VER
Vascular & Endovascular Review

Nutritional and Physico-Chemical Analysis of Flaxseed Flour, Barley Flour and Rice Bran Oil For Production of Nutraceutical

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Received: 5. 09.2025 *Revised: 18.09.2025* *Accepted: 20.10.2025*

ABSTRACT

Nutraceuticals are the foodstuffs with a primary aim of granting health benefits much more than what can be achieved by normal foods and diets. Barley has numerous beneficial health and nutritional properties in cereals, such as enhancing the health and betterment of digestive and immunity system. Consumption of beta glucan-rich barley enhances several cardiovascular risk factors. Gamma Oryzanol, present only in rice bran is effective in moderation of blood serum cholesterol and decrease triglycerides in hyperlipidemic patients. Among all, the most active lignan compound, SDG (secoisolariciresinol diglucoside), which is extracted and concentrated from flaxseed has great promise in providing therapeutic effects on certain hormone dependent cancers, cardiovascular diseases, diabetes as well as other immune-mediated disorders.

How to Cite: Geetanjali Gupta, Sarvesh Rustagi, (2025) Nutritional and physico-chemical analysis of flaxseed flour, barley flour and rice bran oil for production of Nutraceutical, Vascular and Endovascular Review, Vol.8, No.7s, 184-188.
DOI: *https://doi.org/10.15420/ver.2025.08.07.184-188*

INTRODUCTION

The broad range of nutraceutical products obtainable in the market today, can be categorised into numerous broad categories based on their origin, function, and regulatory standards. These consist mainly of functional foods, dietary supplements, and medical foods (Agarwal et al., 2020). Each category offers different functions i.e adding specific nutrition to curing a particular ailment. Barley a staple crop in ancient India, it is celebrated as a sacred grain and major barley growing states are Rajasthan, Uttar Pradesh, Haryana, Punjab. They are found at several regions such as in Madhya Pradesh, Uttrakhand, Himachal Pradesh, Jammu and Kashmir, West Bengal, Chhattisgarh and Sikkim (Kumar et al., 2014). The coarse-grains production declined, reflecting reduced production of mainly barley in Australia and less barley and sorghum in the United States. Coarse grains such as sorghum and barley, which are expected to grow up to 327 metric tonnes by 2027 of which the largest share will come from developing countries. Flaxseeds have high potential of production and is cultivated in more than 50 countries but the two main flaxseed exporting countries are the Canada and China. Rice bran oil is a source of a natural antioxidant called gamma oryzanol. Diets that were abundant in rice bran oil were found to decrease serum total cholesterol and LDL cholesterol. Diets that contained canola and maize oil were capable of decreasing HDL cholesterol. The higher content of unsaponifiables in rice bran oil was the reason for its comparable cholesterol-lowering properties to canola oil and maize oil.

MATERIALS AND METHODOLOGY

Raw Ingredients

The selected three main ingredients rice bran oil, flaxseed, and barley were purchased from certified suppliers from Dehradun, Uttarakhand, to ensure the quality and safety of the food product development.

Nutritional analysis of various sources

Protein Content

Protein content of the extruded products was evaluated according to the Kjeldahl Method (AOAC 976.05). An accurate weight of around 1 g of each sample was weighed and introduced into a digestion flask. Concentrated sulfuric acid (H_2SO_4) 15 mL and a catalyst mixture of potassium sulfate and copper sulfate to promote digestion were added to this. The mixture was heated at a temperature of 370°C until a clear solution was obtained, indicating complete digestion of organic matter. The solution was cooled down and diluted with distilled water, and it was neutralized using a 40 % sodium hydroxide (NaOH) solution after digest. The neutralized digest was distilled to liberate ammonia, which was collected in a boric acid solution. Titration was performed with 0.1N hydrochloric acid (HCl) and an appropriate indicator before use, to determine the ammonia content.
The nitrogen content of the sample was calculated using the formula:

$$\text{Nitrogen (\%)} = \frac{(Volume\ of\ HCl) \times Normality \times 14}{Weight\ of\ Sample\ (g)}$$

Then, protein content was calculated by multiplying the nitrogen percentage by the conversion factor of 6.25, which assumes that proteins contain about 16% nitrogen. Protein Calculation Formula Used:

$$Protein\ (\%) = Nitrogen(\%) \times 6.25$$

Fat Content

The content of the extruded products was determined by Soxhlet Extraction Method (AOAC 920.39). The prepared samples (2–3 g of powdered blend) were stuffed in thimble and mounted in Soxhlet assembly. Hexane (200 mL) was the solvent with extraction performed for 6–8 hours, until no more oil was extracted. The residual fat was left after evaporating the solvent, and its weight was determined. Fat content was calculated using the formula:

$$Fat\ (\%) = \frac{Weight\ of\ Fat\ Residue\ (g)}{Weight\ of\ sample\ (g)} \times\ 100$$

Dietary Fiber Content

The fiber content of the extruded products was analyzed by Enzymatic-Gravimetric Method (AOAC 985.29). For each sample, 1 g was weighed out into a beaker and reacted successively with enzymes (α-amylase, protease, and amyloglucosidase) to hydrolyze the starch and protein. The residue was filtered and washed with ethanol, followed by drying at 105°C and mineral content correction in muffle furnace (525°C). Total dietary fiber was determined by the formula:

$$Fiber\ (\%) = \frac{Weight\ of\ Residue\ (g) - Ash\ (g)}{Weight\ of\ sample\ (g)} \times\ 100$$

Moisture: The moisture content was estimated by the oven-drying method. A known amount of flour is put into an oven at 105 °C for 24 h to remove the water. The sample is then dried, weighed again, and moisture content is determined as:

$$Moisture\ content\ (\%) = \frac{Initial\ Mass - Final\ Mass}{Initial\ Mass} \times\ 100$$

Ash content: A standard method of gravimetric analysis is used to analyze the ash content in the provided formulations. Initially, each sample has a known weight of flour and is dried at a relatively low temperature, to evaporate the moisture. Then, after moisture removal, the sample is placed in a muffle furnace at a temperature of around 550°C for hours. This process burns away the organic material and leaves only inorganic mineral content, otherwise known as ash. The ash is then weighed, and the ash content (as a percentage of the original sample weight) is calculated. The unique compositions of the various formulations reveal insights in this analysis as they relate to their mineral content.

$$\textbf{Ash content (\%)} = \frac{Weight\ of\ ash\ (g)}{Initial\ weight\ of\ sample\ (g)} \times 100$$

Physio-chemical analysis of various sources

pH: For pH analysis, 10 g of sample was weighed and homogenized in 90 mL of distilled water. pH of extruded products was measured using a calibrated digital pH meter. Accurately standardize the pH meter using buffer solutions (e.g. 0, pH 4.0 and 7.0) before use. This is a method which indicates the level of acidity or base compound within the product, influencing its stability, taste and durability.

Total Soluble Solids (TSS): 100 mL distilled water to extract soluble portion. Filter the solution to achieve a clear one. TSS was measured using a digital or handheld refractometer and record the value in °Brix. Be sure to calibrate the refractometer with distilled water before testing. This course of action measures the high-quality, flavor and texture that relate to dissolved solids (Arora et al., 2024)

Titratable Acidity: To determine titratable acidity of each extruded product, the sample was homogenized with 100 ml distilled water for forming homogeneous solution. Titrate the answer the use of 0.1 N sodium hydroxide (NaOH) with turbidimetrically phenolphthalein because the indicator. titration was performed by adding NaOH dropwise until the solution loses its yellow color to a pale pink which is persistent Take a note of the volume of NaOH consumed and determine the titratable acidity in percentage to that of standard acid like citric acid. It will take on acidity that affects flavor, stability and shelf life (Arora et al., 2024).

RESULTS AND DISCUSSION

Nutritional Analysis

Nutritional analysis is performed to assess protein, fat, and fiber levels in the formulations to ensure they have dietary adequacy and health benefits.

Protein: The protein content of the different sample formulations is calculated based on the contribution of each ingredient as mentioned in the Table 1. For calculating protein, firstly we calculated Nitrogen content by the formula outlined in methodology.

Table 1. Protein content of individual ingredients

Ingredients	Nitrogen Content (g/100g)	Protein content (g/100g)
Wheat Flour	2.0	2.0×6.25=12.0
Flaxseed Flour	2.928	2.928×6.25=18.3
Barley Flour	1.6	1.6×6.25=10.0
Rice Bran Oil	0.0	0.0×6.25=0.0

The control sample wheat flour, recorded a protein content of 12.0 g/100g; through it the incorporation of flaxseed flour (18.3 g/100g) and barley flour (10.0 g/100g) (Andersson et al., 1999) resulted in a higher protein content.

Fat Content: The fat content of each individual ingredient is represented in Table 2. The control sample wheat flour, recorded a fat content of 1.5 %; flaxseed flour 42% (Noreen et al., 2024) and barley flour 2%.

Table 2: Fat content (%) of each ingredient

Ingredients	Sample weight (g)	Extracted fat residue (g)	Fat content (%)
Rice Bran Oil	100	100	$\frac{100}{100} \times 100 = 100$
Flaxseed Flour	2	0.84	$\frac{0.84}{2} \times 100 = 42$
Barley Flour	3	0.06	$\frac{0.06}{3} \times 100 = 2$
Wheat Flour	2	0.03	$\frac{0.03}{2} \times 100 = 1.5$

Fiber: The fibrous content of each individual ingredient (Table 3). The value of fiber varies from 0 to 27.3% (RBO 0%, wheat flour 2.4%, barley 17.3% (fiznzer et al., 1975) and flaxseed (27.3%).

Table 3: Fiber content of individual Ingredients

Ingredients	Sample weight (g)	Fiber Residue (g)	Ash content (%)	Fiber content (%)
Rice Bran Oil	100	0	0	$\frac{0-0}{100} \times 100 = 0.0$
Flaxseed Flour	2	0.546	0.024	$\frac{0.546-0.024}{2} \times 100 = 27.3$
Barley Flour	3	0.519	0.030	$\frac{0.519-0.03}{3} \times 100 = 17.3$
Wheat Flour	2	0.048	0.000	$\frac{0.048-0.00}{2} \times 100 = 2.4$

Moisture: Moisture content of each formulation was estimated based on the contribution of each ingredient's moisture content (Table 4).

Table 4: Moisture content of each ingredient

Ingredients	Initial weight (g)	Dry Weight (g)	Moisture content (g)	Moisture content (%)
Rice Bran Oil	100	100	0	$\frac{100-100}{100} \times 100 = 27.3$
Flaxseed Flour	100	94	6	$\frac{100-94}{100} \times 100 = 6$
Barley Flour	100	90	10	$\frac{100-90}{100} \times 100 = 10$
Wheat Flour	100	86	14	$\frac{100-86}{100} \times 100 = 14$

Ash: The ash values of the sample formulations increased gradually, which was probably due to the added higher ash content shares of flaxseed flour and barley flour (Raj et al., 2023) into the formulations. It is a crucial parameter as it indicates the mineral constitution of a product and its effect on health benefits and processes, and as a product its texture. Ash content of each ingredient is shown in the Table 5 below.

Table 5: Ash content of each ingredient

Ingredients	Initial Sample Weight (%)	Weight of Ash (g)	Ash Content (g/100g)
Rice Bran Oil	100	0	$\frac{0}{100} \times 100 = 0$
Flaxseed Flour	100	3.7	$\frac{3.7}{100} \times 100 = 3.7\%$
Barley Flour	100	2.8	$\frac{2.8}{100} \times 100 = 2.8$
Wheat Flour	100	1.6	$\frac{1.6}{100} \times 100 = 1.6$

Physio-chemical analysis of various sources

pH: pH values for individual ingredients are as shown in Table 6:

Table 6: pH value of individual Ingredients

Ingredients	pH
Rice Bran Oil	7.0
Flaxseed Flour	6.0
Barley Flour	5.5
Wheat Flour	6.2

The total soluble solids (TSS): TSS Content represents the number of dissolved solids in a liquid, primarily sugars, salts, acids and other soluble compounds. Each sample's TSS content is calculated based on the individual ingredient contributions and its TSS content as shown in the Table 7.

Table 7: TSS content of each ingredient based on soluble compounds

Ingredients	Sugars (g/100g)	Salts (g/100g)	Amino acids (g/100g)	Other Compounds (g/100g)	TSS solid (g/100g)
Rice Bran Oil	0	0	0	0	0 g
Flaxseed Flour	2.25	0.9	0.9	0.45	4.5 g
Barley Flour	3.6	2.0	1.6	0.8	8.0 g
Wheat Flour	0.48	0.36	0.24	0.12	1.2 g

Titrable Acidity (TA): TA of all the ingredients (g/100g) as shown in Table 8 mentioned below.

Table 8: Titrable Acidity (TA) content of each ingredient

Ingredients	g/100g Titrable acidity
Rice Bran Oil	0
Flaxseed Flour	0.08
Barley Flour	0.15
Wheat Flour	0.12

CONCLUSION

Focusing on the ingredient composition, wheat flour (72.4%) seemed to be solely responsible for the formulation, whilst barley flour (14%) also contributed significantly to the ingredients. Rice bran oil (5%) and flaxseed flour (7.4%) improve the nutritional value of beneficial fatty acids. The average protein content is 15.66 g/100 g, further highlighting the high protein profile of the formulations. As for the components, wheat flour made up the largest share (72.4%) followed by barley flour (14%), flaxseed flour (7.4%), and rice bran oil (5%). And if you consider the nutritional profile, these ingredients play a role in fat content, too. Flaxseed and rice bran oil provide healthy unsaturated fatty acids, barley and wheat flour texture and bulk. The utilization of ingredient composition was a significant contributor to fiber enrichment. Most of the formulation was wheat flour (72.4%), barley flour (14%), flaxseed flour (7.4%), and rice bran oil (5%) Barley flour and flaxseed also significantly added to dietary fiber, thus increasing product nutritional properties.

The pH values were influenced by the main components, i.e.: rice bran oil (5%), flaxseed flour (7.4%), barley flour (14%), and wheat flour (72.4%). The high variability of moisture content among samples most likely comes from the incorporation of water retentive hydrophilic components flaxseed and barley flour, which increased water binding. The ash content was affected by the type of flour (wheat, barley, rice bran with the value of 724 % was the highest in wheat flour, barley (14%), as well as by the oil used rice bran oil (5%). TSS based on the proportion of flaxseed flour, barley flour, and wheat flour was estimated to be 4.5, 8.0 and 1.2 g/100g. There was a noticeable factor variation in several ingredients (flaxseed flour, barley flour, wheat flour) with value 0.08, 0.15 and 0.12 g/100g suggesting that the type of ingredient used has a statistically significant effect in determining the titrable acidity.

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