



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

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### 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,  $\gamma$ -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, Dhupal, 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  $\alpha$ -amylases acting on  $\alpha$ -(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  $\gamma$ -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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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  $\alpha$ -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  $\alpha$ -amylase (AU) was considered as the amount of enzyme required to release one  $\mu$ mol of maltose per min. The falling number (Perten Instruments, FN 1800; USA) is an indirect measure of  $\alpha$ -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  $1.1 \pm 0.05$  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 $\times$  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

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