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Effect of Different Wheat Sprouting Conditions on the Characteristics of Whole-Wheat Flour

Running title: Effect of Controlled Sprouting on Whole-Wheat Flour

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SUMMARY

Research background. Controlled sprouting promotes physiological and biochemical changes in whole grains, enhancing their nutritional status and offering technological advantages for breadmaking as an alternative to traditional whole grains. This study aimed to find sprouting conditions for Klein Valor wheat variety grains (*Triticum aestivum L.*) that would enhance nutritional value without markedly affecting gluten proteins, which are essential in wholegrain baked goods.

Experimental approach. Unsprouted and sprouted whole-wheat flour were assayed for chemical and nutritional composition, enzymatic activity, and pasting properties of flour suspensions.

Results and conclusions. This bioprocess allowed us to obtain sprouted whole-wheat flour with varying levels of component modification. Sprouting at 25 °C resulted in a notable increase in enzymatic activity and metabolic processes, particularly α-amylases, which significantly affect the starch matrix and the associated pasting properties. Additionally, there was a lesser but still notable effect on the structure of cell walls and the protein matrix due to the activation of endoxylanases and

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proteases. In contrast, sprouting at 15 and 20 °C for 24 h allowed for better process control, as it promoted nutritional improvements such as a higher content of free amino acid groups, free phenolic compounds, and antioxidant capacity, along with a lower content of phytates. Additionally, it provided techno-functional advantages due to the moderate activation of α-amylase and xylanase. A moderate decrease in peak viscosity of sprouted whole-wheat flour suspensions relative to control flour was observed, while protein degradation was not widely extended.

Novelty and scientific contribution. Sprouted whole-wheat flour obtained under milder sprouting conditions with moderate enzymatic activity may be promising and interesting ingredients for formulating wholegrain baked goods with enhanced nutritional profiles and techno-functional properties. This approach could avoid the use of conventional flour improvers, thereby positively impacting consumers' acceptance and facilitating the adoption of clean labels.

Keywords: bioactive compounds; enzymatic activity; pasting properties; sprouted whole-wheat flour; techno-functional ingredient

INTRODUCTION

Wheat is the most irreplaceable food crop for breadmaking for most of the world's population. Whole-wheat products have received widespread interest due to their nutritional benefits, including dietary fibre, vitamins, antioxidants, and other health-promoting compounds (*1*). Despite their benefits, the consumption of whole wheat remains below recommended levels in many countries. Additionally, the incorporation of high levels of fibre into cereal-based products remains a technological challenge due to the need to maintain acceptable dough rheological properties and sensory attributes. Several pre-treatments have been proposed to counteract negative effects, such as particle size reduction (*2*), pearling process (*3*), enzymatic treatment (*4)*, and fermentation (*5*). In this context, the implementation of the sprouting process as a natural and cost-effective method to enhance nutritional status (*6,7*) and acceptability of whole grains (*1*) is an emerging interest. This physiological event starts when grains meet suitable environmental conditions: certain hydrolytic enzymes are activated and break down the storage macromolecules for developing seedling (*8*). The amylases rapidly degrade the starch, making it more digestible, and consequently, increasing the bioavailability of reducing sugars. However, an excessive accumulation of hydrolytic enzymes, often seen in preharvest sprouting, represents a negative aspect from a technological point of view, since it affects flour functionality. Xylanases degrade arabinoxylans of cell walls – increasing the availability of micronutrients (*9*) – and proteases hydrolyse proteins – increasing peptides and amino acids availability (*1*). The metabolic activity also promotes polyphenols biosynthesis and raises their

antioxidant capacity (*8*). Additionally, this bioprocess increases phytase activity and enhances the absorption of essential minerals in the gastrointestinal tract (*10*).

Recent studies have reported that controlled sprouting improved the breadmaking performance of common wheat in terms of specific volume, crumb structure and softness during storage when added to white bread (*11–13*). These positive effects were attributed to the natural enzymes that might decrease or completely replace the quantity of commercial enzymes frequently added to bread formulation (*11,14*). In addition, better sensory attributes like flavour and taste were also reported after the use of sprouted grains (*15*). In this way, careful control of sprouting conditions is necessary to achieve a good balance between nutritional advantages and technological performance of wheat flour for breadmaking (*9*). Sprouted flour could be also considered as whole grain, according to the definition outlined by Whole Grains Council (*16*), when preserving all the bran, germ, endosperm and vegetative parts as long as sprout growth does not exceed grain length.

As it is well-known, when compared to refined flour, whole-wheat flour exhibits deficiencies in functionality as the result of gluten dilution caused by a high content of insoluble arabinoxylans, which interferes with the gluten network and the presence of phytates, which negatively impact the bioavailability of minerals. Therefore, understanding the effects of controlled sprouting on protein aggregation, soluble fibre, and phytic acid content in wheat is of paramount importance. For this reason, this study assesses the impact of controlled wheat sprouting on the chemical composition, hydrolytic enzyme activities, and pasting properties of resulting sprouted whole-wheat flour. The objective is to study the effects of sprouting on various nutritional and technological properties which provide essential elements for producing wholegrain bread with improved nutrition and good technological quality.

MATERIALS AND METHODS

Materials and chemicals

We used the commercial wheat variety Klein Valor (*Triticum aestivum L.*) and grains from the same lot sprouted under controlled conditions in the laboratory. Wheat was supplied by Estación Experimental Agropecuaria Marcos Juárez del INTA (Córdoba, Argentina), harvested in 2020 and originated from a cultivation area located at the following geographic coordinates: 32° 43’ S, 62° 6’ W. Klein Valor variety is a corrector wheat with very strong gluten according to the genetic quality classification established by the Winter Cereal Committee of the National Seed Commission (Argentina) (*17*). All the chemicals used in this study were of analytical grade.

Sprouting conditions

Wheat grains were initially surfaced sterilized in 1 % sodium hypochlorite solution for 5 min. The grains were subsequently steeped in potable tap water for 24 h at 18 °C (1:2 water ratio). The drained samples were placed in trays and were put in a germination chamber (*Servicios Mecatrónicos; Córdoba, Argentina*) at different temperature conditions (15, 20 and 25 °C) and a relative humidity of 95 % in dark. Samples for analysis were taken after 18, 24, and 48 h. The degree of sprouting (DoS) of 100 grains and their average was determined visually by classifying the length of the coleoptile and radicles of grains (0-7) according to Krapf *et al.* (*18*). The wheat sprouting conditions were selected based on preliminary tests (*19*). The sprouted grains were dried in a stove with air circulation at 50 °C for 20 h to limit the amount of water for enzyme activity. Finally, sprouted grains (including vegetable parts) were milled in a cyclonic mill (Cyclotec™ 1093; Foss, Hillerød, Denmark) using a 1-mm mesh sieve to obtain sprouted whole-wheat flour (SWF). Unsprouted whole-wheat flour under the same milling and granulometry conditions was used as a control (USWF).

Chemical characterization of flour samples

Moisture levels, protein (N x 5.7), lipid and ash contents of USWF and SWF were assessed by AACC standard methods 44-15.02 (*20*), 46-12.01 (*21*), 30-26.01 (*22*) and 08-01.01 (*23*), respectively. Briefly, moisture content was determined by weighing the sample prior to and after drying for 2 h at 130 °C (Dry oven model 33 600 D060602, Memmert, Germany). Protein content (Kjeldahl method) was determined after their digestion with concentrated H_2SO_4 (Sintorgan, Buenos Aires, Argentina). Digest (Raypa digestor, Barcelona, Spain) was used for sample digestion and Distillation (VELP UDK126A) for the distillation (Scientifica, Milan, Italy). Determination of total lipid was done by Soxhlet extraction with petroleum ether (Sintorgan, Buenos Aires, Argentina). After the extraction, lipid content was determined by weighing. Ash content was determined by weighing the sample prior to and after igniting for 2 h at 600 °C (Indef model 332, Córdoba, Argentina). Total starch content was determined according to the AACC Method 76-13.01 (*24*), with a Total Starch Assay Kit (Megazyme Ltd, Wicklow, Ireland) at 510 nm with UV-Vis Spectrometer (JASCO model V-730, Mary's Court Easton, USA) considering the reducing sugar content of each sample to correct the results. The reducing sugar content was determined using DNS (3,5 dinitrosalicylic acid) method described by Bustos *et al*. (*25)* at 545 nm with UV-Vis Spectrometer. Soluble sugars were extracted and determined by HPLC-RI system (Shimadzu, Japan), equipped with a Luna® Omega 3 µm SUGAR 100 Å column (Phenomenex, USA) following the modified method by Losano Richard *et al*. (*26*). The components were eluted using acetonitrile 75 % (v/v) as mobile phase with a flow rate of 1.2 mL/min. The oven temperature was 35 °C. The total run time was 25 min/sample Quantification was based on a standard curve generated by using commercial standards at five different concentrations. The content of water-

soluble pentosans was quantified following the orcinol-HCl method described in Lancetti *et al*. (*27*) at 670 nm with UV-Vis Spectrometer. All the analyses were conducted at least in duplicate, and the concentrations were based on dry matter (d.m.).

Enzymatic activity of flour samples

α-amylase activity

α-amylase activity of flour samples was measured by the Amylazyme-method (Megazyme, Ireland) (*28*). USWF and SWF samples (0.2 g) were weighed accurately into glass test tubes and incubated at 60 °C with sodium maleate buffer (5.0 mL, 100 mM, pH=6.0, 5 mM $CaCl_2$). After 5 min continuous stirring, an Amylazyme tablet was added. The reaction was allowed to continue for exactly 5 min and was stopped with TRIS solution (6.0 mL, 2 % *m*/*V*, pH=9.0) under vigorous stirring on a vortex mixer. After filtration through a filter paper, the filtrate was measured (590 nm) against a reaction blank, prepared by adding TRIS solution before the Amylazyme tablet. Activities were calculated from the standard curve supplied with the Amylazyme kit (Megazyme, Bray, Ireland) and were expressed in α-amylase units (AU) per gram.

Endoxylanase activity

Endoxylanase activity in flour samples was measured by XylX6 method (Megazyme, Ireland) according to Mangan *et al*. (*29*). To obtain the enzyme extracts, 50 mL of sodium acetate buffer solution (100 mM, pH=4.5) was added to USWF and SWF samples (1 g) in an Erlenmeyer flask and stirred for 15 min at room temperature. The resulting suspensions were clarified by centrifugation (1000×*g*, 10 min) and then diluted until a suitable concentration of endo-xylanase for the assay is achieved. Aliquots of the diluted extract solution were analysed for xylanase activity as below. Aliquots (0.05 mL) of the extract solutions were incubated with XylX6 reagent (0.05 mL) at 40 °C and reactions were terminated after 10 min by the addition of Tris buffer (1.5 mL, 2 % *m*/*V*, pH=10.0). Absorbance of the reaction solutions and the reagent blank was measured at 400 nm. Activities are expressed in xylanase units (XU) per gram.

Impact of controlled sprouting on proteins

The number of peptide bonds cleaved by endo-protease activity was evaluated indirectly as a function of the amount of free amino groups by the OPA (*o*-phthalaldehyde) method according to Perri *et al*. (*30*) at 340 nm in the spectrophotometer using serine as a calibration standard. The results were expressed as μmol serine/mg protein. Changes in protein extractability under reducing and non-reducing conditions were measured with size exclusion high-performance liquid chromatography (SE-

HPLC) using a LC-2010 system (Shimadzu, Kyoto, Japan) equipped with Biosep-SEC-S4000 column (pore size 500 Å, Phenomenex, Torrance, CA, USA), following the method described by Nivelle *et al.* (*31*). The protein extractability (PE) in an SDS-containing medium under non-reducing conditions was calculated from the area under the SE-HPLC chromatogram of a sample. PE was later expressed as a percentage of the total area obtained when extracting the samples under reducing conditions. In addition, SE-HPLC profiles were divided into four fractions according to the protein classification proposed by Ohm *et al*. (*32*): F1 (5.0-7.1 min), F2 (7.1–9.2 min), F3 (9.2–10.6 min), and F4 (10.6-14.0 min). Absorbance area percent of each eluted protein fraction were calculated based on the total absorbance area.

Pasting properties of flour suspension

A Rapid Visco Analyzer instrument (RVA series 4500, Perten Instruments, USA) was used to prepare the flour suspensions and follow the apparent viscosity profile of the samples as a function of temperature and time. To carry out the assay, 5 g of flour (14 % moisture basis) were suspended in 25 mL of deionized water and placed into the aluminium canisters. RVA Standard 1 Method (*33*) was applied. The parameters recorded were peak (PV), peak time (T), final (FV), breakdown (BV), and setback viscosity (SV).

Impact of controlled sprouting on bioactive compounds and phytic acid

As reported in Bustos *et al*. (*34*), extractions for total polyphenols of USWF and SWF were made by mixing 1g of flour sample with 5 mL of acetone: water (70:30) with 0.1 % HCl. Free phenolic acids were determined colorimetrically by the Folin-Ciocalteau method using gallic acid as a calibration standard (*35*); the results were expressed as mg gallic acid per 100 grams. The reducing activity was determined by the Ferric ion Reducing Antioxidant Power (FRAP) assay, following Podio *et al*. (*36*), using Trolox as standard. The results were expressed as mg of Trolox equivalent per 100 grams. $ABTS^{\circ+}$ radical cation scavenging activity was measured according to Podio *et al.* (*36*) using Trolox as standard, and the results were expressed as mg of Trolox equivalent per 100 grams. Determination of phytic acid phosphorus content was conducted according to Haug and Lantzsch's Photometric method, as modified by Raboy *et al.* (*37*). Phytic acid dodecasodium salt was used as calibration, and the results were expressed as mg phytic acid phosphorus per gram.

Statistical analysis

The statistical analysis was performed using InfoStat software v. 1.0 (*38*). The results were evaluated by ANOVA and compared by Di Rienzo, Guzmán and Casanoves (DGC) multiple-

comparison test at a significance level of 0.05 to identify significant effects of sprouting temperature and time on response variables.

RESULTS AND DISCUSSION

Effect of sprouting time and temperature on degree of sprouting

The number fractions of the grains with different degrees of sprouting are shown in Fig. 1. The average degree of sprouting is represented with diamonds. After 18 h, differences in sprouting progress among various temperatures were visible, but only 50 % of grains were sprouted at 15 and 20 °C. Sprouting conditions at 25 °C for 18 h allowed us to obtain the same average DoS (2-3) as that reached at 15 and 20 °C after 48 h, which indicates an already developed embryo emerging from the seed coat. In particular, the sprouting process performed at 25 °C was the most prominent because it favoured the increase of the metabolic rate in grains. After 24 and 48 h of incubation at this temperature, about 40 % and 100 % of the grains were found to have a DoS greater than 4, characterised by radicles longer than a full grain. Although sprouting under these conditions was faster, it was less controlled, inducing greater changes in the grain. Moreover, even these grains can no longer be considered whole grains according to the Whole Grains Council (*16*). The metabolic rate of grains sprouted at 15 and 20 °C decrease considerably, although these conditions lead to a more homogeneous sprouting.

Effect of controlled sprouting on starch

During the sprouting process, α-amylases were *de novo* synthesized in wheat grains with consequent degradation of starch into dextrins and short-chains with reducing potential (Table 1). Evaluated sprouting conditions have a substantial effect on α-amylase activity, as found in other studies (*39,40*). In USWF, a very low α-amylase activity was found (0.14 AU/g). This activity significantly ($p<0.05$) raised between 3-30 times during controlled sprouting following a linear trend with DoS, and sprouting temperature was the most influential factor. Sprouting performed at 15 °C for 18 h was not enough to raise α-amylase activity, but after 24 and 48 h of sprouting, this activity increased 3 and 15 times compared to USWF, respectively. In addition, these samples exhibited a slight decrease of about 10 % in starch content and an increase of 40–60 % in the content of reducing sugars. This rising trend with sprouting time was also observed at 20 °C, promoting α-amylase activity up to 15 times more than USWF. Consequently, a reduction of 12–15 % of starch content and an increase of 50–90 % of the reducing sugars were detected. The rate of increase of α-amylase activity at 25 °C practically doubled that obtained at 15 °C. SWF 15 °C/48 h and 20 °C/48 h presented equivalent α-amylase activities. Sprouting at 25 °C for 48 h produced the highest increase of α-

amylase activity (3.92 AU/g), which is 30 times higher than USWF. The degradation of the starch content reached up to 30 %, while the release of reducing sugars was up to 3 times greater than that found in USWF.

The sugar profile of flour samples was influenced by sprouting conditions (Table 2). In general, glucose and sucrose levels were notably higher in all SWF samples compared to USWF and followed similar patterns. Sucrose content was positively associated with higher DoS and α-amylase activity. Regardless of temperature, longer sprouting times (48 h) favoured the increase in maltose content, while fructose content only increased 4 times when sprouting was performed at 25 °C for 48 h. Relative abundance of each sugar remained constant among samples. Sucrose was the dominant sugar, representing about 60 % of total sugar content. Benincasa *et al*. (*8*) also reported different effects in response to sprouting time, with sucrose as the dominant source of carbohydrate during the early phase of wheat sprouting (24–48 h). This is because sucrose plays a crucial role in carbohydrate transport during early wheat sprouting (*40*). Different sprouting conditions led to varied sugar profiles in SWF, which may impact yeast fermentation in doughs and reduce fermentation times. Additionally, a more varied sugar profile could add natural sweetness to products when sprouted wheat flour is used, which could help manufacturers reduce levels of added sugar in wheat-based products. In the case of whole wheat bread, bitterness usually lingers at the end of mastication and can be considered a negative attribute (*12*). Therefore, a higher content of reducing sugars in SWF may limit or mask bitterness perception.

The hydrolysis of starch resulting from α-amylase activity also had an impact on the pasting properties of flour suspensions (Fig. 2). Concomitantly with the increase of α-amylase activity, significant reductions in viscosity were observed during both heating (peak viscosity) and cooling (final viscosity) steps, which produced a substantial decrease in the swelling, gelatinization, and gelation capacity of SWF. Already after 18 h of sprouting at 15 °C, a reduction of almost 25 % in peak viscosity of SWF suspension was evidenced, while the decline in viscosity reached up to 80-90 % in suspensions of SWF obtained after 48 h of sprouting. A temperature of 25 °C had the greatest impact on the structure of starch during sprouting. Similar trends of viscosity reduction were found in Grassi *et al.* (*41*). This loss of viscosity could be mainly attributed to the lower proportion of starch content, as α-amylases break the α-1,4 glycosidic bonds among glucose molecules throughout the chains during sprouting, and then degrade starch granules during the pasting step (*42*). The peak time for all SWF suspensions was observed to decrease by about 50 % with the increase of α-amylase activity. Trough, breakdown, final viscosity, and setback values of all SWF suspensions showed similar changes as the sprouting process progressed. In general, at increasing DoS of grains, the changes in the characteristic shape of the RVA curves of SWF suspensions became more evident and rather

linear in the final stage of the test. According to Silva Oliveira *et al.* (*42*) the increase in hydrolysed amylose and amylopectin molecules resulted in less interactions between starch chains. Hence, high temperatures and long incubation times caused changes in the starch structure, which could negatively affect the functionality of flours. Conversely, SWF obtained under milder sprouting conditions with moderate α-amylase activity could favour the development of loaf volume before the settling of the crumb structure during breadmaking when these flours are incorporated into the bread formulation. Moreover, the production of maltodextrins through α-amylase action could promote an anti-staling effect on wheat bread crumb by diminishing amylopectin retrogradation when it is enriched with SWF (*12*). Consequently, the enrichment with these SWFs could potentially enhance the bread-making performance of certain flours in place of conventional improvers, *i.e.* enzymatic improver, malt or maltogenic α-amylase.

Effect of controlled sprouting on non-starchy polysaccharides

Besides starch, non-starch polysaccharides of bran also play an important role structurally and functionally in cereal grains. From the results in Table 1, it was evident that the chosen sprouting conditions significantly ($p<0.05$) favoured the increase of endoxylanase activity and followed a linear trend with DoS. Cell wall degradation is a critical step in sprouting, as these cell walls form a physical barrier for α-amylases and proteases to access and degrade their intracellular starch and protein substrates. However, endoxylanase activity increases slowly during the first 48 h compared to the incremental rate of α-amylase activity because these enzymes are often produced late in the sprouting process (*41*). The endoxylanase activity of USWF was low (0.29 XU/g). As reported by Benincasa *et al*. (*8*), controlled sprouting conditions increased the endoxylanase activity between 1.3 and 3 times compared to USWF, except for the 15 °C/18 h sprouting condition (Table 1). Consequently, insoluble pentosans were hydrolysed, resulting in a two-to three-fold increase in water-soluble pentosans content (Table 1). This finding is highly significant in whole wheat-based products because it does not interfere with the gluten network. The degradation of the xylan backbones affects the molecular mass, solubility, and physicochemical properties of pentosans (*11*). Comparable results in sprouted cereals were obtained by Cardone *et al.* (*13*). Thus, SWF with enough xylanase activity can be used as a replacement of commercial microbial xylanases, which are frequently added to improve processing parameters and wheat-based products quality such as crumb elasticity and softness. In addition, these SWF could also enhance the nutritional profile of products due to the fact that non-starch polysaccharides belong to the dietary fibre fraction (*43*).

Effect of controlled sprouting on proteins

A slight increase (5–10 %) in protein content compared to USWF was observed after sprouting at 20 and 25 °C. Similarly, after 24 h of sprouting, regardless of temperature, SWF samples showed a slight increase in ash content (10–15 %) and lipid content (5–15 %) compared to USWF, respectively. However, as observed by Olaerts (*44*), these increases were due to the loss of matter through respiration and starch hydrolysis (Table S1).

Apart from the changes in starch and non-starch molecules due to sprouting, the protein matrix was also affected by endo-proteolytic enzymes. Proteases break down high molecular mass proteins into smaller subfractions and free amino acid groups, which are crucial for cellular metabolism (*45*). However, the degradation of protein in wheat flour plays an important role in the deterioration of breadmaking quality, even more than starch (*12*), because sprouting impacts flour functionality by decreasing dough elasticity and strength (*14*). During sprouting, the amount of free amino groups increased 2–3 times in relation to USWF. A high level of proteolysis was evidenced after longer sprouting times (48 h) at 20 °C. These results align with previous studies by Koehler (*46*) and Žilić (*39*). Koehler (*46*) reported that gliadins were strongly degraded at 20 °C, while glutenins were affected at 25 °C. Moreover, Naumenko *et al*. (*47*) showed that considerable gluten decomposition required sprouting times longer than 100 h.

SE-HPLC chromatograms exhibited qualitative changes in the molecular mass distribution of the proteins extracted in buffer containing SDS under reducing and non-reducing conditions (Fig. 3a and Fig. 3b). A slightly increase (15–30 %) in PE with respect to USWF extracts were detected (Fig. S1) when the sprouting process was performed at 20 °C/48 h and 25 °C, irrespective of time. This could indicate incipient changes in protein structure by proteolytic activity. Additionally, a small shift from higher molecular mass proteins to lower molecular mass proteins was observed for both reducing and non-reducing conditions (after all bonds were broken) as the sprouting process progressed (Fig. 3a and Fig. 3b). Simsek *et al*. (*48*) observed similar results after wheat sprouting for 48–72 h. After 48 h of sprouting at 20 °C and 18 h at 25 °C, the absorbance area of high-molecular-mass (HMM) proteins, the major components of F1, exhibited a substantial reduction of about 30 %. However, when converted to absorbance area percentages (Table S2), the F1 area fraction of extracted proteins under reducing conditions represented a low proportion of the total area; this was probably due to the limited discriminating power of the analysis concerning the fractions eluting first (*44*). As observed by Ohm *et a*l. (*32*), the reduction of the F1 area may indicate glutenin degradation. This is critical, as high molecular mass proteins are essential for gluten strength, especially in whole-wheat products, where the gluten matrix is diluted by bran and germ presence (*49*). However, Cardone *et al*. (*13*) reported that the decrease in gluten aggregation properties in sprouted whole grain flour did not adversely impact breadmaking performance. This was attributed to unique protein aggregations found

in their study. No changes in gliadins (F2) absorbance area percentages of SWF extracts were evidenced, suggesting no degradation. As a result of the degradation of the F1 fraction, an absorbance area percentage (F3), corresponding to lower molecular mass protein fragment or albumins, increased slightly during sprouting. Conversely, the absorbance area percentage corresponding to globulins (F4) did not vary among SWF extracts. Sprouting conditions at 15 °C for 24 h and 20 °C for 18–24 h favoured a moderate increase of α-amylase and xylanase. This maintained essential polymeric proteins of the gluten network, suggesting good breadmaking potential. Additionally, this controlled depolymerization can favour dough extensibility from flours with a high ratio of tenacity to extensibility, such as those usually obtained from Argentine wheat, thereby reducing mixing time and improving loaf volumes (*50*). In addition, it could benefit the development of desirable products of the Maillard reaction (*1*) and enhance protein digestibility (*6*).

Effect of controlled sprouting on free polyphenol content and antioxidant capacity

Results showed that free phenolic content significantly increased (20–50 %) after controlled sprouting compared to USWF, except for 15 °C/18 h sprouting condition (Table 3). Sprouting at 20 and 25 °C after only 18 h was enough to favour an increase of FPC in SWF extracts. In general, FPC was more affected by sprouting time than by temperature. Antioxidant activity of SWF extracts was also positively affected by sprouting. Pitzschke *et al*. (*49*) suggested that antioxidants may play a crucial role in seed dormancy breakage and sprouting to scavenge the reactive oxygen species and serve as protectors against environmental changes during growth. Sprouting conditions affected the antioxidant capacity values obtained using both the FRAP and ABTS methods, with more evident differences in $ABTS^{\circ+}$ values. The highest FRAP values were found when sprouting was performed at 25 °C for 48 h, which was approximately 45 % higher than that observed in USWF extract. Also, by the ABTS method, the highest antioxidant capacity values were observed in SWF extracts obtained at 20 and 25 °C after 48 h of sprouting, which was approximately 4 times higher than that noted in the USWF extract. Increase of both FPC and the antioxidant capacity could be related to the release of bound phenolics attached to lignin or arabinoxylans from cell walls resulting from endoxylanases activation. It could also be due to the *de novo* biosynthesis of polyphenols - in order to maintain homeostasis- and to the increase in the content of free -SH groups in wheat grains during sprouting (*51*). Thus, SWF characterized by moderate enzyme activity also presented an enhancement of their nutritional value.

Effect of controlled sprouting on phytate content

Another nutritional benefit promoted by controlled sprouting includes the activation of phytases, which are responsible for the release of the stored inorganic phosphorus, myo-inositol, and chelated divalent cations for seedling growth (*10*). The sprouting temperature was a critical factor in reducing phytic acid (Table 3). Sprouting at 20 and 25 °C favoured the reduction of 15–20 % and 30–40 % of this antinutrient, respectively. The results were in line with those previously found by Olaerts and Courtin (*9*) in sprouted wheat. In this way, controlled sprouting process could result in a greater availability of minerals of wholegrain baked products.

CONCLUSIONS

Sprouting conditions used in this study affected the physicochemical composition and nutritional profile of the resulting sprouted whole-wheat flour as a consequence of hydrolytic enzymes activation. In general, sprouting temperature of 25 °C resulted in an exacerbated increase of the enzymatic activity and metabolic processes. These conditions severely affected the associated starch matrix and pasting properties, and to a lesser extent, the structure of cell walls and the protein matrix. Sprouting performed at 15 and 20 °C for 24 h allowed for a better control of the process, since it promoted a nutritional improvement and provided techno-functional advantages due to the moderate activation of α-amylase and xylanase making them better food material than the unsprouted wheat grains. Additionally, gluten proteins were mostly not modified. This is crucial for good quality in whole-wheat baked products as a consequence of gluten dilution by the presence of bran and germ. In conclusion, sprouted whole-wheat flours obtained under milder sprouting conditions with moderate enzymatic activity may represent promising and interest ingredients for formulating wholegrain baked goods with improved nutritional profiles, avoiding the use of conventional flour improvers with a positive impact on consumers' acceptance and facilitating the adoption of clean label.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHORS' CONTRIBUTION

Navarro José Luis, Losano Richard Pedro and Steffolani María Eugenia wrote the manuscript with critical input and corrections by León Alberto Edel. Moiraghi Malena and Mariela Bustos did the final editing. All authors contributed to locating and to interpreting the literature sources.

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REFERENCES

1. Lemmens E, Moroni AV, Pagand J, Heirbaut P, Ritala A, Karlen Y, *et al*. Impact of cereal seed sprouting on its nutritional and technological properties: A critical review. Compr Rev Food Sci F. 2019;18(1):305–28. https://doi.org/10.1111/1541-4337.12414
2. Navarro JL, Moiraghi M, Quiroga FM, León AE, Steffolani ME. Effect of wholewheat flour particle shape obtained by different milling processes on physicochemical characteristics and quality of bread. Food Technol Biotechnol. 2020;58(3):325336. https://doi.org/10.17113/ftb.58.03.20.6766
3. Navarro JL, Biglione C, Paesani C, Moiraghi M, León AE, Steffolani ME. Effect of wheat pearling process on composition and nutritional profile of flour and its bread-making performance. Int J Food Sci Technol. 2022;57:249–57. https://doi.org/10.1111/ijfs.15401
4. Marti A, Bottega G, Casiraghi MC, Faoro F, Lametti S, Pagani MA. Dietary fibre enzymatic treatment: A way to improve the rheological properties of high-fibre-enriched dough. Int J Food Sci Technol. 2014;49:305–7. https://doi.org/10.1111/ijfs.12293
5. Perri G, Miisci A, Montemurro M, Pontonio E, Verni M, Rizzello CG. Exploitation of sprouted barley grains and flour through sourdough fermentation. LWT. 2023;187:15326.

https://doi.org/10.1016/j.lwt.2023.115326

6. Singh AK, Rehal J, Kaur A, Jyot G. Enhancement of attributes of cereals by germination and fermentation: A review. Crit Rev Food Sci. 2015;55:1575–89. https://doi.org/10.1080/10408398.2012.706661
7. Guzmán-Ortiz FA, Castro-Rosas J, Gómez-Aldapa CA, Mora-Escobedo R, Rojas-León A, Rodríguez-Marín ML, *et al*. Enzyme activity during germination of different cereals: A review. Food Rev Int. 2018;35:177–200. https://doi.org/10.1080/87559129.2018.1514623
8. Benincasa P, Falcinelli B, Lutts S, Stagnari F, Galieni A. Sprouted grains: A comprehensive review. Nutrients. 2019;11(2):421. https://doi.org/10.3390/nu11020421
9. Olaerts H, Courtin CM. Impact of preharvest sprouting on endogenous hydrolases and technological quality of wheat and bread: A review. Compr Rev Food Sci F. 2018;17(3):698–713. https://doi.org/10.1111/1541-4337.12347
10. Albarracín M, Dyner L, Giacomino MS, Weisstaub A, Zuleta A, Drago SR. Modification of nutritional properties of whole rice flours (*Oryza sativa L*.) by soaking, germination, and extrusion. J Food Biochem. 2019;43(7):e12854. https://doi.org/10.1111/jfbc.12854
11. Marti A, Cardone G, Nicolodi A, Quaglia L, Pagani MA. Sprouted wheat as an alternative to conventional flour improvers in bread-making. LWT. 2017;80:230–236. https://doi.org/doi:10.1016/j.lwt.2017.02.028
12. Marti A, Cardone G, Pagani MA, Casiraghi MC. Flour from sprouted wheat as a new ingredient in bread-making. LWT Food Sci. Technol. 2018;89:237–243. https://doi.org/10.1016/j.lwt.2017.10.052
13. Cardone G, D'Incecco P, Casiraghi MC, Marti A. Exploiting milling by-products in bread-making: The case of sprouted wheat. Foods. 2020;9(3):260. https://doi.org/10.3390/foods9030260.
14. Liu T, Hou GG, Cardin M, Marquart L, Dubat A. Quality attributes of whole-wheat flour tortillas with sprouted whole-wheat flour substitution. LWT. 2017;77:1–7. https://doi.org/10.1016/j.lwt.2016.11.01
15. Johnston R, Martin JM, Vetch JM; Byker-Shanks C, Finnie S, Giroux MJ. Controlled sprouting in wheat increases quality and consumer acceptability of whole wheat bread. Cereal Chem. 2019;96:866–77.

https://doi.org/10.1002/cche.10187

16. Whole Grains Council, 2008. Definition of Sprouted Grains. Available from: https://wholegrainscouncil.org/wholegrains-101/whats-wholegrain-refined-grain/sprouted-wholegrains/definitions-sprouted-grains
17. Winter Cereal Committee of the Nationals Seed Commission - CONASE, Argentina (2020). Available from: https://www.argentina.gob.ar/inase/red-variedades-de-trigo
18. Krapf J, Arysanto A, Walther G, Flöter E. Effect of sprouting conditions on the properties of direct expanded extruded wheat. J Food Process Eng. 2019:E13123. https://doi.org/10.1111/jfpe.13123
19. Navarro JL, Rodriguez MD, Moiraghi M, León AE, Steffolani ME. Effects of controlled sprouting on nutritional and viscosity profile of whole wheat flour, in Proceedings of the 2nd International Electronic Conference on Foods - "Future Foods and Food Technologies for a Sustainable World", 15–30 October 2021, MDPI: Basel, Switzerland. https://doi.org/10.3390/Foods2021-11063
20. AACC Method 44-15.02. Moisture - Air-oven methods. St. Paul, MN, USA: American Association of Cereal Chemists (AACC) International; 2010. https://doi.org/10.1094/AACCIntMethod-44-15.02
21. AACC Method 46-12.01. Crude protein – Kjeldahl method, boric acid modification. St. Paul, MN, USA: American Association of Cereal Chemists (AACC) International; 2010. https://doi.org/10.1094/AACCIntMethod-46-12.01
22. AACC Method 30-25.01. Crude fat in wheat, corn, and soy flour, feeds, and mixed feeds. St. Paul, MN, USA: American Association of Cereal Chemists (AACC) International; 2010. https://doi.org/10.1094/AACCIntMethod-30-25.01
23. AACC Method 08-01.01. Ash - Basic method. St. Paul, MN, USA: American Association of Cereal Chemists (AACC) International; 2010. https://doi.org/10.1094/AACCIntMethod-08-01.01
24. AACC Method 76-13.01. Total Starch Assay Procedure (Megazyme Amyloglucosidase/alpha-Amylase Method). St. Paul, MN, USA: American Association of Cereal Chemists (AACC) International; 2010. https://doi.org/10.1094/AACCIntMethod-76-13.01
25. Bustos MC, Vignola MB, Pérez GT, León AE. *In vitro* digestion kinetics and bioaccessibility of starch in cereal food products. J Cereal Sci. 2017;77: 243–50. https://doi.org/10.1016/j.jcs.2017.08.018

26. Losano Richard P, Steffolani ME, Allende MJ, Carreras J, León AE. By-products of the classification of chickpea as an alternative in the production of hummus. Int J Food Sci Technol. 2020;56(4):1759–65. https://doi.org/10.1111/ijfs.14801

27. Lancetti R, Salvucci E, Moiraghi M, Pérez GT, Sciarini LS. Gluten-free flour fermented with autochthonous starters for sourdough production: Effect of the fermentation process. Food Biosci. 2022;47:101723. https://doi.org/10.1016/j.fbio.2022.101723

28. AACC Method 22-05.01. Alpha amylase – Cereal and microbial α-amylases. St. Paul, MN, USA: American Association of Cereal Chemists (AACC) International; 2010.

29. Mangan D, Cornaggia C; Liadova A, McCormack N, Ivory R; McKie VA, Ormerod, A; McCleary BV. Novel substrates for the automated and manual assay of endo-1,4-β-xylanase. Carbohydr Res. 2017;445:14–22. https://doi.org/10.1016/j.carres.2017.02.009

30. Perri G, Coda R, Rizzello CG, Celano G, Ampollini M, Gobbetti M, *et al*. Sourdough fermentation of whole and sprouted lentil flours: In situ formation of dextran and effects on the nutritional, texture and sensory characteristics of white bread. Food Chem. 2021;355:129638. https://doi.org/10.1016/j.foodchem.2021.129638

31. Nivelle MA, Bosmans GM, Delcour JA. The impact of parbaking on the crumb firming mechanism of fully baked tin wheat bread. J Agric Food Chem. 2017;65(46):10074–83. https://doi.org/10.1021/acs.jafc.7b03053

32. Ohm J, Lee CW, Cho K. Germinated wheat: Phytochemical composition and mixing characteristics. Cereal Chem. 2016;93:612–7. https://doi.org/10.1094/CCHEM-01-16-0006-R

33. Palavecino PM, Penci MC, Calderón-Domínguez G, Ribotta PD. Chemical composition and physical properties of sorghum flour prepared from different sorghum hybrids grown in Argentina. Starch – Stärke. 2016;68(11-12):1055–64. https://doi.org/10.1002/star.201600111

34. Bustos MC, Rocha-Parra D, Sampedro I, de Pascual-Teresa S, León AE. The Influence of different air-drying conditions on bioactive compounds and antioxidant activity of berries. J Agric Food Chem. 2018;66:2714–23. https://doi.org/10.1021/acs.jafc.7b05395

35. Bustos M, Vignola M, Paesani C, León A. Berry fruits-enriched pasta: effect of processing and in vitro digestion on phenolics and its antioxidant activity, bioaccessibility and potential bioavailability. Int J Food Sci Technol. 2020;55:2104–12. https://doi.org/10.1111/ijfs.14453

36. Podio NS, Baroni MV, Wunderlin DA. Relation between polyphenol profile and antioxidant capacity of different Argentinean wheat varieties. A Boosted Regression Trees study. Food Chem. 2017;232:79–88. https://doi.org /10.1016/j.foodchem.2017.02.123

37. Raboy V, Johnson A, Bilyeu K, Brinch-Pedersen H, Cichy K, Hurrell RF, *et al*. Evaluation of simple and inexpensive high-throughput methods for phytic acid determination. JAOCS. 2017;94(3):353–62. https://doi.org/10.1007/s11746-016-2946-y

38. InfoStat Statistical Software, v. 2018, Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Córdoba, Argentina; 2018. Available from: https://www.infostat.com.ar.

39. Žilić S, Janković M, Barać M, Pešić M, Konić-Ristić A, Hadži-Tašković Šukalović V. Effects of enzyme activities during steeping and sprouting on the solubility and composition of proteins, their bioactivity and relationship with the bread making quality of wheat flour. Food Funct. 2016;7(10):4323–31. https://doi.org/10.1039/c6fo01095d

40. Zhang Q, Pritchard J, Mieog J, Byrne K, Colgrave ML, Wang J, *et al*. Overexpression of a wheat α-amylase type 2 impact on starch metabolism and abscisic acid sensitivity during grain germination. Plant J. 2021;108:378–93. https://doi.org/10.1111/tpj.15444

41. Grassi S, Cardone G, Bigagnoli D, Marti A. Monitoring the sprouting process of wheat by non-conventional approaches. J Cereal Sci. 2018,83:180–7. https://doi.org/10.1016/j.jcs.2018.08.007

42. Silva Oliveira MAE, Saldanha Coimbra PP, Galdeano MC, Piler Carvalho CW, Takeiti CY. How does germinated rice impact starch structure, products and nutritional evidences? – A review. Trends Food Sci Technol. 2022;122:13–23. https://doi.org/10.1016/j.tifs.2022.02.015

43. Bistriche Giuntini E, Hoffmann Sardá FA, Wenzel de Menezes E. The effects of soluble dietary fibers on glycemic response: An overview and futures perspectives. Food. 2022;11(23):3934. https://doi.org/10.3390/foods11233934

44. Olaerts H, Roye C, Derde LJ, Sinnaeve G, Meza WR, Bodson B, Courtin CM. Evolution and distribution of hydrolytic enzyme activities during preharvest sprouting of wheat (*Triticum aestivum*) in the field. J Agric Food Chem. 2016;64(28):5644–52. https://doi.org/10.1021/acs.jafc.6b01711

45. Dong Y, Karboune S. A review of bread qualities and current strategies for bread bioprotection: Flavor, sensory, rheological, and textural attributes. Compr Rev Food Sci Food Saf. 2021;20(2);1937–81. https://doi.org/10.1111/1541-4337.12717

46. Koehler P, Hartmann G, Wieser H, Rychlik M. Changes of folates, dietary fiber, and proteins in wheat as affected by germination. J Agric Food Chem. 2007;55:4678–83. https://doi.org/10.1021/jf0633037

47. Naumenko N, Potoroko I, Kalinina I, Fatkullin R, Ivanisova E. The influence of the use of whole grain flour from sprouted wheat grain on the rheological and microstructural properties of dough and bread. Int J Food Sci. 2021;7548759. https://doi.org/10.1155/2021/7548759

48. Simsek S, Ohm JB, Lu H, Rugg M, Berzonsky W, Alamri MS, *et al*. Effect of pre-harvest sprouting on physicochemical changes of proteins in wheat. J Sci Food Agric. 2014;94(2):205–12. https://doi.org/10.1002/jsfa.6229

49. Pitzschke A, Fraundorfer A, Guggemos M, & Fuchs N. Antioxidative responses during germination in quinoa grown in vitamin B-rich medium. Food Sci Nutr. 2015;3(3):242–51. https://doi.org/10.1002/fsn3.211

50. Gómez A, Ferrero C, Calvelo A, Añón MC, Puppo MC. Effect of mixing time on structural and rheological properties of wheat flour dough for breadmaking. Int J Food Prop. 2011;14(3):583–98. https://doi.org/10.1080/10942910903295939

51. Mencin M, Abramovič H, Jamnik P, Mikulič Petkovšek M, Veberič R, Terpinc P. Abiotic stress combinations improve the phenolics profiles and activities of extractable and bound antioxidants from germinated spelt (*Triticum spelta L.*) seeds. Food Chem. 2021;344:128704 https://doi.org/10.1016/j.foodchem.2020.128704

Table 1. Proximal composition and enzymatic activity of USWF and SWF obtained at different sprouting conditions

Samples	Sprouting conditions	Ash/%	Proteins/%	Lipids/%	Starch/%	Reducing sugars/%	Water-soluble pentosans/%	α-amilase activity/(AU/g)	Xylanase activity/ (XU/g)	Free amin. groups/ (μmol serine/mg protein)
USWF		(1.59±0.03)^{a}	(12.32±0.03)^{a}	(2.22±0.05)^{a}	(67.18±0.40)^{e}	(2.04±0.09)^{a}	(0.61±0.02)^{a}	(0.14±0.01)^{a}	(0.28±0.01)^{a}	(0.19±0.00)^{a}
SWF	15°C/18h	(1.68±0.09)^{a}	(12.36±0.05)^{a}	(2.23±0.11)^{a}	(63.32±2.10)^{d}	(2.09±0.18)^{a}	(0.81±0.04)^{a}	(0.19±0.01)^{a}	(0.29±0.02)^{a}	(0.28±0.01)^{b}
	15°C/24h	(1.82±0.03)^{b}	(12.21±0.38)^{a}	(2.30±0.06)^{b}	(60.78±1.74)^{c}	(2.85±0.23)^{b}	(1.08±0.12)^{b}	(0.40±0.03)^{b}	(0.52±0.02)^{c}	(0.30±0.00)^{c}
	15°C/48h	(1.80±0.03)^{b}	(12.48±0.10)^{a}	(2.39±0.05)^{b}	(59.96±1.75)^{c}	(3.24±0.29)^{b}	(1.26±0.01)^{c}	(2.15±0.01)^{e}	(0.76±0.06)^{f}	(0.45±0.00)^{f}
	20°C/18h	(1.78±0.04)^{b}	(12.66±0.08)^{b}	(2.14±0.06)^{a}	(58.81±1.11)^{c}	(2.99±0.21)^{b}	(1.12±0.00)^{b}	(0.65±0.09)^{c}	(0.44±0.03)^{b}	(0.31±0.00)^{c}
	20°C/24h	(1.78±0.01)^{b}	(12.88±0.19)^{b}	(2.32±0.05)^{b}	(55.94±0.95)^{b}	(3.04±0.07)^{b}	(1.29±0.12)^{c}	(1.26±0.05)^{d}	(0.59±0.02)^{d}	(0.35±0.01)^{d}
	20°C/48h	(1.75±0.07)^{b}	(12.78±0.09)^{b}	(2.45±0.05)^{b}	(57.76±1.52)^{c}	(3.92±0.12)^{c}	(1.40±0.00)^{c}	(2.05±0.11)^{e}	(0.84±0.03)^{g}	(0.48±0.01)^{g}
	25°C/18h	(1.69±0.01)^{a}	(12.80±0.13)^{b}	(2.15±0.09)^{a}	(54.52±1.78)^{b}	(3.77±0.04)^{c}	(1.11±0.00)^{b}	(2.82±0.00)^{f}	(0.37±0.03)^{b}	(0.41±0.00)^{e}
	25°C/24h	(1.78±0.01)^{b}	(13.14±0.07)^{c}	(2.35±0.05)^{b}	(55.34±0.64)^{b}	(4.19±0.30)^{c}	(1.55±0.08)^{d}	(2.92±0.09)^{f}	(0.68±0.03)^{e}	(0.44±0.01)^{f}
	25°C/48h	(1.79±0.00)^{b}	(13.22±0.04)^{c}	(2.57±0.03)^{c}	(48.08±1.17)^{a}	(5.38±0.06)^{d}	(81.67±0.16)^{d}	(3.92±0.03)^{g}	(0.82±0.00)^{g}	(0.40±0.00)^{e}
MS (T)		0.001	1.48*	0.01	228.35*	9.19*	0.47*	18.22*	0.10*	0.02*
MS (t)		0.02	0.14	0.27*	39.56*	4.70*	0.57*	7.39*	0.17*	0.04*

USWF=unsprouted wheat flour, SWF=sprouted wheat flour, MS=mean of squares, T=temperature, t=time. Different letters within a column indicate significant differences according to the DGC test ($p \leq 0.05$). Significance indicator: * $p<0.05$

Table 2. Sugar composition by HPLC-IR

Samples	Sprouting conditions	Sugar profile			
		Fructose/%	Glucose/%	Sucrose/%	Maltose/%
USWF		(0.03±0.00)^{a}	(0.01±0.00)^{a}	(0.46±0.00)^{a}	(0.19±0.00)^{a}
SWF	15°C/18 h	(0.07±0.02)^{a}	(0.17±0.04)^{b}	(0.73±0.05)^{b}	(0.16±0.03)^{a}
	15°C/24 h	(0.05±0.01)^{a}	(0.17±0.09)^{b}	(0.70±0.02)^{b}	(0.13±0.00)^{a}
	15°C/48 h	(0.05±0.01)^{a}	(0.29±0.03)^{b}	(0.99±0.03)^{c}	(0.30±0.01)^{b}
	20°C/18 h	(0.05±0.00)^{a}	(0.17±0.05)^{b}	(0.67±0.07)^{b}	(0.18±0.04)^{a}
	20°C/24 h	(0.06±0.02)^{a}	(0.21±0.06)^{b}	(0.75±0.03)^{b}	(0.21±0.05)^{a}
	20°C/48 h	(0.12±0.05)^{a}	(0.18±0.03)^{b}	(1.33±0.04)^{d}	(0.34±0.03)^{b}
	25°C/18 h	(0.09±0.01)^{a}	(0.15±0.06)^{b}	(0.96±0.02)^{c}	(0.23±0.01)^{a}
	25°C/24 h	(0.10±0.01)^{a}	(0.24±0.00)^{b}	(1.33±0.01)^{d}	(0.32±0.01)^{b}
	25°C/48 h	(0.17±0.10)^{b}	(0.39±0.22)^{c}	(1.56±0.23)^{e}	(0.41±0.07)^{c}
MS (T)		0.01	0.02	0.77*	0.04*
MS (t)		0.01	0.05	0.83*	0.09*

USWF=unsprouted wheat flour, SWF=sprouted wheat flour, MS=mean of squares, T=temperature, t=time. Different letters within a column indicate significant differences according to the DGC test ($p \leq 0.05$). Significance indicator: * $p<0.05$

Table 3. Free phenolic content, antioxidant capacity and phytic acid content of USWF and SWF

Sample	Sprouting conditions	FPC as GA/ (mg/100g)	FRAP as TE/ (mg/100g)	ABTS as TE/ (mg/100g)	PA as PAP/ (mg/g)
USWF		(64.70±4.31)^{a}	(1.12±0.01)^{a}	(1.17±0.24)^{a}	(17.27±0.94)^{c}
SWF	15°C/18h	(69.95±0.01)^{a}	(1.15±0.03)^{a}	(1.46±0.08)^{a}	(16.17±0.77)^{c}
	15°C/24h	(89.08±2.24)^{c}	(1.16±0.03)^{a}	(2.62±0.24)^{b}	(15.98±1.43)^{c}
	15°C/48h	(94.13±2.39)^{c}	(1.37±0.04)^{b}	(3.50±0.04)^{b}	(15.68±0.32)^{c}
	20°C/18h	(75.84±5.75)^{b}	(1.28±0.11)^{b}	(1.37±0.07)^{a}	(14.52±0.63)^{b}
	20°C/24h	(86.02±8.04)^{c}	(1.32±0.11)^{b}	(3.05±0.20)^{b}	(13.70±0.91)^{b}
	20°C/48h	(91.19±2.61)^{c}	(1.47±0.03)^{b}	(4.52±0.54)^{c}	(14.37±0.07)^{b}
	25°C/18h	(80.60±1.66)^{c}	(1.35±0.01)^{b}	(3.10±0.07)^{b}	(12.21±0.65)^{a}
	25°C/24h	(96.68±4.29)^{c}	(1.46±0.04)^{b}	(3.30±0.10)^{b}	(11.25±0.59)^{a}
	25°C/48h	(90.36±1.83)^{c}	(1.63±0.11)^{c}	(4.62±0.39)^{c}	(10.50±0.13)^{a}
MS (T)		31,51	1791,12*	0,70*	65.25*
MS (t)		278,00*	1652,43*	2,63*	2.11

USWF=unsprouted wholewheat flour, SWF=sprouted wholewheat flour, MS=mean of squares, T=temperature, t=time, FPC=free polyphenol content, GA=gallic acid, FRAP=ferric reducing antioxidant power, ABTS°⁺=2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), TE=Trolox equivalents, PA=phytic acid, PAP=phytic acid phosphorus. Different letters within a column indicate significant differences according to the DGC test ($p \leq 0.05$). Significance indicator: * $p<0.05$

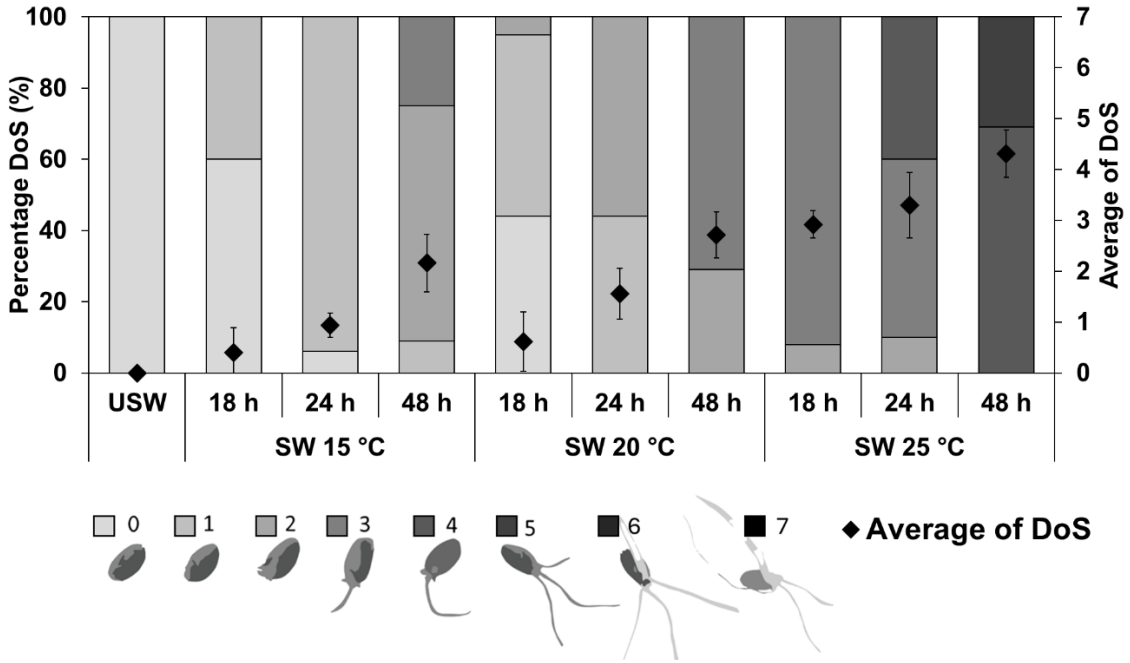

Fig. 1. Effect of controlled conditions on the degree of sprouting of wheat grains and their average degree of sprouting, indicating the visible length of the coleoptile and radicles. USW=unsprouted wheat, SW=sprouted wheat. The average degree of sprouting is represented with diamonds

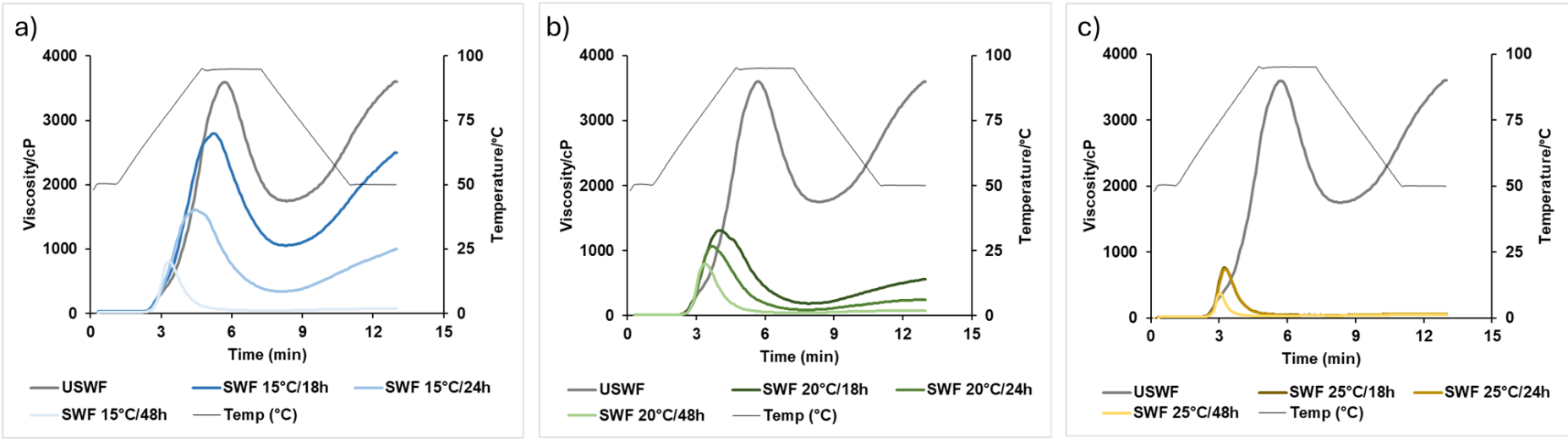

Fig. 2. Pasting properties of unsprouted flour (USWF) and sprouted wholewheat flour (SWF) suspensions obtained at different temperatures: a) 15 °C, b) 20 °C, c) 25 °C, for 18, 24 and 48 h

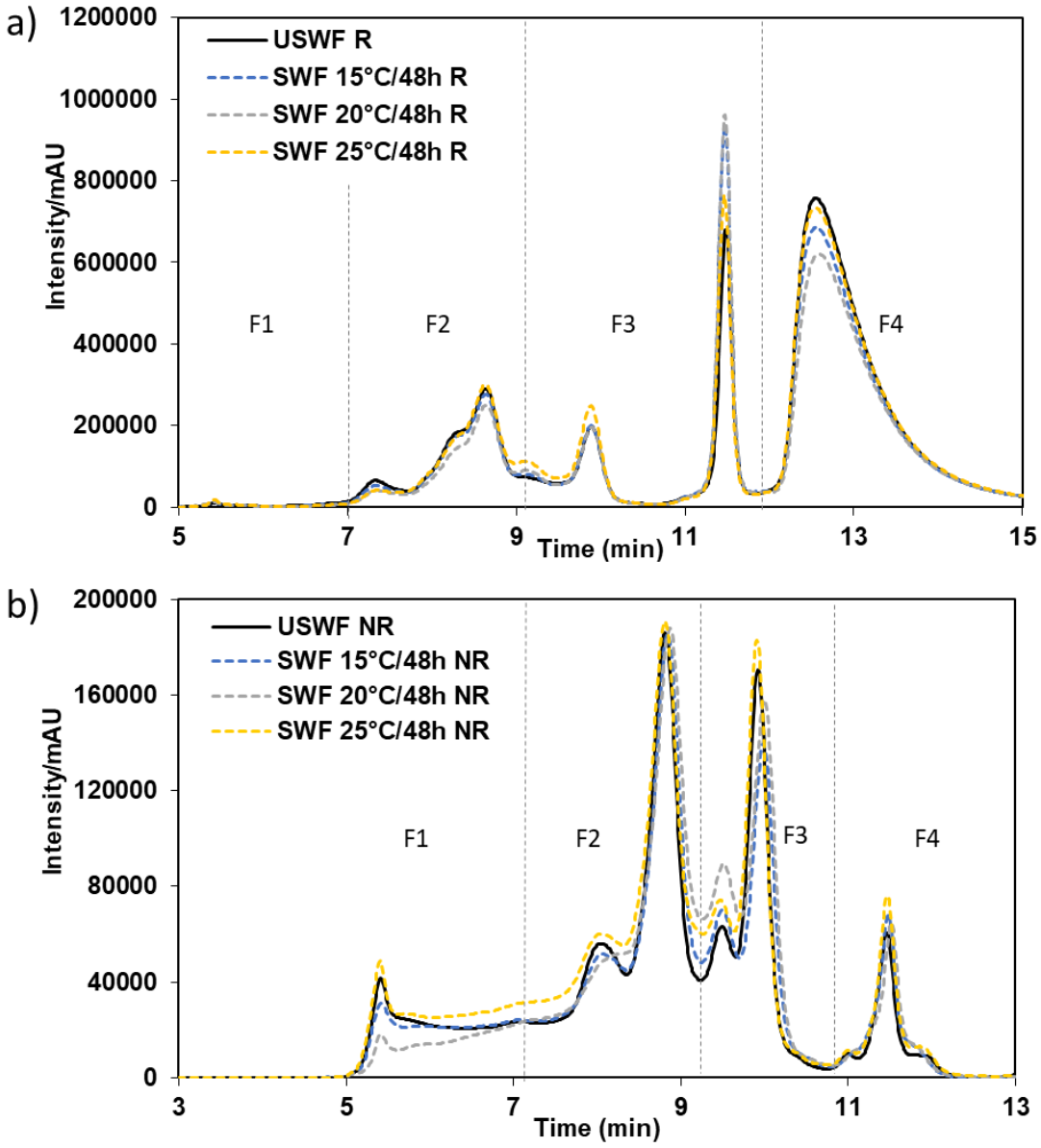

Fig. 3. SE-HPLC chromatograms of flour proteins extracted from USWF (full black lines), and SWF (dotted coloured lines) obtained at 15 °C/48 h, 20 °C/48 h, and 25 °C/48 h under reducing (a) and non-reducing (b) conditions. mAU=arbitrary unit

Supplementary material

Table S1. Effect of controlled sprouting on dry matter losses of initial mass of wheat grains

Sample	Sprouting conditions	Dry matter loss/%
SWF	15°C/18h	(7.78±2.02)[a]
	15°C/24h	(7.76±1.14)[a]
	15°C/48h	(8.42±1.47)[a]
	20°C/18h	(7.70±2.69)[a]
	20°C/24h	(12.25±0.81)[a]
	20°C/48h	(9.86±1.61)[a]
	25°C/18h	(10.53±2.57)[a]
	25°C/24h	(11.42±0.33)[a]
	25°C/48h	(14.58±2.87)[b]

SWF=sprouted wheat flour. Different letters within a column indicate significant differences according to the DGC test ($p \leq 0.05$)

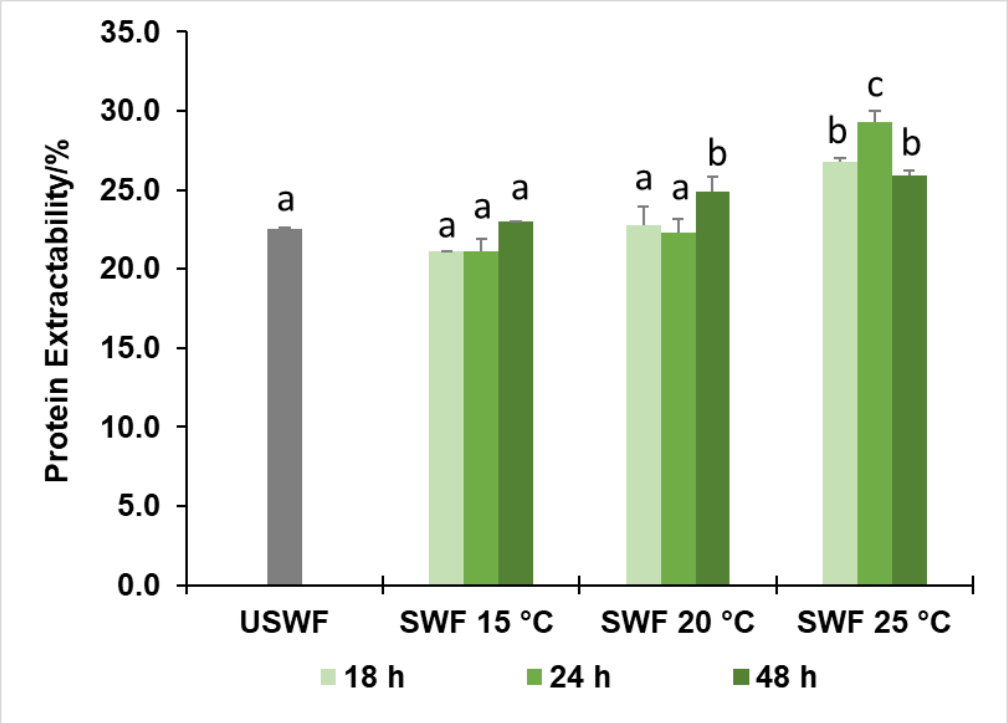

Fig. S1. Protein extractability (PE) in SDS-containing medium under non-reducing conditions

Table S2. Size-exclusion HPLC absorbance area percent values for proteins extracted from USWF and SWF under reducing and non-reducing conditions

		Non-reducing conditions				Reducing conditions			
Sample	Sprouting conditions	F1/%	F2/%	F3/%	F4/%	F1/%	F2/%	F3/%	F4/%
USWF		(18.59±0.17)^{b}	(45.14±1.13)^{a}	(27.81±0.55)^{a}	(8.46±0.75)^{a}	(3.19±0.04)^{b}	(23.25±0.71)^{a}	(11.21±0.19)^{a}	(62.35±0.95)^{a}
SWF	15°C/18h	(19.85±1.88)^{b}	(45.43±0.30)^{a}	(26.51±2.33)^{a}	(8.21±0.16)^{a}	(2.85±0.01)^{b}	(23.31±0.03)^{a}	(15.83±0.51)^{b}	(58.02±0.47)^{a}
	15°C/24h	(18.52±0.46)^{b}	(47.20±1.90)^{a}	(27.62±0.04)^{a}	(6.67±1.48)^{a}	(2.76±0.10)^{b}	(23.21±0.72)^{a}	(17.13±2.73)^{b}	(56.90±1.91)^{a}
	15°C/48h	(16.81±1.15)^{b}	(44.34±1.45)^{a}	(28.58±1.09)^{a}	(10.27±1.51)^{b}	(2.61±0.19)^{b}	(22.47±1.39)^{a}	(15.54±0.07)^{b}	(59.38±1.51)^{a}
	20°C/18h	(17.39± 0.82)^{b}	(46.64±0.67)^{a}	(28.31±0.37)^{a}	(7.66±0.22)^{a}	(2.78±0.17)^{b}	(23.22±0.72)^{a}	(15.51±0.78)^{b}	(58.49±0.11)^{a}
	20°C/24h	(17.22±1.53)^{b}	(45.79±0.59)^{a}	(28.98±1.85)^{a}	(8.00±0.27)^{a}	(2.71±0.01)^{b}	(23.36±0.10)^{a}	(15.77±0.61)^{b}	(58.16±0.50)^{a}
	20°C/48h	(14.16±0.73)^{a}	(47.57±3.27)^{a}	(31.99±0.47)^{b}	(8.71±0.08)^{a}	(1.87±0.62)^{a}	(19.22±5.88)^{a}	(17.79±0.62)^{b}	(61.12±5.87)^{a}
	25°C/18h	(15.09±1.67)^{a}	(43.35±2.34)^{a}	(33.55±3.66)^{b}	(8.01±0.35)^{a}	(2.16±0.23)^{a}	(23.39±0.07)^{a}	(18.24±0.68)^{b}	(55.61±0.98)^{a}
	25°C/24h	(15.64±0.06)^{a}	(45.93±0.05)^{a}	(29.41±0.02)^{a}	(9.01±0.13)^{a}	(2.12±0.15)^{a}	(23.32±0.53)^{a}	(19.84±1.54)^{b}	(55.38±0.86)^{a}
	25°C/48h	(20.74±0.05)^{b}	(43.43±0.34)^{a}	(27.11±0.07)^{a}	(8.73±0.22)^{a}	(2.15±0.11)^{a}	(25.52±1.09)^{a}	(12.45±0.66)^{a}	(59.87±0.32)^{a}

USWF=unsprouted wholewheat flour, SWF=sprouted wholewheat flour. Different letters within a column indicate significant differences according to the DGC test ($p \leq 0.05$)