https://doi.org/10.17113/ftb.61.03.23.8016 preliminary communication

Effect of Ultrasound and Hydrothermal Treatment on Digestible and Antioxidant Properties of Wholegrain Wheat Flour with Different Amylose Content§

Running head: Effect of Treatment on Flour Digestibility and Antioxidants

Valentina Nikolić[1*], Slađana Žilić[1], Marijana Simić[1], Vesna Kandić[1] and Primož Titan[2]

[1]Maize Research Institute Zemun Polje, Slobodana Bajića 1, 11185 Belgrade-Zemun, Serbia
[2]Research Genetics and Agrochemistry Ltd., Krog, Brodarska 27, 9000 Murska Sobota, Slovenia

Received: 29 November 2022
Accepted: 6 July 2023

SUMMARY

Research background. The consumption of cereal wholegrain flour contributes to an increased intake of dietary fibres and phenolic compounds beneficial to human health. However, there are some drawbacks regarding wholegrain flour, such as poor baking performance and lower technological quality. Applying ultrasound and hydrothermal treatments may provide new possibilities for the modification and improvement of the baking- and bio-functionality of flours as well as the quality of baked goods.

Experimental approach. The wholegrain flours of six wheat varieties with different amylose content were investigated. The initial chemical composition and the viscosity profiles of flours were

*Corresponding author:
Phone: +381648406448
E-mail: valentinas@mrizp.rs

§Paper was presented at the 11th Central European Congress on Food and Nutrition CEFood2022, Čatež ob Savi, Slovenia, 27-30 September 2022

assayed. The flour samples were subjected to ultrasound treatment for 10 minutes at frequency 30 kHz and temperature of 40 ˚C, as well as to hydrothermal treatment on a magnetic stirrer with heating for 3 minutes after reaching the boiling point. The treatments were carried out in order to determine their influence on the investigated digestible and antioxidant properties of the flours. A multistep enzymatic *in vitro* digestibility protocol for the simulation of the food digestion process in the human gastrointestinal tract was applied on the untreated and treated wholegrain flour samples. Content of total free phenolic compounds and total antioxidant capacity were determined as well.

Results and conclusions. Hydrothermal treatment positively influenced the digestibility of the wholegrain flours, especially in waxy wheat genotypes compared to high amylose ones, which can be attributed to the formation of resistant starch. The hydrothermal treatment had an overall negative impact on the antioxidant capacity of the flour samples, while ultrasound, in general increased the analytical values of the total free phenolic compounds by enhancing their extractability. These findings can provide valuable guidelines in the development of new wholegrain wheat foods.

Novelty and scientific contribution. To the best of our knowledge, this kind of investigation of the effect of ultrasound and hydrothermal treatment on the digestibility and antioxidant properties has not yet been conducted on wholegrain wheat flours with different amylose content. Waxy and high-amylose wheat varieties are considered novel raw materials due to their unique properties in bread making, such as improved bread texture and increased dietary fibre content.

Keywords: wholegrain wheat flour; ultrasound; hydrothermal treatment; *in vitro* digestibility; phenolic compounds; antioxidant capacity

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops consumed worldwide as a staple in a broad range of food products (*1,2*). The preponderance of wheat-based foods is produced from refined flour that lacks some essential nutrients lost during the milling process after removing the bran and germ (*3*). The prevalence of refined grain foods in the diet has been associated with an increase in health problems and diseases such as type 2 diabetes, coronary atherosclerosis, chronic cardiovascular disease, colon cancer, and obesity, as shown by epidemiological studies (*4*). Whole wheat is rich in nutrients, particularly dietary fibre, B-vitamins, minerals, as well as phytochemicals such as phenolic acids, flavonoids, carotenoids, and tocopherols, all potentially beneficial to human health (*1*).

Most of these compounds are located in the aleurone layer which gives this fraction the highest antioxidant activity, followed by bran and germ. The majority of the bioactive components in bran are attached to fibre, allowing them to pass through digestion in the gastrointestinal tract and arrive intact in the colon, where they create an antioxidant environment. Nevertheless, research has shown that the solubility and activity of bound phenolic compounds increases during digestion processes (*5*).

The grain processing sector is currently gaining momentum with research on the possible applications of various heat and non-thermal procedures, including ultrasonic and hydrothermal treatments, to boost the bio- and baking functionality of flours and enhance the final quality of baked goods (*6*). Ultrasound, for instance, represents a promising green approach that has found its application in the food processing sector since it increases yield and enhances extraction rates of different bioactive compounds and nutrients, which reduces water and energy waste. Ultrasound may either improve or impair food quality depending on the processing variables (frequency, amplitude, treatment time), as well as the food sources (*7*). The distinctive functional characteristics of wheat flour for food manufacturing such as bread loaf volume, starch pasting and rate of starch digestion are greatly influenced by the interactions between the two major macromolecular components, starch and protein (*8*). Starch granules are composed of two main constituents: amylose, primarily a linear polymer of (1→4)-linked α-D-glucopyranosyl units with some minor branching, and amylopectin, a high-molecular-weight branched polymer with α-(1→4) and α-(1→6) linkages that produce branch points (*2,9*). Since only α-glucosidic linkages are present in starch molecules, amylolytic enzymes produced by the human digestive system may be able to break them down. However, the gelatinized cooked starch may upon cooling retrograde to resistant starch, a form not hydrolysed by α-amylase that is not digested in the small intestine (*9*).

The physicochemical and functional properties of starch, the processing properties of flour, the digestibility, and the edible quality of the finished products are all influenced by different amylose-amylopectin ratios and the structure of the starch granules (*2,10*). The proportions of amylose and amylopectin in starches vary depending on the cultivar; whereas, normal wheat starches typically contain 22–35 % amylose and 65–78 % amylopectin, whereas waxy (amylose-free) wheat starches usually have about 100 % amylopectin (*11*). Genotypes having a 16–22 % amylose percentage can be categorised as partial waxy, according to Van Hung *et al.* (*12*). Waxy wheat is acknowledged as a novel raw material for the bakery sector because of its special qualities in bread preparation, such as its capacity to delay staling due to a reduced rate of starch retrogradation (*13*). Studies by Hung *et al.* (*13*) and Regina *et al.* (*14*) indicate that starches of the "high-amylose" wheat genotypes developed by lowering the activity of starch branching enzymes (SBE) have certain nutritional and health-promoting advantages due to slower digestion caused by the formation of resistant starch. The processing of "high-amylose" flour may produce more resistant starch than normal and waxy starch, opening the door to the development of novel food products with higher dietary fibre levels (*15,16*).

In general, both internal factors (protein, amylose, dietary fibre, and lipid content) and external factors (germination, processing, cooking, and starch retrogradation) can affect the digestibility of a cereal-based food product (*17*). The findings of *in vitro* studies have shown that the amylose content, length distributions of the amylopectin and amylose chains, and granular shape are all related to the digestion of starches (*9*). The digestibility and absorption of nutrients such as starch in grains may also be delayed or even prevented in the small intestine by other components, including protein and cell wall matrices, which can be entrapped within starch granules, and lipids, which can form complexes with amylose, leading to lower rapidly absorbed glucose levels (*18*).

The main focus of this study was to investigate the effect of ultrasound and hydrothermal treatment on enzymatic *in vitro* digestibility, antioxidant capacity and the content of total soluble free phenolic compounds of wholegrain wheat flours with different amylose share. Prior to the treatments and simulation of the digestion processes, the chemical composition, total free phenolic compounds, antioxidant capacity, and the viscosity of the wholegrain wheat samples were assessed for comparison and better statistical evaluation (Fig. S1).

MATERIALS AND METHODS

Chemicals

All chemicals and enzymes used in this study were of analytical and high performance liquid chromatography (HPLC) grade. The chemicals used were: ethanol, 95-96 %, p.a. (Zorka Pharma-Hemija d.o.o, Šabac, Serbia); methanol, 99.8%, HPLC grade (JT Baker, Phillipsburg, NJ, USA); Folin-Ciocalteu phenol reagent (F9252) of analytical grade (2N); ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (A1888-5G) (Sigma-Aldrich Company, St. Louis, MO, United States); and sodium carbonate, anhydrous, 99.5 %, p.a. (Loba Chemie, Pvt. Ltd., Mumbai, India). The enyzmes used for the *in vitro* digestion protocol were all produced by Sigma-Aldrich Company (St. Louis, MO, United States), namely: Pepsin from porcine gastric mucosa (P7000-25G); Bile extract porcine B8631-100G; Pancreatin from porcine pancreas (P1750-25G); Protease from Streptomyces griseus (P5147-1G) and cellulolytic enzyme mixture Viscozyme L (V2010-50ML). Distilled water obtained on a laboratory distillation unit type D-8-SLO (Elektron, Banja Koviljača, Serbia).was used for the analyses

Plant material

Six genotypes of wheat (*Triticum aestivum* L.) with different amylose content (0-36.5 %), diverse genetic background and geographic origin were used in this study. All the investigated genotypes were grown in the experimental field of the Maize Research Institute Zemun Polje, Belgrade, Serbia, (44˚52´N, 20˚19´E, 81m asl), in the 2021/2022 growing season. Standard cropping practices were applied in order to provide the plants with adequate nutrition and to keep the plots disease- and weed-free. Wholegrain wheat flour was obtained by grinding in a laboratory mill (Perten MILL 120 CE, Perten Instruments, Hägersten, Sweden) for fine sample preparation (mesh 0.5 mm).

Ultrasound treatment

Wholegrain wheat flour samples (10g) were mixed with water at the weight ratio (hydromodule) 1:3, placed in glass flasks and subjected to ultrasound treatment. The ultrasound treatment was performed in an ultrasound water bath (Model: UZ 4P 220/115V; power 100W; Iskra, Ljubljana, Slovenia) at frequency 30 kHz, temperature of 40 ˚C for 10 minutes. After the ultrasound treatment, the mixtures were transferred to Petri dishes and dried in a ventilation oven (Memmert UF 55, Memmert GmbH + Co.KG, Schwabach, Germany) at 40 °C overnight, and ground in a laboratory mill (Perten MILL 120 CE, Perten Instruments, Hägersten, Sweden) afterwards. The dry matter content of the samples was determined using the conventional drying method at 105 °C to a constant mass. The experiments were conducted in duplicates.

Hydrothermal treatment

Wholegrain wheat flour samples (10g) were mixed with water at the mass ratio (hydromodule) 1:6, in glass flasks. The magnet was added to the mixture and placed on a preheated hot plate (t=200 °C) with magnetic stirring (ARE Heating Magnetic Stirrer, VELP Scientifica, Usmate Velate MB, Italy) and subjected to hydrothermal treatment (cooking). The samples were cooked for 3 minutes after reaching the boiling point, while constantly stirring manually with a glass stirring rod. The mixtures were transferred to Petri dishes and dried in a ventilation oven (Memmert UF 55, Memmert GmbH + Co.KG, Schwabach, Germany) at 40 °C overnight, and ground in a laboratory mill (Perten MILL 120 CE, Perten Instruments, Hägersten, Sweden) afterwards. The dry matter content of the samples was determined using the conventional drying method at 105 °C to a constant mass. The experiments were conducted in duplicates.

Analysis of protein and starch in initial (non-treated) wholegrain flour

The Kjeldahl method was used to determine the protein content on the BÜCHI Kjeldahl System (AutoKjeldahl Distillation Unit K-350 and Speed Digester K-439, BÜCHI Labortechnik, Switzerland), where the total nitrogen was multiplied by 5.7 (*19*). The Ewers method was used to determine the starch content (*20*) on a polarimeter (UniPol L 2020, Schmidt + Haensch GmbH & Co., Berlin, Germany). According to the colorimetric method developed by McGrance *et al.* (*21*) the amounts of amylose and amylopectin were calculated after the absorbance of the samples was measured on a spectrophotometer (Agilent 8453 UV-visible Spectroscopy System, Agilent Technologies, Inc., Santa Clara, California, United States) at 600 nm. All results are shown as percentages of dry matter (dm). The analyses were performed in two replications.

Analysis of dietary fibre in initial (non-treated) wholegrain flour

Using the Fibertec system FOSS 2010 Hot Extractor (FOSS Tecator, Hoeganaes, Sweden), the Van Soest detergent method modified by Mertens (*22*) was applied to quantify the amount of hemicellulose, cellulose, and lignin (ADL). Cellulose content was calculated as the difference between ADF and lignin contents, while hemicellulose content was determined as the difference between NDF and ADF contents. Each result is presented as a percentage per dry matter (dm). The analyses were performed in two replications.

Pasting properties of initial (non-treated) wholegrain flour

Changes in the apparent viscosity of aqueous suspensions were analysed in order to obtain the pasting curves of the investigated whole-grain wheat flour. Suspensions containing 8 % starch (total mass of 500 g) were heated in a viscograph (Brabender Viscograph, model PT 100, C.W. Brabender Instruments, Inc., Duisburg, Germany) from 25 to 95 ˚C at a rate of 1.5 ˚C/min. The suspensions were thermostated at 95 ˚C for 30 minutes, cooled to 50 ˚C, and kept for another 10 minutes. The analyses were performed in two replications according to the official method (*23*), and the viscosities were expressed in Brabender units (BU).

Extraction of total soluble free phenolic compounds in non-treated and thermally treated wholegrain flour

Extracts for the detection of total soluble free phenolic compounds were obtained by continuous shaking of 0.5 g of wholegrain flour sample in 10 mL of 70 % (*V*/*V*) $ethanol_{(aq)}$ for 30 min at room temperature on a horizontal shaker (MLW Thys 2, VEB MLW Labortechnik Ilmenau, Germany). After the centrifugation at 11200×*g* for 5 min (Dynamica Velocity 18R Refrigerated Benchtop Centrifuge, DKSH New Zealand Limited, New Zeland), the supernatant was used for the detection of total phenolic compounds. Five mL of extracts were evaporated under the N_2 stream at 30 °C to dryness (Reacti-Therm nitrogen evaporator system 18821, Thermo Fisher Scientific Inc., United States), and final residues were dissolved in methanol (1.5 mL) . Prior to the analyses the extracts were kept at a temperature of -70 °C. All extractions were performed in duplicates.

Analysis of total soluble free phenolic compounds in non-treated and thermally treated wholegrain flour

The total soluble free phenolic content was determined by the Folin–Ciocalteu assay and expressed as mg of gallic acid equivalent (GAE) per kg of dm (*24*). Fifty to 150 µL of the extract were transferred into the test tubes and filled up to 500 µL with water. After adding Folin–Ciocalteu reagent (250 µL of the 0.2 M) and 1.25 mL of 20 % aqueous Na_2CO_3 solution to the tubes, samples were vortexed (VORTEX ZX3, VELP Scientifica Srl, Usmate, Italy). Spectrophotometer Agilent 8453 was used for measurements of the absorbances of the mixtures at 750 nm after 40 min. The analyses were performed in duplicates.

Analysis of total antioxidant capacity of non-treated and thermally treated wholegrain flour

The antioxidant capacity of the wholegrain wheat flour was measured according to the direct or QUENCHER method using the ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt reagent (*25*). The absorbance was measured at wavelength 734 nm on a spectrophotometerAgilent 8453, and the total antioxidant capacity was expressed as the Trolox equivalent (TE) antioxidant capacity (TEAC) and given as mmol of Trolox per kg of dm (mmol TE/kg). The analyses were done in two replications.

In vitro multistep enzymatic digestion protocol

In order to determine the potential wholegrain wheat flour digestibility for human consumption, as a function of processing conditions, an *in vitro* multi-step digestion procedure was applied. The method consisting of oral, gastric, duodenal, and colon phases (*26*,*27*) was performed without the attempt to quite mimic gastrointestinal digestion. The samples obtained after the *in vitro* digestion were filtered through qualitative filter paper, air dried in a ventilation oven for 2 h and subsequently dried at 105 °C for 4 h, to constant mass. The samples were weighed and the calculation was done according to the Eq. 1:

$$\text{Digestibility}(\%)=((m_0-m_d)/m_0)\cdot 100 \qquad /1/$$

where m_0 is the mass of absolutely dry sample before digestion; m_d is the remaining (undigested) mass of absolutely dry sample.

Statistical analysis

The data were reported as a mean ± standard deviation of two independent repetitions (n=2). Statistical analyses were performed in Minitab (v. 19.2.0) Statistical Software (*34*). Significance of differences between samples was analysed by the Tukey's test. Differences between the means with probability $p < 0.05$ were accepted as statistically significant. The principal component analysis (PCA) was used to visually display relationships among the observed parameters. A positive correlation between two parameters was represented by an acute angle between them, while a negative correlation was represented by an obtuse angle.

RESULTS AND DISCUSSION

Characterization of the initial wholegrain wheat flours - Chemical composition and viscosity profiles

The initial chemical composition and fibre content of the investigated wholegrain wheat flours is shown in Table 1.

Table 1

The overall starch content ranged from 64.10 % (Titan SBE I) to 69.34 % (Apache). Based on the amylose content the wheat genotype Apache (13.90 %) could be categorized as normal, and Zemunska rosa and with 19.00 % amylose, with normal amylose content could be subcategorized as "partial waxy". Previous studies have indicated that "partial waxy" (16-22 % amylose) wheat is the most suitable for the production of noodles traditionally used in Asian cuisine (*12*). The two genotypes obtained by decreasing the activity of starch branching enzymes (SBE I and SBE II) analysed in this study Titan SBE I and Titan SBE II had 36.50 % and 28.00 % amylose, respectively. The highest amylose-amylopectin ratio, an indicator of low glycemic index, was determined in the high-amylose genotype Titan SBE I (0.57). The results indicate that genotype Titan SBE I could be classified as "high-amylose", given that it contained over 35 % amylose, while genotype Titan SBE II did not manifest elevated amylose content. Nevertheless, the pasting curve of Titan SBE II shown in Fig. 1 exhibits transitional properties of this genotype, which are within the range between normal and high-amylose starch flour. The starch of wheat genotypes Waxy1 and W1 waxy did not contain amylose, i.e. it consisted of 100 % amylopectin. The content of total protein, the second abundant macronutrient in wheat, determined in the wholegrain flour samples varied significantly among genotypes, between 11.32 % (Zemunska rosa), and 16.23 % (Titan SBE II) (Table 1). These results indicate that high amylose wheat, on average, has the highest protein content, followed by waxy wheat, and wheat with regular amylose content, and are consistent with previously reported findings (*29*). Proteins, including non-gluten (15-20 %) and gluten proteins (80-85 % of total wheat protein), play a key role in determining the aptitude of wheat flour to be processed into different food products by affecting their functional properties (*8*). Similar total protein contents determined on bread and durum wheat varieties were reported earlier *(31,8).* Findings of Li *et al. (32)* indicate that the increased protein content in native high amylose wheat flour, that our results confirm as well, and thermal stability of the starch in noodles prepared with this type of flour lead to lower digestibility and consequently enhances the resistance against α-amylase digestion. Waxy wheat cultivars are considered to be responsible for the prolonged shelf-life of baked goods, without the use of wheat gluten. However, high amylose wheat varieties with significantly higher amylose contents (55 to 65 %) have been produced depending on the enzyme that controls the amylose synthesis (*33*).

Furthermore, significant differences regarding the fibre composition of the investigated genotypes were noticed (Table 1). Flour of genotype with normal amylose content, Zemunska rosa with the highest insoluble fibres share, *i.e.* NDF content (73.26 %), also had a very high percentage of hemicellulose (β-glucans and arabinoxylans) (66.94 %). On the other hand, the waxy wheat genotype W1 had the lowest contents regarding all analysed dietary fibres, which was not the case with Waxy1 genotype that showed significantly higher fibres content. Researchers Morita *et al.* (*29*) reported a higher fibre content in flour of high amylose and waxy wheat genotypes compared to the regular wheat flours. According to our results, high amylose Titan SBE I and Waxy1 genotypes showed the highest content of insoluble lignin (ADL) fibres.

The apparent viscosity of aqueous flour suspensions varied, as illustrated by the pasting curves in Fig. 1.

Figure 1

Lower amylose content genotypes like Apache (13.90 %) and Zemunska rosa (19.00 %) had a similar trend for the emergence of the peak and final viscosities. A high activity of α-amylase, which causes the enzymatic breakdown of the starch molecules in the endosperm into simple sugars, may have induced low viscosity levels. This type of flour, which is commonly produced by grinding sprouted wheat, has a reduced ability for gas retention and might affect how well bread can be baked (*34*). The transition between flour with a normal and high amylose content may be seen in the pasting curve of Titan SBE II (28.00 % amylose). Pasting curve of high amylose Titan SBE I genotype (36.50 % amylose) showed a constant increase without reaching the peak viscosity during heating nor thermostating of the aqueous flour suspensions. Peak viscosity was attained relatively quickly; breakdown and end viscosities were very high. The Waxy1 and W1 genotypes' flour displayed pasting curves typical of waxy wheat. According to Blazek and Copeland (*35*)*,* the pasting curves of the waxy wheat genotypes are often characterised by high peak viscosities and low final viscosities in comparison to normal amylose content wheat flours, which is consistent with our findings. The results are in accordance with previous findings (*31*).

Effect of thermal treatments on in vitro digestibility of the wholegrain wheat flours

Results of the multistep enzymatic *in vitro* digestion protocol are depicted in Fig. 2a.

Figure 2

The digestibility of the untreated samples ranged from 26.55 % in Zemunska rosa, a normal amylose content genotype, to 35.49 % in Titan SBE I, a high amylose genotype. After the ultrasound treatment, the highest increase in the digestibility, compared to the untreated same flour sample, was noticed in the wholegrain flour of the wheat genotype Apache (about 13.5 %), while an amylopectin Waxy1 genotype showed a significant decrease of digestibility (about 9 %). Furthermore, there was no linear correlation between ultrasonic treatment and the *in vitro* digestibility of the samples (Fig. 2a). In addition to the mentioned, the digestibility of wholegrain flour of wheat genotype W1 and Titan SBE I remained unchanged after ultrasonic treatment in comparison with that of untreated flour (33.56 % vs 33.08 % and 34.98 % vs 35.49 %).

Contrary, the hydrothermally treated - cooked wholegrain wheat flour samples all exhibited high increase in digestibility compared to the untreated same flour sample. In cases of flour obtained from waxy genotypes digestibility increase was the highest (W1 showed double increase and Waxy1 a 1.9

times increase), followed by standard wheat varieties (Zemunska rosa 1.9 times, and Apache 1.7 times), while high-amylose wheat genotypes showed the lowest digestibility increase (Titans SBE II 1.4 times, and Titan SBE I 1.3 times). The limited increase of digestibility of the flours obtained from SBE genotypes may be explained as a result of resistant starch formation that occurs during cooling with retrogradation of starch (*17*). However, the resistant starch content was not measured in our study. The effect of hydrothermal treatment on the improved digestibility of wholegrain wheat flour can be attributed to a great extent to the gelatinization of starch present in large amounts in this material, making it more readily available for enzymatic degradation (*36*). Similarly, a study by Kiers *et al. (37)* showed that after cooking and subsequent malt saccharification, the proportion of the digested white maize increased by approximately three times, from 25.5 to 63.6 %, while cooking raised the overall digestibility of the soy bean from 36.5 to 44.8 % and the digestibility of the cowpea from 15.4 to 40.9 %, respectively. Considering that hydration patterns of any substrate play a crucial role in enzymatic processes, and therefore digestibility, it is possible that soft wheat cultivars, with better hydration patterns, may be more digestible than the hard varieties. Even though endosperm texture depends upon genetic background, the interaction between genotype and environment is of great importance. Endosperm texture cannot categorically always be marked as "hard" or "soft", but somewhere in the spectrum. Protein contents of hard wheat are about 15 %, while those of soft wheat varieties are closer to 10 % (*38*). Although durum wheat, as representatives of hard wheat cultivars, were not included in our experiment, the results confirm that flours of the high-protein, high-amylose genotypes Titan SBE I and Titan SBE II have in general a lower digestibility after cooking (46.58 and 46.21 %, respectively) compared to the flours of the Zemunska rosa and waxy wheat varieties (51.56, 66.54, and 67.43 %, respectively) with a lower protein content. In addition to protein, the content and structure of starch in cereals has, it could be said, a crucial role in digestibility of grain/wholegrain flour. Because of their stable and ordered semi-crystalline structure, starch granules are not soluble in water at room temperature. However, when subjecting native starch to thermal treatments such as cooking, α-amylase inhibitors and α-amylase itself become denatured, the ordered structure becomes disrupted, different processes such as granule swelling, lixiviation of amylose and disorganization of amylopectin take place (*38*). Research has shown that hydrothermal treatments such as heat-moisture treatment and annealing can increase the slowly digestible and resistant starch fraction in starches from different plant sources without destroying the granular structure. Even though a reduction in starch digestibility would presumably need to be accompanied by a reduction in protein digestibility as a consequence of the starch/protein matrix, it seems there is no evidence to this (*38*).

Foods with a higher amylose content retrograde more quickly and to a greater extent even when the crystalline structure is broken down during heating. Our results showed that flours with 100 % amylopectin starch were on average 30 % more digestible after ultrasonic treatment than flours with containing 36 and 28 % amylose. When the amylose level is raised, more dietary resistant starch is formed (*33*). In addition, lipids may aggregate with more amylose or longer branch chains during heating and cooling. Wu *et al.* (*39*) showed that phenolic compounds attach to dietary macromolecules such proteins, lipids, and carbohydrates blocking the digestive enzymes (α-amylase and amyloglucosidase) by chemical interactions that cause the enzymes to precipitate, which lowers their activity on the digestion of carbohydrates. Aggregates of polymeric glycoside complexes form between phenolic compounds and sugars which may affect the absorption of phenolics. Through delayed carbohydrate digestion and prolonged digesting time, the polyphenol-carbohydrate interaction may decrease the release and absorption of glucose (*40*).

Effect of thermal treatments on content of total soluble free phenolic compounds in the wholegrain wheat flours

Phenolic compounds are predominantly located in bran which is, hence, responsible for the overall antioxidant capacity of wheat (*5*). The majority of phenolic compounds (about 95 %) are bound to cell wall polysaccharides and referred to as dietary fibre-phenolic compounds (*41*). In our research, the content of free soluble phenolic compounds was measured. The changes in the phenolic compounds detected prior and after thermal treatments are shown in Fig. 2b. Among the untreated wholegrain wheat samples, high-amylose genotype Titan SBE I showed the highest content of total free phenolics (655.26 µg GAE/g dm), while genotype Titan SBE II had the lowest content of these bioactive components (479.99 µg GAE/g dm). A study by Adom *et al.* (*42*) reported that free phenolic contents of the untreated flour of wheat varieties ranged from 119.61 to 201.25 µmol GAE/100g of grain, as well as that the ratio of bound to free phenolic content ranged from 2.5 to 5.4 times higher on average in the investigated wheat genotypes. The ultrasound treatment positively influenced the content of total soluble free phenolic compounds in all of the wholegrain wheat samples, except the amylopectin W1, genotype, conversely the content of soluble free phenolics decreased in all samples except the Waxy1 during cooking. Compared to the untreated wheat flour, the content of free phenolic compounds, that may be declared bioavailable, was increased by about 27, 15, 6, 21 and 3 % after ultrasound treatment of Zemunska rosa, Apache, Titan SBE I, Titan SBE II and Waxy1 flours, respectively. This result can be caused by the hydrolysis of bound phenolic compounds that contributes to the increase of the free phenolics content after hydrothermal treatment. On the other hand, as a result of thermal degradation, the soluble free phenolic compounds losses during the cooking at around 80 °C for 3 min of Zemunska rosa, Apache, Titan SBE I, Titan SBE II and W1 flours were 8, 39, 21, 23 and 5 %, respectively. Some studies reported that bioactive compounds, including anthocyanins and ascorbic acid, were shown to degrade following extended ultrasonic treatment, while heat treatment and extrusion manifested diverse effects on barley, rye, triticale, oat, sorghum and millet (*43*). Cui *et al.* (*43*) reported that despite an increase seen in the first two hours, phenolic compounds started to degraded after a certain period in a time-dependent way. Sęczyk *et al.* (*44*) reported that hydrothermal treatment showed negative impact on the phenolic contents of the investigated flour samples, in comparison with their initial amount. Our results also indicate that the flours of the waxy genotypes were the most thermally stable for the content of soluble free phenolic compounds.

Effect of thermal treatments on antioxidant capacity of the wholegrain wheat flours

The changes in the antioxidant capacity determined before and after the ultrasonic and hydrothermal treatments are shown in Fig. 2c. Results show that the antioxidant capacities did not vary significantly among the wheat genotypes. However, the average antioxidant capacity of non-treated flour of bread wheat genotypes (Zemunska rosa and Apache) was the lowest and amounted to 32.64 mmol TE/kg. The average antioxidant capacity of flour of high-amylose and waxy wheat genotypes was 36.59 and 34.75 mmol TE/kg, respectively. Žilić *et al.* (*5*) The reported antioxidant capacity values of bran and debranned wheat flour obtained from different bread and durum wheat varieties ranged from 6.78 to 19.55, and from 27.81 to 37.57 mmol TE/kg, indicating that the majority of antioxidant potential of the grain is concentrated within the bran. In addition, Adom *et al.* (*42*) found that bound phytochemicals contributed >82 % of the overall antioxidant activity on average, among 11 investigated wheat varieties. According to our study ultrasound treatment increased the antioxidant capacity of the flours, although not statistically significant. Similarly, in a study by Cui *et al.* (*43*) the interdependence between the thermal treatment and antioxidant capacity was inconclusive. Slight increase in total antioxidant capacity of wheat flours after ultrasound treatment could be explained by the increased release of bound phenolic compounds as mentioned above. The results of Žilić *et al.* (*45*) showed that wheat grain phenolic compounds in the bound form, as dominant, exert lower antioxidant capacity in comparison with its hydrolysed and isolated free forms in the analysed extracts. In contrast to ultrasound treatment, after cooking of flour samples, its antioxidant capacity was decreased by about 28, 30, 20, 22, 23 and 5 % in relation to that in untreated flour of genotypes Zemunska rosa, Apache, Titan SBE I, Titan SBE II, W1 and Waxy1, respectively. In these samples antioxidant capacity correlates very well with a reduction in content of total soluble free phenolic compounds in them.

Principal components analysis

The graphical presentation of the principal components analysis results is shown in Fig. 3.

Figure 3

The principal components analysis showed that amylose content was in high negative correlation with digestibility after the hydrothermal treatment, conversely, amylopectin was in high positive correlation with this parameter. Starch content negatively influenced digestibility after ultrasound treatment. Protein content was very strongly correlated to digestibility after ultrasound treatment, in a significant correlation with the digestibility of the untreated flours. In contrast, no considerable interrelation was found between hydrothermally treated samples' digestibility (cooking) and protein content. ADF content showed a significant correlation with the antioxidant capacity of primarily untreated wholegrain flour samples, followed by cooked and ultrasonicated samples. Interrelation between protein content and antioxidant capacities of the untreated, and to a lesser extent treated samples, was also noticed.

CONCLUSIONS

The present study highlights some of the advantages and disadvantages of different treatments on the digestive and antioxidant properties of wholegrain wheat flour. Hydrothermal treatment positively influenced the digestibility of the whole-wheat flours, especially in waxy genotypes compared to high amylose ones, which can be explained by the formation of resistant starch. The hydrothermal treatment had an overall negative impact on the antioxidant capacity of the flour samples, while ultrasound, in general positively influenced the total free phenolic compounds content. The application of ultrasound and hydrothermal treatments may provide new possibilities for the modification and improvement of the baking- and bio-functionality of flours as well as the quality of baked goods. At the moment, more investigation is still required to fully understand how different pre-treatments, components of a food's complex structure, interactions between its macronutrient contents, and digestibility impact the antioxidant properties of wholegrain wheat flour. In this regard, the results obtained in this research can be useful for further studies focusing on the effects of pre-treatments on the bio-functional properties of wholegrain flours.

AKNOWLEDGEMENTS

We dedicate this article to the memory of our dear colleague Dr. Dejan Dodig who sadly passed away before it was published. Thank you for all the contribution, selfless collegial help, and friendly advice you gave us during years of work together on previous studies and articles that gave our research endeavours more meaning.

FUNDING

This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200040).

CONFLICT OF INTEREST

The authors declare there are no conflicts of interest regarding this article.

SUPPLEMENTARY MATERIALS

All supplementary materials are available at: www.ftb.com.hr.

AUTHORS' CONTRIBUTION

Valentina Nikolić wrote the first draft of the manuscript, collected, analysed and interpreted the data. Slađana Žilić conceived and designed the research and did final approval of the version to be published. Marijana Simić contributed to acquisition and analysis of data. Vesna Kandić carried out the field experiments and prepared the samples for the research. Primož Titan selected and provided the starting plant material for the study. All authors read and approved the final manuscript.

ORCID ID

V. Nikolić https://orcid.org/0000-0002-5834-6683

S. Žilić https://orcid.org/0000-0001-8299-9185

M. Simić https://orcid.org/0000-0002-6719-7982

V. Kandić https://orcid.org/0000-0003-1999-2030

P. Titan https://orcid.org/0000-0003-4171-1354

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Table 1. Chemical composition of initial wholegrain wheat flours (before thermal treatment)

	Genotype					
Composition w/%	Zemunska rosa	Apache	Titan SBE I	Titan SBE II	W1	Waxy1
Starch	(67.74±0.53)[b]	(69.34±0.52)[a]	(64.10±0.00)[d]	(58.78±0.31)[e]	(69.15±0.00)[a]	(66.07±0.00)[c]
Amylose	(19.00±0.00)[c]	(13.90±0.50)[d]	(36.50±0.50)[a]	(28.00±0.50)[b]	(0.00±0.00)[e]	(0.00±0.00)[e]
Amylopectin	(81.00±0.00)[c]	(86.01±0.50)[b]	(63.50±0.50)[e]	(72.00±0.50)[d]	(100.00±0.00)[a]	(100.00±0.00)[a]
Protein	(11.32±0.04)[e]	(12.10±0.19)[d]	(14.87±0.06)[b]	(16.23±0.05)[a]	(12.58±0.09)[d]	(14.21±0.20)[c]
NDF	(73.26±0.34)[a]	(67.06±0.28)[b]	(63.66±0.14)[c]	(68.54±0.04)[b]	(48.13±0.56)[d]	(66.86±0.74)[b]
ADF	(6.32±0.64)[b]	(4.53±0.07)[c]	(10.39±0.27)[a]	(10.08±0.35)[a]	(5.24±0.33)[bc]	(10.70±0.54)[a]
ADL	(3.49±0.08)[b]	(2.65±0.42)[bc]	(7.37±0.64)[a]	(1.87±0.04)[c]	(2.14±0.22)[c]	(6.64±0.10)[a]
Hemicellulose	(66.94±0.98)[a]	(62.53±0.35)[b]	(53.27±0.41)[e]	(58.47±0.39)[c]	(42.89±0.23)[f]	(56.16±0.20)[d]
Cellulose	(2.84±0.71)[b]	(1.89±0.35)[b]	(3.02±0.91)[b]	(8.21±0.38)[a]	(3.11±0.11)[b]	(4.06±0.45)[b]

Values are presented as mean (n=2) ± standard deviations. Means followed by the same letter within the same row are not significantly different (α=0.05 %). NDF-neutral detergent fibre, ADF-acid detergent fibre, ADL-lignin.

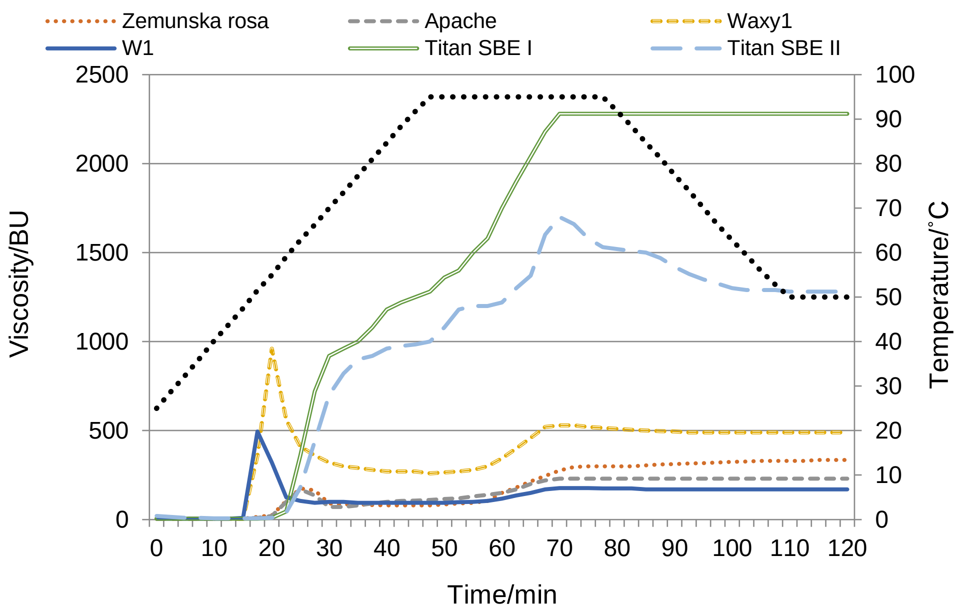
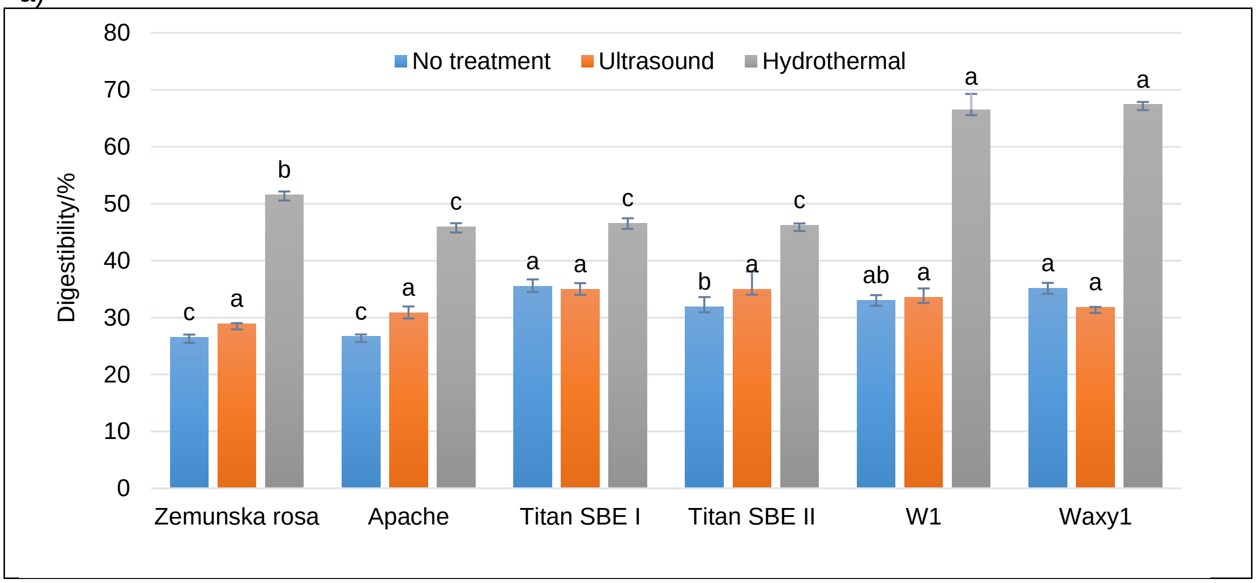
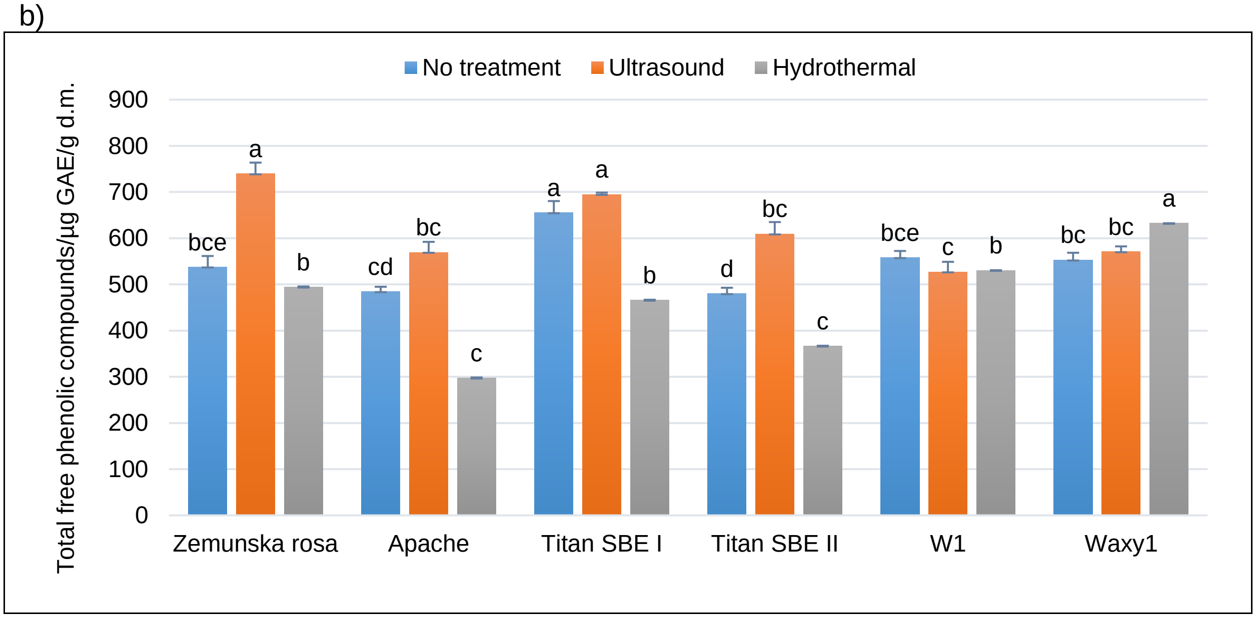


Fig. 1. Pasting profiles (viscosity curves) of initial wholegrain wheat flours (before the treatments).

a)


No treatment
Ultrasound
Hydrothermal
Digestibility/%
Zemunska rosa
Apache
Titan SBE I
Titan SBE II
W1
Waxy1

b)


No treatment
Ultrasound
Hydrothermal
Total free phenolic compounds/μg GAE/g d.m.
Zemunska rosa
Apache
Titan SBE I
Titan SBE II
W1
Waxy1

c)

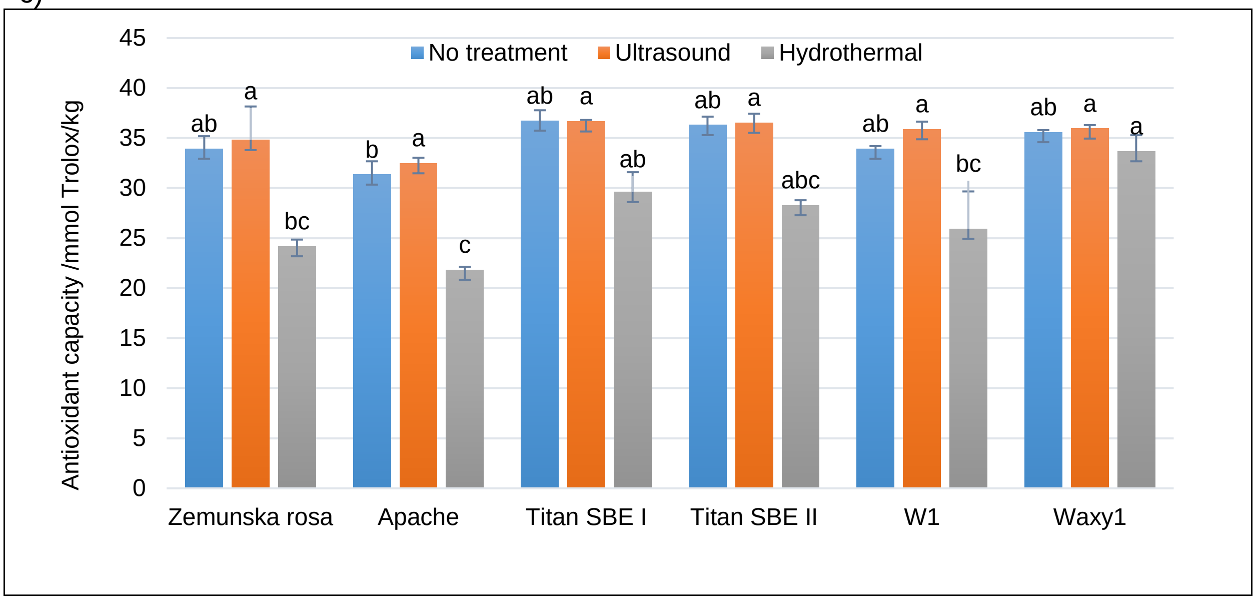
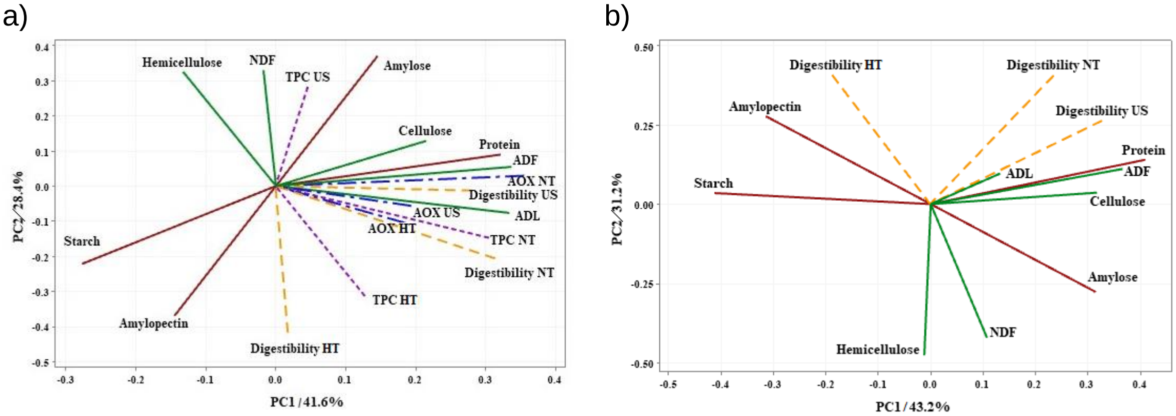


Fig. 2. Digestibility (a), total soluble free phenolic compounds (b), and antioxidant capacity of wholegrain wheat flour (c) before and after thermal treatments. Values are presented as mean (n=2) ± standard deviations. Means followed by the same letter within the same genotype are not significantly different (α=0.05 %).

a) b)



c)

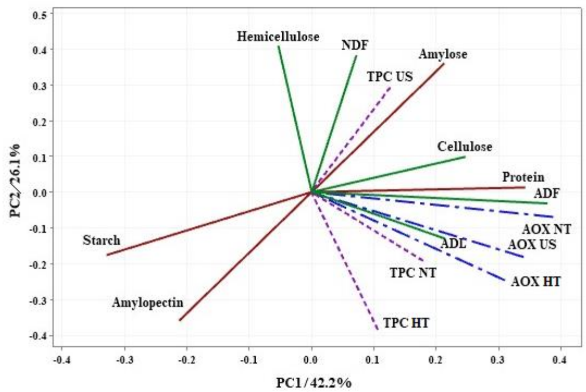


Fig. 3. Principal component analyses. Genotype by trait bi-plot showing the interrelationship amongst initial chemical composition of wholegrain wheat flour and digestibility, as well as antioxidant properties of the flour after thermal treatments a), and separately, amongst initial chemical composition of wholegrain wheat flour and digestibility of the flour after thermal treatments b), amongst initial chemical composition of wholegrain wheat flour and antioxidant properties of the flour after thermal treatments c). NDF-neutral detergent fibre, ADF-acid detergent fibre, ADL-lignin, TCP-total soluble free phenolic compounds, AOX-antioxidant capacity, NT- not treated, US-ultrasound, HT-hydrothermal treatment.

SUPPLEMENTARY MATERIAL

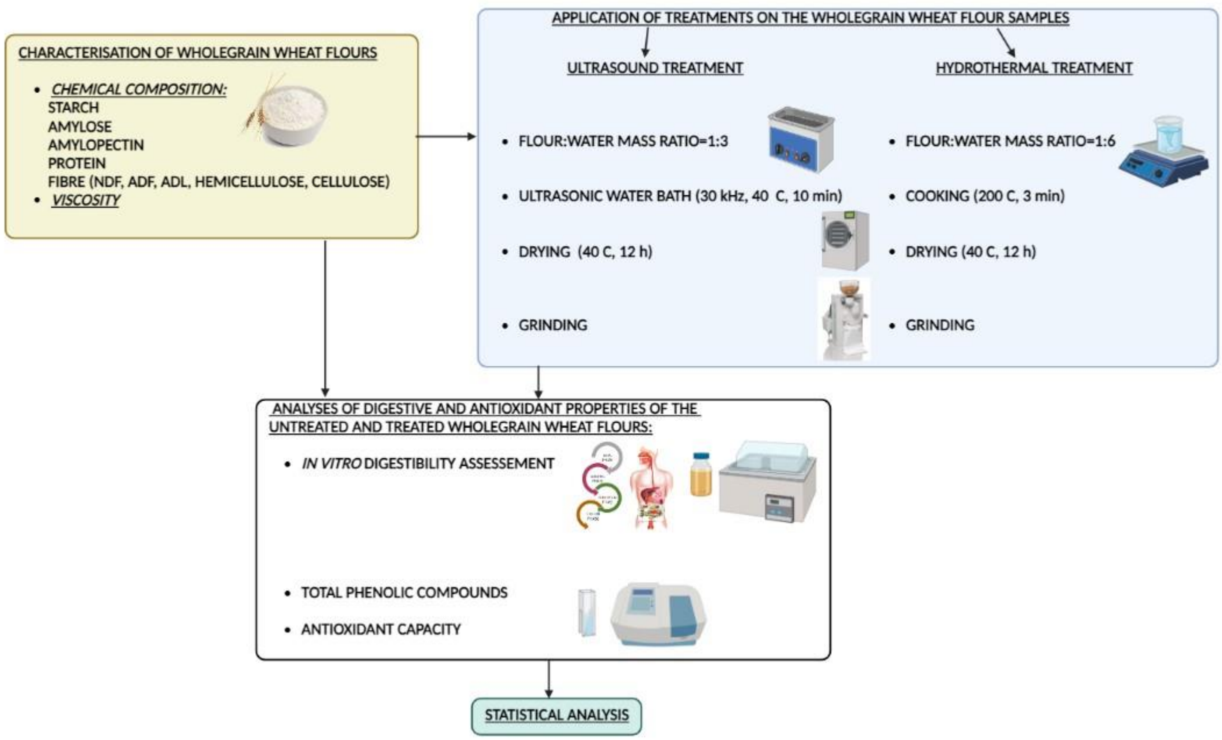


Fig. S1. Schematic illustration of the processes applied in this study