Wheat germ stabilization by heat-treatment or sourdough fermentation: effects on dough rheology and bread properties

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Abstract:

The aim of this work was to evaluate the effects of wheat germ - stabilized by toasting or by sourdough fermentation – on dough and bread properties. Doughs were produced by adding increasing amounts of each type of stabilized germ, starting with the current recommended level of 3g/100g up to 20g/100g. Sourdough fermentation ensured the presence of lactic acid bacteria (LAB) in amounts comparable to those found in conventional sourdough. The acidification induced by LAB inactivates lipase and lipoxygenase, as does the toasting process. These results decreased the phenomena of rancidity, as demonstrated by the low development of hexanal during storage. Fermentation significantly decreased the content of glutathione, responsible for the deterioration of the rheological characteristics and workability of dough containing high levels of germ. Dough enriched with fermented germ exhibited high stability during mixing and development. Positive effects associated with the use in bread-making of germ stabilized by fermentation have been detected both in fresh bread (high specific volume) and in bread samples stored up to 4 days in controlled conditions of humidity and temperature. Finally, the sensory consumers' test confirmed that the addition of fermented germ did not diminish the liking of the sample.

Keywords: wheat germ; sourdough fermentation; rheological properties; bread quality; sensory acceptability

Abbreviations: RWG, raw wheat germ; TWG, toasted wheat germ; SWG, sourdough wheat germ; FWG, fermented wheat germ; WF, wheat flour.

1. Introduction

Wheat germ, together with bran, is the main milling by-product of wheat. It is an excellent source of vitamins E and B, dietary fiber, essential amino acids, and functional phytochemicals such as flavonoids and sterols. Despite these interesting compositional traits, wheat germ is rarely used for human consumption. First of all, it is quickly subjected to rancidity during storage due to its large amount of unsaturated fats as well as the presence of hydrolytic and oxidative enzymes (Sjövall, Virtalaine, Lapveteläinen & Kallio, 2000). Secondly, the presence of germ negatively affects the technological quality of flour and above all, dough stability. Several efforts have been made, indeed, for stabilizing and improving germ shelf-life. All the approaches have aimed at inactivating its enzymatic activities, with particular attention to lipase and lipoxygenase. This can be achieved directly, using thermal treatments to eliminate enzyme activity, or indirectly, by creating adverse conditions for their action (e.g. by acidification, oxygen elimination, etc.). Until the 1980s, heat-treatments (toasting, hot air process, and pressure-extrusion) were the only methods for retarding rancidity (Haridas Rao, Kumar, Ranga Rao & Shurpalekar, 1980). In addition, extrusion-cooking and microwave heating have been reported to be rapid and interesting approaches for enzyme inactivation (Matucci et al., 2004). Nevertheless, heat treatments may be expensive and responsible for a decrease in nutritional value. Moreover, some researchers emphasized the worsening of the rheological properties associated with germ integration of 10g/100g and more (Gómez, González & Oliete, 2012; Pomeranz, 1987). In addition, it should be considered that there may be little nutritional benefit from germ enrichment considerably less than 10g/100g.

Recently, lactic acid bacteria were isolated from wheat germ and used as starters for preparing fermented wheat germ on lab-scale (Rizzello, Nionelli, Coda, De Angelis & Gobbetti, 2010). The wheat germ stabilized by sourdough fermentation was then used for preparing 4g/100g germ enriched

bread (Rizzello, Nionelli, Coda, Di Cagno & Gobbetti, 2010). Despite that, there is little information on the effect of sourdough fermented wheat germ on the rheological properties of dough.

The main objectives of the present work were (1) to evaluate the suitability of germ fermentation - that was carried out on an industrial scale by using sourdough from bread-making - of improving germ shelf life; (2) to understand the improvement of the rheological properties of germ-enriched dough associated with sourdough fermentation. The effects of the addition of wheat germ on bread characteristics were also investigated, with particular attention not only to the conventional objective qualitative indices but also to sensory parameters.

2. Materials and Methods

2.1 Germ fermentation

The germ samples produced and analyzed in this work were provided by Molino Quaglia S.p.A. (Vighizzolo D'Este, Padova, Italy). Raw wheat germ (RWG) - separated from common wheat kernels during industrial milling – was stabilized by two different approaches. The toasted wheat germ (TWG) was stabilized by the process adopted by the company using a fluid bed dryer (190 °C; 60 s) (Aeromatic, Basel, Switzerland). Germ stabilization by fermentation was carried out by the same company using sourdough from bread-making. In particular, artisanal wheat sourdough (100 kg; pH = 4.2; total titrable acidity = 10.3 ml NaOH 0.1 mol L^{-1}/10g; total bacteria count = $8*10^8$ cfu/g; LAB = $7*10^8$ cfu/g; yeast = $8*10^7$ cfu/g) was mixed with raw germ (500 kg) and water (100 kg), and the mix was fermented for 24 hours at 20°C in an industrial fermenter (Agriflex, Forlì, Italy). After that, an aliquot of raw germ (500 kg) was added to the pre-fermented mass and it was fermented for 12 hours at 20°C. This refreshment step was repeated daily for 7 days to obtain the amount of sourdough germ (SWG; moisture content: 33g/100g) necessary for the experimental plan. SWG was finally dried

resulting in dried fermented germ (FWG; moisture content: 9.2g/100g) using the same conditions applied for TWG.

Samples were finely ground (<250 μm) in a laboratory mill and stored at room temperature until analysis.

2.2 Chemical characterization

Moisture, ash, starch, proteins and fat were determined in duplicate according to the approved methods AACC 44-15, 08-12, 76-13, 46-12, and 30-10, respectively (AACC, 2001). The amount of total dietary fibre was determined according to the gravimetric enzymatic method proposed by Prosky, Asp, Schweizer, DeVries & Furda, 1998). Sugar content was determined according to Zygmunt *et al.* (1982). Water activity (aw) was measured by an electronic hygrometer (Aqua Lab, CX-2 – Decagon Devices, Pullman, WA), based on the determination of the dew point and previously calibrated with standard solutions of LiCl and NaCl of known activity (prepared by High-Purity Standards for Decagon Devices). Total titratable acidity (TTA) was determined on 10 g of wheat germ homogenized with 90 mL of distilled water and expressed as the amount mL of 0.1 mol L^{-1} NaOH to get a pH of 8.5. The pH value was determined by a Crison GPL22 (Crison Instruments, Alella, Barcelona, Spain). Total glutathione content was determined according to Tietze (1969).

2.3 Microbiological analysis

Ten grams of each sample was aseptically weighed and suspended in a sterile bag, mixed with 90 mL of sterile 0.85% trypton salt solution and homogenized with a Stomacher Calworth 400 Circulator (PBI International, Milan, Italy) at 230 min^{-1} for 1 min. Tenfold progressive dilutions were prepared and the following microbiological determinations were performed: *i)* Total Bacterial Count (TBC) by pour plates on Plate Count Agar (PCA) (VWR GmbH, Darmstadt, Germany), incubation at

30 °C for 48 h (ISO 4833, 2003); *ii)* Total Lactic Acid Bacteria (LAB) by pour plates on de Man Rogosa Sharpe agar MRS; Merck, Darmstadt, Germany incubation under anaerobic conditions (gas pack) at 30 °C for 48 h (De Man, Rogosa & Sharpe, 1960); *iii)* yeasts by spread technique on Yeast Glucose Chloramphenicole (YGC) incubation at 30 °C for 48 h (ISO 6611, 1992). All microbiological analyses were carried out in duplicate, and the results were expressed as the mean CFU per gram.

2.4 Enzymatic activities

The enzymatic activities were controlled on the raw and stabilized samples.

2.4.1 Lipase activity

The lipase activity was measured using the automatic Mettler DL 25 titrator with pH stat (Mettler-Toledo Ltd., Leicester, UK). A sample aliquot of 500 mg was dispersed in 15 mL of distilled water, and adjusted to pH = 7. After the addition of substrate tributyrin (0.1 mL), the titrator corrected the pH variation induced by the action of the enzyme for 10 minutes, with aliquots of 5 mmol L^{-1} NaOH. A blank test was carried out by measuring the change in pH of a suspension of germ only without the addition of the substrate tributyrin. All determinations were performed in triplicate and the lipase activity expressed in micromoles of NaOH consumed per minute, which are equivalent to micromoles of ester bond hydrolyzed.

2.4.2 Lipoxygenase activity

The lipoxygenase activity was measured using the method described by Guerrieri & Cerletti (1989) which provides for the determination of conjugated dienes formed from linoleic acid by spectrophotometric reading. A sample aliquot of 10 mg was added to 3 mL of reaction medium consisting of sodium-phosphate buffer 50 mmol L^{-1}, pH 7.0, 0.5 mM linoleic acid and Tween 20

95 0.05%. After incubation at 20 °C for 2 minutes, the reaction was stopped and 4mL of ethanol were
96 added. After centrifugation (1800 x g for 2 min), the absorbance of the supernatant was measured at
97 340 nm against the phosphate buffer, within 20 min. All determinations were performed in triplicate
98 and the results were expressed as μmol linoleate min^{-1} $g_{d.b.}^{-1}$.
99
100 2.5 Determination of hexanal
101 RWG, TWG, and FWG samples were stored for 80 days at room temperature. The volatile
102 compounds were extracted at different storage times by using the static headspace technique coupled
103 by gas chromatographic analysis. Samples were weighed (1.0 ± 0.01 g) in 20 mL glass vials sealed by
104 silicon/Teflon septa and aluminum crimp tops and stored at -25°C. At the end of the storage, all vials
105 were analyzed at the same time using a static headspace analyzer (HS40 Headspace, Perkin Elmer
106 Italia). The wheat germ was conditioned at 90°C for 30 min in a HS40 Headspace (Perkin Elmer,
107 Italia). An aliquot of the vapor phase was transferred through a transfer line to a gas chromatograph
108 equipped with a flame ionization detector (Clarus 600, Perkin Elmer, Italia). Compounds were resolved
109 on a Supelco capillary column Innowax (30 m X 0.53 mm), under the following conditions: injection
110 port temperature, 250°C; helium pressure, 30 kPa; oven temperatures, 40 °C for 2 min then 5 °C/min to
111 220 °C and final isothermal for 15 min. The helium flow was set at 1 mL min^{-1}. Peak identification of
112 hexanal was carried out by comparing retention time with that of the standard (Sigma Chemical Co.,
113 Italy). The results were expressed as arbitrary units of hexanal area.
114
115 2.6 Rheological properties
116 Toasted or fermented germ was added at 3g/100g, 10g/100g and 20g/100g replacement levels to
117 a commercial wheat flour of good bread-making properties (protein: 14g/100$g_{d.b.}$; alveographic W: 380
118 * 10^{-4} J; alveographic P/L: 0.55) provided by Molino Quaglia S.p.A. Dough prepared from the same

wheat flour was prepared as a control. The rheological properties of wheat flour and flour-germ blends were evaluated as described below.

2.6.1 Mixing properties

The Brabender Farinograph-E test was used for measuring water absorption properties according to the standard AACCI Method 54-21A (AACC, 2001), using a 50g-mixing bowl. Besides water absorption, dough development time, stability, and mixing time were also evaluated. Measurements were performed in triplicate.

2.6.2 Gluten aggregation properties

The gluten aggregation properties of blends were measured by using a GlutoPeak (Brabender GmbH and Co KG, Duisburg, Germany). An aliquot of an 8 g sample was dispersed in 10 mL of distilled water. The sample temperature was maintained at 35 °C by circulating water through the jacketed sample cup. The paddle was set to rotate at 2500 min^{-1} and each test ran for 5 min. Torque was expressed in Brabender equivalents (B.E.). Peak maximum time (PMT; s) and the area under peak, expressed in B.E. and equivalent to energy, were considered. Measurements were performed in triplicate.

2.7 Bread-making

Wheat flour or wheat-germ blends were mixed with compressed yeast and salt, each comprising 1.5g/100g of the total mixture, and previously dissolved in water. The amount of water added to each formulation varied according to the farinographic water absorption index, previously determined. For each formulation, the ingredients were mixed in an automatic spiral mixer (Bomann, Clatronic s.r.l., Italy), for 12 min. Immediately after mixing, the dough was left to rest for 15 min at room temperature.

143 After that, the dough was divided into portions of 300 g, molded into cylinder shapes, put in baking
144 pans (8×15×5 cm) and left to rest for 45 min in a proofing chamber at 30 °C and 70% RH. Samples
145 were baked for 35 min at 205 °C in a oven (Self Cooking Center®, Rational International AG), with
146 vapor injection in the first instants of baking. Two hours after removing the samples from the oven they
147 were packaged in perforated OPP film and stored at controlled conditions (20 °C, 60% RH) for four
148 days. For each sample, two baking experimental tests were performed and four loaves were obtained
149 from each baking test.
150
151 2.8 Bread texture
152 Crumb texture characteristics were assessed using a testing machine (Z005, Zwick Roell, Ulm,
153 Germany), equipped with a 5 kN load cell. To evaluate hardness, the three central slices (1.5 mm thick)
154 of two loaves were compressed to 40% of their height by using a 30 mm diameter cylindrical
155 aluminum probe and a test speed of 2 mm/s. Crumb hardness was measured (n = 6) after 0 (two hours
156 after baking), 2 and 4 storage days and expressed as the load (N) at 30% strain.
157
158 2.9 Sensory consumers' test
159 Seventy-five habitual bread consumers (39% males, 61% females, 19–67 years, mean age 26)
160 participated in the study carried out at the Food and Wine Sensory Laboratory of the University of
161 Gastronomic Sciences (Bra, Italy). They had seen or received an invitation and volunteered based on
162 their interest and availability. All tests were conducted in individual booths under white light, social
163 interaction was not permitted. Subjects participated in a session organized in two sub-sessions. In the
164 first sub-session, participants evaluated a set of six breads (3g/100g TWG, 10g/100g TWG, 20g/100g
165 TWG, 3g/100g FWG, 10g/100g FWG and 20g/100g FWG). In the second sub-session, the reference
166 sample (WF) was tested separately to limit the contrast effect (Meilgaard, Civille & Carr, 2006) due to

the difference of color existing among the control bread and the other six samples. The samples of the first sub-session were presented to the subjects in random order. All seven samples (2.5 cm cubes) were presented in blind condition in coded, clear disposable plastic cups (237 mL) hermetically sealed with a clear plastic lid. For each bread, the consumers rated the appearance, aroma, taste, flavor, texture and overall liking on a nine-point scale ranging from 1 (extremely dislike) to 9 (extremely like) (Peryam & Pilgrim, 1957). Consumers were required to rinse their mouth with still water during a 60 seconds rest interval between samples.

2.10 Statistical analysis

Analysis of variance (ANOVA) and significant correlations were performed adopting the least significant difference (LSD) and Pearson correlation analysis procedure, respectively. Data were processed by Statgraphic Plus for Windows v. 5.1. (StatPoint Inc., Warrenton, VA, USA).

As far as the sensory analysis was concerned, liking data (overall acceptability, liking for appearance, aroma, taste, flavor and texture) from consumers were independently submitted to a two-way mixed ANOVA model (fixed factor: sample; random factor: subject) with a Fisher LDS post hoc test considered significant for $p < 0.05$. Overall acceptability data expressed by all 75 subjects were submitted to the principal component analysis in order to obtain an internal preference map for explorative purposes. To investigate potential segments of consumers with different bread preferences, the broken stick criteria (Todeschini, 1998) was used, whereby the first five principal components were selected to limit overloaded information and noise implied in components with low variance and analyzed by cluster analysis applying an Euclidean distance metric and a Ward method of linkage (XLSTAT software, version 2011.3.02, Addinsoft, Paris, France). Liking data expressed by the two segments of consumers (Cluster 1 and Cluster 2) were independently treated with a two-way ANOVA

model, with Fisher LDS post hoc test considered significant for $p \leq 0.05$. ANOVA was performed using the FIZZ Calculations software version 2.46A (Biosystèmes, Courtenon, France).

3. Results and discussion

3.1 Characterisation of germ samples

The main chemico-physical indices of germ samples are summarized in Table 1. As expected, the chemical composition of samples, in terms of protein, carbohydrate, lipid and fibre, did not change much after thermal stabilisation. On the other hand, the soluble/insoluble fibre ratio increased after sourdough fermentation; this aspect is of great interest, from the nutritional point of view, as soluble dietary fiber has been associated with certain health benefits like maintenance of normal blood cholesterol levels due to its ability to form viscous solutions in the intestine (Kumar, Sinha, Makkar, De Boeck, & Becker, 2012). As expected, the fermentation resulted in a decrease in the total content of sugars, and in particular of sucrose and raffinose, most likely due to microbic methabolism, confirming the results of a previous work (Rizzello, Nionelli, Coda, De Angelis & Gobbetti, 2010). In contrast, the fermented sample showed a higher content of fructose, probably resulting from the hydrolysis of sucrose, as compared to the raw or toasted germ.

Lipase and lipoxygenase activities favor sensitivity to oxidation, and the consequent destruction of essential fatty acids and vitamins (Sjövall, Virtalaine, Lapveteläinen & Kallio, 2000). Both toasting and sourdough fermentation were effective in decreasing lipase and lipoxygenase activities (Table 2), in agreement with previous studies (Rizzello, Nionelli, Coda, De Angelis & Gobbetti, 2010). Indeed, due to low pH and high TTA (Table 2) during fermentation, lipase activity was not detectable in SWG and FWG samples. Lipoxygenase activities of RWG also markedly decreased after sourdough fermentation and it completely disappeared in TWG.

214 The perceived rancidity of wheat germ, during its storage, is attributed to the accumulation of
215 aldehydes from lipid oxidation (Sanches-Silva, Lopez-Hernandez & Paseiro-Losada, 2005). Hexanal is
216 one of the main volatile compounds responsible for off-flavor, therefore it is considered to be an
217 indicator of the state of lipid oxidation (Pastorelli et al., 2007; Sanches-Silva, Lopez-Hernandez &
218 Paseiro-Losada, 2005; Piergiovanni & Limbo, 2004). The accumulation of hexanal in headspace during
219 wheat germ storage is shown in Fig. 1. Hexanal markedly increased during storage of RWG: in only 10
220 days its amount increased about threefold. After the same time of storage, the quantity of hexanal found
221 in both FWG and TWG was one third lower and it kept almost constant during the whole storage
222 period (90 days), confirming the good stabilization action of both toasting and sourdough fermentation.
223
224 3.2 Microbiological analyses
225 The microbiological analysis of wheat germ samples are reported in Table 2. In RWG the TBC
226 is around 5 log CFU g^{-1} and consists principally of aerobic spore forming bacteria. Their development
227 is greatly limited by the low value of aw of the product analysed (aw<0.6). The microbial composition
228 of TWG is very similar to that of RWG, despite a slight increase in TBC. The microbial composition
229 drastically changed in SWG. After sourdough fermentation, LAB become the most important microbial
230 population, constituting the totality of the TBC: lactic acid produced by their metabolic activity is
231 responsible for the increase in acidity. The yeast population also increased in SWG.
232 The drying step of SWG sample, critical in ensuring a product's long shelf-life, is associated with a
233 decrease in nearly 4 logarithmic orders of LAB. However, even after heat treatment, FWG had a total
234 charge higher than that observed in TWG.
235
236 3.3 Effect of stabilization treatments on the rheological properties of dough

Very low amounts (3-4g/100g) of wheat germ are usually used in bread-making because higher concentrations (e.g. 6-8g/100g) dramatically affect the processing quality of the flour (Gómez, González & Oliete, 2012; Pomeranz, 1987). In this work, very high percentages (up to 20g/100g) of germ were added.

3.3.1 Mixing properties

The effects of incorporation of TWG and FWG on mixing characteristics were determined by the farinographic test and shown in Table 3. Incorporation of stabilized germ caused an increase in water absorption capacity, regardless of the type of treatment. In particular, water absorption increased approximately by 1.5, 2, and 5 percentage points, when 3g/100g, 10g/100g, and 20g/100g of germ was added to flour, respectively. The increase in water absorption is believed to be related to the presence of protein - mainly globulin fractions (Zhu, Zhou & Qian, 2006) - and fibre in germ. Gelatinisation of the residual starch in wheat germ during the heat-treatment has to be considered, too (Gómez, González & Oliete, 2012).

Addition of increasing quantities of germ gradually decreased the dough development time (Table 3), confirming previous studies (Srivastava, Sudha, Baskaran & Leelavathi, 2007). At the initial 3 g/100g of germ addition, the decrease in dough development time was more evident when TWG was used instead of FWG. As expected, the addition of wheat germ greatly affected dough stability time, which was reduced from 17.9 min (control) to 3 min at 20g/100g addition of TWG. The results confirmed the weakening effect of germ addition on the rheological characteristics of the dough described by Pomeranz (1987). However, the negative effect of germ addition was more pronounced for TWG and the differences between TWG and FWG were most noticeable at high germ addition levels (10 and 20g/100g).

Differences in farinographic indices among TWG and FWG-enriched dough samples are likely related to the changes associated with fermentation of wheat germ components and of their competition for water. Moreover, stability is known to be related to the quality of the protein matrix, which is easily damaged by the addition of other ingredients, due to gluten dilution and the presence of reducing agents, such as glutathione, which weaken the gluten network by breaking disulphide bonds (Every, Morrison, Simmons & Ross, 2006). High amounts of glutathione were found in RWG (6.52 ± 0.02 μmol/g). The toasting treatment was not efficacious in decreasing the glutathione content, since the TWG contained 6.34 ± 0.01 μmol/g of glutathione. On the contrary, fermentation significantly decreased the level of this component (1.17 ± 0.03 μmol/g). LAB, which are present in high amounts in FWG, use the glutathione as aminoacid sources for their growth (Hullett & Stern, 1941), thus accounting for the better results obtained with FWG.

3.3.2 Gluten aggregation properties

The GlutoPeak test (GPT) is a new instrument for testing gluten quality. The GPT uses shear force to mix the ingredients (flour or flour blends and water) uniformly and measures their consistency and the torque associated with the mixing. As the macromolecular aggregation develops, the shear force increases as well as the energy required for the mixer. The GPT indices of flour and germ-enriched blends are shown in Fig. 2. PMT is indicative of the time required for gluten to aggregate and exhibit maximum torque on the spindle. The addition of germ significantly decreased the PMT of wheat flour ($p<0.05$), and the effect was greater when high percentages of germ were added. A similar trend was also observed in the presence of cellulosic fibre, as a consequence of the weakening of the gluten network (Goldstein, Ashrafi & Seetharaman, 2010). The results here reported showed that the PMT is negatively correlated to the farinographic water absorption ($p<0.01$; $R = - 0.88$): the higher the water absorption of sample, the faster the gluten aggregation. The area under the peak is an indicator of

284 the amount of work required to mix and form gluten. The presence of germ significantly decreased this
285 parameter ($p<0.05$), suggesting a negative effect on gluten aggregation properties.

286 The negative effect of germ addition on gluten aggregation properties was less pronounced
287 when FWG was used instead of TWG. The effects of fermentation on gluten aggregation kinetics are
288 primarily due to proteins, and the proteolytic effect of fermentation on albumins - accounting for 34.5%
289 of the total proteins in germ (Zhu, Zhou & Qian, 2006) - should be taken into account. The type of
290 treatment may also have influenced the competition of protein, lipids, and fibre for water. Huschka,
291 Challacombe, Marangoni & Seetharaman (2011) demonstrated that when water is strongly bounded,
292 gluten would require more time and energy for aggregation.

293 Last but not least, the decrease in pH and likely the increase in lactic acid concentration in FWG during
294 sourdough fermentation could account for the higher energy measured in FWG blends, compared to
295 TWG-enriched flour (Fig 2). The effect of pH and lactic acid in gluten aggregation strength have been
296 demonstrated by Melynk (2001). Changes in gluten aggregation strength was attributed in that study to
297 protein unfolding and subsequent hydrogen bond crosslinking due to addition of hydrogen ions and
298 lactic acid molecules. Moreover, structural changes with pH decreases below the isoelectric point of
299 gluten also resulted in charge repulsion and protein unfolding that increased gluten aggregation torque
300 (Melynk, 2001).

301

302 3.4 Bread Texture

303 Changes in crumb hardness during storage are reported in Fig. 3. After baking, an increase in
304 firmness was observed when wheat germ was added, even in the smallest quantity (3g/100g),
305 confirming previous studies (Gómez, González & Oliete, 2012). Significant differences ($p<0.05$) were
306 observed according to the kind of germ treatment, especially at high percentages (10 and 20g/100g):
307 FWG-enriched breads exhibited less hardness compared to TWG-breads. Bread texture properties

cannot be related to bread crumb moisture nor to water activity, as no significant differences were observed among the samples (data not shown). Firmness during storage (two and four days) provided information about the rate of bread-hardening and, therefore, of their shelf-life. During storage, the differences among the samples appeared more pronounced (Fig. 3). In literature it has been reported that fermentation promotes - through lipid hydrolysis - the production of mono and diglycerides, which is by time confirmed the effectiveness in slowing the staling of bread (Williams & Pullen, 2007).

3.5 Sensory acceptability

Liking data expressed by all 75 consumers for overall acceptability, appearance, aroma, taste, flavor and texture of bread samples are reported in Table 4. Surprisingly, no significant differences ($p>0.05$) were found among products in terms of overall acceptability, highlighting the fact that consumers did not show a clear preference for any of the tested breads. In other words, consumers neither clearly preferred the reference sample (100g/100g wheat flour) nor disliked the experimental products, suggesting that the type of germ treatment and the level of germ addition did not negatively impact on consumer judgment. This result could be considered positive taking into account that the degree of familiarity with a food greatly influences its acceptability by the consumer. Generally, the average ratings for liking all samples corresponds approximately to the central value of the scale (5 = neither like nor dislike), considered in this work as the minimum acceptable value. Perhaps the quite low preference ratings resulted from the tendency of untrained subjects (such as the consumers selected for the testing) to use the middle section of a category scale (Meilgaard, Civille & Carr, 2006).

Applying the cluster analysis to the preference data, two groups of consumers were obtained: the first consisting of 21 subjects (28%), namely Cluster 1; the second consisting of 54 subjects (72%), namely Cluster 2. The average ratings for liking expressed by the two clusters of consumers for appearance, aroma, taste, flavor and texture of the seven tested breads are reported in Table 4. Regarding the overall

acceptability and liking for the five evaluated sensory modalities, both clusters did not discriminate among the WF bread and the enriched products with the lower levels of germ, regardless of the germ treatment (3g/100g FWG and 3g/100g TWG). On the contrary, interesting differences were noticed among breads prepared using higher levels of germ addition. In particular, Cluster 1 preferred samples of 10g/100g FWG and 20g/100g TWG which obtained average ratings for overall liking equal to 6.1 and 5.8 respectively, higher ($p < 0.05$) than WF bread (4.8). The highest overall liking shown for sample 10g/100g FWG was associated with the highest mean values of liking observed for taste, flavour and texture. These findings are in agreement with Rizzello, Nionelli, Coda, Di Cagno & Gobbetti (2010) who used lactic acid bacteria – previously isolated from wheat germ - as starters for preparing lab-scale fermented wheat germ. Cluster 2 preferred the highest addition of fermented germ, scoring the sample of 20g/100g FWG with an average value (5.3) not significantly different ($p < 0.05$) from sample WF (5.0) and higher than sample 10g/100gFWG (4.2). Also in this case, the high overall rating is explained by the highest average ratings of liking for taste, flavor and texture. Both clusters did not discriminate among products in terms of liking for appearance confirming the results observed for all of the subjects. Concerning odor, cluster 1 did not discriminate among samples whereas Cluster 2 indicated a decrease in liking compared to the WF, when 10 and 20g/100g of TWG were used in the bread formulation (as already observed for data pertaining to the total of consumers).

4. Conclusion

Germ fermentation by using wheat sourdough is a valid alternative to conventional wheat germ stabilisation by toasting, since it is effective in decreasing the enzymatic activities that are responsible for rancidity and the limited shelf life of products containing wheat germ. The action of microorganisms promotes a significant reduction in the initial content of glutathione, which is associated with the weakening of the gluten network. Fermentation allows the addition of high

356 percentages of germ (up to 20g/100g), resulting in a more stable and easier to process product than the
357 toasted germ enriched dough. Using fermented wheat germ retards bread staling - keeping the crumbs
358 soft during storage - and induces some changes in bread sensory properties, without altering consumer
359 preferences.
360
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364
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444

Figure captions

Fig. 1. Hexanal in headspace during germ samples storage.

raw wheat germ; toasted wheat germ; fermented wheat germ

Fig. 2. Effect of germ treatment on PMT (triangle) and area (circle).

Toasted wheat germ; Fermented wheat germ (grey line).

Fig. 3. Changes in crumb hardness of bread samples during storage for 4 days.

WF; 3g/100g TWG; 3g/100g FWG; 10g/100g TWG; 10g/100g FWG; 20g/100g TWG; 20g/100g FWG. FWG, fermented wheat germ; TWG, toasted wheat germ; WF, wheat flour. Percentage values indicate the percentages of wheat germ added.

Table 1. Characterisation of wheat germ samples.

	RWG	TWG	SWG	FWG
Moisture (g/100g)	10.5 ± 0.05	5.2 ± 0.62	33.1 ± 0.60	9.2 ± 0.49
Aw	0.583 ± 0.008	0.405 ± 0.002	0.970 ± 0.001	0.497 ± 0.001
Protein (g/100g db)	33.0 ± 0.0	34.2 ± 0.4	-	34.1 ± 0.0
Lipid (g/100g db)	11.0 ± 0.7	11.9 ± 0.9	-	11.8 ± 1.1
Total Fibre (g/100g db)	12.8 ± 0.3	11.6 ± 0.3	-	12.2 ± 0.5
Insoluble fibre	11.3 ± 0.1	8.9±0.1	-	7.5 ± 0.1
Soluble fibre	1.5 ± 0.2	2.7±0.2	-	4.8 ± 0.3
Starch (g/100g db)	13.9 ± 1.4	13.3 ± 0.3	-	15.7 ± 3.2
Sugars (g/100g db)	17.8	19.6	-	11.4
glucose	0.7±0.0	0.4±0.0	-	0.3±0.0.
fructose	0.3±0.1	0.2±0.0	-	1.1±0.1
sucrose	10.2±0.9	12.7±0.1	-	5.4±0.0
maltose	0.7±0.0	0.3±0.1	-	0.3±0.1
raffinose	5.9±0.9	6.0±0.1	-	4.3±0.1

db = dry basis

RWG, raw wheat germ; TWG, toasted wheat germ; SWG, sourdough wheat germ; FWG, fermented wheat germ.

Table 2. Chemical, enzymatic, and microbial characteristics of wheat germ samples.

	RWG	TWG	SWG	FWG
Lipase activity ($\mu mol\ min^{-1}\ g_{db}^{-1}$)	2.16 ± 0.02	n.d	n.d	n.d
Lipoxygenase activity ($\mu mol\ min^{-1}\ g_{db}^{-1}$)	10.6 ± 0.02	n.d.	4.2 ± 0.19	2.2 ± 0.01
pH	6.6 ± 0.09	6.6 ± 0.2	4.9 ± 0.01	5.0 ± 0.04
Total titratable acidity (mL NaOH 0.1 $mol\ L^{-1}$ / 10g)	6.0 ± 0.49	5.3 ± 0.28	13.5 ± 0.25	13.4 ± 0.21
Total Bacteria Count (cfu/g)	$8* 10^3$	$4* 10^4$	$1.6*10^9$	$1.9*10^5$
LAB (cfu/g)	10^2	$< 10^3$	$1.7*10^9$	$1.8*10^5$
Yeast (cfu/g)	<10	$< 10^2$	$5*10^3$	10^2

n.d., not detectable
db = dry basis
RWG, raw wheat germ; TWG, toasted wheat germ; SWG, sourdough wheat germ; FWG, fermented wheat germ.

Table 3. Effect of germ treatment on mixing properties evaluated by Farinograph

Germ enrichment (g/100g)	Water Absorption (g/100g)		Development time (min)		Stability (min)	
	TWG	FWG	TWG	FWG	TWG	FWG
0	63.5a		18c		17.9g	
3	65.1b	65.2b	5.9a	6.7a	8.7e	16.4f
10	65.8b	65.6b	6.3a	6.4a	5.4c	6.8d
20	68.8c	68.4c	7.2b	7.5b	2.8a	4.1b

Means with a different letter for each index are significantly different (LSD, $p<0.05$).
TWG, toasted wheat germ; FWG, fermented wheat germ.

Table 4. Effect of germ treatment on bread liking expressed by all consumers, Cluster 1 (n = 21) and Cluster 2 (n = 54).

Treatment	Overall			Appearance			Odour			Taste			Flavour			Texture	
	All	Cl 1	Cl 2	All	Cl 1	Cl 2	All	Cl1	Cl 2	All	Cl 1	Cl 2	All	Cl 1	Cl 2	All	Cl 1
WF	4.9a	4.8cd	5.0a	5.5a	5.2a	5.7a	5.2ab	5.5a	5.1ab	5.0a	5.0bcd	5.0ab	4.8a	4.8bc	4.8abc	4.7a	4.2d
3g/100g TWG	5.0a	5.2bc	4.9ab	5.6a	6.1a	5.5a	5.0ab	5.3a	4.9abc	5.0a	5.0bcd	5.0ab	5.0a	5.3ab	4.9ab	5.0a	5.4ab
3g/100g FWG	4.8a	5.2bc	4.7abc	5.3a	5.5a	5.3a	5.2ab	5.1a	5.2ab	5.1a	5.3abc	5.1ab	5.0a	5.3ab	4.9ab	5.0a	5.1bc
10g/100g TWG	4.5a	4.8cd	4.4bc	5.6a	5.8a	5.5a	4.5c	5.2a	4.2d	4.6a	4.5d	4.6bc	4.4a	4.4c	4.4bc	4.6a	4.8bcd
10g/100g FWG	4.7a	6.1a	4.2c	5.4a	5.8a	5.2a	5.1ab	5.9a	4.8bcd	4.8a	5.8a	4.4c	4.8a	5.8a	4.4bc	5.0a	6.1a
20g/100g TWG	4.6a	5.8ab	4.2c	5.6a	6.1a	5.4a	4.8bc	5.8a	4.4cd	4.7a	5.6ab	4.3c	4.6a	5.6a	4.2c	4.7a	5.5ab
20g/100g FWG	5.0a	4.3d	5.3a	5.7a	5.5a	5.8a	5.3a	5.1a	5.4a	5.0a	4.7cd	5.2a	5.0a	4.5c	5.2a	5.1a	4.5cd

Means with a different letter in the same column are significantly different (LSD, $p<0.05$).

WF, wheat flour; TWG, toasted wheat germ; FWG, fermented wheat germ. Percentage values indicate the percentages of wheat germ added.

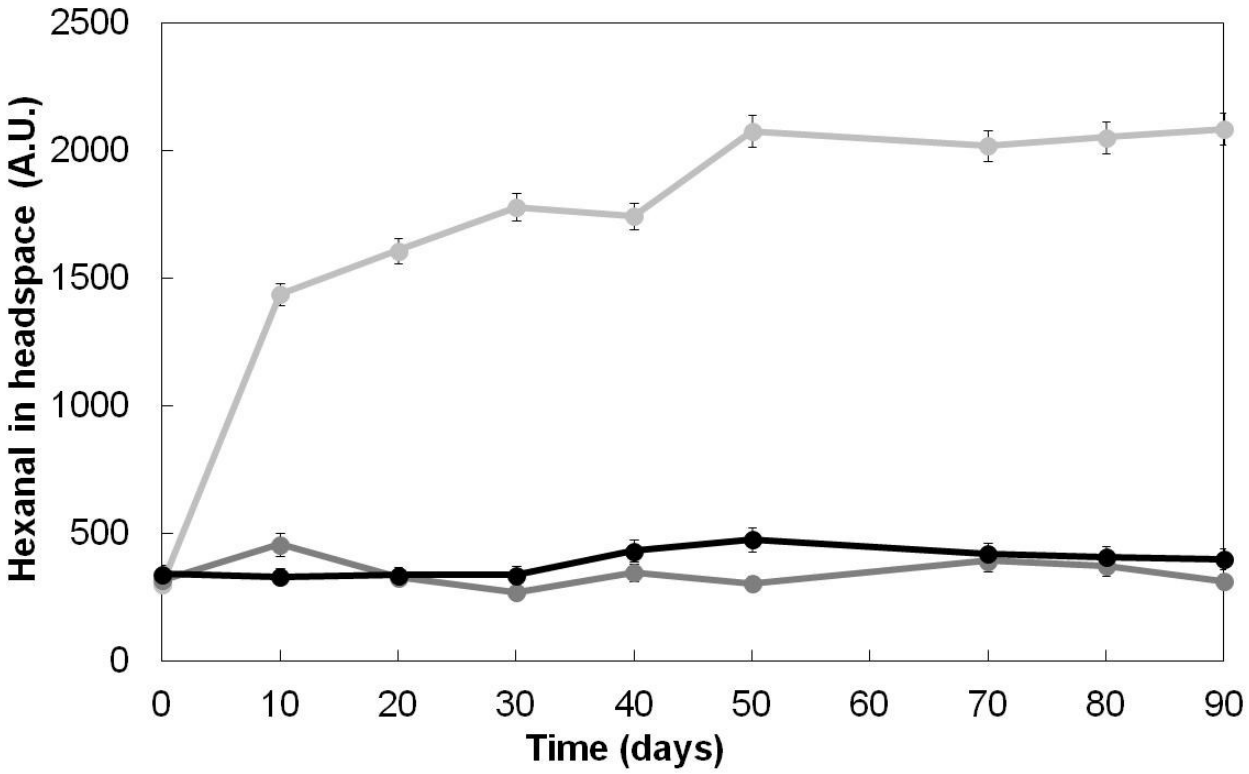
Hexanal in headspace (A.U.)
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
70
80
90
Time (days)

Fig. 1.

445

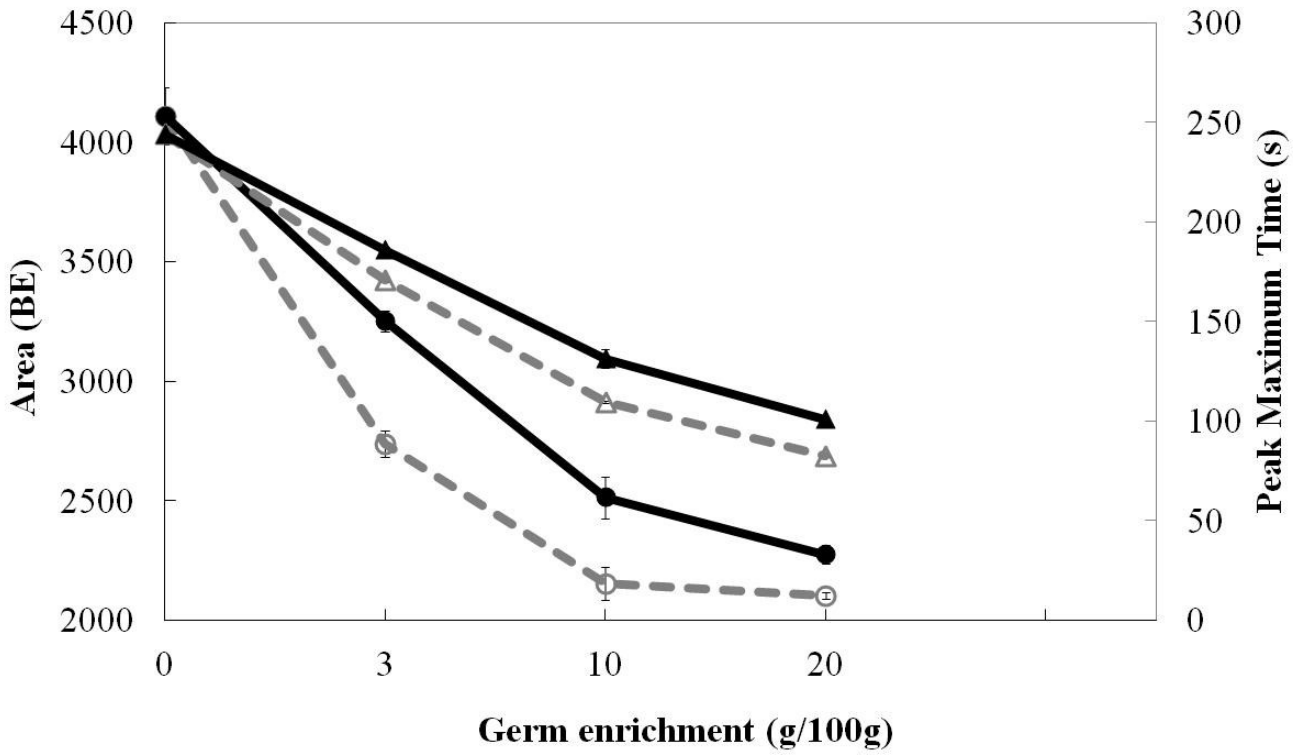
4500
4000
3500
3000
2500
2000
Area (BE)
300
250
200
150
100
50
0
Peak Maximum Time (s)
0
3
10
20
Germ enrichment (g/100g)

Fig. 2.

446

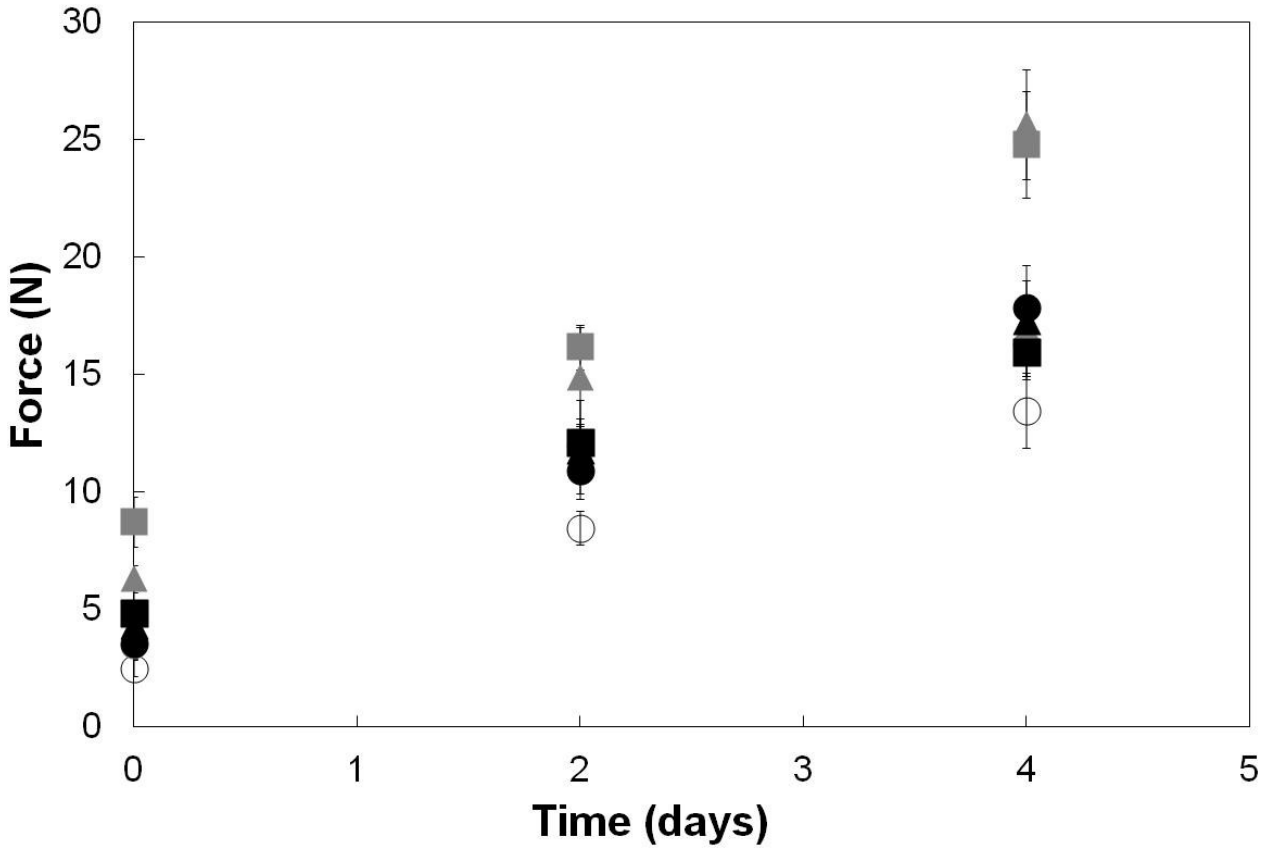

Fig. 3.