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Influence of Cryogenic Grinding on the Nutritional and Antinutritional Components of Rapeseed Cake

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Abstract: We investigated the influence of cryogenic grinding on the quality of rapeseed cake. Rapeseed cake is a good source of valuable proteins (30%) and oil (14%), with a balanced fatty acid composition and a fair amount of sterols, which may reduce the risk of cardiovascular diseases. However, the presence of antinutritive compounds prevents its use as a food source. Grinding under cryogenic conditions is much more efficient than grinding at room temperature in terms of particle size reduction. The additional cryogenic grinding of the cake had little effect on the nutritional components, as phytosterols and soluble dietary fiber increased slightly. It had no effect on insoluble dietary fiber, polyphenols, and tannins. Prolonged grinding time, both at room and subzero temperatures, reduced the total amount of glucosinolates by 34 and 43%, respectively. However, the reduction in undesirable components is not sufficient to use cryogenic grinding as the sole treatment for the cake, but it could be a good pretreatment for chemical or biological treatments.

Keywords: rapeseed cake; cryogenic grinding; dietary fibre; polyphenols; glucosinolates

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1. Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), demand for food will roughly double by 2050 due to the growing world population. One of the biggest challenges will be meeting the growing demand for meat, as it is estimated that meat production will have to increase by over 200 million tons per year [1]. Population pressures aside, environmental considerations and production efficiencies underscore the importance of new high-quality sources of protein for human nutrition, such as plants, for both developed and developing countries. And rapeseed could be a good local source. Rapeseed is used to produce high-quality oil, but the by-products of this production, rapeseed meal and cake, have a high protein value. The balance and bioavailability of amino acids and the digestibility of proteins are comparable to those of soybean meal [2,3]. In fact, rapeseed cake and meal are the second most important feed source after soybean meal [4] and have the chance to be an economical and sustainable source of protein in food [2]. In addition, research has shown that rapeseed meal has good water and oil binding capacity as well as very good emulsifying and foaming properties, which are of great importance for incorporation into novel functional foods [5]. However, despite decades of research, development of various technologies, and large-scale manufacturing, there are still no commercially available rapeseed cake products for human consumption [2]. The use of rapeseed meal and cake in human nutrition is limited by the presence of antinutritional components such as glucosinolates, phenolic compounds, tannins, phytates, and hulls [6].

The main concern when using rapeseed meal and cake as food ingredients are glucosinolates. This is a large group of sulphur-containing secondary plant metabolites found

in all economically important varieties of Brassica. There is great diversity among glucosinolates, and more than 120 different compounds have been identified [7]. Although some glucosinolates possess antibacterial and antifungal properties as well as anticancer activity, the antinutritional effects of glucosinolates and their degradation products present in rapeseed meal and cake have limited the use of rapeseed by-products as a food source [8]. They are known to reduce animal growth, cause iodine deficiency, and cause hypertrophy of liver, kidney, and thyroid [7]. In addition, these compounds provide a bitter taste and sulphurous aroma due to their degradation products. Therefore, one of the most important breeding objectives is to reduce the glucosinolate content in the seed to trace amounts [8].

Commercial rapeseed cakes are rich in phenolic compounds, especially derivatives of sinapic acid, which account for about 80–99% of total phenols, mainly in the form of esters and glucosides. Although they are known to be potent free radical scavengers and antioxidants [9], their content in rapeseed meal and cake is up to 30 times higher than in by-products from other seeds. These high concentrations of phenolic compounds may contribute to the dark colour, bitter taste, and astringency of rapeseed meal. In addition, phenols and their oxidized products can form complexes with essential amino acids, enzymes, and other substances, lowering the nutritional value of meal and cake. Another group of phenolic constituents found in canola are condensed tannins. Tannins are complexed phenolic compounds formed by polymerization of flavan-3-ols or flavan-3,4-diols [10]. Similar to phenolic acids, they also have antioxidant activity [11], and they are also of concern due to their ability to bind with proteins. Tannins form complexes with lysine and methionine, making them nutritionally unavailable. Therefore, the phenolic compounds and tannins, as well as their concentrations, are important factors when canola meal is considered a protein source in food formulations [10].

Great efforts have been made to reduce undesirable components in rapeseed meal and cake and to reduce their antinutritional effects, which would allow their use in human nutrition. In recent decades, new varieties with lower glucosinolate and hull content (and thus insoluble dietary fibre—IDF) have been developed. Phytates are removed by enzymatic methods, while various extraction methods are used for phenolic compounds. New techniques are also being explored, such as ultrasound, pulsed electric field, and micronization, but so far none of these techniques are being used commercially [2]. Micronization technology is developing rapidly as it not only improves the functional and sensory properties of the ground material but also increases the bioavailability of nutrients and the rate of biological and chemical reactions. Various methods can be used to reduce particle size [12]. It has been reported that cryogenic grinding increases the production of fine particles and reduces the energy consumption for grinding the materials by increasing their brittleness. This could be very important for oily materials such as cake and meal residues after oil extraction, as the extremely low temperatures in the grinder solidify the oils, causing the materials to crumble easily [13]. In our research group, experiments were performed on millet bran [14] and pumpkin seed cake [15] to investigate the effects of cryogenic grinding on the composition, functional properties, and extractability of nutritive compounds. The aim of this study was to determine the effects of cryogenic grinding on the content and composition of the nutritional and antinutritional components of rapeseed cake and its fitness as a treatment or pre-treatment for further use of rapeseed by-products in food formulations.

2. Materials and Methods

2.1. Rapeseed Cake

Rapeseed cake was obtained as a by-product in the laboratory production of virgin rapeseed oil. Rapeseed was grown in the experimental field of the Faculty of Agriculture, University of Zagreb. Prior to oil extraction, the seeds were ground, heated at 80 °C for 30 min, and the oil was extracted by double pressing with a Komet screw press (model CA/53, Monforts and Reiners, Rheydt, Germany). The obtained rapeseed cake was ground using

a laboratory disc mill (Buehler—Miag, Helvoirt, The Netherlands) and stored at −20 °C until further analysis.

2.2. Grinding of the Cake

Additional grinding of the cake was performed using the ball mill "CryoMill" (Retsch, Haan, Germany). The rapeseed cake (8 g) together with 12 metal balls (10 mm in diameter) were placed in a 50 mL metal jar in which grinding was performed. Grinding in this type of mill is performed by the radial oscillations of the jar, which cause high friction and impact. A constant flow of liquid nitrogen flows through a hollow double wall of the grinding jar before and during the grinding process, maintaining the temperature at −196 °C. Samples were ground for 2, 4, 8, and 12 min without liquid nitrogen cooling (at room temperature—RT) and with liquid nitrogen cooling (cryogenic cooling—CC). The experiments with cryogenic cooling were performed with automatic pre-cooling. In this setting, grinding starts after a countdown of 3 min, which is initiated when the temperature sensor indicates that the surface of the jar has cooled down to −190 °C.

2.3. Particle Size Distribution

The particle size distribution of the ground rapeseed cake was determined using a laser diffraction particle size method as previously described by Benković et al. [16] with some modifications. Briefly, the analysis was performed using the Mastersizer 2000 particle size analyzer connected to the Scirocco 2000 dry disperser (Malvern Instruments, Worchester shire, UK) operating at a feed pressure of 1 bar and a laser obscuration of 2–6%. The feed rate was adjusted between 50 and 100% during the measurement to ensure that the laser obscuration was within the defined range. Six different particle size parameters were determined: d (0.1), d (0.5), d (0.9), Sauter diameter (D_{32}, span, and specific surface area.

2.4. Basic Quality Parameters of the Cake

Rapeseed cake quality parameters were determined using standard methods. The moisture and volatile matter content were determined by drying to a constant mass according to the standard ISO 665:2000 method [17]. The oil content was determined by the Soxhlet reference method according to ISO 659:2009 [18]. Crude protein content and total ash were determined according to the grain analysis methods ISO 20483:2013 [19] and ISO 2171:2007 [20], respectively.

2.5. Extraction of the Non-Polar Components

The extraction of the nonpolar components of rapeseed cake was carried out according to the method described by Li et al. [21] with some minor modifications. The sample (2 g) was placed in a 50-mL plastic tube along with 20 mL of hexane. The test tube was placed horizontally on the orbital shaker (IKA MS 3 basic, IKA-Works, Staufen im Breisgau, Germany) and shaken for 30 min. After extraction, the phases were separated by centrifugation for 10 min at 5000 rpm (Rotina 380, Hettich GmbH, Kirchlengern, Germany). The extraction process was repeated 2 more times. The extracts were combined and then filtered through a sintered glass filter (pore size 10–16 µm), and the solvent was evaporated under reduced pressure at 40 °C using a rotary evaporator (Heidolph, Schwabach, Germany). Nonpolar extracts were used for the determination of fatty acid composition and sterol content and composition, while defatted rapeseed cakes were used for the extraction of phenolic components, tannins, dietary fibre, and glucosinolates.

2.6. Fatty Acid Composition

The fatty acid composition was determined by gas chromatography after transmethylation of the nonpolar dry extracts with methanolic potassium hydroxide [22]. The analysis of the prepared methyl esters was performed according to the method described in our previous research [23]. Briefly, 1 µL of methyl esters were injected into an Agilent Technologies 6890N Network GC system (Santa Clara, CA, USA) with flame ion-

ization detector. Fatty acid methyl esters were separated on a DB-23 capillary column (60 m × 0.25 mm × 0.25 µm; Agilent Technologies). The injector and detector temperatures were set at 250 °C and 280 °C, respectively. The Owen temperature was programmed to increase by 7 °C/min from 60 °C to 220 °C, where it was held for 17 min. The split ratio was 30:1, and helium was used as the carrier gas with a constant flow of 1.5 mL/min. The fatty acid methyl esters were identified by comparing their retention times with those of commercial standards. The content of each fatty acid is expressed as a percentage of total fatty acids.

2.7. Content and Composition of Sterols

The sterol composition was determined according to the standard ISO 12228-1:2014 method for animal and vegetable fats and oils [24] with α-cholestanol as the internal standard. After saponification of the oily extract of rapeseed cakes, followed by extraction and purification of the sterol fraction, the sterols were silylated with 100 µL of pyridine-hexamethyl-disilazane-chlorotrimethylsilane solution (5/2/1, $v/v/v$). The prepared silylated sterol fraction (1 µL) was injected into an Agilent Technologies 6890N Network GC system (Santa Clara, CA, USA) equipped with an Agilent Technologies 5973 Inert Mass Selective Detector and an Agilent DB-17MS capillary column (30 m × 0.32 mm × 0.25 µm). The split ratio was 13.3:1. Helium was used as the carrier gas at a constant flow rate of 1.5 mL/min. The temperature of the injector was set at 290 °C. The temperature of the oven was programmed to increase by 6 °C/min from 180 to 270 °C and remained at the maximum temperature for 30 min. The transfer line temperature was set at 280 °C. Sterols were identified from an internal mass spectral library created using the β-sitosterol, campesterol, and stigmasterol standards and the NIST 2 and NIST 5 online libraries. All sterols were quantified using an internal standard method. The concentration of total sterols was calculated based on the mass of the cake, taking into account the amount of extracted oil from each sample.

2.8. Determination of Dietary Fibre

Insoluble and soluble dietary fibre and total dietary fibre were determined according to the official AOAC method [25] using the Megazyme assay kit. After extraction of insoluble dietary fibre (IDF) and dietary fibre soluble in water but precipitated in 78% aqueous ethanol (SDFP), dietary fibre soluble in water and not precipitated in 78% aqueous ethanol (SDFS) was analysed using a Shimadzu HPLC system with RID detector. Prior to injection, samples were deionized with a freshly prepared mixture of Amberlite FPA 53 and Ambersep 200 resins (1/1, w/w). The deionized sample (20 µL) was injected onto the METACARB 67C column (300 mm × 6.5 mm; Agilent Technologies, Santa Clara, CA, USA) heated to 80 °C. The components were separated on a Shimadzu HPLC system by isocratic chromatography using calcium disodium EDTA hydrate solution (50 mg/L) as the mobile phase at a flow rate of 0.5 mL/min for 30 min. Quantification of low molecular weight soluble dietary fibres was performed by comparing their peak with the peak of the internal standard (D-sorbitol).

2.9. Extraction of the Phenolic Components

The extraction of the free and bound phenolic components from the rapeseed cakes was performed according to the modified method described by Martini et al. [26].

For the extraction of free phenolic compounds, 100 µL of an internal standard solution (3,5-dichloro-4-hydroxibenzoic acid, 5 mg/mL) was added to 0.5 g of the defatted rapeseed cake, homogenized for 15 s (IKA MS 3 basic, IKA-Works, Staufen im Breisgau, Germany) with 3 mL of an 80% ethanolic solution (v/v), and then placed in an ultrasonic bath (Sonorex, Berlin, Germany) for 10 min. After centrifugation at 4000 rpm for 15 min (Rotina 380, Hettich GmbH, Kirchlengern, Germany), the supernatants of the three successive extractions were combined in a 10-mL flask and made up to the mark with ethanol solution

(80%, v/v). The crude rapeseed cake residue after extraction of the free phenolic compounds was used to extract the bound phenolic compounds.

After discarding the ethanol extract, 100 µL of the internal standard solution was again added to the rapeseed cake, and the samples were hydrolyzed with 4 mL of 2 M sodium hydroxide for 4 h. After 4 h, the pH of the mixture was adjusted to pH 2 ± 0.1 (Jenway pH meter, London, UK) to deionize the released phenolic compounds. The extraction was performed three times, the first time with 4 mL of ethyl acetate, and the second and third times with 2 mL of ethyl acetate, as described for the extraction of free phenolics. The supernatants were combined, the solvent was evaporated under nitrogen, and the dry extract was dissolved in 10 mL of methanol.

The content and composition of free and bound phenolic compounds were determined by HPLC using a diode array detector and a Kinetex C18 column (150 mm × 4.6 mm × 2.6 µm, Phenomenex, Torrance, CA, USA) under chromatography conditions published by Panić et al. [27]. All extracts were filtered through a PVDF filter with a pore size of 0.20 µm before injection (5 µL) into the system. Chromatograms of the phenolic compounds were recorded at a wavelength of 280 nm. Retention times and spectral data of the external standards were compared to identify the individual components. Detected peaks whose retention times did not match any of the external standards used, but whose UV spectra matched the UV spectra of the phenolic compounds (hydroxycinnamic acids or hydroxybenzoic acids), were classified as non-identified phenolic compounds. The internal standard mentioned above was used for the quantification of the identified and non-identified phenolic compounds.

2.10. Extraction and Determination of the Tannins

Tannins were determined according to the method published by Price et al. [28]. Defatted rapeseed cake (1 g) was weighed into a 50-mL plastic test tube, and 15 mL of acetone (70%, v/v) was added. Extraction was performed using a laboratory homogenizer (IKA T18 Ultraturrax, IKA-Works, Staufen im Breisgau, Germany) for 1 min at 10,000 rpm. The supernatant was separated using a centrifuge (10 min, 5000 rpm) (Rotina 380, Hettich GmbH, Kirchlengern, Germany), and the acetone solution was evaporated using a rotary evaporator (Heidolph, Schwabach, Germany) at 40 °C under reduced pressure. The dry extract was dissolved in 10 mL of methanol and filtered through a 0.20 µm PVDF filter.

For spectrophotometric determination of tannin concentration, the vanillin reagent was prepared by mixing equal amounts of a 1% vanillin solution in methanol and an 8% solution of HCl in methanol and stabilized overnight. 0.5 mL of the tannin extract was transferred into the spectrophotometric cuvette, and 2.5 mL of vanillin reagent was added. After incubation at 30 °C for 20 min, the absorbance was measured at 500 nm against the blank (0.5 mL extract + 2.5 mL 4% HCl solution in methanol) (Specord 50 plus, Analytik Jena, Germany). Tannin concentration was calculated using the calibration curve of catechin (10–1000 µg/mL).

2.11. Determination of Glucosinolates

The content of glucosinolates was determined according to the standard ISO method [29] using HPLC with sinigrin monohydrate (potassium allyl-glucosinolate monohydrate) as an internal standard. After extraction and desulfation of glucosinolates, an Agilent Technology HPLC 1200 system with DAD detector and Phenomenex C18 column (Kinetex 150 mm × 4.6 mm, 2.6 µm, 100 Å) was used for separation and identification of each component. The prepared sample (20 µL) was injected into the system. The mobile phases for the separation of glucosinolates were water (solvent A) and 20% (v/v) acetonitrile solution in water (solvent B), and the gradient used was as follows: 0–1 min 100% A; 1–21 min 100–0% A; 21–26 min 0–100% A; 26–31 min 100% A. The flow rate was 1 mL/min throughout the run. The column temperature was 30 °C. Chromatograms were recorded at a wavelength of 229 nm, and UV spectra were also recorded throughout the analysis time. The identification of each component was performed by comparing the relative retention

times with those in the aforementioned ISO method, and for their quantification the internal standard was used, taking into the account response factors for each component given in the same ISO standard method.

2.12. Statistical Analysis

Rapeseed cake was ground in three replicates. All analyses were performed in triplicate on all ground rapeseed cake samples and on the original cake as a control, except for the analysis of basic quality parameters, which were determined only on the original rapeseed cake. Results are given as mean ± SD. To estimate the effects of time and grinding conditions and their interactions on the nutritional and antinutritional components of rapeseed cake, the two-way ANOVA was used in combination with Tukey's multiple comparison tests. Statistical analyses were performed using XLSTAT 2023 software solution (Lumivero: Denver, CO, USA) with the statistical significance threshold set at $p = 0.05$.

3. Results and Discussion

3.1. Quality of the Rapeseed Cake

The quality of rapeseed cake is determined by the composition of macro- and micronutrients and the presence of desirable and undesirable components. Rapeseed cake produced in our laboratory contained 9.5% water, which was due to the addition of water during the production process (Table 1). The oil content of the cake produced was 14%, a result comparable to cakes produced in industry [30]. Since rapeseed cake contains a relatively high amount of oil, the composition of these nonpolar components is important for the incorporation of the by-product in food formulations. The fatty acid composition of the cake affects not only the nutritional value but also the oxidative stability of the cake and the products in which it is used. The fatty acid composition of the residual oil in rapeseed cake is shown in Table 1. According to the Codex Standard for Named Vegetable Oils [31], it can be classified as low erucic acid rapeseed, with oleic acid being the predominant fatty acid and a significant amount of linoleic and linolenic acid in the ratio 2.8:1. Due to its favourable fatty acid composition, rapeseed oil is beneficial to health from various aspects: it reduces the risk of cardiovascular disease, improves lipid and glucose metabolism, and has a positive effect on atherosclerosis and inflammation [32].

Table 1. Quality parameters, composition of the non-polar fraction and dietary fiber of laboratory produced rapeseed cake (RC).

Parameter		RC
Quality parameter (%)	Water	9.5 ± 0.1
	Oil	14.1 ± 0.3
	Protein	29.5 ± 0.4
	Ash	6.4 ± 0.3
Fatty acid (% of total)	C14:0	0.1 ± 0.0
	C16:0	5.1 ± 0.0
	C16:1	0.4 ± 0.0
	C17:1	0.1 ± 0.0
	C18:0	1.9 ± 0.1
	C18:1	64.4 ± 0.3
	C18:2	19.1 ± 0.0
	C18:3	6.8 ± 0.2
	C20:0	0.6 ± 0.0
	C20:1	1.1 ± 0.1
	C22:0	0.3 ± 0.0

Table 1. *Cont.*

Parameter		RC
Sterol (% of total)	Brassicasterol	11.6 ± 0.5
	Campesterol	29.4 ± 1.0
	Campestanol	1.1 ± 0.0
	Stigmasterol	0.7 ± 0.1
	β-sitosterol	51.5 ± 0.5
	Δ5-avenasterol	3.5 ± 2.3
	Δ7-avenasterol	0.8 ± 0.4
	Δ7-stigmasterol	0.1 ± 0.1
	Δ-5,24-stigmastadienol	1.3 ± 0.1
	Total sterols (mg/100 g)	148 ± 6
Dietary fiber (%)	IDF	25.7 ± 0.6
	SDFP	3.0 ± 0.1
	SDFS	2.0 ± 0.0

IDF—insoluble; SDFP—soluble in water but precipitated in 78% ethanol; SDFS soluble in water and not precipitated in 78% ethanol.

In addition, the sterols of rapeseed oil are known as assistants in lowering the risk of cardiovascular disease. Based on the mass of the cake, the rapeseed cake produced in this experiment contained 148 mg/100 g of sterols (Table 1). The composition of sterols is typical of low erucic rapeseed [31] with β-sitosterol as the dominant sterol (51.5%), followed by campesterol (29.4%) and brassicasterol (11.6%).

The analyzed rapeseed cake contained 6.4% ash. It has already been reported that rapeseed cake and meal are good sources of available calcium, iron, manganese, and selenium. Although they are high in phytate, they are also one of the richest sources of non-phytate phosphorus [33]. However, the most valuable components of rapeseed cake and the reason for numerous research studies to incorporate it in human nutrition are its proteins, which have a balanced amount of essential amino acids, are rich in sulfur-containing amino acids, and have good functional properties. Their digestibility is reported to be as high as 84%, while egg and milk proteins have a digestibility of 94% and 95%, respectively. Rapeseed cake proteins have good solubility, emulsification, foaming, and stabilization properties. To date, there are several rapeseed protein products that have been approved by the U.S. Food and Drug Administration (FDA) as safe (GRAS) or by the European Food Safety Authority (EFSA) as novel foods and can be used in a variety of bakery products, fruit and vegetable juices, egg substitute products, and processed meats [2]. Rapeseed cake produced and used in this study contained nearly 30% crude protein (Table 1).

Regardless of the enumerated advantages, the use of rapeseed cake as a food source is limited by the presence of antinutritional components. One of these components are dietary fibres, which accounts for the largest portion of rapeseed cake, 30.7%. However, dietary fibre can also have some beneficial properties that depend mainly on their nature. Khajali and Slominski, in their review [33], reported the average values of several different studies, and the amounts of total dietary fibre were comparable to the results of this study. The vast majority of the dietary fibre fraction, more than 83%, was insoluble dietary fibre (IDF) (Table 1). This is the undesirable fraction, consisting mainly of non-starch polysaccharides (cellulose and a variety of non-cellulose polysaccharides) and polyphenolic lignin, components resistant to hydrolysis by enzymes in the digestive tract. Their antinutritional properties are reflected in their inhibitory effect on the digestibility of nutrients [34,35]. However, soluble dietary fibre (SDF), which represent a desirable but small proportion of rapeseed dietary fibre (about 17% in the present study), could have a beneficial effect on total and LDL cholesterol levels [36].

Polyphenolic compounds are also among the components with ambiguous characteristics. They are known for their antioxidant and free radical scavenging activities and potential health benefits [37]. However, high concentrations of phenolic compounds, as found in our study, where phenolic compounds accounted for 2.1% of the produced cake

(Table 2), have negative effects on the nutritional value and organoleptic properties of rapeseed cake. Phenolic compounds are known to be responsible for the dark colour, bitter taste, and astringency of rapeseed meal. As mentioned earlier, phenolic compounds and their oxidised products can also interact with amino acids, enzymes, and other food components [6]. Free phenols accounted for nearly 70% of the total phenols in this study (Table 2). Of these, free phenolic acids accounted for less than 15%, and the remainder were esterified phenolic compounds. The dominant phenolic compound was sinapine, choline ester of sinapic acid (607 mg/100 g). In contrast to previously reported results [10], sinapic acid was not the predominant free phenolic acid in the produced cake. The reason for this could be due to the production of oil and cake, i.e., the conditioning of the seeds, which leads to the decarboxylation of sinapic acid and the formation of canolol, decreasing the concentration of sinapic acid in the cake. The absence of a carboxyl group gives canolol a more lipophilic character than its precursor, which is why it accumulates in the oil [38] and its concentration in the cake was low (9 mg/100 g). The bound phenolic compounds represent a smaller fraction of phenolics and accounted for slightly more than 30% of the total phenolic compounds. Among them, sinapic acid was the dominant compound. It accounted for 80% of the bound phenolic compounds. Minor phenolic compounds were ferulic acid, p-coumaric acid, and syringaldehyde. Tannins are another group of phenolic compounds. Their concentration in rapeseed cake was slightly lower than 0.5%. This amount is lower than those reported in the literature [39], but the concentration of tannins should be further reduced because they can form complexes with proteins and proteolytic enzymes in the gastrointestinal tract, which negatively affects the nutritional value of the cake [35].

Table 2. Composition of phenolic compounds and glucosinolates of laboratory produced rapeseed cake (RC).

Parameter		RC
Free phenolic compound (mg/100 g)	Gallic acid	27 ± 0
	Chlorogenic acid	75 ± 9
	Ferulic acid	65 ± 2
	Sinapic acid	85 ± 2
	p-coumaric acid	97 ± 1
	Syringaldehyde	14 ± 6
	Canolol	9 ± 1
	Sinapine	607 ± 188
	Non identified	825 ± 22
	Total	1927 ± 51
Bound phenolic compound (mg/100 g)	Ferulic acid	32 ± 0
	Sinapic acid	688 ± 4
	p-coumaric acid	10 ± 0
	Syringaldehyde	4 ± 0
	Non identified	132 ± 1
	Total	865 ± 4
Tannins (mg/100 g)		489 ± 36
Glucosinolate (µmol/g) *	Glucoiberin	0.1 ± 0.1
	Progoitrin	6.9 ± 0.7
	Glucoraphinin	0.5 ± 0.0
	Gluconapoleiferin	0.4 ± 0.0
	Gluconapin	4.0 ± 0.0
	4-hidroxyglucobrassicin	0.3 ± 0.1
	Glucobrassicanapin	1.1 ± 0.0
	Glucobrassicin	0.5 ± 0.0
	4-metoxygucobrassicin	0.1 ± 0.0
	Total	14.0 ± 0.8

* Expressed on the mass of defatted and dry rapeseed cake.

The concentration of glucosinolates in the produced cake was low, only 14 µmol per g of defatted and dry cake. For a seed to be classified as a canola or 00-rapeseed variety, the concentration of glucosinolates should be less than 30 µmol/g [40]. The major glucosinolates detected were progoitrin (6.9 µmol/g), gluconapin (4.0 µmol/g), and glucobrassicanapin (1.1 µmol/g) (Table 2). All other compounds detected (gucoiberin, epi-progoitrin, glucoraphanin, gluconapoleiferin, glucobrasicin, 4-hidroxyglucobrassicin, 4-metoxyglucobrassicin) are present at concentrations less than 1 µmol/g. This composition is typical for Brassica napus cultivars [35]. Although the produced cake contains low concentrations of glucosinolates, and most of which are aliphatic glucosinolates, which mainly have a negative effect on the organoleptic properties of the cake, their concentration should be reduced due to degradation products such as goitrin, a degradation product of progoitrin, which has antithyroid activity [35].

3.2. Influence of the Grinding on the Particle Size Distribution

Particle size is one of the most important properties affecting the flow properties of powders used as raw materials, as well as the textural and sensory properties of the products in which powders are used. In general, during the grinding process, feed particles are broken down into smaller particles that become more reactive due to a larger specific surface area, which is beneficial in processes involving heat and mass transfer [41,42]. The effects of grinding time as well as cooling with liquid nitrogen on particle size are shown in Table 3.

Table 3. Particle size distribution parameters determined for the rapeseed cake (RC = sample before additional grinding; RT = room temperature; CC = cryogenic cooling).

Sample	d(0.1) (µm) $^{\$,£,§}$	d(0.5) (µm) $^{\$,£,§}$	d(0.9) (µm) $^{\$,£,§}$	D_{32} (µm) $^{\$,£,§}$	Span $^{\$,£,§}$	Specific Surface Area $^{\$,£,§}$ (m^2/g)
RC	128.2 ± 3.8 a	393.6 ± 9.0 a	881.6 ± 19.7 a	238.8 ± 6.0 a	1.9 ± 0.0 g	0.010 ± 0.0006 h
2RT	60.1 ± 0.3 b	273.4 ± 2.0 b	770.9 ± 6.2 b	130.6 ± 0.9 b	2.6 ± 0.0 d	0.019 ± 0.0000 g
4RT	48.5 ± 1.0 c	229.4 ± 7.1 c	698.4 ± 20.3 c	108.6 ± 2.1 c	2.8 ± 0.0 c	0.022 ± 0.0006 f
8RT	41.3 ± 0.5 d	202.4 ± 5.0 d	669.7 ± 13.6 c	95.1 ± 1.1 d	3.1 ± 0.0 b	0.026 ± 0.0006 e
12RT	33.5 ± 0.4 f	174.9 ± 5.0 e	615.2 ± 10.6 d	79.7 ± 1.1 ef	3.3 ± 0.0 a	0.031 ± 0.0006 d
2CC	39.6 ± 2.9 de	164.2 ± 2.8 e	360.4 ± 1.5 e	83.1 ± 4.2 e	1.9 ± 0.0 fg	0.029 ± 0.0015 d
4CC	35.5 ± 0.1 ef	137.4 ± 0.1 f	288.2 ± 1.1 f	75.1 ± 0.2 f	1.8 ± 0.0 h	0.033 ± 0.0000 c
8CC	20.7 ± 0.4 g	92.3 ± 0.2 g	204.9 ± 0.7 g	49.8 ± 0.5 g	2.0 ± 0.0 f	0.049 ± 0.0006 b
12CC	13.8 ± 0.2 h	61.8 ± 1.2 h	148.8 ± 1.0 h	34.3 ± 0.5 h	2.2 ± 0.0 e	0.071 ± 0.0012 a

$^{\$}$ Grinding time had significant influence ($p \leq 0.05$); $^{£}$ grinding temperature had significant influence ($p \leq 0.05$); § interaction of the grinding time and temperature had significant influence ($p \leq 0.05$); values with different letters in each column are statistically different ($p \leq 0.05$) according to Tukey's multiple comparison tests.

The rapeseed cake was ground for 2, 4, 8, and 12 min with cryocooling (samples CC) or without cooling at room temperature (samples RT). For RT samples, particle size decreased with grinding time, ending after 12 min with a 44.4% decrease from the original median particle size. The grinding duration affects the particle size of different crops [43], but it is important to emphasize that the effect of grinding duration also depends on the properties of the ground material [41]. For samples CC, the reduction in particle size was significantly higher ($p < 0.05$) than for samples RT. For example, the median particle size decreased to 164 µm after only 2 min of grinding, which according to the Tukey test is comparable to the particle size measured for the sample RT after 12 min of grinding (175 µm). The need for shorter grinding times is very important when considering the economics of particle size reduction, as it also means that less energy is required and only a small amount of liquid nitrogen is needed to speed up the process. After 12 min of grinding under liquid nitrogen, a significant reduction in particle size was achieved, with the resulting particles being only 15% of the original size.

Span and specific surface area of powders affect the flow and caking properties of powders [44]. The samples without cooling (RT) had a larger distribution span, ranging from 2.6 to 3.3 and increasing significantly with increasing grinding time. This was an indication that while longer grinding time resulted in a smaller median particle size, it also resulted in particles with different particle diameters and a larger spread from the median diameter. Powders with larger spans often exhibit poor flow properties that are difficult to control [41]. The effect of increasing the span was not as pronounced at CC. The span of the initial sample before grinding (1.9) was comparable to the values obtained after grinding for two minutes with cryogenic cooling, with a slight increase in span for sample 12CC (2.2). The specific surface area increased from 0.010 m^2/g to 0.031 m^2/g without cooling and to 0.071 m^2/g with cooling. Again, the positive effect of cooling is particularly pronounced, as the specific surface area increases sevenfold, whereas without cooling it increases only threefold. As with the median particle size, the same increase in specific surface area was obtained with 12 min of RT grinding as with only 2 min of CC grinding. The smaller particle size of rapeseed cake and distribution span of particle size, as well as the increase in specific surface area of samples grinded under cryogenic conditions, are in accordance with the results of previous research [13–15]. Pre-cooling rapeseed cake with liquid nitrogen solidifies the oil since the melting point of rapeseed oil ranges from −20 to −10 °C [40]. In addition, cooling the system during the grinding absorbs the heat generated during the grinding operation and maintains the desired low temperature [13]. This embrittles the material, which allows finer grinding and a more uniform particle size.

3.3. Influence of the Grinding on the Rapeseed Cake Quality

As expected, grinding had no effect on the fatty acid composition of the residual oil in the produced rapeseed cake (Table 4). The grinding process was much too short, and the final temperatures of the cake in the experiments conducted at room temperature were far too low for oxidation processes to occur. Sterol content and sterol composition, on the other hand, were influenced by the grinding process (Table 5). Stigmasterol was under direct influence of grinding temperature ($p < 0.05$), with generally higher amounts in CC samples according to Tukey test. The levels of brassicasterol and Δ5-avenasterol were also affected by grinding temperature, but also by the duration of the process and the interaction of the two factors. Time and the interaction of time and temperature had significant effects on the content of campesterol, Δ-5,24-stigmastadienol, and concentration of total sterols, while β-sitosterol, the dominant sterol in rapeseed cake, was affected only by the interaction of the factors studied. According to the Tukey test, the highest amounts of total sterols were found in the sample CC ground for twelve minutes. These values were slightly higher but comparable to those of the RT samples ground for 8 and 12 min, respectively. Longer mechanical treatment by the ball mill probably resulted in greater disruption of the cell structure and thus better availability and extractability of the sterols. Using wheat bran as an example, it has been shown that cryogenic grinding causes destruction of the cell wall, resulting in the release of the cell contents and thus higher bioavailability of certain compounds. It also facilitates the interaction between the compound and the solvent and the extraction of the desired components [45].

Table 4. Fatty acid composition of rapeseed cakes additionally grinded at room temperature (RT) and with cryogenic cooling (CC).

Fatty Acid (% of Total)	Sample							
	2RT	4RT	8RT	12RT	2CC	4CC	8CC	12CC
C14:0	nd *	0.1 ± 0.0	nd *	nd *	nd *	nd *	nd *	nd *
C16:0	5.1 ± 0.0	5.1 ± 0.0	5.1 ± 0.1	5.1 ± 0.0	5.1 ± 0.0	5.1 ± 0.2	5.1 ± 0.0	5.2 ± 0.0
C16:1	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
C17:1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C18:0	1.9 ± 0.0	2.0 ± 0.0	2.0 ± 0.2	2.0 ± 0.1	1.9 ± 0.0	1.9 ± 0.0	1.9 ± 0.1	1.9 ± 0.0

Table 4. *Cont.*

Fatty Acid (% of Total)	Sample							
	2RT	4RT	8RT	12RT	2CC	4CC	8CC	12CC
C18:1	64.3 ± 0.6	64.3 ± 0.4	64.2 ± 0.1	64.2 ± 0.2	64.2 ± 0.6	64.2 ± 0.3	64.2 ± 0.2	63.9 ± 0.4
C18:2	19.1 ± 0.3	19.1 ± 0.0	19.2 ± 0.2	19.2 ± 0.0	19.2 ± 0.2	19.1 ± 0.1	19.1 ± 0.0	19.4 ± 0.4
C18:3	6.8 ± 0.0	6.8 ± 0.2	6.8 ± 0.1	6.8 ± 0.1	6.8 ± 0.0	6.9 ± 0.0	6.9 ± 0.2	6.8 ± 0.1
C20:0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
C20:1	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.1	1.0 ± 0.0	1.0 ± 0.2	1.0 ± 0.0
C22:0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.1

* not detected.

Table 5. Content and composition of sterols determined in rapeseed cakes additionally grinded at room temperature (RT) and with cryogenic cooling (CC).

Sterol (% of Total)	Sample							
	2RT	4RT	8RT	12RT	2CC	4CC	8CC	12CC
Brasicasterol $^{\$,£,§}$	11.0 ± 0.0 c	11.4 ± 0.5 bc	11.8 ± 0.2 ab	11.7 ± 0.0 ab	11.5 ± 0.3 bc	12.1 ± 0.2 a	11.4 ± 0.2 bc	11.6 ± 0.4 ab
Campesterol $^{\$,§}$	27.2 ± 0.4 c	28.9 ± 1.4 ab	30.0 ± 0.2 a	29.1 ± 0.0 ab	28.7 ± 0.5 abc	28.6 ± 0.7 abc	28.8 ± 1.1 bc	28.2 ± 0.9 bc
Campestanol	1.1 ± 0.0	1.1 ± 0.1	1.1 ± 0.0	1.2 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.1	1.1 ± 0.0
Stigmasterol $^{£}$	0.7 ± 0.0 ab	0.7 ± 0.0 ab	0.5 ± 0.4 b	0.7 ± 0.0 ab	0.7 ± 0.0 ab	0.7 ± 0.0 ab	0.8 ± 0.1 a	0.9 ± 0.0 a
β-sitosterol §	51.8 ± 0.6 b	51.2 ± 0.9 ab	52.3 ± 0.4 a	51.3 ± 0.4 ab	51.8 ± 0.7 ab	52.8 ± 1.8 a	51.1 ± 1.0 ab	51.7 ± 0.6 ab
Δ5-avenasterol $^{\$,£,§}$	5.8 ± 1.1 a	4.7 ± 2.2 ab	2.2 ± 1.0 bc	3.2 ± 0.1 bc	3.5 ± 1.0 bc	1.1 ± 0.2 c	4.7 ± 2.3 ab	3.6 ± 1.8 bc
Δ7-avenasterol	0.6 ± 0.1	0.7 ± 0.6	0.8 ± 0.2	1.0 ± 0.0	0.9 ± 0.4	1.2 ± 1.5	0.8 ± 0.4	1.1 ± 0.1
Δ7-stigmasterol	0.3 ± 0.1	nd *	nd *	nd *	0.1 ± 0.1	0.7 ± 0.2	0.1 ± 0.1	0.2 ± 0.1
Δ-5,24-stigmastadienol $^{\$,§}$	1.4 ± 0.1 ab	1.2 ± 0.0 b	1.2 ± 0.1 ab	1.4 ± 0.0 ab	1.3 ± 0.1 ab	1.2 ± 0.0 ab	1.4 ± 0.2 a	1.3 ± 0.1 ab
Total sterols (mg/100 g) $^{\$,§}$	138 ± 6 b	143 ± 3 b	149 ± 8 ab	147 ± 2 ab	149 ± 3 ab	140 ± 7 c	145 ± 8 b	157 ± 5 a

* Not detected; $^{\$}$ grinding time had significant influence ($p \leq 0.05$); $^{£}$ grinding temperature had significant influence ($p \leq 0.05$); § interaction of the grinding time and temperature had significant influence ($p \leq 0.05$); values with different letters in each row are statistically different ($p \leq 0.05$) according to Tukey's multiple comparison tests.

The content of IDF was not affected by the grinding process and particle size reduction, while it significantly affected soluble dietary fiber (SDF) (Figure 1). The amount of SDFP was significantly affected by the grinding time and the interaction of grinding time and temperature. According to the Tukey test, their amount increased with increasing grinding time until 8th minute, both with and without cryogenic cooling, and then decreased after 12 min. Samples ground for 12 min contained significantly lower amounts of SDFP than samples prepared in shorter procedures. The increase was also observed by Liu et al. [46] on an orange peel. The reason could be the release of soluble fibres from stable complexes with a longer grinding time, while further grinding leads to mechanical destruction of the molecules and consequently to a decrease in their amount. This hypothesis is confirmed by the fact that the number of SDFS increased slightly (by about 8%) after 12 min of grinding with cryogenic cooling. Both SDFP and SDFS were significantly affected by the duration of the grinding process, but SDFS were also affected by the temperature of the process, with cryogenic grinding yielding a slightly higher value. Čukelj Mustač et al. [14] also reported an increase in SDFS of millet bran with decreasing particle size.

Grinding process had a significant effect on the concentration of free and bound phenolic compounds, but not as much as expected. Several authors reported no significant effect of cryogenic grinding on phenolic compounds [14,45,47], but the results of our previous studies on pumpkin seed cake showed a significant effect of grinding time and grinding conditions on total phenolic content. Grinding the pumpkin cake for 12 min under cryogenic conditions in the aforementioned study resulted in 36% more extracted phenolic compounds [15]. The results of this study were somewhat different. In the present study, the duration of grinding treatment had a significant effect on the concentrations of gallic, ferulic, sinapic, and *p*-coumaric acids, as well as on sinapine and consequently on total free phenolics (Table 6). A significant effect of grinding temperature was found for the concentration of all free phenolic compounds except for canolol. According to the Tukey test, the concentration of sinapine, the dominant phenolic compound in rapeseed cake, and

the total amount of free phenolics were significantly higher in the RT samples than in the CC samples. The total amount of bound phenols was also significantly affected by the grinding conditions applied, but an opposite trend to the free phenolics was observed (Table 7). The Tukey test showed that the concentration of bound phenolics was significantly higher in the cryogenically ground samples. Interestingly, a very small scatter is observed between the results of total phenolics, and free and bound phenols combined (2783 ± 127 mg/100 g). This suggests that the increase in free phenolics is due to the damage to the cell walls caused by grinding and the release of phenolic compounds bound to the cell wall material, and that the heat generated during grinding at room temperature favours this process.

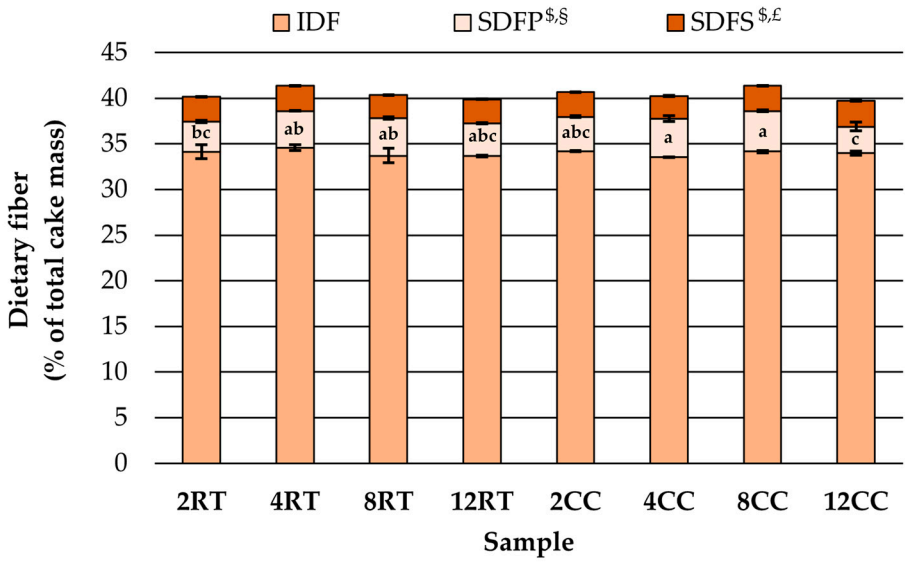

Figure 1. Dietary fiber content and composition of rapeseed cake ground at room temperature (RT) and with cryogenic cooling (CC) expressed as % of defatted and dried cake (IDF—insoluble dietary fiber; SDFP—soluble in water but precipitated in 78% ethanol; SDFS soluble in water and not precipitated in 78% ethanol; $ grinding time had significant influence ($p \leq 0.05$); £ grinding temperature had significant influence ($p \leq 0.05$); § interaction of the grinding time and temperature had significant influence ($p \leq 0.05$); values with different letters are statistically different ($p \leq 0.05$) according to Tukey's multiple comparison tests.

Table 6. Content and composition of free phenolics determined in defatted and dry rapeseed cake (RC) and cakes ground at room temperature (RT) and with cryogenic cooling (CC).

Phenolic Compound (mg/100 g)	Sample							
	2RT	4RT	8RT	12RT	2CC	4CC	8CC	12CC
Gallic acid $,£	58 ± 11 ab	47 ± 15 abc	68 ± 11 a	59 ± 1 ab	43 ± 6 bc	38 ± 13 bc	62 ± 11 ab	32 ± 8 c
Chlorogenic acid £	84 ± 4 a	84 ± 4 a	84 ± 4 a	77 ± 3 ab	75 ± 5 ab	76 ± 1 ab	79 ± 6 ab	72 ± 2 b
Ferulic acid $,£	76 ± 3 a	69 ± 4 ab	74 ± 3 a	69 ± 3 ab	70 ± 5 ab	67 ± 2 ab	71 ± 5 ab	62 ± 5 b
Sinapic acid $,£	97 ± 3 a	93 ± 7 a	98 ± 7 a	91 ± 3 a	90 ± 5 ab	87± 0 ab	92 ± 6 a	78 ± 8 b
p-coumaric acid $,£	112 ± 6 a	106 ± 4 ab	108 ± 6 ab	100 ± 4 ab	100 ± 8 ab	102 ± 5 ab	105 ± 8 ab	95 ± 8 b
Syringaldehyde £	18 ± 1	19 ± 2	19 ± 2	18 ± 1	15 ± 4	16 ± 3	19 ± 1	16 ± 1
Canolol	9 ± 2	9 ± 0	8 ± 1	8 ± 1	8 ± 2	7 ± 1	8 ± 1	9 ± 1
Sinapine $,£	913 ± 49 a	809 ± 44 ab	882 ± 25 a	798 ± 55 ab	826 ± 52 ab	816 ± 31 ab	830 ± 73 ab	710 ± 78 b
Non identified $,£	954 ± 23 a	791 ± 65 bcd	974 ± 2 a	827 ± 30 bc	876 ± 70 ab	816 ± 12 bc	690 ± 26 d	731 ± 56 cd
Total $,£	2322 ± 70 a	2027 ±145 bc	2225 ± 53 ab	2047 ± 92 abc	2102 ±145 ab	2025 ± 35 bc	2115 ± 142 ab	1804 ± 167 c

$ grinding time had significant influence ($p \leq 0.05$); £ grinding temperature had significant influence ($p \leq 0.05$); values with different letters in each row are statistically different ($p \leq 0.05$) according to Tukey's multiple comparison tests.

Table 7. Content and composition of bound phenolic compounds determined in defatted and dry rapeseed cake (RC) and cakes ground at room temperature (RT) and with cryogenic cooling (CC).

Phenolic Compound (mg/100 g)	Sample							
	2RT	4RT	8RT	12RT	2CC	4CC	8CC	12CC
Ferulic acid [£]	25 ± 1	28 ± 2	30 ± 1	26 ± 3	28 ± 1	29 ± 4	28 ± 1	33 ± 2
Sinapic acid [$,£,§]	413 ± 14 c	502 ± 21 c	533 ± 6 bc	427 ± 37 c	535 ± 48 bc	646 ± 33 ab	515 ± 10 bc	694 ± 48 a
p-coumaric acid [$,£]	7± 0 c	8 ± 1 b	8 ± 0 bc	7 ± 0 c	9 ± 1 ab	10 ± 0 a	9 ± 1 ab	9 ± 1 ab
Syringaldehyde	4 ± 1	3 ± 1	5 ± 0	3 ± 0	4 ± 0	5 ± 0	4 ± 0	4 ± 0
Non identified [$,£,§]	93 ± 7 c	114 ± 3 c	110 ± 1 c	101 ± 8 c	182 ± 21 a	137 ± 12 bc	92 ± 14 c	168 ± 24 ab
Total [$,£,§]	541± 8 e	656 ± 22 cde	685 ± 4 cd	565 ± 48 de	758 ± 10 bc	826 ± 25 ab	647 ± 23 cde	907 ± 26 a

[$] grinding time had significant influence ($p \leq 0.05$); [£] grinding temperature had significant influence ($p \leq 0.05$); [§] interaction of the grinding time and temperature had significant influence ($p \leq 0.05$); values with different letters in each row are statistically different ($p \leq 0.05$) according to Tukey's multiple comparison tests.

The changes in tannin concentration in ground rapeseed cakes are shown in Figure 2. According to the results, the initial rapeseed cake contained lower amounts of tannins (Table 2) than all other samples. The results show that a longer grinding time improves the extractability of tannins ($p \leq 0.05$). It is observed that better extraction is obtained at higher temperatures, but the grinding temperature was not found to be significant. Tukey test also showed that there was no significant difference in tannin concentration between samples. Similar to phenolic compounds, tannins are also released from their complexes by cell disruption.

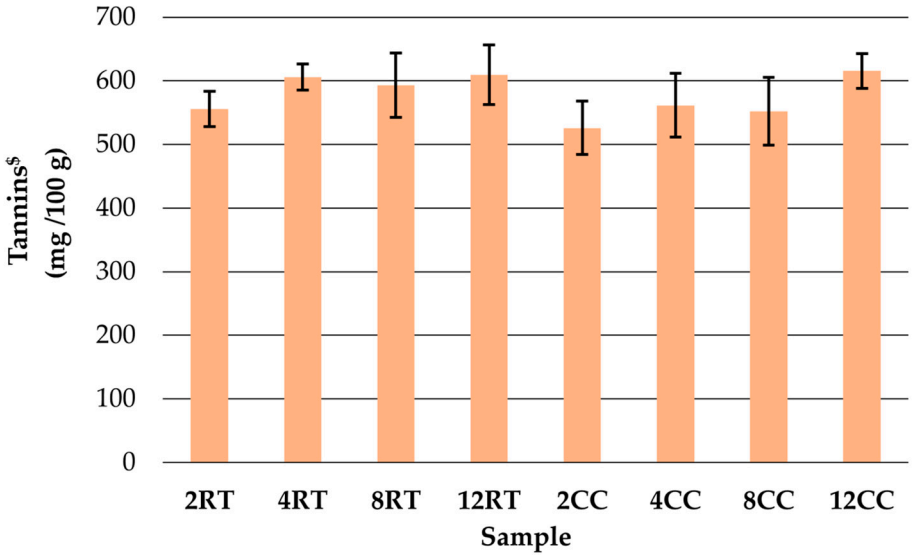

Figure 2. Tannin content cakes grinded at room temperature (RT) and with cryogenic cooling (CC); [$] grinding time had significant influence on the concentration of tannins ($p \leq 0.05$).

As mentioned above, glucosinolates are considered the major antinutritive constituents of rapeseed cake. Their degradation products, including goitrin, inhibit iodine uptake and the biosynthesis of thyroid hormones T3 and T4, leading to hypothyroidism and subsequent enlargement of the thyroid gland [35]. The concentration and composition of glucosinolates in rapeseed cake are shown in Table 8. The concentration of major glucosinolates (progoitrin, gluconapin, glucobrasicanapin) and glucoraphanin decreased with the duration of the grinding process, according to Tukey test ($p \leq 0.05$). This resulted in a 34% decrease in total glucosinolates after 12 min of grinding at room temperature and 43% after 12 min of cryogenic grinding. Wang et al. [48] reported that a larger surface area of ground horseradish correlated positively with the concentration of isothyocianates, which may be attributed to more extensive hydrolysis of glucosinolates by endogenous myrosinase. Although we achieved a significant reduction in glucosinolate concentration,

the remaining concentrations are still too high, requiring further processing of the cake to use it as a food source.

Table 8. Content and composition of glucosinolates determined in defatted cakes ground at room temperature (RT) and with cryogenic cooling (CC).

Glucosinolate (μmol/g)	Sample							
	2RT	**4RT**	**8RT**	**12RT**	**2CC**	**4CC**	**8CC**	**12CC**
Glucoiberin	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	tr *	tr	tr
Progoitrin $^{\$,\S}$	5.0 ± 0.4 ab	5.2 ± 0.4 ab	5.2 ± 0.6 ab	4.8 ± 0.7 ab	5.9 ± 1.3 a	5.1 ± 0.2 ab	5.0 ± 1.4 ab	4.4 ± 0.6 b
Epi-progoitrin	nd **	0.1 ± 0.1	0.1 ± 0.1	nd **	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	nd **
Glucoraphinin $^{\$}$	0.4 ± 0.2	0.2 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.1	0.2 ± 0.0
Gluconapoleiferin	0.2 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	0.3 ± 0.1	0.3 ± 0.0
Gluconapin $^{\$,\S}$	2.9 ± 0.5 a	2.5 ± 0.3 ab	2.4 ± 0.3 ab	2.5 ± 0.2 ab	2.9 ± 0.7 a	2.4 ± 0.3 ab	2.6 ± 0.4 ab	2.1 ± 0.4 b
4-hidroxyglucobrassicin	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
Glucobrassicanapin $^{\$}$	0.8 ± 0.2 a	0.7 ± 0.1 ab	0.7 ± 0.1 ab	0.7 ± 0.0 bc	0.8 ± 0.2 a	0.7 ± 0.1 ab	0.8 ± 0.2 bc	0.6 ± 0.1 c
Glucobrassicin $^{\$,\S}$	0.6 ± 0.2 a	0.3 ± 0.1 ab	0.2 ± 0.0 ab	0.3 ± 0.0 ab	0.3 ± 0.0 ab	0.3 ± 0.0 ab	0.1 ± 0.0 b	0.2 ± 0.1 ab
4-metoxyglucobrassicin $^{\$,\S}$	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	tr
Total $^{\$,\S}$	10.6 ± 0.7 a	9.6 ± 0.7 ab	9.5 ± 1.2 ab	9.3 ± 0.9 ab	10.8 ± 2.3 a	9.5 ± 0.4 ab	9.4 ± 1.3 ab	8.0 ± 1.3 b

* traces (<0.05) ** not detected; $^{\$}$ grinding time had significant influence ($p \leq 0.05$); § interaction of the grinding time and temperature had significant influence ($p \leq 0.05$); values with different letters in each row are statistically different ($p \leq 0.05$) according to Tukey's multiple comparison tests.

4. Conclusions

Cryogenic grinding is a much more efficient method of reducing particle size than room temperature grinding. Grinding the rapeseed cake with a ball mill for only 2 min at −196 °C resulted in the same particle size as grinding for 12 min at room temperature. Twelve minutes of cryogenic grinding resulted in particles about 6.5 times smaller and a specific surface area 7 times larger compared to the starting material.

Micronization of the rapeseed cake had some effect on the nutritional and antinutritional components, but not to the extent we expected. On the positive side, grinding the cake had no effect on the fatty acid composition of the cake, but the cake ground for 12 min under cryogenic conditions had an increased content of phytosterols. Soluble dietary fibre content also slightly increased with decreasing particle size. However, the content of insoluble dietary fibre was not affected. The mechanical destruction of the cell walls combined with the heat generated during grinding at room temperature resulted in the release of the bound phenolics and thus an increase in the total free phenolic compounds in these samples. The same occurred with the tannins, making them more extractable and consequently resulting in higher tannin concentrations after longer grinding times. However, the best results were obtained in reducing the components that raise the most concerns, glucosinolates. Room temperature grinding reduced glucosinolate content by up to 34%, and even better results were obtained under cryogenic conditions: 43% less after 12 min.

Although these results are promising, they are not sufficient to recommend micronization as the sole treatment for rapeseed cake. It could be used as a pre-treatment for chemical or biological treatments to reduce antinutritive components, since cell disruption and increase in specific surface area increases the extractability and availability of undesirable components. These studies should also investigate whether the high cost of the cryogenic process is justified, as it did not significantly affect the antinutritional components of rapeseed cake.

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References

1. Wiebe, K. FAO's Director-General on How to Feed the World in 2050. *Popul. Dev. Rev.* **2009**, *35*, 837–839. [CrossRef]
2. Campbell, L.; Rempel, C.; Wanasundara, J. Canola/Rapeseed Protein: Future Opportunities and Directions—Workshop Proceedings of IRC 2015. *Plants* **2016**, *5*, 17. [CrossRef] [PubMed]
3. Hald, C.; Dawid, C.; Tressel, R.; Hofmann, T. Kaempferol 3-O-(2‴-O-Sinapoyl-β-Sophoroside) Causes the Undesired Bitter Taste of Canola/Rapeseed Protein Isolates. *J. Agric. Food Chem.* **2019**, *67*, 372–378. [CrossRef] [PubMed]
4. USDA ERS Oil Crops Yearbook. Available online: https://www.ers.usda.gov/topics/crops/soybeans-oil-crops/oil-crops-sector-at-a-glance/ (accessed on 7 February 2023).
5. Khattab, R.Y.; Arntfield, S.D. Functional Properties of Raw and Processed Canola Meal. *LWT Food Sci. Technol.* **2009**, *42*, 1119–1124. [CrossRef]
6. Shahidi, F.; Naczk, M. An Overview of the Phenolics of Canola and Rapeseed: Chemical, Sensory and Nutritional Significance. *J. Am. Oil Chem. Soc.* **1992**, *69*, 917–924. [CrossRef]
7. Tripathi, M.K.; Mishra, A.S. Glucosinolates in Animal Nutrition: A Review. *Anim. Feed Sci. Technol.* **2007**, *132*, 1–27. [CrossRef]
8. Szydłowska-Czerniak, A.; Bartkowiak-Broda, I.; Karlović, I.; Karlovits, G.; Szłyk, E. Antioxidant Capacity, Total Phenolics, Glucosinolates and Colour Parameters of Rapeseed Cultivars. *Food Chem.* **2011**, *127*, 556–563. [CrossRef]
9. Thiyam, U.; Stöckmann, H.; Schwarz, K. Antioxidant Activity of Rapeseed Phenolics and Their Interactions with Tocopherols during Lipid Oxidation. *J. Am. Oil Chem. Soc.* **2006**, *83*, 523–528. [CrossRef]
10. Kozlowska, H.; Naczk, M.; Shahidi, F.; Zadernowski, R. Phenolic Acids and Tannins in Rapeseed and Canola. In *Canola and Rapeseed*; Springer: Boston, MA, USA, 1990; pp. 193–210.
11. Amarowicz, R.; Naczk, M.; Shahidi, F. Antioxidant Activity of Crude Tannins of Canola and Rapeseed Hulls. *J. Am. Oil Chem. Soc.* **2000**, *77*, 957. [CrossRef]
12. Chen, T.; Zhang, M.; Bhandari, B.; Yang, Z. Micronization and Nanosizing of Particles for an Enhanced Quality of Food: A Review. *Crit. Rev. Food Sci. Nutr.* **2018**, *58*, 993–1001. [CrossRef]
13. Singh, K.K.; Goswami, T.K. Cryogenic grinding of cloves. *J. Food Process. Preserv.* **2007**, *24*, 57–71. [CrossRef]
14. Čukelj Mustač, N.; Novotni, D.; Habuš, M.; Drakula, S.; Nanjara, L.; Voučko, B.; Benković, M.; Ćurić, D. Storage Stability, Micronisation, and Application of Nutrient-Dense Fraction of Proso Millet Bran in Gluten-Free Bread. *J. Cereal Sci.* **2020**, *91*, 102864. [CrossRef]
15. Balbino, S.; Dorić, M.; Vidaković, S.; Kraljić, K.; Škevin, D.; Drakula, S.; Voučko, B.; Čukelj, N.; Obranović, M.; Ćurić, D. Application of Cryogenic Grinding Pretreatment to Enhance Extractability of Bioactive Molecules from Pumpkin Seed Cake. *J. Food Process. Eng.* **2019**, *42*, e13300. [CrossRef]
16. Benković, M.; Srečec, S.; Špoljarić, I.; Mršić, G.; Bauman, I. Flow Properties of Commonly Used Food Powders and Their Mixtures. *Food Bioprocess Technol.* **2013**, *6*, 2525–2537. [CrossRef]
17. *ISO 665:2000*; Oilseeds—Determination of Moisture and Volatile Matter Content. International Organization for Standardization: Geneva, Switzerland, 2000.
18. *ISO 659:2009*; Oilseeds—Determination of Oil Content (Reference Method). International Organization for Standardization: Geneva, Switzerland, 2009.
19. *ISO 20483:2013*; Cereals and Pulse—Determination of the Nitrogen Content and Calculation of the Crude Protein Content—Kjeldahl Method. International Organization for Standardization: Geneva, Switzerland, 2013.
20. *ISO 2171:2007*; Cereals, Pulses and by-Products—Determination of Ash Yield by Incineration. International Organization for Standardization: Geneva, Switzerland, 2007.
21. Li, T.; Beveridge, T.; Drover, J. Phytosterol Content of Sea Buckthorn (*Hippophae rhamnoides* L.) Seed Oil: Extraction and Identification. *Food Chem.* **2007**, *101*, 1633–1639. [CrossRef]
22. *ISO 12966-2:2017*; Animal and Vegetable Fats and Oils—Gas Chromatography of Fatty Acid Methyl Esters—Part 2: Preparation of Methyl Esters of Fatty Acids. International Organization for Standardization: Geneva, Switzerland, 2017.
23. Kraljić, K.; Stjepanović, T.; Obranović, M.; Pospišil, M.; Balbino, S.; Škevin, D. Influence of Conditioning Temperature on the Quality, Nutritional Properties and Volatile Profile of Virgin Rapeseed Oil. *Food Technol. Biotechnol.* **2018**, *56*, 562–572. [CrossRef]

24. *ISO 12228-1:2014*; Determination of Individual and Total Sterols Contents—Gas Chromatographic Method—Part 1: Animal and Vegetable Fats and Oils. International Organization for Standardization: Geneva, Switzerland, 2014.
25. AOAC. *AOAC Official Method 2011.25 Insoluble, Soluble, and Total Dietary Fiber in Foods*; AOAC: Rockville, MD, USA, 2012.
26. Martini, D.; D'Egidio, M.G.; Nicoletti, I.; Corradini, D.; Taddei, F. Effects of Durum Wheat Debranning on Total Antioxidant Capacity and on Content and Profile of Phenolic Acids. *J. Funct. Foods* **2015**, *17*, 83–92. [CrossRef]
27. Panić, M.; Stojković, M.R.; Kraljić, K.; Škevin, D.; Redovniković, I.R.; Srček, V.G.; Radošević, K. Ready-to-Use Green Polyphenolic Extracts from Food by-Products. *Food Chem.* **2019**, *283*, 628–636. [CrossRef]
28. Price, M.L.; Van Scoyoc, S.; Butler, L.G. A Critical Evaluation of the Vanillin Reaction as an Assay for Tannin in Sorghum Grain. *J. Agric. Food Chem.* **1978**, *26*, 1214–1218. [CrossRef]
29. *ISO 9167-1:1992*; Rapeseed—Determination of Glucosinolates Content—Part. 1: Method Using High-Performance Liquid Chromatography. International Organization for Standardization: Geneva, Switzerland, 1992.
30. Leming, R.; Lember, A. Chemical Composition of Expeller-Extracted and Cold-Pressed Rapeseed Cake. *Agraarteadus* **2005**, *16*, 103–109.
31. WHO; FAO. *Codex Standard for Named Vegetable Oils*; Food and Agriculture Organization of the United Nations/World Health Organization: Rome, Italy, 1999.
32. Hammad, S.; Pu, S.; Jones, P.J. Current Evidence Supporting the Link Between Dietary Fatty Acids and Cardiovascular Disease. *Lipids* **2016**, *51*, 507–517. [CrossRef] [PubMed]
33. Khajali, F.; Slominski, B.A. Factors That Affect the Nutritive Value of Canola Meal for Poultry. *Poult. Sci.* **2012**, *91*, 2564–2575. [CrossRef] [PubMed]
34. Kaczmarek, P.; Korniewicz, D.; Lipinski, K.; Mazur, M. Chemical Composition of Rapeseed Products and Their Use in Pig Nutrition. *Pol. J. Nat. Sci.* **2016**, *31*, 545–562.
35. Slominski, B.A.; Jia, W.; Rogiewicz, A.; Nyachoti, C.M.; Hickling, D. Low-Fiber Canola. Part 1. Chemical and Nutritive Composition of the Meal. *J. Agric. Food Chem.* **2012**, *60*, 12225–12230. [CrossRef]
36. Brown, L.; Rosner, B.; Willett, W.W.; Sacks, F.M. Cholesterol-Lowering Effects of Dietary Fiber: A Meta-Analysis. *Am. J. Clin. Nutr.* **1999**, *69*, 30–42. [CrossRef]
37. Leopoldini, M.; Marino, T.; Russo, N.; Toscano, M. Antioxidant Properties of Phenolic Compounds: H-Atom versus Electron Transfer Mechanism. *J. Phys. Chem. A* **2004**, *108*, 4916–4922. [CrossRef]
38. Kraljić, K.; Škevin, D.; Pospišil, M.; Obranović, M.; Neđeral, S.; Bosolt, T. Quality of Rapeseed Oil Produced by Conditioning Seeds at Modest Temperatures. *J. Am. Oil Chem. Soc.* **2013**, *90*, 589–599. [CrossRef]
39. Naczk, M.; Amarowicz, R.; Sullivan, A.; Shahidi, F. Current Research Developments on Polyphenolics of Rapeseed/Canola: A Review. *Food Chem.* **1998**, *62*, 489–502. [CrossRef]
40. Przybylski, R.; Mag, T.; Eskin, N.A.M.; McDonald, B.E. Canola Oil. In *Bailey's Industrial Oil and Fat Products*; Shahidi, F., Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2005; pp. 61–121. ISBN 9780471678496.
41. Barbosa-Canovas, G.; Ortega-Rivas, E.; Juliano, P.; Yan, H. *Food Powders*; Food Engineering Series; Kluwer Academic/Plenum Publishers: New York, NY, USA, 2005; ISBN 978-0-306-47806-2.
42. Wang, H.; Mustaffar, A.; Phan, A.N.; Zivkovic, V.; Reay, D.; Law, R.; Boodhoo, K. A Review of Process Intensification Applied to Solids Handling. *Chem. Eng. Process. Process Intensif.* **2017**, *118*, 78–107. [CrossRef]
43. Hossen, M.S.; Sotome, I.; Takenaka, M.; Isobe, S.; Nakajima, M.; Okadome, H. Effect of Particle Size of Different Crop Starches and Their Flours on Pasting Properties. *Japan J. Food Eng.* **2011**, *12*, 29–35. [CrossRef]
44. Carpin, M.; Bertelsen, H.; Dalberg, A.; Bech, J.K.; Risbo, J.; Schuck, P.; Jeantet, R. How Does Particle Size Influence Caking in Lactose Powder? *J. Food Eng.* **2017**, *209*, 61–67. [CrossRef]
45. Hemery, Y.; Chaurand, M.; Holopainen, U.; Lampi, A.M.; Lehtinen, P.; Piironen, V.; Sadoudi, A.; Rouau, X. Potential of Dry Fractionation of Wheat Bran for the Development of Food Ingredients, Part I: Influence of Ultra-Fine Grinding. *J. Cereal Sci.* **2011**, *53*, 1–8. [CrossRef]
46. Liu, Y.; Wang, L.; Liu, F.; Pan, S. Effect of Grinding Methods on Structural, Physicochemical, and Functional Properties of Insoluble Dietary Fiber from Orange Peel. *Int. J. Polym. Sci.* **2016**, *2016*, 6269302. [CrossRef]
47. Rosa, N.N.; Barron, C.; Gaiani, C.; Dufour, C.; Micard, V. Ultra-Fine Grinding Increases the Antioxidant Capacity of Wheat Bran. *J. Cereal Sci.* **2013**, *57*, 84–90. [CrossRef]
48. Wang, J.; Zhang, M.; Devahastin, S.; Liu, Y. Influence of Low-Temperature Ball Milling Time on Physicochemical Properties, Flavor, Bioactive Compounds Contents and Antioxidant Activity of Horseradish Powder. *Adv. Powder Technol.* **2020**, *31*, 914–921. [CrossRef]