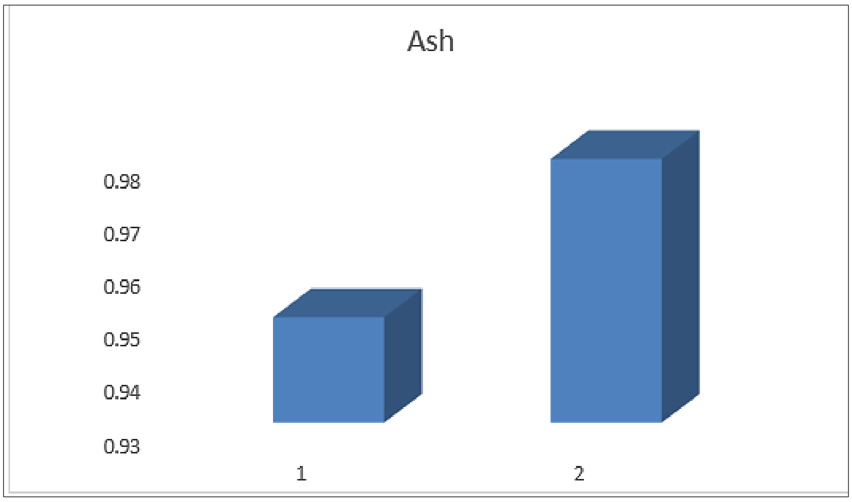

Fig. 4. Ash content comparison of T0 and T3 pizza base samples

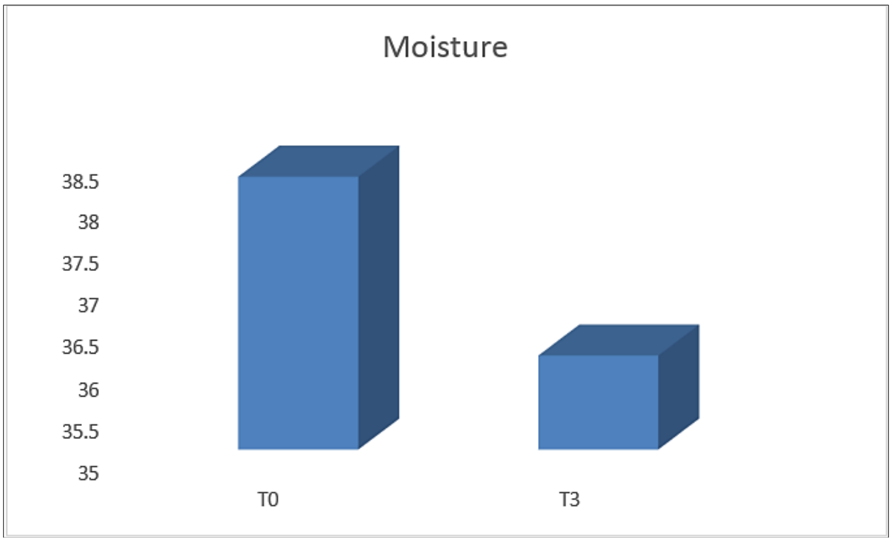

Fig. 5. Moisture content comparison of T0 and T3 pizza base samples

4. CONCLUSIONS

On the basis of present study, it can be concluded that the developed value-added pizza base T_3 is more nutrient rich than the control pizza base T_0. It has higher amount of protein and fiber, whereas lower amount of fat, moisture, and higher ash content as compared to control pizza base sample T_0. The developed pizza base samples were subjected to sensory evaluation. The Nine-point hedonic rating scale method was used for organoleptic evaluation of the products. The results showed that the among all the sample T_3 (20.51%:20.51%:15.38%:7.6%), has been the most acceptable with regards to all sensory parameters.

5. RECOMMENDATION & SUGGESTION

In present scenario when there is rise in non-communicable disease such as obesity, diabetes and high blood pressure due to lack of nutrients in daily food routine the developed value-added pizza base T3 may be helpful. Thus, it can be a replacement for the conventional refined wheat flour pizza base which can cause serious health issues.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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