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Chemical Composition and Properties of Wheat Sprouts with Reference to 1-Octacosanol

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Abstract

The aim of this work was to examine the chemical properties of extracts obtained from wheat sprouts, with particular emphasis on the content of 1-octacosanol and phenolic acids, which have antioxidant properties. This study focuses on examining the content of many nutrients in cereals, which potentially increase the functional qualities of food and enrich the daily human diet. Applied UV-Vis technique allowed for analysis of the content of natural dyes in sprouts, which confirmed the presence of β-carotene, chlorophyll *a* and chlorophyll *b*. The antioxidant activity of obtained extracts and pure 1-octacosanol at various concentration levels was compared. Applications of TLC showed the presence of many polyphenolic compounds like chlorogenic acid in wheat sprouts. The presence of 1-octacosanol in tested sprouts has been confirmed. Qualitative analyses of samples containing 1-octacosanol were performed using spectroscopic techniques FT-IR. A rapid method for identifying this compound in plant origin samples has been developed.

Keywords

Wheat Sprouts, Polyphenols, Policosanol, Extraction, Thin-Layer Chromatography, Spectroscopy

1. Introduction

Grain is one of the basic foods consumed by people living in almost every corner of the Earth. As an ingredient of food products, various species of cereals are used in many cultures, as they are the basic source of carbohydrates in the diet. They have many important nutritional values, but also have a cleansing effect on the

body of unnecessary products, which is related to the high fiber content. There are many types of cereals; the most important are wheat, rice, maize, barley, oats, rye, millet and buckwheat. Cereals are the most important source of food. They are easy to store and are processed into a wide range of foods, both for humans and for animals. The cultivation and processing of cereals is an important part of the economy related to food production [1]-[3]. For humanity, cereal plants were and are the first and most important food as we know it. Since the dawn of time, these plants have been known to fill the stomach. Cereal grains are a staple food, but also have valuable nutritional values. Over the years, the methods of processing cereals have been improved, which is confirmed by primitive tools for grinding grain found by archaeologists. The first cakes began to be made from ground grain and then with the development of new skills, various types of bread were made [3] [4]. Cereals and cereal products are very important in our diet, which is confirmed by the food pyramid, where they find their high second place. Cereal plants are a rich source of carbohydrates, fiber, vitamins, minerals, fats and proteins. Eating of good quality bread highly satisfies daily requirements for certain nutrients, such as proteins (about 30%), carbohydrates (nearly 50%) and vitamins B (50% - 60%) [5] [6].

It is commonly known, that cereal grains are the main source of many nutrients that humans provide in their diets. However, sprouted cereal grains are considered to have exceptional nutritional value. The production of sprouts is simple and additionally allows for obtaining a product rich in desired ingredients. During germination of cereal grains, a number of chemical changes occur, resulting in increased activity of hydrolytic enzymes and improved quality of nutrients and bioactive compounds of cereals, thus increasing the content of proteins, amino acids, sugars and vitamins [7]-[9]. Cereal sprouts are a special case as a source of antioxidants, which show numerous beneficial effects and functions for the human body. Biologically active compounds found in sprouts participate in metabolic processes; they strengthen the immune and antioxidant systems, affect the cardiovascular, digestive, nervous and respiratory systems. Many of these ingredients have preventive and sometimes even healing properties. According to the botanical definition, plant sprouts are the part of the embryo containing the bud and root bud [7]. Briefly, these are germinated plant seeds and foods, which contain high concentration of nutrients, such as phenols, minerals or vitamins. Also, they affect the reduction in anti-nutritional factors, which increases the bioavailability of minerals. This results in the fact, that cereal sprouts are used to increase the nutritional value of certain food products [7].

Wheat (*Triticum* L.) is considered the most important of all types of cereals, because it is the most common crop in the world [10]-[18]. It is grown on every continent, in different climates and soil conditions. Wheat crops in the world are arranged in such a way that each month the harvest takes place in a different place. Example wheat harvest lasts in India from February to May, in Poland it lasts from the second half of July to the end of the first half of August, and in Brazil from

December to January [10]. Wheat is cultivated all over the world and in various climatic conditions; it is most adapted to a temperate climate (with rainfall from 30 to 90 cm) [12]. Wheat has been known for at least 10,000 years [11]. The oldest known varieties of wheat are *Triticum turgidum* ssp. dicoccoides; *Triticum araraticum*; *Triticum dicocoides*, have been grown in the areas of Ancient Egypt, Israel, Türkiye, etc. At that time, this plant was a self-pollinating, annual grass, which farmers began to cultivate, improving it by planting it in subsequent seasons. The oldest varieties of wheat planted by farmers from year to year resulted in larger seeds and husks that did not disintegrate for self-sowing. One of the oldest varieties of wheat is still cultivated all over the world, but today this plant looks slightly different than in biblical times [11]. This plant has long and slender leaves and stems, which are hollow in most strains. At the end of the stalk, there are inflorescences, which in cereals are called ears. Depending on the variety, they consist of 20 to 100 small flowers, which are gathered in two to six groups of flowers. The flower structures of grasses are called spikelets. In the spike, there are two husks (upper and lower), which are usually of similar length. The shapes of the husks can be different—bone, pointless or sharpened [14]. The fruit of cereals is a kernel. It consists of a series of tissues with a specific composition and structure. More precisely, it is made up of 80% - 85% endosperm, 2% - 3% embryos and 13% - 17% bran. It resembles more a seed than a fruit, due to its hardened, thin and practically completely connected to the seed. Colloquially, the fruit of cereals is called grain. Usually, wheat kernels are oval and elongated in shape.

A characteristic feature of the grain is the outermost layer of the seed coat. A caryopsis is a dry, non-cracking fruit that is a single-seeded achen. It is made of dead and capable of developing cells. It is the grain of wheat that is the source of food. The endosperm of wheat is mainly composed of starch and proteins. The embryo, on the other hand, is rich in lipids and oxidative and hydrolytic enzymes (lipase, protease and lipoxidase). However, it is the embryo that is the most important part of the grain [19]-[28]. It contains about 26% protein, 17% sugars and 10% oil, which contains valuable fatty acids such as omega-6 and omega-3 and microelements. In addition, there is a high content of vitamin E in the embryo in the form of tocopherols and tocotrienols [15]. Many varieties of wheat are grown in Europe, because the genus Triticum is assigned to both domesticated and wild species. Currently, thousands of varieties are known, but the most important of them are cultivated ones, namely hard wheat (*Triticum durum*) [15] [18] and common wheat, called bread wheat (*Triticum aestivum* L.). However, in Poland species like spelt wheat (*Triticum spelta* L.), Polish wheat (*Triticum polonicum* L.) [16] and emmer wheat [13] [17] are also cultivated.

The aim of this study is the qualification of extracts obtained from wheat grain and sprouts, with particular emphasis on an antioxidant activity, related to the presence of polyphenolic compounds in the tested extracts. Attention was also paid to the presence of 1-octacosanol (see **Figure 1**), which belongs to aliphatic alcohols.

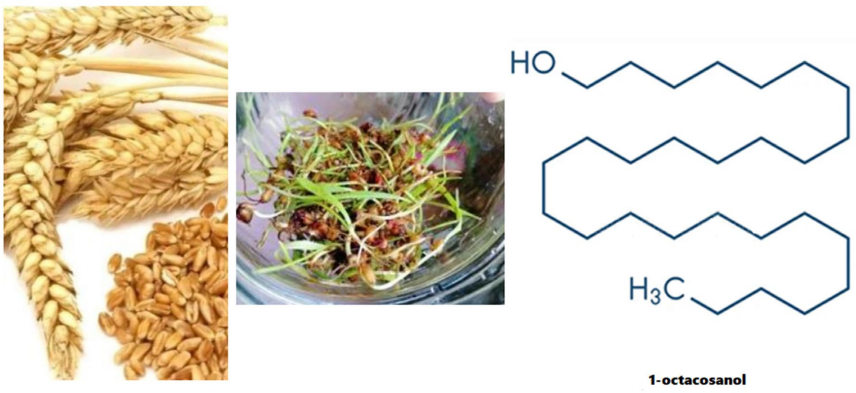

Figure 1. The structure and presence of 1-octacosanol.

Mentioned above compound is a main component of policosanol, a mixture of long chain alcohols extracted from plant waxes. 1-Octacosanol is a long-chain fatty alcohol and a component of plant waxes, commonly found in the waxy cuticle of cereal grains such as wheat. Moreover, this compound occurs in the plant origin food products like sugarcane (*Saccharum officinarum* L.) molasses, buckwheat (*Fagopyrum esculentum*) husk, propolis (or bee glue). The consumption of dietary supplements containing policosanol is safe and well tolerated for a human organism, but it can affect the functions of the nervous system, as well as transport and oxygen capture in the human body and is effective at lowering the blood cholesterol (reduction of low-density lipoprotein—LDL levels) [29].

2. Materials and Methods

2.1. Equipment and Reagents

The spectrophotometric measurements were performed by usage of UV-Vis and a spectrophotometric a plate reader. This apparatus was used for qualitative analysis of standard's solutions and plant extracts. Chromatographic analyses were realized using an HPTLC system, equipped with a Visualizer. TLC analysis of standards and plant extracts was eluted in a DS-L horizontal chamber. TLC plates coated with silica gel on aluminum foil and plastic backings were also used. Visualization was performed under UV light.

For extraction procedure, liquid nitrogen was applied. The next solvents and reagents were used for elution: methanol, ethanol, acetone, distilled water, and mixtures such as acetone-chloroform-water (80:20:10, v/v/v) and hexane-dimethyl ether-acetic acid (85:15:2, v/v/v). Reagents included: DPPH in methanol and Folin-Ciocalteu reagent, and a sodium carbonate solution. Methanolic solutions of various standards were also prepared, including gallic acid, vanillic acid, ferulic acid, p-coumaric acid, 3,4-dihydroxybenzoic acid, p-hydroxybenzoic acid, synapic acid, syringic acid, chlorogenic acid, quercetin, rutin, 1-octacosanol. Water was purified using a standard laboratory water purification system. In **Table 1**, the details about instrumentation, reagents, and standards used for spectrophotometric, chromatographic, and phytochemical analysis are presented.

Table 1. Analytical equipment, solvents, and chemical standards employed in the phytochemical characterization of plant extracts.

Category	Item/Reagent	Supplier/Manufacturer/Location	Purpose/Notes
Spectrophotometry	UV-Vis Spectrophotometer (Helios Gamma)	Thermo Fisher Scientific, Waltham, MA, USA	Qualitative analysis of standard solutions and plant extracts
	Multiwell Plate Reader (Varioscan)	Thermo Scientific, Waltham, MA, USA	
Chromatography (HPTLC)	HPTLC System (Linomat V, Visualizer, VisionCATS v2.0)	CAMAG, Muttenz, Switzerland	Chromatographic analysis
	TLC Chamber (DS-L horizontal)	Chromdes, Lublin, Poland	TLC analysis of standards and plant extracts
	TLC Plates Kieselgel 60 F254 (on Al foil or plastic)	Merck, Darmstadt, Germany	Used for TLC separation; UV visualization at λ = 254 nm and λ = 366 nm
FT-IR Analysis	FT-IR Vertex 70V with Hyperion 1000 microscope	Bruker Optics GmbH & Co. Ettlingen, Germany	FT-IR spectra of wheat grain and 1-octacosanol standard
Sample Preparation	Liquid Nitrogen	Air Products, Toruń, Poland	Sample freezing/grinding during extraction
Solvents	Methanol, Ethanol 96%, Acetone, Distilled Water	Avantor S.A., Gliwice, Poland	General extraction solvents
	Acetone:Chloroform:Water (80:20:10 v/v/v)	—	Solvent mixture for extraction/TLC
	Hexane:Dimethyl Ether:Acetic Acid (85:15:2 v/v/v)	—	Solvent mixture for chromatography
	n-Hexane 95%	Sigma-Aldrich, Steinheim, Germany	Chromatographic separation
Reagents	DPPH (0.02 mg/mL methanolic solution)	Sigma-Aldrich, Steinheim, Germany	Antioxidant activity assay
	DPPH· (2,2-diphenyl-1-picrylhydrazyl)	Sigma-Aldrich, St. Louis, MO, USA	Synthetic free radical for antioxidant tests
	Folin-Ciocalteu Reagent (F-C)	Sigma-Aldrich, Steinheim, Germany	Total phenolic content assay
	Sodium Carbonate (Na_2CO_3, 20% solution)	Avantor S.A., Gliwice, Poland	Used in Folin-Ciocalteu assay
Standards (Phenolic Compounds)	Gallic acid, Vanillic acid, Ferulic acid, p-Coumaric acid, 3,4-DHB, p-HB, Synapic acid, Syringic acid, Chlorogenic acid	Sigma-Aldrich, Steinheim, Germany (or "Niemcy")	Methanolic standard solutions for calibration/comparison
Standards (Flavonoids)	Quercetin, Rutin (rutoside)	Sigma-Aldrich, Steinheim, Germany	
Standard (Waxy Alcohol)	1-Octacosanol	Sigma-Aldrich, Steinheim, Germany	Standard for wax component analysis
Water Purification	MilliQ RG System	Millipore Intertech, Bedford, MA, USA	Production of distilled/deionized water

2.2. Samples

Grain—common wheat (*Triticum aestivum* L.), variety Euforia obtained from a local farmer from Kuyavian-Pomeranian Voivodeship (Poland) and were cultivated during this experiment. To obtain sprouts the germination process lasted 30 days. Firstly 10.01 g of wheat grains were weighed, were poured into the greenhouse where the process took place. Then, in order for the wheat to undergo the swelling process, it was rinsed several times with prepared water, after which the greenhouse was filled with water. The greenhouses were covered with sterile gauze to prevent unwanted impurities from getting into the wheat grains and to allow air from entering the cropping. This stage was repeated the next day. After two days, when the beans swelled and obviously cracked, the water was drained. The wheat was then rinsed several times during the day with distilled water. The action was repeated for the next few days until the sprouts were fully developed. In general, the applied germination conditions were as follows: temperature 22˚C, light regime 200 lx (12 h per day), watering schedule 2 times per day, growth vessel conditions (humidity 50% - 70%, CO_2 400 ppm). During these experiments two number of independent germination batches were performed. Only fully developed sprouts were harvest. The stages of growth of wheat sprouts are presented in Figure 2.

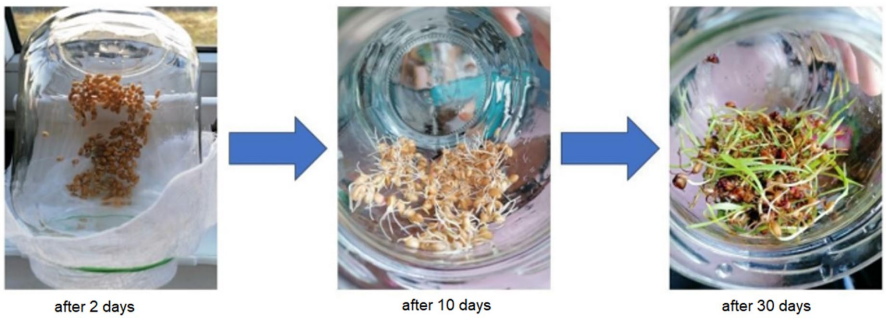

Figure 2. Wheat germinated sprouts in different stages of growing.

2.3. Extraction Method

Some of sprouts samples were put into a mortar, infused with liquid nitrogen, and then rubbed. In each five falcons tubes 250 mg of crushed sprouts was weighed. Then, 2 mL of solvent was added to each tube. There were use various solvents as follows: pure methanol; 50% methanol (mixture: methanol:distilled water (1:1) v/v); pure ethanol; 50% ethanol (mixture: ethanol:distilled water (1:1) v/v); pure acetone. The tubes were placed in a shaker during 24 hours, the solvents were evaporated so that only the sludge remained was weighed. The extraction efficiency was then calculated. For further analysis obtained extracts were dissolved in 1 mL of methanol and then filtered into glass vials through syringe filters to remove the undissolved sediments. To analyze octacosanol in anlyzed samples, it was necessary to perform a re-extraction, this time in a non-polar solvent, therefor n-hexane was used. Extraction was carried out for both on wheat grains (10 g of

grains + 12 mL of n-hexane) and on wheat sprouts (2 × 250 mg of sprouts + 3 mL of n-hexane). Extract samples were analyzed against a solution of 1-octacosanol in n-hexane (20 mg 1-octacosanol in 5 mL n-hexane solution, c = 4 mg/mL).

2.4. Analytical Methods

The scheme of proposed steps of analytical procedure is presented in Figure 3.

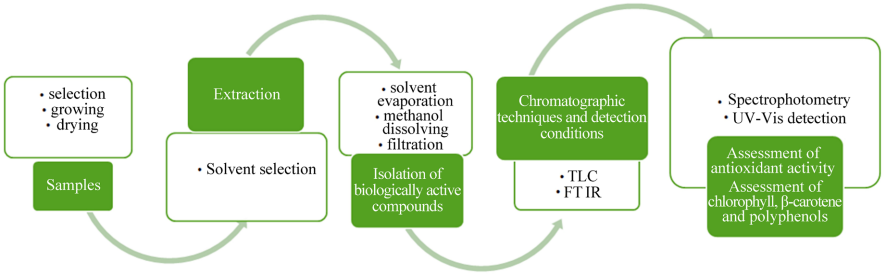

Figure 3. Analytical procedure applied in experimental section.

2.4.1. Determination of Total Polyphenols Content per Gallic Acid Equivalent

In order to carry out the analysis, the F-C reagent was used, which is a mixture of solutions of sodium tungstate (Na_2WO_4), sodium molybdate (Na_2MoO_4), bromine water, lithium sulfate (Li_2SO_4) and concentrated hydrochloric and phosphoric acids. The reaction with the F-C reagent consists in the reversible reduction of molybdenum (VI) to molybdenum (V) by polyphenolic compounds in an alkaline reaction.

Briefly, 12 µL of each extract and the same amount of methanol were taken as a blank sample. Then 188 µL of distilled water and 12 µL of F-C reagent were added to each extract and blank sample. The rehearsals were suspended for 8 minutes. After this time, 38 µL of 20% Na_2CO_3 was added and incubated for 30 minutes to make them react. In the next step, the absorbance of the samples was measured at a wavelength of λ = 765 nm. Each measurement was performed in 3 times repetitions. The total content of polyphenols was calculated as a gallic acid equivalent. Calibration parameters are presented in Table 2.

Table 2. Calibration parameters for gallic acid equivalent.

Concentration range $\left[\frac{mg}{mL}\right]$	Calibration curve	R^2
0.05 - 0.15	y = 5.452x − 0.1573	0.9988

2.4.2. Determination of the Total Antioxidant Capacity of Plant Extracts Using a DPPH· Reagent

Evaluation of the antioxidant activity of the crude methanolic extracts was performed. For the preparation of DPPH solution, 2 mg of the pure substance was dissolved in 100 mL of methanol. Next, 5 µL of the methanolic extracts was added to 245 µL of DPPH solution and this mixture was kept in the dark for 30 min.

After this procedure, the absorbance of the mixture was measured using a spectrophotometer at wavelength λ = 517 nm. For control experiments, the absorbance of pure DPPH solution was also measured, using methanol instead of plant extract.

The measured values were used to calculate the radical scavenging antioxidant activity of the extracts using the following formula (1):

$$RSA = \frac{A_{DPPH} - A_{sample}}{A_{DPPH}} \cdot 100\% \quad (1)$$

where: RSA—radical scavenging activity, A_{sample}—absorbance of sample, A_{DPPH}—absorbance of DPPH methanolic solution.

2.4.3. Determination of *β*-Carotene, Chlorophyll *a* and Chlorophyll *b* Content in the Tested Extracts

To determine the content of natural plant dyes in wheat sprouts extracts (formula (2)), 200 µL of each extract was taken and then absorbance was measured at the wavelengths presented Table 3.

Table 3. Parameters to calculate the content of individual natural plant dyes.

Parameters	*β*-carotene	chlorophyll *a*		chlorophyll *b*	
Wavelength λ [nm]	449	643	667	643	667
Molar absorption coefficient $\varepsilon \left[\frac{\mathrm{dm}^3}{\mathrm{mol}\times\mathrm{cm}}\right]$	152,021	16,600	50,800	36,268	1071
Optical path length l [cm]	0.5233	0.5233		0.5233	
M $\left[\frac{\mathrm{g}}{\mathrm{mol}}\right]$	536.88	893.5		907.48	

$$A = \varepsilon c l \Rightarrow c = \frac{A}{\varepsilon l} \Rightarrow c = \frac{A_{sprouts} - A_{blank}}{\varepsilon l} \quad (2)$$

where: A—absorbance, c—molar concentration.

2.4.4. Chromatographic Analysis

The thin-layer chromatography—TLC was used to analyze wheat sprouts extracts. Applied procedure steps (see Figure 4) were as follows:

1) preparation of TLC plate with a proper stationary phase (silica gel) and scan made by Vizualizer;
2) application of each extract by use of a semi-automatic sample applicator designed for precise and reproducible application of samples onto TLC plates;
3) development of chromatograms in a chromatographic chamber; two mixture of mobile phase were proposed acetone:chloroform (trichloromethane):water (80:20:10 v/v/v) and in case to determine 1-octacosanol the mixture contains hexane:dimethyl ether:acetic acid (85:15:2 v/v/v);

4) drying and possibly derivatization using DPPH· reagent;
5) visualization of obtained chromatograms and data analysis.

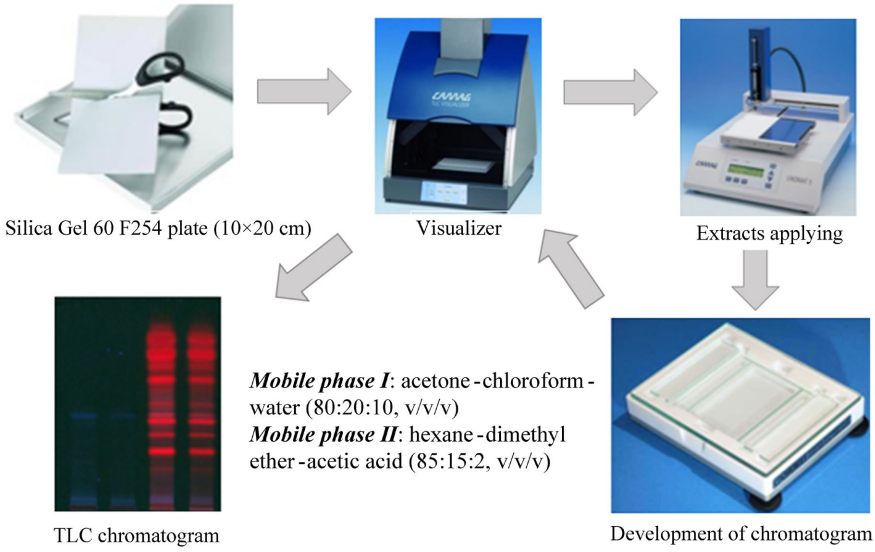

Figure 4. Steps of qualitative analysis by TLC.

2.4.5. FT-IR Analysis

The tests of samples were carried out on dry remains of extracts obtained after extraction, without their prior dissolution. Samples and 1-octacosanol standard were directly applied to the crystal of the ATR attachment, without additional chemical preparation, and then pressed in accordance with the recommendations of the instrument manufacturer. Infrared spectra recorded using an infrared spectrometer with Fourier transform (FT-IR Vertex 70 V), equipped with a diamond crystal single-reflection ATR adapter, operating in vacuum conditions. Photo diode type detectors provided spectral detectivity at all light levels. A detector was used for detection RT-FOR TGS. The analysis was carried out in the range of 4000 - 400 cm^{-1}. Background spectra were recorded before each measurement series and automatically subtracted from the sample. Data analysis and processing was carried out using software OPUS 7.5 (Bruker).

2.5. Statistical Analysis

IBM SPSS Statistics software (version 21) was utilized for hierarchical clustering and correlation analyses. Microsoft Excel 2016 and Microsoft PowerPoint 2010 were employed to prepare and assemble composite figures.

3. Results and Discussion

In general, this study focused on wheat, which is widely distributed and widely traded worldwide due to the specific properties of its protein. The wide distribution and adaptability of wheat is a reason to apply it as a diet component for human as well as for animal feed [30]. Recently, is observed growing positive perceptions about sprouted cereals, which have resulted in new food product and

beverage. Also, the impact of cereal seed sprouting on its nutritional and technological properties is an interesting scientific problem [31]-[36]. On the other hand, cereal sprouts including wheat are special case as a source of antioxidants, which show numerous beneficial effects and functions for the human body. Biologically active compounds found in sprouts participating in metabolic processes, they strengthen the immune and antioxidant systems, affect the cardiovascular, digestive, nervous and respiratory systems. Many of these ingredients have preventive and sometimes ever healing properties.

The one of main aims of this work was to examine the chemical properties of extracts obtained from wheat sprouts and particular emphasis on the content of phenolic acids having antioxidant properties. The content of many nutrients in cereals, which potentially increase the functional qualities of food and enrich the daily human diet has been examined. Methods for extracting compounds, especially phenolic acids, from wheat sprouts were used, along with chromatographic and spectroscopic techniques to analyze the quantitative and qualitative components in wheat sprout (*Triticum aestivum* L.) extracts. Important properties of extracts obtained from wheat sprouts, with particular emphasis on the content of selected phenolic acids and 1-octacosanol have been presented. The qualitative and quantitative composition of wheat sprouts gown in laboratory conditions was characterized. Moreover, the methodologies for the extraction and determination of phenolic acids and 1-octacosanol in extracts from wheat grains and sprouts based on classical and modern extraction methods as well as chromatographic and spectroscopic techniques used in qualitative and quantitative analysis have been presented. In our study, the initial stage of investigations was to determine the efficiency of extraction. The results obtained for samples subjected to complete evaporation of excess solvent are shown in Figure 5.

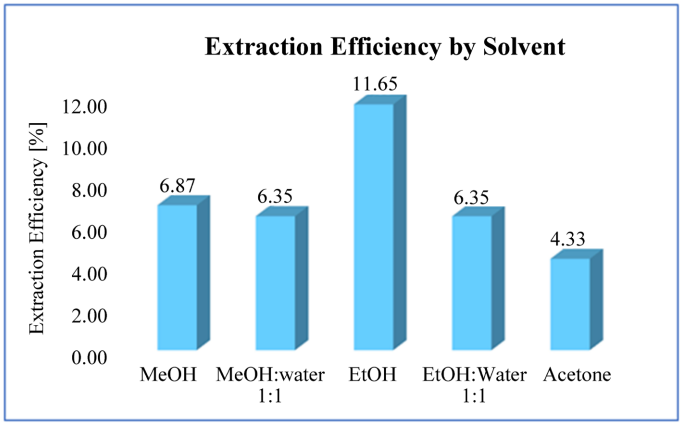

Figure 5. Extraction efficiency of wheat sprout components using maceration (24 h) with different solvents: methanol, methanol-water (50:50), ethanol, ethanol-water (50:50), and acetone.

The extraction efficiency has been calculated according to the following formula (3):

$$Extraction\ efficiency = \frac{m_{extract}}{m_{sprouts}} \cdot 100\% \quad (3)$$

where: m—dry mass of obtained extracts and wheat sprouts respectively.

Obtained results show that the average of extraction efficiency was 7.11%. The extraction efficiency depends the kind of solvent or mixture used. The highest efficiency 11.65% was observed after ethanol used. The lowest efficiency of 4.33% was achieved by acetone. For other solvents the extraction efficiency was similar ca. 6.5%. The most effective solvent used in this experiment pure ethanol (96%) was found. On the other hand, the weakest solvent was acetone, because the law effectiveness extraction of components from wheat sprouts was observed. The research therefore shows that ethanol is the best choice among the solvents used for the sample preparation of wheat sprouts.

The next step was to evaluate the total content of polyphenolic compounds in obtained extracts. We have confirmed that sprouts are enriched in bioactive compounds including polyphenols as biologically active. The results available in literature confirm the importance of polyphenolic acids as antioxidants [37]-[45].

The results of total concentration of polyphenols in wheat sprouts' extracts after maceration in methanol; methanol:water (50:50); ethanol; ethanol:water (50:50); acetone have been presented in **Figure 6**.

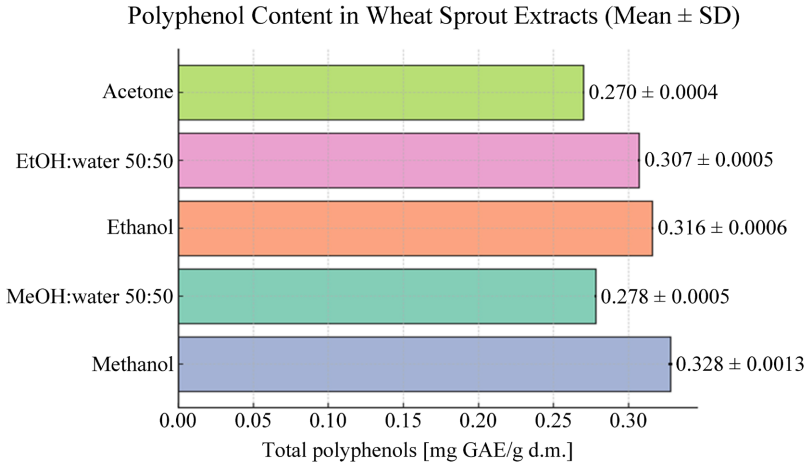

Figure 6. Total polyphenols content in wheat sprouts extracts (expressed as gallic acid equivalents, [mg GAE/g d.m.]) following maceration with various organic solvents.

Average content of polyphenolic compounds in the analyzed wheat sprouts, which was determined by the Folin-Ciocalteu reagent, falls within the range of 0.27 - 0.33 mg/g. Average it amounted to 0.3 (mg GAE)/g. The highest content of polyphenols was for the methanol extract (0.3282 ± 0.0013 mg/g), which is interesting, because the highest extraction efficiency was for the ethanol extract. However, wheat sprouts methanol extract also has the highest standard deviation from the results. The second highest result of the total content of polyphenolic compounds, similar to that for the ethanol extract, whose polyphenol content was

0.3166 ± 0.0006 mg/g, was shown by 50% ethanol extract (0.3075 ± 0.0005 mg/g). The lowest total polyphenol content was shown by wheat sprouts extract in acetone (0.2700 ± 0.0004 mg/g). In this case, it is combined with the lowest extraction efficiency. In addition, interestingly, this extract has a standard deviation closest to zero, compared to the others. On the other hand, the ethanolic extract from wheat sprouts is closest to the average value of the total content of polyphenolic compounds. The highest concentration of these compounds was shown by the methanol extract, which is interesting, because the highest extraction efficiency was obtained for the ethanol extract. On the other hand, the lowest content, which is additionally consistent with the lowest extraction efficiency, was found in wheat sprouts extracted with acetone. However, the results of the total content of polyphenolic compounds in each of the extracts are very similar, which cannot be said about the efficiency of extraction. The antioxidant activity of the tested extracts is closely related to the content of polyphenolic compounds. The radical scavenging activity (RSA) is calculated on the basis of presented above formula (1). Since the analyses for the blank have been performed, the absorbance result for the blank should be subtracted from the absorbance results obtained for the extracts in order to obtain the actual result of the concentration of polyphenols in the extracts. RSA is the capacity of compounds (antioxidants) to neutralize harmful free radicals by donating an electron or hydrogen atom, thereby preventing oxidative damage to cells and biomolecules. Mentioned process involves antioxidants intercepting unstable free radicals, stopping chain reactions that lead to cell damage, and can be measured using methods like the DPPH· assay, where a colored radical solution is decolorized by antioxidants. Antioxidant properties were tested using a multifunctional reader. The antioxidant activity of tested wheat sprouts extracts, which was determined using the synthetic DPPH· and calculated as the RSA value is presented in Figure 7. Obtained RSA values were in the range of 6% - 13%, then the average was 10.03%.

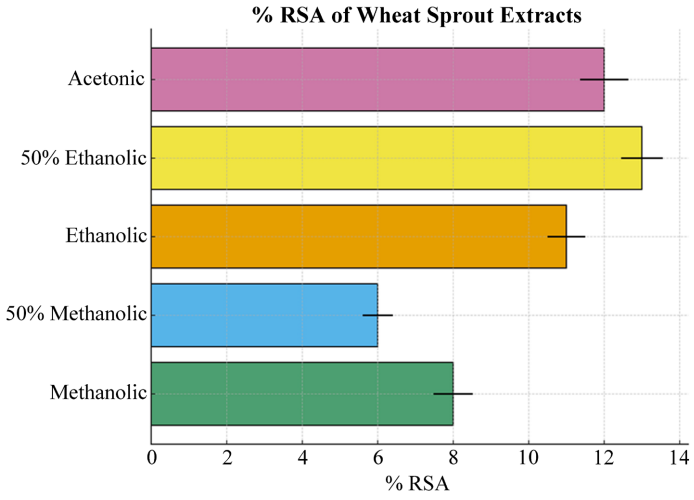

Figure 7. RSA of wheat sprouts' extracts after maceration in various organic solvents by use of DPPH· assay.

The highest RSA score was 13.07 ± 0.55%. It was obtained for the extract sample from sprouts in a 50% ethanol solution. Interestingly, the second-place results of an antioxidant ability is boasted by the extract with the lowest extraction efficiency what refers to acetone as a solvent and it was 12.03 ± 0.64%. The antioxidant capacity for the tested wheat sprouts does not depend on the type of solvent used for the extraction. The highest RSA was obtained for extracts, where 50% ethanol solution was used, while the lowest results was observed after 50% methanol solution was applied for the extraction. It can be assumed that the antioxidant activity of tested extracts is due not only to polyphenol compounds that are well soluble in methanol, but also to other components that have higher solubility in ethanol and acetone. Similar studies were performed for 1-octacosanol solutions with different concentrations of this analyte. We compared RSA for 1-octacosanol solutions in the concentrations range from 0.4 to 1.0 mg/mL (see Figure 8). Considering the obtained results, we noticed the low antioxidant activity of this long-chain alcohol. Obtained RSA did not exceed 6%. Although 1-octacosanol itself exhibits relatively low antioxidant activity, the results indicate its functional potential, especially at higher concentrations, which may be important from the point of view of functional foods and supplementation.

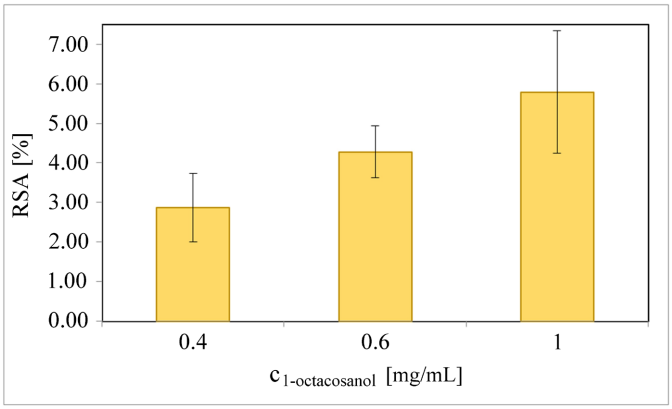

Figure 8. Antioxidant activity of 1-octacosanol (concentration range 0.4 - 1.0 mg/mL).

An application of spectroscopic methods gives possible to determine the content of natural dyes in wheat sprouts (Figure 9). This analysis showed that the tested wheat sprouts contained β-carotene and chlorophylls a and b. Wheat sprouts showed the highest concentration of chlorophyll *b* among the tested dyes (avg. 0.5401 mg/g) with a maximum for a wavelength equal to λ = 667 nm, of which the highest content was shown by ethanol extract, in which the chlorophyll *b* content was 0.9347 ± 0.0135 mg/g. On the other hand, the sprouts had the least β-carotene. Overall, the highest concentrations of plant dyes are attributed to ethanol extract (96% ethanol), which translates into the highest extraction efficiency among the tested solvents. However, the lowest content of dyes does not translate into extraction efficiency at all. Correlation analysis was run based on the amount of dyes detected, as presented in Figure 9 below.

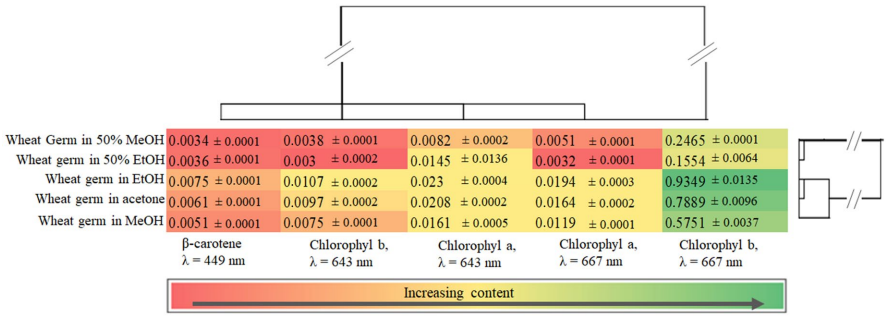

Figure 9. The content of natural dyes in wheat sprouts after maceration in various organic solvents (the content is presented with ± SD, expressed in mg/g).

The Pearson moment correlation was performed to evaluate the relationships between samples and their levels of significance. The correlation matrix was constructed based on hierarchical cluster analyses using the "average linkage between groups" method within the "squared Euclidean distance" interval, as presented in **Figure 9**. The hierarchical cluster analysis based on the solvent used (vertical part) resulted in two main clusters of equal significance, which were further divided into three subclusters with varying levels of significance. In the heat map shown in **Figure 9**, the correlation matrix is highlighted according to the clustering pattern. Regarding the correlation values, strong positive correlations were observed among the investigated samples ($r(4) = 0.998$, $p = 0.01$ to $r(4) = 0.879$, $p = 0.05$), as well as instances of no correlation. For the three detected dyes (horizontal part of the dendrogram), the hierarchical cluster analysis formed two clusters with different levels of significance, one of which was further split into three subclusters sharing the same level of significance.

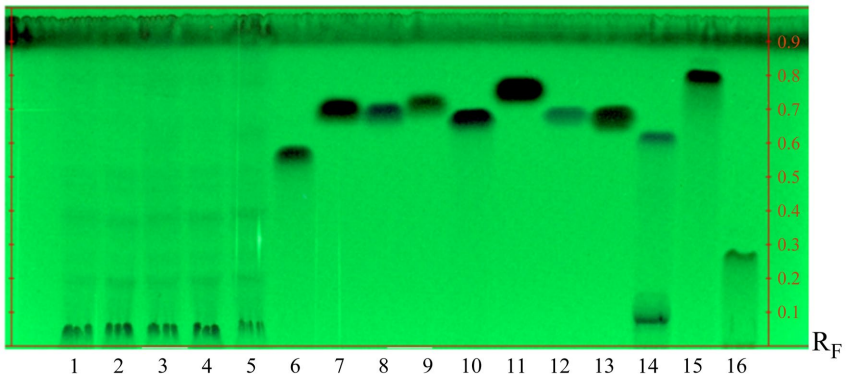

Figure 10. Visualization of chromatograms for extract samples with standards at wavelength $\lambda = 254$ nm. Order of chromatograms: 1) wheat sprouts methanolic extract; 2) wheat sprouts in 50% methanolic extract; 3) wheat sprouts in ethanolic extract; 4) wheat sprouts in 50% ethanolic; 5) wheat sprouts extract in acetone; 6) gallic acid (1 mg/mL); 7) vanillic acid (1 mg/mL); 8) ferulic acid (1 mg/mL); 9) p-coumaric acid (1 mg/mL); 10) 3,4-DHB (1 mg/mL); 11) p-HB (1 mg/mL); 12) synapic acid (1 mg/mL); 13) syringic acid (1 mg/mL); 14) chlorogenic acid (1 mg/mL); 15) quercitin (1 mg/mL); 16) rutin (1 mg/mL).

The results of the analysis of wheat sprouts extracted with various solvents by

use of TLC are presented in Figure 10. Obtained chromatograms show the presence of many components from the polyphenol group (flavonoids and polyphenolic acids) in the tested samples, particularly the content of chlorogenic acid, rutin and quercetin. Additionally, the analysis of TLC showed the presence of chlorophyll in the composition of the sprouts.

As can be seen in Figure 10, a significant amount of different polyphenolic compounds was detected at a wavelength of λ = 254 nm. However, the visualization of the chromatogram did not have the desired effect to be able to accurately identify each compound. Probably the reason for this is the low concentration of some polyphenolic compounds in the analyzed extracts. Therefore, in order to achieve the best possible effect enabling the identification of individual components found in wheat sprouts, the DPPH· reagent (derivatization after the development of chromatograms) was used. As a result of the reaction of DPPH· with polyphenolic compounds, derivatives with particularly high fluorescence at a wavelength λ = 366 nm are formed (see Figure 11).

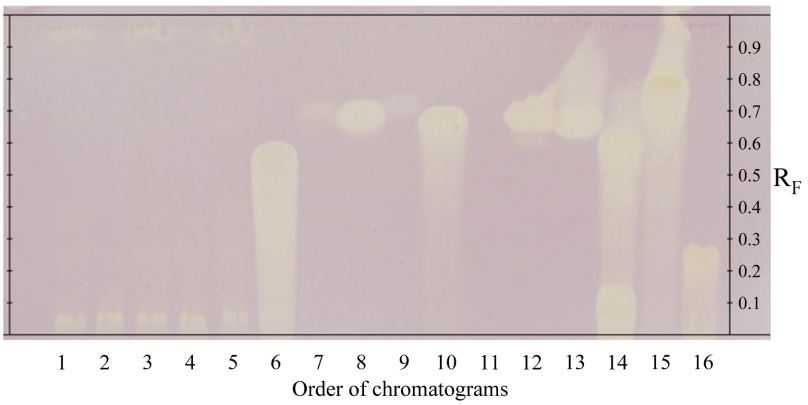

Figure 11. Visualization of samples under visible radiation after the application of DPPH· reagent.

The use of the DPPH reagent enabled better visibility of individual analytes on each chromatogram, where previously individual chromatograms were almost invisible. The results of analyses obtained with the use of TLC for wheat sprouts extracted with various solvents showed the presence of many components from the polyphenol group (flavonoids and polyphenolic acids) in the tested samples. In most cases, the most intense bands (the highest concentrations of polyphenolic compounds) showed ethanol extract (96% ethanol) and acetone extract. In analyzed extracts mainly, polyphenols were detected. Often at a concentration exceeding the concentration of the reference substance being tested, also at a comparable concentration or much lower. As example vanillic acid as well as syringic acid were not detected. It has been confirmed that among polyphenols in tested extracts the potentially highest concentrations of the following analytes were observed: chlorogenic acid (spot No. 14 on the plate, concentration of this acid in wheat sprouts extracts is much higher than 1 mg/mL), quercetin and rutin (spots

No 15 and 16, respectively, which are found in the highest concentration in ethanolic and acetone extracts, low concentration than standards), gallic acid (spot No 6, seen in 50% ethanol extract), p-coumaric acid (No 9) and 3,4-DHB (No 10) in acetone extracts were found in a similar concentration as for the standards' solutions. The follow-up of mentioned study is needed. Due to the presence of many compounds from the polyphenol group the analysis of extracts by high-performance liquid chromatography will applied in the next study.

Germinating seeds are characterized by an intensive course of metabolic processes, which can be controlled towards the production of specific biologically active compounds. Therefore, in our work, we paid attention to the chemical changes that occur in seeds during germination. One of the interest wheat components, which is found on the surface of grains, but also in sprouts is 1-octacosanol. The most visible band of 1-octacosanol was obtained at a wavelength λ = 366 nm (see Figure 12). This component was detected in each of the analyzed hexane extracts (both sprouts and wheat grain extracts). However, in sprouts (spots No 1 and 2) the concentration is clearly higher than in wheat grains (spots No 3 and 5). Qualitative analysis of the tested extracts confirms the presence of the determined compound. Estimating the total content (concentration) of 1-octacosanol in the extracts under study would also require the use of more advanced chromatographic techniques. As it is confirmed by available reference, the conditions and time of cultivation of wheat, from which polycosanol can be obtained, affect its composition, including the presence of 1-octacosanol [46].

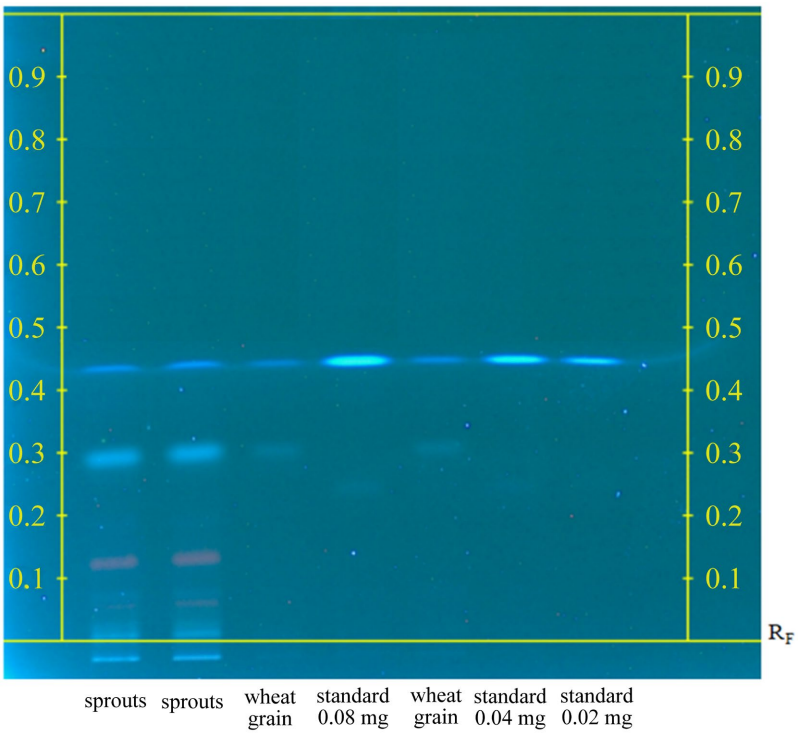

Figure 12. Visualization of TLC chromatograms of wheat sprouts and grains hexanoic extracts and 1-octacosanol standards (4 mg/mL) at wavelength λ = 366 nm.

A comparison of the FT-IR spectra for both the standard of 1-octacosanol and the individual samples is presented in Figure 13. The characteristic absorption bands along with the assignment to the appropriate functional groups for the standard and the analyzed natural samples have been compared. The obtained results after FT-IR analysis provided evidence for the presence of 1-octacosanol in analyzed samples. However, more specific confirmatory criterion and detailed spectroscopic analysis could be provided. The observed bands corresponded to the characteristic vibrations of hydroxyl, alkyl and carbonyl groups, confirming the presence of long-chain aliphatic alcohols, sugars and other bioactive compounds such as flavonoids or polyphenols. The missing some strands in the spectrum of pure 1-octacosanol (e.g. in the range of 1750 - 1500 cm^{-1}) confirmed its chemical purity of the tested standard.

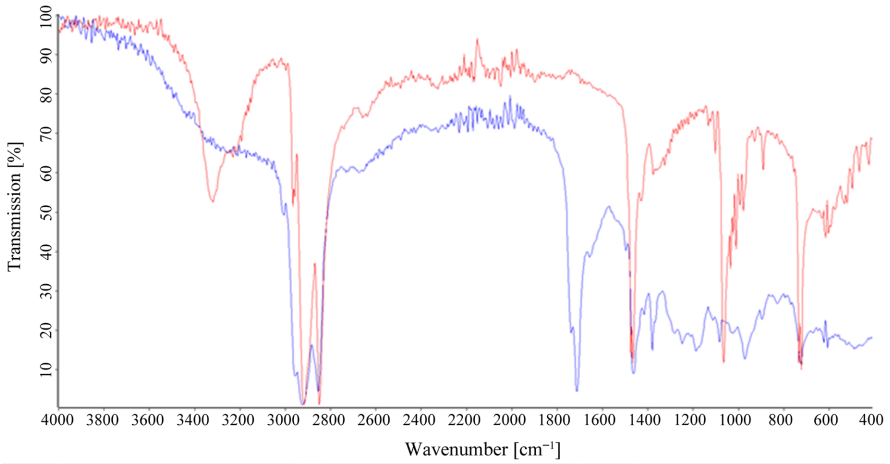

Figure 13. Example FT-IR spectra of wheat grain extract (blue) compared to 1-octacosanol standard (red).

4. Conclusions

An important component found both in sprouts and the top waxy layer covering wheat grains is 1-octacosanol. This compound is the main component of policosanol, a mixture of primary fatty alcohols with high molecular weight commonly found in eucalyptic plant waxes. It can also be found on the leaves of most cereals. TLC analysis can be applied to detect the content of 1-octacosanol in wheat in wheat grains and sprouts. However, the content of this alcohol—1-octacosanol is clearly higher in sprouts than in wheat grains.

Mentioned study conducted on sprouts of wheat (*Triticum aestivum* L.) made it possible to demonstrate the diversity of components like polyphenols, chlorophylls, carotenoids and to determine the effectiveness of various solvents for the separation of these groups of compounds. The most effective solvent for the extraction was 96% ethanol, but the extraction efficiency of mentioned ingredients did not always correlate with their content. Due to the potential use of the obtained plant extracts as dietary supplements, it is advisable to consider only extracts based on ethanol or 50% ethanol. Ethanol is strongly preferred over meth-

anol in laboratory applications primarily due to its significantly lower toxicity profile. While both alcohols are central nervous system depressants, ethanol is safely metabolized by the human body into harmless byproducts, whereas methanol converts into highly lethal toxins. The study confirmed the diversity of composition and antioxidant potential of sprouts' extracts, which is correlated into their potential health benefits. Due to the content in obtained extracts, some components like 1-octacosanol and other antioxidant nutrients potentially increase the functional qualities of food and enrich the daily human diet and sprouts of wheat could be applied as medical plants.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Owens, G. (2001) Cereals Processing Technology (Vol. 53). CRC Press. https://doi.org/10.1533/9781855736283

[2] Serna-Salvidar, S.O (2016) Cereal Grains: Properties, Processing, and Nutritional Attributes. CRC Press. https://doi.org/10.1201/9781439882092

[3] Belitz, H.-D., Grosh, W. and Schieberle, P. (2009) Food Chemistry. 4th Revised and Extended Edition. Springer-Verlag, 670-742.

[4] Venske, E., dos Santos, R.S., Busanello, C., Gustafson, P. and Costa de Oliveira, A. (2019) Bread Wheat: A Role Model for Plant Domestication and Breeding. *Hereditas*, **156**, Article No. 16. https://doi.org/10.1186/s41065-019-0093-9

[5] McKevith, B. (2004) Nutritional Aspects of Cereals. *Nutrition Bulletin*, **29**, 111-142. https://doi.org/10.1111/j.1467-3010.2004.00418.x

[6] Kulp, K. (2000) Handbook of Cereal Science and Technology, Revised and Expanded. CRC Press, 385-387. https://doi.org/10.1201/9781420027228

[7] Waliat, S., Arshad, M.S., Hanif, H., Ejaz, A., Khalid, W., Kauser, S., *et al.* (2023) A Review on Bioactive Compounds in Sprouts: Extraction Techniques, Food Application and Health Functionality. *International Journal of Food Properties*, **26**, 647-665. https://doi.org/10.1080/10942912.2023.2176001

[8] Miyahira, R.F., Lopes, J.D.O. and Antunes, A.E.C. (2021) The Use of Sprouts to Improve the Nutritional Value of Food Products: A Brief Review. *Plant Foods for Human Nutrition*, **76**, 143-152. https://doi.org/10.1007/s11130-021-00888-6

[9] Benincasa, P., Falcinelli, B., Lutts, S., Stagnari, F. and Galieni, A. (2019) Sprouted Grains: A Comprehensive Review. *Nutrients*, **11**, Article 421. https://doi.org/10.3390/nu11020421

[10] Singh, S.K., Kumar, S., Kashyap, P.L., Sendhil, R. and Gupta, O.P. (2023) Wheat. In: Ghosh, P.K., Das, A., Saxena, R., Banerjee, K., Kar, G. and Vijay, D., Eds., *Trajectory*

of 75 *Years of Indian Agriculture after Independence*, Springer, 137-162. https://doi.org/10.1007/978-981-19-7997-2_7

[11] de Sousa, T., Ribeiro, M., Sabença, C. and Igrejas, G. (2021) The 10,000-Year Success Story of Wheat! *Foods*, **10**, Article 2124. https://doi.org/10.3390/foods10092124

[12] Hasanuzzaman, M., Nahar, K. and Hossain, M.A. (2019) Wheat Production in Changing Environments. Springer.

[13] Avni, R., Nave, M., Barad, O., Baruch, K., Twardziok, S.O., Gundlach, H., *et al.* (2017) Wild Emmer Genome Architecture and Diversity Elucidate Wheat Evolution and Domestication. *Science*, **357**, 93-97. https://doi.org/10.1126/science.aan0032

[14] Lachuga, Y., Alabushev, A., Pakhomov, V., Ionova, E. and Khliystunov, V. (2019) Physico-Mechanical Characteristics of Connections and Biological Features of Separating Grain from Ear. *IOP Conference Series: Earth and Environmental Science*, **403**, Article 012050. https://doi.org/10.1088/1755-1315/403/1/012050

[15] Durante, M., Lenucci, M.S., Rescio, L., Mita, G. and Caretto, S. (2012) Durum Wheat By-Products as Natural Sources of Valuable Nutrients. *Phytochemistry Reviews*, **11**, 255-262. https://doi.org/10.1007/s11101-012-9232-x

[16] Mirek, Z., Piękoś-Mirkowa, H., Zając, A. and Zając, M. (2002) Flowering Plants and Pteridophytes of Poland. Checklist. In: Szafer, W., Ed., *Biodiversity of Poland*, Institute of Botany, Cracow, 21-230.

[17] Peng, J., Sun, D. and Nevo, E. (2011) Wild Emmer Wheat, 'Triticum Dicoccoides', Occupies a Pivotal Position in Wheat Domestication Process. *Australian Journal of Crop Science*, **5**, 1127-1143.

[18] Tidiane Sall, A., Chiari, T., Legesse, W., Seid-Ahmed, K., Ortiz, R., van Ginkel, M., *et al.* (2019) Durum Wheat (*Triticum durum* Desf.): Origin, Cultivation and Potential Expansion in Sub-Saharan Africa. *Agronomy*, **9**, Article 263. https://doi.org/10.3390/agronomy9050263

[19] Doll, H. (1977) Storage Proteins in Cereals. In: Muhammed, A., Aksel, R. and von Borstel, R.C., Eds. Genetic Diversity in Plants. *Basic Life Sciences*, Vol. 8, Springer.

[20] Niroula, A., Khatri, S., Timilsina, R., Khadka, D., Khadka, A. and Ojha, P. (2019) Profile of Chlorophylls and Carotenoids of Wheat (*Triticum aestivum* L.) and Barley (*Hordeum vulgare* L.) Microgreens. *Journal of Food Science and Technology*, **56**, 2758-2763. https://doi.org/10.1007/s13197-019-03768-9

[21] Grimm, B. and Porr, R.J. (2006) Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications. Springer, 1-26.

[22] Shewry, P.R. and Hey, S.J. (2015) The Contribution of Wheat to Human Diet and Health. *Food and Energy Security*, **4**, 178-202. https://doi.org/10.1002/fes3.64

[23] Fattal-Valevski, A. (2011) Thiamine (Vitamin B1). *Journal of Evidence-Based Complementary & Alternative Medicine*, **16**, 12-20.

[24] Bashir, R., Iqbal, S., Awais, M., Afzal, B., Shakoor, M.B. and Iqbal, M. (2023) Thiamine (Vitamin B1) Helps to Regulate Wheat Growth and Yield under Water Limited Conditions by Adjusting Tissue Mineral Content, Cytosolutes and Antioxidative Enzymes. *Plant Growth Regulation*, **101**, 629-642. https://doi.org/10.1007/s10725-023-01045-6

[25] Goyer, A. (2010) Thiamine in Plants: Aspects of Its Metabolism and Functions. *Phytochemistry*, **71**, 1615-1624. https://doi.org/10.1016/j.phytochem.2010.06.022

[26] Biller, J. and Ferro, J.M. (2014) Neurologic Aspects of Systemic Disease: Part II—Hydrosoluble Vitamins. Elsevier, 891-914.

[27] Zheng, J., Wang, X., Wu, B., Qiao, L., Zhao, J., Pourkheirandish, M., *et al.* (2022)

Folate (Vitamin B9) Content Analysis in Bread Wheat (*Triticum aestivum* L.). *Frontiers in Nutrition*, **9**, Article ID: 933358. https://doi.org/10.3389/fnut.2022.933358

[28] Gharibzahedi, S.M.T. and Jafari, S.M. (2017) The Importance of Minerals in Human Nutrition: Bioavailability, Food Fortification, Processing Effects and Nanoencapsulation. *Trends in Food Science & Technology*, **62**, 119-132. https://doi.org/10.1016/j.tifs.2017.02.017

[29] Chen, J.T., Wesley, R., Shamburek, R.D., Pucino, F. and Csako, G. (2005) Meta-Analysis of Natural Therapies for Hyperlipidemia: Plant Sterols and Stanols versus Policosanol. *Pharmacotherapy*: *The Journal of Human Pharmacology and Drug Therapy*, **25**, 171-183. https://doi.org/10.1592/phco.25.2.171.56942

[30] Shewry, P.R. (2009) Wheat. *Journal of Experimental Botany*, **60**, 1537-1553. https://doi.org/10.1093/jxb/erp058

[31] Lemmens, E., Moroni, A.V., Pagand, J., Heirbaut, P., Ritala, A., Karlen, Y., *et al.* (2018) Impact of Cereal Seed Sprouting on Its Nutritional and Technological Properties: A Critical Review. *Comprehensive Reviews in Food Science and Food Safety*, **18**, 305-328. https://doi.org/10.1111/1541-4337.12414

[32] Nonogaki, H., Barrero, J.M. and Li, C. (2018) Editorial: Seed Dormancy, Germination, and Pre-Harvest Sprouting. *Frontiers in Plant Science*, **9**, Article ID: 1783. https://doi.org/10.3389/fpls.2018.01783

[33] Joshi, R. (2018) Role of Enzymes in Seed Gemination. *International Journal of Creative Research Thoughts*, **6**, 1481-1485.

[34] Weitbrecht, K., Müller, K. and Leubner-Metzger, G. (2011) First off the Mark: Early Seed Germination. *Journal of Experimental Botany*, **62**, 3289-3309. https://doi.org/10.1093/jxb/err030

[35] Han, C. and Yang, P. (2015) Studies on the Molecular Mechanisms of Seed Germination. *Proteomics*, **15**, 1671-1679. https://doi.org/10.1002/pmic.201400375

[36] Bewley, J.D. and Black, M. (1994) Seeds: Physiology of Development and Germination. 2nd Edition, Springer Science & Business Media, 1-115. https://doi.org/10.1007/978-1-4899-1002-8

[37] Kumar, N. and Goel, N. (2019) Phenolic Acids: Natural Versatile Molecules with Promising Therapeutic Applications. *Biotechnology Reports*, **24**, e00370. https://doi.org/10.1016/j.btre.2019.e00370

[38] Verma, B., Hucl, P. and Chibbar, R.N. (2009) Phenolic Acid Composition and Antioxidant Capacity of Acid and Alkali Hydrolysed Wheat Bran Fractions. *Food Chemistry*, **116**, 947-954. https://doi.org/10.1016/j.foodchem.2009.03.060

[39] Verma, S., Singh, A. and Mishra, A. (2013) Gallic Acid: Molecular Rival of Cancer. *Environmental Toxicology and Pharmacology*, **35**, 473-485. https://doi.org/10.1016/j.etap.2013.02.011

[40] Graf, E. (1992) Antioxidant Potential of Ferulic Acid. *Free Radical Biology and Medicine*, **13**, 435-448. https://doi.org/10.1016/0891-5849(92)90184-i

[41] Boz, H. (2015) *P*-Coumaric Acid in Cereals: Presence, Antioxidant and Antimicrobial Effects. *International Journal of Food Science & Technology*, **50**, 2323-2328. https://doi.org/10.1111/ijfs.12898

[42] Kiliç, I. and Yeşiloğlu, Y. (2013) Spectroscopic Studies on the Antioxidant Activity of P-Coumaric Acid. *Spectrochimica Acta Part A*: *Molecular and Biomolecular Spectroscopy*, **115**, 719-724. https://doi.org/10.1016/j.saa.2013.06.110

[43] Pandi, A. and Kalappan, V.M. (2021) Pharmacological and Therapeutic Applications of Sinapic Acid—An Updated Review. *Molecular Biology Reports*, **48**, 3733-3745.

https://doi.org/10.1007/s11033-021-06367-0

[44] Meng, S., Cao, J., Feng, Q., Peng, J. and Hu, Y. (2013) Roles of Chlorogenic Acid on Regulating Glucose and Lipids Metabolism: A Review. *Evidence-Based Complementary and Alternative Medicine*, **2013**, Article ID: 801457. https://doi.org/10.1155/2013/801457

[45] Naveed, M., Hejazi, V., Abbas, M., Kamboh, A.A., Khan, G.J., Shumzaid, M., *et al.* (2018) Chlorogenic Acid (CGA): A Pharmacological Review and Call for Further Research. *Biomedicine & Pharmacotherapy*, **97**, 67-74. https://doi.org/10.1016/j.biopha.2017.10.064

[46] Ra, J., Woo, S., Lee, K., Lee, M.J., Kim, H.Y., Ham, H.M., *et al.* (2020) Policosanol Profiles and Adenosine 5'-Monophosphate-Activated Protein Kinase (AMPK) Activation Potential of Korean Wheat Seedling Extracts According to Cultivar and Growth Time. *Food Chemistry*, **317**, Article 126388. https://doi.org/10.1016/j.foodchem.2020.126388