Journal homepage: http://fens.usv.ro/index.php/FENS
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XX, Issue 3 - 2021, pag. 247 - 255

FOOD AND ENVIRONMENT SAFETY

EXTRACTION AND COMPARATIVE STUDY OF PHENOLIC COMPOUNDS FROM TENDER AND TREATED WHEAT GERM AND THEIR ANTIOXIDANT POWER

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Received 23th June 2021, accepted 29th September 2021

Abstract: *Wheat is the second most consumed cereal for human consumption after rice due to the presence of high calories. Wheat germ (2-3% of the grain) can be separated as a by-product when milling wheat. It is considered an important by-product and can be used in various applications such as food, and other biological purposes. It is considered a nutrient rich in vitamin and antioxidant included, vitamin B, minerals, polyphenols and flavonoids. The goal of our work is to estimate the total polyphenol composition and the evaluation of antioxidant potential present in pure and Industrial common wheat germ. The manual dissection was carried out to produce pure wheat germ and based on the co-product of the flour mill for the treated wheat germ. The results obtained show that the total polyphenols represent values of 0.074 ± 0.001 mg (EAG) / g and 0.062 ± 0.001 mg (EAG) / g for the pure wheat germ and the treated wheat germ, respectively. A similarity in flavonoids was noted for the two varieties of common wheat (pure and treated) with a value of 0.022 ± 0.002 mg of EC / g DM. The evaluation of the antioxidant activity, show that the treated wheat germ has the best activity against the radical DPPH with an IC50= 0.051 mg / ml compared to the pure wheat germ with IC50 = 0.361 mg / ml. Wheat germ can be used as a protective agent, as a rich source of bioactive compounds with beneficial health effects.*

Keywords: *Common wheat, wheat germ pure, polyphenols, Antioxydant activity*

1. Introduction

Cereals play an important role in human nutrition, either for baking or as a raw material for baking flour. Botanically, they belong to the *Gramineae* family, which includes wheat, maize, rice, oats, etc., and are used in the production of flour, barley, millet, sorghum and rye. Wheat is one of the main cereals and food ingredients in the world because of its ability to be milled into flour. Wheat is an omnipresent cereal in the Mediterranean diet due to its energetic and nutritional value. Nowadays, Western countries are more and more interested in adding a functional ingredient naturally enriched with bioactive molecules, interesting to incorporate in food formulas. Cereals are considered as a main source of human and animal nutrition [1] according to [2] their production reaches up to 2 billion tons. Wheat germ (WG) is widely recognized as a nutritious raw material to be incorporated into food product formulations or as a food in its own right. Typical applications are in sprout-fortified bread, snacks and supplements, for breakfast cereals and for

DOI: https://doi.org/10.4316/fens.2021.027

the production of wheat germ oil. Wheat germ, containing about 8% to 14% oil (10% on average), is mainly used in the food, medical and cosmetic industries as a source of oil [3]. Wheat germ, a nutritious byproduct of milling constituting 2.5 - 3.0 g / 100g of the cereal box is separated in a fairly pure form by using a germ separator or by appropriate adjustments in milling techniques [4]. Wheat germ (embryonic axis and scutellum) accounts for about 2.5 to 3.8% of the total seed weight and is an important by-product of the milling industry [5]. Is part of the wheat grain (*Triticum vulgaris*) which contains the embryo of the future plant. It could therefore serve as a cheap source of raw material for food and oleochemical industries, and also give stable oil for various uses and applications, including shampoos, soaps and by-products,and salads and cooking oils. Therefore, wheat germ, with its inherent nutritional value, could be a good alternative to processed vegetable oils in food products [6]. It is a major by-product of the wheat milling industry and is considered natural source of highly concentrated nutrients [7]. It is widely recognized as a nutritious raw material to be incorporated into food product formulations or as food in its own right. Typical applications are in sprout-enriched bread, snack foods and cereal supplements for breakfast and for the production of wheat germ oil. The latter, containing about 8% to 14% oil (on average 10%), is mainly used in the food, medical and cosmetic industries as a source of oil [8].

The stored raw sprout develops a rancid and bitter taste in a short time, due to the activity and unsaturated fat content of fresh sprouts. The low stability of the raw germ limits its uses; this problem could be overcome either by inactivating enzymes or by creating unfavorable conditions for enzymatic activity by appropriate means [9]. The objective of this study is to evaluate by a comparative study some phytochemical parameters in polyphenols and flavonoids for pure soft wheat germ and treated wheat germ. The antioxidant potential was also evaluated in this study.

2. Matherials and methods

2.1. Plant material

In this study, a comparative study of the nutritional value of treated soft wheat germ and pure soft wheat germ (ARZ variety) by the analysis of phenolic compounds, flavonoids and the study of their antioxidant activity by the DPPH test. For this experimental study, wheat germ samples are obtained in two different ways depending on the process of use; for pure soft wheat germ, it is degermination, a process obtained by a manual dissection method, while the treated germ comes directly from mills with advanced milling In our experimental part, all the analyses and the extraction method were carried out at the immunology and general biochemistry laboratories of the University of Djillali Liabes of Sidi bel abbés. The plant material of our study includes two varieties of soft wheat, the ARZ variety from ITGC of Sidi bel abbés for the study of pure wheat germ and the soft wheat treated by L'O.A.I.C of Sidi bel abbés for the study of treated wheat germ which was treated at the Habour mill in Oran (table 1). The wheat germ obtained must be kept at a temperature of 5° C in the fridge, in order to extend its shelf life and avoid rancidity (rotting) until the time of analysis.

Table 1.
Pure and processed common wheat

Variety And Origin		
Pure common wheat	ARZ	ITGC of sidi bel abbés
Soft wheat Treaty	Treaty	Treaty by O.A.I.C; Habour. Oran

2.2. Extraction of soluble phenolic compounds

The sample preparation is done by maceration: a mass of 30g of each sample is macerated in 100ml of 70% ethanol, after the sample is stirred for 24 hours; the mixtures have been separated by simple filtration. The extracts are then evaporated to dryness using an evaporator of about 45°C. The extract will complete drying in the oven for 24 hours; so that the extract does not lose its phenolic compounds with water during evaporation. The conservation is done in the refrigerator.

2.3. The determination of total polyphenols in the extract

2.3.1. Determination of total phenolic content

Using the Folin-Ciocalteu reagent, a volume of 200 µl of each extract was added, with a mixture decrypted [10] of 1 ml of Folin-Ciocalteu reagent diluted 10-fold, and 800 µl of a 7.5% sodium carbonate (Na_2CO3) solution. The tubes are shaken and stored for 30 min. The absorbance is read at 765 nm using a UV spectrophotometer (the level of polyphenols was expressed in microgram equivalent of gallic acid per milligram of extract (µg EAG/mg E). A calibration curve was carried out in parallel under the same operating conditions using gallic acid at different concentrations (0 to 1000 µg/ml).

2.3.2. Determination of flavonoid content

The protocol used is based on the one described by [11], with some modifications. In a glass hemolysis tube, 0.5 ml of extract was mixed with 2 ml of distilled water and 0.15 ml of 5% NaNO2. After 6 minutes, 0.15 ml 10 % AlCl3 was added. After 6 minutes a volume of 2 ml 4% NaOH was added to the medium and adjusted with 0.2 ml distilled water. The absorbance is read off at 510 nm after stirring and incubation for 15 min. A methanolic quercitrin solution has been prepared. Daughter solutions prepared from the stock solution at different concentrations between 100 and 900 µg/ml will be used to draw the calibration curve.

2.4. Antioxidant Activity

2.4.1. DPPH Radical scavenging activity

The DPPH radical scavenging activity was measured according to the protocol described by [12].A 50 µl solution of each methanolic extract at different concentrations was added to 1.95 ml of DPPH (0.025g/l). At the same time, a negative control is prepared by mixing 50µl of methanol with 1.95 ml of the methanolic solution of DPPH. The absorbance reading is taken against a blank prepared for each concentration at 515 nm after 30 min incubation in the dark and at room temperature.

The positive control is represented by a solution of a standard antioxidant, ascorbic acid, whose absorbance was measured under the same conditions as the samples and for each concentration. DPPH radical scavenging in percentage was calculated as follows:

DPPH (%)=100 (A (control) - A (sample)) / A control

Where: DPPH (%) is the percentage reduction in DPPH, (Acontrol) is the absorbance of the negative control, and (Asample) is the absorbance of the sample. The results were expressed as the average of three measurements obtained for each sample. The IC50 value that determined the concentration that reduced the DPPH radical by 50% was revealed graphically for each extract from the percentage DPPH reduction versus concentration curve

2.5. Statistical analysis

All results were performed in three replicates. The results are expressed as average ± standard deviation. The data (the averages plus at least standard deviation as well as representations) are statistically analyzed using the statistical software EXCEL 2010.

3. Results and discussion

3.1. Yield in dry extract

Extraction is a very important step in the isolation and identification of phenolic compounds. Extraction methods depend on the extraction yield of the phenolic compounds. Ethanol extracts (70% ethanol), recovered after evaporation, and was weighed to determine the resulting dry weight. These extracts contain the total polyphenols. Extractions of the different cereals for the wheat varieties allowed us to calculate the yield of each extract. The table below shows the results for 30 g of dry plant matter expressed as a percentage. The results obtained are presented in Table 2.

Table 2.
Physical parameters (yield, color, and physical appearance) in dry extract

Sample Variety	Treated Common Wheat Germ	Pure Tender Wheat Germ (ARZ)
Physical appearance	Sticky gel	Sticky gel
Colour	Pale Yellow	Dark Yellow
Return (%)	20.6	12.73

3.2. Result of total phenolic and flavonoid

The dosage was carried out on alcoolic extracts f wheat germ after passing through an oven to eliminate the solvent in order to obtain dry extracts. The resulting dry weight contains the total polyphenols which are determined by the Folin-Ciocalteu test. The content of phenolic compounds in each extract was calculated from the calibration curve (Y= 8.7218x-0.002), of which y is the absorbance and X the concentration of the gallic acid solution), our results were expressed in milligrams gallic acid equivalent per gram of dry matter (EAG/g dm) and the correlation coefficient (R^2=0.997) the optical density measurement was performed at a wavelength of 760 nm. The results obtained are presented in Table 3.

Table 3

Polyphenol content expressed in mg EAG/g dry matter.

Variety	Pure Common Wheat Germ (ARZ)	Treated Common Wheat Germ
Total polyphenol content in mg EAG/g	0.074±0.001	0.062±0.001

The results obtained from the wheat germ extracts (pure wheat germ and treated wheat germ analysed reveal that pure wheat germ (ARZ) is richer in total phenolic compounds
0.074±0.001mg (EAG)/g than treated wheat germ (secondarily) with a content of 0.062±0.001mg (EAG)/g.
The main reason for looking for flavonoids in wheat germ is due to the fact that flavonoids are very well known for their antioxidant capacity.The flavonoid content of each extract was calculated from the calibration curve (Y=4.6153x-0.0118), of which y is the absorbance and X the concentration of the catechin solution) our results were expressed in milligrams catechin equivalent per gram of dry matter (mg EC/g dm), the optical density measurement was performed at a wavelength of 510nm. The results obtained are presented in Table 4.

Table 4

Flavonoid content expressed in mg EAG/g dry matter.

Sample Variety	Pure Common Wheat Germ (ARZ)	Treated Common Wheat Germ
Flavonoid content expressed in mg EC/g dm	0.022±0.002	0.021±0.002

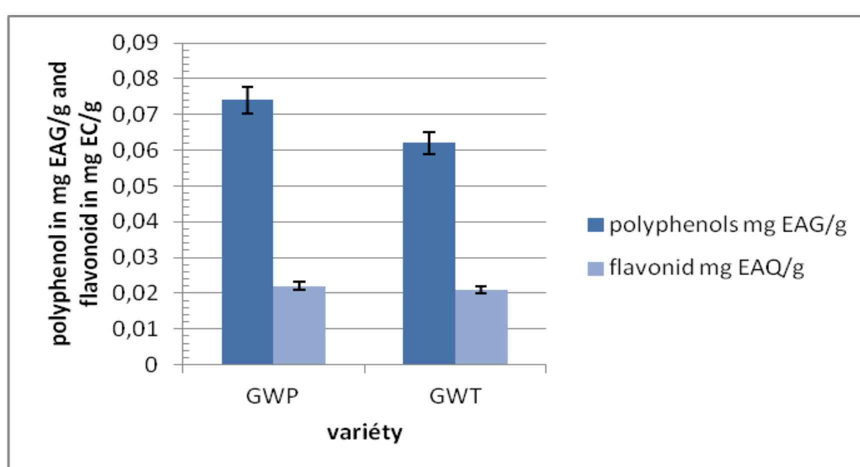

Fig.1. Polyphenol and flavonoid content of wheat
t germ from different varieties of soft wheat, GWP: Pure wheat germ, GWT: Processed Wheat Germ

3.3. Free radical scavenging DPPH. (2, 2-diphenyl-1-picrylhydrazyl)

The natural antioxidant activity depends on various parameters, such as reaction mechanism, isolation procedures, purity of active compounds, as well as the test system and substrate to be protected by the antioxidant.

In our case, we evaluated the samples by DPPH radical scavenging activity in order to estimate and measure their antioxidant activities. The anti-radical capacity cannot be measured directly but by monitoring the effect of reactivity in vitro. This activity has been evaluated by measuring the DPPH radical scavenging power according to the method described by [13]

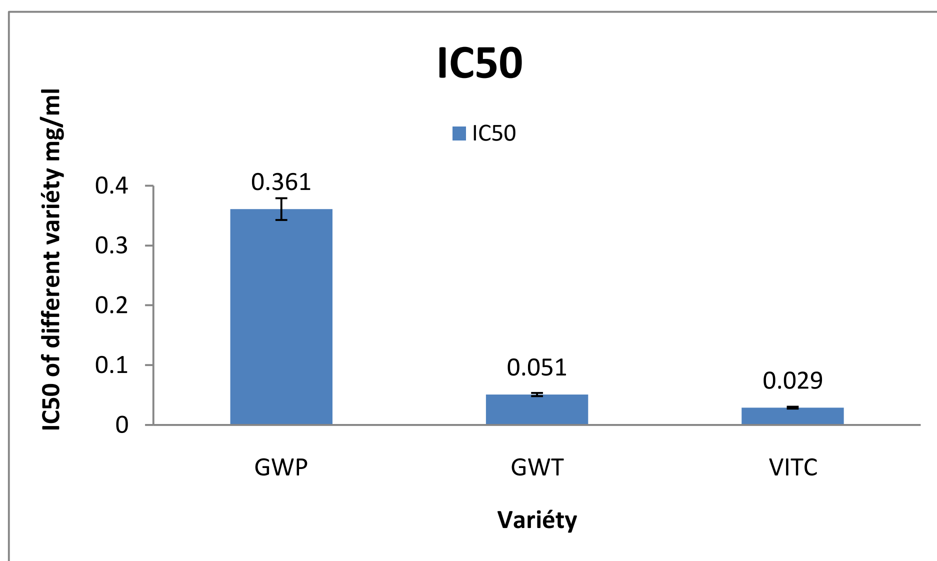

Fig 2. Antioxidant power of different extracts of soft wheat germ.
GWP: Pure wheat ger, GWT: Processed Wheat Germ

Our results on the anti-radical activity presented in the curves were expressed by measuring the effective concentration IC50; the concentration that traps (inhibits) 50% of the DPPH radical of each extract and ascorbic acid. This IC50 value is inversely proportional to the antioxidant power, each time the IC50 of the extract is close to the IC50 of the ascorbic acid. The stronger the antioxidant activity in the extra it From the comparison of IC50 of each variety studied and according to the results obtained with that of ascorbic acid, we notice that IC50 of the pure and treated wheat germ extracts are higher than IC50 of ascorbic acid with 0.029 mg/ml (used as a reference antioxidant), 0.051 mg/ml and 0.361 mg/ml, for the pure wheat germ extract (ARZ) and the treated wheat germ, respectively. The antioxidant capacity of a compound is higher when its IC50 is small, so the antioxidant capacity of ascorbic acid is higher than that of the extract of two wheat germs. In addition, the results also show that this same capacity is higher for pure wheat germ than for treated wheat germ.Wheat germ is a by-product of wheat milling that has the potential as a food ingredient, a source of high quality protein, minerals, vitamins and antioxidants. Several studies have indicated the superiority of wheat germ over other milling products as a rich nutritional supplement, [14].

The results of assaying total polyphenols and flavonoids in both wheat germ varieties show that wheat germ extracts contain moderate amounts of total polyphenols, particularly in the pure germ of the ARZ variety.

No differences were found in flavonoids in the two germs used in our case. The marked values showed that the total polyphenol content. Represents 0.84 mg/g wheat germ. In a study by [15] indicate that, ethanol extraction gave a polyphenol content ranging from 0.038 mg/g to 1.6 mg/g, from which he considers values ranging from average to good. Our results are consistent with his work, hence the presence of considerable amounts of polyphenols in wheat germ, which are less present compared to other wheat grain tissues as indicated by several studies [16], studied the distribution of polyphenols in the milling function of wheat, and about 73% of the phenolic compounds in grains were found in wheat bran. This indicates that the wheat germ is less rich in polyphenols. This result is also confirmed by [17] who found that bran layers had the highest content of total phenolic compounds (total polyphenols and flavonoids).

Concerning flavonoids, the flavonoids in cereals were mainly concentrated in the outer layers of the grain compared to other parts of wheat grain [18]. A content of 0.45 mg/g to 1.07 mg/g and which vary according to the types of flavonoid compounds [19].

Several parameters intervene and influence the quality and quantity of its compounds, the variety and/or species, the extraction method used as well as the extraction solvent can vary quantitatively the yield quality of the grain. The study by [20] Show that flavonoid values in wheat germ are varied (changed) by the extraction solvent used. In addition, the quantity of polyphenols is considerable between the two varieties of the same species, it is less important compared to the tissues of the peripheral layers (aleurone layer, testa and hyaline layer). In the work for [21] confirm that the phenolic content that can be recovered in different samples of cereals is influenced by the polarity of the extraction solvents and the solubility of this compound in the solvent used for the extraction process. Also [22] confirm that several factors can affect the content, composition and stability of phenolic compounds in wheat products depending on the ingredients, industrial or domestic processing.

Anti-oxidant properties are measured and highlighted by measuring the effective concentration (IC50). The results highlighted by the DPPH test. The IC50 values obtained range from 0.029 mg/ml to 0.36 mg/ml with ascorbic acid used as (reference antioxidant) with IC50=0.029 mg/g. The antioxidant power of our extracts between them reveals that treated wheat germ is the most active with an IC50=0.051mg/ml followed by pure wheat germ with an IC50=0.3 mg/ml. Comparison of the effective concentrations (IC50) of each of these two extracts shows that IC50 of our studied varieties and ascorbic acid (used as a reference antioxidant) are close, indicating that the anti-radical activity of the DPPH trapping of our varieties is very important (very strong) with the highest value of ascorbic acid.

Several researches have been devoted to study the wheat germ and its derivatives and have proven that this part has a great antioxidant capacity through the presence of natural bioactive compounds. The highest free radical scavenging activity in wheat germ is a value of 0.42 mg/ml in 70% ethanol extract according to a study conducted by [23]. A similar trend has been observed in the study of antioxidant activity by [24]. Our organism therefore reacts constantly to this permanent production of free radicals and we distinguish at the cell level two lines of defense unequally powerful to detoxify the cell [25]. These are exogenous molecules. Unlike antioxidant enzymes, an

antioxidant molecule traps a single free radical. To be able to function again, this antioxidant molecule must therefore be regenerated by other systems [26].

4. Conclusion

Wheat germ (WG) is widely recognized as a nutritious raw material to be incorporated as a food ingredient and for the production of wheat germ oil. Is a nutrient by-product of the milling process and is a natural source of highly concentrated nutrients. The composition total polyphenols and flavonoids in both wheat germ varieties gave that wheat germ extracts contain moderate amounts of total polyphenols, particularly in the pure germ of the ARZ variety. The antioxidant power reveals too that treated wheat germ is the most active that IC50 are close, indicating that the anti-radical activity of the DPPH trapping of our varieties is very important (very strong)

Different factors can influence the value of natural compounds present in the germ, preservation and storage methods, the method of growing the grain, the environment, exposure to heat and light, extraction methods and the solvent used may vary and affect the yield and value of the germ. To this end, it is interesting to extend our research toother varieties and other cereal species and to carry out further study based on advanced research techniques in order to improve nutritional and phytochemical quality.

5. Acknowledgments

The authors acknowledge the University of Sidi Bel Abbes, Biology Department at Sidi Bel Abbes for the financial support of this research

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