Effects of Grape Peels Addition on Mixing, Pasting and Fermentation Characteristics of Dough from 480 Wheat Flour Type

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Bulletin UASVM Food Science and Technology 75(1)/2018
ISSN-L 2344-2344; Print ISSN 2344-2344; Electronic ISSN 2344-5300
DOI: 10.15835/buasvmcn-fst: 0021

Abstract
In this study, white grape peels were incorporated in wheat flour at levels of 3, 5, 7 and 9\% (w/w), with different particle sizes (large, L > 500 mm, medium, 200 mm > M < 500 mm and small fractions, S < 200 mm). The effects of the addition of grape peels flour (GPF) to wheat flour on the viscoelastic properties of dough and both mixing and proofing behaviour is presented. By GPF addition an increase of water absorption and a decrease of dough stability were recorded, whereas dough development time is remarkable influenced by the particle sizes. Alpha-amylase activity and gelatinization temperature increases by the increased of GPF addition level. The presence of GPF at different levels and particle sizes in dough affected fermentation behaviour of dough with GPF. The whole study shown that grape peels can be used as ingredients in breadmaking in order to improve bread quality.

Keywords: alpha-amylase activity, dough, empirical rheological properties, grape peels flour, particle size, fermentation process, wheat flour

Introduction
Grapes processing into wine or grape juice generates large amounts of grape pomace, by-products which represents approximately 20-25\% of the weight of the grape pressed (Yu and Ahmedna, 2013). Grape pomace includes peels, seeds and residual pulp (González-Centeno et al., 2014) after grape juice extraction by pressing. This by-product is usually used as a soil fertilizer or as animals feed (Acun and Gül, 2013). However, grape pomace has a great potential to be valorized as a food ingredient due to the valuable compounds such as dietary fiber and antioxidant compounds (Sant’Anna et al., 2012) which add value to the final product. Also, proteins, lipids, carbohydrates, vitamins and minerals are present in the composition of grape pomace (Sousa et al., 2014) and can provide opportunities for improving nutritional value of food products. Recent studies (Lavelli et al., 2014; Pasqualone et al., 2014) reported that the use of grape pomace as a source of dietary fiber in various foods showed an increase in dietary fiber content in the developed products. Fiber is an important ingredient associated with prolonged gastric emptying, and slower transit time through the small intestine, modifying the trend and the extent of starch digestion (Angioloni and Collar 2011).

Grape peels, the major component of grape pomace are a valuable source of nutrients with great potential to be used as a functional ingredient in different food products. Grape peels contain polysaccharides, cellulose and hemicelluloses, acidic pectin substances, proteins, fats, minerals, sugars, phenolic compounds (Karovičová et al., 2015; Mendes et al., 2013; Pinelo et al., 2006),
several organic acids and flavors (Arvik, 2012). The concentration of these compounds depends on several factors, such as grape varieties, time of harvest, geographic origin, climatic and cultivar conditions (Rockenbach et al., 2011; Deng et al., 2011). The partial replacement of refined wheat flour, deficient in some essential nutrients, by flours made from fruit pomace have been the subject of significant research during the last years. Grape peels have more micronutrients, such as minerals (Moncalvo et al., 2015) and antioxidants (Deng et al., 2011) than white flour. Previous studies (Lavelli et al., 2015; Sant’Anna et al., 2014) have shown that grape fiber is used to improve baked goods, because fiber can lower blood sugar, decrease cholesterol and may even prevent colon cancer (Karovičová et al., 2015) probably due to the combined effect of dietary fiber and antioxidants. In addition, due to the polyphenols content, grape peels can extend shelf life when are used as an ingredient in food product. Grape peel incorporation in frequently consumed food like white wheat bread could help to overcome the fiber deficit and represent a good alternative to improve the nutritional value of refined wheat grain. The partial replacements of wheat flour with grape peels flour (GPF) can significantly affect the technological behaviour of wheat flour dough, and therefore the bread quality.

The knowledge of changes that occur during mixing, pasting and dough fermentation is essential for bread processors in order to make the adequate adjustment during the bread making process. The impact of grape peels at different levels addition on wheat flour dough technological process previously studied (Bono, 2014; Walker et al., 2014) can be explored in this work. This investigation intends to complete the researches previously made done by and evaluate the effect of replacing wheat flour with grape peels flour at four levels (3, 5, 7 and 9%) and at of different particle sizes (large, medium and small) on the rheological characteristics of the composite flour dough during mixing, pasting and fermentation process. The correlation between the alpha-amylase activity of composite flour blends and dough pasting characteristics was also established.

**Materials and methods**

A commercial wheat flour of 480 type (harvest 2016) obtained from S.C. Dizing S.R.L. (Brusturi, Neamţ, Romania) were used in this investigation. The wheat flour characteristics were analyzed according to Romanian or international standard methods: moisture (ICC method 110/1), ash content (ICC 104/1), protein content (ICC 105/1), wet gluten (ICC 106/1), gluten deformation index (SR 90:2007), falling number (ICC 107/1). The following results were obtained: 14.10% moisture, 0.47% ash, 10.8% protein, 1.1% fat content, 27.8% wet gluten, 2.5 mm gluten deformation index, and 370.5 s falling number.

White wine grape pomace, *Vitis vinifera* L. provided from the viticulture center Jariştea, Odobeşti ecosystem were used in this study. Grape peels were manually separated from dried grape pomace in open air, ground in a domestic blender, and sieved through a Retsch Vibratory Sieve Shaker AS 200 basic (Haan, Germany) to obtain three different particle sizes: large, L > 500 mm, medium, 200 mm > M < 500 mm and small fractions, S < 200 mm. The grape peels flour (GPF) were fractioned to allow a rapid and homogeneous dispersion in the food matrix and to evaluate the potential influence of particle size on the wheat flour dough rheological characteristics during all the technological process. According to the standard analysis methods, the following analytical parameters of grape peels were determined: moisture (ICC method 110/1), protein content (ICC 105/1), fat content (SR EN ISO 659:2009), and ash, as a percentage of the dried substance (SR ISO 2171:2009). Protein content was calculated adopting 6.25 as a conversion factor. Fiber content was determined by Near Infrared Reflectance (NIR) spectroscopy technology, a non-destructive and rapid technique (Fărcaş et al., 2014; Restaino et al., 2009).

Twelve samples were formulated by incorporating each fraction of GPF at four levels (3, 5, 7 and 9%) in wheat flour of 480 type and a sample without GPF as control. The tests applied to samples to determine corresponding properties were carried out at least in duplicates and the results are reported as average value ± standard deviation (SD). Alpha-amylase activity of formulated samples were determined using a Falling Number device (Perten Instruments, Sweden) according to ICC method 107/1. Dough mixing was conducted on a Brabender Farinograph®–E (Brabender OHG, Duisburg, Germany) with a 300 g capacity, according to AACC method 54-21 (AACC, 2000).
Farinograph characteristics, water absorption (WA, %), dough development time (DT, min), dough stability (ST, min) and degree of softening (SDg) at 10 min were recorded. The pasting properties of the wheat flour blend with GPF were assessed using the Brabender Amylograph®–E (Brabender OHG, Duisburg, Germany) according to ICC method 126/1. The parameters measured were gelatinisation temperature (Tg, ºC), peak viscosity (PVmax, BU) and temperature at peak viscosity (Tmax, ºC). Dough development during fermentation process was recorded with a Rheofermetometer F3 (Chopin Rheo, Villeneuve-La-Garenne Cedex, France) which indicates: dough maximum height (Hm, mm), dough development at the end of the test (h, mm), the time at which the dough reaches the maximum height (T1, min), maximum height of gaseous production (H’m, mm), the time required to reach H’m (T’1, min), the time at which gas starts to escape from dough (Tx), total carbon dioxide volume production (Vt, mL), volume of the CO\textsubscript{2} retained in the dough at the end of the test (Vr, mL) and gas retention coefficient (Rc, %).

The statistical analysis was performed with Microsoft Excel 2007 and the Statistical Package for Social Science (Version 16.0, SPSS Inc., Chicago, IL, USA). Significant levels were considered at p < 0.05.

Results and discussion

The analytical characteristics of wheat flour and GPF used in this study are shown in Table 1. The results highlight that the wheat flour used in this study is a strong one for bread making. The falling number value of 370.50 s indicates that it has a low alpha-amylase activity (Codină, 2012). GPF is a good source of fiber due to the amount of total dietary fiber (28.50%) which was higher than that of the other nutrients, leading to the conclusion that it is as a major component of the grape peels of this study, in quantitative terms. This result was consistent with the result obtained by Deng et al. (2011) for Muller Thurgan variety. Grape peels, by-products derived from fruit processing, contain a better insoluble/soluble fiber ratio, and also have better functional properties than those obtained from cereal processing (Grigelmo-Miguel et al., 1999). Also, grape peels have better quality dietary fiber due to the presence of bioactive compounds such as polyphenols (Călinoiu et al., 2017; Iuga et al., 2017; Pedroza et al., 20015; Mildner-Szkudlarz et al., 2013; Deng et al., 2011) which have a high antioxidant capacity, leading to health-promoting effects and other properties in various biological and food systems (Aizpurua-Olaizola et al., 2015; Fontana et al. 2013). These polyphenols may be classified as cell-wall phenols and non-cell-wall phenols. With respect to the cell-wall phenolics, these are reported to be bound to polysaccharides by hydrophobic interactions and hydrogen bonds, mainly to celluloses and hemicelluloses, but the latter ones are, on their turn, tightly linked to lignin. The non-cell-wall phenols include phenols confined in the vacuoles of plant cells and phenols associated with the cell nucleus (Pinelo et al., 2006). Grape peels fiber as well known as “antioxidant dietary fiber” is a product which provides fiber along with soluble and insoluble antioxidants (Saura-Calixto, 1998).

Table 1. The analytical characteristics of wheat and grape peels flour (mean ± SD)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Wheat flour</th>
<th>Grape peels flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>14.10 ± 0.04</td>
<td>5.80 ± 0.02</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>10.80 ± 0.18</td>
<td>7.44 ± 0.14</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>1.10 ± 0.02</td>
<td>3.18 ± 0.05</td>
</tr>
<tr>
<td>Fiber (%)</td>
<td>0.40 ± 0.07</td>
<td>28.50 ± 0.10</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.47 ± 0.01</td>
<td>3.70 ± 0.08</td>
</tr>
<tr>
<td>Wet gluten (%)</td>
<td>27.80 ± 0.16</td>
<td>-</td>
</tr>
<tr>
<td>Gluten deformation (mm)</td>
<td>2.50 ± 0.08</td>
<td>-</td>
</tr>
<tr>
<td>Falling number (s)</td>
<td>370.50 ± 16.26</td>
<td>-</td>
</tr>
</tbody>
</table>
Studies on fiber functional properties highlight that swelling, water and fat retention capacity results showed great variability depending on the grape variety analysed (González-Centeno et al., 2010). The functional properties of the grape peel such as, swelling, water holding capacity, water hydration capacity and fat adsorption capacity are expected to influence starch gelatinization and gluten development, dough rheological and pasting properties of the flours blended with GPF, in agreement to the results obtained for other by-products (Ktenioudaki et al., 2013).

The Farinograph characteristics e.g., water absorption, dough development time, stability and degree of softening were influenced during mixing of wheat flour with different levels of GPF at different particle sizes. As it can be seen from the Figure 1, the replacement of wheat flour with GPF leads to an increase in the Farinograph water absorption, mainly due to the high fiber content of grape peels. Grape peels contain high proportion of dietary fiber, mostly as cellulose, which has a unique organizational structure (Lu and Hsieh, 2012), made up of small spherical microcrystals, not fibers as found in wood or cotton. Due to their structure, grape peels lead to a better incorporation and coating in mixtures. The chemical structure of the fibers added, the association between molecules, the size of the particle and the porosity of the fibers are the parameters which determined variation in water absorption (Thebaudin et al., 1997). An increase in water absorption was reported with the addition of cellulose in wheat flour dough (Poran et al., 2008).

Moreover, grape peels can be used to enrich wheat flour with more acids, mostly tartaric and malic, that help in conditioning rapid-rise of the dough's.

A decrease of SDg parameter with the increase level of GPF addition was obtained. Also, particle size has a relevant influence on SDg. The small (S) particle size of GPF lead to a lower decrease of dough weakness compared with large (L) and medium (M) particle sizes. This fact can be due to the presence of fiber from grape peels, which has the ability to increase the viscosity of the dough, L and M particle size more increasing more viscosity as compared to S particle size. Probably, S particle size presented a higher amount of soluble fibers comparatively with insoluble fiber. Cappa et al., (2015) reported an increase of soluble to insoluble dietary fiber ratio with decreased of particle size. The softening degree at 10 min shows that the incorporation of small particles (S) led to a weakening effect on wheat flour dough, while the large particles (L), a strengthened one.

Regarding the dough stability (Figure 2), the results showed a decreased. Type and flour particle size of used flours affect dough stability. Addition of GPF at different levels decreased dough stability proportional with the increase level of
GPF, the results being similar with those obtained by Bono (2014). At the same particle size, the dough stability decreases with the increase level of GPF incorporated in wheat flour, while for the same level of GPF added in wheat flour, a decrease of dough stability proportional with the particle sizes decrease was obtained.

The differences observed in the analytical characteristics among wheat flour and grape peels were highlighted by the evident variability in the Amylograph curve characteristics. The gelatinization temperature (Tg) increased with increase in GPF content from 0% to 9%, while particle size did not shows a noticeable modify on Tg. This behaviour can be due to the effect of varying gelatinization temperature of the fiber fractions. Peak viscosity increased with increase in GPF in the blends, while the temperature at peak viscosity has an unnoticeable modify. The presence of GPF determined higher peak viscosities according to the result obtained by Bono (2014). The fibers from GPF appeared to interfere with the starch matrix during the cooling, since the increase in viscosity of the samples formulated was less if compared to that of white wheat flour. Comparatively with large and medium particle sizes, the higher peak viscosities and lower tendencies to retrogradation were evident for small particle size. Particle size has a very clear effect for each level of GPF addition. The higher level represents in fact the higher peak viscosity and the lower tendency to retrogradation.

Addition of GPF at different levels and particle size significantly affected the alpha-amylase activity of the flour blends. The results highlight higher value for the gelatinization peak viscosity in the GPF-wheat flour blends comparatively with the control sample. GFP addition decreased the FN value, indicating an increase level of alpha-amylase activity in dough system.

The correlation between alpha-amylase activity of flour blends and dough pasting characteristics related to starch gelatinization process is shown in Figure 3. It was found that the first two principal components (PCs) explain 98.32% of the total variability, with PC1 explaining 88.65%. The plot loadings show along PC1 axis, a close relationship between peak viscosity (PVmax) and gelatinization temperature (Tg) for all the particle size (L, M and S) of GPF addition in wheat flour. Therefore, significant correlations at the 0.05 level were obtained between Tg_L and PVmax_L (r = 0.955), between Tg_M and PVmax_M (r = 0.856) and between Tg_S and PVmax_S (r = 0.968). The first component PC1 underlines the opposition between peak viscosity parameter and falling number (FN) index. A significant negatively correlations was found between FN_L and PVmax_L (r = -0.935), FN_M and PVmax_M (r = -0.952), and between FN_S and PVmax_S (r = -0.959). The second component PC2 distinguishes...
the temperature at peak viscosity and peak viscosity characteristics, which are opposed.

The evolution of dough characteristics throughout the fermentation process is continuously registered by the Rheofermentometer device giving information about the effect of GPF on dough development, gas production and gas retention in the sample formulated. Dough heights (Hm), when supplemented with GPF, decreased compared to the wheat flour dough. Probably, the interactions between gluten and the fibrous fraction from GPF prevent the free expansion of wheat dough during proofing. The time to reach the maximum dough development (T1) was not affected by GPF.

Concerning the volume of gas production during the fermentation (Vt), we noticed a decrease when added GPF at different levels and particle size, while in case of medium particle size addition at levels of 3% (3M) and 5% (5M), they were higher compared with the control sample (C).

**Figure 3.** Loading plot of first two principal components based on falling number (FN) index and Amylograph characteristics peak viscosity (PVmax), temperature at peak viscosity (Tmax) and gelatinization temperature (Tg) for the particle size large (L), medium (M) and small (S) of GPF

**Figure 4.** Effect of GPF on rheofermentogram characteristics dough maximum height (Hm) and dough development at the end of the test (h)
However, the gas retained volume in dough and the end of test (Vr) (Figure 5) increased by incorporation of GPF at different levels and particle size, probably produced by means of a balanced gluten network strength. The gas retention coefficient (Rc) percent increased as function of particle size and level addition. The relationship between gas production and retention, when dough is supplemented with GPF at different levels and particle size, indicate an increased retention coefficient compared to the wheat dough.

The maximum height of the gaseous curve (H’m) is affected by the GPF incorporation in wheat flour dough (Figure 6). With respect to the time of maximum gas formation (T’1) and the time at which gas starts to escape (release) from dough (Tx) were decreased by GPF addition. Therefore, the addition of GPF can probably conduct to the disruption of gluten protein network, the fibrous component from GPF acting as points of weakness or stress concentrations within the expanding dough cell walls. The levels and particle size of GPF influence the magnitude of this phenomenon.

Large and medium particle size showed positive influences up to at level of 7% on H’m while small particle size at level of 7% and 9% showed a negative effect. Probably when the amount of sugar from GPF-wheat flour is high, the osmotic pressure of the system is increased.

Conclusion
The addition of GPF to wheat flour, at different levels and particle sizes changed the mixing, pasting and fermentation characteristics of the wheat flour dough, probably due to the combined action of compounds from grape peels. The GPF that was use used to replace the wheat flour had higher fiber contents which
influence the pasting characteristics, farinograph and reofermentometer properties of the blends especially at substitution levels beyond 5% and made them to be significantly different from that of white wheat flour. The proofing ability of composite flour dough is strongly influenced by the particle size. The deviation in rheological behaviour is believed to be associated with the phytochemical components in the fractions of GPF which enrich white wheat flour.

The replace of wheat flour with GPF cause a decrease in alpha-amylase activity with the increase level of GPF addition and the decrease of GPF particle dimension while the peak viscosity increase. Our results indicated significant negative correlations between falling number index and peak viscosity parameter for all particle size of GPF added in wheat flour.

The data obtained provide important information for selecting specific particle size and addition level of GPF in white wheat flour that could be useful to produce GPF-enriched bread with a desired quality.

Acknowledgments. This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI – UEFISCDI, project number PN-III-P2-2.1-BG-2016-0136, within PNCDI III.

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