
Formulation and Quality Evaluation of Barley-Based Functional Instant Noodles

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Abstract

The current study was carried out to create functional instant noodles by combining barley and whole wheat composite flour to improve the nutritional and functional value of traditional instant noodles. Barley was chosen because of its abundance of β -glucan, dietary fiber, and bioactive substances with antioxidant potential. Using varying ratios of barley and whole wheat flour, composite flour formulations (N2, N3, and N4) were created and their proximate and physicochemical qualities assessed. Formulation N4 was chosen for noodle development because it had the best overall nutritional profile and functional qualities. Proximate composition, phytochemical screening, in vitro antioxidant activity, microbiological quality, cooking properties, texture profile, and sensory acceptance were all assessed for the produced noodles. When compared to traditional noodles, proximate analysis showed enhanced nutritional composition with moisture (57.18%), protein (14.54%), fat (13.62%), ash (1.6%), fiber (3.42%), and carbs (9.82%). Terpenoids were not present, however flavonoids, tannins, saponins, and phenolic substances were, according to qualitative phytochemical screening. With total phenolic content (7.42 mg GAE/g), total flavonoid content (6.82 mg QE/g), total tannin content (6.60 mg TAE/g), and excellent DPPH radical scavenging activity, in vitro antioxidant study demonstrated considerable bioactive potential. Rehydration rate (89%), water absorption capacity (89%), cooking loss (12%), and appropriate texture qualities were among the acceptable cooking and quality features shown by functional evaluation. The product stayed below acceptable safety levels, according to microbiological investigation, although a small amount of contamination emphasizes the significance of more hygienic processing. With an overall score of 7.05 on a nine-point hedonic scale, the sensory evaluation demonstrated strong consumer approval. The findings show that adding barley greatly improves antioxidant, functional, and nutritional qualities without impairing sensory quality. All things considered, instant noodles made from barley and whole wheat offer a more nutritious option than regular noodles and show great promise for growth as a useful convenience food for consumers who are health-conscious.

Keywords: Barley-based Instant Noodles, Instant Noodles, Barley, Functional Noodles, Antioxidants, Fiber.

Introduction

Instant noodles are a staple cuisine in many Asian nations. The global market for instant noodles is estimated to be worth \$55.5 billion in 2023 and is projected to reach \$93.77 billion by 2032, indicating their widespread popularity (SkyQuest, 2025). These numbers point to the fact that the market for instant noodles and its consumption across the world are increasing rapidly. In the last 10

years, the consumption of instant noodles in Pakistan has also grown particularly amongst young adults and children. However, mostly instant noodles available in local supermarkets are prepared from refined wheat flour, which typically contains refined carbohydrates, additives, and sodium, while being low in many essential nutrients, especially fiber, due to which health concerns are rising among consumers. Despite being popular, consumers are becoming more health-conscious and demand healthier alternatives that not just offer convenience, but also benefits to the health, or at the very least no negative impact on the health. To overcome this nutritional gap while maintaining ease of convenience, this study aims to develop functional instant noodles by using barley flour with the combination of whole wheat flour. Barley flour was favored on account of its rich composition of bioactive compounds such as phytosterols, lignans, dietary fiber, saponins and carotenoids that enhance the nutritional composition of the noodles (Idehen et al., 2017) whereas wheat flour was used to maintain the quality and desired texture (Chillo et al., 2008). This project study is aimed at exploring the formulation and processing along with the consumer acceptability of this product, aiding in the rise of convenience-based and nutritious food (Boukid 2022).

Instant noodles are made from water, salt, wheat, starch, and kansui. Kansui is an alkaline mineral water that occasionally contains phosphoric acid along with sodium and potassium carbonates (Sikander et al., 2027).

Majorly, instant noodles contain carbohydrate, which comes from the flour, but they do not contain a significant amount of protein, fiber, vitamins, and minerals (Olorunsogo et al., 2023). This poor nutritional profile of instant noodle is what is linked to malnutrition and metabolic disorders when consumed for the long term. Many studies have been conducted to improve this issue of poor nutritional content of noodles, with two main approaches being used: fortifying noodles with essential micronutrients to enhance the overall nutritional profile, or by substituting the primary ingredient of instant noodles with a healthier, nutritious alternative (Ayustaningwarno et al., 2025). The latter is a more promising solution for increasing the nutritional value of noodles.

One grain, which is highly underutilized but very well known for its health benefits beyond basic nutrition, is barley. Barley offers a great option for improving the instant noodles nutritional profile. It is also very easily available in the local market, with Pakistan producing 40,000 tonnes of barley annually according to the DAWN newspaper (2023). When talking about the nutritional profile of barley, it contains 10 to 20% protein, 65 to 68% starch, and 11 to 34% fiber, with other smaller amounts of fat, minerals, and also antioxidants.

Barley is widely grown and recognized as one of the most significant cereal crops, after wheat, corn, and rice (Chen et al., 2020). Most probably location of barley cultivation retrieved back to 10000 BC is a fertile crescent region. (eastern Iraq, southern Turkey & western Iran) (Hamaker, 2008). In Ethiopia, China and Korea, Barley growth was also crop up (Hamaker, 2008). Barley contains nutraceutical compounds these are β -glucan, polyphenol, tocopherols and phytosterols. Additionally, barley's β -glucan is crucial for improving glycemic management and lowering cholesterol. Furthermore, by dealing type 2 diabetes & cardiovascular diseases β -glucan made Barley a potent digestive component (Cavallero et al., 2002). Furthermore, phenolic chemicals and flavonoids found in barley function as antioxidants, aid in the battle against oxidative stress, and may reduce the risk of diseases like cancer (Idehen et al., 2017). The reduction of visceral fat pile and body weight has been associated with Barley in diet (Aoe et al., 2017). Barley flour is obtained by roller milling of blocked or pearled barley. In addition,

The global demand for the instant noodle market shows the potential of functional instant noodles formulation which are not only "empty calories", but also allows the addition of health benefits and cause no long-term harm to human health. Supermarkets are carrying an increasing range of barley food products. Barley food products are more prominent in supermarkets, online and health food stores. This is in conjunction with the worldwide "whole grain trend" and concerns with sustainable

agriculture.

Methods and Materials

This chapter discusses the methods and the materials used for various testing and analysis done on the formulated noodles. It also discusses the process of formulation of the barley and whole wheat noodles. The formulated noodles made from barley and whole wheat flour were subjected to proximate analysis, phytochemical analysis, invitro analysis, microbial analysis, and various physiochemical analysis. The composite flour of barley and whole wheat used for product formulation was also subjected to proximate and physiochemical analysis.

Materials

The instant noodles were prepared with ingredients that were procured from the local market. The flours were purchased from the local flour shops in North Karachi. All chemical reagents and equipment used for testing and analysis were of analytical grade provided by the premises of JUW, Nazimabad, Karachi, Pakistan.

Proximate Analysis of Flour

In order to assess the moisture content, crude protein, ash content, carbohydrate content, and crude fat content using standardized analytical techniques, the flour samples were made in three different ratios (50:50, 60:40, and 70:30). Before a product was developed, the basic nutritional makeup of these various wheat mixes was ascertained.

Total Moisture Content

The oven drying method, as described in AACC Method 44-15A, was used to assess the moisture content of the composite flour (barley and whole wheat flour). A petri dish containing approximately 5 gm of the flour sample was put in the dry oven (Electrothermal Constant-temperature Dry Box REX-C700) at 105 °C for three hours in order to dry it. The dry sample was reweighed after chilling in a desiccator, and the formula was used to calculate the moisture content based on the weight difference (Czaja et al., 2020).

$$\% \text{ Moisture} = \frac{W_2 - W_1}{W_1} \times 100$$

Where,

W1 = Initial wt. of sample before drying.

W2 = Final wt. of sample after drying

Crude Protein Content

The crude protein in the composite flour was estimated using the Kjeldahl method (AOAC Method 981.10). 5 gm of the flour sample were digested with concentrated sulfuric acid at 420°C using a catalyst (potassium sulfate, K₂SO₄) for about two hours. The digested sample was then neutralised and ammonia released was titrated with 0.1 N hydrochloric acid (HCl). The conversion factor of 6.25 was adopted and total nitrogen content converted into crude protein content (Mæhre et al., 2018).

$$\% \text{ Nitrogen} = (V - B) \times N \times 1.4007 / \text{Wt. Of sample}$$

Where,

V = Volume of acid used for the sample (mL)

B = Volume of acid used for the blank (mL)

N = Normality of the acid

1.4007 = Factor based on the atomic weight of nitrogen

W = Weight of the sample (g)

$$\% \text{ Protein} = \text{Nitrogen \%} \times (6.25)$$

Where,

6.25 = Conversion factor

Crude Fat Content

The composite flour's crude fat content was determined using the solvent extraction method. The sample of flour (10 gm), 60 mL petroleum ether, and 40 mL distilled water were mixed in a separating funnel. The fat was extracted by refluxing the sample for four hours. The fat was dried in an oven at 105°C to evaporate the solvent and then weighed before it was dried. It was then weighed again after being cooled (Ansari, 2022).

$$\% \text{ Fat} = \frac{\text{weight of fat obtained}}{\text{initial weight of sample}} \times 100$$

Total Ash Content

AACC Method 08-0 was used to find the composite flour's total ash content via a muffle furnace (Type SX). In order to eliminate all biological stuff and turn the residue into ash, about 5 gm of the sample were thermally treated in the pre-weighed crucible and heated to 550 °C for four hours. The crucible was reweighed after cooling it in the desiccator (Czaja et al., 2020).

$$\% \text{ Ash} = \frac{W_2 - W_1}{\text{weight of sample (g)}} \times 100$$

Where,

W1 = Empty crucible weight

W2 = Crucible with ash

Crude Fiber Content

The acid and alkali digestion method was used to calculate the composite flour's crude fibre content. For thirty minutes, 200 mL of 0.255 N sulfuric acid was heated with about 5 gm of the flour sample. The residue was filtered, cleaned, and then heated for an additional half hour in 200 mL of 0.313 N sodium hydroxide (Ansari, 2022). After the residue was dried, weighed, and burned, the amount of ash was deducted.

$$\% \text{ Fiber} = \frac{\text{weight of residue after digestion and ashing(g)} - \text{Ash weight(g)}}{\text{weight of sample (g)}} \times 100$$

Carbohydrate Content

The carbohydrate content of composite flour was determined by using differential method with the following formula:

$$\% \text{ Carb} = 100 - (\% \text{ protein} + \% \text{ moisture} + \% \text{ fat} + \% \text{ ash} + \% \text{ fiber})$$

Physiochemical Analysis of Flour

To ascertain the degree of bioactive phytochemical compound distribution, which is pertinent to the characteristics of the raw materials used to make the flours used for the study, phytochemical analysis was performed on the flour samples. Similar to how antioxidants exhibit advantageous qualities for the development of health, the phytochemical compound constitutions examined comprised phytochemical compound groups with proven health benefits. To determine the composition of phytochemical compounds of the appropriate raw materials used in the study, different phytochemical extraction methods could be adopted.

SDS (Sodium Dodecyl Sulfate) Sedimentation

The SDS sedimentation test was carried out using the standard procedure. 50 mL of the measured barley and whole wheat flour was taken in 100 mL graduated cylinders. Distilled water (50 mL) was added to the content and shaken rigorously for 15 seconds for 2, 4 and 6 minutes. The graduated cylinder was then topped up with 50 mL of the sodium dodecyl sulphate-lactic acid solution prepared by mixing 1.2 N lactic acid (LA) and 3% sodium dodecyl sulphate (SDS). After slowly inverting the sample ten times for two minutes at a time, it was given forty minutes to settle peacefully. During the quiescent period, the sediment formed was measured which is settle in the bottom of the graduated cylinder to evaluate the gluten strength (Xia et al., 2022).

AWRC (Apparent Water Retention)

A redesigned conceptual framework served as the foundation for the AWRC assay. First, a piece of whole wheat composite flour and 1 gm of barley flour were placed in a 15 mL centrifuge tube. In this step, a thorough mixing process was used. Ten mL of distilled water were put to a centrifuge tube, and it was vortexed for one minute. The sample was centrifuged (LABCEN 60 table top low speed centrifuge) at 3000 rpm for 15 minutes after being allowed to settle for 30 minutes at room temperature. After the sediments were separated, the residue's weight was recorded to evaluate the AWRC computation (Methe et al., 2024).

$$\text{AWRC} = \frac{\text{Wet weight of hydrated flour} - \text{Dry weight of flour}}{\text{Dry weight of flour}} \times 100$$

Solvent Retention Capacity (SRC)

- **Sodium Carbonate (Starch Damage)**

The SRC test was being carried out using the American Association of Cereal Chemists (AACC) method. The amount of 2.5 gm barley and whole wheat composite flour was transferred to a 15 mL centrifuge tube. Then 15 mL of sodium carbonate was added to the centrifuge tube. The samples were mixed for 5 seconds in the vortex and left for 20 minutes; and then again mixed for 5 seconds every 5 seconds after 5 minutes. It was then centrifuged for 15 minutes at 1000 rpm. The pellet was not disturbed and the supernatant was removed gently after resting the tube. The test tube was left on absorbent paper for 10 minutes. After that, the weight of the sample was recorded to determine the SRC value. The Sodium carbonate SRC value reflected the starch damage of flour. Thus bigger values will indicate greater damage (Labuschagne et al., 2021).

$$\text{Damage Starch} = \frac{\text{Wet weight of hydrated flour} - \text{Dry weight of flour}}{\text{Dry weight of flour}} \times 100$$

- **Sucrose (Fiber/Pentosan Content)**

The water holding capacity of sucrose-soluble components of barley-wheat composite flour known as arabinoxylans (pentosans) and soluble fiber was determined by using the sucrose solvent retention capacity (SRC) test. During this test, 13 mL 8% (w/v) sucrose solution was mixed with 2.5 gm of composite flour in a 15 mL centrifuge tube, vortexed for a few seconds, and left to stand at room temperature for 20 minutes with occasional stirring. After centrifuging for 15 minutes at 1000 rpm, the supernatant was removed. To eliminate excess water, the hydrated pellet was transferred to absorbent paper. Using the weight of the hydrated pellet, the sucrose SRC value, which displays the pentosan and soluble fiber fraction's water absorption capacity, was calculated (Labuschagne et al., 2021).

$$\text{Fiber/Pentosan Content} = \frac{\text{Wet weight of hydrated flour} - \text{Dry weight of flour}}{\text{Dry weight of flour}} \times 100$$

- **Lactic acid**

To a 15 mL centrifuge tube was added 1.5 g sample and 7.5 gm of 5% lactic acid solution. The centrifuge tube was shaken for 5 seconds. Then the centrifuge tube was shaken every five minutes for 20 minutes. Then the centrifuge tube was spun for 15 minutes at 1000 rpm. After the liquid had drained from the centrifuge tube, it was then inverted onto a filter paper for ten minutes. After that, the centrifuge tube was weighed using a gel (Labuschagne et al., 2021).

$$\text{SRC} = \frac{\text{Wet weight of hydrated flour} - \text{Dry weight of flour}}{\text{Dry weight of flour}} \times 100$$

- **Water**

A sample of 1.5 gm was weighed into a 15 mL centrifuge tube with 7.5 mL distilled water. The tube was shaken for 5 seconds to mix. The centrifuge tube was then shaken every five minutes for 20 minutes. The centrifuge tube was then spun in a centrifuge (1000 rpm) for 15 minutes. The centrifuge tube was inverted on the filter paper for 10 minutes once the liquid had drained from the centrifuge tube. Then the centrifuge tube was weighed in the gel (Labuschagne et al., 2021).

$$\text{SRC} = \frac{\text{Wet weight of hydrated flour} - \text{Dry weight of flour}}{\text{Dry weight of flour}} \times 100$$

Gelatinization Temperature

The temperature at which starch granules irreducibly absorb water to create a gel is called the gelatinization temperature (GT), which is important in the cooking and textural properties of noodles. In this experiment, 2 g of composite flour and 15 mL of distilled water was mixed in a 50 mL graduated cylinder. The suspension was gradually heated (about 1°C per minute) while being stirred. GT was considered to be the temperature at which the mixture started to become sticky and gel-like (Wang et al., 2018). This is because, according to the reports on starch gelatinization of cereal starches, the method accurately defines the thermal functional properties of starch granules.

Bulk Density

For bulk density, the 50 gm noodle flour were poured into a 100 mL measuring cylinder, and the cylinder tapped until constant volume. Bulk density was calculated using the following formula as suggested by Morantes et al. (2020).

$$\text{Bulk Density (g/cm}^3\text{)} = \frac{\text{Weight of sample}}{\text{Volume occupied}}$$

Noodle Preparation

Arora et al. (2017) 's Method of instant noodle preparation was used with slight variation. The dough was made using a blend of barley and whole wheat flour, with barley being 70% of the flour content and whole wheat being 30%. Other minor ingredients were added to the dough along with water for hydration and were uniformly mixed. After kneading, the dough was left to rest to promote further hydration. The dough was crumbly and was compressed into continuous sheets. The sheeter was used for sheeting and cutting of noodles. Following sheeting and cutting, steaming was done to partially cook the noodles at 100 °C for 3-5 minutes, crucial for rehydration and texture. The steamed noodles were cooled, proportioned, and then partially dehydrated in the dry oven, followed by deep frying which was done in canola oil at 140 °C for 60 seconds to remove moisture and preserve the texture and quality of noodles. After being allowed to cool at room temperature, the fried noodles were sealed in airtight plastic containers for later analysis.

Table 1: Ratios in composite flour of barley and whole wheat

Sample Name	N1	N2	N3	N4
Flour Concentration	Control Sample	50% barley and 50% whole wheat	60% barley and 40% whole wheat	70% barley and 30% whole wheat

Proximate Analysis of Product

The prepared instant noodles were assessed to proximate analysis to evaluate their nutritional quality after processing by using similar methods which used for the raw materials. Proximate analysis of product included total moisture, crude fat, crude protein, total ash, crude fiber content, and carbohydrate content. For all these tests, the same methods as described in section 2.2 were followed.

Qualitative Phytochemical Screening of Prepared Noodles

Flavonoid

- **Extract Preparation of Sample**

The noodles were crushed into a fine powder using a mortar and pestle. This step aids further extraction. A conical flask with 50 mL of 80% methanol was filled with around 2.5 g of the powdered material. To maximize the solubility of flavonoid compounds, the conical flask was submerged in a

shaking water bath at 25 °C for 60 minutes. Filter paper was used to separate the solid materials from the mixture after the extraction procedure. For additional testing, the clear filtrate was gathered in amber vials and called the qualitative flavonoid extract (Li, 2024).

- **Lead Acetate Test**

2 mL of the prepared noodles extract was taken and mixed with 1 mL of lead acetate 10% solution. The formation of a yellow precipitate showcases the presence of flavonoids (Maheshwaran et al., 2024).

- **Ferric Chloride Test**

2 mL of methanolic noodle extract were mixed with three drops of a 5% ferric chloride solution. The green color was observed which indicate presence of flavonoid compound (Maheshwaran et al., 2024)

- **Alkaline Reagent Test**

2 mL of methanolic noodle extract were mixed with five drops of 10% NaOH. Yellow intense color was observed. Afterwards, in the sample mixture 5 drops of 10% HCL were added. The color disappearance was observed which indicated flavonoid presence (Maheshwaran et al., 2024)

2.6.2 Terpenoid

- **Salkowski's Test**

In this test, 5 gm of extracted flour were combined with chloroform, followed by a few drops of strong sulfuric acid. The appearance of a reddish-brown color indicates presence of terpenoids (Kaviya et al., 2023).

- **Liebermann-Burchard Test**

After dissolving around 5% of the flour extract in the chloroform, a few drops of strong sulfuric acid and acetic anhydride were added to the test tube. A greenish or violet color was observed, which indicates the presence of terpenoids (Theeba et al., 2015).

- **Vanillin Sulfuric Acid Test**

5 gm of noodle extract was mixed with some drops of vanillin reagent (vanillin in acetic acid) in test tube. A few drops of concentrated sulfuric acid were added. The presence of terpenoids in the flour sample was verified by a purple or reddish hue (Wutsqa et al., 2021).

Tannin

- **Ferric Chloride Test**

A few drops of 5% ferric chloride solution were added to the noodles extract. The appearance of a greenish-black or dark blue-black color indicates the presence of tannins due to the formation of complex between iron ions and phenolic hydroxyl groups.

- **Gelatin Test**

Another method in which tannins were tested was with the Gelatin Test. This test is induced by the protein precipitating action of tannins. The extract was mixed with a 1% gelatin solution that included 10% sodium chloride for this test. Since the development of insoluble complexes with proteins is a well-known technique for the qualitative screening of phenolic compounds, the presence of tannins in the sample can be verified by the formation of a white precipitate. (Maheshwaran et al., 2024).

- **Lead Acetate Test**

The lead acetate test was also used to further establish the presence of tannins. In this test, when 10% solution of lead acetate is added to this mixture, a white or yellowish precipitate is formed if tannins have been added to the mixture. This is due to tannins (chelate ions forming insoluble complexes) from the plant, widely cited in the phytochemical screening literature.

Saponin

- **Standard Foam Test**

In this test, 3 gm of dry crushed noodles were added to 300 mL of hot distilled water in accordance with the method described by El Aziz et al. (2019) to detect the presence of saponin. After filtering and cooling, this combination was placed in the refrigerator for a full day. Following a 24-hour period, 5 mL of the extract were extracted from the prior filtrate and agitated for two minutes with an equivalent volume of water.

- **Wet Foam Test**

In this method the extract prepared in the previous test was taken into small amount and diluted by distilled water then shaken vigorously for 2 minutes to check about formation of stable solution. Foams at the top.

- **Dry Foam Test**

For this test, 0.5 gm of powdered dry smashed noodles and 5 mL of distilled water were combined and boiled in a hot water bath. 3 drops of olive oil were then added, and kept by shaking the mixture in a vigorous manner, to check for emulsification to occur.

Phenolic Compounds

- **Ferric Chloride Test**

Three drops of ferric chloride (5%) and 2 mL of methanol extract were added to the test tube. For one to two minutes, the mixture was left to stand at room temperature. A color change from bluish green to dark black is a sign of phenolic compounds from the interaction of ferric ions and the hydroxyl groups. (Muhamad et al., 2022; Maheshwaran et al., 2024;)

- **Lead Acetate Test**

The prepared instant noodles were tested for phenolic chemicals using the Lead Acetate Precipitation technique. Three to five drops of 10% lead acetate (reagent) were added to a 5 mL sample of methanolic extract. Because the lead ion reacted with the hydroxyl group of the phenolics, the white precipitate formed, indicating that the test was positive for the presence of phenolics. (Kalauni et al., 2024).

- **Gelatin Test**

The precipitation test using gelatin was used to detect tannin-type phenolics of the formulated instant noodles. The test was done using a methanolic extract of noodles, 1% gelatin, 10% sodium chloride solution and standard lab glassware After adding 5 mL of methanol extract to 5 mL of sodium chloride-containing 1% gelatin solution, the mixture was allowed to come to room temperature. The existence of tannin-type phenolic compounds was demonstrated by the production of a white precipitate. The formation of insoluble complexes has taken place because of the reaction between proteins and tannins. This approach is commonly applied for phytochemicals to measure plants and food extracts. (Kamarudin et al., 2021).

In Vitro Quantitative Analysis of Product

Extract Preparation of Sample

The initial step was the preparation of extract for the quantification of the product (TPC, TFC, TAC, TTC and DPPH radical scavenging activity) in vitro. Methanol (16 mL) was mixed with 2 gm of the ground noodles. The methanol used contained 1% hydrochloric acid (HCL). This was allowed to stand at room temperature for a day to help extract phytochemicals and antioxidants. Then, it was centrifuged at 4000 rpm for 15 minutes. The supernatant was then collected and kept at 4°C for further analysis.

Total Phenolic Content (TPC)

TPC was determined by Folin-Coicaltu method. The method described by Li et al. (2015) was used to mix 0.5 mL of the extracted sample to 5 mL of Folin-Coicaltu reagent. Following the addition and mixing of 4 mL of saturated sodium carbonate, it was left at room temperature for two hours. After

two hours, the absorbance of the mixture at 765nm was measured using a spectrophotometer (JENWAY 6305 UV/Vis Spectrophotometer). The absorbance value was then compared with the standard curve prepared with Gallic acid.

$$C = \frac{c \times V}{m}$$

Where,

C = Total phenolic contents (mg/gm plant extract, in GAE)

c = Concentration of gallic acid (mg/mL)

V = Volume of extract (mL)

m = Mass of sample (gm)

Total Flavonoid Content (TFC)

Li et al.'s (2015) method was adopted for the determination of the total flavonoid content. A mixture of 0.5 mL of the extract produced, 2 mL distilled water, and 0.15 mL 5% sodium nitrite (NaNO₂) was mixed and left at room temperature for five minutes. Then 0.15 mL of 10% aluminum chloride was added, and the reaction mixture was allowed to stand for another five minutes at room temperature. Finally, 1 mL of the 1 M solution of sodium hydroxide (NaOH) was added, and the mixture was allowed to stand for another 15 minutes. The total flavonoids content of the extract was calculated and expressed as mg quercetin equivalent per gm of dry extract (mg QE/gm DE) using the standard curve and the absorbance of this solution was read with the aid of the spectrophotometer at 415 nm.

Total Tannin Content (TTC)

The method proposed by Negi et al. 2018 was used for the total tannin content (TTC). This involved the Folin-Denis method of adding 50 µL of sample extract to a solution of distilled water and making it up to 7.5 mL. Then, 0.5 mL of Folin-Denis's reagent and 1 mL of sodium carbonate were added to it. The sample's absorbance was obtained at 700 nm after its volume was adjusted to 10 mL with distilled water. The results were expressed as µg of tannic acid equivalent (TAE) per mg of extract, which was based on tannin standard curve using tannic acid.

Total Anthocyanin Content (TAC)

The anthocyanin content was determined with the pH differential method and also the method developed by Shiau et al. (2023). For this, 1 mL of extract was mixed with 4 mL of acidic pH 1.0 buffer and another 1 mL of extract was mixed with a less acidic buffer, pH 4.5. Both of these solutions were read with a spectrophotometer at 520 and 700 nm. The noodles' TAC was calculated according to the formula below:

$$\text{TAC } (\mu\text{g/g, DB}) = \frac{A \times F \times \text{MW} \times 1000 \times V}{\epsilon \times x \times W}$$

Where,

$$A = [(A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 1.0} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 4.5}];$$

F = Dilution factor

MW = 449.2 g/mol of cyanidin-3-glucoside (standard anthocyanin used as a reference)

V = Volume of extract (mL)

ε = 26,900 L/mol.cm, molar extinction coefficient of cyanidin-3-glucoside

x = Path length of cuvette (cm)

W = Sample weight (g)

DB = Dry weight basis

DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The DPPH radical scavenging activity was determined by the technique outlined by Arora et al. (2017) to measure antioxidant activity. Using this approach, 1 mL of DPPH solution (80 µg/mL in

ethanol) was mixed with 0.2 mL of noodles extract. The solution was let to remain for a while in order for the antioxidants, if they were present, to react with the DPPH radicals and begin neutralizing them. The resultant solution was then centrifuged at 3000 rpm for 15 minutes. Following centrifugation, 0.5 mL of the supernatant is collected and combined with 1 mL of ethanol in a cuvette. The absorbance of this solution is measured at a wavelength of 517 nm using a UV-Visible spectrophotometer. Additionally, a blank sample was made with just ethanol and DPPH and no antioxidant extract. The spectrophotometer was used to measure this blank sample in the same manner. The formula written below was used to estimate the radical scavenging activity as a percentage of DPPH:

$$\% \text{ DPPH inhibition} = \frac{AB-AS}{AB \times 100}$$

Where,

AB = absorbance of the blank (no antioxidants)

AS = absorbance of the sample (with antioxidants)

Microbial Testing of Prepared Noodles

The end product's microbiological quality, safety for human eating, and sanitary ranking from the standpoint of processing and storage of microorganism indicators were all assessed using microbial analysis of the noodles. The purpose of this investigation was to identify any pathogenic or spoiling microorganisms that might be present in the prepared noodles as well as to calculate the load of pathogenic microbes. All microbiological examination were performed under aseptic condition of laboratory using appropriate culture media and incubation.

Total Plate Count (TPC)

According to Alshubaith et al. (2025), the pour plate method was used as the standard procedure to determine the TPC of noodles. A sterile stomacher bag holding 225 mL of 0.1% buffered peptone water was aseptically filled with a 25 g sample of noodles. Then the mixture was homogenized for 2 min to create a mixture microbial suspension. This homogenized mixture was then used to create a series of ten-fold dilutions using the same diluent.

After the samples were diluted, they were plated in duplicate using Plate Count Agar (PCA), using the pour plate method. For 48 hours, the plates were kept between 30 and 35°C. After that, plates with between 30 and 300 colonies were chosen and tallied. The results were expressed in colony-forming units per gm (CFU/g) of the sample, as describes by Alshubaith et al. (2025).

$$\text{Number of Colonies} = \frac{\text{Dilution Factor}}{\text{Volume Plates(mL)} \times \text{Dilution factor}}$$

Coliform Count

The Most Probable Number (MPN) as described by Akhigbemidu et al (2015), was used to assess the coliform count. 5 gm of the noodle sample was aseptically blended in 45 mL of sterile buffered peptone water resulting in a 1:10 dilution. Aliquots from the series of ten-fold dilutions were inoculated in tubes of MacConkey broth. The inoculated tubes were incubated at 37°C for 24-48 hours. Coliform-positive tubes were characterised by a colour change and gas production. The numbers of positive tubes from each dilution were used to estimate the number of coliforms per gm of sample from the standard MPN-table.

Staphylococcus Aureus

Isolation and enumeration of the *Staphylococcus aureus* in the noodle samples was done using the selective culture media and biochemical confirmation tests as described in the article of Mairi et. al. (2025). In order to enhance micro-organism growth, 25 g of noodles was mixed in 225 mL of sterile buffered peptone water and incubated at 37°C for 24 hours.

After incubation, a loopful of the enhanced culture was redressed on the Baird-Parker Agar (BPA)

and incubated for 24-48 hours at 37°C. The black, shiny colonies with clear halos were presumed as *Staphylococcus aureus* colonies as a result of tellurite reduction and lecithinase production. These colonies were further identified using the Gram's staining (gm), Coagulase (an enzyme that converts fibrinogen to fibrin) and a Coagulase test (Coagulase test is a major characteristic of *Staphylococcus aureus*) (Mairi et al. 2025).

$$S. aureus (CFU/g) = \frac{\text{Number of confirmed colonies} \times \text{Dilution factor}}{\text{Volume plated (mL)}}$$

Yeast and Mold Count

For yeast and mold count, sterile saline 0.85% was used to homogenize 25 gm samples for 5 minutes before sampling. 225 mL was used. Following serial dilutions of 10-fold dilutions, the respective dilutions were plated in duplicate. Yeast and mold were grown on selective agar at 28°C for 5 days. The range of 15 to 150 CFU/plate was used to count the colonies and the results were mentioned in CFU/gm (Yang et al., 2022).

$$\text{Number of Colonies} = \frac{\text{Dilution Factor}}{\text{Volume Plates}}$$

Final Noodle Quality Analysis

This section presents the methodological framework for evaluation of the final quality of the instant noodles made from N4 composition of flour. The noodles' texture, structural features, and cooking performance were all examined in the lab. All tests were done in controlled conditions for consistency and reliability. The experimental procedure described in this section is a structural approach to evaluate the quality of noodles and the results obtained are discussed in the next chapter.

Rehydration Rate

Five gm of dried sample were soaked in one hundred mL of hot water for ten minutes in order to measure the water absorption of the noodles. Following the procedure described by Singh et al. (2017), the noodles sample was strained to remove any remaining water after soaking, and it was then weighed once more to determine whether the increase in weight was caused by hydration. The percentage of rehydration was determined using the weight difference.

$$\% \text{ Rehydration Rate} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Cooking Loss

A 25 gm samples of dried noodles was cooked in 300 mL distilled water until the core turned transparent. According to the protocol determined by Tuta et al. (2017), the cooking water was filtered, remaining solids dried till constant weight values and the weight value was calculated to determine the loss of solids. Cooking loss was estimated by using the formula:

$$\% \text{ Cooking Loss} = \frac{\text{Weight of solids in cooking water}}{\text{Initial weight of noodles}} \times 100$$

Texture Profile Analysis (TPA)

Using a texture analyser (AMETEK CT3 1 000) fitted with a 36mm round aluminium probe, the noodle underwent 70% compression with approach and return speed defined by standardized methods. Cooked noodles were analysed for textural properties, including firmness, elasticity, cohesiveness, and stickiness were studied. The testing was carried out according to established standardised protocols for noodle texture (Sun et al., 2019; Wang et al., 2022).

Water Absorption Capacity (WAC)

Fresh noodles 2 gm were boiled in 30 mL hot water for 3-5 minutes, using the method described by Morantes et al. (2020). After the excess water was removed, the noodle was weighed. Level of hydration was calculated using following formula.

$$\% \text{ Water Absorption} = \frac{\text{Weight after cooking} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Swelling Index

The swelling capacity was calculated by comparing original volume of 1 gm dried noodle before and after boiling, as described by Singh et al. (2017). The formula below was used to calculate the swelling capacity:

$$\text{Swelling Index (mL/g)} = \frac{\text{volume after swelling} - \text{Original volume}}{\text{Weight of dry sample}}$$

Color (L*, a*, b*)

The CR-20 device (Konica Minolta, Japan) was used to measure the color attribute of the cooked noodle samples using the CIE Lab* color system. For each sample, lightness (L*), redness/greenness (a*), and yellowness/blueness (b*) values were measured five times. Whiteness index was also calculated to help in visual analysis, consistent with other reported instrumental color analysis methods (Wang et al., 2022).

Cooking Time

To determine the cooking time, 25 gm of noodles in 300 mL of distilled water. After 30 seconds intervals, noodle samples were compressed between glass slides to observed internal consistency until the opaque center disappeared as described by Tuta et al. (2017).

PH Measurements

After blending 10 gm of cooked noodles in 100 mL of deionized water for five minutes, the pH value of the cooked noodles was determined. The produced mixture was allowed to come to room temperature for half an hour. The pH was measured using a digital pH meter that has been calibrated using the technique suggested by Morantes et al. (2020).

Breaking Strength

Breaking strength was assessed using a Texture analyzer. A cooked noodle sample was compressed until the breaking point was reached. The peaked force before ruptured was recorded. As per Singh et al. (2017).

Stickiness

The surface stickiness of boiled noodles was evaluated through a TA-XT2i Texture Analyzer fitted with a flat aluminum probe and a stickiness fixture. Stickiness of boiled noodles was analysed using a texture analyser with a flat probe and stickiness fixture. The force required to separate the noodle from the probe after compressing to 70% of the original height was measured. This method is in parallel with other validated methods for noodle stickiness and adhesion analysis (Cai et al., 2024).

Noodle Strand Uniformity

Measurement of noodle strand width and length were taken to assess uniformity using a digital micrometer for accurate measurement. The degree of uniformity was calculated through standard deviation analysis, as described in the method by Singh et al. (2017).

Sensory Evaluation of Prepared Noodles

Twenty panelists, comprising teachers and students from the university, were recruited for the sensory evaluation of cooked instant noodles with barley integration. The sensory panelists were randomly given a sample of noodles (approximately 60 gm) in a clear glass bowl with a three-digit number on it. A 9-point hedonic sensory evaluation scale was used to assess the noodle sample's texture, color, flavor, scent, and general attractiveness. The scale had a range of 1 to 9, where:

9 = Like extremely

8 = Like very much

7 = Like moderately

6 = Like slightly

5 = Neither like nor dislike

4 = Dislike slightly

3 = Dislike moderately

2 = Dislike very much

1 = Dislike extremely

Statistical Analysis of Prepared Noodles

The data of 9 point hedonic sensory scale for the prepared noodles were statistically analysed. Inferential and descriptive statistics included the mean, standard deviation, one-sample t-test and Pearson's correlation analysis. These were used to determine the association between different sensory attributes and the comparison of mean score of each attribute with the neutral score and among themselves. P-value less than 0.05 was used for all statistical tests.

Results and Discussion

In addition to statistical analysis, proximate analysis, phytochemical analysis, microbiological analysis, in vitro analysis, and sensory assessment were performed on the prepared noodles manufactured from barley and whole wheat flour. The goal of this study was to create a nutraceutical instant noodle with better quality and functional qualities by using barley flour, which has a high β -glucan and dietary fiber content.

Proximate Composition of Composite Flour and Developed Noodles

Proximate analysis of the composite flour (barley and wheat) using three different formulations (N2, N3 and N4) showed that nutritional quality of composite flour has significantly improved by the inclusion of barley. Moisture content varied between 10.2% and 11.6% where N4 had lowest moisture content, implying higher stability and shelf life during storage (Meherunnahar et al., 2023). Crude fiber content ranged from 2.70% to 3.41% and the addition of barley flour as a source of fiber is a noteworthy addition (Bhatt et al 2023). Fat content also increased (1.8-2.6%) and N4 contained an acceptable level of fat for instant noodles with enhanced texture and taste. Similarly, ash content also increased (2.2-3.6%) suggesting more minerals, and carbohydrates decreased (68.7% to 66.19%) due to high protein and fiber content of the flour mixtures (Pavana et al., 2019). Similarly, protein content also increased from 12.92% (N2) to 14.0% (N4), demonstrating that incorporation of barley flour increased the protein content due to β -glucan and amino acid profile (Bayomy et al., 2022). But carbohydrate content was reduced (68.7% to 66.19%) because of high protein and fiber content in the blends. Regarding the finished product (N4 noodles), cooked moisture content (57.18%) is typical for cooked noodles (Marciniak-Lukasiak et al., 2024). The protein content (14.56%) of the final product validates the development of fortified noodles Arora et al., 2017). Ash (1.6%) and crude fiber (3.42%) content were within normal limits while fat content (13.62%) was comparatively higher due to the frying process, plays a major role in improving palatability but needs to be tackled for stability against rancidity (Wang et al., 2022).

Table 2: Proximate Analysis of Flour

Formulation	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Fiber (%)	Carbohydrates (%)
Control (100%)	12.0	1.5	13	1.0	2.50	72.0
N2	11.6	1.8	12.92	2.2	2.70	68.7
N3	10.6	2.0	13.74	3.4	3.28	67.1
N4	10.2	2.6	14.00	3.6	3.41	66.19

Table 3: Proximate Analysis of Final Noodles

Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Fiber (%)	Carbohydrates (%)
57.18	13.62	14.54	1.6	3.42	9.82

Physicochemical Analysis of Composite Flour

Physicochemical Analysis results showed that as barley proportions were increased, functional properties were affected. SDS sedimentation values dropped from 24 mL (N2) to 18.5 mL (N4) due to weaker gluten quality from dilution of wheat gluten proteins (Rani et al., 2021). Absorbed water retention capacity (AWRC) from 60% to 65%, suggested improved water-holding capacity due to arabinoxylans properties and β -glucan content in barley (Rani et al., 2021). Solvent retention capacity (SRC) measures showed increased pentosans and water absorption and lactic acid SRC indicated good gluten function in N4 (Kweon et al., 2011). This showed the starch gelatinisation temperature between 53°C and 56°C, suggesting balanced starch properties during cooking (Kweon et al., 2011). Bulk density (0.64 g/cm³) corresponded to the porous nature of the whole grain flours, providing better water absorption and processing.

Table 4: Physicochemical Analysis of Flour

Formulation	Water (CSRC) (%)	Gelatinization Temperature (°C)	SDS (mL)	AWRC (%)	SRC Sodium Bicarbonate (%)	SRC Sucrose (%)	SRC Lactic Acid (%)	Bulk Density (g/cm ³)
N2	96	53	24	60	69.20	113.60	95	0.71
N3	90	56	22	62	70.60	124.00	88	0.66
N4	102	54	18.5	65	72.00	140.00	80	0.606

Phytochemical Composition and Antioxidant Activity

Phytochemicals and antioxidant activity Qualitative phytochemical analysis indicated that flavonoids, tannins, phenolics and saponins were detected in the noodles while terpenoids were not (Deng et al., 2024). The husk's presence is responsible for the presence of bioactive compounds as it was rich in polyphenols. The result of the DPPH radical scavenging activity (50.66-78.65% with increasing concentration) showed the potential antioxidant capacity. This is associated with hydrogen donating ability of phenolic and flavonoid molecules (De Paula et al., 2017). The flavonoid content (6.82 mg QE/g), tannin content (6.60 mg TAE/g) and total phenolic content (7.42 mg GAE/g) were higher than many reported values in raw barley, possibly due to the modification caused during the process which helps in higher extraction of bound phenolics (Montalbano et al., 2016). The results indicate that barley enriched noodles can be used as functional foods due to their antioxidant activity.

Table 3: Qualitative Phytochemical Test

Test	Flavonoid	Terpenoid	Tannin	Saponin	Phenolic Compounds
Result	Present	Absent	Present	Present	Present

Table 4: Invitro Quantitative Analysis Results

Test	TPC	TFC	TTC
Results	7.42 mg GAE/g	6.82 mg QE/g	6.60 mg TAE/g

Table 5: DPPH scavenging activity

Concentration (%)	% Inhibition
0	0.00
2	50.56
4	62.92
6	74.16
8	76.40
10	78.65

Microbiological Characteristics of Developed Noodles

In microbial analysis, the total plate count was found to be 6,400 CFU/mL, a moderate count. *Staphylococcus aureus* was detected, showing possible contamination that might have occurred during processing or handling, however, there were only low numbers of coliform bacteria (Yang et al., 2022). The presence of a relatively high number of yeasts and molds (6,000 CFU/g) could be the consequence of improper drying or storage. This suggests the need for better hygiene practices, proper drying and controlled storage conditions to maintain product safety and quality and to extend product shelf life.

Table 6: Microbial Testing

Test	Dilution	Observation
Total Plate Count	10 ⁻¹	64 colonies
Coliform Count	10 ⁻¹	2 tubes positive
	10 ⁻²	0 tubes positive
<i>Staphylococcus Aureus</i>	-	Positive
Yeast & Mold Count	10 ⁻¹	60 colonies

Noodle Cooking and Functional Quality

The presence of beta glucan in noodles depicted that the noodles produced had excellent water holding capacity (89 of water holding and rehydration capacity) (Wu et al., 2025).

Cooking loss was 12% which was in the permissible range of noodles fortified with fiber. The values obtained in the texture profile analysis i.e. Hardness (325 g), springiness (0.85), and chewiness (203 g) indicate a tough and chewy texture as previously reported (Zhu et al., 2024).

Stickiness was found to be 0.42 N which is acceptable and can be handled easily. High swelling index after heating has shown that the starch has successfully gelatinized. After cooking, slight discolouration occurred, which is in line with other cereal-based products (Brennan et al, 2013). The optimum cooking time was determined to be seven minutes, and the pH (6.0) of the instant noodle

product confirmed its chemical stability.

Table 7: Physicochemical & Cooking Properties

Rehydration Rate (%)	Water Absorption Capacity (%)	Cooking Loss (%)	Swelling Index (Before Boiling, mL/g)	Swelling Index (After Boiling, mL/g)	Bulk Density (g/cm ³)	Optimal Cooking Time (min)	pH
89	89	12	1.5	2.5	0.64	7	6

Table 8: Textural & Structural Properties

Hardness (g)	Springiness	Chewiness (g)	Breaking Strength (N)	Stickiness (N)	Strand Width (cm)
325	0.85	203	1.85	0.42	0.0701 ± 0.00661

Table 9: Color Properties,

Color (Uncooked)	L* (Uncooked)	Color (Uncooked)	a* (Uncooked)	Color (Uncooked)	b* (Uncooked)	Color (Cooked)	L* (Cooked)	Color (Cooked)	a* (Cooked)	Color (Cooked)	b* (Cooked)
16.72		17.57	0.09			15.6		15.3		1.97	

Sensory and Statistical Assessment

For the 9-point hedonic scale for sensory evaluation, the total acceptability score was 7.05, or “like moderately,” indicating good consumer acceptance. Texture, fragrance, and general acceptance were shown to be highly significant ($p < 0.001$) by statistical analysis. Despite having the highest mean score, color was not statistically significant—possibly because different people have different visual perceptions. Strong positive correlations between texture and color and overall acceptance were found by correlation analysis, indicating that these characteristics are important in influence consumer preference.

Table 12: Descriptive statistics of hedonic sensory evaluation

Sensory Attribute	N	Mean	Standard Deviation
Color	20	7.15	0.75
Aroma	20	6.85	0.49
Texture	20	6.70	0.73
Taste	20	6.75	0.79
Overall Acceptability	20	7.00	0.73

Table 13: One-sample t-test comparing mean hedonic scores with neutral value of 5

Sensory Attribute	Mean	t-value	p-value	Significance
Color	7.15	-0.25	≥ 0.05	Not significant
Aroma	6.85	10.04	< 0.001	Significant
Texture	6.70	10.66	< 0.001	Significant
Taste	6.75	12.63	< 0.001	Significant
Overall Acceptability	7.00	-	-	-

Conclusion

To develop a healthier product alternative to wheat noodles, this study has been successfully focused on product formulation and quality control of functional instant barley noodles. The addition of barley flour effectively improved the nutritional value of the product by increasing the total dietary fiber, β -glucan, and some important micronutrients which has many potential health benefits, including better absorption, bowel movement and reduction in the incidence of chronic health problems. The instant noodles had proper moisture content, cooking and texture properties where observed through the physicochemical analysis. There were slight variations in physiological properties of formulated noodles compared to the control. The sensory evaluation study showed this formulation was acceptable to consumers of flavor, texture, color and overall acceptability. The instant noodles were processed using safe hygienic practices and microbial examination confirmed that it was safe to eat. From this study, it is apparent that barley can be incorporated into instant noodles to yield a functional food product while not compromising much on quality. This research provides the opportunity to improve development and commercialisation of functional food products via the demonstrated benefits of barley noodles as a healthy alternative.

Ethical Statement

Practical ethical research practices and safe laboratory practices were observed in carrying out this research. Experiments were conducted in a safe and sterile setting to guarantee safety and accuracy. Sensory assessment was done through the voluntary nature of the panelists and consent was sought from them before commencing the study. There was no physical, chemical or biological damage to the participants involved. The data presented in this study were honestly collected, interpreted and reported without any fabrication, falsification or manipulation. A full reference was made to every source in order to avoid plagiarism.

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