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Investigating the addition of enzymes in gluten-free flours — The effect on pasting and textural properties

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ABSTRACT

A wide range of enzymes (fungal amylase, esterase, hemicellulase, glucose oxidase and transglutaminase) were added to gluten-free flours (buckwheat, corn and rice) at different concentrations (0, 1, 3, 5, 10 g/100 kg flour) to investigate the effects on pasting properties of flour and texture profiles of the flour gels. Concerning the pasting properties, fungal amylase enzyme consistently affected the flour properties. Breakdown of complex starch molecules into simpler sugars due to the enzyme activity decreased the overall viscosity parameters (at least trough viscosity by 50%, breakdown viscosity by 80%, final viscosity by 60% and setback viscosity by 67%) during the application of heating, shearing and cooling cycle. Increasing enzyme concentration decreased these values further due to increase in the rate of hydrolysis. Texture profiles of the flour gels showed good relation with pasting properties. Decreasing viscosity of gluten-free gels by enzymes caused reduction in the firmness of the gels. The hardest gel was found as corn flour at the highest amylase enzyme concentration (10 g/100 kg flour). However, the highest springiness, resilience, cohesiveness and adhesiveness value was observed for buckwheat starch gel.

1. Introduction

Gluten is the key ingredient found in the grains such as wheat, barley and rye. As a structure-building protein, it directly affects the bread quality by giving elasticity to dough, helping it rise and gives the final product a chewy texture. However, people with celiac disease must adhere to what is commonly referred to as a gluten-free diet which generally involves the products made by buckwheat, corn and rice flour. Baking without gluten causes many problems including a crumbling bread texture, poor color, a weak or poorly developed dough structure and other quality defects making its replacement a major technological challenge for the food industry. To improve the baking performance of gluten-free flours, starches (Milde, Ramallo, & Puppo, 2012; Pongjaruvat, Methacanon, Seetapan, Fuongfuchat, & Gamonpilas, 2014; Tsatsaragkou, Gounaropoulos, & Mandala, 2014), hydrocolloids (Korus, Witczak, Ziobro, & Juszczak, 2015; Mariotti, Pagani, & Lucisano, 2013; Moreira, Chenlo, & Torres, 2013), enzymes (Hamada, Suzuki, Aoki, & Suzuki, 2013; Mohammadi, Azizi, Neyestani, Hosseini, & Mortazavian, 2015), sourdough (Campo, del Arco, Urtasun, Oria, & Ferrer-Mairal, 2015; Moroni, Dal Bello, Zannini, & Arendt, 2011; Novotni, Cukelj, Smerdel, Bituh, Dujmic, & Curic, 2012) and acidic food additives (Blanco, Ronda, Pérez, & Pando, 2011) can be added to formulations (Capriles & Arêas, 2014). In this study focus is given to starch and enzymes since they have critical functions in bread making process.

Starch is one of the most important ingredients and functions as pillars of dough structure. It acts as a thickener, water binder, emulsion stabilizer and gelling agent. Rotsch (1954) studied the role of starch in bread-making process and showed that bread could be exclusively prepared from starch and gel-forming substances, since starch gelatinization increased the volume and product consistency by mimicking the gluten viscoelastic properties. Therefore, investigation of pasting properties of starch and textural properties of its gel plays a critical role in the formulation

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of dough in good quality.

Use of enzymes in the formulation of gluten-free products has many advantages since (i) they are considered as clean label compounds, (ii) they can be used as best and safest alternative to chemical compounds, (iii) they do not remain active after bread making process due to their protein structure being denatured during baking, and (iv) they can add various improvements in dough handling properties, fresh product quality and shelf life. The enzymes most frequently used in bread making are the α-amylases from different origins (cereal, fungal and microbial) (Rosell, Rojas, & Benedito de Barber, 2001), which increase bread volume, improve crumb grain, crust and crumb color, and contribute to flavor development. The hydrolases of non-starch polysaccharides occurred as a result of enzyme activity usually bring positive effects on dough and bread characteristics (Haros, Rosell, & Benedito, 2002). Hemicellulase activity is reported to improve gluten elasticity and final bread quality giving better bread volume and crumb porosity in the whole wheat bread (Jimenez & Martinez-Anaya, 2000, 2001). Crosslinking enzymes (transglutaminase and glucose oxidase) have been proposed as processing aids for improving gluten-free bread quality (Gujral, Haros, & Rosell, 2004; Gujral & Rosell, 2004a, 2004b). Gel structure and pasting properties of gluten-free flours might be improved by the addition of such enzymes.

Based on the disadvantageous position of gluten-free flours and positive effects of starches and enzymes on dough formation, the aim of this study was to investigate the effect of different enzyme addition (fungal amylase, esterase, hemicellulase, glucose oxidase and transglutaminase) on pasting and textural properties of gluten-free flours (buckwheat, corn and rice flours). In the production of gluten-free products, the interaction of enzymes with starch molecules has been scarcely explored. Moreover, although benefit of using enzymes has been observed in the overall dough structure, how these benefits occur has not been understood thoroughly due to the involvement of many molecules and their various interactions with each other in bread making process. Texture is one of the major criteria that consumers use to judge the quality of foods and the most abundant molecule is starch in flours; therefore, studying the effect of enzymes on the pasting properties of flours and textural properties of their gels will be beneficial to reveal the importance of starch–enzyme interaction in bread making process.

2. Materials and methods

2.1. Materials

Buckwheat flour (891 g dry matter/kg, 198 g protein/kg, 26.5 g fat/kg and 16.6 g ash/kg) was procured from Fitmek, Hadef Glutensiz Ekmek Industry and trade limited company, Izmir, Turkey; corn (890 g dry matter/kg, 71.2 g protein/kg, 38 g fat/kg and 6.6 g ash/kg) and rice (950 g dry matter/kg, 93.5 g protein/kg, 16.5 g fat/kg and 4.5 g ash/kg) flours were from Aro-Tech Industry and trade limited company, Izmir, Turkey. The enzymes (brand Mühlenchemie) were obtained from SternIngredients, İzmir, Turkey. The specifications of the enzymes used and their selling aim in the bakery industry are summarized in Table 1.

2.2. Methods

2.2.1. Pasting properties of flour/enzyme combinations

The influence of different enzymes addition at different concentrations on the pasting properties of buckwheat, corn and rice flours was determined using a Rapid Visco Analyser (Perten RVA 4500, Australia). For this aim, amount of flour was adjusted to 14 g flour/100 g mixture depending on its moisture content. For each flour type, 28 g flour and water mixture was put into RVA canisters. Enzyme was added to this mixture at different concentrations (1, 3, 5, 10 g enzyme/100 kg flour) with respect to flour amount. After being heated of the dispersions to 50 °C at a rate of 10 °C/min and stirring at that temperature at 160 rpm for 10 s, they were held at 50 °C for 1 min. Then they were heated to 95 °C at a rate of 13.16 °C/min and maintained at 95 °C for 5 min. Then the gel was cooled to 50 °C for 6.18 min. Thermocline windows software (Perten RVA 4500, Australia) was used to calculate pasting parameters, namely, peak viscosity, trough viscosity, breakdown viscosity, final viscosity, setback viscosity, peak time and pasting temperature.

2.2.2. Textural properties of gels

The prepared gels kept in the canisters covered with parafilm were stored at 25 °C for 3 h prior to measurement of textural properties. Texture profile analyses (TPA) of the gels prepared with different flour and enzyme combinations were performed using a texture analyzer (TAXT2i, Stable Micro Systems Ltd., Surrey, England) equipped with 49 N load cell and SMSP/25P probe. Strain, test speed and trigger force was selected respectively as 25%, 1 mm/s and 0.049 N. Hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience parameters were calculated using Texture Exponent Software.

2.3. Statistical analysis

All of the analyses mentioned were repeated three times with two replications. The results were expressed as mean ± standard deviation. Two way ANOVA was conducted using JMP 5.0.1 (Version 5.0.1a, SAS Institute, Inc., Cary, USA) to determine if the effect of enzyme type and concentration on pasting and texture properties of the samples was significant ($\alpha = 0.05$).

3. Results and discussion

3.1. Effect of enzyme addition on pasting properties of gluten-free flours

The pasting properties of buckwheat, corn and rice flour treated with different enzymes and enzyme concentrations are depicted in Figs. 1–3, respectively. All pasting parameters calculated using those figures and their corresponding statistical analyses are also shown from Tables 2–4. As a general overview, among the studied enzymes (fungal amylase, esterase, hemicellulase, glucose oxidase and transglutaminase), only fungal amylase and its different concentrations significantly affected the pasting characteristics of gluten-free flours in a consistent manner ($P < 0.05$). Hemicellulase and glucose oxidase seemed to have an influence on rice flour. However, a clear trend was not observed with different concentrations of the enzymes on the pasting properties of the flour.

As can be seen from Tables 2–4, the concentration of fungal amylase negatively correlated with peak viscosity. Peak viscosity is attributed to the swelling power of the starch granules during heating stage before their physical breakdown due to shear (Ragaee & Abdel-Aal, 2006). Amylase enzyme has a function of catalyzing the hydrolysis of starch into sugars. Starch occurs as semi-crystalline granules composed of two polymers of glucose, called amylose and amylopectin (Zhu, 2015). Degree of starch hydrolysis depends on many factors such as damaged starch content, granule size, gelatinization temperature and enzyme properties. The best condition for the fungal amylase enzyme used in this study is at 30 °C. Based on these facts, it can be deduced that damaged starch granules should exist in the all samples as the reducing effect of the fungal amylase enzyme on the peak viscosity was observed before the gelatinization temperatures. Therefore, hydrolysis of amylose