Effects of cellulolytic treatment conditions on dietary fiber content of grape pomace and use of enzyme-treated pomace in cookie making

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Abstract. Grape pomace is a rich source of dietary fibers and phenolic compounds; it has been added to bakery products to enhance their dietary fiber content and antioxidant activity. Nevertheless, the Insoluble Dietary Fiber (IDF) content of grape pomace is significantly higher than the Soluble Dietary Fiber (SDF) content, the IDF/SDF ratio of bakery products was much higher than the recommended value of 3:1 from the dietetic associations. In this research, grape pomace was treated with cellulase preparation to partially convert IDF to SDF. The appropriate conditions of the cellulolytic treatment were as follows: initial moisture content of grape pomace of 7.5 g water/g dry basis, cellulase concentration of 6 U/g dry basis and treatment time of 1 h. Under these conditions, the SDF content increased by 20% and the IDF/SDF ratio decreased by 23.2%. Then, the enzyme-treated grape pomace (ETGP) and untreated grape pomace (UGPM) were separately mixed with wheat flour for cookie formulation; the grape pomace ratio was 20% of the composite flour weight. The use of ETGP or UGPM significantly improved the content of total, insoluble and soluble dietary fiber, total phenolics, anthocyanin of cookies as well as their antioxidant activity measured by DPPH and FRAP assays. Cookies supplemented with ETGP or UGPM had increased hardness and reduced brightness. The enzymatic treatment of grape pomace did not affect the total dietary fiber and antioxidant activity of cookies; however, their IDF/SDF ratio decreased to 12% and the value of 3.7 was close to the recommended ratio from the dietetic associations. Furthermore, the preference scores of ETGP added cookies and UTGP added cookies were higher than that of the control cookies without grape pomace addition.

1. Introduction
Cookies are widely available and consumed all around the world. In 2017, the sales of cookies accounted for 54% of the bakery market in Vietnam. Cookies contain a high amount of starch, sugar and lipid but lack dietary fiber and bioactive compounds [1]. The development of cookies with dietary fiber and antioxidants has attracted attention. In our country, high fiber and antioxidant cookies have
been produced from whole wheat flour. As wheat has been imported, it is crucial to find out potential sources of natural dietary fibers and antioxidants for cookie making.

Grape is one of the most widely grown fruits in the world, with approximately 79.1 million tons, of which more than 75% is used for wine and juice processing [2]. The pomace, which is the by-product of wine and juice production, includes skin, seeds and residual pulp and accounts for about 20% of the grape mass. Grape pomace is primarily used for cattle feed or fertilizer. This by-product contains 50-75% total dietary fibers, in which SDF, mostly pectin, accounts for 1-10% of total dietary fiber, and IDP includes cellulose, hemicellulose and lignin. Moreover, grape pomace also contains grape phenolic compounds with antioxidant activity [3]. From the point of view of human nutrition, SDF of the pomace may reduce blood sugar response and plasma cholesterol, prevent intestinal inflammation as well as act like a prebiotic. Furthermore, IDF may prevent constipation and improve the conditions of diabetic patients [4].

It is reported that grape pomace was added to bakery products to increase their dietary fiber content and antioxidant activity [5, 6]. However, the IDF/SDF ratio of supplemented cookies was approximately 4.7% and this value was much higher than the recommended value of the dietetic associations [5]. According to the dietetic associations, the IDF/SDF ratio of food products should be nearly 3:1 to improve the health benefits of the both fiber fractions [4]. Treatment of grape pomace with cellulase preparation can partially convert IDF into SDF [7]. However, the use of the enzyme-treated grape pomace in bakery production has not been considered. Moreover, a comparison of the quality of bakery products with enzyme-treated grape pomace and untreated grape pomace has not been reported.

In this research, grape pomace was treated with cellulase preparation to increase SDF content. The objective of the study was to determine appropriate conditions of the cellulytic treatment as well as to compare the quality of three cookie samples: cookies supplemented with enzyme-treated grape pomace, cookies added with untreated grape pomace and cookies without grape pomace addition.

2. Materials and methods

2.1. Materials

Grape pomace: Red grape berries (Red cardinal variety) were bought from Thai An Agricultural General Service Cooperative, Ninh Thuan Province, Vietnam. Fresh grapes were rinsed and air dried. The grapes were juiced and the obtained pomace was collected and evenly divided into polyethylene bags. The bags were then sealed and stored in a freezer (-20°C) for experimentation.

Baking ingredients: Wheat flour with 8% gluten (Dai Phong flour Co. Ltd, Vietnam), fresh chicken egg with 9-15% protein (Ba Huan Co. Ltd, Vietnam), diet sugar isomalt with 98% carbohydrate (Vikymobi Ltd., Vietnam), unsalted butter with 84% lipid (Pilot, Australia), acesulfame potassium with 98.8% purity (Vitasweet, China), refined salt with 98% sodium chloride (Mien Nam salt Inc., Vietnam); vanilla flavoring (Rayner’s, UK); baking powder (Alsa, France).

Chemicals used for analysis were purchased from Sigma-Aldrich (USA); Cellulast 1.5L with endo 1,4-beta glucanase activity was used in the enzymatic treatment of grape pomace; Termamyl with amylase activity and Alcanase 2.5L with protease activity were used for the fiber quantification; all enzyme preparations were provided by Brentag Vietnam Co.Ltd. One unit (U) of endoglucanase activity is defined as 1 μmol of glucose released from carboxymethylcellulose per minute under the assay conditions.

2.2. Experimental design

2.2.1. Cellulolytic treatment of grape pomace

Dissolving an appropriate amount of cellulase preparation in distilled water at 50°C, the enzyme solution was then added to the grape pomace for desirable moisture content and enzymatic activity. The mixture was incubated at 50°C in a 2L bioreactor at the mixing rate of 75 rpm. At the end of the
enzymatic treatment, the mixture temperature was increased to 95°C and kept for 10 min for enzyme deactivation. The obtained mixture was then dried in a convection dryer at 55-60°C to achieve 7-8% moisture content. Finally, the mixture was deseeded, pulverized and sieved through a 40-mesh screen to collect enzyme-treated grape pomace powder (EGPP). The investigated factors of the pomace treatment were water content of the pomace (3.5, 5.5, 7.5, 9.5g water/ g dry basis), cellulase concentration (0, 3, 6, 9, 12 U/g dry basis) and cellulolytic time (0, 1, 2, 3, 4 hours). For the untreated grape pomace powder (UGPP), fresh grape pomace was defrosted and subsequently dried, deseeded, pulverized and sieved under the same conditions for EGPP. Both EGPP and UGPP were sampled to determine proximate composition and compared.

2.2.2. Cookies making
EGPP and UGPP were separately mixed with wheat flour for cookie making. The ratio of EGPP or UGPP was 20% of the mixed flour weight. The control sample with grape pomace addition was also done under the same condition.

Other ingredients of cookie formulation including 305g fresh chicken eggs, 467g unsalted butter, 310g of isomalt, 4.4g refined salt, 10.6g of baking powder, 4g of vanilla, 87mL water were added to 1kg of wheat flour and grape pomace powder mixture [8].

Egg whipping (both egg yolk and white) was performed in the mixer (Model M8, UNIE) for 4 min at the mixing speed of 200 rpm; acesulfame potassium, isomalt, NaCl, vanilla flavour and baking powder were subsequently added to the egg mixture and mixed for 4 min; the butter was then added and mixed for 5 min. The wheat flour-SCG powder mixture was finally added and mixed for 2 min at 100 rpm. The dough was sheeted to 4 mm of thickness and cut into 35 mm in diameter using a cookie cutter.

The cookies were baked within 2 stages: the first stage was performed at 175°C for 14 min while the second stage was done at 150°C for 4 min. After baking, the cookies were cooled down to room temperature and put into polyethylene bags, vacuum-sealed to remove air.

2.3. Analytical methods
2.3.1. Proximate composition
Proximate composition of grape pomace, wheat flour and cookies was analyzed using AOAC methods [9]. The moisture was determined by AOAC 930.15 method. Lipid was quantified according to AOAC 960.39 method. Protein was estimated following AOAC 984.13 method. Ash was measured by AOAC 930.30 method. The soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) content were determined following AOAC 993.19 and AOAC 991.42 methods, respectively. The total dietary fiber (TDF) content was the sum of SDF and IDF. The amount of carbohydrate was calculated by subtracting the percentage of moisture, protein, lipid and ash content.

2.3.2. Antioxidant content and activity
Total phenolic content was determined by a spectrophotometric method using Folin-Ciocalteau reagent; the results were expressed in mg Gallic Acid Equivalent per 100g dry basis (mg GAE/100g dry basis) [10]. The total anthocyanin content (TAC) was determined following the pH differential method [10]. Antioxidant activity was estimated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. The results were expressed in micromole Trolox equivalent per 100g dry basis (μM TE/100g dry basis) [10].

2.3.3. Physical properties of grape pomace, wheat flour and cookies
Water and oil holding capacity of grape pomace and wheat flour were measured by a method described by Jia, et al. (2020) [11]. Diameter and thickness of cookies were evaluated using a method described by Mishra (2012) [12]. Hardness of cookies was estimated using a texture profile analyzer (Model 5543, Instron, USA) and 3 points measuring method reported by Aziah (2012) [13].
Instrumental colors were determined using CIELAB system, the results were expressed by L* (brightness), a* (from green to red) and b* (from green to yellow). The total color difference (ΔE) between the SCG added cookies and the control cookie was calculated by a formula previously described elsewhere [8].

2.3.4. Sensory evaluations of cookie products

Overall acceptability of cookies was measured using the method of Mudgil et al (2019) [14], with a maximum score point of 9 based on preference, from like to dislike. The participants were up to 60 people.

2.4. Statistical analysis

All experiments were triplicated. The results of this research were expressed as mean ± standard deviation. One-way analysis of variance and Tukey's comparison test with significance level at p < 0.05 were performed on Minitab 16.

3. Results and Discussion

3.1. Cellulolytic treatment of grape pomace

The effects of initial moisture content on the content of fiber fractions of grape pomace are showed in Figure 1.

![Figure 1](image_url)

**Figure 1.** Effects of initial moisture content on the content of insoluble dietary fiber IDF (A), soluble dietary fiber SDF (B), ratio of IDF/SDF (C) and total dietary fiber TDF (D) of grape pomace. The values with different letters in the same figure are significantly different (P <0.05). The enzyme concentration was 3 U/g dry basis and the treatment time was 2 h.

At cellulase concentration of 3 U/g dry basis and treatment time of 2 h, when the initial moisture content increased from 3.5 to 7.5 g water/g dry basis, the SDF content increased by 7.8% while the
IDF and TDF content was almost constant. An increase in the water content of the substrate improved the cellulolysis catalyzed by cellulase preparation. In principle, enzyme molecules readily interacted with substrate molecules and converted the insoluble beya glucan into soluble fibers, which had shorter chain. Therefore, the IDF:SDF ratio gradually decreased from 5.3 to 4.9. Further increase in water content from 7.5 to 9.5 g water/g dry basis decreased the content of SDF, IDF and TDF by 2.8%, 11.9% and 10.4%, respectively. Since the reduction of SDF was greater than that of IDF, the ratio of IDF:SDF was reduced to 4.4. The high amount of water in the mixture enhanced hydrolysis of both soluble and insoluble fibers even though the hydrolysis time and enzyme concentration remained constant. The soluble fiber was broken down into short-chain oligosaccharide molecules. It should be noted that oligosaccharides with polymerization index less than 9 could not be precipitated in ethanol 78% by volume, so they could not be quantified [15]. As a result, the soluble fiber content of the grape pomace was reduced. Similar results were reported in a previous study on the effects of moisture content on fiber hydrolysis by cellulase preparations. According to Yang et al. (2010), when the dry matter and water ratio of grape pomace changed from 1:10 to 1:15, the soluble fiber content increased from 10.9% to 12.3%. Nevertheless, the change in the dry matter and water ratio of the grape pomace from 1:15 to 1:20 decreased the soluble fiber content by 11.1% [16]. Thus, the appropriate water amount of grape pomace was 7.5 g water/g dry basis since the highest soluble fiber content and the least change in total fiber content were achieved.

The effects of cellulase concentration on the content of different fiber fractions of grape pomace are illustrated in Figure 2.

**Figure 2.** Effects of cellulase concentration on the content of insoluble dietary fiber IDF (A), soluble dietary fiber SDF (B), ratio of IDF:SDF (C) and total dietary fiber TDF (D) of grape pomace. The values with different letters in the same figure are significantly different (P <0.05). The initial moisture content of grape pomace was 7.5 g water/g dry basis and the treatment time was 2 h.
At the initial moisture content of 7.5 g water/g dry basis and treatment time of 2 h, when increasing cellulase concentration from 0 to 6 U/g dry basis, the amount of SDF increased by 20.8% while the IDF content decreased by 5.2%, the TDF content declined by 1.4% and the IDF/SDF ratio was reduced by 21.6%. Theoretically, the higher the enzyme concentration, the higher the insoluble fiber content that can be converted into low molecular weight products, leading to an increased content of the soluble fiber. Nevertheless, increase in enzyme concentration from 6 to 12 U/g dry basis, the content of SDF, IDF and TDF tended to be decreased. At the cellulase concentration of 12 U/g dry basis, the content of IDF, SDF and TDF decreased by 5.0%, 10.6% and 6.0%, respectively, compared to that at the enzyme concentration of 6 U/g dry basis. Previously, Chamorro et al. (2012) performed the treatment of red grape pomace with cellulase preparation of Laminex ® and found that increase in enzyme concentration enhanced the content of various sugars including rhamnose, arabinose, xylose and galactose in the pomace [16]. Recently, Krusir et al. (2020) reported a gradual decrease in total fiber content when increasing the cellulase concentration in the treatment of grape pomace from wine making [17]. Our experimental results show that at cellulase concentration of 6 U/g dry basis, the soluble fiber content achieved maximum while the IDF/SDF ratio was the lowest; this value was therefore used in the subsequent experiments.

The effects of treatment time on the content of different fiber fractions of grape pomace are demonstrated in Figure 3.

Figure 3. Effects of treatment time on the content of insoluble dietary fiber IDF (A), soluble dietary fiber SDF (B), ratio of IDF/SDF (C) and total dietary fiber TDF (D) of grape pomace. The values with different letters in the same figure are significantly different (P <0.05). The initial moisture content of grape pomace was 7.5g water/g dry basis and the cellulase dosage was 6 U/g dry basis.

At the initial moisture content of 7.5 g water/g dry basis and the cellulase dosage of 6 U/g dry basis, after 1 h of hydrolysis, the SDF content increased by 19.7% while the IDF content decreased by 4.5%.
Further increase in treatment time decreased the content of both SDF and IDF. Specifically, the SDF content of the grape pomace treated for 4 h was 11.7% lower than that treated for 1 h. It is suggested that prolonged time generated more hydrolytic products with low molecular weight, which were lost during the quantification of soluble fiber content [18]. The lowest IDF/SDF ratio of the grape pomace was recorded at the treatment time of 1 h and this value was 20.2% lower than that of the untreated grape pomace. Thus, the treatment time with cellulase preparation was chosen to be 1 hour.

Table 1 presents the proximate composition, antioxidant activity and physical properties of the untreated grape pomace, enzyme-treated grape pomace and wheat flour. The treatment of grape pomace with cellulase preparation lowered the IDF and TDF contents by 5.6% and 1.5%, respectively, whereas the SDF content increased by 20%. Consequently, the IDF/SDF ratio of the pomace decreased by 23.2%. In general, the content of dietary fibers of both grape pomace powder samples was superior to that of wheat flour, but the IDF/SDF ratio of wheat flour was lower. The decrease in total fiber content in grape pomace after the enzymatic treatment was responsible for the slight increase in protein and lipid content in grape pomace. Wheat flour was richer in protein and lipid than grape pomace. Moreover, the enzymatic treatment of grape pomace did not change the mineral content. Grape pomace was richer in minerals than wheat flour.

Table 1  Proximate composition, antioxidant activity and physical properties of main materials for cookie making (% dry basis).

|                        | Untreated grape pomace powder (UGPP) | Enzyme-treated grape pomace powder (EGPP) | Wheat flour |
|------------------------|-------------------------------------|------------------------------------------|-------------|
| Moisture content (%)   | 9±0.1a                              | 9.5±0.1b                                 | 12.3±0.2c   |
| Protein (% dry basis)  | 8±0.1a                              | 8.2±0.1b                                 | 11.2±0.1c   |
| Lipid (% dry basis)    | 4.4±0.1b                            | 4.7±0.2c                                 | 2.1±0.1a    |
| Ash (% dry basis)      | 4±0.1b                              | 4.1±0.1b                                 | 0.5±0.1a    |
| Starch (% dry basis)   | nd                                  | nd                                       |             |
| Carbohydrate (% dry basis) | 83.6±0.1b                             | 83±0.1a                                   | 86.2±0.1c   |
| Soluble fiber (% dry basis) | 8.0±0.1b                              | 9.6±0.1c                                  | 1.3±0.1a    |
| Insoluble fiber (% dry basis) | 44.4±0.2c                             | 41.9±0.1b                                 | 1.6±0.1a    |
| Total fiber content (% dry basis) | 52.3±0.1c                             | 51.5±0.1b                                 | 2.9±0.2a    |
| IDF/SDF                | 5.6±0.1c                            | 4.3±0b                                    | 1.3±0.1a    |
| Anthocyanin (mg/100g dry basis) | 27.8±0.4b                             | 26.7±0.4a                                 | nd          |
| Total phenolic content (mg GAE/100g dry basis) | 4968±26c                           | 4840±17b                                  | 231±6a      |
| Antioxidant activity by DPPH assay (μM TE/100g dry basis) | 44216±56c                         | 43957±90b                                  | 262±13a     |
| Antioxidant activity by FRAP assay (μM TE/100g dry basis) | 27268±46c                          | 26980±75b                                  | 140±11a     |
| Water holding capacity (g water/g dry basis) | 3.75±0.1b                          | 3.63±0.06a                                 | 0.89±0.03a  |
| Oil holding capacity (g oil/g dry basis) | 1.60±0.02c                          | 1.52±0.06b                                 | 0.90±0.02a  |
| L                      | 44.3±0.3b                           | 41.3±0.2a                                 | 91.4±0.1c   |
| a                      | 6.1±0.1b                            | 5.6±0.1b                                  | 4.8±0b      |
| b                      | 15.2±0.3c                           | 11.1±0.1a                                 | 12.8±0b     |
| ΔE                     | 48.5±0.4b                           | 51.3±0.2c                                 | 0±0         |

Values with different letters within the same row are statistically different (p < 0.05).
The enzymatic treatment also slightly reduced the total phenolic and anthocyanin content as well as the antioxidant activity of the grape pomace. The water and oil holding capacities of the enzyme-treated grape pomace powder were slightly lower than those of the untreated grape pomace. This was due to a decrease in total fiber content. In terms of instrumental color, the enzymatic treatment enhanced the darkening of the pomace. It can be explained that the cellulolysis produced oligosaccharides with reducing groups which react with free amino groups of the protein (Maillard reaction) during the pomace drying, resulting in darkened product [19]. The formation of melanoidin slightly reduced $a^*$ (red color) and $b^*$ values (yellow color) of grape pomace.

### 3.2. Comparison of quality of cookie samples

At the ratio of 20% UGPP or EGPP in the cookie formulation, the change in proximate composition of the product is illustrated in Table 2.

The moisture content of three cookie samples was approximately 4% in accordance with TCVN 5909-1995. It can be noted that the moisture content of UGPP and EGPP supplemented cookies was both higher than that of the control sample. This can be explained that the addition of grape pomace powder to the cookie recipe increased the dietary fiber content of the product. The hydroxyl group (-OH) of the fiber could bind with water, reducing the amount of water that can be evaporated during the baking.

|                         | Control cookies | Cookies supplemented with enzyme-treated grape pomace powder | Cookies supplemented with untreated grape pomace powder |
|-------------------------|-----------------|-------------------------------------------------------------|-------------------------------------------------------|
| Moisture content (%)    | 3.8 ± 0.1$^a$   | 4.0 ± 0.1$^b$                                              | 4.1 ± 0.1$^b$                                         |
| Protein (% dry basis)   | 8.5 ± 0.1$^c$   | 8.3 ± 0.1$^b$                                              | 8.0 ± 0.1$^a$                                         |
| Lipid (% dry basis)     | 23.2 ±0.1$^a$   | 25.2 ± 0.1$^b$                                              | 25.3 ± 0.1$^b$                                         |
| Ash content (% dry basis)| 1.1 ± 0.1$^a$   | 1.6 ± 0.1$^b$                                              | 1.5 ± 0.1$^b$                                         |
| Starch (% dry basis)    | 53.8 ± 0.2$^b$  | 38.2 ± 0.2$^a$                                              | 38.5 ± 0.3$^a$                                         |
| Carbohydrate (% dry basis)| 67.2 ± 0.1$^d$ | 65.0 ± 0.1$^b$                                              | 65.2 ± 0.2$^b$                                         |
| Soluble fiber (% dry basis)| 0.8 ± 0.1$^a$   | 2.1 ± 0.0$^c$                                              | 2.0 ± 0.0$^b$                                         |
| Insoluble fiber (% dry basis)| 1.1 ± 0.0$^a$   | 7.9 ± 0.1$^b$                                              | 8.1 ± 0.1$^c$                                         |
| Total dietary fiber (% dry basis)| 1.9 ± 0.1$^a$   | 10.0 ± 0.1$^b$                                              | 10.1 ± 0.2$^b$                                         |
| IDF/SDF ratio           | 1.5 ± 0.2$^a$   | 3.7 ± 0.1$^b$                                              | 4.2 ± 0.0$^c$                                         |
| Anthocyanin (mg/100g dry basis) | nd               | 7.8±0.2$^a$                                     | 8.1±0.3$^a$                                           |
| Total phenolic content (mgGAE/100g dry basis) | 94±13$^a$           | 459±9$^b$                                      | 463±10$^b$                                           |
| DPPH (µMTE/100g dry basis) | 234±32$^a$          | 2981±94$^b$                                    | 2947±165$^b$                                         |
| FRAP (µMTE/100g dry basis) | 126±5$^a$           | 2617±118$^b$                                   | 2646±117$^b$                                         |

Values with different letters within the same row are statistically different ($p < 0.05$).

The protein content of the control sample was slightly higher than that of the samples with EGPP and UGPP. This difference is due to various protein contents of wheat flour (11.2%), EGPP (8.2%) and UGPP (8.0%). The lipid and ash content of both UGPP and EGPP supplemented cookies were higher than that of the control sample due to the higher lipid and ash content of UGPP and EGPP. Moreover, the use of grape pomace in the cookie recipe increased the fiber content of the product. Grape pomace powder had a better oil holding capacity than wheat flour, thereby limiting oil loss.
during the rolling and cutting of the cookie dough. As a result, the lipid content of grape pomace supplemented cookies was higher than that of the control cookies.

The starch content of EGPP and UGPP-supplemented cookies were both lower than that of the control cookies since starch was not detected in grape pomace powder. In contrast, the total fiber content of EGPP and UGPP-supplemented cookies were roughly 5.3 times higher than that with UGPP. The difference in fiber content of the ingredients led to the difference in fiber content of cookies. On the other hand, the total fiber content of EGPP and UGPP-supplemented cookies was statistically similar even though the total fiber content of these two types of grape pomace was slightly different. This can be explained that the ratio of grape pomace used in the cookie formulation (20%) was not high enough to generate a significant difference in total fiber content among the two cookie samples. The total fiber content of the two samples with grape pomace were both greater than 6%, consistent with the standard for fiber content in high fiber food [20]. The enzymatic treatment resulted in a 12% reduction in IDF:SDF ratio, which was close to the recommended value of 3:1 of the dietetic associations [4].

Table 2 reveals that the total anthocyanin and phenolic contents of UGPP and EGPP-supplemented cookies were not different and were superior to those of the control sample. Similar results were reported by Szukudlarz et al. (2013) when white grape pomace was added to the biscuit recipe [5]. According to these authors, the total phenolic content of biscuits which was supplemented with 20% white grape pomace was 334 mg GAE/100g dry basis while the control sample had a total phenolic content of 85 mg GAE/100g dry basis. In our study, the antioxidant activities of the cookies with UGPP and EGPP evaluated by DPPH and FRAP assays were also statistically similar and were 12.6 and 20.7 times, respectively, superior to those of the control cookies.

Physical properties and overall acceptability of all cookie samples are presented in Table 3. The hardness of UGPP and EGPP-supplemented cookies were similar and lower than that of the control sample. The addition of grape pomace to the cookie recipe reduced the gluten content of the cookie dough, leading to a decreased hardness of cookies [21]. Reduction in hardness of bakery products was also reported in the studies of Kuchtová et al. (2018) [21] and Szkudlarz et al. (2013) [5] when wheat flour was partially replaced by red and white grape pomace powder in the baking recipe.

| Table 3. Physical properties and overall overall acceptability of cookies |
|---------------------------------|---------------------------------|---------------------------------|
| Control cookies                | Cookies supplemented with enzyme-treated grape pomace powder | Cookies supplemented with untreated grape pomace powder |
| Hardness (N)                   | 2123± 318<sup>b</sup>           | 1508 ± 246<sup>a</sup>          | 1583 ± 343<sup>a</sup>          |
| Diameter (mm)                  | 35.7 ± 0.1<sup>a</sup>          | 35.7 ± 0.1<sup>a</sup>          | 35.8 ± 0.5<sup>a</sup>          |
| Thickness (mm)                 | 7.1 ± 0.1<sup>a</sup>           | 7.1 ± 0.1<sup>a</sup>           | 7.2 ± 0.1<sup>a</sup>           |
| Spread factor (SF)             | 5.0 ± 0.0<sup>a</sup>           | 5.1 ± 0.0<sup>a</sup>           | 4.9 ± 0.1<sup>a</sup>           |
| L*                              | 60.6 ± 0.5<sup>c</sup>          | 39.7 ± 0.1<sup>a</sup>          | 42.6 ± 0.2<sup>b</sup>          |
| a*                             | 5.8 ± 0.1<sup>b</sup>           | 4.7 ± 0.2<sup>a</sup>           | 5.7 ± 0.3<sup>b</sup>           |
| b*                             | 33.4 ± 0.3<sup>c</sup>          | 14.5 ± 0.1<sup>a</sup>          | 17.8 ± 0.5<sup>b</sup>          |
| ΔE                             | 0.0 ± 0.0<sup>a</sup>           | 28.2 ± 0.4<sup>c</sup>          | 23.8 ± 0.8<sup>b</sup>          |
| Overall acceptability          | 5.83±1.57<sup>a</sup>           | 6.5±1.43<sup>b</sup>            | 6.03±1.48<sup>ab</sup>          |

Values with different letters within the same row are statistically different (p < 0.05).

In addition, the diameter, thickness and SF value of the three cookie samples were statistically similar. According to Szkudlarz et al. (2013) [5], when partially replacing wheat flour with white
grape pomace, the diameter and SF value of cookies increased while their thickness decreased. Another study of Kuchtová et al. (2018) [21] showed that the diameter and thickness of cookies reduced while their SF value increased when red grape pomace was used. The variation of these physical properties of cookies may depend on the proximate composition and physical properties of different grape pomace samples, which were originated from various grape varieties and processing conditions [5]. In this study, the grape pomace ratio of 20% did not generate a change in diameter, thickness and SF value of the cookie samples.

Color is an important property since color change can be clearly recognized by consumers. The lightness of UGPP and EGPP-supplemented cookies was lower than that of the control [22]. It can be explained that grape pomace was darker than wheat flour. Moreover, Maillard and caramelization reactions that happen during the baking process may cause the bakery product to become darker. Besides, the color of the cookie sample supplemented with EGPP was darker than that added with UGPP since the enzymatic treatment released oligosaccharides with reducing radicals. Thus, Maillard reaction was enhanced during the baking, and the EGPP-added biscuits had the lowest L* value. The color difference between the EGPP added cookies and the control was greater than that between the UGPP added cookies and the control.

Table 3 shows that the addition of EGPP and UGPP to cookie formulation significantly improved the sensory score of the product. According to the panelists, the balance of sweet and sour taste of both cookie samples with grape pomace was highly preferred in comparison with the sweet taste of the control cookie. The combined sweet and sour taste was due to residual organic acids in the grape pomace. It can be noted that the overall acceptability of the EGPP added cookies and UGPP supplemented cookies was similar.

4. Conclusion
The appropriate conditions of the enzymatic treatment of grape pomace were initial moisture content of 7.5g water/g dry basis, cellulase concentration of 6U/g dry basis, treatment time of 1 h. Under these conditions, the soluble dietary content (SDF) of grape pomace increased by 20% while the IDF/SDF ratio reduced from 5.6 to 4.3. The addition of grape pomace to the cookie recipe significantly improved dietary fiber content and antioxidant activity of the product. Besides, the hardness of cookies also increased and their color was darker than that of the control. Although the use of EGPP did not change the total fiber content and antioxidant activity of the product in comparison with that of UGPP, the IDF/SDF ratio of cookies with EGPP was closer to the recommended value suggested by the dietetic associations. Furthermore, the overall acceptability of cookies with UGPP or EGPP was higher than that of the control sample. Future in-vivo study is essential to clarify the role of IDF/SDF ratio of cookies on human health.

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5. References
[1] Mishra N and Chandra R, 2012, Development of functional biscuit from soy flour & rice bran, *International Journal of Agricultural Food Science*, vol. 2, no. 1, pp. 14-20.
[2] FAOSTAT.2021. *Crops and livestock products.* Available: https://www.fao.org/faostat/en/#data/QCL
[3] Iuga M and Mironeasa S, 2020, Potential of grape byproducts as functional ingredients in baked goods and pasta, Comprehensive Reviews in Food Science and Food Safety vol. 19, no. 5, pp. 2473-2505.

[4] Yu J and Ahmedna M, 2013, Functional components of grape pomace: their composition, biological properties and potential applications, International Journal of Food Science & Technology vol. 48, no. 2, pp. 221-237.

[5] Mildner-Szkudlarz S, Bajerska J, Zawirska-Wojtasiak R, and Górecka D, 2013, White grape pomace as a source of dietary fibre and polyphenols and its effect on physical and nutraceutical characteristics of wheat biscuits, Journal of the Science of Food and Agriculture, vol. 93, no. 2, pp. 389-395.

[6] Theagarajan R, Malur Narayanaswamy L, Dutta S, Moses J A, and Chinnaswamy A, 2019, Valorisation of grape pomace (cv. Muscat) for development of functional cookies, International Journal of Food Science & Technology, vol. 54, no. 4, pp. 1299-1305.

[7] Dumitru M and Jurcoane Ş, 2017, Researches concerning the enzymatic action of byproduct grapes, Scientific Bulletin, Series F, Biotechnologies vol. 21, p. 206.

[8] Lâm T N C, Trần T T T, Tôn N M N, Lê N Đ D, and Lê V V M, 2019, Nghiên cứu hưởng của ti lệ bã malt và bột mì đến chất lượng bánh quy giàu chất xơ Tạp chí Nông nghiệp và phát triển nông thôn, vol. 22, pp. 72-79.

[9] AOAC, 2000, A. International, Ed. Official Methods of Analysis, 12th ed. Gaithersburg, MD, USA.

[10] Nielsen S S, 2017, Food Analysis. Springer International Publishing.

[11] Jia M et al., 2020, Physical quality and in vitro starch digestibility of biscuits as affected by addition of soluble dietary fiber from defatted rice bran, Food Hydrocolloids, vol. 99, p. 105349.

[12] Mishra N and Chandra R, 2012, Development of functional biscuit from soy flour & rice bran, International Journal of Agricultural and Food Science, vol. 2, no. 1, pp. 14-20.

[13] Noor Aziah A, Mohamad Noor A, and Ho L-H, 2012, Physicochemical and organoleptic properties of cookies incorporated with legume flour.

[14] Mudgil D, Barak S, and Khatkar B S, 2017, Cookie texture, spread ratio and sensory acceptability of cookies as a function of soluble dietary fiber, baking time and different water levels, LWT, vol. 80, pp. 537-542.

[15] McCleary B V et al., 2012, Determination of insoluble, soluble, and total dietary fiber (CODEX definition) by enzymatic-gravimetric method and liquid chromatography: collaborative study, Journal of AOAC International, vol. 95, no. 3, pp. 824-844.

[16] Chamorro S, Viveros A, Alvarez I, Vega E, and Brenes A, 2012, Changes in polyphenol and polysaccharide content of grape seed extract and grape pomace after enzymatic treatment, Food Chemistry, vol. 133, no. 2, pp. 308-314.

[17] Krusir G, Sagdeeva O, Malovanyy M, Shunko H, and Gnizdovskyi O, 2020, Investigation of Enzymatic Degradation of Solid Winemaking Wastes, Journal of Ecological Engineering, vol. 21, no. 2, pp. 72-79.

[18] Dai F-J and Chau C-F, 2017, Classification and regulatory perspectives of dietary fiber, Journal of food and drug analysis, vol. 25, no. 1, pp. 37-42.

[19] Martins S I, Jongen W M, and Van Boekel M A, 2000, A review of Maillard reaction in food and implications to kinetic modelling, Trends in food science & technology, vol. 11, no. 9-10, pp. 364-373.

[20] 2006, European Parliament & Council, Regulation (EC) No 1924/2006 of the European Parliament and the of the Council on nutrition and health claims made on foods. Off. J. Eur. Union, pp. 9–25.

[21] Kuchtová V, Kohajdová Z, Karovicova J, and Lauková M, 2018, Physical, textural and sensory properties of cookies incorporated with grape skin and seed preparations, Polish Journal of Food and Nutrition Sciences, vol. 68, no. 4.
[22] Acun S and Gül H, 2014, Effects of grape pomace and grape seed flours on cookie quality, *Quality Assurance and Safety of Crops & Foods*, vol. 6, no. 1, pp. 81-88.