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## Use of durum wheat clear flour in vital gluten and bioethanol production

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### ABSTRACT

During milling of durum wheat (*Triticum durum*) into semolina used for pasta processing, certain amount of clear flour (5–15%) with low economic value is obtained. This study aimed at determining the suitability of durum clear flours for vital gluten and bioethanol productions. The durum clear flours were wet-processed into vital gluten by three wet-milling methods, namely dough-washing, dough-water dispersion and flour-water dispersion. Vital glutes with acceptable purities (71.0–82.1% protein, Nx5.7, dm), yields (9.8–14.3%, dm) and recoveries (48.7–76.8%) were achieved by the dough-water and flour-water dispersion methods. However, vital gluten by the dough-washing method could not be isolated satisfactorily. The dough mixing and breadmaking qualities of vital glutes from the clear flours were found comparable to the commercial vital gluten. The carbohydrate-rich remnants of the clear flours upon isolation of glutes were subjected to enzymatic hydrolysis and yeast fermentation, leading to ethanol yields and conversion efficiencies of 32.2–33.5% (g/g, based on clear flour solids) and 80.5–87.6%, respectively. In conclusion, except for the dough-washing method, vital gluten and bioethanol with acceptable purities, yields, recoveries and qualities can be produced by the dough-water and flour-water dispersion methods.

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### 1. Introduction

When durum wheat (*Triticum durum*) is milled into semolina that is used for pasta production, a substantial amount of clear flour (5–15%) with low economic value is obtained (Sissons et al., 2008; Yağcı and Göğüş, 2008). Durum clear flour is mostly used for feed because it is not suitable for breadmaking due to its weaker gluten, yellowish color, excessive starch damage and improper granulation (Özen et al., 1986; Kemahloğlu and Ünal, 2001). Search has been underway to discover new areas of value-added usage for this by-product. In this respect, possible uses of durum clear flours at relatively low levels (5–20%) were reported in extruded snack foods (Yağcı and Göğüş, 2009a; 2009b, 2009c), various breads (Özen et al., 1986; Kılıç, 1999), sausage fillers, low-quality pasta or noodles (Sissons et al., 2008). Processing of durum clear flour into vital gluten and bioethanol also seems a promising approach,

which constituted the goal of this study.

Vital wheat gluten, along with starch, is produced commonly from coarsely ground wheat flour by wet-milling technology (Sayaslan, 2004; Van Der Borgh et al., 2005; Wrnkowska, 2016) and finds applications in food, feed and other industries (Magnuson, 1985; Maningat et al., 1994; Ortolan and Steel, 2017). In the food industry, vital gluten is mostly used in the bakery products to increase their protein contents and/or quality (Maningat et al., 1994; Marchetti et al., 2012; Ortolan and Steel, 2017).

More than 15 processes were developed for wet-milling of wheat; however, only four of them starting with flour instead of kernel had industrial application, namely the Martin, hydrocyclone, Alfa-Laval/Raisio and high-pressure disintegration processes (Sayaslan, 2004; Van Der Borgh et al., 2005; Wrnkowska, 2016). Being the oldest one, the Martin wet-milling process is heavily dependent on gluten agglomeration properties and uses large amount of water, whereas the other three are relatively newer wet-milling processes are less dependent on gluten agglomeration properties with a lesser amount of water requirement.

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Laboratory-scale methods imitating the industrial processes were designed by Godon et al. (1983), Weegels et al. (1988), Meuser et al. (1989), Bergthaller et al. (1998) and Sayaslan et al. (2012). Among the laboratory-scale wet-milling methods, the dough-washing represents the industrial Martin process, the dough-water dispersion imitates the industrial hydrocyclone process, while the flour-water dispersion symbolizes the industrial Alfa-Laval/Raisio and high-pressure disintegration processes (Sayaslan, 2004; Van Der Borght et al., 2005; Sayaslan et al., 2012). In this study, these laboratory wet-milling methods were used to isolate vital gluten from durum clear flours and the remnants rich in starch were subjected to enzymatic hydrolysis and yeast fermentation to produce ethanol.

Due to decreasing fossil fuels and their environmental risks, search for alternative bioenergy sources has increased over the last decades (Guo et al., 2015). Among the renewable bioenergy sources, productions of biodiesel from fat/oil and bioethanol from carbohydrates have been steadily growing with enormous economic value (Demirbaş, 2008; Ho et al., 2014). In many countries, such as the USA and the EU, legislations and new targets were put into action to increase the shares of renewable bioenergy sources (Shrestha and Gerpen, 2010; Walker, 2010; Guo et al., 2015). Corn and sugar cane are the largely used sources for bioethanol, while rapeseed, soybean and palm oils for the biodiesel production in the world (Demirbaş, 2008; Sanchez and Cardona, 2008). However, as corn and sugar cane are main food and feed sources, utilization of lignocellulosic materials, food wastes and by-products has been under investigation as alternatives for bioethanol production (Kim et al., 2011; Kiran et al., 2014; Yang et al., 2014; Karmee, 2016).

The purpose of this study was to investigate the suitability of durum clear flour, a by-product of semolina production, for the co-production of vital gluten and bioethanol through different wet-milling methods.

## 2. Materials and methods

### 2.1. Materials

The durum clear flours were obtained from three different semolina mills in Turkey (Kongıda, Karaman; Selva, Konya; Nuh, Ankara) and they were randomly coded as A, B and C. A relatively weak bread flour (base or control flour) and commercial vital wheat gluten, which were used in comparing the qualities of isolated glutes from the clear flours, were supplied by Kongıda A.Ş. (Karaman, Turkey). The enzymes (thermostable  $\alpha$ -amylase, amyloglucosidase, cellulase, hemicellulase) and chemicals used in ethanol production were purchased from Biais (Turkey) and Sigma-Aldrich (Germany).

### 2.2. Methods

#### 2.2.1. Vital gluten isolation from durum clear flours by dough-washing method

Vital gluten isolation from the durum clear flours was carried out by the laboratory dough-washing wet-milling method described by Sayaslan et al. (2010, 2012) with some modifications. In brief, clear flour (50 g, 14% mb) was mixed to a dough at optimum water absorption level for optimum time in the Chopin-mixolab device (Chopin, France). The optimally kneaded dough was placed in a beaker containing 75 ml of distilled water and rested for 30 min at room temperature. The rested dough was first manually washed under running water (50 ml/min) for 3 min over a 63- $\mu$ m screen. Partially washed gluten was then divided into two equal portions and further washed on the Glutomatic system (Erkaya, Turkey) at 50 ml/min water rate for 2 min. The isolated wet gluten

was frozen, freeze-dried, milled to pass 0.3-mm screen and saved for quality assessment. The aqueous phases obtained throughout the gluten isolation process, which contains mainly starch and water-soluble solids, were collected and saved for ethanol production.

#### 2.2.2. Vital gluten isolation from durum clear flours by dough-dispersion method

Vital gluten isolation from the durum clear flours through the dough-water dispersion approach was performed by the laboratory wet-milling method described by Sayaslan et al. (2010, 2012) with some modifications. Briefly, clear flour (50 g, 14% mb) was mixed to a dough at optimum water absorption level for optimum time in the mixolab instrument (Chopin, France). The dough, together with 75 ml of distilled water, was placed in 300-ml cup of Waring blender and rested at room temperature for 30 min. The rested dough was dispersed in the blender at full speed for 1 min and then spinned in a swinging-bucket centrifuge at 2500  $\times$  g for 15 min. Four fractions from top to bottom were separated, namely supernatant fraction containing water-soluble solids, damaged starch plus hemicellulose, wet gluten and starch fractions. Of these fractions, the wet gluten was washed by the Glutomatic system at a water rate of 50 ml/min for 2 min, freeze-dried and milled as previously described for quality evaluation. The other three fractions and the liquid phases obtained throughout the gluten isolation were combined and saved for ethanol production.

#### 2.2.3. Vital gluten isolation from durum clear flours by flour-water dispersion method

Vital gluten isolation from the durum clear flours through the flour-water dispersion method was carried out by the laboratory wet-milling method of Sayaslan et al. (2010, 2012) with some modifications. In summary, clear flour (50 g, 14% mb) plus 75 ml of distilled water at 35 °C were placed in a 300-ml capacity centrifuge tube and homogenized at 6000 rpm for 2 min using a rotor-stator with 37 mm in diameter. The dry matter content of the dispersion was adjusted to about 27% with additional water and centrifuged at 2500  $\times$  g for 15 min in a swinging-bucket centrifuge. As in the dough-dispersion method, four fractions were separated. Of these fractions, the wet gluten was placed in a beaker containing 75 ml of water and rested for 20 min to mature gluten. Finally, the wet gluten was washed by the Glutomatic system at a water rate of 50 ml/min for 2 min, freeze-dried and milled for quality evaluation. The other three fractions and the liquid phases obtained throughout the gluten isolation process were combined and saved for ethanol production.

#### 2.2.4. Ethanol production from starch-rich remnants of vital gluten isolation

The starch-rich aqueous remnants, which were collected and saved during vital gluten isolation from the clear flours, were subjected to enzymatic hydrolysis and yeast fermentation for ethanol production. The dry matter contents of the aqueous remnants were first adjusted to 6.5% (about 5% fermentable carbohydrates) with additional water and then converted to ethanol following the procedure of Zhao et al. (2009a, 2009b) with some adjustments to wet-milling process. For this purpose, the starch-rich aqueous remnant (about 700 ml) was first placed in a 1-L erlenmeyer flask. Then, thermostable  $\alpha$ -amylase (30  $\mu$ l, from *Bacillus licheniformis*,  $\geq$ 300 U/g, Sigma-Aldrich, Germany) and  $\text{KH}_2\text{PO}_4$  (150 mg) were added. Finally, the erlenmeyer content was first incubated at 95 °C for 5 min and then at 86 °C for 90 min in a shaking water bath set at 100 rpm. Upon completion of starch gelatinization and  $\alpha$ -amylase liquefaction, the erlenmeyer content was cooled down to room temperature and its pH was adjusted to

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