The influence of Cabernet Sauvignon wine grape pomace powder addition on the rheological and microstructural properties of wheat dough

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ABSTRACT
Grape seed powder (GSP), grape skin powder (GSKP), or grape pomace powder (GPP) was introduced as partial substitution for wheat flour (WF) with variable percentages by weight (0–20 wt%), and the resultant addition effects on the rheological and microstructural properties were studied. GPP and GSP significantly ($p < .05$) reduced the water absorption but three types of grape powders could increase the development time and dough stability. Furthermore, all kinds of grape powders with various incorporations could significantly ($p < .05$) enhance the strength of dough systems. Grape powders decreased the storage ($G'$) and loss ($G''$) moduli, while the tan $\delta$ increased with the increasing of GSP/GSKP/GPP addition levels. The microstructural observation of composite doughs suggested that the integrity and continuity of gluten networks were destroyed in the presence of grape powders. Based on the above results, the GSP addition levels of 10% to 15%, GSKP/GPP of 5% to 10% are recommended.

Influencia de la adición de polvo de orujo de vino Cabernet Sauvignon en las propiedades reológicas y microestructurales de la masa de trigo

RESUMEN
En el presente estudio se introdujo polvo de semilla de uva (GSP), polvo de piel de uva (GSKP), o polvo de orujo de uva (GPP), para sustituir parcialmente la harina de trigo (WF) en porcentajes variables en peso (0–20%), estudiándose los efectos resultantes de dicha adición en las propiedades reológicas y microestructurales. Aunque el GPP y el GSP redujeron significativamente ($p<.05$) la absorción de agua, los tres tipos de polvo de uva aumentaron el tiempo de desarrollo y la estabilidad de la masa. Además, todos los tipos de polvo de uva adicionados en cantidades diversas mejoraron significativamente ($p<.05$) la resistencia de los sistemas de masa. Asimismo, los polvos de uva disminuyeron los módulos de almacenamiento ($G'$) y de pérdida ($G''$), mientras que con el incremento de los niveles de adición de GSP/GSKP/GPP se produjo el aumento del tan $\delta$. La observación de la microestructura de las masas compuestas permitió constatar que, en presencia de los polvos de uva, se destruyeron tanto la integridad como la continuidad de las redes de gluten. Los resultados anteriores llevan a recomendar niveles de adición de GSP a 10% a 15%, y de GSKP/GPP de 5% a 10%.

1. Introduction
Grape represents as a major fruit crop around the world and accounts for more than 10% of the global fruit planting area, with an approximate annual production of 25.7 million tons. In winemaking industry, approximately 20 wt% of the feedstock is transformed into grape pomace as a major by-product (Laufenberg et al., 2003), which consists mainly of the residue of grape and stem in fermentation, along with some yeast cells. To date, efficient utilization of the grape pomace consistently draws researchers’ attention from both academia and industry, as it holds the potential to boost the economy.

It is well documented that dietary fiber is the main component of dried grape pomace, with concentrations ranging from 43% to 75% (Gül et al., 2013; Martínez-Meza et al., 2021; Spinei & Oroian, 2021), and followed by lipids (14%–17%) (Gül et al., 2013; Mironesea et al., 2016) and proteins (6%–15%) (Gazzola et al., 2014). In contrast to the above-mentioned components, mineral content shows obvious variation, owing to the inherent different growing environment and/or brewing process. In terms of the phenolic compounds in grape pomace, substantial efforts have been devoted to figuring out their composition and thus providing the foundation for potential application (Peralbo-Molina & Luque de Castro, 2013; Teixeira et al., 2014). Anthocyanins and flavanols are the major phenolic compounds in grape pomace, and grape seeds and skins contain 60% and 17%, respectively (Girard et al., 2016). The inherent color of anthocyanins can be used to improve the sensory properties of food products. In view of this, we can assume that grape pomace rich in active substances in line with people’s demand for modern healthy food, which is promising for food fortification. However, the addition of different grape powders on dough quality is particularly important.

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Grape pomace is practically identified as a low-value byproduct, and primarily used as animal feed component or feedstock for fertilizer in many small- and medium-sized enterprises, which underestimates its promising utilization value. Currently, grape pomace is receiving increased attention for its higher phenolic compounds content, and numerous studies suggest that grape phenolic compounds have positive function in the prevention of chronic degenerative diseases, such as atherosclerosis, cancer, cardiovascular disease, and type 2 diabetes, among others (Ferri et al., 2016; Jara-Palacios et al., 2015; Kadouh et al., 2016; Nunes et al., 2016). In this scenario, many researchers tentatively add grape pomace into food as functional component, with varying purpose from improving the sensory qualities of food to increasing the nutritional value. In light of a large number of previous studies, one can envision that grape pomace could be an excellent ingredient in food processing (e.g. bread and cake-making dough) for nutrition enhancement (Mironesaca et al., 2012). However, most of the studies focused on the extraction of active substances from grape pomace and its antioxidant activity (Manca et al., 2020; Ricucci et al., 2021; Stuart et al., 2020). There is a lack of systematic research on the application of grape pomace in food fortification and the effects of grape seeds, grape skins and grape pomace addition on dough properties. According to our previous findings, there was great difference in the chemical compositions of various grape powders, where dietary fiber, protein and polysaccharide content related to water absorption varied among three types of powders (Lou et al., 2021). These indicators have a certain impact on the formation of dough and the quality of the product, therefore, the addition amount of grape powder is significantly important. In view of this, this study focused on the effects of GSP, GSKP, or GPP addition on the thermal-mechanical properties, rheological properties, and the microstructure of wheat dough, aiming to provide a foundation for the application of grape pomace in wheat-flour foods.

2. Materials and methods

2.1. Chemicals

Alkaline protease solution (100 U/mg) was purchased from Shanghai Lanji Biotechnology Co., Ltd. (Shanghai, China). High temperature-resistant alpha-amylase solution (30 U/mg) and glycosylase solution (100 U/mg) were purchased from Shanghai Ruji Bio-Technology Co., Ltd. (Shanghai, China). 2-(4-Morpholino) ethanesulfonic acid (MES) and tris-(hydroxy-methyl)aminomethane (TRIS) were bought from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Folin&Ciocalteu’s Phenol (FC) reagent was purchased from Aladdin (Shanghai, China). All chemicals are analytical grades.

2.2. Sample preparation

Cabernet Sauvignon grape, grown in Huailai city (2019, Hebei, China), was used as feedstock and fermented with 1 wt% Saccharomyces cerevisiae (Lalvin strain D-254) at 25°C for 7 days. The grape pomace (GP) was collected under operating conditions of 25°C, 75 rpm by a laboratory-scale extruder (Shanghai Yulushiye Instrument Co., Ltd., Shanghai, China), and subsequently washed with water for 4–5 times. For grape skins (GSK) and grape seeds (GS), they were manually gathered. The GP, GSK, and GS were placed outside and dehydrated to around 65 wt%, and then transferred to an air-dry oven at 50°C for 80 h. The dried GP, GSK, and GS were crushed by a laboratory-scale pulverizer (Shanghai Zhikai Powder Machinery Manufacturing Co., Ltd., Shanghai, China) and then screened to gather the powder with diameters less than 120 mesh (i.e. 0.125 mm). Low-gluten wheat flour with 13.7% protein, 12.3% moisture, and 2.8% fat was purchased from COFCO Co., Ltd. (Beijing, China). As for the composite flour preparation, GPP, GSKP, or GSP was homogeneously mixed with wheat flour with varying additional levels (i.e. 5 wt%, 10 wt%, 12.5 wt%, 15 wt% and 20 wt%, based on the author’s previous study). Doughs were prepared using a laboratory-scale kneader by adding water to the above thoroughly mixed composite powder, dough-mixing time was 8 min, and the amount of water added was calculated by the water absorption measured by Mixolab.

2.3. Color measurement

The color measurement of composite flour was carried out using an ADCI-60-C Hunter Colorimeter (Hunter Associates Laboratory Inc., Reston, USA) equipped with the CIE L*, a*, and b* color system. Where L* indicates whiteness (100) or blackness (0), a* indicates red (positive) or green (negative) and b* indicates yellow (positive) or blue (negative). The colorimeter was calibrated using a standard.

2.4. Particle size measurement

The particle size distribution of composite flour was measured by a Malvern laser particle analyzer (Mastersizer 3000, Malvern, Britain). Typically, 1.5 g sample was dispersed in absolute ethanol to form a suspension liquid featuring a shading coefficient of 9.80% and a refractive index of 1.333. The results are expressed in the manner of DS0 and D90.

2.5. Composition determination of GPP, GSP, and GSKP

The content of moisture, ash, crude protein, and crude fat of the as-prepared sample (i.e. GPP, GSP, and GSKP) were determined by the methods 44–15, 08–01, 46–11, and 30–10 approved by AACC (AACC International, 2000). The total sugar content of sample was determined using a direct titration method (Dubois et al., 1956). The total dietary fiber (TDF) content were measured by the enzymatic-gravimetric method (AOAC Official Method 991.43 [17th ed.], 2000).

2.6. Rheological properties of dough

Thermo-mechanical properties. The thermo-mechanical properties of composite dough with varying GPP, GSKP, or GSP percentages were evaluated using a Mixolab 2 (Chopin Technologies, Villeneuve la-Garenne, Paris, France). The analysis method was primarily based on standard “Chopin+” protocol according to ICC No.173, while some operating parameters were slightly modified according to the published method (Mironesaca et al., 2012). In brief, the total weight of composite powder was 75 g, the water addition was estimated based on previous studies by the authors until the target torque reached 1.05 ~ 1.15 Nm, the mixing speed was 80 rpm, the target torque was 1.1 Nm, and the
temperature profile was as follows: keeping at 30°C for 8 min, heating to 90°C at a rate of 4°C/min and holding for 7 min, and then cooling to 50°C at a rate 4°C/min (the total time was 45 min).

Dynamic rheological properties. The dynamic viscoelastic measurement of the samples was evaluated at 25 ± 0.1°C on a Haake MARS III controlled-stress rheometer (Haake, Germany) equipped with a 1 mm gap parallel-plate sensor system. The WA of the sample was determined after kneading for 8 min by a KitchenAid flour kneader, and then the sample was transferred to the plate of the rheometer, on which the redundant dough was scraped off. Specifically, the exposed dough was covered with mineral oil to avoid any water losses. The test parameters were referred to a published method (Correa et al., 2010). Frequency sweep test was conducted as a function of frequency ranging from 0.1 to 10 Hz.

2.7. Microstructure of dough

Microstructure of composite dough sample was observed using a scanning electron microscope (FEI, Hitachi, USA) according to a published method proposed by Nie et al. (Nie et al., 2019). Briefly, samples with different addition levels of GPP, GSP, or GSKP were cut into slices with a diameter of 2 ~ 3 mm and a thickness less than 5 mm, and then subjected to freeze drying. The dried dough slices with natural fracture surfaces passed through conductive resin, were coated with gold, and then scanned under 20 kV voltage and low vacuum condition.

2.8. Statistical analysis

All experiments were conducted at least in duplicate, and the corresponding results were statistically analyzed by SPSS version 22.0 software (v.22, SPSS, Chicago, IL, USA). The significant differences between means were evaluated by using one way ANOVA, and a Tukey’s multiple range test was applied for the multiple comparisons among experimental means (p < .05).

3. Results and discussion

3.1. The general composition of GSP, GSKP, and GPP

Table 1 shows the general composition of GSP, GSKP, and GPP. Dietary fiber is the main component of dried wine pomace ranging from 59% to 68%, which plays a positive role in maintaining normal intestinal function and lowering blood pressure (Bartolomé et al., 1995), and the content in GSP is significantly higher than that in GSKP (p < .01). This result is consistent with the report by Göl et al. (2013) With regard to crude protein, GSKP contains higher content than that in GSP (p < .01). Contrarily, the content of crude fat in GSP is richer than GSKP. The moisture content of GPP is 5.86 g/100 g, which is the highest among these three samples (i.e., GSP, GSKP, and GPP). As the moisture and water activity of raw-food material largely dictate the quality of finished food (Bugusu et al., 2001), it is reasonable to claim that maintaining a rational shelf life necessitates appropriate dehydration to lower moisture to a certain level.

| Component                  | GSP (%) (DW) | GSKP (%) (DW) | GPP (%) (DW) |
|----------------------------|--------------|---------------|--------------|
| Crude protein              | 8.75 ± 0.03^a| 16.31 ± 0.02^a| 13.62 ± 0.03^b|
| Crude fat                  | 20.92 ± 0.06^a| 10.66 ± 0.10^a| 17.46 ± 0.04^b|
| Ash                       | 2.98 ± 0.11^a| 3.95 ± 0.05^a| 6.02 ± 0.14^a|
| Moisture                   | 3.69 ± 0.21^a| 4.51 ± 0.18^a| 5.86 ± 0.25^a|
| TDF                       | 68.53 ± 0.29^a| 59.38 ± 0.33^a| 65.20 ± 0.28^a|
| Total carbohydrate         | 57.04 ± 0.48^a| 50.25 ± 0.55^a| 51.66 ± 0.71^a|

*GSP: grape seed powders; GSKP: grape skin powders; GPP: grape pomace powders; TDF: total dietary fiber.

All data are expressed as mean ±SD (n = 3). Different latin letters within the same column indicate significant differences among samples (lowercase and uppercase letters represent p < 0.05 and p < 0.01, respectively).

3.2. Color measurement

All grape powders had great influence on wheat flour color (supplementary Table 1). Composite flours with GPP, GSP, or GSKP addition exhibited decreased L* and b* but increased a* values, indicating that these additives could decrease brightness and increase dark rose color. GSKP behaved a higher degree influence on the L*, a*, and b* values than that of GPP and GSP.

3.3. Particle size

As shown in Table 2, the average particle diameters of the wheat flour at D50 and D90 are 96.52 μm and 242.50 μm, respectively. 10 wt% GPP addition results in substantial

| Addition level (wt%) | D50 (μm) | D90 (μm) |
|----------------------|----------|----------|
| GSP                  | 2.98 ± 0.11^a | 29.66 ± 1.05 | 126.40 ± 3.12 |
| GPP                  | 10       | 35.49 ± 2.12^b | 151.66 ± 1.74^b |
| GSKP                 | 10       | 26.48 ± 1.42^d | 117.60 ± 2.56^d |

* WF: wheat flour; GSP: grape seed powders; GSKP: grape skin powders; GPP: grape pomace powders.

The values are mean ±SD of three independent determinations. Means followed by different alphabetic superscripts within a column are significantly (p < 0.05) different.

* WF: harina de trigo; GSP: polvos de semillas de uva; GSKP: polvos de piel de uva; GPP: polvos de orujo de uva.

Los valores representan la media ±DE de tres determinaciones independientes. Las medias seguidas de distintos superíndices alfabéticos dentro de una columna son significativamente diferentes (p < 0.05).
The decrease in gluten size. This may be related to the release of water molecules or the existence of secondary activity in dough. Particle size has a significant negative effect on WA, indicating that with the decrease of particle size, WA increases, which is due to the larger surface area of the particles that adsorb more water. These results are consistent with the observations reported by Ahmed et al. (2019) and Drakos et al. (2017) for quinoa, rye and barley flours. When the GPP addition level is 10 wt%, DDT suddenly increased (about 2 – 3 times), which was related to particle size. DDT increased significantly in the composite flour with large particle size, revealing that it took a long time between the amount of water added and the time when the dough reached the optimal elastic and viscous properties. The increase of DDT can be explained by adding non-glue flour that affects gluten quality. Particle size affected negatively related ST, suggesting the increase of gluten network which due to the fact that the composite dough with smaller particle size could maintain a longer time of mechanical processing in bread making process. This increase may be related to some components in the particle size componentizes, because tannin can interact with gluten through disulfide bonds (Girard et al., 2016) to increase dough ST.

### 3.4. Thermo-mechanical properties

Mixolab can measure the protein and starch properties of sample under the dual influence of mechanical shear stress and temperature. C1, C2, and slope-α can be used to characterize the thermo-mechanical properties of the sample protein (Table 4), and C3, C4, C5 to delineate thermo-mechanical properties of the starch component (see Table 5). The results suggested that GSKP, GSP, and GPP had significant effects on WA of the corresponding composite samples. For the dough with GSP addition at the studied levels, the WA was lower than that of the control group (p < .05), and the samples with GPP additions also exhibited slightly lower WA. This result is contrary to other studies because the addition of fibers or extracts leads to higher WA.

#### Table 3. Effects of different particle sizes of WF incorporated with GSP/GSKP/GPP on Mixolab parameters.

| Samples | Addition level (wt%) | Parameters |
|---------|---------------------|------------|
|         | D50 (μm) | C2 (Nmin) | DDT* (min) | ST* (min) | C1-C2 (Nm) | Slope-α | C2 (Nm) |
| WF*     | 0        | 96.52 ± 2.38 | 0.436 ± 0.005 | 4.207 ± 0.025 | 6.367 ± 0.045 | 64.0 ± 1.00 |
| GSKP*   | 10       | 86.73 ± 1.57 | 0.338 ± 0.001 | 4.053 ± 0.011 | 5.407 ± 0.025 | 61.5 ± 0.56 |
| GPP*    | 10       | 75.25 ± 1.30 | 0.361 ± 0.002 | 3.230 ± 0.036 | 5.677 ± 0.021 | 62.0 ± 0.42 |
| GSP*    | 10       | 62.86 ± 2.53 | 0.385 ± 0.002 | 3.153 ± 0.015 | 6.603 ± 0.005 | 62.5 ± 0.24 |

* WF: wheat flour; GSP: grape seed powders; GSKP: grape skin powders; GPP: grape pomace powders; DDT: dough development time; ST: dough stability; WA: water absorption.

The values are mean ± SD of three independent determinations. Means followed by different alphabetic superscripts within a column are significantly (p < 0.05) different.

#### Table 4. Effect of different addition levels of GSP/GSKP/GPP on protein thermomechanical properties.

| Samples | Addition level (wt%) | WA* (%) | DDT* (min) | ST* (min) | C1-C2 (Nm) | Slope-α | C2 (Nm) |
|---------|---------------------|--------|------------|-----------|------------|---------|---------|
| WF*     | 0                   | 67.5 ± 1.10 | 3.17 ± 0.05 | 6.20 ± 0.21 | 0.772 ± 0.10 | -0.078 ± 0.04 | 0.413 ± 0.03 |
| GSP*    | 5                   | 66.5 ± 0.05 | 4.85 ± 0.11 | 9.70 ± 0.24 | 0.640 ± 0.04 | -0.148 ± 0.01 | 0.438 ± 0.02 |
| GPP*    | 10                  | 63.6 ± 0.06 | 2.60 ± 0.02 | 10.40 ± 0.13 | 0.430 ± 0.02 | -0.068 ± 0.00 | 0.667 ± 0.12 |
| GSP*    | 10                  | 62.0 ± 0.05 | 1.35 ± 0.01 | 6.60 ± 0.34 | 0.347 ± 0.02 | -0.038 ± 0.00 | 0.789 ± 0.22 |

* WF: harina de trigo; GSP: polvos de semillas de uva; GSKP: polvos de piel de uva; GPP: polvos de orujo de uva; DDT: tiempo de desarrollo de la masa; ST: estabilidad de la masa; WA: absorción de agua.

Las medidas seguidas de distintos superíndices alfabéticos dentro de una columna son significativamente diferentes (p < 0.05).
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Los valores representan la media ±DE de tres determinaciones independientes. Las medias seguidas de distintos superíndices alfabéticos dentro de una columna son significativamente diferentes (p < 0.05).

(Boubaker et al., 2016; Huang et al., 2016; Park et al., 2012). On one hand, this phenomenon can be ascribed to GSP and GPP lack gluten and polysaccharides attributable to higher WA. On the other hand, lipids from GPP and GSP may partially coat the starch granules and gluten proteins, decreasing the WA during mixing (Mironesea & Codina, 2019). However, GSKP addition increased the WA of the composite sample, as the WA increased by 9.65% when 20 wt% GSKP was added, which is due to the high protein content in GSKP and the intrinsic character of dietary fiber. Furthermore, the protein in GSP is abundant in alanine and lysine, which are responsible for WA (Zhao et al., 2011).

DDT and ST are basically used as indicators of dough strength, which dictates the firmness of baked food products. In addition, this firmness is generally used as an indicator of product quality, and Hoye and Ross (2011) have reported that bread firmness increased with GSP addition. In this work, GPP, GSP, and GSKP displayed a great influence on DDT and ST, as shown in Table 4. Dough with GSKP addition showed the longest DDT, and followed by GPP and GSP. For GSKP, DDT increased from 3.17 min to 9.03 min with the addition level of 12.5 wt%, suggesting that the appropriate level of dried grape pomace can be used to enhance the dough strength and increase the agitation resistance of composite dough, therefore elevating its processing performance, as well as endowing a good gas holding capacity. Among the investigated GSP and GSKP addition levels, the maximum DDT showed at 12.5 wt%. This increasing of dough strength could be resulted from the phenolic substances in grape powders, and the positive correlations between total phenolic substances and ST have already been reported by Anil (2017). However, the dilution of gluten protein in the presence of GPP would lead to decreased dough strength, and it became the dominant effect when the addition level surpassed 12.5 wt%, rendering deteriorated ST.

Torque C2 values correlate to the weakening degree of protein under mechanical force and thermal treatment, and they inversely correlated to the dough mechanical stability (Operea et al., 2018). Several studies have suggested that C2 values can be used to predict the volume, external shape, and internal pore structure of biscuits (Mills et al., 2003; Montemayor-Mora et al., 2018). As shown in Table 4, when compared to the control group, the C2 values of samples with GSKP and GPP addition s initially decrease then increase with increasing addition levels, the lower C2 for GSKP and GPP with addition levels less than 10 wt% dough expressed higher protein weakening whereas pure wheat dough exhibited higher C2 indicating lower protein weakening. Which could be explained by the reduced gluten content and the destruction of the gluten network (Nikolic et al., 2013). However, when addition level exceeded 10 wt%, the opposite occurred, which could be probably ascribed to the higher content of phenolic substances (Anil, 2017). A similar trend can be observed in the case of the difference between peak C1 and C2 values (C1 - C2), which characterized the degree of protein weakening due to heat (Saavedra et al., 2021). The lowest C1- C2 values were determined for the composite dough containing 20 wt% GSKP, and this result was highly consistent with C2 value. This phenomenon could also be ascribed to high phenolic substances content (Anil, 2017).

Physicochemical properties of starch in composite wheat dough that evaluated by Mixolab included peak torque (C3), cooking stability (C4/C3), and setback (C5-C4), which were recorded at the heating temperature above 60°C. The peak torque (C3) values are related to the starch gelatinization process (Operea et al., 2018). An increase of C3 value was observed with the increased level of GSKP addition (see Table 5), and it was highly significant (p < .05). This indicated that the viscosity of dough increased after GSKP addition, which might be due to due to the quick rupture of starch granules leading to lower pasting temperatures and to higher paste consistency (Kohajdová et al., 2018). In the case of dough samples with GSP and GPP addition, the slightly decreased C3 value when the addition level was higher than 10 wt% (p > .05) suggested the decrease of gelatinization capacity of the composite dough sample, which was likely due to the slight increase in the quantity of free water in the dough, which consequently intensified starch hydrolysis because of the increased activity of amyrase in the flour mix (Kohajdová et al., 2018; Mironesea et al., 2012). The formation of amylase-lipid complexes, the
amount of amylose leached, and the free water competition between the leached amylose and ungelatinised granules are the three main factors that affect the starch gelatinisation process (Woodbury et al., 2021). The difference between the C5 and C4 values, which represent starch degradation (Rosell et al., 2010), have a significant decreasing trend with the increasing addition level for all three additives in contrast to the control group. The lower values (C5-C4) obtained for GSKP indicate a higher resistance to degradation (Hadnádev et al., 2013). The results showed that the rheological properties of dough play an important role in the production of cookies, as they determine the attributes and characteristics that are used to assess their quality (Kohajdová et al., 2018). A decrease in C4/C3 value compared to the control group was observed (see Table 5), indicating that GSP could potentially reduce cooking stability of the composite flour, which has been verified by our previous study using composite flour to make noodles and determine the cooking characteristics. However, the difference was not significant among different GPP or GSKP addition levels. In terms of the dough temperature during starch gelatinization, the addition of GSP, GSKP, or GPP decreased the T-C3 value, which reached minimum at the GSP addition level of 20 wt%. The presence of GSP, GSKP, or GPP promoted the gelatinization of starch was highly consistent with the existing reports by Silvia Mironesoa et al. (2012). The increased a-amylolytic activity of composite flour resulted in the highest T-C3 values, as the addition of GPP in wheat flour aggravated the deterioration of starch (starch decomposition) and increased the number of fermentable sugars in dough, which caused the decrease of the maximum viscosity gelatinization temperature and the viscosity of the formed dough gel (Codiná et al., 2012). With regard to the dough temperature (T-C4) in the active period of starch decompositi,

3.5. Dynamic rheological properties of dough

It is well known that the viscoelasticity of dough depends on its three-dimensional network originated from the interaction between gluten and starch or non-starch polysaccharides (Yang et al., 2020; Yang et al., 2020; Yuan et al., 2016). Dynamic rheological properties of dough can not only characterize its physical properties, such as adhesiveness and kneading resistance, but also determine the elasticity, hardness, volume, and color. The effect of GSP, GSKP, and GPP addition on the dynamic rheological properties of wheat dough was studied by oscillatory frequency scanning mode, and the storage modulus (\(G'\)), loss modulus (\(G''\)), and tangent value of loss angle (\(\tan \delta = G''/G'\)) were depicted as a function of frequency (Figure 1).
of frequency, as shown in Figure 1. Both G' and G'' showed an increasing trend with increased frequency within the range from 1 to 10 Hz can be observed for all samples, the same result with the report by luga et al. (2019) who emphasized this behavior was because of the presence of binding agents in the mix flour doughs and attractive forces between starch granules are predominant. The G’ values of all samples were higher than that of G'', indicating that the elastic properties of the doughs dominated their viscous properties (Khoigani et al., 2017), and all values of loss tangent tanδ were determined less than 1, indicating a typical dynamic rheological profile of weak gel with pronounced elastic properties (Ptaszek et al., 2009). G’ and G’’ had a downward trend with the increasing GSP or GPP addition level compared to the control group, as shown in Figure 1a,b,g,h), which was contrary to the results reported by Nie et al. (2019) for wheat dough supplemented with Flammulina velutipes powder. The decrease of moduli might be attributed to different interactions between starch granules with different particle sizes of GSP (Moreira et al., 2014).

Regarding the samples with GSKP addition, the elastic modulus (G') of all samples were higher than the viscous modulus (G''), and thus led to the tanδ < 1. G’ and G’’ increased in the whole range of frequencies, in dependency on the GSKP concentration and particle size, suggesting a solid elastic-like behavior of the composite flour dough formulations. As compared with the control group, the G’ and G’’ values of the composite samples decreased significantly with the increasing GSKP addition level, and reached to the lowest value when addition level was 12.5 wt% then increased gradually, and reached the maximum point at the addition level of 20 wt%, still lower than that of the control group, this fluctuation can be explained by the difference in hydration capacity between flour mixtures. This reduction was more prominent at a higher frequency. In addition, the different interactions between starch granules and GSP particle sizes also have an influence, and the structure and chemical composition of their particle sizes may be related to this variations (luga et al., 2019). As shown in Figure 1c,f,l), all dough samples with grape powder supplementation behaved much more liquid-like than the pure wheat dough because their tan δ was higher than the control. This was probably due to the dietary fiber in grape powders, which could strengthen the WA ability of dough. The significantly increased water promoted swelling of wheat flour starch and proteins, and thus increase the viscosity of the dough (Nie et al., 2019). These results suggested that grape powders addition had weakened the gluten network structure of dough. This might be due to the easy formation of hydrogen bonds between polysaccharides from grape powder and water molecules (Lerbret et al., 2005), which destroys the formation of gluten network in the dough (Peng et al., 2017). In addition, The oxidation resistance of polyphenols in grape pomace is not conducive to the processing of flour. Its reduction destroys the formation of disulfide bonds of gluten protein molecules, hinders the crosslinking and aggregation of gluten proteins, and brings adverse effects on the gluten network structure (Han & Koh, 2011). However, although the addition of grape pomace polyphenols destroyed the most important disulfide bond in gluten network structure, the Mixolab experiments showed that dough strength still enhanced (C2 value increased). The reason might be attributed to that some phenolic substances such as tannic acid from GPP forms a new cross-linking with gluten protein, which increases gluten strength (Zhang et al., 2010).

3.6. Microstructure of dough

The microstructures of pure wheat dough and composite dough with varying GSP, GSKP or GPP addition levels were observed by SEM (see Figures 2–4). As shown in the micrographs of the control group (0 wt% addition), starch granules (SG) were tightly wrapped and evenly distributed in a complete and continuous gluten network composed of gluten film (GF) and gluten strand (GS) (Struck et al., 2018). According to the report by Jekle and Becker (2011), the protein cross-linking network is formed during kneading and adding water, mainly through hydrogen bond, ionic bond, hydrophobic bond and covalent bond interaction. Higher magnification (2000x) clearly showed that gluten formed a continuous silk or sheet network structure. With the increasing GSP, GSKP, or

Figure 2. Scanning electron micrographs of composite dough supplemented with GSP at various levels. SG: starch granule; GS: gluten strand; GF: gluten film.

Figura 2. Micrografías electrónicas de barrido de la masa compuesta suplementada con GSP a diferentes niveles. SG: gránulo de almidón; GS: hebra de gluten; GF: película de gluten.
GPP addition level, the integrity and continuity of protein network structure were destroyed by large holes to varying degrees. At the GSP/GSKP/GPP addition level of 5 wt%, the amount of GF that could be found at high magnification was significantly reduced and the GS became thinner than that of the control group. In addition, at the addition level of 15 wt%, more SG exposed, some fibrous or bony structures could be clearly observed, while the network structures of filamentous or lamellar gluten proteins in all composite samples suffered from further broken. Although some portions of the GF could be observed in the dough with high level grape powders addition, they could not completely wrapped the SG, which appeared less embedded in the gluten network than those in the control sample, indicating that the addition of GSP/GSKP/GPP destroyed the network structure of wheat gluten proteins, and that increasing the addition level led to more deterioration, which is in line with the results of dynamic rheological dough analyses. However, the Mixolab results showed that certain amounts addition could increase dough strength, taking the dilution effect of additive and the weakening effect related to the reducibility of phenolic compounds into consideration, these phenomena might be due to the formation of new crosslinking between polyphenols and gluten protein, which dominated the scenario of gluten strength increasing. Hence, it necessitates more efforts to elucidate the underlying mechanism.

It can be seen from the microstructure changes of the dough that the dilution of wheat protein with >15 wt% flour substitution is too high, and does not effectively in integrating of grape powder in the wheat dough. The elasticity and ductility of the dough become poor due to the limitation of gluten by fiber and phenolic substances, which will lead to a decrease in gas retention, thus a decrease in bread volume.

4. Conclusion
This study investigated the effects of different addition levels of GSP, GSKP, and GPP on the rheological properties
and microstructures of wheat dough. The results of Mixolab analysis showed that GSP and GPP could reduce the water absorption of dough and improve the development time and stability of dough, while the addition of GSKP was inconsistent with the addition of GSP. In addition, the addition of three kinds of grape powders in different ranges could enhance the strength of dough. The dynamic oscillation experiment showed that in the whole frequency range, the addition of three grape powders reduced G′ and G″, but increased tanδ. The microstructure of dough samples showed that the addition of grape flour decreased the integrity and continuity of gluten network in dough. Overall, the addition of grape seed and grape skin powder had a significant effect on the quality of dough. Combined with rheological parameters (the dough strength is weaker when <10% GSKP/GPP was added; >10%, stronger doughs. For GSP, <15%, stronger doughs; >15%, weaker doughs) and microstructure results, and relatively high addition levels of 10% to 15% for GSP, 5% to 10% for GSKP/GPP seem promising to be successfully applied to wheat flour foods. The detailed addition levels in line with product characteristics requirements should be further developed. This work may provide fundamental insight into the application of grape pomace in food fortification, such as enrichment with polyphenols, fortification with minerals, improvement of fatty acid profile, and enrichment with fiber, among others.

Disclosure statement
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