



## Nutritional and antioxidant profiling of Algerian rapeseed seeds (*Brassica napus* L.) and its cold-pressed by-products

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### KEY CONTRIBUTION

Cold extraction enriched rapeseed cakes with proteins and minerals, particularly potassium, optimizing their nutritional value for animal feed. Acetone and methanol allowed targeted extraction of phenolic and flavonoid compounds, enhancing antioxidant activity, particularly in seeds. Finally, the discovery of gossypin, an antioxidant flavonoid, enriches the functional profile of Algerian rapeseed.

### ABSTRACT

This study investigated the "Zitna" variety of rapeseed seeds (*Brassica napus* L.) cultivated in Algeria and their by-product cakes used for animal feed after cold extraction. Physicochemical parameters, mineral profile, phenolic compounds (extracted with acetone, methanol, ethanol, and water), and antioxidant activity by DPPH free radical scavenging were analysed. The methanolic extract of the Zitna variety were analysed using HPLC. The results showed minor differences in physicochemical composition. The protein content was  $3.79 \pm 0.01$  mg/mL in the seeds and  $4.92 \pm 0.04$  mg/mL in the cakes, reflecting an increased protein concentration in the by-products due to the extraction process. Mineral concentrations were determined using the flame atomic absorption spectroscopy (FAAS) technique, which revealed that cakes had higher levels of essential minerals than the seeds and the most abundant element was potassium (K) with a concentration  $(27.2 \pm 0.8 \text{ mg/L})$ . The highest total phenolic content (TPC) was observed in acetone extracts, with seeds having  $67.7 \pm 4.3 \text{ mg GAE/g}$  and cakes  $61.3 \pm 1.1 \text{ mg GAE/g}$ . Methanol extracts showed the highest total flavonoid content (TFC), with seeds containing  $23.5 \pm 0.6 \text{ mg QE/g}$  and cakes  $18.5 \pm 0.6 \text{ mg QE/g}$ . Antioxidant activity, measured by  $IC_{50}$  values, was higher in seeds compared to cakes, with acetone extracts having  $IC_{50}$  values of  $6.5 \text{ mg/mL}$  for seeds and  $14.2 \text{ mg/mL}$  for cakes. The negative correlations were detected between  $IC_{50}$  and phenolic contents in seeds ( $-0.98$ ;  $-0.97$ ) and cakes ( $-0.98$ ;  $-0.990$ ) respectively. The chromatogram of the 'Zitna' rapeseed extract showed that sinapic acid is the major compound, with antioxidant activity enhanced by the novel discovery of gossypin, a flavonoid with antioxidant and anti-inflammatory properties. These findings demonstrate that cold-pressed extraction of Algerian rapeseed seeds preserves and concentrates valuable nutritional and antioxidant properties in the by-products, making them suitable for various industrial applications.



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## Introduction

After soybeans, rapeseed (*Brassica napus* L.) is the second-largest oilseed crop in the world. It contains beneficial substances with proven health and nutritional benefits, such as phenols and fatty acids (Guirrou et al., 2023). In recent years, rapeseed production has seen notable growth, far outpacing that of many other oilseed crops, with an increase of around 50%. According to the Food and Agriculture Organisation of the United Nations (FAO), Canada is the world's leading producer of rapeseed, followed by China, India, France, and Australia (Goyal et al., 2021).

Rapeseed (*Brassica napus* L.) is a plant that is mostly farmed for its oil and belongs to the oleaginous cinerous species (Zago et al., 2015). Considerable waste and by products are produced in the process of getting vegetable oils.

These vegetable oil industry leftovers are significant because they include high value-added chemicals and are a great source of bioactive ingredients like antioxidants (Multescu et al., 2022). A thorough knowledge of the rapeseed microstructure is necessary to address these challenges. Light microscopy (LM) and scanning electron microscope (SEM) were used to study the surface topography and detailed histology of the seeds (Tileuberdi et al., 2024). The anatomy and histology of the seeds can be used to assess decortication and other processing techniques, such as milling. Rapeseed exhibits moderate tolerance to salinity; however, high concentrations of soluble salts significantly limit its production in arid and semi-arid regions. Consequently, effective management of saline soils is crucial to sustain the rapeseed production in these areas (Naveed et al., 2020).

Mineral elements are abundant in oilseeds. In general, adequate mineral consumption is required for humans to have a vibrant existence (Ibourki et al., 2021). Furthermore, due to the fact that dietary minerals are engaged in several pathways in fundamental biological processes, maintaining their equilibrium in the human body is extremely important (Grillo et al., 2019).

Antioxidants are substances that have the ability to eliminate free radicals due to their hydrogen redox donor and singlet oxygen quencher properties. Phytochemicals such as phenolics, flavonoids and tannins have antioxidant potential (Nawaz et al., 2018).

Although synthetic antioxidants are available on the market, natural antioxidant products are still preferred because they are considered safer and have fewer side effects. Given its advantageous nutritional qualities and significantly higher phenolic content concentration as compared to other oilseeds, canola (*Brassica napus* L.) is highly sought after for processing into vegetable oils and animal feed (Jun et al., 2014).

Today, the oilseed sector is facing new challenges to develop new processing and value-adding technologies and valorisation technologies (Zago et al., 2015). Phenolic chemicals are similarly abundant in rapeseed meal. In fact, as some researches have shown, (Cai and Arntfield, 2001), tested procedures for the extraction of phenolic compounds from rapeseed seeds and cakes showed that the best yield of phenolic compounds (102.1  $\mu\text{mol}$  GAE /g meal of total phenolic content) was obtained when the extraction was carried out with a methanol/water solution (70/30, v/v) at 75°C for 20 minutes. The majority of research on the phenolic components of rapeseeds has employed the HPLC method (Cai and Arntfield, 2001; Thiyam et al., 2006).

This work aims to contribute to the study of the structural composition and nutritional variability of rapeseeds supplied by ITGC from Sidi Bel Abbes in Algeria and their co-products from mechanical extraction.

## Materials and methods

### Chemicals and reagents

Folin-Ciocalteu reagent, gallic acid (GA) ≥99%, DPPH (2,2-diphenyl-1-picrylhydrazyl), 95%, sodium nitrite ( $\text{NaNO}_2$ ) ≥97%, sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) ≥99.5%, aluminium chloride ( $\text{AlCl}_3$ ) ≥98%, and all organic solvents (methanol, 99.9%, ethanol 99.8%, acetone, 99.9%, and nitric acid 65%,) were purchased from Sigma Aldrich, Germany.

### Plant material

The plant material used in this study was composed of an Algerian rapeseed variety "ZITNA" provided in appropriate quantities by the Technical Institute of Field Crops (ITGC) of Sidi Bel Abbes and stored in vacuum-sealed 2.5 kg bags at 4°C. This ensures that the same sample of raw material was available throughout the trials, thus eliminating the effects of variety, season, and harvest related to the variability of the raw material "rapeseed".

### Optical microscopic observation (O.M.)

Microscopic analysis of rapeseed was carried out using an optical (or photonic) microscope at three levels of magnification (x10, x40, and x100). In order to prepare the slides, the seeds were dehydrated using a series of organic solvents, then embedded in kerosene, cut with a microtome, and stained with hematoxylin (Stanley et al., 1976). Observations were made using an optical microscope (Optika B-350), and photos were captured using a digital camera (Optikam B5).

### Physicochemical analyses of rapeseed and cake

All the tests were performed in triplicate and the means were reported.

### Determining Moisture Content

The moisture content is determined by raising the product's (about  $5\text{g} \pm 0.01\text{g}$  each) temperature to approximately  $105^\circ\text{C} \pm 2^\circ\text{C}$  while maintaining a nearly constant mass in an isothermal oven at atmospheric pressure (AOAC, 1995). The water and volatile matter content, expressed as a percentage by mass, is calculated using the following formula:

$$H\% = \frac{M1 - M2}{M1 - M0} \times 100 \quad (1)$$

$M0$  = the mass, in grams, of the empty capsule,

$M1$  = the mass, in grams, of the capsule and test sample before drying,

$M2$  = mass, in grams, of the capsule and test portion after drying.

Dry matter rate (ms %) is given by the following formula:

$$Ms \% = 100 - H\% \quad (2)$$

### Determining ash content

A 2 g test portion is incinerated at  $550 \pm 15^\circ\text{C}$  in an electrically heated muffle furnace until a practically constant weight is obtained (Audigié et al., 1984).

The crude ash content, expressed as a percentage by mass, is calculated using the following formula:

$$Mc\% = \frac{M2-M0}{M1-M0} \times 100 \quad (3)$$

M0 is mass in grams of the incineration capsule,

M1 is mass in grams of the incineration capsule loaded with the test sample,

M2 is mass in grams of incineration capsule loaded with ash.

#### *Protein Content (PC)*

To determine the protein concentration in rapeseed and rapeseed meal, the Bradford spectrometric method was used (Kalaydzhiev et al., 2018). This method is based on the colour change of Coomassie Blue upon binding with basic amino acids and hydrophobic residues of amino acids present in the proteins. The method relies on the change in absorbance at 595 nm observed after the addition of 5 mL of acidic Coomassie Brilliant Blue G-250 solution to 100 µL of the diluted extract. After homogenization and incubation for 5 to 30 minutes in the dark, the absorbance of the protein-dye complex at 595 nm allows for precise quantification of the protein content in the sample. Concentrations were calculated from the linear curve using BSA (bovine serum albumin) as standard at 1 g/L expressed in mg/mL.

#### *Determining of raw fibre (Weende method)*

The determination of gross cellulose was performed according to Weende the AOAC (Sullivan et al., 1993). Samples were dried in an oven at 105-110°C for a few hours. A 1g sample was ground to a fine powder using a Moulinex grinder, sieved at 1 mm, and placed in a glass crucible with a fritted glass bottom. Next, 150 mL of 1.25% sulfuric acid and 3-5 drops of n-octanol as an anti-foam agent were added to the sample. The mixture was boiled for 30 minutes. After boiling, the sulfuric acid was drained, and the solid samples were washed three times with 30 mL of hot distilled water. Following the sulfuric acid treatment, 150 mL of 1.25% preheated potassium hydroxide and 3-5 drops of an anti-foam agent were added. The samples were again boiled for 30 minutes. After this treatment, the contents of the crucible were washed three times with 25 mL of acetone. The dry weight of the samples was determined after drying in an oven at 105°C for an hour and cooling in a desiccator. To determine the ash content, the samples were combusted for 4 hours at 550°C in a high-temperature oven. After cooling for an hour in a desiccator, the residue was weighed. The raw fibre content was then calculated using the formula:

$$\% \text{ raw fibre} = \frac{F1-F2}{F0} \times 100 \quad (4)$$

F0 is the dry sample weight,

F1 is the weight of the crucible with residue,

F2 is the weight of the crucible with residues close to ashes.

This method is based on the solubilisation of non-cellulosic compounds in sulfuric acid and potassium hydroxide solutions.

#### *Oil Content (OC)*

The principle involves performing an extraction with an organic solvent using a 250 mL Soxhlet apparatus. The flour is defatted by the passage of solvents. It is estimated that the extraction will be

completed after 6 hours (AOAC, 1995). Two solvents used are hexane and petroleum ether. Once the extraction is finished, the solvents are removed using a rotary evaporator, and the extraction yield (oil) is calculated by gravimetry.

#### *Determination of essential minerals Na, K, Mg, Zn and Fe in the Brassica napus L. seed and cake samples*

The biological system requires certain metals, including Fe, Zn, K, Na, and Mg (Soylak et al., 2004). The tests were carried out by (Rodier et al., 1996) for the Na, K, Mg, Zn, and Fe contents. For mineralization, a 0.5 mg sample of seed and cake mix it with 25 mL of water, 6 mL of hydrochloric acid, and 2 mL of nitric acid, at to boiling for 120 minutes, let cool, filter through a 0.45 µm filter, then dilute the filtrate to 100 mL. The analyses were carried out at the Scientific Directorate of the Technical Research Center for Physico-Chemical Analyses (CRAPC). The concentrations of sodium (Na), potassium (K), magnesium (Mg), iron (Fe), and zinc (Zn) were determined by further analysis using an Agilent 240FS/240Z flame atomic absorption spectroscopy (FAAS). Ranges of standard curves (5 points) were selected corresponding to the expected concentrations for all the elements present in the *Brassica napus* L. seed and cake samples. The concentrations are expressed in mg/L. Additionally, they are described as the mean and standard deviation.

#### *Secondary metabolites of rapeseed seed and cake*

##### *Extract Preparation*

The extraction method applied to rapeseed and cake powders involves cold maceration. A 10 g quantity of powder was macerated in 100 mL of solvents for 24 hours, using various solvents (acetone, ethanol and methanol) at 70% and aqueous solutions. After this period, the mixture was filtered and the solvent removed by rotary evaporator at 45°C (Hussain et al., 2022).

##### *Extraction yield calculation*

The yield is expressed as a percentage relative to the weight of the starting material and is determined by the following equation:

$$R\% = \frac{P_1}{P_0} \times 100 \quad (5)$$

R is yield in %,

P1 is the mass of the extract after evaporation of the solvent in g,

P0 is the mass of the plant sample in g.

##### *Determination of TPC*

The Folin-Ciocalteu method stated by (Singleton and Rossi, 1965) was used to determine the total phenolic contents of rapeseed extracts. After 200 µL of the various extracts are mixed with 1 mL of 10-fold diluted Folin reagent and 0.8 mL of 7.5% sodium carbonate. After 30 minutes of incubation at room temperature, total polyphenols were measured by absorbance at 765 nm using a UV-visible Spectrophotometer (Optizen POP/KLAB, South Korea). A calibration curve was run simultaneously under the same experimental conditions, using gallic acid at various concentrations. Results are expressed in milligram equivalents of gallic acid per gram (mg GAE/g).

*Determination of TFC*

The technique used to identify the flavonoids involved the development of an extremely stable combination between aluminium chloride and the oxygen atoms found on the flavonoids' carbons 4 and 5. The procedure followed is as outlined in (Zhishen et al., 1999). For the flavonoid content of samples, 500 µL of each extract was mixed with 1500 µL of deionized water (Milli-Q system, Millipore, France), followed by the addition of 150 µL of 5% sodium nitrite. The mixture was incubated in the dark at room temperature for 5 minutes. After this incubation, 150 µL of 10% aluminium (III) chloride (AlCl<sub>3</sub>) were added, and the mixture was reincubated under the same conditions for 6 min. In the next step, 500 µL of 4% NaOH was added to the mixture, followed by stirring to homogenize. Absorbance was determined at 510 nm against a blank. At the same time, a calibration curve was run using quercetine as a positive control, with results expressed as milligram equivalents of quercetin per gram (mg QE/g).

*DPPH radical scavenging activity assay*

The antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method based on the quantification of free radical scavenging activity of the extracts described by (Samarth et al., 2008), with some modifications. A methanolic solution of DPPH (0.2 mM) was prepared. Various solvent fractions of seed extracts were diluted with methanol and added to 0.8 mL of the DPPH solution, then incubated in darkness for 30 minutes. The absorbance was measured against a blank at 517 nm using an Optizen POP/KLAB spectrophotometer. The percentage inhibition of DPPH free radicals was calculated using the following formula:

$$\text{Inhibition of DPPH\%} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (6)$$

The results were presented as the mean of three measurements for each sample. The IC<sub>50</sub> value represents the concentration of the sample that can scavenge 50% of the DPPH free radicals. It was determined graphically for each extract from the curve showing the percentage reduction of DPPH relative to concentration.

*HPLC analysis*

The HPLC analysis was performed using an Agilent 1260 Infinity system (Agilent technologies, USA) with a UV/Vis detector set at 254 nm and controlled by Chem Station software. Sample injection was done with a 20 µL injection loop. The chromatographic separation was carried out on a C18 column from KNAUER, measuring 250 mm in length, with an internal diameter of 4.6 mm and a particle size of 5 µm. The mobile phase consisted of two solvents: P.A: Water containing 1% acetic acid, P.B: Methanol (HPLC grade).

The gradient program started with 95% P.A and 5% P.B at 0 minutes, then gradually changed to 5% P.A and 95% P.B at 55 minutes. This gradient was maintained for 2 minutes before returning to the initial condition of 95% P.A and 5% P.B at 59 minutes. Compound detection was performed at a wavelength of 254 nm, with a constant flow rate of 1 ml/min. This setup is ideal for the separation of both polar and non-polar compounds, allowing precise and reproducible analysis under the described conditions. Phenolic compounds present in the methanolic extract of rapeseed were identified by comparing their retention times and UV spectra obtained from HPLC analysis with those of reference standards and literature data.

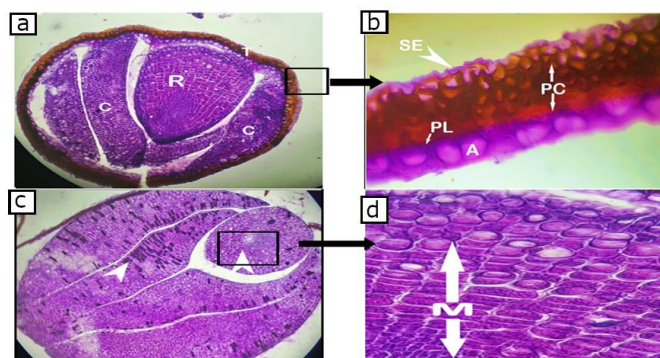
### Statistical analysis

All test were carried out in triplicate, and results were expressed as mean  $\pm$ SD (standard deviation). Data were analysed by student's test and the two way analysis of variance (ANOVA) followed by Sidak's multiple comparisons test to assess the comparison between mean values at the significance level 5 % ( $p \leq 0.05$ ). Correlations between items were assessed by the Pearson correlation test ( $r$ ). Comparison of polyphenol concentrations and  $IC_{50}$  of *Brassica napus* L. seeds and cakes was also carried out. The data analysis was performed using GraphPad Prism 8.0.2 statistical software.

## Results and discussion

### Microscopic observation of rapeseed seeds

Many previous studies have examined and focused on the structure and composition of rapeseed seeds to research and have precise information on the identification and distribution of nutrients in the different compartments of the seed (Yiu et al., 1982). Optical microscopy analysis of rapeseed seeds revealed their maturity, and several authors (Barthet et al., 2011; Carré and Loison, 2021) have studied their complete structure, identifying the seed coat (tegument) and the embryo (Figure 1). The tegument completely encloses the embryo but not the endosperm, and the two mature cotyledons surround the radical (Figure 1a). This encapsulation indicates that the seeds are fully developed and ready for germination under appropriate conditions. The tegument of rapeseed is composed of several layers: the epidermis, the palisade layer, and the aleurone layer (Figure 1b). The epidermis is the outermost layer providing protection. The palisade layer, which occupies a significant portion of the tegument's width, consists of elongated, tightly packed cells providing structural integrity and it defends against physical damage and pathogens. The aleurone layer, located beneath the palisade layer, comprises a single line of cells, but represents a significant portion of the tegument. This structure is consistent with the observations of Hu et al. (2013).



**Figure1.** Cross section of rapeseed observed under an optical microscop (x40magnification). a) rapeseed: (R) radicle, (T) tegument or testa(C) cotyledons. b) Tegument composed: (SE) sub-epidermis, (PL) pigment layer, (A) aleurone layer and (PC) palisade cells. c) glycoprotein abundant in cotyledons. d)(M) meristematic tissue (deficient in glycoprotein).

The cells within these layers contain various essential components. Oil bodies, found abundantly in the cells of the aleurone layer, radicle, and cotyledons, are spherical and vary in size, with the majority being small. They occupy a large part of the cell surface in cotyledonary cells, a moderate part in aleurone cells, and a smaller part in radicular cells. Alongside oil bodies, protein bodies are observed in the same cells, contributing to the seeds nutritional content. Glycoproteins are abundant in certain cells, particularly those corresponding to the cotyledons (Figure 1c), indicating richness in

protein-carbohydrate complexes. We also noted that the meristematic tissue is deficient in glycoprotein (Figure 1d).

The nutrient distribution is also notable. Cotyledonary cells are rich in oil and protein bodies, indicating their role in providing energy and nutrients during seed germination (Hu et al., 2013). Aleurone cells, while containing moderate amounts of oil bodies, play a crucial role in storing proteins and enzymes that aid in seed germination and early seedling growth. Radicular cells, though having fewer oil bodies compared to cotyledonary and aleurone cells, still contribute to the overall nutrient storage within the seed (Yiu et al., 1982). In summary, the microscopic analysis of rapeseed seeds reveals a detailed and structured composition. The seed coat, with its multiple protective layers, encases the embryo, which is rich in nutrient reserves. Oil and protein bodies, along with glycoproteins, are distributed throughout the cotyledons, radicle, and aleurone layer, providing essential nutrients for the seed's development and germination (Stanley et al., 1976). The complex structure and nutrient density of rapeseed make it a valuable crop for various applications.

### *Results of physicochemical analyses of rapeseed and cake*

#### *The moisture content results*

The moisture content value of rapeseed seeds and cakes is significant as it provides insight into the product's stability during storage (Mazaheri et al., 2019). The higher the moisture content, the faster the constituent elements degrade during the storage of raw materials and by-products. The average moisture content of our samples is  $3.6 \pm 0.6\%$  for seeds and  $4.8 \pm 0.8\%$  for cakes, with 96.4% and 95.2% dry matter (DM) content, respectively, which enhances product preservation and commercialization. Our results are lower than those reported by (Öztürk et al., 2022), by 7.4%, and higher than those found by (Gagour et al., 2022). The variation in moisture content observed between our samples and the literature could be attributed to differences in genetics, storage conditions, temperature, cultivation regions, and various other factors among oilseeds (Kirkegaard et al., 2018). The moisture content in rapeseed oilcake increases after oil extraction due to water absorption by the solid matrix. The oilcake's porous structure allows it to retain more moisture from the environment.

**Table 1.** Results of physicochemical composition of rapeseed seed and cake: Values are expressed as mean  $\pm$  SD.

Parameters	Seeds	Cakes
Moisture content (%)	$3.63 \pm 0.59$	$4.81 \pm 0.77$
Dry matter	96.37	95.19
Ash content (%)	$3.82 \pm 0.06$	$6.11 \pm 0.41$
Protein content (mg/mL)	$3.79 \pm 0.01$	$4.92 \pm 0.04$
Fiber (NDF) (%)	$0.18 \pm 0.02$	$0.25 \pm 0.01$
Oil yield (%)		
Hexane	44.20	30.3
Petroleum ether	37.72	28.34

After oil extraction, the cake structure may be more porous and able to absorb more water from the environment. Solid residues can retain moisture more effectively than whole seeds, which are more compact and less permeable. Processing conditions during extraction, such as temperature and



pressure, can affect the moisture content of oilcake. For example, if extraction takes place at high temperature, this may result in an initial loss of moisture, but once cooled, the oilcake may reabsorb moisture from the ambient air.

#### *Ash content*

The mineral content of rapeseed seeds and cakes was determined after incineration, and the resulting greyish ash represents various mineral substances. The ash content of rapeseed seeds and cakes is  $3.82 \pm 0.06\%$  and  $6.11 \pm 0.41\%$ , respectively. These results are similar to those obtained by (Alhomodi et al., 2021) and (Jaafar et al., 2019), which reported  $3.8 \pm 0.12\%$ , and to those reported by Gagour (2022) for seeds. However, the ash content of cakes is lower than that reported by (Laguna, 2019), which is  $7.9 \pm 1.0\%$ . Some variations in the values are strongly linked to agro-ecological conditions across countries (Hussain et al., 2022). The increase in total ash in rapeseed meal compared to seed after oil extraction can be explained by the following reasons: The oil extracted from rapeseed is mainly lipid-based and contains no inorganic matter. Removing this oily component means that the inorganic substances initially present in the seeds are concentrated in the solid residue. In summary, the increase in total ash in rapeseed meal after oil extraction is explained by the relative concentration of inorganic components (ash) in the solid residue once the oil has been removed.

#### *Protein Content (PC)*

Proteins, whatever their origin, all have the same role to play in the body. They have a structural role, because they are involved in most of our physical functions (Mansouri et al., 2018). Proteins are located in the cotyledons and the embryonic axis of the seed, with only a small amount present in the integument (Jithender et al., 2019). The protein content of *Brassica napus* L. seeds was  $3.79 \pm 0.009$  mg/mL and  $3.92 \pm 0.04$  mg/mL for cakes by the Bradford method (Table 1). The protein content in cakes is marginally higher than in seeds. Rapeseed is often processed for its oil, and the remaining seed meal (cakes) is used as a protein-rich animal feed. The slightly lower protein content in seeds could be due to the presence of more oil, which is extracted during processing. After oil extraction, the remaining cake has a higher protein concentration. This is explained by the fact that the process of oil extraction likely concentrates the protein content in the cake. The protein content obtained seems quite strong and it agrees with previous studies that reported values of 20.12% and 29.22 in rapeseed seed of Zhang et al. (2019). According to (Leming and Lember, 2005) the crude protein content of cold-pressed rapeseed cake was 30.6%.

#### *Determination of crude fibres*

Fibres are one of the undesirable and antinutritional factors of rapeseed. A high fibre content reduces its energy value (Axentii and Codină, 2024). Fibre consists of biopolymers such as cellulose, non-cellulosic polysaccharides, and lignin, which decrease meal digestibility. It is mainly located in the husks of rapeseed, and the dehulling the seeds can help reduce fibre content. Crude fibre plays an important role in nutrition, contributing to digestive health and offering various physiological benefits. It has several health advantages, including reducing the risk of chronic conditions such as heart disease, type 2 diabetes, and certain cancers, particularly colon cancers (Benguella et al., 2022). Fibre is mostly found in the seed coats of rapeseed (Laguna, 2019). In this study, we compared the crude fibre content in whole rapeseed and oilcake obtained after oil extraction. The results indicate that crude fibre is significantly higher in oilcake ( $0.25 \pm 0.01\%$  DM) than in whole seeds ( $0.18 \pm 0.02\%$  DM). The significant increase in crude fibre in the meal compared to whole seeds is primarily due to the concentration effect.

During oil extraction, a large portion of the seed mass, mainly oil, is removed. Since oil does not contain fibre, the remaining fibrous components in the solid residue (cake) become more concentrated. According to Jiang et al. (2015) there was a substantial difference in crude fibre content between black-seeded and yellow-seeded rapeseed meal (4.56% vs. 8.86% DM). The crude fibre content dropped to 3-5% in dehulled rapeseeds, which have a higher oil content and lower crude fibre content. A comparison between the twin-screw press to the conventional single-screw press shows a higher compression ratio in the former (Huang et al., 2005). Additionally, Leming and Lember (2005) reported that crude fibre in cold-pressed rapeseed cake was 13.1%. This characteristic makes rapeseed cakes particularly valuable for nutritional and industrial applications where a high fibre content is desirable.

### *Oil yield*

A key factor in determining how well oilseeds are used to produce oil is their oil content. Oil yield is a critical component of a seed's worth since it determines the seed's monetary value in the commercial oilseed trade. The yields of the two samples (Table 1) at different solvent hexane and petroleum ether respectively were 44.20% 37.72% (seeds) and 30.3% 28.34% (oilcake). The results clearly indicated that our oilseeds could be considered an excellent alternative source of oil and the n hexane is the best solvent for oil extraction. Moreover, this study on oil content was similar to those published by (Kirkegaard et al., 2018), who reported an average oil content of 42.6 to 48.8% in Australian rapeseed, and (Radić et al., 2021), who reported an oil content of 45.96% in rapeseed grown in Bosnia-Herzegovina.

Our results are higher than those reported by (Öztürk et al., 2022), which is 35.70%, and those found by (Alhomodi et al., 2021) which is  $40.6 \pm 0.3\%$ , and (Gagour et al., 2022), which is  $38.80 \pm 0.50\%$ . The variations in oil content reported in the literature can generally result from various factors, such as climatic conditions, soil and crop management practices, genetic differences between species, disparities in cultivation and storage conditions, and analytical methods.

### *Determination of essential minerals Na, K, Mg, Zn and Fe*

In the present study, a comparative analysis of the mineral element contents in cakes and rapeseeds seeds grown in Tessala region, Sidi Bel Abbes, Algeria (Latitude: 35° 14' 33.46"N; longitude: 0° 46' 11.71"E). The rapeseed cake showed a higher content of mineral elements than rapeseed grains, including K ( $27.20 \pm 0.80$  vs  $25.32 \pm 0.76$ ), Mg ( $13.52 \pm 0.29$  vs  $10.64 \pm 0.23$ ), Na ( $3.92 \pm 0.21$  vs.  $3.27 \pm 0.17$ ), Fe ( $0.22 \pm 0.02$  vs.  $0.18 \pm 0.02$ ) and Zn ( $0.29 \pm 0.20$  vs.  $0.28 \pm 0.19$ ). To determine the percentage increase of minerals present in cakes compared to seeds, we calculate the relative difference between the two and express it as a percentage.

**Table 2.** Mineral composition of rapeseed seeds and cakes in mg/L: Values are expressed as mean  $\pm$  SD for three replicates.

Minerals	Seeds	Cakes
Fe	$0.18 \pm 0.02$	$0.22 \pm 0.02$
K	$25.32 \pm 0.76$	$27.20 \pm 0.80$
Na	$3.27 \pm 0.17$	$3.92 \pm 0.21$
Mg	$10.64 \pm 0.23$	$13.52 \pm 0.29$
Zn	$0.28 \pm 0.19$	$0.29 \pm 0.20$

The Potassium (K) mineral is an essential element for plants, involved in the regulation of various physiological functions (Johnson et al., 2022). The cakes show a 7.4% increase in potassium content compared to the seeds, which can be attributed to the concentration of this mineral after oil extraction. While Magnesium (Mg), is necessary for photosynthesis and other enzymatic processes (Rodrigues et al., 2021), showed a significant increase of 27.1% in the cakes. This increase is probably due to the concentration of the mineral in the residue after extraction. On the other hand the Sodium (Na) although present in lower quantities than potassium or magnesium, shows an increase of 20% in cakes compared to seeds. This increase may be related to the natural concentration after removal of the oil. Concerning Iron (Fe), is crucial for the formation of chlorophyll and the transport of oxygen (Gupta, 2014). The meals show 24.3% increase in iron content, indicating that iron, although present in small quantities, becomes more concentrated in the residue. The Zinc (Zn) is involved in many enzymatic processes and plant growth (Nandal and Solanki, 2021). Although the difference between cakes and seeds is less pronounced for zinc, there is still a slight increase of 4.8% in cakes. The samples analysed contained significant amounts of important minerals essential for human nutrition (Table 2). In this study, the potassium levels were the highest, followed in descending order by magnesium and sodium. Trace elements of iron and zinc were present in similar content ranges in the two samples analysed.

Other authors have reported comparable results (Gagour et al., 2022; Jaafar et al., 2019). In rapeseed, K was the highest represented macro element, containing  $7936.53 \pm 63.87$  mg/kg. Conversely, Fe was the lowest macro element found in the examined samples, typically less than  $30.93 \pm 2.80$  mg/kg. Studies conducted by Jiang et al. (2015) have shown that, when comparing the mineral composition of black-seeded and yellow-seeded rapeseed meal, Fe (91.02 mg/kg) was found to be greater in the former. Similarly, the K content was slightly higher in black-seeded rapeseed meal (12,161.50 mg/kg) compared to yellow-seeded rapeseed meal (12,157.70 mg/kg). Differences in mineral compositions can occur due to several factors, including the genetic properties of the plant species and the environmental conditions in which it is grown. The increase in mineral content in rapeseed cakes compared to seeds after oil extraction can be explained for the following reasons when oil is extracted from rapeseed, the extracted oil makes up a significant proportion of the total seed mass. This oil contains virtually no minerals. By removing this oily fraction, the remaining components (including minerals) become more concentrated in the solid residue, i.e. the oilcake. According to our findings, rapeseed cakes might make a worthwhile source of minerals. These results have important implications for the use of cakes as a nutritional supplement and for the improvement of agricultural practices.

#### *Secondary metabolites of rapeseed seed and cake*

Phenolics compounds can be extracted using various solvents and extraction methods, with solvent extraction being the most frequently employed technique for isolating natural antioxidants. The characteristics of the solvents are crucial in the extraction of antioxidant compounds.

**Table 3.** Phenolic (mg GAE/g) and antioxydant compounds of rapeseed seed and cake.

Extracts	Yields (%)		TPC (mg GAE/g)		TFC (mg QE/g)		IC <sub>50</sub> (mg/ml)	
	Seeds	Cakes	Seeds	Cakes	Seeds	Cakes	Seeds	Cakes
<b>Acetone 70%</b>	11.6	10.7	67.69±4.29	61.3±1.12	18.12±0.74	11.41±0.99	6.46	14.24
<b>Mathanol 70%</b>	9.4	9.9	58.51±0.98	52.02±0.43	23.54± 0.58	18.53±0.66	7.31	18.32
<b>Ethanol 70%</b>	9.8	10.07	50.36±1.18	33.41±0.60	8.48 ± 0.83	6.08±0.43	8.50	22.14
<b>Aqueous</b>	15.07	14.7	30.63±2.59	22.11±0.63	2.31 ± 0.65	1.86±0.41	12.62	27.6

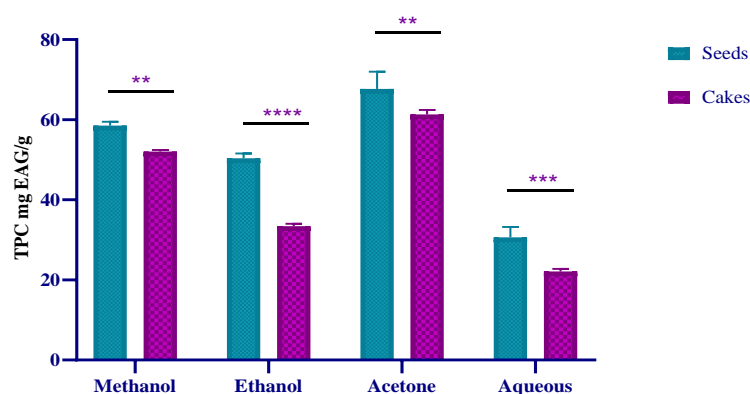
### Extraction yields

In our study four solvents (methanol, ethanol, acetone 70%, and Aqueous) were used to extract the phenolic compounds from the seeds and cakes of *Brassica napus* L. Table 2 summarizes the results obtained, including the extraction yield and the content of total polyphenols, and total flavonoids.

Solvent extraction power significantly affects yield. The aqueous extract had the highest yield: 15.07% for seeds and 14.7% for cake. This aligns with Hussain et al. (2022), where the water extract was the most effective, yielding 40–50% from canola meal. The acetonic extract followed with 11.6% for seeds and 10.7% for cake. Methanolic and ethanolic extracts had lower yields. In all cases, seeds yielded more than cakes

The solvent has a significant impact on yield. This is due to differences in polarity. Water, the most polar solvent, extracts a wide range of phenolic compounds, including the most polar ones. Acetone, with intermediate polarity, extracts less polar compounds than water. Methanol and ethanol, being less polar, mainly extract the least polar phenolic compounds.

### Total polyphenol content



**Figure 2.** The Total polyphenols contents of different solvents fractions of *Brassica napus* L. Values are expressed as mean SD of three replicates. \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ .

### Optimization of total polyphenols in rapeseed seeds and meals using different solvents

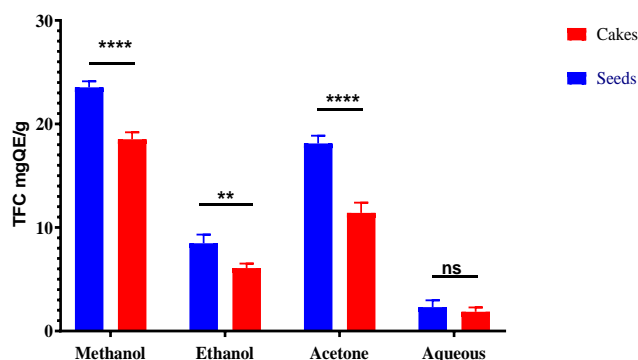
The best solvent for removing total polyphenols from rapeseeds was determined to be acetone 70%, which was followed by methanol 70%, ethanol 70%, and aqueous solution. This makes sense given the solvents polarity and capacity to solubilize phenolic chemicals. For each solvent, the polyphenol contents in the cakes are systematically lower than those in whole seeds, although the difference varies depending on the solvent used. Acetone and methanol 70% showed the lowest reductions in total polyphenols in the cakes compared to whole seeds, indicating better polyphenol retention during oil extraction with these solvents. For example, with 70% acetone, the polyphenol content decreases by 9.4% (from 67.69 to 61.3 mg GAE/g), while with 70% methanol, the decrease is 11.1% (from 58.51 to 52.02 mg GAE/g). The 70% ethanol and the aqueous solution show greater reductions in polyphenols in the cakes. With 70% ethanol, the polyphenol content decreases by 33.6% (from 50.36 to 33.41 mg GAE/g) and with the aqueous solution, the decrease is 27.8% (from 30.63 to 22.11 mg GAE/g). This could be due to the lower efficiency of these solvents in extracting and preserving polyphenols in solid residues. The observed differences can be attributed to the solubility of the polyphenols in each solvent and the ability of these solvents to penetrate the seed matrix and extract the phenolic

compounds. More polar solvents, such as acetone and 70% methanol, are more effective at solubilizing a wide range of polyphenols, resulting in higher yields.

To maximize the extraction of polyphenols from rapeseeds, 70% acetone appears to be the solvent of choice, closely followed by 70% methanol. These solvents make it possible to preserve a significant proportion of polyphenols in the cakes, which is advantageous for the valorisation of residues. Cakes, despite slightly reduced polyphenol content, remain a rich source of these compounds. Their use in the food, cosmetic or nutraceutical industry could be explored, in particular by using solvents that maximize the retention of polyphenols. The results show that the total polyphenol content varies significantly depending on the solvent used. The hydro-acetonic extract contains the highest amount of polyphenols in hexane-extracted flours. Our results are consistent with those of Hussain et al. (2022) for cakes and are also similar to those obtained by Jun et al. (2014) for seed. The TPC content varies from  $20.0 \pm 0.7$  to  $71.9 \pm 0.4$   $\mu\text{g}$  GAE/mg and from 38.50 to 63.95 mg GAE, respectively. However, our results are superior to those found by Gagour et al., (2022) on rapeseeds where the TPC content of 70% methanolic extract is  $38.49 \pm 0.22$  mg GAE/g DM.

#### Total flavonoid content

According to our results, we observe that for methanol and acetone, the difference between seeds and cakes is statistically very significant. This indicates that these two solvents extract more flavonoids from seeds than from cakes. However, for ethanol, the difference is statistically significant, even though the values are identical. This could indicate lower variability between replicates. As for the aqueous extract, the difference is not statistically significant; indicating that aqueous extraction of flavonoids is similar for seeds and cakes.



**Figure 3.** The flavonoid content of different solvent fractions of *Brassica napus* L. Values are the mean  $\pm$  SD of three replicates. ns indicates non-significant difference, \*\*\*\* indicate significant difference at  $p < 0.0001$ , \*\*  $p < 0.01$ .

Methanol 70% is the most effective solvent for extracting flavonoids from rapeseed (*Brassica napus* L.) grown in Algeria, with high contents of  $23.54 \pm 0.58$  mg QE/g for seeds and  $18.53 \pm 0.66$  mg QE/g for cakes. Acetone 70% is also effective but slightly less so, while ethanol 70% and aqueous extraction are less efficient. Seeds contain more flavonoids than cakes, likely because cakes are residues from oil extraction. These results are similar to those obtained by (Gagour et al., 2022) which determined the TFC value of the seeds which varied  $22.84 \pm 0.46$  mg QE/g DM. According to El-Beltagi et al. (2011), the TFC in seeds ranges from  $18.14 \pm 0.22$  to  $29.5 \pm 0.46$  mg QE/g. However, our results are lower than those found by (Jun et al., 2014) on rapeseeds produced in North Dakota, USA in 2007 where the contents vary between  $09.7 \pm 0.3$   $\mu\text{g}$ /mg for aqueous extract and  $34.9$   $\mu\text{g}$ /mg for methanol 80%.

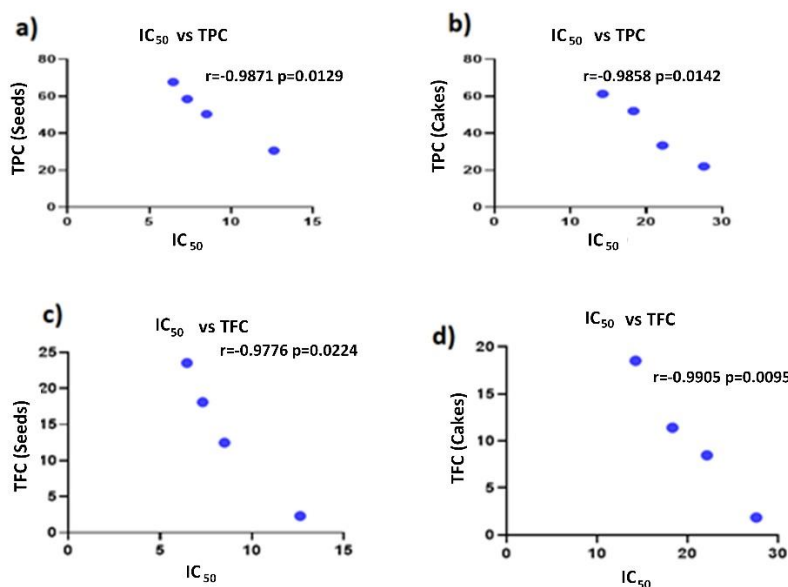
The extracted flavonoids can be used as natural antioxidants in the pharmaceutical and food industries, thereby adding value to the by-products of rapeseed oil production. To maximize the extraction of flavonoids from rapeseed, methanol 70% would be the solvent of choice, closely followed by acetone 70%. Although the cakes contain fewer flavonoids than the seeds, they can still be an interesting source of these compounds, especially if extracted with effective solvents like methanol.

#### Antioxidant activity evaluation

We evaluated the antioxidant activity of the seeds and meal of *Brassica napus* L. using various methanolic, ethanolic, acetonic at 70%, and aqueous extracts through the DPPH method. The degree of discoloration from violet to light yellow indicates the recovery potential of the extract and its ability to donate hydrogen. The results of this method revealed that all solvent fractions exhibited significant DPPH scavenging activity. The  $IC_{50}$ , which is the concentration of the extract needed to eliminate 50% of the radicals, serves as an indicator of an extract's activity. The lower the  $IC_{50}$ , the higher the scavenging activity. The  $IC_{50}$  of the acetone extract is the lowest for both seeds (6.46 mg/ml) and meal (14.24 mg/ml), indicating superior activity, very close to that of the vitamin C standard, followed by the methanol and ethanol extracts (7.316 mg/ml for seeds and 18.32 mg/ml for meal, and 8.5 mg/ml for seeds and 22.14 mg/ml for meal, respectively). In contrast, the aqueous extract shows the lowest activity with  $IC_{50}$  values of 12.62 mg/ml for seeds and 27.6 mg/ml for meal. These results confirm a positive correlation with polyphenol content: the richer an extract is in polyphenols, the lower the  $IC_{50}$ , indicating higher recovery activity. Similar results were found by (Öztürk et al., 2022), with the DPPH value of the seeds varying between  $2.82 \pm 0.03$  mmol/kg and  $3.30 \pm 0.00$  mmol/kg. According to studies conducted by (Jun et al., 2014) the  $EC_{50}$  values of 80% methanol extract from rapeseed varieties were 693  $\mu$ g/ml.

#### Correlation analysis of phenolics compounds and antioxidant activity

Based on the analysis of phenolic content and antioxidant activity of rapeseed seeds and cakes, correlation coefficients between antioxidant activity and total phenolic/flavonoid content were examined (Figure 4).



**Figure 4.** Correlation between polyphenolic components and antioxidants  $IC_{50}$ : (a-b) seeds and cakes phenols against DPPH, respectively; (c-d) seeds and cakes flavonoids against DPPH, respectively.

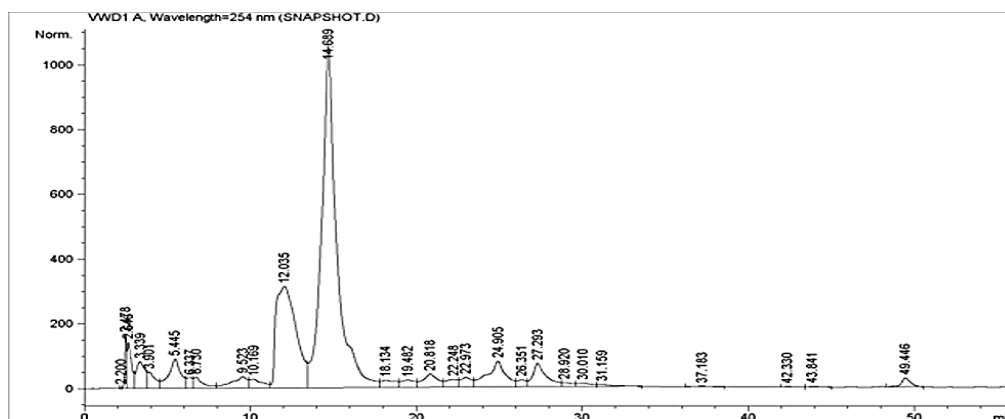
We observed strong negative correlations between phenolic and flavonoid contents in seeds ( $r = -0.9871$  and  $r = -0.9776$ ) and cakes ( $r = -0.9858$  and  $r = -0.9905$ ) respectively, consistent with previous findings (Pajak et al., 2014). These correlations indicate that the antioxidant indices are closely correlated with flavonoid and phenolic contents, suggesting that these compounds play a significant role in rapeseed antioxidant activity. These results align with previous reports showing that antioxidant activity is associated with flavonoid and polyphenol contents in the metabolic organs of *Brassica napus* L. (Farg et al., 2013).

The study by Teh et al. (2014) also reported that canola seed cakes contain more polyphenols than hemp and flaxseed cakes, and these polyphenols were positively correlated with antioxidant capacity. Although this study showed a positive correlation (likely using a different antioxidant activity index), our study shows a significant negative correlation with  $IC_{50}$ , indicating increased antioxidant activity with lower  $IC_{50}$  values. Our correlation is therefore similar to the results of Wang et al. (2018) for rapeseed-based products.

In summary, the correlation is negative in all cases, indicating that higher levels of phenolic and flavonoid compounds are associated with better antioxidant activity against DPPH for rapeseed seeds and cakes.

### HPLC Analysis

The chromatogram obtained in Figure 5 showed that among the 27 peaks in the chromatogram and the 34 standards used for identification, 7 peaks were identified as corresponding to the selected standards at 254 nm. The most abundant compound is sinapic acid, with a retention time of 14.889 minutes and a peak area of 19.8024 and this is consistent with Yang et al. (2015) revealed that sinapine was the predominant compound among all samples, ranging from 29.74 to 52.24 mg/g DW and accounting for an average of 79% of the total phenolic content across four rapeseed varieties cultivated on Jeju Island using HPLC analysis. It is followed by gallic acid, which has a retention time round to 5.5 min and an area of 3.4362.



**Figure 5.** HPLC chromatogram of 70% methanolic extract of phenolic compound from rapeseed seed (Zitna) flour recorded at 254 nm. Sinapic acid (SA), ascorbic acid (AA), coumaric acid (CA), Galic acid (GA), Butylated hydroxytoluene (BHT), Gossypin, 5hydroxyflavone and other non-identified phenolic constituents.

Another compound detected is gossypin, with a peak area of 3.1137 and a retention time of 27.293 min. Dihydroxyflavone appears next, with a peak area of 0.3444 and a retention time of 31.159 minutes. Finally, BHT was detected at 49.446 minutes with a minimal peak area of 0.8164, along with ascorbic acid at a retention time of 2.646 minutes and a peak area of 1.824. Sharma et al. (2017) stated that the major phenolic compounds observed in *Brassica juncea* included gallic acid, ferulic acid, sinapic acid, *p*-

coumaric acid, *p*-hydroxybenzoic acid, and caffeic acid, with sinapic acid being the predominant compound across all samples. Also, Khattab et al. (2010) found that the sinapine (the choline ester of sinapic acid) was identified as the major phenolic constituent in extracts of canola seeds, meals, and press cakes, comprising 69.76–87.15% of the total phenolic content across various canola fractions.

Analysis of the methanolic extract of our rapeseed variety 'Zitna' reveals that sinapic acid is the most abundant compound, suggesting high antioxidant activity, as this acid is well-known for its antioxidant properties in plants, according to Chew (2020). Gallic acid, another potent antioxidant, is the next most concentrated compound. The presence of gossypin, a flavonoid with known antioxidant and anti-inflammatory effects, further enhances the bioactivity of the extract. Ascorbic acid (vitamin C), though present in lower quantities, also contributes to the extract's antioxidant profile. Finally, BHT, a synthetic antioxidant commonly used as a preservative, was found in low concentration, indicating it is not a dominant natural compound in this sample.

The extract demonstrates strong antioxidant activity, attributed to compounds like sinapic and gallic acids, with additional support from other molecules such as ascorbic acid. This composition highlights the potential health benefits and stability of the 'Zitna' rapeseed variety.

## Conclusions

This study reveals that rapeseed seeds and their cakes, cultivated in Algeria, constitute an exceptional source of minerals. In addition to being rich in proteins, oil, and fiber, these seeds exhibit significant variability in their polyphenol content depending on the solvent used for extraction. The results demonstrate that the choice of solvent is crucial in polyphenol extraction, with 70% acetone and methanol being the most effective solvents. These solvents are thus recommended for applications requiring high polyphenol content. Furthermore, a thorough comparison of the physicochemical parameters of the analyzed seeds with the available literature data has highlighted significant differences. These variations can be attributed to several factors such as the geographical origin of the seeds, technological processing methods, as well as specific nutritional benefits and practical uses in each context. These differences are expected and illustrate the diversity and richness of the properties of Algerian rapeseed. In conclusion, rapeseed cultivated in Algeria offers numerous advantages and could be considered a valuable and appropriate ingredient for various industrial applications. Its richness in nutrients and bioactives compounds, combined with its adaptability to different processing methods, makes it a promising resource for the food industry and beyond. The results of this study provide valuable insights for optimizing the use of Algerian canola in diverse contexts, thereby enhancing its potential as a strategic crop for both local and international economies.

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**Conflicts of Interest:** The authors declare no conflict of interest.



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