ORGANOLEPTIC EVALUATION AND IMPROVEMENT OF BREAD USING DIFFERENT YEAST STRAINS

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Abstract:

Background: Dough samples were collected from various bakeries in Surat, Gujarat, India, and a commercial yeast strain was procured from a local market for comparative analysis. The isolates were identified based on the morphological characteristics of the yeast cells. Further investigations were conducted to assess baking performance, temperature tolerance and cultural characteristics of the yeast strains. Additionally, the nutritional composition and organoleptic properties of bread prepared using the isolated yeast strains were evaluated.

Results: The bread samples were prepared using whole wheat flour, and doughs inoculated with different yeast strains were compared to identify the most nutritionally beneficial option. Based on the superior performance of the selected yeast strain, bread with optimized texture, flavor, and enhanced nutritional value was developed, highlighting its potential health benefits.

Conclusion: The color development of the bread crust and crumb varied across the different yeast strains. Bread produced with the optimized strain demonstrated higher protein content and reduced carbohydrate levels. Furthermore, the incorporation of chia seeds was explored to enhance the overall nutritional profile of the bread.

Introduction:

Yeast has been used for thousands of years in the production of food and beverages. Saccharomyces cerevisiae, commonly known as baker's yeast, is a sugar-fermenting fungus naturally found in the environment and widely used in the preparation of breads (Menaik et al., 2011). In baking, baker's yeast serves as a leavening agent by converting fermentable carbohydrates in the dough into carbon dioxide which causes the dough to expand and results in lighter, softer bread. Although S. cerevisiae used in baking is closely related to brewer's yeast, it represents a different strain typically optimized for baking purposes.

The rising of bread dough is primarily attributed to the production of carbon dioxide through the fermentation of flour carbohydrates by active baker's yeast, though other yeasts and bacteria may also contribute to this process (Tadayon, 1978). Freshly baked bread is characterized by a brown, crisp crust, a pleasant roasted aroma, fine slicing properties, a soft, elastic crumb texture, and a moist mouthfeel (Giannou et al., 2003).

This study aimed to investigate the presence and diversity of yeast strains used as leavening agents in Indian breads (Tadayon, 1976). Dough samples were collected from various bakeries in Surat, Gujarat, India, and yeast strains were isolated and characterized using cultural,

morphological, and biochemical analyses. The baking performance of these strains was also evaluated to assess their potential in bread production.

MATERIALS AND METHODS

Collection of samples

Dough samples were collected from different bakeries in Surat, Gujarat, India. The exact geoFigureical coordinates (latitude and longitude) of Umrao's Bakery and Modi Bakers, from which the samples were obtained, are presented in Plate 1 (A) and (B). The collected dough samples were stored in sterile jars with tightly sealed screw-top lids and promptly transported to the laboratory to maintain sterility and sample integrity.

(A) (B)

Plate 1: (A) Umrao's bakery and (B) Modi bakers, Surat, Gujarat, India.

Additionally, three commercial yeast samples were procured from the local market in Surat, Gujarat, India, as shown in Plate 2: (1) Instant Dry Yeast (New Arife Lamould, Surat, Gujarat, India), (2) Active Dry Yeast (Blue Bird, Surat, Gujarat, India), and (3) Moist Yeast (Prakash Bakery, Surat, Gujarat, India).

(A) (B) (C)

Plate 2: Yeast purchased from the local market of Surat, Gujarat, India
(A) Instant dry yeast (B) Active dry yeast and (C) Moist yeast

Isolation of Yeast

To ensure sterility, the workspace was disinfected with 70% ethanol prior to initiating the procedure. For the isolation of yeast strains, a small portion of each dough sample was collected

using a sterile loop, suspended in 1 mL of distilled water, thoroughly mixed, and streaked onto Sabouraud Dextrose Agar (SDA) plates (composition: dextrose 4 g, peptone 1 g, agar 1.5 g, distilled water 100 mL; pH 5.6).

Sample Code	Source
Saccharomyces cerevisiae Control	Department of Biotechnology, VNSGU, Surat, Gujarat, India
Y1	Umrao's bakery, Surat, Gujarat, India
Y2	Modi bakers, Surat, Gujarat, India
Y3	Local market (Instant dry yeast), Surat, Gujarat, India
Y4	Local market (Active dry yeast), Surat, Gujarat, India
Y5	Local market (Moist yeast), Surat, Gujarat, India

Table 1: Sample Code and their Sources

The inoculated plates were incubated at 28 °C for 48 hours. Following incubation, colonies were examined for morphological characteristics and microscopic appearance. All experimental samples were appropriately coded, as summarized in Table 1.

Identification of Yeast Cells

Under bright-field microscopy at high magnification, the yeast cells were observed to be oval or egg-shaped, often exhibiting budding structures on some cells (Hassan et al., 2018). On SDA plates, the isolates typically formed small, creamy to white colonies. Colonies with a shiny white appearance were indicative of yeast, whereas non-shiny or dull white colonies were also considered potential yeast isolates (Okerentugba et al., 2016).

Temperature Tolerance Test

The thermal tolerance of the yeast isolates was assessed by inoculating them onto SDA medium and incubating the plates at four different temperatures which were 25 °C, 30 °C, 37 °C and 45 °C for 72 hours (Tsegaye et al., 2018).

Baking Test

The bottom of a beaker was filled with dough and covered with Bio-cling film, leaving two to three small perforations for aeration. To prevent drying, the dough was allowed to rise until it reached a volume of 50 mL. The dough was then mixed with the specific yeast strain, compressed back to its original volume, and again allowed to rise to a volume of 50 mL. The baking strength of the sample was determined by summing the times recorded for the first and second rises (Tadayon, 1976).

Bread Preparation

Dough preparation was carried out under aseptic conditions using fully sterilized bowls using procured culture from dough already collected from different bakeries, as outlined in Table 2. Whole wheat flour (Ashirwad Whole Wheat Flour, India) served as the base ingredient for all formulations. Five distinct yeast strains were evaluated along with a control containing Saccharomyces cerevisiae. Ingredients were mixed in six separate sterilized bowls, labelled Y1, Y2, Y3, Y4, and Y5 for the experimental strains, and C for the control.

Ingredients	g/mL
Flour (Aashirwad whole wheat flour, Indian Brand))	100
Yeast	2
Salt (Tata Company Ltd.)	2
Warm Water	60-70
Vinegar	0.1
Oil (Tirupati, Indian Brand)	3
Jaggery	4

The baking strength of each formulation was assessed using the standard baking strength test, which measures carbon dioxide production during dough fermentation (Tadayon, 1976). Dough balls prepared from each sample were allowed to ferment in beakers at room temperature, and gas production was quantified after 30 minutes. The fermented dough was then baked in a preheated oven at 230 °C for 20 minutes. Following baking, the bread samples were cooled at room temperature for 30 minutes before being collected for proximate composition analysis and organoleptic evaluation.

Proximate Analysis

The protein content of the bread samples was determined using the Folin–Lowry method (Protocol, 1994), as outlined in Table 3.

Sr. No.	BSA Concentration (µg/mL)	BSA (1 mg/mL)	Distilled water (mL)	Alkaline copper (mL)	FC reagent (mL)
1.	-	0.0	1.0	5	0.5
2.	100	0.1	0.9	5	0.5
3.	200	0.2	0.8	5	0.5
4.	400	0.4	0.6	5	0.5
5.	600	0.6	0.4	5	0.5
6.	800	0.8	0.2	5	0.5
7.	1000	1.0	0.0	5	0.5

Table 3: Protein by Folin-Lowry Method (O.D. at 600 nm, Protocol, 1994)

Sr. No.	Glucose Concentration (µg)	Working Standard solution (2 mg/mL)	Distilled Water	DNSA Reagent (mL)	15 Minutes in water bath at 100°C
B	0.00	0.0	1.0	3	
1	200	0.2	0.8	3	
2	400	0.4	0.6	3	
3	600	0.6	0.4	3	
4	800	0.8	0.2	3	
5	1000	1.0	0.0	3	

Table 4: Carbohydrate by DNSA method (O.D. at 540 nm, Himedia, 2010)

Carbohydrate content was evaluated using the DNSA method (Himedia, 2010), described in Table 4. Additionally, the ash and moisture contents of the samples were measured (Moisture, 1999).

To Determine Organoleptic Evaluation of Bread

The organoleptic attributes of the bread samples, including color, taste, texture, flavor, and aroma, were evaluated. Each bread sample was served in coded dishes labeled as Control, Y2, Y3, Y4, Y5, and Y6 to ensure anonymity during assessment. To minimize sensory bias, panelists were provided with room-temperature water to rinse their mouths before and after each evaluation (Chikwendu et al., 2015).

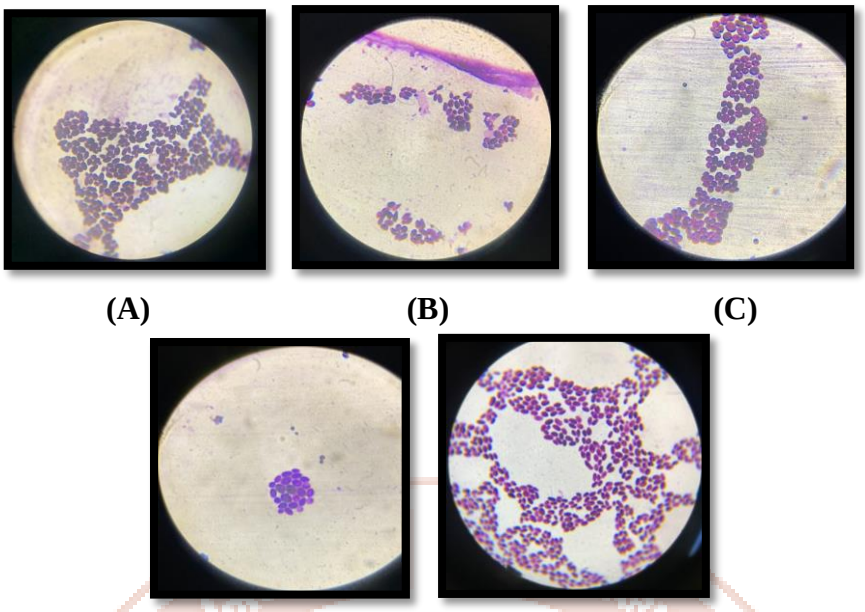
Flavorings and for Health Benefits

Flaxseeds and Chia seeds were sprinkled over the dough prior to baking as a decorative and nutritional enhancement, following the standard bread preparation protocol. These seeds were incorporated due to their high nutritional value, including a rich content of omega-3 fatty acids, dietary fiber, essential minerals and antioxidants. Regular consumption of Flaxseeds and Chia seeds has been associated with multiple health benefits, such as improved cardiovascular health, better digestive function, potential weight management support and maintenance of healthy skin, hair, nails and bones. Additionally, these seeds may contribute to lowering cholesterol levels and blood pressure, thereby enhancing overall wellness.

RESULTS AND DISCUSSION

Identification of Yeast Cells

As shown in Plate 3, Gram staining was performed to identify the isolated yeast strains. The physical characteristics of the isolated strains are summarized in Table 5 (Okerentugba et al., 2016).



(A) (B) (C)

(D) (E)

Plate 3: Results of Gram's staining (A) Y1, (B) Y2, (C) Y3, (D) Y4 and (E) Y5

Sample	Color	Nature	Elevation	Edge	Size	Shape
Control	Creamy white	Smooth & Dull	Raised	Entire	Intermediate	Round
Y1	Cream	Smooth & Shiny	Convex	Entire	Intermediate	Round
Y2	Cream	Smooth & Shiny	Pulvinate	Entire	Intermediate	Round
Y3	White	Smooth & Shiny	Convex	Entire	Intermediate	Round
Y4	Cream	Smooth & Shiny	Convex	Entire	Small	Round
Y5	Cream	Smooth & Shiny	Convex	Entire	Small	Round

Table 5: Colony Morphological Characterization

To examine the morphological properties of the yeast cells, samples were observed under a compound microscope at 100 X magnification (Hassan et al., 2018).

Temperature Tolerance Test

To gain a clearer understanding of yeast behavior, a temperature tolerance assay was conducted on the isolated strains. Since temperature plays a critical role in influencing fermentation efficiency and yeast metabolism, the isolates were evaluated for growth performance across a range of incubation temperatures. The growth and inhibition profiles of the isolates at different temperatures are summarized in Table 6.

Sample	Temperature 25°C	30°C	37°C	45°C
Control (*S. cerevisiae*)	+++	+++	+++	+++
Y1	+++	+++	+++	-
Y2	+++	+++	+++	-
Y3	++	+++	+++	-
Y4	+++	+++	+++	-
Y5	+++	+++	+++	+

Table 6: Temperature Tolerance Test

+++ = Intensive growth, ++ = Moderate growth, + = Poor growth, - = No growth

Intense growth was observed between 30°C and 37°C, with 37°C supporting the most robust growth and metabolic activity in all isolates. In contrast, growth was significantly reduced at 45 °C, indicating thermal inhibition under elevated temperature conditions.

Baking Strength

The comparative baking strength of the isolated yeast strains is presented in Plate 4. Strains Y1 and Y2 exhibited lower baking activity compared to the control, whereas strains Y3, Y4, and Y5 demonstrated significantly higher baking strength, indicating superior leavening potential.

Sample	First Rise	Second Rise
Control (S. *cerevisiae*)	+++	+++
Y1	++	+
Y2	+	-
Y3	+++	+++
Y4	+++	+++
Y5	+++	+++

Table 7: Baking Strength

+++ = Intensive growth, ++ = Moderate growth, + = Poor growth, - = No growth

The baking strength of the yeast strains was assessed based on their leavening ability during the first and second rise phases of the dough as per shown in Table 7. The control strain (S. cerevisiae) demonstrated intensive growth (+++) in both rises, establishing a benchmark for comparison.

Among the isolates, Y3, Y4, and Y5 exhibited comparable performance to the control, showing intensive leavening (+++) during both the first and second rises, indicating their strong fermentation potential and suitability for bread production. In contrast, Y1 displayed moderate growth (++) in the first rise but showed a reduced leavening ability (+) in the second.

Making Bread Using Different Kind of Yeast

The comparative baking strength of the isolated yeast strains is presented in Plate 4. Strains Y1 and Y2 exhibited lower leavening ability, resulting in breads with suboptimal volume and irregular shape. In contrast, breads produced with strains Y3, Y4, and Y5 demonstrated superior baking performance, characterized by well-risen loaves with uniform shape, desirable texture, and a pleasant aroma, indicating their strong leavening potential and suitability for bread production.

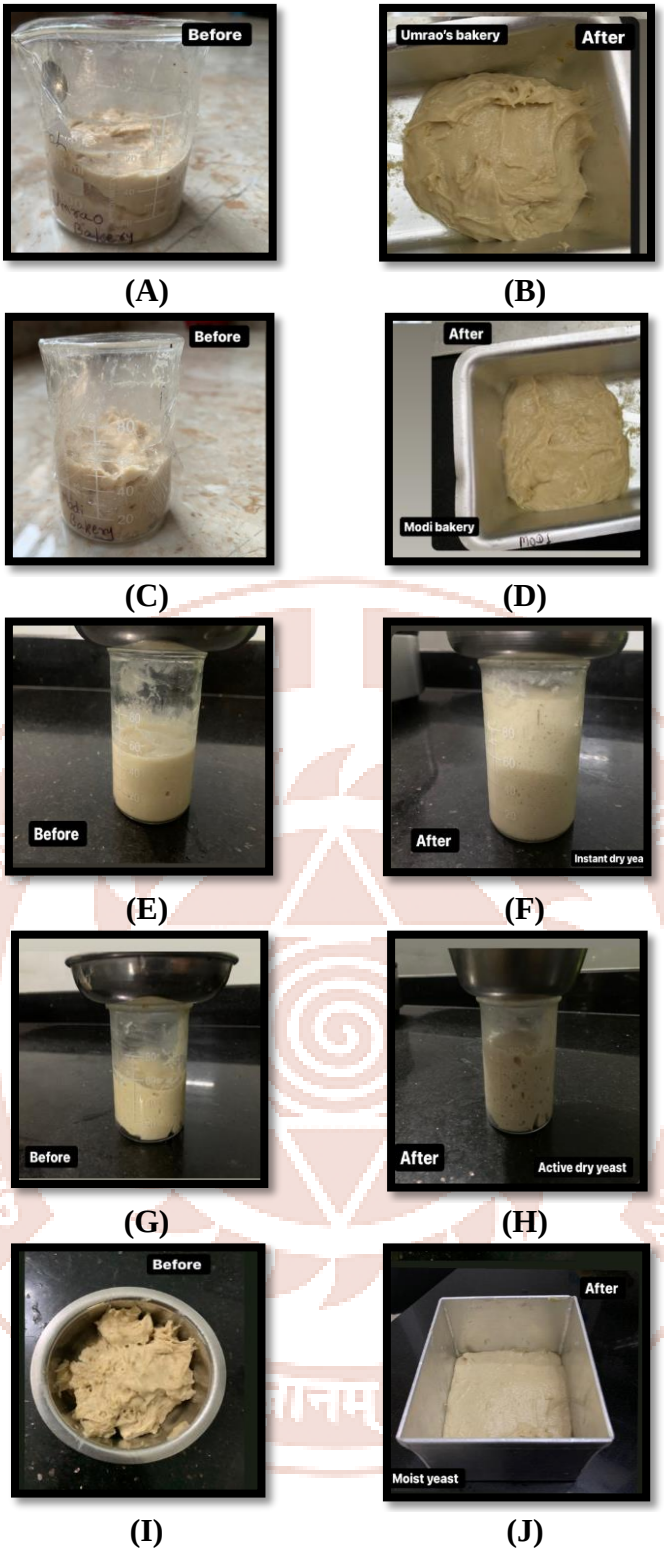


Plate 4: Baking strength; (A) Y1-Before, (B) Y1-After; (C) Y2-Before, (D) Y2-After; (E) Y3-Before, (F) Y3-After; (G) Y4-Before, (H) Y4-After; (I) Y5-Before, (J) Y5-After

Proximate analysis

Figure 1 illustrates the protein content of the breads prepared with different yeast strains. Strains Y3, Y4, and Y5 exhibited comparatively higher protein levels, whereas Y1 and Y2 showed lower protein concentrations. Overall, the protein content of the samples ranged from 300 to 1000 g/mL, with Y3 recording the highest value at 942.663 g/mL. The elevated protein levels are attributed to the use of whole wheat flour, which naturally contains more protein compared to refined all-purpose flour. Among all samples, Y3 and Y4 demonstrated the highest protein contents, at 942.663 g/mL and 967.924 g/mL respectively (Protocol, 1994).

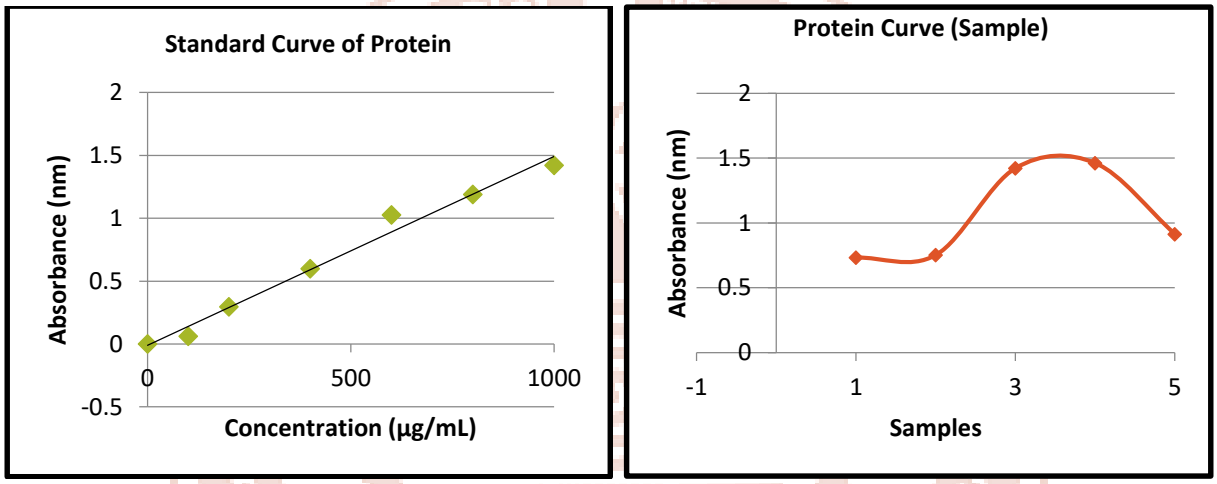


Figure 1: Standard Protein Curve

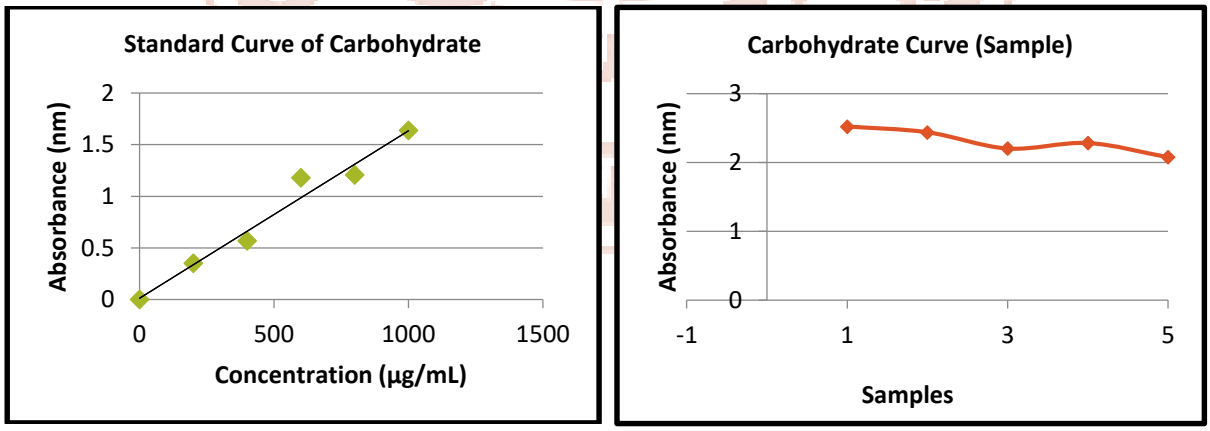


Figure 2: Carbohydrate Curve

As shown in Figure 2, the carbohydrate content of the bread samples ranged from 1200 to 1500 g/mL. Y5 exhibited the lowest carbohydrate concentration, closely followed by Y3, whereas Y1 and Y2 displayed relatively higher carbohydrate levels of 1504.707 g/mL and 1455.686 g/mL, respectively. The use of jaggery as a natural sweetener contributed to the carbohydrate profile of the samples. From a nutritional perspective, samples such as Y3 and Y5, with comparatively lower carbohydrate concentrations, may be preferable. Since starch is the most abundant carbohydrate in bread, its concentration remains a significant factor influencing both nutritional value and texture (Himedia, 2010).

Selection of Final Product

The final bread formulations were selected from samples Y3 and Y4, which exhibited higher protein concentrations of 942.663 g/mL and 967.924 g/mL, respectively. Additionally, sample Y5, characterized by a comparatively lower carbohydrate concentration of 1243.654 g/mL, was also chosen for the final product. These selected breads were subjected to detailed analyses to evaluate their organoleptic attributes, including color, aroma, texture and taste, as well as their nutritional composition, such as protein, carbohydrate, moisture and ash contents.

The combination of higher protein content and balanced carbohydrate levels in these samples highlights their potential as nutritionally superior bread products. The organoleptic evaluation further revealed that these formulations produced breads with desirable sensory properties, making them both health-promoting and consumer-acceptable options.

Organoleptic Evaluation

The bread produced exhibited a relatively small loaf volume, a rough crust and darker crumbs, accompanied by a slightly bitter taste. This can be attributed to the lower gluten content of whole wheat flour compared to all-purpose flour. Since gluten plays a critical role in providing structure and elasticity to dough, reduced gluten levels tend to yield loaves that are flatter and denser. Despite these limitations, the overall texture of the product was superior to that of the other bread samples tested. However, the low gluten content in the blended flours likely hindered optimal dough development, contributing to the observed variations in texture and structure. Additionally, the flavor profile of the product was slightly different from conventional breads (Chikwendu et al., 2015).

Proximate Analysis of Product

Protein content, carbohydrate value, moisture content, and ash content were the four parameters analyzed to evaluate the final bread product. The protein content, determined using the Folin–Lowry method, was found to be the highest (799.967 g/mL). The use of whole wheat flour contributed significantly to the elevated protein levels in the product. The carbohydrate content was recorded at 1146.793 g/mL, which was comparatively lower due to the inclusion of jaggery as the sweetener instead of refined sugar (Chikwendu et al., 2015).

The product's moisture content was 21.04%, indicating a relatively short shelf life, as foods with higher moisture content are more prone to microbial spoilage. The ash content of the product was 6.96%, which is valuable for nutritional proximate analysis and indicates the total mineral content present in the bread (Moisture, 1999).

(A) **(B)** **(C)**

Plate 5: Final Product of Bread (A) Whole wheat bread (B) Flaxseeds bread (C) Whole wheat bread

Overall, the nutritional profile of the bread demonstrates an enhanced protein value, balanced carbohydrate content, and acceptable mineral composition. Plate 5 shows the finished loaf of bread, representing the optimized combination of nutritional and yeast qualities.

CONCLUSION

The study conlcudes that yeast isolated from dough exhibited promising potential characteristics for bread production. The findings also confirmed that different yeast strains influenced the color development of the bread crust and crumb, indicating their significant role in fermentation quality and appearance. This observation further suggests that yeasts from different regional fruits could thrive in diverse habitats, benefiting from unique nutrient sources that support their growth and metabolic activities.

From a nutritional standpoint, the bread produced in this study demonstrated higher protein content, attributed to the use of whole wheat flour, and a lower carbohydrate content due to the incorporation of jaggery as the sweetener. To further enhance the nutritional profile, flax and chia seeds were added. These seeds are well-known for their richness in protein, omega-3 fatty

acids, antioxidants, dietary fiber, and essential minerals. Their inclusion not only boosts the functional value of the bread but also contributes to several health benefits, such as reducing the risk of heart disease, helping regulate blood pressure, stabilizing blood sugar levels, lowering cholesterol, and supporting healthy weight management.

These findings indicate that combining naturally isolated yeast strains with nutrient-dense ingredients like whole wheat flour, jaggery and functional seeds could pave the way for developing healthier, protein-rich, and fiber-packed bread varieties suitable for commercial baking applications.

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