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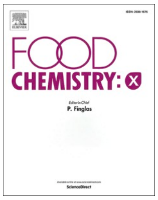

Nutritional composition, *in vitro* carbohydrates digestibility, textural and sensory characteristics of bread as affected by ancient wheat flour type and sourdough fermentation time

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ABSTRACT

This study aimed to investigate the effect of ancient wheat flour type and sourdough fermentation time on the nutritional, textural and sensorial properties of fiber-rich sourdough bread. The proximate composition, minerals, carbohydrates, organic acids, volatiles, total phenolic content, simulated gastrointestinal digestion, textural and sensorial characteristics were investigated. Bread's minerals, total phenolics, cellulose contents and radical scavenging activity variations clearly indicates an increasing trend with sourdoughs fermentation time. Compared to maltose and glucose, fructose was predominant in all bread samples. Sourdough fermentation time and wheat type had non-significant influence on fructose content from digested fraction. Excepting emmer bread, fermentation time increased *in vitro* digestibility values for tested samples. The crumb textural parameters (hardness, gumminess, chewiness, cohesiveness and springiness index) were positively influenced by fermentation time. The specific clustering of the analysed characteristics distinguished emmer bread from other samples in terms of volatile compounds, textural and overall acceptability, being preferred by panellists.

1. Introduction

Sourdough fermentation is increasingly used for bakery products, particularly in large-scale bread manufacturing. It is generally accepted that the sourdough microbiota determines the bread' distinct flavour and quality, as well as delay aging and prevent microbial spoilage. Furthermore, numerous studies stated that sourdough fermentation enhances the nutritional profile of bread due to the lactobacilli metabolism's ability to generate novel useful molecules such as peptides, amino acid derivatives, and *exo*-polysaccharides and to increase the bioavailability of minerals and phytochemicals (Arora et al., 2021; Chiş et al., 2020; D'Amico et al., 2023). However, the final bread properties, including technological, biochemical, sensorial, and nutritional aspects, are affected also by the type of grain, the type of microbial starters, and other ingredients added to the dough. Moreover, the sourdough process's parameters (temperature, time), significantly influence the bread quality (Canesin & Cazarin, 2021).

Even when made from whole grains, bakery products which are the major carbohydrate source of the daily diet, have high glycaemic indices (Demirkesen-Bicak, Arici, Yaman, Karasu, & Sagdic, 2021). A high number of studies claimed that sourdough fermentation reduces the bakery products' glycaemic response. One of the most frequent explanations is the dough pH reduction to 3.5–4.0 which can stimulate the development of resistant starch through retrogradation after the heat action during baking. This type of starch lowers the blood glucose level due to limited digestion (Canesin & Cazarin, 2021). It is considered that 3–5% of total starch transits through the digestive system undigested (De Angelis et al., 2007; Hefni, Thomsson, & Witthöft, 2021). Nevertheless, the degree of gelatinization, porosity, as well as bread storage time could significantly influence the resistant starch content (D'Amico

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et al., 2023).

In addition, it is well established that the amount and type of bread's dietary fiber affects intestinal viscosity, gastrointestinal transit, and starch's digestion and absorption degree. The viscous qualities of the soluble fiber are advantageous for reducing the glucose amount absorbed in the intestine (Gil-Cardoso et al., 2021; Srichamroen & Chavasit, 2011). Additionally, this fiber contributes to the microbial synthesis of short-chain fatty acids (SCFAs), which are known to influence metabolism and insulin sensitivity, reducing the risk of developing diabetes and becoming overweight (D'Amico et al., 2023). Importantly, recent studies emphasized that other phytochemicals included in whole grains, notably phenolic compounds, played a role in how fiber affects health outcomes (Amoako & Awika, 2016; Kan, Capuano, Fogliano, Oliviero, & Verkerk, 2020).

The relationship between the microbial strain type and the substrate composition is a deciding element in the production of bread using sourdough technology, according to metabolomics research (Păucean et al., 2021; Şerban et al., 2023). During sourdough fermentation proteins, carbohydrates, phenolic acids, and other compounds undergo specific transformation, leading to improved texture, volume, shelf life, flavour, and nutritional value.

In recent years, ancient grain species have gained interest from both researchers and consumers due to their superior chemical composition and potential health benefits. Spelt (*Triticum aestivum* L. subsp. *spelta*), einkorn (*Triticum monococcum* L. subsp. *monococcum*), emmer (*Triticum turgidum* L. subsp. *dicoccum*) and Khorasan wheat (*Triticum turgidum* L. subsp. *turanicum*) are the most studied and used ancient wheat species. Generally, the ancient wheat species are richer in proteins than common wheat, while between the carbohydrates and lipids contents only slighter differences were recorded (Şerban, Păucean, Man, Chiş, & Mureşan, 2021; Shewry & Hey, 2015). Moreover, it was reported that einkorn had a lower proportion of total and resistant starch as compared to soft wheat. However, interestingly, the number of slower-digesting amylose molecules was found greater than the number of amylopectin molecules, which decreased blood glucose and insulin levels after meals and prolonged feelings of fullness (Dinu, Whittaker, Pagliai, Benedettelli, & Sofi, 2018).

Concerning the bioactive components, these wheat species stood out for their high content of polyphenols, carotenoids, fibers, B vitamins, and antioxidants (tocopherols, tocotrienols) (Arzani & Ashraf, 2017; Şerban et al., 2021; Shewry & Hey, 2015). As expected, higher amounts of these bio-compounds are found in whole grains and wholemeal flours, compared to refined ones. Moreover, it is considered that their nutritional quality is derived from the interplay of all chemical compounds rather than from each one alone (Bordoni, Danesi, Di Nunzio, Taccari, & Valli, 2017).

Consequently, this study aimed to investigate the effect of three ancient wheat (AW) (*i.e.*, einkorn, spelt, emmer) flour type and *Lactiplantibacillus plantarum (Lp)* ATCC 8014 strain sourdough fermentation time on the nutritional, textural and sensorial properties of fiber-rich bread.

2. Materials and methods

2.1. Materials

All wholemeal flours (common wheat, einkorn, spelt, emmer, rye), inulin, compressed baking yeast (Pakmaya), sunflower oil, salt, sugar and Mung bean were procured from local stores in Romania. *Lactiplantibacillus plantarum* ATTC 8014 (*Lp*) was delivered by Microbiologics (Minnesota, USA), while all chemicals and reagents were purchased from Sigma Aldrich (Germany), Honeywell (USA), and Chempur (Poland). Mung bean flour was obtained by grinding to a fine flour (<300 μm) on a Grindomix (GM200) laboratory mill at 10.000 rot min^{-1} for 50 s.

2.2. Bread baking

Sourdough bread formulations (expressed for 1 kg of mixed flours obtained from rye flour and one of the analysed wheat flours) and samples coding are given in Table 1. Common wholemeal wheat flour was used for the control bread sample. For each bread sample obtained from einkorn spelt, emmer, and common wheat (W) flour a corresponding sourdough made from the same flour was used. From each sourdough, at three moments of fermentation (0, 12, 24H) samples were collected and used in breadmaking.

Raw materials were prepared by sifting the flours, activating the yeast and bringing the water to 25 °C. Then in a dough mixer (KitchenAid® Precise Heat Mixing Bowl., USA) the kneading stage of all ingredients was carried out for 4 min at medium speed and 3 min at high speed. Sourdoughs were obtained by fermenting einkorn, spelt, emmer, W flours with *Lp*, as previously described by Şerban et al. (2023). The dough was initially fermented at 4 °C for 2 h, then at 22 °C for 30 min. Each sample was divided (250 g), shaped, and introduced in the tray. The final proof of the dough was carried out for 45 min, at 25 °C and 80% relative humidity and baked at 210 °C for 30 min in an electric conventional Zanolli oven, equipped with a proofer (Zanolli SRL, Italy). Cold fermentation of dough enhances the production of extracellular polysaccharises by LAB, limits starch interactions with water and alters gluten water absorption, improving dough and bread's textural properties and forming more volatile compounds for a pleasant aroma (Dongdong, Xing, Yingqi, & Shuncheng, 2023; Xu et al., 2020). All samples were made in triplicate.

2.3. Proximate chemical composition

The bread samples were analysed to determine their levels of ash (AOAC International method 923.03), protein (Kjeldahl method, AOAC International method 920.87), and crude cellulose (ISO 5498:1981).

2.4. Total starch content

Starch Assay Kit (Sigma Aldrich) was used for determination. Briefly, 1 g of the grounded sample was solubilized with 20 mL of dimethyl sulfoxide and 5 mL of 8 M HCl and incubated for 30 min at 60 °C. A volume of 50 mL of deionized water was added and pH was adjusted to 4–5 (5 N NaOH). The solution was cooled down and diluted to 100 mL with deionized water. The hydrolysis of starch to glucose was catalyzed by amyloglucosidase. Aliquots of 25 mL were centrifuged (Eppendorf AG 5804, Germany) at 7155*g* (10 min), extracting 0.1 mL of the supernatant to continue the analysis. A glucose standard was run starting at this point. Finally, the absorbance was read at 340 nm and converted to total starch content using the calculation equations according to the reference method from the kit's technical information. A control sample was also run in parallel.

Table 1
Sourdough bread formulations.

Materials	P_0	P_1	P_2	P_3
Rye flour, g	400	400	400	400
Wheat flour, g	600			
Einkorn flour, g		600		
Spelt flour, g			600	
Emmer flour, g				600
Sourdough, mL	200	200	200	200
Yeast (*Saccharomyces cerevisiae*), g	20	20	20	20
Inulin, g	100	100	100	100
Mung bean flour, g	50	50	50	50
Water, mL	715	715	715	715
Sunflower oil, mL	10	10	10	10
Salt, g	20	20	20	20
Sugar, g	5	5	5	5

P_0 – control sample, P_1 – einkorn bread, P_2 – spelt bread, P_3 – emmer bread.

2.5. Total free amino acids content (TFA)

The method adapted after Kowalska, Szłyk, and Jastrzębska (2022), using ninhydrin was applied. Shortly, 1 mL extract was homogenized with 0.5 mL phosphate buffer solution (pH 8.04), 0.5 mL ninhydrin 2% and 0.8 mg/mL of tin (II) chloride. The mixture was placed in the laboratory oven at 105 °C (10 min) and after cooling 10 mL distilled water was added. The absorbance was read at 570 nm (spectrophotometer Shimadzu 1700, Japan). Determination of TFA was performed with standard calibration procedure using alanine solutions. For calibration curve eight calibration solutions of alanine in the range of 10–600 μg/mL were used.

2.6. Determination of macro/microelements by atomic absorption spectrophotometry (AAS)

Macro/microelements (mg/kg) were identified using AAS (Varian 220 FAA, Germany) based on method described by (Şerban et al., 2023). Samples (3 g) were calcinated for 10 h at 500 ± 100 °C in a furnace (Nabertherm B150, Germany). The residue was treated with 5 mL of HCl 6 mol/L and subsequently dissolved in 20 mL of HNO_3 0.1 mol/L.

2.7. Determination of carbohydrates, organic acids, and ethanol content by HPLC-RID

The method described by (Şerban et al., 2023) was used. Briefly, the compounds were eluded for 25 min (mobile phase H_2SO_4 5 mM at a flow rate of 0.6 mL/min, column temperature $T = 80$ °C, and RID temperature $T = 35$ °C). The OpenLab—ChemStation system from Agilent Technologies, USA, was utilized to analyze the results.

Finally, the substances were identified by comparing the acquired retention time to the standard values for the analysed compounds (Sigma-Aldrich, Germany).

2.8. Determination of volatile organic compounds by ITEX/GC–MS technique

The CombiPAL AOC-5000 autosampler was utilized with the ITEX/GC–MS as described by Păucean et al. (2019). 1 g of bread (crumb and crust), was sealed and was heated to 60 °C. The headspace phase's volatile chemicals were desorbed thermally in the gas chromatograph injector after being adsorbed into a Tenax carbon fiber. Aromatic compound separation was carried out using a GCMS QP-2010 mass spectrometer (Shimadzu Scientific Instruments, Japan) on a ZB-5 ms capillary column with dimensions of 30mx0.25mmi.d.x0.25m (film thickness). By comparing the mass spectra of each chromatographic peak with the NIST27 and NIST147 libraries, and only considering compounds that showed a resemblance of at least 85%, volatile chemicals were identified.

2.9. Analysis of Total free phenolic compounds (TPC)

The method described by Chiş, Păucean, Man, Vodnar et al. (2020) was used. Shortly, 1 g of each bread was homogenized with 100 mL of acidified methanol (85:15, v/v, MeOH:HCl) and dried at 40 °C using a vacuum rotary evaporator (Laborota 4010, Germany). The total phenols content was assessed using the Folin-Ciocâlteu colorimetric technique. The absorbance was measured at 760 nm. The results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g product.

2.10. Radical scavenging activity by DPPH assay (RSA)

DPPH method (1,1-Diphenyl-2-picrylhydrazyl) was used. Briefly, a UV/visible spectrophotometer Shimadzu 1700 was used to measure the absorbance at 515 nm after mixing 0.1 mL of each methanolic extract with 3.9 mL of DPPH solution. RSA was calculated as described previously by Chiş, Păucean, Stan, and Mureşan (2018).

2.11. Simulated gastrointestinal digestion

The standardized static *in vitro* digestion approach for foods recommended by the INFOGEST® network (Minekus et al., 2014) and Brodkorb et al. (2019) was used. *In vitro* digestion of samples and a blank sample was performed. Minekus et al. (2014) instructions were followed to prepare the simulated fluids. An aliquot from each phase was then collected and freeze-dried with a protease inhibitor (Pefabloc SC, Sigma-Aldrich, St. Louis, MO, USA). Enzymes' concentrations utilized were calculated according to the manufacturer's instructions. For each sample maltose, glucose, and fructose concentrations were determined by HPLC-RID, as was described previously in Section 2.7.

In vitro digestibility (IVD) (%) was calculated according to Batista et al. (2017). In the blank assay, no sugars were detected.

2.12. Crumb texture profile analysis (TPA)

TPA was performed on 4 cubes (25x25x25 mm) sampled from the centre of each bread and four parameters were determined (hardness, cohesiveness, springiness, chewiness). For TPA, CT3 Texture Analyzer (Brookfield Engineering Labs, USA) which was equipped with a 10 kg load cell and the TA11/1000 cylindrical probe (25.4 mm diameter), being set at a 40% target value, a test speed of 1 mm/s, a 5 s recovery time, and 2 cycle count. Texture Pro CT V1.6 software was used for parameters calculation (Man et al., 2019).

2.13. Sensory analyses

The Hedonic test in 9 points was used. A number of 11 panellists (5 males and 6 females with ages ranging from 18 to 35 years, students and staff from the Faculty of Food Science and Technology Cluj-Napoca) evaluated the bread samples from the point of view of appearance, texture, smell, taste, aroma, and overall appreciation. The analyses were carried out 1–3 h after baking. The ethics guidelines of the university were followed and each panelist gave his/her consent to take part in the research and for their information to be used.

2.14. Statistical analyses

For the interpretation of scientific data, Duncan multiple comparison test (SPSS version 19 software version 19; IBM Corp., Armonk, NY, USA) was applied. Different lowercase letters indicate significant differences ($p < 0.05$) between samples (P_0, P_1, P_2, P_3) at the same fermentation time, and different uppercase letters indicate significant differences between the same sample at different fermentation time (0,12, 24H). All analyses were made in triplicate.

Unscrambler software (version 10.5.1; CAMO Software AS, Oslo, Norway) was used for the Principal Component Analysis (PCA), and MetaboAnalyst software (version 5.0; Xia Lab at McGill University, Quebec, QC, Canada) was used to conduct Hierarchical Cluster Analysis (HCA) and Heatmap Visualization.

3. Results

3.1. Proximate composition and macro/microelements content

The proximate composition of bread samples obtained with common wheat (P_0), einkorn (P_1), spelt (P_2), and emmer (P_3) have been presented in Fig. 1. The bread samples' total starch content ranged from 32.5% to 39.1%, with highest values at 12H fermentation. The fermentation time significantly influenced the starch content of bread samples. P_3 recorded the highest starch contents comparing to other samples, while its protein contents were the lowest compared to P_1 and P_2 and significantly different ($p < 0.05$).

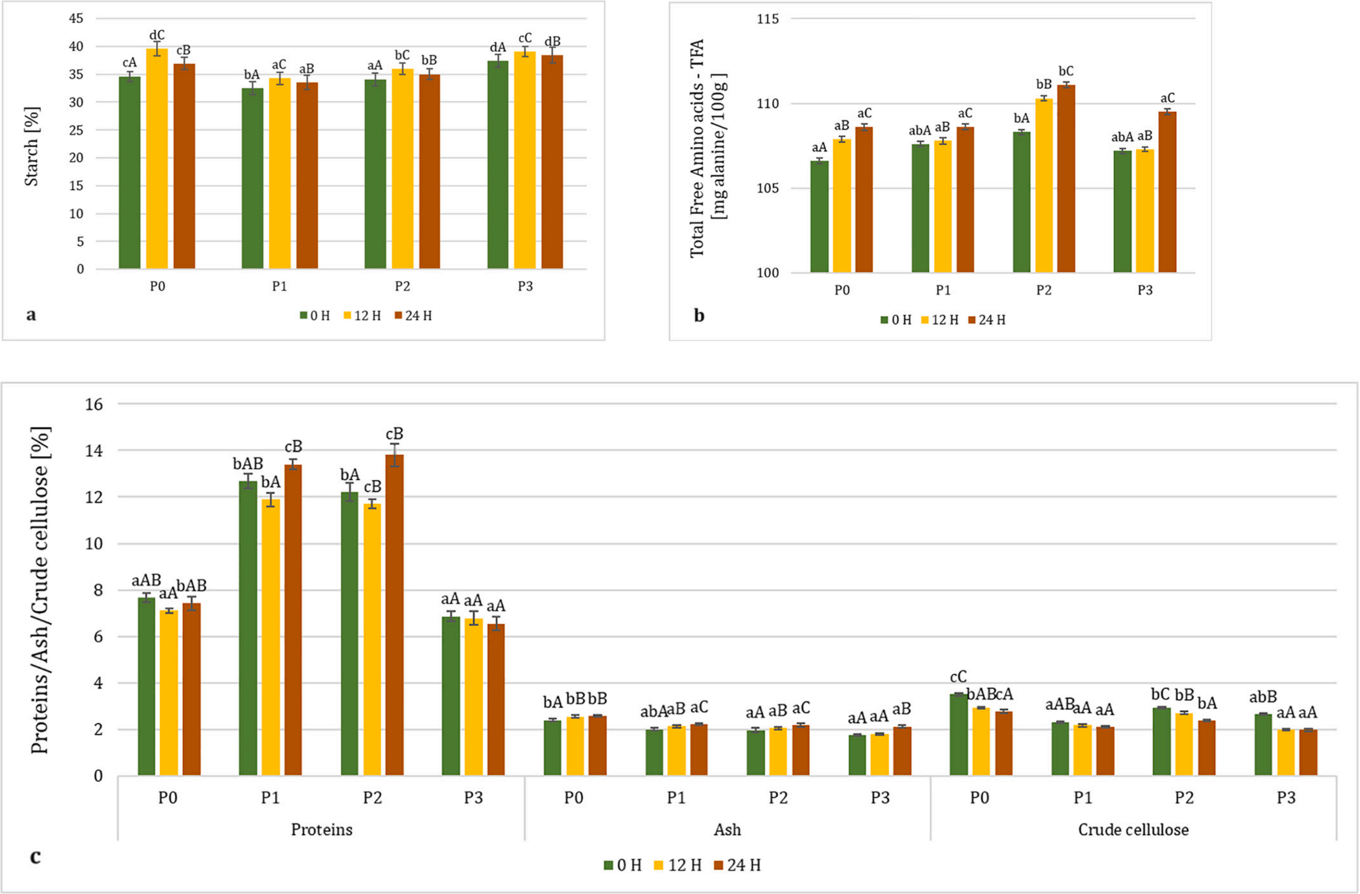

Fig. 1. Influence of sourdough fermentation time on proximate composition of bread samples obtained with common wheat (P_0), einkorn (P_1), spelt (P_2), and emmer (P_3). Different lowercase letters indicate significant differences between samples (P_0, P_1, P_2, P_3) at the same fermentation time, and different uppercase letters indicate significant differences between the same sample at different fermentation time (0,12, 24H).

Moreover, P_3 obtained with sourdough fermented for 24H reached the second highest value for TFA, after P_2 at the same moment. In addition, TFA of P_3 obtained with 12H fermented sourdough had the lowest level, indicating a higher degree of proteolytic activity. This also could explain the P_3 low content of protein. Noteworthy, TFA of emmer sourdough had almost doubled after 12H of fermentation (data not shown), amino acids being necessary for the microbial dynamic of lactobacilli.

Increases in the solubility of minerals throughout the fermentation process led to a rising tendency of the ash content. Furthermore, the variation of the minerals' contents (Table S1) as influenced by the sourdough fermentation time, clearly indicates an increasing trend for all determined elements, with the highest values for 24H fermented sourdough. The mineral profile of AW breads showed increased values for K, Ca, Mg, Zn, Mn, and Fe. P_2 was rich in K, while P_1 in Ca, Mg, Cu, Zn, Cu, Mn, Fe. However, P_0 recorded the highest values for K, Ca, and Fe, when 24H fermented sourdough was used. The differences between AW breads could be related to the initial mineral content of the raw flours, the soil composition, the type of microorganisms, and the fermentation time. The common wheat flour used in this study had a different provenance and producer than AW, thus explaining the differences. For all tested samples cellulose decreased slightly as the sourdough fermentation time increased, due to enzymatic hydrolysis which generated the fibers' solubilization.

3.2. Simulated gastrointestinal digestion - in vitro digestibility (IVD) and maltose, glucose, fructose digestion

The fermentation time limits of 0 and 24H were used for this analysis.

Fructose was the major free carbohydrate found in all sourdough bread samples, compared to maltose and glucose. The highest concentrations were recorded for 24H of fermentation. Fructose content ranged from 7.53 mg/g in $P_3$24H to 14.39 mg/g in $P_0$24H, while $P_1$24H and $P_2$24H recorded 10.89 mg/g and 11.42 mg/g fructose, respectively. Compared to fructose levels, maltose was found in concentrations of 4.07 to 5.82 times lower in samples with 24H fermentation. The exception was $P_3$24H where maltose content was 1.42 times lower than fructose. Concerning glucose concentrations, the fructose to glucose ratio ranged from 4.4 ($P_2$24H) to 4.96 ($P_0$24H). Fructose/glucose ratios in $P_1$24H and $P_3$24H were superior, at 6.4 and 6.54, respectively.

Fig. 2. a, b, and c show the variations of maltose, glucose, and fructose concentrations from undigested bread samples *vs* digested and residual fractions. As could be noticed in all samples, maltose, glucose, and fructose concentrations were higher in the residual fraction. Increased sourdough fermentation time led to lower maltose level in digested fractions of P_1, P_2, P_0.

Per contra, the concentration of maltose in digested fraction of $P_3$24H bread was significantly higher ($p < 0.05$) as compared to $P_3$0H. The highest concentration of maltose in the digested fraction was found in $P_0$0H (184.9 mg/g), followed by $P_2$0H (171.25 mg/g) and $P_1$0H (166.85 mg/g). The maltose ratios from residue and digested fraction ranged from 1.54 to 1.77, with the lowest value for P_3 and the highest value for

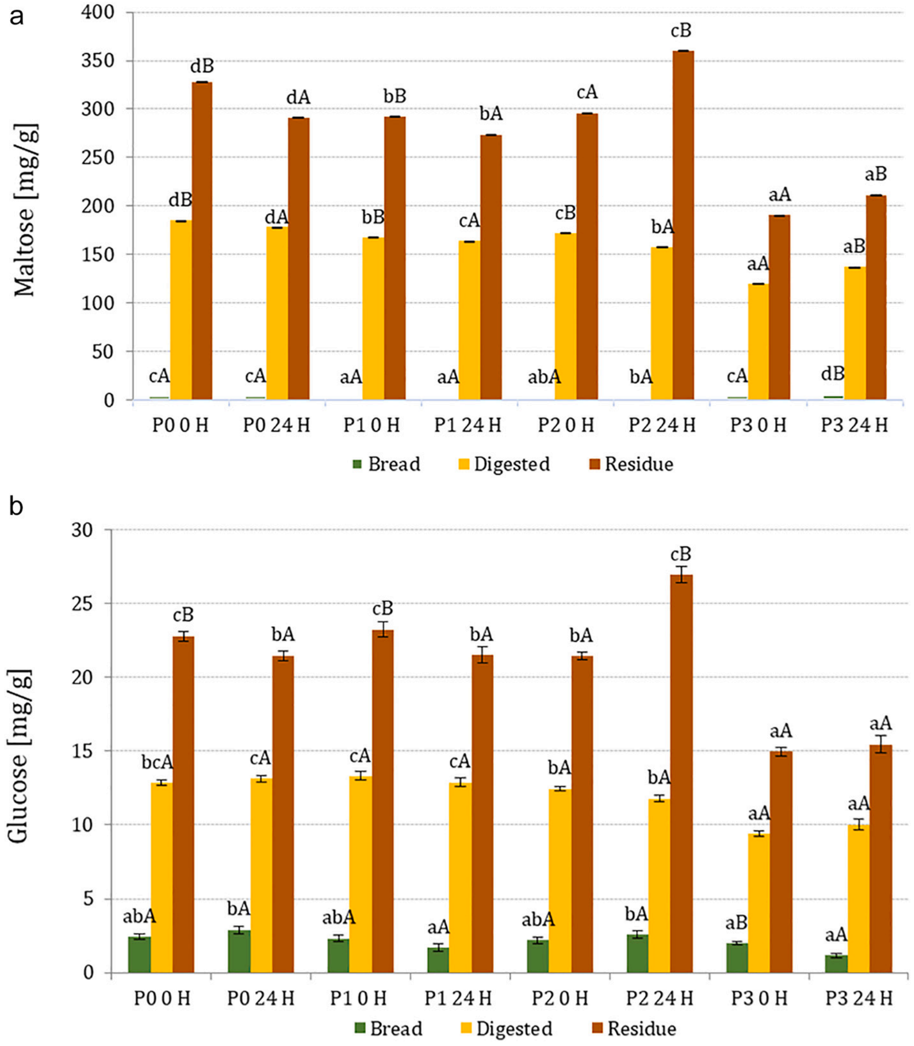

Fig. 2. Content of maltose (a) glucose (b), fructose (c) from undigested/digested/residue fractions after simulated gastrointestinal digestion and % IVD (d). Different lowercase letters indicate significant differences between samples (P_0, P_1, P_2, P_3) at the same fermentation time, and different uppercase letters indicate significant differences between the same sample at different fermentation time (0,12, 24H).

P_0. For glucose content, the sourdough fermentation time did not significantly influence the results, values varied from 11.79 mg/g ($P_2$24H) to 13.13 mg/g ($P_0$24H). The glucose ratios between the residual and digested fractions ranged from 1.54 ($P_3$24H) to 2.28 ($P_2$24H). Similarly, sourdough fermentation time and wheat type had non-significant influence on fructose content from digested fraction. The highest contents of fructose were recorded in $P_0$0H and $P_0$24H. The fructose content of the digested and residual fractions of P3 followed a similar pattern to the quantities of maltose and glucose. The fructose ratios between the residual and digested fractions were very close to those found for glucose. The average fructose/glucose ratio in digested bread samples was 2.1, while for undigested bread samples it was around 4.68.

According to the methodology used, IVD (%) represents the proportion of the digested sample from the undigested product. Fig. 2.d shows that excepting the emmer bread, all samples recorded higher IVD for 24H fermentation, with values ranging from 78.82% (P124H) to 82.38% (P024H). P324H had high IVD values recording an average of 85.18%, while P30H had higher value (86.53%) but without significant differences ($p > 0.05$). For the other bread samples, extended fermentation time resulted in a significant increase in the IVD values compared to bread samples obtained with unfermented sourdough.

3.3. Total free phenolic content (TPC) and DPPH radical scavenging activity (RSA)

The evolution of TPC and RSA in AW bread is presented in Fig. 3. (a, b).

The highest TPC contents were recorded in the bread with 24H of sourdough fermentation and ranged from 125 mg GAE/100 g dw (dry weight) in P_3 to 149 mg GAE/100 g dw in P_1. TPC content of $P_2$24H was 142 mg GAE/100 g dw, while $P_0$24H recorded 135 mg GAE/100 g dw.

Generally, bread made from flours with the highest beginning level of total phenolic acids showed the greatest increase in TPC (Fig. 3.a). But no less important, fermentation time significantly ($p < 0.05$) increased the level of TPC; moreover, a similar RSA trend being indicated in Fig. 3.b. $P_1$24 H showed 37.81% RSA, being placed in the first position with a 5.77% increment *vs* $P_1$0H. $P_2$24H registered 36.24% RSA and was situated in the second place followed by $P_0$24H (31.88%) and finally $P_3$24H (31.55%).

3.4. Organic acids and ethanol contents

The variations of organic acids and ethanol contents are shown in Fig. 4.

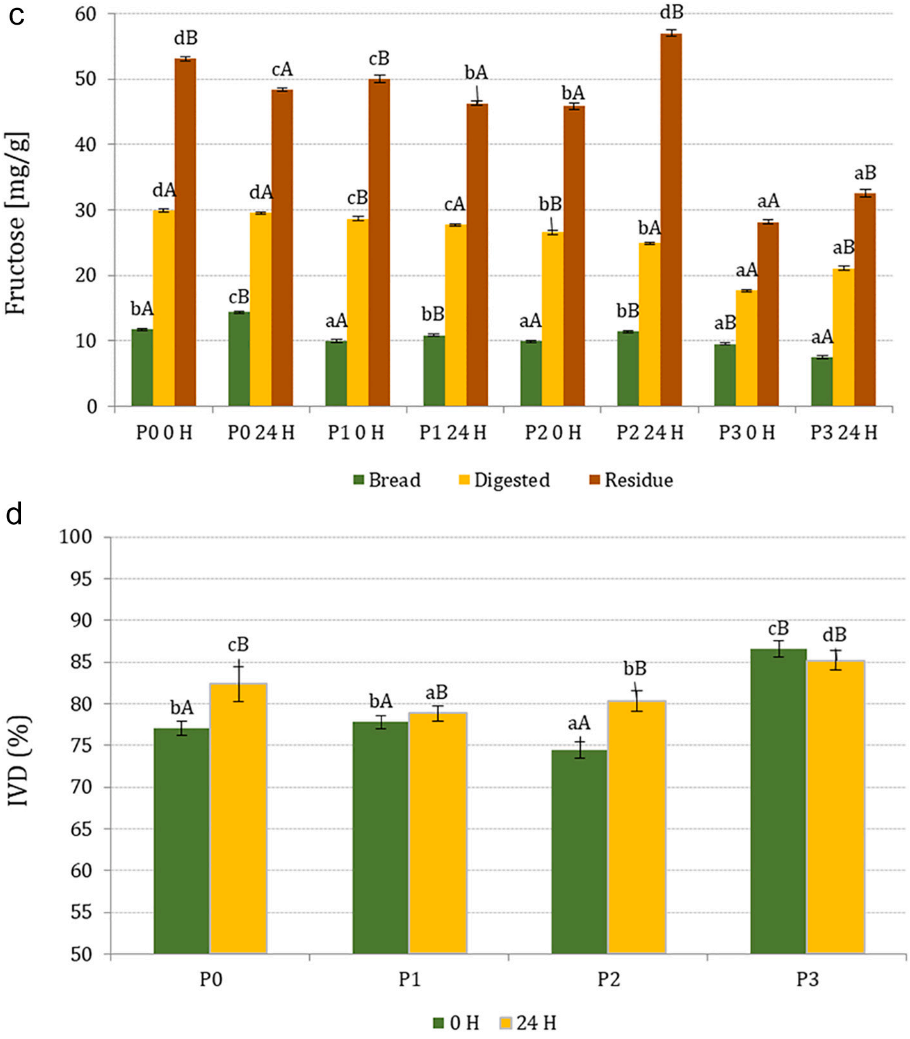

Fig. 2. (*continued*).

As expected, increased fermentation time resulted in higher lactic acid contents. The highest concentration of lactic acid was recorded in $P_1$24H (1.13 mg/g), followed by $P_3$24H and $P_0$24H, but without significant differences ($p > 0.05$). Acetic acid was detected in lower concentrations than lactic acid and only in P_0 and P_1 with increasing values for 24H of fermentation. $P_1$24H recorded the highest value 0.55 mg/g. Moreover, as the fermentation time increases, a rapid increase in lactic acid is recorded and the acetic acid content remains low.

Particularly, citric acid was present in all bread samples including breads obtained with unfermented sourdough, indicating its presence in AW flours. The highest concentration of citric acid was found in $P_0$24H (1.52 mg/g), followed by $P_2$24H. $P_1$24H and $P_3$24H also revealed high amounts of citric acid, close to 0.9 mg/g. During the fermentation process, the citric acid amount fluctuated having lower values after 12H of fermentation, probably, due to its metabolization by *Lp* through the citrate pathway.

Concerning ethanol, it was recorded in all samples with 12 and 24H of fermentation. The low ethanol content is related to the cold fermentation of dough which was used to improve texture but decreased bakery yeast metabolism and consequently its main metabolites.

3.5. *Volatile Organic Compounds (VOCs)*

In the present case, 24 VOCs were identified, of which 7 are alcohols, 7 aldehydes, 3 ketones and 7 other compounds (Table S2). 1-Butanol, 3-methyl was recorded in the highest concentrations in all bread samples, ranging from 41.96% to 60.98%. Fermentation time had significant influence on its content. 1-Butanol, 2-methyl was recorded in the highest concentrations in $P_0$0H but there was no significant impact of fermentation duration on all samples. 1-Hexanol concentrations differed significantly ($p < 0.05$) between samples with unfermented sourdough, but fermentation time influence was not significant ($p > 0.05$). The major aldehyde was hexanal, ranging from 7.03 to 27.42%. Benzaldehyde, a product of Maillard reactions, was recorded in small concentrations in all samples as well as other carbonyl compounds. Furan, 2-penthyl ranged between 1.8 and 3.8%, being the result of sugar thermal degradation during baking. Limonene was found in high concentrations ranging between 10.1 and 17.3%, especially in P_1, P_2, P_0 at 0H. The highest concentration was recorded in $P_2$12 H, followed by $P_0$24H. $P_3$0H had the lowest content of limonene (1.51%) but increased significantly with the fermentation time.

3.6. *Texture profile*

The highest values of crumb hardness were recorded for $P_0$0H, followed by $P_2$0H and $P_3$0H (Table S3). Sourdough fermentation time significantly decreased ($p < 0.05$) the value of crumb hardness for all tested samples. The highest crumb cohesiveness was found for $P_1$24H followed by $P_2$24H. Cohesiveness was not significantly influenced by fermentation time. Gumminess decreased for all samples as the

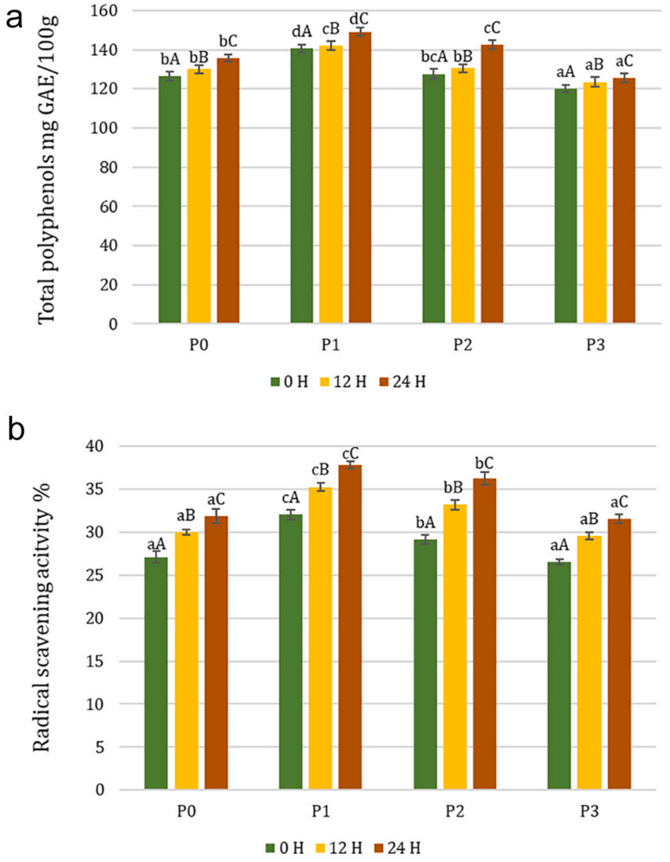

Fig. 3. Influence of sourdough fermentation time on TPC and RSA of bread samples with wheat (P_0), einkorn (P_1), spelt (P_2), and emmer (P_3). Different lowercase letters indicate significant differences between samples (P_0, P_1, P_2, P_3) at the same fermentation time, and different uppercase letters indicate significant differences between the same sample at different fermentation time (0,12, 24H).

sourdough fermentation time increased, the values were recorded for $P_0$0H. Chewiness decreased with extended fermentation time in all crumb samples and significant differences between samples were found ($p < 0.05$). AW breads returned lowest values for chewiness than common wheat bread. The control sample (P_0) recorded the highest values for crumb springiness followed by P_2. Prolonged fermentation increased the springiness index in all samples. Significant differences ($p < 0.05$) were found between the springiness index and resilience of AW and W bread.

3.7. Colour parameters and sensory analysis

The colour parameters (chromatic coordinates) (L*, a*, b*) of bread samples were not significantly influenced by sourdough fermentation time (Table S4). Prolonged fermentation times led to the increment of colour parameters. Generally, P_3 had the highest values for L*, a*, and b*, followed by P_1 and P_2.

Although there were perceived little variations in the degree of preference for the bread samples, no significant differences ($p < 0.05$) were found. Overall acceptance of bread samples was in the range of 6.0–7.09 from a maximum of 9 points, with the lowest values for P_2 and the higher acceptance for P_3. Sourdough fermentation time negatively influenced the odor and the aroma of all breads when fermentation has been prolonged to 24H.

3.8. Effect of experimental factors (ancient wheat flour and fermentation time) on the nutritional, textural and sensorial characteristics of bread

Based on the 73 parameters determined in this experiment (Supplementary data), after a weighted standard deviation pre-treatment applied for providing a relative significance to each value, a PCA and HCA using a heatmap (Fig. 5 a,b.) were performed. The analysis may provide a deeper insight of the effect of factors -flour type and fermentation time- on the bread's nutritional and sensorial characteristics. Fig. 5a. shows that the two principal components (PC-1, PC-2) and their scores explain 28% and 21% of the range in data variations and indicate a clear separation between bread samples, both due to sourdough fermentation time and the type of flour. Obviously, the plot indicates a clear separation for emmer bread samples from the others wheat species bread. This aspect could be noticed from HCA also, indicating low levels of ash and minerals (K, Mg, Ca, Fe, Zn, Mn), proteins, total amino acids, glucose, total polyphenols, and RSA for emmer sample comparing to the other samples. On the contrary, starch and maltose contents were much higher than in other samples.

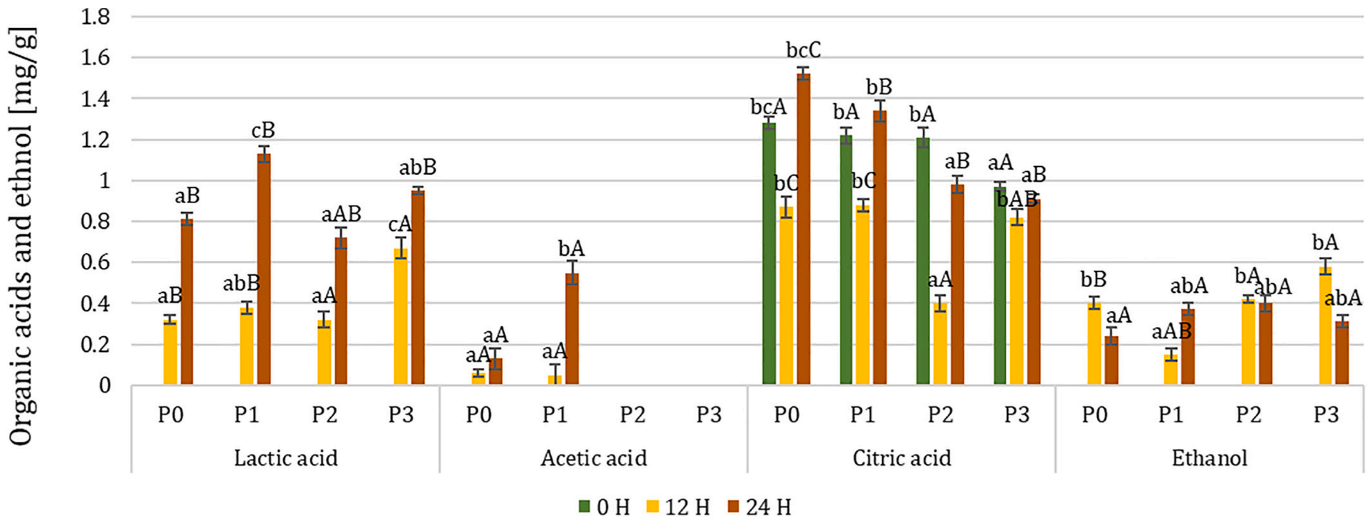

Fig. 4. Influence of sourdough fermentation time on organic acids and ethanol contents in bread samples with wheat (P_0), einkorn (P_1), spelt (P_2), and emmer (P_3). Different lowercase letters indicate significant differences between samples (P_0, P_1, P_2, P_3) at the same fermentation time, and different uppercase letters indicate significant differences between the same sample at different fermentation time (0,12, 24H).

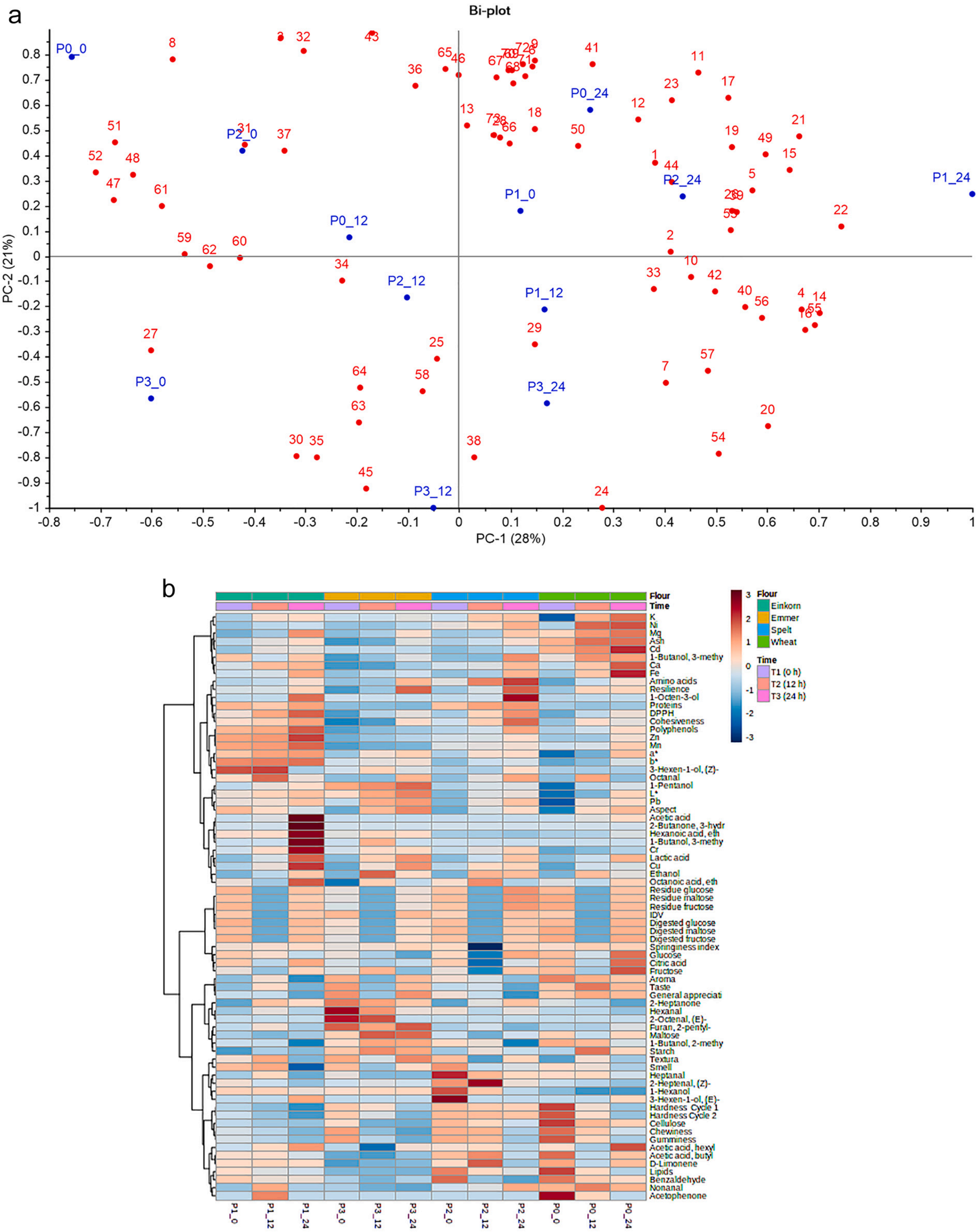

Fig. 5. PCA biplot of the nutritional, textural and sensorial parameters of bread samples (a) and Heat map and HCA of the nutritional, textural and sensorial parameters of bread samples (b). The colours represent the compounds' level, starting from light blue (low level) to white to dark red (high level). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Sourdough technology is focusing on nutritional benefits such as lower glycemic index, improved fiber solubility, mineral bioavailability, and increased antioxidant capacity. It also enhances sensorial and textural properties through sourdough fermentation. However, creating fiber-rich bread with a balanced nutritional, technological, and sensory quality remains a challenge. Fiber-rich bread lags behind white bread due to technological quality criteria like unattractive crumb colour, decreased volume, underdeveloped crumb structure, and crumbly texture. The raw ingredients used may also cause changes in aspect, colour, texture, odor, and taste, potentially impacting customer acceptance. Therefore, more attention is needed to develop fiber-rich bread products with a balanced nutritional, technological, and sensory quality.

4.1. Nutritional characteristics of AW bread samples

The starch content of AW bread showed the highest values after 12H of fermentation and decreased after 24H. The common wheat bread had the highest starch content after 12H but the extended fermentation time determined the highest starch content values in emmer bread. The high starch content of emmer bread is explained primarily by a greater starch amount of emmer flour (65.9%) compared to einkorn (62.2%) and spelt flours (61.6%) (Kulathunga, Reuhs, Zwinger, & Simsek, 2021). However, the high values recorded for 12H of fermentation might be due to the differences between starch granules in terms of size and degree of embedding into the protein matrix. For instance, in einkorn flour, small starch granules and thinner cell walls confer more accessibility for amylase compared to large granules and thick cell walls. Moreover, emmer showed the highest firm embedding followed by einkorn and spelt (Kulathunga et al., 2021; Şerban et al., 2021).

All examined samples showed an increase in fiber solubilization as fermentation length increased. The highest cellulose content was recorded for W bread, while the lowest value was recorded for emmer bread. Recent research shows that sourdough fermentation significantly impacts the chemical and physical properties of fiber, converting insoluble to soluble fiber and reducing insulinemic and glycemic responses (Fernández-Peláez, Paesani, & Gómez, 2020; Graça, Lima, & Raymundo, 2021).

The protein content variation was comparable for all analysed samples, with a slight decrease for 12H of fermentation, followed by a small rise for 24H. The lowest protein content of emmer bread is partially explained by its higher amount of starch and insoluble fibers (Kulathunga et al., 2021). Nevertheless, TFA variation shows an upward relationship with sourdough fermentation time in all examined samples. Protein decomposition by endogenous and microbial proteases increases TFA content, while amino acids impact bread's nutritional, aroma, total phenolics and antioxidant activities. Colosimo et al. (2020) reported enhanced concentrations of branched aminoacids, bioactive peptides and antioxidant activity for spelt sourdough.

The variation of the bread minerals' contents clearly indicates an increasing trend with sourdoughs fermentation time for all tested samples. The sourdough fermentation time linked with the raw AW chemical composition improved the AW breads' mineral profile (Kraska, Andruszczak, Gawlik-Dziki, Dziki, and Kwiecińska-Poppe (2020), Van Boxstael et al. (2020), Şerban et al. (2023). Furthermore, several research on whole grain sourdough fermentation have shown that the majority of phytic acid is degraded and that high mineral bioavailability is promoted (Fernández-Peláez et al., 2020; Lopez et al., 2003).

Concluding, the proximate composition of tested AW bread indicates that the starch content fall within the typical values for fiber-rich bread, ranging from 33 to 39% (Hallström, Sestili, Lafiandra, Björck, & Östman, 2011; Scazzina, Del Rio, Pellegrini, & Brighenti, 2009; Demirkesen-Bicak et al. (2021). Nowadays, a lower starch content may indicate a superior dietetic advantage, considering the physiological implications of high starchy diets in humans (Oh, Gilani, & Uppaluri, 2023). In addition, Mung beans and inulin were added to the AW bread recipe, in alongside employing whole flours, to improve the fiber content. The consumption of insoluble fiber has numerous health benefits, including a lower risk of chronic diseases (such as diabetes, cardiovascular disease, obesity, and certain types of cancer) in humans, as well as the promotion of gut microbiota growth (Chinma et al., 2022). Furthermore, the presence of other bioactive compounds such as minerals, proteins and free aminoacids contribute to a balanced nutritional composition of sourdough AW bread.

Compared to maltose and glucose, fructose was the predominant free carbohydrate present in all sourdough bread samples. The high fructose content of AW bread samples might derive from the kernel's fructan content since wholemeal flours were used, as well as from inulin and sucrose that were used in bread formulation. Commonly, sugar-rich breads have higher contents of fructose than glucose, the last being primarily consumed by yeast and/or lactobacilli during dough fermentation (Gélinas, Mckinnon, & Gagnon, 2016).

Fermentation time influenced distinctively the free carbohydrates concentrations of emmer bread compared to the other samples. Increased maltose content were recorded in 24H fermented sourdough emmer bread while in the other samples maltose decreased in extended fermented sourdough bread. Oppositely, glucose and fructose contents from emmer bread recorded the same decreasing trend like the other AW bread when prolonged fermentation was used. Thus, emmer's starch distinctive techno-functional characteristics could explain these results (Kulathunga et al., 2021). Noticeable, the high maltose, glucose, fructose amounts of W bread indicate high amylolytic activity possible due to specific treatments at milling as well as specific interventions during cultivar breeding.

With respect to the *in vitro* digestion, the patterns of variance in maltose, glucose, and fructose seen in AW bread maintained in both the digested and residual fractions. The sourdough fermentation time did not considerably affected the carbohydrates' amounts in these fractions. Again, emmer and W samples were situated at opposite poles. Excepting emmer bread, fermentation time increased IVD values for tested samples.

AW breads' digestibility could have been influenced by its high content of fiber and polyphenols. During processing and storage, starch can interact with a variety of dietary ingredients, including lipids, proteins, fibers, and polyphenols among others (Batista et al., 2017). Fibers and polyphenols hinder starch enzymatic breakdown reducing its digestibility (Chinma et al., 2022). For instance, dietary fibers slow down α-amylase action in a starch-α-amylase-dietary fiber system (Srichamroen & Chavasit, 2011). Moreover, a recent study (Ahmed, Thomas, & Khashawi, 2020) found that inulin-enriched bread reduced intestinal starch breakdown, particularly on slowly digested starch, demonstrating positive effects. This might indicate that no depolymerization of inulin occurs throughout the stomach and ileal stages of digestion. In addition, polyphenols can inhibit α-amylase and α-glucosidase, altering the glycemic response to carbohydrates (Kan et al., 2020). Polymeric polyphenols present in sufficient concentration can combine with protein and starch to create massive complexes or polyphenol-coated particles thus slowing the carbohydrates digestion (Amoako & Awika, 2016). Thus, *in vitro* dry matter (IVD) of sourdough „crostini" enriched with microalgae was found to be above 85% (Niccolai et al., 2019), while for wheat crackers values ranged within 78.3–93% (Batista et al., 2017).

However, the almost double concentrations of fructose compared to glucose from digested fractions of AW bread could be considered a digestive issues for individuals sensitive to diets high in fructose (Gélinas et al., 2016; Pejcz et al., 2021). It was also stated that consuming fructan-rich prebiotics like inulin may cause health concerns (Kumar, Prashanth, & Venkatesh, 2015), while foods with higher glucose content may increase gastrointestinal absorption of fructose (Gibson & Shepherd, 2010). However, it is worth noting that to take 10 g fructans, one would need to eat the equivalent of 1–2 kg of bread per day, since such goods contain just 0.5% fructans. Fortunately, fructans leftovers in long-fermentation bread did not produce significant wheat sensitivity or digestion issues (Gélinas et al., 2016).

TPC contents and RSA of analysed bread samples showed an evident increasing trend with the fermentation time. Einkorn bread had the highest TPC and RSA after 24H of fermentation but extended time significantly increased these parameters for each bread type. Spelt and emmer bread had comparable TPC and RSA values and closer to W bread.

Research on polyphenol levels in ancient and modern wheat cultivars shows contradictory results. Shewry and Hey (2015) stated that AW has a comparable phenolic content to wheat, with total ferulic acid being the most prevalent. However, some studies show lower amounts of TPC in einkorn, spelt, and emmer flours (Ivanišová, Ondrejovič, & Šilhár, 2012; Lachman et al., 2012; Zrcková et al., 2019), while others highlight AW's superior bioactivity (Van Boxstael et al., 2020). These variations could be attributed to factors such as growth location, agronomic practices, and environmental conditions (Dapčević-Hadnađev et al., 2022). Wholemeal flour bakery products, such as sourdough mini-baguette and dark wheat bread, showed close TPC values with our results, ranging from 129.56 to 147.09 mg GAE/100 g (Catană, Catană, and Burnete (2022); Man et al. (2021). Modern wheat also reported close values of RSA (27.1%–47.5%) Han and Koh (2011).

Several processes occurring during the breadmaking procedure including TPC release from cell walls, degradation by flour and microbial enzymes like esterases, heat decomposition during baking and recurrent binding by surrounding carbohydrates and proteins. On the other hand, factors such as bread formulation, flour extraction, baking temperature, and duration have been found to affect the antioxidant activity in bakery goods (Shen, Chen, & Li, 2018). Wholemeal flours exhibited higher TPC and RSA than refined ones due to the presence of brans and germs, which bring phenolic acids (ferulic and caffeic acid), as well as phytic acid, vitamin E, and selenium, known with antioxidant potential (Hirawan, Ser, Arntfield, & Beta, 2010; Yu, Nanguet, & Beta, 2013). During fermentation the hydrolysis of antioxidant linkages releases antioxidants that scavenge free radicals. In addition, sourdough fermentation enhances antioxidant capacity through extractible phenols (Banu, Vasilean, & Aprodu, 2010) and antioxidant peptides, increasing TPC and RSA through acidification and hydrolysis of complex forms. Furthermore, *Lp* reduces ferulic acid content in wheat sourdough, exhibits high tolerance to phenolic acids, and positively influences aroma and antioxidant activity (Skrajda-Brdak, Konopka, Tańska, & Czaplicki, 2019); (Rodríguez et al., 2009).Thus, the metabolic activity of lactobacilli is of great importance for both TPC and antioxidant activity (Pejcz et al., 2021). Regarding baking, increasing temperature and duration may produce the release of insoluble conjugated bound phenolic compounds, resulting in increased antioxidant activity of bread (Han & Koh, 2011). Melanoidin compounds formed during baking are also partially responsible for the antioxidant potential (Shen et al., 2018).

4.2. Aroma, colour and textural characteristics of AW bread samples

Extended fermentation time resulted in higher contents of lactic acid for all AW bread, while acetic acid, was detected in wheat and einkorn breads, only. Lactic and acetic acids are the main organic acids produced through the heterofermentative metabolism of *Lp*. AW breads' lactic acid concentrations were close to those reported by Hadaegh, Seyyedain Ardabili, Tajabadi Ebrahimi, Chamani, and Azizi Nezhad (2017) for wheat bread with 20% sourdough fermented with *Lp*. Even if, acetic acid was found only in common wheat and einkorn bread, it might be possible that part of the acetate formed during metabolic pathways remained bound in esters like acetic acid, hexyl ester, and acetic acid, butyl ester, as the results for VOCs showed (Table S2) and contributing to the bread aroma profile. On the other hand, when lactobacilli have low amounts of fermentable carbohydrates available, the production of lactate is shifted to transforming citrate (Gobbetti & Gänzle, 2013). Sometimes citrate is used as an energy supplier (Teleky, Martău, Ranga, Chețan, & Vodnar, 2020). Thus, for all AW bread lower citric acid amounts were found after 12H of fermentation. But only for wheat and einkorn, extended fermentation led to higher citric acid concentrations. Spelt and emmer bread recorded slight lower contents of citric acid when 24H fermented sourdough was used. However, high concentrations of citric acid found in AW tested samples can improve bread aroma and textural characteristics (crumb hardness and resilience), according to Filipčev, Šimurina, and Bodroža-Solarov (2014); Kokawa et al. (2017).

Excepting einkorn bread, fermentation time decreased ethanol content for AW bread. Both *Lp* and baking yeast produced ethanol, with significantly higher concentrations after 24 h of fermentation compared to the corresponding sourdough (Șerban et al., 2023). Both the fermentation time and the type of wheat influenced the ethanol concentrations, as well as the dough's cold fermentation applied in this study. The high concentrations of ethanol in bread samples could be an explanation for the lower contents of acetic acids (Martău, Teleky, Ranga, Pop, & Vodnar, 2021) or for the absence of acetic acid in spelt and emmer samples where ethanol was in higher amounts. Fermentation of wheat sourdough bread with LAB and yeast led to close findings as those reported by (Păucean, Vodnar, Socaci, & Socaciu, 2013; Sidari, Martorana, Zappia, Mincione, & Giuffrè, 2020; Teleky et al., 2020). Nevertheless, ethanol was not detected when VOCs analysis was performed, probably due to the sensitivity of the analytical equipment. Ethanol content may fluctuate by evaporation or due to the participation of different reactions leading to short-chain alcohols, SCFAs, esters, and carbonyl compounds (De Luca et al., 2021; Pico, Bernal, & Gómez, 2015), thus explaining the differences between samples.

Compared to the corresponding sourdoughs' content of VOCs (Șerban et al., 2023), fewer compounds were determined in bread samples. This is an expected result since part of these compounds evaporate during baking or are transformed into other compounds. 3-methyl-1-butanol, 2-methyl-1-butanol and 1-Hexanol are considered typical VOCs in wheat bread and were found in the highest concentrations in all tested samples. Their amounts slightly fluctuated with the fermentation time. Generally, higher amounts of alcohols are formed when bread is fermented with sourdough and yeast. The main VOC identified, 1-Butanol, 3-methyl, was found positively correlated to wheat bread aroma (Rehman, Paterson, & Piggott, 2006). Hexanal, the primary aroma compound from wheat flour, was identified as the main aldehyde in all AW bread. However, lipoxygenase catalyzes lipid oxidation, leading to the formation of aldehydes and esters. Unsaturated linoleic and linolenic acids are degraded by hydroperoxides and converted into hexanal during baking (Pico et al., 2015). AW and einkorn, richer in fatty acids than common wheat, contains high unsaturated fatty acids, making hexanal the lipids' oxidation product with the highest odor activity value in bread crumb (Birch, Petersen, & Hansen, 2014; Hidalgo, Brandolini, & Ratti, 2009). Heptanal, octanal, nonanal, and 2-heptenal were also reported as products of lipids oxidation (Birch et al., 2014), but in all AW bread samples, they were found in small amounts. Ethyl hexanoate and ethyl octanoate, even if very volatile, have an important contribution to the wheat bread aroma profile because they possess pleasant fruit odours (Birch et al., 2014; Pico et al., 2015). In the present study, they were recorded in very small concentrations (<1%) in all tested bread samples. However, limonene was detected in considerable concentrations in all AW bread. Limone has been linked to carotenoid content (Chiş, Păucean, Man, Mureşan, et al., 2020), and as AW is rich in carotenoids (Șerban et al., 2021) this might explain the accumulation of limonene in bread. Limonene favourably improves the bread taste by having a sweet, citrus flavour.

With respect to textural parameters, crumb hardness, gumminess, chewiness and springiness index were positively and significantly influenced by fermentation time. On crumb's cohesiveness and resilience fermentation time had no significant influence, but their values increased with the fermentation time. Gluten quality and quantity significantly influence textural parameters in bread. AW flours produce softer doughs with lower elasticity and higher extensibility compared to wheat (Șerban et al., 2021). The high-molecular-weight fraction of glutenin macropolymer (HMW-GMP) predicts bread volume. Common wheat and spelt have similar peak HMW-GMP numbers but differ from emmer and einkorn. Low GLA/GLUT ratios result in higher textural quality (Geisslitz, Longin, Scherf, & Koehler, 2019). This could explain the differences between AW bread. On the other hand, the effect of sourdough fermentation on crumb hardness is attributed to acidification which impacts the solubility of the structure-forming components such as gluten and starch and to the improvement of gas retention in dough (Jitrakbumrung & Therdthai, 2014). Novotni, Čukelj, Smerdel, and

Ćurić (2013) showed that in sourdough wholemeal wheat bread, lactic acid formation progressively contributed to a more elastic gluten structure and a softer crumb, while acetic acid was the cause of shorter and harder gluten. Since in our study, acetic acid was absent or in small concentrations, this explains the improvement of hardness, springiness index with lactic acid accumulation. Furthermore, Tomić et al. (2023), found that sourdough fermentation increases cohesiveness, which is crucial for preventing product breakdown during mastication. Thus, AW flours sourdough can significantly reduce the effort needed to chew the bread crumb before swallowing. Moreover, it is obvious from the sensorial analysis results that spelt bread had the best texture profile compared to einkorn and emmer bread. Callejo, Vargas-Kostiuk, and Rodríguez-Quijano (2015) found higher crumb elasticity in spelt bread, but poor extensibility due to high GLA/GLUT ratio. In addition, spelt bread acidity was higher, potentially resulting in better volume and crumb grain compared to the other samples. Einkorn bread had the lowest hardness, chewiness, and gumminess, but as other studies are reported, contradictory results were found. Regarding emmer, poor textural qualities were observed, results confirmed by a recent study performed by Zamaratskaia, Gerhardt, and Wendin (2021).

Sourdough fermentation time did not influence chromatic parameters of AW bread crumb. Nevertheless, the analysis's findings are attributable to the kind of flour. Hence, wholemeal flours are rich in bran particles, resulting in darker breadcrumbs. A higher a* value is associated with the presence of dark-coloured bran fractions in the wholemeal flour (Wójtowicz et al., 2020). In turn, the minerals and carotenoid amounts were correlated with yellow pigment content (Popov-Raljić, Mastilović, Laličić-Petronijević, & Popov, 2009). AW bread's yellowish intensity (b*) may be attributed to its high carotenoid content, with einkorn having the highest concentration of carotenoids (Belcar, Sobczyk, & Sekutowski, 2021; Çakır, Arıcı, Durak, & Karasu, 2020).

4.3. Characteristics of AW bread type as affected by the factors

PCA and HCA analysis were performed to better understand the differences between AW bread. It was noticed that emmer bread clearly distinguished from the other samples. Starting from the parameters pointed out in Fig. 5 (a,b), it could be stated that emmer's lower minerals and protein content may slow down the microbial metabolism, at least for 12H of sourdough fermentation. Probably the high amylolytic activity released large amounts of maltose which was used up to 12H by the yeast for ethanol production and then as the availability of glucose increased, *Lp* produced lactic acid (as it could be seen in bread with prolonged fermentation). In addition, in 24H fermented sourdough emmer breads' TFA were higher and part of them acted as VOCs precursors. Thereby, in P_3 were registered elevated concentrations of pentanol, hexanol, 1-butanol-3 methyl, 1-butanol-2 methyl, octanone, 2 heptanone, 2 octenal, furan pentyl, compounds which significantly influenced the sensorial scores for taste and aroma. Moreover, P_3 recorded better results for the sensorial analysis from all tested samples and this is attributed to the textural parameters, also. Thus, HCA indicates lower hardness and chewiness and higher resilience and cohesiveness compared to the other bread samples, being the results of both initial chemical composition of emmer flour and the effect of sourdough fermentation. Concerning the *in vitro* digestion, even if medium digestion level of maltose, glucose, fructose was recorded in emmer bread samples (0, 24H), the IDV revealed elevated scores. These results are probably due to the high content of starch and the strength of its interactions with other compounds like proteins which significantly influenced the enzymatic action during digestion.

On the other hand, P_1 was characterized by a group of VOCs (dark red for prolonged fermentation) composed of acetic acid, 2-butanone, 3 hydroxy, hexanoic acid, ethyl ester, 1-butanol, 3-methyl, octanoic acid and lactic acid which likely influenced the lowest scores for aroma and taste of this sample, giving pronounced acidic notes.

The textural parameters of P_0 were positively influenced by sourdough fermentation time, and this explain the high score obtained by these samples especially for texture and aspect. Moreover, as HCA indicates a clusterisation of several volatiles like D-limonen, benzaldehyde, acetic acid-buthyl, acetic acid-hexyl, nonanal in the case of P_0. These compounds are giving fruity and floral notes and positively influenced the panellists' perception for aroma and taste.

The negative influence of prolonged fermentation is due probably to the presence of several alcohols (*e.g.*, 1-penthenol) and aldehydes (*e.g.*, hexanal, heptanal) which were recorded in increased concentrations in prolonged fermented sourdough breads and can give sour, fermented aroma. However, results for odor and aroma of AW bread ranged between 6.18 and 7.09 indicating good consumer acceptability which is the consequence of the presence of volatile compounds related to fruity aroma.

5. Conclusion

Both AW flours' specific chemical composition and the sourdough fermentation time significantly influenced the nutritional, textural and sensory properties of the tested samples. Sourdough fermentation time enhanced the content of bioactive compounds, the textural and sensory characteristics being also improved. Excepting emmer bread, *in vitro* digestibility of bread samples increased with the fermentation time and even if fructose content was higher in digested fractions compared to glucose, the concentration is considered not too high for a rational diet. A different properties' profile was revealed in the case of emmer bread as a results of the experimental factors interaction. Emmer bread had the highest *in vitro* digestibility and specific acidic profile, VOCs, texture parameters thus being preferred by panellists.

CRediT authorship contribution statement

Adriana Păucean: Writing – review & editing, Writing – original draft, Supervision, Project administration, Conceptualization. **Larisa-Rebeca Șerban:** Writing – original draft, Investigation, Data curation, Conceptualization. **Maria Simona Chiș:** Visualization, Validation, Software, Investigation, Formal analysis. **Vlad Mureșan:** Visualization, Validation, Software, Methodology. **Andreea Pușcaș:** Investigation, Formal analysis. **Simona Maria Man:** Methodology, Formal analysis. **Carmen Rodica Pop:** Methodology. **Sonia Ancuța Socaci:** Methodology, Formal analysis. **Marta Igual:** Visualization, Software, Investigation, Formal analysis. **Floricuța Ranga:** Methodology, Investigation, Formal analysis. **Ersilia Alexa:** Visualization, Investigation, Formal analysis. **Adina Berbecea:** Validation, Formal analysis. **Anamaria Pop:** Validation, Formal analysis.

Declaration of competing interest

The authors declare they have no financial interests. The authors have no competing interests to declare that are relevant to the content of this article.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101298.

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