

Comparative Nutritional Analysis of *Neurospora Crassa*-Derived Mycoprotein Bread and Conventional All-Purpose Flour Bread

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The rise in bread consumption across Asia, alongside carbohydrate-dense staples such as rice, has intensified dietary imbalances, particularly protein deficiencies. This study explores the potential of mycoprotein, derived from *Neurospora crassa*, to enhance the nutritional profile of bread. Mycoprotein, a sustainable fungal protein, is rich in high-quality protein and dietary fiber, offering a promising solution to nutritional gaps in carbohydrate-dominant diets. Bread formulations with varying levels of mycoprotein inclusion (0–40%) were analyzed for macronutrient composition, caloric distribution, and amino acid profiles. Results indicate a significant increase in protein content, from 8.13 g/100 g at 0% to 15.69 g/100 g at 40% inclusion, and dietary fiber, which rose from 1.7 g/100 g to 15.43 g/100 g. Concurrently, carbohydrate levels declined, demonstrating mycoprotein's potential to reduce glycaemic load. While essential amino acid profiles improved, certain amino acids, including methionine and cysteine, remained limited, suggesting the need for complementary protein sources. This research highlights mycoprotein's capability to address protein and fiber deficiencies while offering a sustainable alternative to conventional bread ingredients. These findings support its application in developing nutritionally enriched, environmentally friendly food products for regions experiencing dietary transitions.

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Introduction

Asia's growing appetite for bread signals a profound shift in dietary habits, marked by an accelerating embrace of processed, convenience foods. In China, for instance, the bakery industry expanded by 26% between 2015 and 2020, driven by a growing demand for white bread [1]. Similarly, in Japan, household expenditure on bread surpassed that of rice for the first time in 2011, indicating a substantial departure from traditional dietary patterns [2]. As bread becomes a more integral component of daily food consumption across Asia, its nutritional composition—characterized by high carbohydrate and low protein content—has become a focal point of concern.

Excessive carbohydrate intake, particularly from refined grains such as those found in conventional bread, has been implicated in the etiology of several metabolic disorders, including obesity, insulin resistance, and an elevated risk of type 2 diabetes [3]. This issue is especially pronounced in many Asian populations, where rice, a staple with high carbohydrate density, already constitutes a significant proportion of the diet. The additional introduction of bread exacerbates this dietary imbalance, leading to a marked increase in carbohydrate consumption without addressing the population's protein requirements. Bread's inherently low protein content, when

combined with a carbohydrate-heavy dietary framework, intensifies the existing challenge of protein insufficiency in these regions [4].

Protein is a critical macronutrient, essential for numerous physiological processes such as metabolic homeostasis, muscle protein synthesis, and tissue repair. Despite its importance, protein intake in many Asian countries remains suboptimal due to the dominance of carbohydrate-rich staples, such as rice and bread, in the diet [4]. This imbalance, characterized by excessive carbohydrate intake coupled with insufficient protein consumption, not only exacerbates the prevalence of metabolic disorders but also complicates efforts to mitigate chronic protein deficiencies—a nutritional concern that is projected to persist and even worsen in the coming decades.

In response to these emerging public health concerns, there has been a growing demand from consumers, alongside government-driven initiatives, to develop food products with enhanced nutritional profiles [5]. These trends have spurred research and development efforts aimed at reformulating bread products to increase their protein and dietary fiber content, with the goal of counteracting the negative health impacts associated with carbohydrate overconsumption and protein insufficiency in traditional bread formulations [5].

One promising approach to improving the nutritional quality of bread involves the incorporation of mycoprotein, a high-protein biomass produced from filamentous fungi, such as *Fusarium*, *Neurospora*, and *Rhizopus* spp. Mycoprotein offers several distinct nutritional advantages: it is rich in high-quality protein, contains a favourable amino acid profile, and is also a significant source of dietary fiber. Studies have demonstrated that mycoprotein can promote digestive health, enhance satiety, and support glycaemic control and weight management [6]. Furthermore, the production of mycoprotein is highly sustainable when compared to traditional animal-based protein sources, requiring fewer natural resources, such as land and water, and generating a substantially lower carbon footprint [7]. In addition to its high protein content, mycoprotein's minimal environmental impact thus positions it as a promising solution to intensifying global food challenges [7].

No	Protein Source	Type	CO2 Emissions
1.	Mycoprotein	Fungi	1.5 kg CO ₂ per kg
2.	Peas	Grain legumes	2.3 kg CO ₂ per kg
3.	Soy	Grain legumes	2.8 kg CO ₂ per kg
4.	Chicken	White Meat	6.9 kg CO ₂ per kg
5.	Tuna	Fish	13 kg CO ₂ per kg
6.	Salmon	Fish	15 kg CO ₂ per kg
7.	Pork	Red Meat	24 kg CO ₂ per kg
8.	Beef	Red Meat	31 kg CO ₂ per kg
9.	Lamb	Red Meat	38 kg CO ₂ per kg

Table 1: CO₂ Emissions of Different Protein Sources (Per Kilogram).

Research Objective

The present research aims to investigate the potential of mycoprotein as a novel ingredient in bread formulations to improve the overall nutritional profile of bread products. While mycoprotein's high protein and fiber content is well-documented, its application in bread remains underexplored within the context of nutritional enhancement. This study will examine how varying levels of mycoprotein incorporation can modify the macronutrient composition of bread, with a particular focus on improving protein content, optimizing the amino acid profile, and increasing dietary fiber levels. Furthermore, the impact of mycoprotein inclusion on the carbohydrate and fat composition of bread will be analysed.

Through a rigorous evaluation of these parameters, this research will provide comprehensive insights into the viability of mycoprotein as a functional ingredient in bread production, with the potential to contribute to improved public health outcomes by addressing the nutritional imbalances prevalent in many Asian diets.

Materials & Formulations

The experiment was designed to assess the effect of mycoprotein inclusion at varying levels on the nutritional profile of bread. Mycoprotein, derived from the filamentous fungi strain *Neurospora crassa* (Fig.1), containing approximately 35-40g of protein and 20-25g of fiber per 100g, was used to replace all-purpose flour in the bread formulations.

Initially, mycoprotein inclusion rates of 0%, 5%, 10%, 15%, and 20% were proposed; however, the design was revised to include higher levels for a more comprehensive assessment. The final inclusion rates of 0%, 10%, 20%, 30%, and 40% were selected.



Figure 1: *Neurospora crassa* derived mycoprotein powder

All samples included all-purpose flour, oil, salt, sugar, yeast, and water, with the water content adjusted based on a standardized parameter. Other minor ingredients, such as bread improver, calcium propionate, and gluten, were incorporated to support structure and shelf-life. The doughs were mixed, rested, proofed, and baked under standardized conditions.

No	Items	0% MYP	10% MP	20% MP	30% MYP	40% MP
1.	Mycoprotein (g)	0.00	31.00	62.00	94.00	124.8
2.	All Purpose Flour (g)	312.00	281.00	250.00	218.00	187.2
3.	Oil (g)	24.00	24.00	24.00	24.00	24.00
4.	Salt (g)	6.00	6.00	6.00	6.00	6.00
5.	Sugar (g)	31.00	31.00	31.00	31.00	31.00
6.	Yeast (g)	5.00	5.00	5.00	5.00	5.00
7.	Water (g)	168.00	168.00	168.00	168.00	168.00
8.	Bread Improver (g)	1.00	1.00	1.00	1.00	1.00
9.	Calcium Propionate (g)	2.00	2.00	2.00	2.00	2.00
10.	Gluten (g)	1.00	1.00	1.00	1.00	1.00
	Total (g)	550.00	550.00	550.00	550.00	550.00

Table 2: Formulations of Bread with Varying Mycoprotein Inclusion Levels. (Per 550g of bread)

Analytical Methods



Figure 2: Breads with mycoprotein inclusion from left to right: 0%, 10%, 20%, 30%, and 40%

The bread samples were sent to Bureau Veritas India, a lab accredited with National Accreditation Board for Testing and Calibration Laboratories (NABL) standards for comprehensive nutritional analysis in order to evaluate the impact of mycoprotein inclusion.

Protein content was measured using the Kjeldahl method, which involved the digestion of samples in acid to release nitrogen, followed by its measurement and conversion to total protein using a nitrogen-to-protein conversion factor.

The amino acid composition of the bread formulations was analysed using high-performance liquid chromatography (HPLC), enabling a precise and comprehensive profile of both essential and non-essential amino acids. This analysis was crucial for evaluating the protein quality of the formulations, particularly given the varying levels of mycoprotein inclusion.

Initially, a complete amino acid profile was planned for each formulation (0%, 10%, 20%, 30%, and 40% mycoprotein inclusion). However, it was determined that a composite analysis of the mycoprotein bread samples (10%, 20%, 30%, and 40%) would be more effective. This approach was chosen because individual analyses at lower inclusion levels would likely yield results below quantifiable limits (BQL) for several amino acids, limiting practical insights. In contrast, the composite analysis provided a robust and quantifiable amino acid profile, serving as a representative proxy for assessing protein quality across formulations.

The dietary fiber content was measured using an enzymatic-gravimetric method. This approach enabled the precise separation and quantification of fiber fractions within the bread samples.

Moisture content was determined using the oven-drying method, where the bread samples were heated at a constant temperature until no further weight loss occurred. This provided an accurate measure of the water content in the bread, which is important for understanding shelf life and texture. Fat content was determined using Soxhlet extraction, which involved dissolving the fat in an organic solvent and quantifying it by evaporation.

Carbohydrate content was determined by difference, subtracting the measured values of protein, fat, fiber, and ash from the total mass. In addition to total carbohydrates, the content and

profile of simple sugars was analysed separately using HPLC to allow for precise assessment of individual sugar components.

The sodium content was measured using atomic absorption spectrophotometry (AAS) or ion-selective electrode (ISE) analysis to determine the precise contribution of sodium from salt and other ingredients. Ash content, representing the total mineral fraction, was measured through gravimetric incineration at 550°C, allowing for precise quantification of inorganic materials in the bread.

Lastly, the total caloric content, calorific content from protein, calorific content from carbohydrates, and calorific content from fat was calculated based on the macronutrient composition, using standard conversion factors (4 kcal/g for protein and carbohydrates, and 9 kcal/g for fat), to provide an estimate of the energy content within the bread samples.

Results & Interpretation

Nutritional Composition

Parameters	0% Inclusion	10% Inclusion	20% Inclusion	30% Inclusion	40% Inclusion
Protein(g)	8.13±0.05	9.83±0.16	11.66±0.21	14.29±0.5	15.69±0.14
Carbohydrates(g)	56±0.01	53.66±0.47	50.33±0.47	47.33±0.58	45.33±0.47
Fat(g)	7.12±0.03	5.83±0.01	5.67±0.03	5.99±0.03	6.6±0.07
Sugars(g)	1.76±0.11	2.97±0.25	4.32±0.16	5.22±0.03	6.48±0.03
Fiber(g)	1.7±0.01	5.3±0.23	9.3±0.2	13.53 ± 0.1	15.43±0.3
Sodium (mg)	1.89±0.01	0.62±0.01	0.77±0.01	0.81±0.01	0.64± 0.01
Ash(g)	5.16±0.08	2.13±0.08	3.12±0.03	3.6 ± 0.01	3.59±0.01

Table 3: Nutritional Composition of Bread with Varying Mycoprotein Levels (per 100g of bread). The results are the calculated mean along with standard deviation (n = 3).

The nutritional analysis of bread formulations with incremental mycoprotein inclusion (0%, 10%, 20%, 30%, 40%) revealed distinct trends in protein, fiber, and carbohydrate content, indicative of mycoprotein's compositional influence.

Protein content exhibited a near-linear increase with rising mycoprotein inclusion, from $8.13 \pm 0.05\text{g}/100\text{g}$ at 0% to $15.69 \pm 0.14\text{g}/100\text{g}$ at 40%. This trend underscores mycoprotein's substantial protein density and suggests its efficacy as a concentrated protein source within the matrix. The proportional increase across inclusion levels indicates that protein contribution from mycoprotein is both consistent and scalable within the bread formulation.

Fiber content increased markedly with higher levels of mycoprotein, from $1.7 \pm 0.01\text{g}/100\text{g}$ at 0% to $15.43 \pm 0.3\text{g}/100\text{g}$ at 40% inclusion. Given mycoprotein's known high fiber content (~30%), this trend suggests effective retention and integration of dietary fiber within the bread matrix. The steep rise in fiber levels as inclusion increases reflects mycoprotein's potential as a primary

fiber contributor that substantially alters the nutritional properties of the formulations.

Carbohydrate content demonstrated an inverse relationship with mycoprotein inclusion, declining from $56 \pm 0.01\text{g}/100\text{g}$ at 0% to $45.33 \pm 0.47\text{g}/100\text{g}$ at 40%. This decrease likely results from the displacement of carbohydrate-rich flour by mycoprotein, which has a comparatively low carbohydrate content. The downward trend indicates a dilution effect on total carbohydrates, suggesting that mycoprotein could support lower-carbohydrate formulations while maintaining nutrient density.

Fat content remained relatively stable across inclusion levels, with only minor fluctuations from $7.12 \pm 0.03\text{g}/100\text{g}$ at 0% to $6.6 \pm 0.07\text{g}/100\text{g}$ at 40%. The limited variation in fat content implies that mycoprotein inclusion does not significantly impact the lipid profile of the formulation, likely due to the low-fat content in mycoprotein itself. This stability suggests that fat levels are more dependent on the base ingredients in the formulation.

Sugar content showed a gradual increase with higher mycoprotein inclusion, from $1.76 \pm 0.11\text{g}/100\text{g}$ at 0% to $6.48 \pm 0.03\text{g}/100\text{g}$ at 40%. This trend may be attributable to the intrinsic sugars in the mycoprotein itself or interactions that elevate sugar content during processing. This increase in sugars with rising mycoprotein levels warrants further analysis to understand the underlying cause, especially given its potential implications for flavour and glycaemic load.

Ash content decreased from $5.16 \pm 0.08\text{g}/100\text{g}$ at 0% inclusion to $3.59 \pm 0.01\text{g}/100\text{g}$ at 40% inclusion. This reduction likely reflects a shift in the mineral composition as all-purpose flour, which has a standardized mineral content, is progressively replaced with mycoprotein.

Lastly, sodium content remained low across most inclusion levels, with an unexpected peak of $0.81 \pm 0.01 \text{mg}/100\text{g}$ at 30% inclusion, possibly due to variations in processing or inherent sodium content in the mycoprotein used to make this particular formulation.

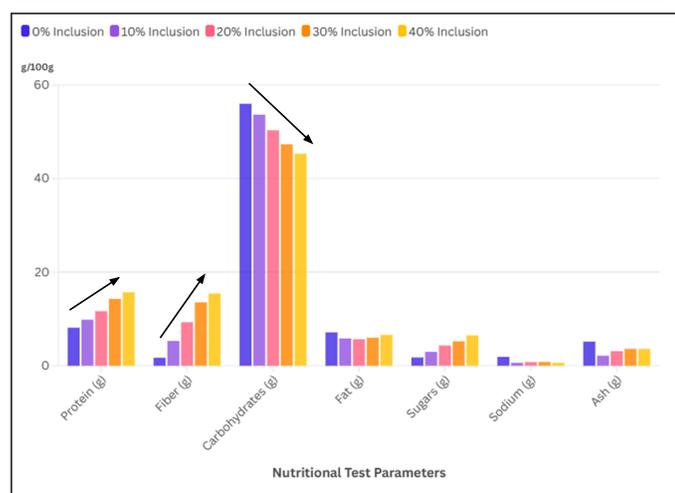


Figure 3: Nutritional Composition of Bread with Mycoprotein Levels (per 100g)

Calorific Distribution

The calorific distribution diagram demonstrates a clear shift in macronutrient contributions to total calories as mycoprotein inclusion increases. Calories from protein rise progressively, reflecting mycoprotein’s substantial protein content, while calories from carbohydrates decrease, indicating a reduction in carbohydrate density as more mycoprotein replaces flour. Calories from fat remain relatively constant, suggesting that fat content is largely unaffected by mycoprotein levels. These results are in accordance with earlier mentioned observations.

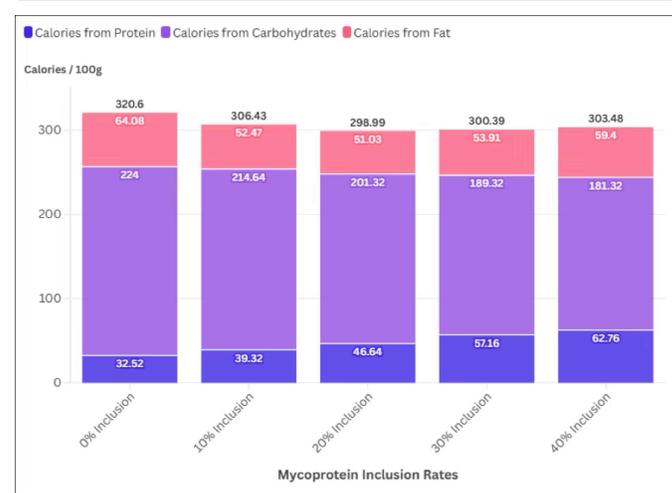


Figure 4: Caloric Distribution in Bread with Varying Mycoprotein Inclusion Levels (per 100g)

Representative Amino Acid Profile of Composite Bread Formulation

The composite amino acid profile of the mycoprotein-enriched bread formulation shows a total amino acid concentration of 10.65g per 100g, which represents a moderate level for a bread product. For comparison, conventional white bread contains approximately 7.61g of total amino acids per 100g [22], highlighting the enhanced amino acid content in the mycoprotein-enriched formulation.

Elevated levels of glutamic acid (2.526g/100g) and aspartic acid (0.636g/100g) are observed, both of which are commonly abundant in fungal-based proteins and these quantities align with the typical amino acid profile of fermented protein products [13]. Several other essential amino acids, including threonine (0.306g/100g), valine (0.428g/100g), isoleucine (0.382g/100g), and leucine (0.609g/100g), are also present in moderate amounts. These amino acids are essential for human health, and their presence in meaningful quantities suggests that the mycoprotein-enriched bread provides a promising essential amino acid profile.

The presence of these essential amino acids in significant quantities suggests that the mycoprotein-enriched bread could potentially contribute meaningfully to dietary protein quality by providing the building blocks necessary for various physiological functions.

However, methionine (0.140g/100g), tryptophan (0.076g/100g), and cysteine (0.290g/100g) are present at

relatively low levels. Their low concentrations suggest that additional protein sources might be required to achieve a fully balanced amino acid profile. The above composition thus indicates that while mycoprotein-enriched bread significantly contributes to protein and essential amino acids, supplementing it with complementary proteins would be required to further optimize the amino acid profile and better fulfil dietary requirements.

Test Parameters	Results	Unit
Aspartic Acid	0.636	g/100g
Glutamic Acid	2.526	g/100g
Serine	0.375	g/100g
Histidine	0.27	g/100g
Glycine	0.418	g/100g
Threonine	0.306	g/100g
Alanine	0.289	g/100g
Arginine	0.63	g/100g
Tyrosine	0.258	g/100g
Cysteine	0.29	g/100g
L-Valine	0.428	g/100g
Methionine	0.14	g/100g
Phenylalanine	0.47	g/100g
Isoleucine	0.382	g/100g
Leucine	0.609	g/100g
Lysine	0.466	g/100g
Proline	0.659	g/100g
Tryptophan	0.076	g/100g
Total Amino Acids	10.65	g/100g

Table 4: Amino Acid Profile of Mycoprotein- Enriched Bread Formulation (g/100g)

The moisture content of the bread formulations increased from 23.48% at 0% inclusion to approximately 28.5–28.9% once any level of mycoprotein was added, regardless of the specific inclusion rate. This suggests that even a small amount of mycoprotein contributes significantly to moisture retention, likely due to its water-binding properties.

The increase in moisture content with mycoprotein inclusion likely enhances crumb softness and extends initial freshness by reducing staling rates through improved water retention in the bread matrix. This elevated moisture, however, could also raise susceptibility to microbial growth, impacting shelf stability. Further studies are needed to assess these effects in detail and these studies fall beyond the scope of this current analysis.

Moisture Content

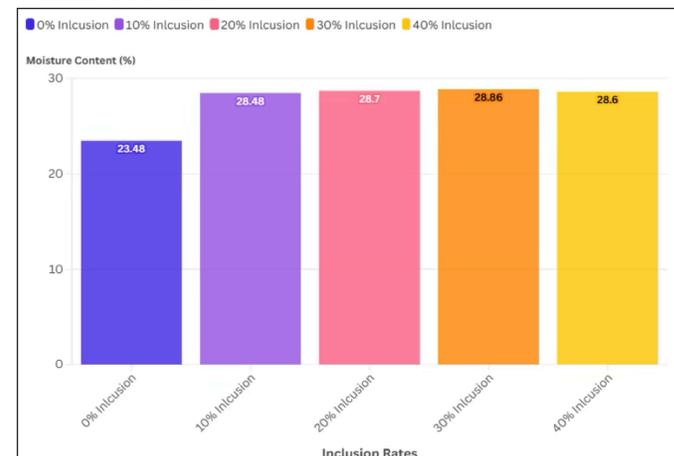


Figure 6: Moisture Content of Bread Formulations with Varying Mycoprotein Inclusion Levels

Discussion & Summary

The growing reliance on refined bread products across Asia, in conjunction with carbohydrate-dense staples such as rice, has exacerbated nutritional imbalances, particularly in protein intake, thereby contributing to the rise of diet-related metabolic disorders [1,2]. This research addresses these concerns by investigating the incorporation of *Neurospora crassa*-derived mycoprotein into bread formulations, with the aim of increasing protein and fiber content while reducing carbohydrate levels.

Mycoprotein, a sustainable protein source derived from filamentous fungi, offers a well-balanced protein composition with a moderate concentration of essential amino acids. Additionally, it is rich in dietary fiber, making it an excellent candidate for enhancing the nutritional profile of various food products. This positions it as an optimal solution for fortifying conventional bread, which is characteristically deficient in both protein quality and fiber [9].

Our experimental analysis demonstrated that the inclusion of mycoprotein in bread formulations led to a substantial increase in total protein content, rising from 8.13g/100g at 0% inclusion to 15.69g/100g at 40% inclusion. The composite amino acid profile revealed an improved essential amino acid composition, with notable contributions from key amino acids such as threonine, valine, isoleucine, and leucine. This enhancement in essential amino acids is especially valuable in bakery formulations, as it helps address common amino acid deficiencies in carbohydrate-heavy diets, where access to high-quality protein sources is often limited [10].

In addition to boosting protein content, mycoprotein inclusion increased dietary fiber levels, with fiber content rising from 1.7g/100g at 0% to 15.43g/100g at 40% inclusion. This increase compensates for the traditionally low fiber content in refined bread products, thus enhancing the overall nutritional value of the bread [12]. Increased dietary fiber in bread could promote gastrointestinal health, enhance satiety, and help regulate glycaemic response. While these health outcomes were not the primary focus of this study, it is hypothesized that

mycoprotein-enriched bread could offer long-term benefits beyond basic nutrition [14].

Moreover, mycoprotein inclusion was associated with a reduction in total carbohydrate content, decreasing from 56g/100g at 0% to 45.33g/100g at 40% inclusion, suggesting that mycoprotein could be a valuable ingredient for developing breads with a potentially lower glycaemic load [18]. Such formulations may be particularly advantageous for consumers managing metabolic health concerns, such as diabetes or insulin resistance, and are seeking lower-carbohydrate options within the bakery sector [19].

While a gradual increase in sugars was observed with higher mycoprotein levels, rising from 1.76g/100g at 0% to 6.48g/100g at 40% inclusion, this increase was modest relative to the overall reduction in total carbohydrates and this trend likely reflects the natural sugars present in mycoprotein.

Despite these promising nutritional improvements, the impact of mycoprotein on dough rheology remains insufficiently understood. Preliminary observations suggest that mycoprotein affects key dough properties—such as viscosity, elasticity, and water retention—but these insights are based on qualitative visual assessments and highlight the need for detailed quantitative analysis. Research on other alternative proteins in bread formulations has shown that both the type and concentration of protein can significantly alter dough behaviour, influencing crucial aspects like texture, structure, and the overall quality of the final bread product [9,10,11].

Optimizing these factors is essential to ensure that mycoprotein-enriched bread is not only nutritionally enhanced but also maintains the desirable sensory qualities that appeal to consumers [13]. Future studies should rigorously investigate these rheological properties, along with comprehensive sensory evaluations (taste, aroma, and mouthfeel), to maximize commercial viability and consumer acceptance [21]. The current research forms the foundation for developing formulations that effectively balance nutritional improvements with desirable sensory attributes [16].

In conclusion, mycoprotein derived from *N. crassa* has demonstrated considerable potential in enhancing the nutritional profile of bread by increasing both protein and fiber content, while concurrently reducing carbohydrate levels. This provides a viable solution to the nutritional challenges prevalent in carbohydrate-centric regions such as Asia [10]. Incorporating *Neurospora crassa*-derived mycoprotein into bread formulations offers a promising pathway to meet Asia's evolving dietary needs, where rising bread consumption reflects a shift towards more processed and convenience foods. This study demonstrates that mycoprotein can enhance the protein and fiber content of bread, addressing common nutritional gaps in carbohydrate-dominant diets such as those in Asia. Additionally, the sustainability of mycoprotein aligns with Asia's growing focus on reducing the environmental impact of food production [4]. By establishing foundational insights into both the nutritional benefits of mycoprotein-enriched bread, this research contributes to the development of innovative, sustainable food products that could cater to the region's health, dietary, and environmental priorities.

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