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Soluble and Insoluble Fiber Content and different Metabolites in Durum Wheat Bran (*Triticum turgidum. Durum*) cultivated in Western Algeria

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Abstract

Wheat bran is considered a widely available by-product in the wheat processing industry. It was recovered from the cereal and derivative food industry "E.R.I.A.D." in Ouled Mimoun, Algeria. This study is aimed at evaluating the physicochemical composition of wheat bran in order to assess the primary and secondary metabolites as well as the different fractions of dietary fibers. The experimental study focuses on the determination of dietary fibers in wheat bran, followed by a physicochemical analysis of the residues of primary and secondary metabolites with a cold ethanol maceration decreasing at 95%, 80%, and 65% for 24 hours. The results of the statistical study showed homogeneity of variances. Wheat bran is rich in dietary fibers, with a content of 39.5% DM, predominantly hemicelluloses (24.73%). Cellulose has a content of 10.19%, while the levels of sodium (Na) and potassium (K) are 3.87 mg/l and 3.44 mg/l, respectively, and protein content is 12.8% DM, flavonoids, and tannins. A more significant result was found to be very significant ($p < 0.00$) during extraction with 65% ethanol, 0.265 and 0.124 mg/l (catechin equiv/100 g). Wheat bran, with its high dietary fiber content and physicochemical properties, appears to fit well within therapeutic strategies for the management of several metabolic and digestive diseases.

Keywords: Wheat bran, physicochemical analysis, dietary fibers, protein, polyphenols.

Introduction

Cereal-based foods are the primary source of nourishment, energy, protein, B vitamins, and minerals for the global population. The role of cereals has changed significantly over time, with their use now spanning human consumption, animal feed, and various non-food applications. Wheat stands out as the most significant food crop for direct human consumption (Sennous *et al.*, 2020). In the food industry,

cereal by-products such as wheat bran can be added to improve the quality of the manufactured foods thanks to their richness in dietary fibers. These fibers, present in plant cell walls and indigestible, are known for their health benefits. Wheat, like other cereals, is a significant source of insoluble dietary fibers. These fibers are mainly found in the grain husk, called wheat bran, which is a by-product of the refining process. Epidemiological and intervention studies have demonstrated the beneficial effects on health of daily consumption of fiber-rich dietary fibers, especially in people with digestive and metabolic problems (Menadi *et al.*, 2021).

1. Materials and methods

Wheat bran It was recovered from the cereal and derivative food industries company “E.R.I.A.D.” in the commune of Ouled Mimoun in the city of Tlemcen, which occupies a prime geographical position within the entire national territory. Wilaya, both border (of Morocco) and coastal, is limited: - To the north, by the Mediterranean Sea; - To the northeast, through the wilaya of Ain Témouchent; - To the east, through the wilaya of Sidi Bel Abbes; - To the west, through Morocco; - In the south, by the wilaya of Naâma (Figure 1), characterized by a Mediterranean type climate, a cold and humid winter and a hot and dry summer. The rainfall remains very irregular and varies from 200 to 500 mm/year according to DPSB (management of budget programming and monitoring).



Figure 1. Map of location and boundaries of the Wilaya of Tlemcen according to ANIREF/Monograph of the Wilaya of Tlemcen

It is obtained following grinding by a mill illustrated in the following figure: (02):

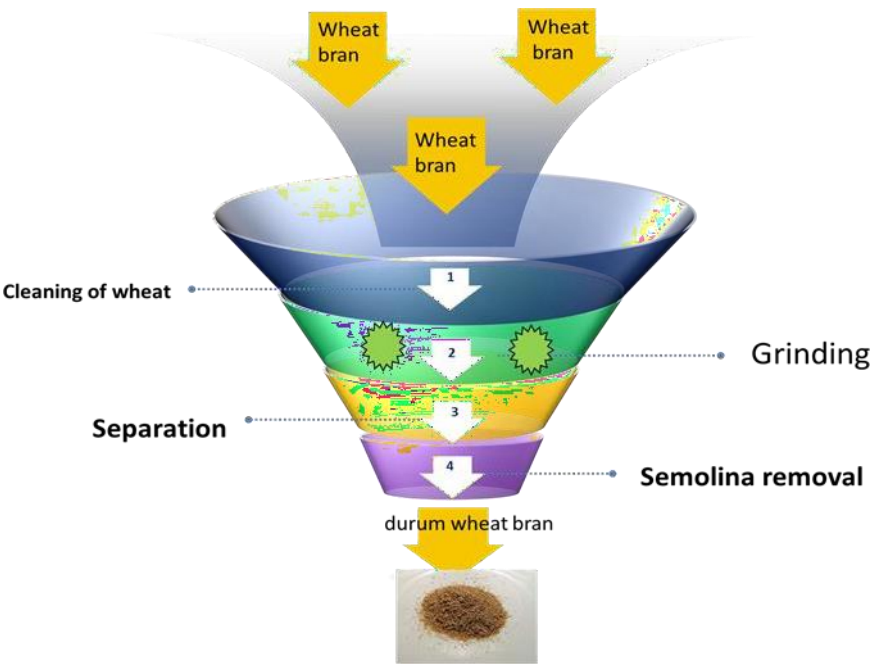


Figure 2: Illustration of dry fractionation of grains into wheat bran

Moisture content (NA 1132/1990)

Drying of a sample at 130°C under normal atmospheric pressure in a "Chopin" oven

Dry matter content (DM%)

It is given by the following formula: $DM\% = 100 - H\%$

Ash content: (ISO 2171,2007)

Incineration of a sample in an oxidizing atmosphere at a temperature of $550 \pm 10^\circ\text{C}$ until complete combustion of the organic matter and obtaining a constant mass. The ash content (AC) relative to the dry matter is calculated by the following formula:

$$AC = ((m_2 - m_0) / (m_1 - m_0)) \times 100 \times 100 / 100 - H$$

(m_0 : mass of the crucible in grams, m_1 : mass of the crucible + the sample in grams before incineration, m_2 : mass of the crucible + the sample in grams after incineration, $H\%$: water content, in mass percentage, of the sample).

Fat content

This involves solid-liquid extraction, using the Soxhlet apparatus (Luque de Castro and García- Ayuso, 1998). The fat content is calculated by the following formula:

$$\text{Fat}\% = (M_2 - M_1) \times 100 / M_0 \times 100: 100 - H$$

(M_0 : Mass of the sample (g), M_1 : Mass of the empty flask (g), M_2 : Mass of the flask and extracted fat (g))

Proteins

Determination of protein concentration by colorimetric assays is based both on the Biuret reaction, in which the peptide bonds of proteins react with copper under alkaline conditions to produce Cu^+ , which then reacts with the Folin reagent, and on the Folin-Ciocalteu reaction, where phosphomolybdate is reduced to heteropoly blue by the copper-catalyzed oxidation of aromatic amino acids. These reactions result in a strong blue color, which partly depends on the content of tyrosine and tryptophan (Waterborg, 2003).

Total fibers "NDF" Van.Soest 1967

This gravimetric technique is based on the extraction of soluble components from cell wall structures using neutral (for NDF) or acidic (for ADF) detergent solutions and weighing the residue after drying using a semi-automatic analyzer (Fibertec type). Neutral fibers correspond to the insoluble residue containing cellulose, hemicellulose, and lignin, or the residue from the cell walls. Acidic fibers correspond to the insoluble residue containing cellulose and lignin, or lignocellulose. Lignin is obtained by treating the ADF residue with 72% sulfuric acid and weighing the dry residue (ADL). The dry residues are incinerated at 550 °C, and the results are expressed relative to dry matter. (Figure 3).

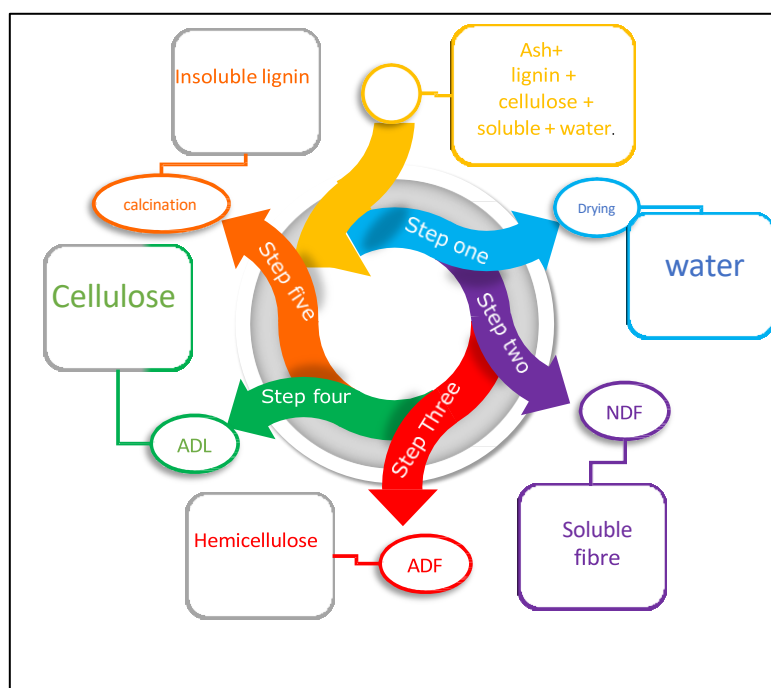


Figure 3. Synthetic sketch of the Van Soest method allowing assay of the biomass composition.

ADF: acid detergent fiber; ADL: acid detergent lignin; NDF: neutral detergent fiber.

Preparation of Detergents

1. Neutral Detergent Fiber (NDF)

For 1L of NDF solution, place 18.61 g of EDTA and 6.81 g of disodium tetraborate in a beaker, add a certain amount of distilled water, and heat until dissolved. Then, add 30 g of sodium lauryl sulfate, 10 ml of ethyl ether, and 4.56 g of anhydrous disodium hydrogen phosphate. Bring the volume up to the mark (1000 ml) with hot distilled water, then stir vigorously.

2. Acid Detergent Fiber (ADF)

For 1L of ADF solution, in a 1-liter beaker, place 20g of Cetyl Trimethyl Ammonium Bromide (CTAB) on a magnetic stirrer to ensure the mixture is homogeneous. Then, carefully add 30 ml of

concentrated sulfuric acid (the addition should be done gently into the beaker placed in a cold water sink).

3. Acid Detergent Lignin (ADL)

The ADF residue is treated with 72% H₂SO₄.

Operating Procedure

Accurately weigh approximately 1 g of sample (dried and ground to 1 mm) in a filter crucible. Proceed in the same manner as described in the Weende method, except that the amount of NDF solution is 200 ml and the extraction duration is one hour. Recover the NDF crucibles after drying them in the oven at 105°C, weigh them, and place them back into the Fibertec beaker containing 200 ml of ADF solution. After one hour of extraction, wash the filters containing the ADF residue with distilled water, followed by acetone. • Dry and weigh the ADF residue, then recover it for treatment with sulfuric acid to extract the ADL residue. Once filtered and rinsed with hot water and then with acetone, the recovered residue undergoes an attack with 72% sulfuric acid for 3 hours at room temperature. The acid is placed directly into the crucibles while frequently stirring with glass rods (the crucibles placed in a glass container should be filled with acid to 3/4).

After this operation, the residue must be filtered again and then rinsed with hot water. The crucible is dried in an oven at 105°C for 12 hours, weighed, and finally ignited at 450°C for 3 hours. (Goering and Van Soest, 1970; AOAC, 1990).

Results Expression

The NDF content is calculated as follows: $\% \text{ NDF} = ((P1-P2) / PE \times DM) \times 100$

(P1: weight of the crucible after drying at 105°C (g), P2: weight of the crucible after combustion (g), PE: sample size (g), DM: % dry matter/ 100).

The ADF content is calculated as follows: $\% \text{ ADF} = ((P1-P2) / PE \times DM) \times 100$

(P1: weight of the crucible after drying at 105°C (g), P2: weight of the crucible after combustion (g), PE: sample size (g), DM: % dry matter/ 100).

Hemicellulose content is calculated as follows: $\% \text{ Hemicellulose} = \% \text{ NDF} - \% \text{ ADF}$

The ADL content is calculated as follows: $\% \text{ ADL} = \% \text{ Lignin} = ((P1-P2) / PE \times DM) \times 100$

There is no specific method for determining the total hemicelluloses (Multon, 1981). Hemicelluloses were obtained by subtracting the values of ligno-cellulose obtained with acid detergent "ADF" from total fibers NDF.

(P1: weight of the crucible after drying at 105°C (g), P2: weight of the crucible after combustion (g), PE: sample size (g), DM: % dry matter/ 100).

Cellulose content is calculated as follows $\% \text{ Cellulose} = \% \text{ ADF} - \% \text{ ADL}$

Determination of Micronutrient Content (k) and (Na)

The levels of the micronutrients potassium (K) and sodium (Na) were determined by flame atomic absorption spectrometry. Samples after combustion of 1 g were diluted 100 times to give concentrations in the absorption range. The reading was carried out with a flame atomic spectrophotometer at a specific wavelength of the element to be measured. We conducted the dosing of the following mineral elements: potassium and sodium (Gueguen and Rombauts, 1961).

Extraction of Plant Material for the Measurement of Secondary Metabolites

The extraction method used on wheat bran powders is a cold ethanol maceration decreasing at 95%, 80%, and 65% for 24 hours as illustrated in figure (4).

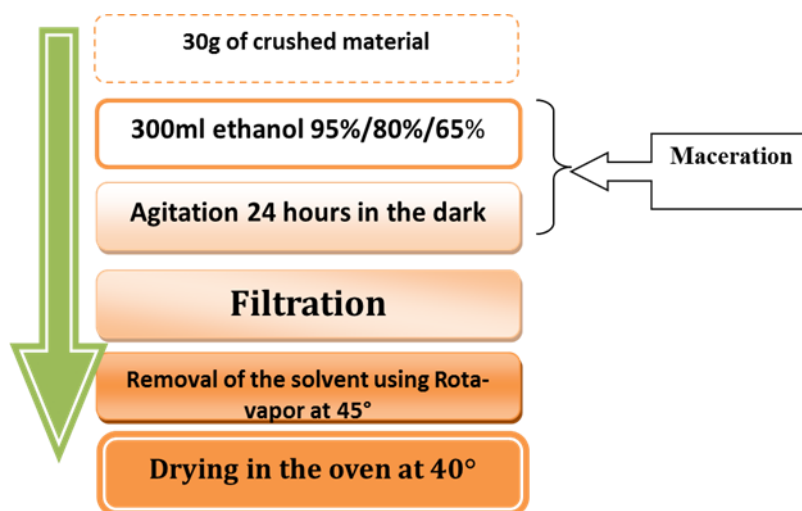


Figure 4. Illustration of extraction of plant material

Phytochemical Tests (Phytochemical Screening)

To highlight the main classes of chemical secondary metabolites in wheat bran, a phytochemical screening using colored reactions or precipitation by specific chemical reagents was carried out on ethanol extracts of wheat bran (Balamurugan *et al.*, 2019).

Polyphenols To 2 ml of each aqueous extract, a few drops of a 2% alcoholic solution of ferric chloride (FeCl_3) are added. Ferric chloride causes the appearance of a dark blue or green color in the presence of polyphenolic derivatives (Balamurugan *et al.*, 2019).

Flavonoids Treatment of 1 ml of ethanol extract with a few drops of concentrated HCl and 0.5 g of magnesium turnings. The appearance of a pink or red color after three (3) minutes reveals the presence of flavonoids (Balamurugan *et al.*, 2019).

Saponins The presence of saponins is highlighted by mixing 10 ml of the aqueous extract with distilled water. After shaking for 15 seconds, the observation of persistent foam indicates a positive reaction (Balamurugan *et al.*, 2019).

Terpenoids 5 ml of the extract solution are added to 2 ml of chloroform, and after simple agitation, a few drops of H_2SO_4 are added. The formation of a brown-red ring at the interface indicates the presence of terpenoids (Balamurugan *et al.*, 2019).

Anthraquinones To 5 ml of extract, a few ml of concentrated H_2SO_4 are added, and then 1 ml of diluted ammonia is added. The appearance of pink confirms the presence of anthraquinones (Balamurugan *et al.*, 2019).

Quinones The test is carried out by mixing the extract and an aqueous KOH solution (10%). The appearance of an aqueous phase turning red after agitation indicates the presence of anthraquinones (Balamurugan *et al.*, 2019).

Tannins 0.250 g of ethanol extract is added to 10 ml of distilled water in a test tube, then brought to a boil and filtered. A few drops of ferrous chloride are added to the filtrate. The observation of a green-brown color (catechic tannins) or midnight blue (gall tannins) indicates the presence of hydrolysable tannins in the extract (Balamurugan *et al.*, 2019). Following the results of the phytochemical tests.

Spectrophotometric dosing was carried out by calibration for the positive constituents as follows:

Measurement of Polyphenols

The measurement of total polyphenols is based on their ability to be oxidized by a mixture of phosphotungstic acid (H₃ PW₁₂ O₄₀) and phosphomolybdic acid (H₃ PMo₁₂ O₄₀), commonly called the Folin-Ciocalteu reagent. These are reduced to blue oxides with maximum absorption at 760 nm. A calibration curve (Figure 5) is prepared in the same way as that of the sample by preparing different concentrations of gallic acid in ethanol-water (50:50, v/v) ranging from 0 to 250 mg/l (Singleton and Rossi., 1965).

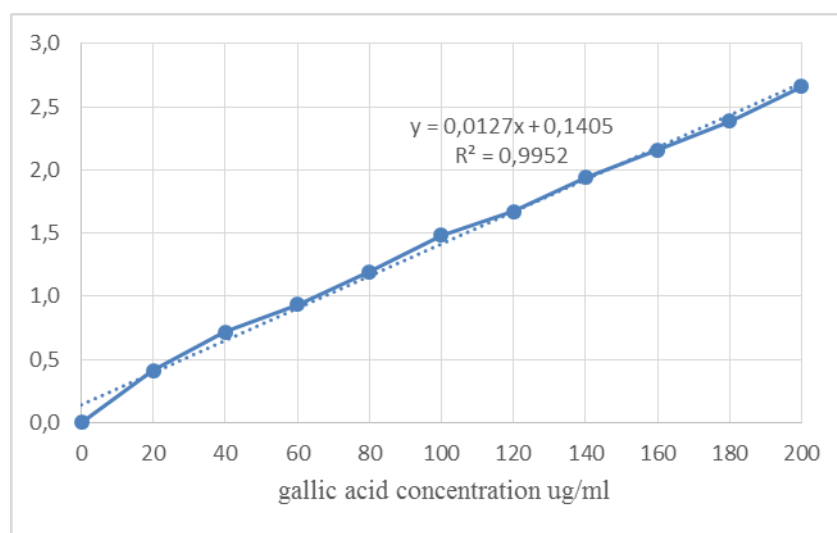


Figure 5. Gallic acid calibration curve

Measurement of Total Flavonoids

The quantification of flavonoids is done by the method adapted by Zhishen *et al.* (1999 using aluminum trichloride (AlCl₃) and sodium hydroxide (NaOH), and the content of flavonols is determined according to the protocol described by Adedapo *et al.* (2008). A calibration curve (Figure 6) is prepared in the same way as that of the sample by preparing different concentrations of quercetin.

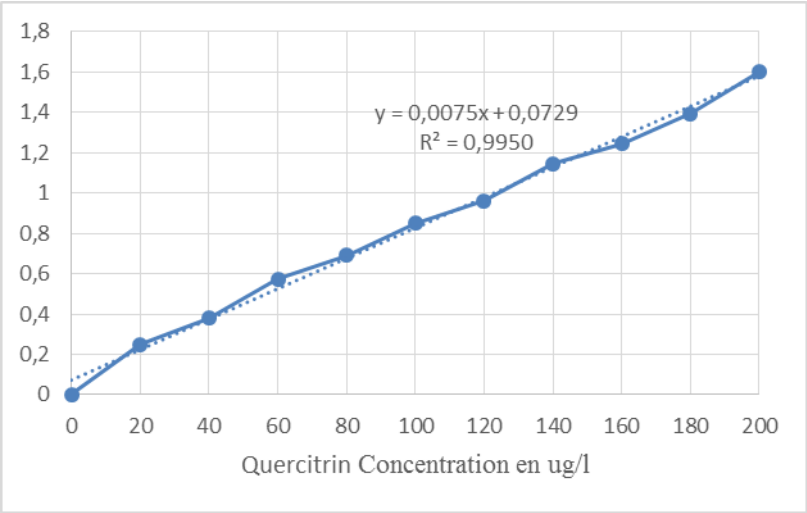


Figure 6. Quercitrin calibration curve

Tannin Measurement

The quantification of condensed tannins is done by the Vanillin method, which has the ability to react with tannin units in an acidic medium (Porice *et al.*, 1978). A calibration curve (Figure 7) is prepared in the same way as that of the sample by preparing different concentrations of vanillin.

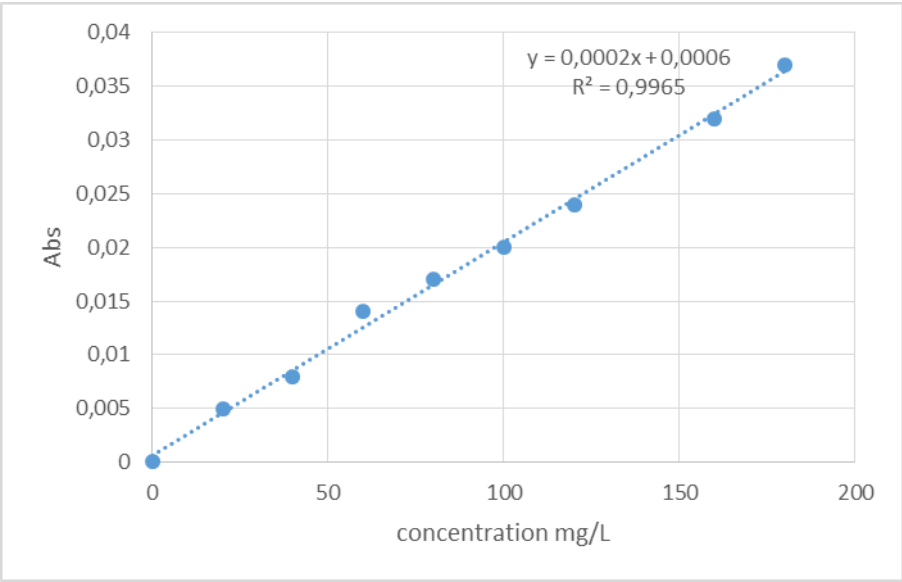


Figure 7. Vanillin calibration curve

3. Results

Composition of durum wheat bran in primary metabolites The results of physico-chemical analyses of wheat bran are presented in table 01 with fiber content in table 2, the contents of polyphenols, flavonoids, flavonols and tannins in tables 3 and figure 8.

Table 1. Results of physicochemical analyzes of wheat bran expressed as a % of dry matter

Analysis	Moisture	Ash	Fat	Protein	[NA] mg/l	[K] mg/l	Total fiber NDF
<i>durum wheat bran</i>	5,44	4,12	5,40	12,80	3.87	3.44	39,50

Table 2. Composition of the different fractions of dietary fiber from wheat bran expressed in % dry matter

Fractions	NDF %	Hemicellulose %	ADF%	Cellulose%	ADL%
<i>durum wheat bran</i>	39,50	24,73	14,77	10,19	4,58

Table 3. Summary table of phytochemical tests (phytochemical screening)

Secondary metabolites	95%	80%	65%
Polyphenols	+++	+++	+++
Flavonoides	+++	+++	+++
Tannins	+	+	+
Terpenoides	-	-	-
Saponosides	+++	+++	+++

Quinones	+	+	+
Anthraquinones	-	-	-

(+++)
(++) High presence / (+) Low presence / (-) absence

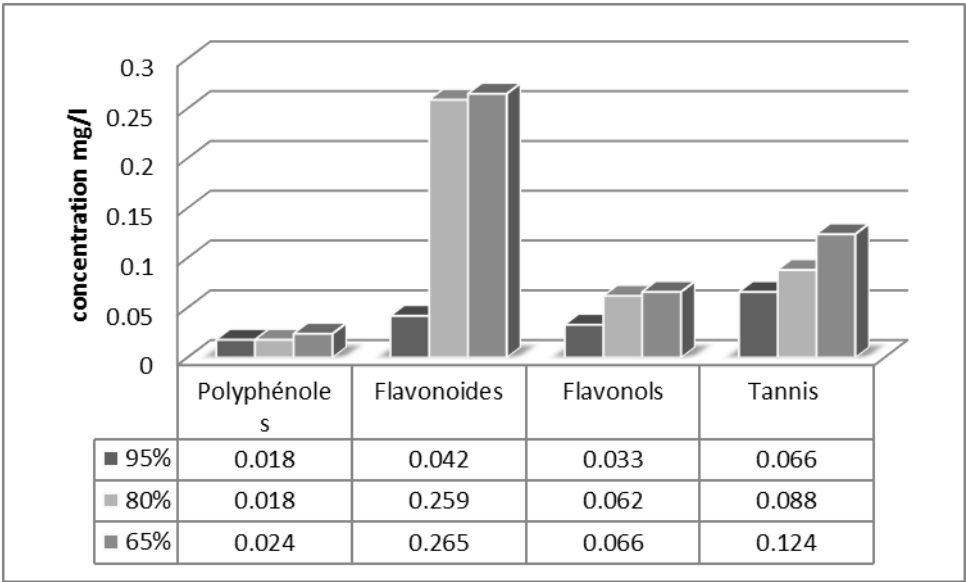


Figure 8. Content of polyphenols, flavonoids, flavonols and tannins in wheat bran in different degrees of extraction

4. Discussion

The physico-chemical analyses conducted on wheat bran, as presented in Table 1, highlight the high presence of dietary fibers compared to other components. Indeed, total fibers, known as "NDF," represent a significant proportion of the dry matter, reaching 39.50% with a predominance of hemicelluloses at 24.73%. Cellulose is also present at a rate of 10.19%, while lignin is less abundant, at a rate of 4.58%.

The water content of wheat bran is a crucial parameter for assessing its stability during storage for commercial purposes. A low water content, as measured in our sample at 5.44%, promotes the preservation of the product during storage. This result is lower than those reported by other studies, notably by Onipeet Jideani and Beswa (2015) and Hernandez-Díaz *et al.* (2007) with levels of 8% and 9.38%, respectively.

Regarding mineral substances, our analysis reveals a rate of 4.12%, similar to that obtained by Boudouma in 2009, at 4.62%. The levels of sodium (Na) and potassium (K) are 3.87 mg/l and 3.44

mg/l, respectively. These values differ from those found in other studies, with higher levels for potassium and sodium.

The protein content in our wheat bran sample is estimated at 12.8% of dry matter, which corresponds to other similar results in the literature. Wheat bran proteins are a cost-effective source of protein, offering different fractions that are nutritionally interesting. They stand out for their amino acid composition compared to flour proteins, which can influence their properties during the production of food products.

As for the fat content, our sample has a rate of 5.40% of dry matter, explained by the significant presence of fats from the wheat germ. This proportion is higher than that obtained in other studies, suggesting variations due to different factors such as grain genotype and growing conditions.

Wheat bran, rich in dietary fibers, is an important by-product of the cereal industry, as confirmed by our study with a concentration of 39.5% of dry matter. This value falls within a similar range to other studies, although variations may be observed depending on growing and production conditions.

Finally, the analysis of secondary metabolites in wheat bran, including polyphenols, flavonoids, flavonols, and tannins, highlights the diversity of compounds present in this food. These compounds can have beneficial effects on health, particularly in terms of physiological effects related to digestion and nutrient absorption.

The crucial role of oxidative stress in various physiological and pathological processes of the body is well known. Secondary metabolites contribute to defense against oxidative stress-induced damage. It is established that significant levels of ferulic acid are found in the bran part of wheat, mainly in insoluble and esterified form to the hydroxyl group C-5 (Wang *et al.*, 2009).

The results of the statistical study showed homogeneity of variances. The results obtained showed that the extraction degree significantly modifies the estimates of the antioxidant properties of wheat bran. In the case of flavonoids and tannins, a more significant result was found to be very significant ($p < 0.00$) during extraction with 65% ethanol, 0.265, and 0.124 mg/l (catechin equiv/100 g). A slight difference is observed in the extraction results of polyphenols and flavonols at different degrees. Many experiments have revealed that the highest concentrations of interesting phytochemical substances are found in the bran/germ fraction of whole wheat grain. Furthermore, the bran/germ fraction of whole wheat flour accounts for 83% of the total phenolic content and 79% of the total flavonoid content (Povilaitis *et al.*, 2015).

A result close to what we observed was found for polyphenols, with a rate of 1.99 mg/g (Abozed *et al.*, 2014; S. Kouider *et al.*, 2020) in the case of extraction with ethanol (70%), whereas extraction with acetone (50%) resulted in a rate of 4.66 mg/g. Another study done by Adom, Sorrells, and Rui (2005) revealed that the flavonoid content of bran/germ fractions in the wheat varieties tested (740–940 μmol of catechin equiv/100 g) was 10 to 15 times higher ($p < 0.01$) than the flavonoid content of the respective endosperms ($p < 0.01$). The presence of flavonoids is higher in whole grain than in bran, the main ones being apigenin-C-diglycosides (flavonols), cyanidin-3-glycosides, and peonidin-3-glycosides (anthocyanins) (Vaher *et al.*, 2010).

Phenolic acids, tannins, and flavonoids are major compounds present in cereals that have been correlated with their antioxidant properties and health benefits. More recent studies have shown that phenolic components of wheat bran, such as feruloyl oligosaccharides, protect against oxidative effects caused by free radicals in human red blood cells (Wang *et al.*, 2009) and prevent oxidative damage to proteins and membrane lipids, thus playing a role in reducing the risk of chronic diseases such as cardiovascular diseases (Zhou and Yu, 2004) by inhibiting LDL, likely by binding to apolipoprotein-B (Liyana-Pathirana and Shahidi, 2007).

According to the theory of Vitaglione, Napolitano, and Fogliano (2008), dietary fibers can act mechanically in the intestine to release beneficial phenolic compounds for health. These compounds could be released by soluble dietary fibers through esterases and then absorbed at various levels in the intestine. Once in the blood, these compounds could exert their beneficial effects on the whole body. Furthermore, the intestinal microbiota would contribute to the continuous absorption of phenolic compounds from dietary fibers (Prückler *et al.*, 2014).

5. Conclusion

In conclusion, our measurements carried out during this first series of tests revealed the richness in dietary fibers and primary and secondary metabolites of wheat bran from our region. From a nutritional standpoint, by-products such as wheat bran derived from wheat processing are rich in nutrients compared to finished products such as semolina and flour. These by-products have a high content of fibers, minerals, vitamins such as B6, thiamine, folate and vitamin E, as well as certain phytochemical compounds, including antioxidants such as phenolic compounds (Shewry, 2009), (Singh, 2011).

Conflict of interest statement

The authors declare that there is no conflict of interest.

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