LWT - Food Science and Technology 90 (2018) 483–490

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Changes in enzymatic activity, technological quality and gamma-aminobutyric acid (GABA) content of wheat flour as affected by germination

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ARTICLE INFO

Keywords:
Wheat
Germination
Enzymes
Bread
GABA

ABSTRACT

The effects of wheat with pre-harvest sprouting (PHS) in the field, and 24, 48 and 72 h in the laboratory-induced germination (IG) were investigated by enzymatic activity, γ-aminobutyric acid (GABA) content, physicochemical, rheological and baking properties. Germination increased the amylolytic activities but did not affect the proteolytic activity. The damaged starch and gluten contents differed between the IGs and PHS flours. By alveography, balanced gluten presented more tenacity with germination increasing. The reduction of farinography values (water absorption, development time and stability), Mixolab parameters (starch gelatinization-C3, resistance amylase-C4 and starch retrogradation-C5) and pasting properties (peak viscosity, breakdown, setback and final viscosity) of germinated flours showed a dough weakening and the capacity for reduction of starch gelatinization. Bread specific volume, firmness, color and bread GABA content were increased in both IGs and PHS flours as compared to non-germinated flour. The germination caused a reduction in gluten strength and protein weakening in the mixing properties. However, it caused an increase in bread volume. There was an increase in the GABA content in flours and bread due to germination.

1. Introduction

Wheat is one of the main cereal crops among the basic food for the world population. The occurrence of rain in the pre-harvest stage for wheat, especially in the seed filling process, causes spike cob germination, also known as pre-harvest sprouting (PHS). This abiotic factor damages the grain yield, as well as the extraction and technological quality of the flour, affecting its application form. This occurs mainly in the production of bread and pasta with a consequent decrease in economic value. The PHS can occur in different countries, for example, Canada, the USA, Australia, European countries, China, Japan, Iran (Malakshah, Dhumal, Pirdashti, & Saptarshi, 2014), and Brazil. The PHS problem occurs in more than 27 million hectares of cereals around the world (Mares & Mrva, 2008). According to Dahal (2012, p. 93), the pre-harvest sprouting of wheat can cause a significant damage, resulting in 30–50% or higher amount of severely damaged grains.

In germination process, initially, the starch is hydrolyzed by the action of amylolytic enzymes, mainly the α-amylases acting on α-(1–4) linkages producing maltose, glucose, dextrins and oligosaccharides (Delcour & Hoseney, 2000). In addition, storage proteins, such as those that are gluten forming (gliadins and glutenins), are also hydrolyzed, and in the more advanced stages of germination, they release free amino acids and peptide chains (Hajnal et al., 2014).

Different factors can affect wheat technological quality, such as protein content, damaged starch content, the particle size of flour and enzymatic activity. The increase in enzymatic activity produced by germination has a detrimental effect on the quality of wheat processing for grinding and baking. The germination process affects the baking properties of flour, but it is also considered as a tool to improve the quality of food and increase the functional potential of health promotion (Cho & Lim, 2016). This occurs because the starch becomes more digestible, there is an increase in amino acid bioavailability and in addition, a large number of bioactive compounds are formed. Among the metabolites formed during germination, there is the γ-aminobutyric acid (GABA), recommended to prevent neurological disorders, such as anxiety (Ohm, Lee, & Cho, 2016). This may also be associated with the

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https://doi.org/10.1016/j.lwt.2017.12.070
Received 12 July 2017; Received in revised form 27 December 2017; Accepted 28 December 2017
Available online 02 January 2018
0023-6438/

prevention or reduction of symptoms of diseases such as Type 2 diabetes, hypercholesterolemia, hypertension, insomnia, depression, Alzheimer's, chronic kidney disease, some cancers (breast, colon, liver), rheumatoid arthritis and thyroid hormone dysfunction (Chalermchaiwat, Jangchud, Jangchud, Charunuch, & Prinyawiwatkul, 2015; Cornejo, Cáceres, Villaluenga, Rosell, & Frias, 2015). Some studies have shown the production of GABA from grain germination (Ohm et al., 2016). Other studies have considered the application of germinated wheat in bakery products as a source of GABA (Cornejo et al., 2015).

However, a study combining the effects of wheat germination on the physicochemical, rheological, baking properties and GABA content of flour from wheat with pre-harvest sprouting, and the comparison of effects of this caused by different stages of germination are still not well elucidated in the literature. Due to the difficulty of obtaining wheat with different stages of pre-harvest germination, without interfering with the growing conditions, it was chosen to simulate (on a laboratory scale) the germination conditions that occur in the field and to compare its effects. For this, a known cultivar was used, having as control a wheat with pre-harvest sprouting and another non-germinated, this was germinated for 3 times. Only the time of germination was varied, until reaching the falling number and amylolytic activity similar to the wheat with pre-harvest sprouting in the field. In this context, the objective in this work was to investigate the effect of germination on the enzymatic activity, physicochemical and rheological properties, and GABA content in the wheat flour and bread made from these flours.

2. Material and methods

2.1. Material

Wheat from the cultivar BRS Marcante (*Triticum aestivum* L.) with pre-harvest sprouting (PHS) in the field (2015 crop year) and grains of the same lot non-germinated (NG, 2013 crop year) or with induced germination (IG) in the laboratory were used. Wheat was supplied by Embrapa Trigo, Passo Fundo, RS, Brazil.

2.2. Germination

The wheat germination process was performed according to Hung, Hatcher, and Barker (2011), with some modifications. The wheat grains were initially immersed in sodium hypochlorite solution (4–6% active chlorine) 1% (v/v) for 15 min to eliminate any microbiological contamination and after washed with water until a pH 7.0. The grains were allowed to stand to remove excess water and when they presented moisture of approximately 30–35% were incubated for germination in a BOD chamber with humidity and temperature control. Germination was carried out for 24, 48 and 72 h at 80% relative humidity, at 15 and 20 °C, at intervals of 12 h at each temperature and absence of light. The samples were dried at 40 °C in a controlled air circulation oven up to 12% (w.b.) moisture. The samples were called of IG24 (induced germination by 24 h), IG48 (induced germination by 48 h) and IG72 (induced germination by 72 h).

2.3. Milling of grains

The wheat grains were conditioned to 15% (w.b.) moisture with distilled water and after 24 h, then milled in an experimental roll mill (Chopin, Moulin CD1, France), following AACC International 26–10.02 method (AACCI, 2010). The flour yield was calculated based on the initial mass of grains and expressed as a percentage.

2.4. Enzymatic activity and chemical composition of the wheat flours

The determination of the total amylolytic activity and the α-amylase enzyme of the wheat flours was performed according to Saman, Vázquez, and Pandiella (2008) and the absorbance was measured at 540 nm (Molecular Devices, SpectraMax 190, Brazil). A unit of total amylolytic activity and α-amylase (AU) was considered as the amount of enzyme required to release one μmol of maltose per min. The falling number (Perten Instruments, FN 1800; USA) is an indirect measure of α-amylase, and it was evaluated according to the AACC International 56–81.03 method (AACCI, 2010). The proteolytic assay was performed according to Hajnal et al. (2014) for samples germinated (IGs and PHS) and non-germinated (NG) wheat flour. The proteolytic activity was measured using azocasein as the substrate and the absorbance was measured at 440 nm (Molecular Devices, SpectraMax 190, Brazil). A unit of proteolytic activity (PU) corresponds to the amount of the enzyme required to promote the change in one unit of absorbance per min.

The levels of moisture at 105 °C, ash at 600 °C, lipids, crude protein, damaged starch and gluten of the wheat flours were determined according to AACC International 44–15.02, 08–12.01, 30–25.01, 46–13.01, 76–33.01 and 38–12.02 methods, respectively (AACCI, 2010).

2.5. Rheological properties of wheat flours

The alveography (Alveograph Chopin, NG model, France) and farinography in a mixing bowl of 50 g (Farinograph Brabender, Typ 820 600, Germany) of the wheat flour were performed according to AACC International 54–30.02 and 54–21.01 methods, respectively (AACCI, 2010). Pasting properties of the wheat flours also were determined using a Rapid Visco Analyser (RVA-3D, Newport Scientific, Australia) equipped with Thermocline for Windows software, version 3.1, according to the AACC International 76–21.01 method (AACCI, 2010).

The mixing and pasting properties by Mixolab (Chopin, Tripette et Renaud, France) were performed according to AACC International 54–60.01 method (AACCI, 2010), using the Chopin + protocol, which is the typical curve shown in *Supplementary material 1*. The amount of water added for the initial consistency was enough to reach 1.1 ± 0.05 Nm. The evaluated parameters from the curves were water absorption (%); DDT - dough development time (min); mixing stability (min); C1 is the force required to reach 1.1 Nm, initial maximum consistence during mixing used for determining the ability to absorb water (Nm); C2 is the protein weakening, minimum value of torsion during mixing and initial heating (Nm); C3 is the starch gelatinization, maximum value (peak) of torsion during heating stage (Nm); C4 is the resistance amylase (Nm); and C5 is the starch retrogradation, stability of hot starch paste (Nm) (Schmiele, Felisberto, Clerici, & Chang, 2017).

The morphology of the flour was observed by the use of optical microscopy with a 40× objective. The microscopy is equipped with a digital camera (Laborana, LAB1002-TC, Brazil) connected to a computer, and the images of each flour were recorded. A small amount of sample was dispersed in 50% glycerol solution and carefully placed on a glass slide, and covered with a cover glass.

2.6. Bread making and evaluation of bread quality

The formulation of the breads consisted of wheat flour (100%), hydrogenated vegetable fat (3%), refined salt (1.75%), ascorbic acid (0.009%), sugar (5%), yeast (3%) and water at 4 °C, based on the water absorption obtained in the analysis of farinography. After, the mass was divided into two fractions of 35.0 g and fermented in a resting chamber (Gelopar, Brazil) at a temperature of 30 °C, relative humidity of 80% for 40 min. The baking of the bread was carried out in an electric conventional oven (Fischer, Plus, Brazil) at 150 °C for 13 min. The bread was cooled to room temperature and analyzed after 1 h. The bread was evaluated by specific volume (by the displacement of millet seeds), firmness using a texturometer (TA.XT.160, Stable Micro Systems, England) and moisture content according to the AACC International

Table 1
Chemical composition, enzymatic activity, falling number, extraction yield and gluten content of the non-germinated and germinated wheat flours.

Parameters	NG	IG24	IG48	IG72	PHS
Total amylolytic activity (AU.g^{-1})	29.49 ± 0.91^{c}	30.88 ± 1.77^{c}	35.93 ± 1.17^{b}	39.52 ± 0.800^{a}	32.13 ± 0.78^{c}
α-amylase activity (AU.g^{-1})	12.62 ± 0.37^{e}	13.23 ± 0.19^{d}	15.78 ± 0.09^{b}	17.52 ± 0.11^{a}	13.84 ± 0.18^{c}
Proteolytic activity (PU.g^{-1})	0.003 ± 0.001^{a}	0.013 ± 0.011^{a}	0.010 ± 0.002^{a}	0.017 ± 0.001^{a}	0.011 ± 0.007^{a}
Falling Number (s)	452 ± 6^{a}	346 ± 4^{b}	153 ± 0^{c}	108 ± 4^{d}	160 ± 4^{c}
Extraction yield (%)	72.0 ± 2.4^{a}	72.1 ± 0.2^{a}	70.9 ± 0.7^{ab}	68.1 ± 0.4^{bc}	65.1 ± 1.8^{c}
Moisture (% w.b.)	14.85 ± 0.10^{ab}	15.11 ± 0.12^{a}	14.43 ± 0.11^{c}	14.65 ± 0.07^{bc}	14.42 ± 0.07^{c}
Ash (% d.b.)	0.52 ± 0.03^{ab}	0.49 ± 0.02^{ab}	0.49 ± 0.01^{ab}	0.47 ± 0.01^{b}	0.58 ± 0.03^{a}
Lipids (% d.b.)	0.72 ± 0.05^{c}	0.71 ± 0.02^{c}	0.79 ± 0.05^{b}	0.78 ± 0.03^{b}	1.00 ± 0.02^{a}
Crude protein (% x 5.7 d.b.)	11.41 ± 0.23^{b}	11.53 ± 0.29^{b}	11.04 ± 0.20^{b}	11.26 ± 0.27^{b}	14.38 ± 0.21^{a}
Damaged starch (% d.b.)	3.03 ± 0.04^{c}	3.64 ± 0.09^{a}	3.06 ± 0.01^{c}	2.66 ± 0.12^{d}	3.39 ± 0.14^{b}
Gluten index	100 ± 0^{a}	100 ± 0^{a}	99 ± 1^{a}	100 ± 1^{a}	93 ± 3^{b}
Wet gluten (% w.b.)	25.47 ± 1.11^{cd}	27.07 ± 0.85^{c}	29.38 ± 1.11^{b}	24.05 ± 1.24^{d}	41.98 ± 0.45^{a}
Dry gluten (% d.b.)	9.39 ± 0.37^{c}	9.69 ± 0.26^{c}	10.59 ± 0.22^{b}	9.96 ± 0.34^{bc}	14.75 ± 0.24^{a}

* Values with the same letter in a line did not differ significantly ($p > .05$). The analytical determinations were performed in triplicate, and the means ± Standard Deviation were reported. NG: non-germinated; IG24: induced germination by 24 h; IG48: induced germination by 48 h; IG72: induced germination by 72 h; PHS: pre-harvest sprouting.

10–05.01, 74–10.02, 44–15.02 methods (AACCI, 2010), respectively. For firmness, the bread was cut to 25 mm thick and the slices size was 25 mm wide x 25 mm long with the removal of the crust. A cylindrical probe of aluminum P/36R (36 mm radius) was used, with a velocity of 10.0 mm s^{-1} and a compression force of 40%. Bread color was measured using a colorimeter (Chromo Meter CR 400, brand Minolta, Japan), coupled to DP-100 processor, with illuminant D65 and 10° angle. The CIE (International Commission on Illumination) evaluation system was used, with color parameters: L * (luminosity of the lightest - 100 to the darkest - 0), a * (color change from green - a-to red a+), and b *; (color change from blue - b-to yellow b+).

2.7. Extraction and evaluation of GABA

The GABA was extracted from flour and bread; the bread was frozen at −81 °C and then freeze-dried. The GABA was extracted from 1 g of a sample and added 5 mL of 90% (v/v) methanol. The mixture was homogenized using a vortex for 10 min, sonicated for 15 min at room temperature and centrifuged at 2500 × g for 10 min. The supernatant was collected and the residue re-extracted twice under the same conditions. The supernatants were mixed (resulting in 15 mL of extract), filtered through a 0.22 μm nylon syringe filter (Chalermchaiwat et al., 2015). Then, 10 mL of the extract were injected in a LC/MS (Liquid Chromatograph/Mass Spectrometer) system on an ultra-fast liquid chromatograph (UFLC, Shimadzu, Japan), equipped with an online degasser, binary pump, diode array detector (DAD), automatic sampler and a high-resolution quadrupole-time-of-flight type mass spectrometer (Maxis Impact, Bruker Daltonics, Germany) with an electrospray ionization source. A Diamond Hydride column (100 mm × 2.1 mm; 2.2 μm) (Microsolv Technology Corporation, USA) for the chromatographic separation was used. The elution system used was based on a linear gradient, using an A solution (aqueous solution of ammonium acetate 0.1 mM) as a mobile phase and a B solution (mixed solution of acetonitrile: ultra-pure water 95:5 in ammonia acetate 0.1 mM). The initial mobile phase was 100% of the A solution, which was linearly increased with B solution up to 5% in 6.0 min. At 6.1 min, the gradient was raised 90% of B solution and held constant up to 10 min. The mobile phase was then restarted to 100% of A solution in 12 min for the next injection. The flow was 0.2 mL min^{-1} and the column oven was maintained at 40 °C.

The mass spectrometer was operated in a positive ESI mode (Electrospray Ionization), where the spectra were acquired over a mass range of m/z 50 to 1200. For the quantification, a calibration curve with an external GABA standard was prepared (Sigma Aldrich, 97% purity) in the concentration range of 7–1000 ηg.mL^{-1}. The QuantAnalysis software (Bruker Daltonics, Germany) was used to process the calibration curves and quantification data. The results were expressed in μg.g^{-1}.

2.8. Data processing

A comparison of the means was processed using a Tukey's test to a 5% level of significance by an analysis of variance (ANOVA). The GABA content of the flours and bread were compared using *t*-test at 5% significance level was used.

3. Results and discussion

3.1. Enzymatic activity of the wheat flours

Germination mainly activates the synthesis of amylolytic enzymes involved in the degradation of starch. The PHS flour was obtained under natural conditions without any environmental control (temperature, moisture, luminosity, cultivation, soil, among other factors), which differs from the controlled induced germination conditions (IGs). As the wheat germination time increased, the activity of the amylolytic enzymes (including α-amylase) increased, and consequently, the falling number of these samples decreased (Table 1). The 24 h induced germination time (IG24) was not enough to cause the activation of the amylolytic enzymes that were studied, causing only a moderate decrease in the falling number. The PHS flour did not show a significant increase in the total amylolytic activity compared to NG; however, an increase in the α-amylase activity resulted in a falling number decreased, confirming the hydrolysis of the starch chains. The deteriorated wheat starch due to PHS damage is caused by the increased activity of hydrolytic enzymes formed during germination or due to altered inherent properties of starch within the grain (Olaerts et al., 2016).

The grain germination affects the enzyme distribution, which migrates from the aleurone into the endosperm, and therefore, differences can be observed in the flour that has been obtained. The amylases are distributed throughout the wheat plants, but mainly in the germ and pericarp of the grains; and, in the presence of water, these migrate to regions that are rich in starch, proteins and lipids, where they initiate hydrolytic processes for the generation of energy for the new plant' formation (Delcour & Hoseney, 2000).

Noda et al. (2003) compared the germination processes natural sprouting (PHS) in the field grain, where temperature and moisture cannot be controlled with an induced germination in laboratory scale, which was performed on mature wheat grains and in optimal germination conditions (grain moisture ≥ 80% and temperature of 30 °C), with started homogeneous and immediate germination. They observed a pronounced increase in α-amylase activity after 4 and 5 days of germination, but PHS presented results of enzymatic activity less

Table 2
Alveography and farinography of the non-germinated and germinated wheat flour.

Rheological properties	NG	IG24	IG48	IG72	PHS
Alveography					
P (mm Hg)	122 ± 1[a]	99 ± 4[b]	64 ± 4[c]	67 ± 1[c]	92 ± 0[b]
L (mm)	80 ± 0[b]	73 ± 3[b]	112 ± 7[a]	115 ± 6[a]	109 ± 5[a]
P/L	1.53 ± 0.02[a]	1.35 ± 0.10[a]	0.57 ± 0.07[c]	0.58 ± 0.04[c]	0.85 ± 0.04[b]
W ($J.10^{-4}$)	379 ± 1[a]	280 ± 1[c]	251 ± 4[e]	268 ± 2[d]	292 ± 1[b]
Elasticity index (%)	66 ± 1[a]	63 ± 0[a]	63 ± 0[a]	63 ± 1[a]	53 ± 2[b]
Farinography					
Water absorption (%)	61.3 ± 0.0[b]	59.6 ± 0.1[c]	57.5 ± 0.4[d]	56.2 ± 0.0[e]	63.7 ± 0.2[a]
DDT (min)	21.9 ± 0.4[a]	12.1 ± 0.1[b]	2.2 ± 0.3[d]	2.4 ± 0.2[d]	8.3 ± 0.9[c]
Stability (min)	31.1 ± 0.9[a]	16.4 ± 0.5[b]	3.4 ± 0.0[d]	3.9 ± 0.1[d]	8.7 ± 0.0[c]
MTI (FU)	7 ± 2[c]	22 ± 5[b]	42 ± 3[a]	41 ± 1[a]	33 ± 1[ab]

*Values with the same letter in a line did not differ significantly ($p > .05$). The analytical determinations were performed in duplicate, and the means ± Standard Deviation were reported. NG: non-germinated; IG24: induced germination by 24 h; IG48: induced germination by 48 h; IG72: induced germination by 72 h; PHS: pre-harvest sprouting; P: tenacity; L: extensibility; P/L: ratio tenacity/extensibility; W: gluten strength; DDT: dough development time; MTI: mixing tolerance index; FU: farinographic units.

intense than those obtained at the laboratory scale. These changes were more intense than in the present work in which the maximum germination time was 3 days (72 h).

On the other hand, germination did not promote proteolytic activity (Table 1). Ichinose et al. (2001) reported similar results, where the germination did not affect the endo-protease activity that did not differ for germinated wheat for up to 96 h. The germination causes degradation of the reserve constituents of the grain and it reduces the extraction rate of the flour (lower flour yield). The induced germination by 72 h (IG72) showed a decrease in the flour extraction rate. The same behavior was observed in the PHS sample. The induced germination by 24 and 48 h (IG24 and IG48) had no effect ($p \leq .05$) on the flour extraction yield (Table 1).

3.2. Physicochemical properties of flour

The results of the chemical composition are shown in Table 1. In all samples (NG, IGs and PHS flours), the moisture content showed values close to 15% and there was no change in the ash content. The induced germination by 24 h (IG24) was not enough to cause changes in the chemical composition of the flour. Germination provided an increase in the lipid content of the IG48, IG72 and PHS flours. This increase can be due to the increase in free lipids, provided by germination. Regarding the protein content, in relation to the NG sample, only the PHS flour showed a 20.8% significant increase of this macronutrient. Induced germination did not affect protein content. The PHS flour presented the highest protein content. This can be due to differences in cultivation among the wheat grains (Rakita et al., 2015). Although damaged starch content differs in the evaluated samples (Table 1), have a variation that cannot be attributed to the germination process, since the NG flour presented a higher damaged starch value than the IG72 flour.

The values of gluten index, wet gluten and dry gluten contents of PHS flour (Table 1) were significantly ($p \leq .05$) different from the other flours (NG and IG's). The PHS flour presented the lowest gluten index and the highest wet gluten content, which can be explained by its low gluten quality and high protein content. The changes observed in the formation of the gluten network after germination are due to the formation of phenolic compounds able of sequestrating the sulfides available for the formation of disulfide bridges. This is important for the formation of mass alveoli or protein oxidation may have occurred (Sullivan, Dahle, & Schipke, 1962; Rosell, Wang, Aja, Bean, & Lookhart, 2003). This change is consistent with studies of Ichinose et al. (2001), who observed differences in gluten quality, even without identifying changes in proteolytic activity.

Hadnadev, Hadnadev, Simurina, and Filipcev (2013) also reported similar results, where the most affected wheat by high temperatures and rainfall prior to the harvest presented the lowest values of gluten index and the highest values of wet gluten content. The degradation of gluten during germination occurs primarily due to peptide bonds hydrolysis. This is followed by the break of secondary bonds, such as ionic, hydrogenic and hydrophobic, known to contribute to the physical structure of gluten (Delcour & Hoseney, 2000).

Although the time of induced germination increased the amylolytic activity, as can be seen by the reduction of the falling number, the increase of the amylolytic activity and the α-amylase activity did not affect the morphology by light microscopy (Supplementary material 2). This can be due to low enzymatic activity to promote changes in the granules. In all treatments, it was possible to observe the homogeneous distribution of large and small granules with aggregate protein. The action of the enzymes may have promoted a greater porosity to the starch granule, which facilitates the entry of water and makes them more fragile to heating, resulting in differences in rheological properties of flours (Pilli, Legrand, Giuliani, Derossi, & Severini, 2009).

3.3. Rheological properties of wheat flour

Changes in the rheological properties of wheat flour caused by the effect of germination are shown in Table 2. The increase in the germination time reduced tenacity (P) and increased extensibility (L) of the flours. As for the P/L ratio (tenacity/extensibility), there was a reduction in values from 1.5 to 0.6, and those with higher tenacity characteristics ($P/L > 1.2$) changed to balanced gluten (P/L of 0.5–1.2). The induced germination by 24 h (IG24) was not able to produce a change in P/L ratio; however, gluten strength (W) was reduced in all germinated samples. The results showed that, even after 72 h of germination (IG72), the wheat remained with a high W ($> 250 \times 10^{-4}$ J).

The knowledge of the mass viscoelastic properties is an essential factor for the determination of the end use of the flours. The flours that present tenacious gluten indicates a strong gluten and are preferred for pasta and bread, whereas the flours with extensible gluten correspond to weak gluten and can be used for the production of cakes and cookies (Melini, Melini, Luziatelli, & Ruzzi, 2017; Sanchez-Garcia, Álvaro, Peremarti, Martín-Sánchez, & Royo, 2015). The effects of germination on the mixing properties were analyzed by farinography test (Table 2). The water absorption of the flours decreased with the increase of the induced germination time. However, PHS flour had a higher water absorption value, which can be attributed to the higher values found in the protein content and the damaged starch of this sample (Table 1). The water absorption capacity depends on enzymatic activity, moisture, fiber (bran), protein and damaged starch contents of the flour (Hallén, Ibanoglu, & Ainsworth, 2004).

The germinated wheat samples (IGs and PHS) had the same effect on both the Dough Development Time (DDT) and the stability (Table 2); the germination caused a decrease in both parameters. Stability time is the point between arrival time and departure time and

generally indicates the strength of flour. The reduction of dough stability time can be attributed to a relative decrease in the wheat gluten (Abera, Solomon, & Bultosa, 2017). Possible reasons for these effects on the rheological properties of dough might include an effective decrease in wheat gluten content, a competition between soluble and insoluble proteins for water (Hallén et al., 2004; Kohajdová, Karovicová, & Magala, 2013). Similar results also were obtained by Hadnadev et al. (2013), who studied the rheological properties of wheat flours affected by different climatic conditions. These authors reported lower values for the DDT and dough stability in wheat flours that were submitted the pre-harvest sprouting. The flours with induced germination by 48 h (IG48) and 72 h (IG72), reached both, reductions of approximately 10 and 9 times for the DDT and stability, respectively.

Germination is capable to promote changes in the proteins forming gluten. This can be due to hydrolysis of intra or intermolecular bonds of gliadins and glutenins or by breaking the disulfide bonds among the involved amino acids (cystines and cysteines). Thus, contributing to the decrease of DDT. Therefore, the germination causes hydrolysis of the gluten network, became gliadins and glutenins weaker and less stable during the prolonged mixing process.

An increase in the Mixing Tolerance Index (MTI) values of the germinated flour were also observed (Table 2). High MTI values correspond to flours that have little resistance to kneading showing that the increase of germination resulted in the weakening of the flours. Hefni and Witthöft (2011) reported that the substitution of wheat flour for germinated wheat flour negatively affected the rheological properties of the dough, causing a weakening and decrease of DDT and stability with the increase of the level of substitution for germinated wheat flour.

The results found for the mixing and paste properties by Mixolab are shown in Table 3. In the same way as for the farinography, when evaluated by Mixolab, the germination resulted in a decreased water absorption, DDT and stability of flour germinated (Table 2). The DDT and stability are related to the first stages of the curve of the Mixolab (C1 and C2). These correspond to the weakening of the proteins due to the mechanical action and the increase of the temperature of the system, resulting in a decrease in the consistency of the dough and consequently in the torque reduction (Koksel, Kahraman, Sanal, Ozay, & Dubat, 2009).

There was a reduction in the C2 values in the germinated flours (IGs and PHS) in relation to NG (Table 3). This result showed that the germination changes the protein quality, as it has been determined in the rheological analyzes shown in Table 2. Koksel et al. (2009) reported that the amount of protein in wheat genotypes did not predict their quality since genotypes with higher protein contents also presented lower values of C2 and gluten strength (W), which indicate a weaker protein binding.

Flours with values of C2 lower than 0.4 Nm indicate a low protein quality and then have a greater recommendation for products such as cakes and cookies (Banu & Aprodu, 2015). The germination caused a reduction in the values of C3, C4 and C5 when compared to the non-germinated flour (Table 3). This corresponds to the main physicochemical changes that occur in the starch structure such as, starch behavior regarding gelatinization (C3), activity amylolytic (C4) and starch retrogradation (C5). Similar results were found by Rakita et al. (2015) evaluating the α-amylase activity and bread-making properties of wheat flours and reported a reduction of C3 with the increase of the enzymatic action.

The C4 value is considered an indirect measure of α-amylase activity, similar to falling number, and presented inversely proportional results. The increase in germination time reduced the C4 value (Table 3). The increase in α-amylase activity decreases the starch gelatinization capacity, as well as reduces the value of C4. The setback is related to the amylase activity, for the larger the difference between the C3, C4 and C5 parameters, the greater the α-amylase activity (Rosell, Collar, & Haros, 2007). The germination activated hydrolytic enzymes such as α-amylase, causing a rapid reduction in the viscosity of the starch suspension due to degradation in simple sugars and oligosaccharides and altered the rheological characteristics of the germinated flours (Table 2).

The pasting properties of wheat flours are shown in Fig. 1. The induced germination process did not promote differences in the paste temperature of flours when compared to the non-germinated flour; however, the PHS flour showed a higher value ($p \leq .05$). This high value is correlated (Pearson's correlation $r = 0.98$) to higher protein content (Table 1) of this sample. These results are in agreement with that reported by Barak, Mudgil, and Khatkar (2013), who observed the relationship of gliadin and glutenin proteins with dough rheology, flour pasting properties and the bread making the performance of wheat varieties. They reported that the protein content has a great influence on the paste temperature of the samples, where the flours with the highest protein contents had the highest paste temperature. On the other hand, all the other parameters evaluated by RVA presented a reduction of their values with the germination process.

The germination for 24 h (IG24) did not promote changes in the breakdown and setback. There was a drastic reduction in final viscosity, which changed from 213.11 RVU in the NG flour to 11.75 and 9.14 RVU, in the flours IG48 and IG72, respectively, representing a reduction of approximately 20 times. Ichinose et al. (2001) studied the pasting properties of the wheat flour with PHS and found that the samples with low levels of α-amylase activity had high values of maximum viscosity (amylograph). However, during germination with an increased α-amylase activity, the maximum viscosity values decreased, indicating that the starch in the wheat flours was rapidly degraded with an increased α-amylase activity due to germination (Ichinose et al., 2001). The germination caused a decline in the peak viscosity for the three IGs flours, with a significant reduction in the final viscosity to values close to 3.3 RVU. Thus, although the gelatinization process started at the same time, the final viscosity decreased to values below 20 RVU for the IG48, IG72 and PHS flours, indicating that the starch gelatinization

Table 3
Mixolab parameters of non-germinated and germinated wheat flours.

Mixolab parameters	NG	IG24	IG48	IG72	PHS
Water absorption (%, w.b.)	60.5	62.4	57.0	55.4	56.2
DDT (min)	8.91 ± 0.41^{a}	2.60 ± 0.17^{e}	1.82 ± 0.00^{c}	1.80 ± 0.04^{c}	5.71 ± 0.06^{b}
Stability (min)	11.34 ± 0.06^{a}	10.59 ± 0.27^{a}	9.72 ± 0.16^{b}	9.46 ± 0.27^{b}	8.46 ± 0.18^{c}
C1 (Nm)	1.13 ± 0.01^{a}	1.07 ± 0.01^{a}	1.11 ± 0.03^{a}	1.10 ± 0.01^{a}	1.13 ± 0.03^{a}
C2 (Nm)	0.62 ± 0.01^{a}	0.45 ± 0.00^{b}	0.39 ± 0.00^{c}	0.38 ± 0.00^{c}	0.39 ± 0.01^{c}
C3 (Nm)	2.01 ± 0.00^{a}	1.82 ± 0.01^{b}	1.56 ± 0.01^{c}	1.47 ± 0.02^{d}	1.32 ± 0.02^{e}
C4 (Nm)	1.76 ± 0.16^{a}	1.40 ± 0.01^{b}	0.60 ± 0.00^{c}	0.40 ± 0.03^{c}	0.64 ± 0.04^{c}
C5 (Nm)	3.24 ± 0.11^{a}	2.40 ± 0.01^{b}	0.97 ± 0.01^{c}	0.59 ± 0.03^{d}	1.12 ± 0.03^{c}

* Values with the same letter in a line did not differ significantly ($p > .05$). The analytical determinations were performed in duplicate, and the means ± Standard Deviation were reported. NG: non-germinated; IG24: induced germination by 24 h; IG48: induced germination by 48 h; IG72: induced germination by 72 h; DDT: dough development time, PHS: pre-harvest sprouting; C1: force required to reach 1.1 Nm; C2: protein weakening; C3: starch gelatinization; C4: resistance amylase; C5: starch retrogradation.

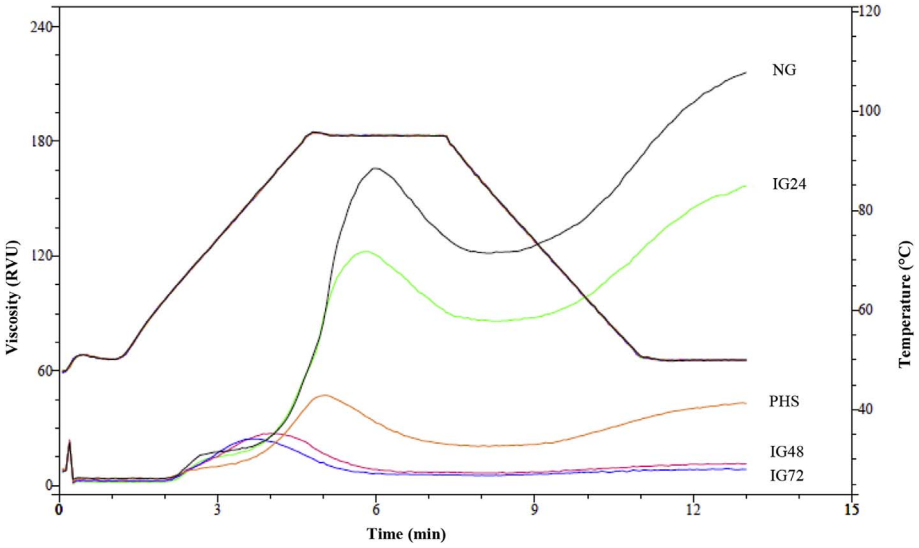

Fig. 1. Pasting properties of germinated and non-germinated wheat flours.

capacity of these flours was decreased.

3.4. Quality characteristics of bread

The effects of germination on baking were evaluated through specific volume, firmness, moisture content and color of the bread (Table 4 and Fig. 2). The germination (IGs and PHS) increased the specific volume and firmness of the bread. The specific volume of bread with germinated flour was around 14% higher than the bread with non-germinated. The bread moisture content ranged from 31.1% to 35.5%, similar values were found by Boita et al. (2016).

The bread germinated firmness increased by around 40% in the IG24, IG48 and PHS samples, and approximately 60% of the samples germinated for 72 h (IG72). The moisture content and flour protein quality can affect the firmness of the IGs and PHS bread. Bread firmness is caused mainly by the formation of cross-links between partially solubilized starch and gluten proteins. In bread, water acts as a plasticizer. The reduction of moisture accelerates the formation of cross-links between the starch and the protein increasing the firmness of the bread. Therefore, the moisture and firmness are closely related (He & Hoseney, 1990). The greater firmness of the bread can be due to gluten weakening, which difficult the gas retention in the wheat flour dough.

For bread color, the brightness results (L*) showed the highest values for the NG flour, which corresponds to lighter flour, whereas the PHS flour had the lowest value corresponding to the darker flour. In relation to the chromaticity coordinate a*, it was observed that the PHS flour had the highest value tending to red hue. The induced germination caused increases in a* value. The high a* values are associated with the decrease in L* that cause browning, as occurred in PHS flour. The PHS bread had a darker crust than the others (Fig. 2), due to the presence of higher reduced sugars and dextrins contents that, combined with free amino acids, favored the occurrence of a Maillard reaction. The germination did not cause changes in the b*values, with the exception of IG48 treatment. According to Ohm, Ross, Peterson, and Ong (2008), the flours that require higher water absorption and have a higher protein content are less bright and redder, which explains what happened in PHS flour, according to the values found for water absorption (Tables 2 and 3) and protein content (Table 1).

3.5. GABA content

The GABA formation is attributed to the decarboxylation of L-glutamate during the grain germination that activates the glutamate decarboxylase enzyme (*Supplementary material 3*). The GABA content of wheat flour and bread is shown in Fig. 3. Germination increased the GABA content in the flours studied (IGs and PHS). The IG24 and IG48 flours showed an increase of about 2.3 times of GABA in relation to the NG flour, while the IG72 and PHS flour increased by 1.6 and 1.3 times, respectively. Cornejo et al. (2015) observed a significant improvement in the GABA content and also an increase in a number of free amino acids with the germination time, varying according to the method and wheat cultivar used. The bread that maintained the highest levels of GABA were those produced with NG (1.15 $\mu g\, g^{-1}$), IG24 (1.80 $\mu g\, g^{-1}$) and PHS (1.57 $\mu g\, g^{-1}$) flours and were statistically equal. On the other hand, there was a significant loss of GABA in all bread with respect to their respective flours IGs and PHS, being the most pronounced decrease in samples with the IG48 (90%) and IG72 (75%).

The high temperatures used during the baking of bread (around 175 °C) resulted in a reduction of the GABA content, which can be attributed to the degradation of free amino acids that are used in the Maillard reaction (Cornejo et al., 2015). Although the level of GABA in the PHS flour was not so high, it was the sample that maintained the

Table 4
Specific volume, firmness, moisture content and color of the breads of flours obtained from wheat non-germinated, with induced germination and pre-harvest sprouting.

Bread characteristics	NG	IG24	IG48	IG72	PHS
Specific volume (mL.g^{-1})	3.61 ± 0.08^{c}	3.79 ± 0.10^{bc}	4.09 ± 0.08^{a}	3.94 ± 0.16^{ab}	3.93 ± 0.14^{ab}
Firmness (g)	205.6 ± 0.7^{c}	280.4 ± 0.6^{b}	292.0 ± 1.6^{b}	332.1 ± 5.8^{a}	289.7 ± 2.7^{b}
Moisture (%, w.b.)	32.4 ± 0.1^{c}	34.9 ± 0.0^{b}	31.1 ± 0.0^{e}	31.6 ± 0.1^{d}	35.5 ± 0.1^{a}
Color					
L*	84.0 ± 0.5^{a}	82.6 ± 0.0^{b}	82.6 ± 0.3^{b}	83.8 ± 0.1^{a}	80.2 ± 0.2^{c}
a*	1.1 ± 0.1^{d}	1.8 ± 0.1^{b}	1.3 ± 0.0^{c}	1.3 ± 0.0^{c}	2.0 ± 0.0^{a}
b*	17.5 ± 0.0^{a}	17.4 ± 0.0^{a}	16.6 ± 0.0^{b}	17.8 ± 0.4^{a}	17.7 ± 0.1^{a}

* Values with the same letter in a line did not differ significantly ($p > .05$). The analytical determinations were performed in triplicate, and the means ± Standard Deviation were reported. NG: non-germinated; IG24: induced germination by 24 h; IG48: induced germination by 48 h; IG72: induced germination by 72 h; PHS: pre-harvest sprouting.

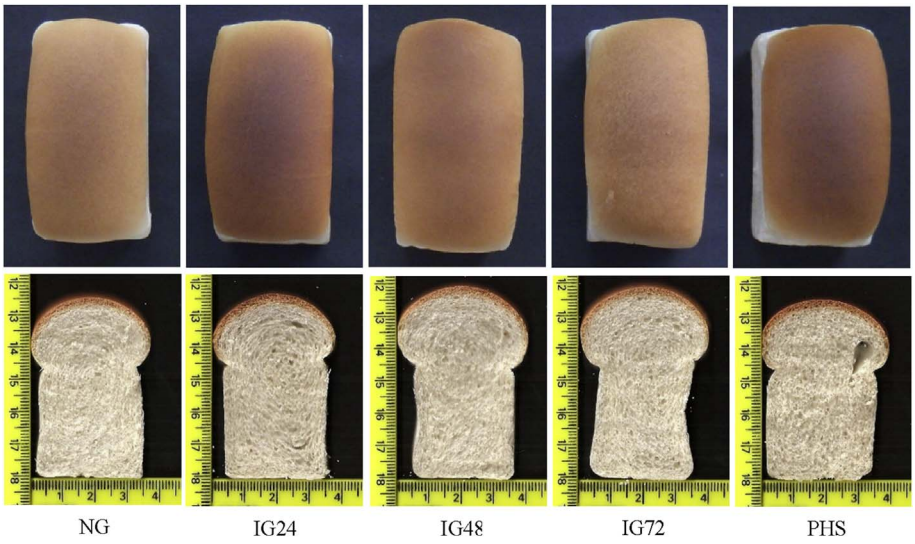

Fig. 2. Macroscopic characteristics of bread made with wheat flour obtained from non-germinated and with the induced germination in the laboratory and in the field n (PHS).

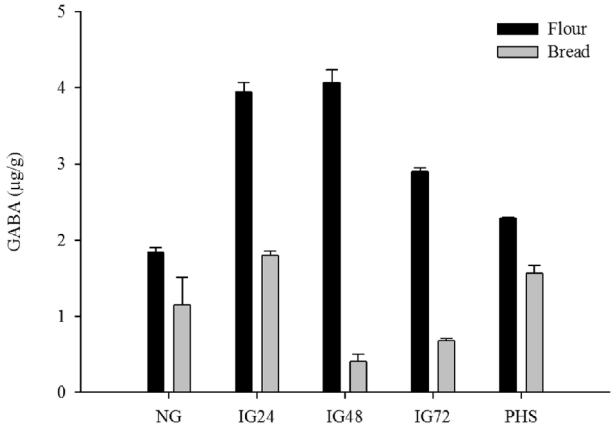

Fig. 3. GABA levels in germinated and non-germinated wheat flour and during bread making.

highest amount of GABA after baking, probably due to the presence of some phytochemical formed during the germination in the field that was responsible for the most stable GABA. In this way, it was possible to observe that the germination is an excellent process to increase the GABA levels in wheat, especially in products consumed *in natura*, unprocessed. The consumption of germinated cereals, rich in bioactive compounds such as GABA, has become popular among health-conscious consumers, besides phenolic compounds and vitamins formed in the germination (Ohm et al., 2016).

4. Conclusions

Induced germination and pre-harvesting of wheat caused activation of amylolytic enzymes but did not affect ($p \leq .05$) the activity of proteolytic enzymes. The germination changed the damaged starch content and the gluten index of the flours, in which the IGs and PHS flours had a lower gluten index and higher wet gluten content as compared to the non-germination flour. The germination also affected the rheological properties, in the viscoelastic properties of the flour were observed a reduction of the tenacity, an increase of the extensibility, and weakening of the gluten strength and in the mixing properties of the mass of germinated wheat flour. Also, there was a reduction of the water absorption, development time and mass stability, an increase of the tolerance index to the mixture, evidencing the weakening of the proteins. In relation to the flour pasting properties, starch hydrolysis occurred, resulting in a reduction in the values of gelatinization capacity, peak viscosity, breakdown, final viscosity and setback. In baking, germination had a beneficial effect, since the bread had higher specific volume and firmness, although a reduction in gluten strength and protein weakening in the mixing properties were observed. As a consequence of the germination, there was an increase in the GABA content in the flour and bread, but part of the GABA was lost during the breadmaking process.

Acknowledgement

The authors would like to thank for the financial support of CAPES-Embrapa Project, Brazil (Grant number 88882.156946/2014-01).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2017.12.070.

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