AN ABSTRACT OF THE THESIS OF

Rebecca M. Walker for the degree of Honors Baccalaureate of Science in Food Science and Technology presented on May 7, 2013. Title: Feasibility of developing wine grape pomace fortified baked goods for health promotion.

Abstract Approved: ____
Yanyun Zhao

Wine grape pomace (WGP) as a source of antioxidant dietary fiber was used to fortify bread and muffins bakery goods. Pinot Noir wine grape pomace (RWGP) and Pinot Grigio wine grape pomace (WWGP) replaced wheat flour at concentration between 5-20% (w/w). The finished bread and muffin products were evaluated for physicochemical, bioactive, and sensory properties. The WGP addition decreased the volume index of bread and muffin by a minimum of 25% and 7%, respectively. The highest total phenolic content (TPC) and radical scavenging activity (RSA) values for fortified bread and muffins were achieved in 15% RWGP with TPC and RSA values of 68.32 mg GAE/ serving (50 g) and 80.70 AAE mg/ serving (50 g), respectively, for bread and 2164 mg GAE/serving (55 g) and 1526 mg AAE/serving (55 g), respectively, for muffins. RWGP 15% breads and muffins also had the highest amount of dietary fiber per serving (6.33 g and 8.44 g, respectively). The sensory evaluation found no difference in overall liking of 5 and 10% RWGP fortified bread and muffin products compared to the control. This study demonstrated that WGP is a viable functional ingredient in bakery goods to increase TPC, RSA, and DF in consumer's diets.

Key words: wine grape pomace, dietary fiber, antioxidants, breads, muffins

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Feasibility of developing wine grape pomace fortified baked goods for health promotion

by

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Rebecca M. Walker, Author

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1. Introduction

Wine grape pomace (WGP), a byproduct from winemaking, constitutes about 20% of harvested grapes (Laufenberg and others 2003). Previous studies have verified this residual product as antioxidant dietary fiber (ADF) (Saura-Calixto 1998; Tseng and Zhao 2012). ADF is classified as being high in dietary fiber and polyphenolic compounds such as anthocyanins and flavanols that have antioxidant properties and potential health benefits. Furthermore, ADF may be utilized as a functional food ingredient because it provides additional health benefits above those of traditional nutrients (Lee and others 2004).

Antioxidants, the first component of ADF, are important compounds that can reduce the amount of reactive oxygen species (ROS) and free radicals in a system and in turn decrease the amount of damage caused by the ROS and free radicals. ROS and free radicals can have many negative effects on the body including lipid oxidation, protein degradation, and damaging DNA, which can result in modification of the DNA and gene expression (Gropper and others; Lee and others 2004). These reactions and modifications in the body are thought to contribute to aging, cancer, heart disease, and many other diseases or disorders (Gropper and others).

Among the more important antioxidant categories are polyphenolic compounds. Polyphenols are a large group of molecules categorized as having an aromatic ring with several hydroxyl groups (Manach and others 2004). These molecules are produced by plants as secondary metabolites and can further be classified based on their structure into phenolic acids, flavonoids, stilbenes, and lignins. A previous study evaluated red WGP for polyphenols and determined 39 types of anthocyanins, hydroxycinnamic acids, catechins, and flavonols present in the WGP (Kammerer and others 2004; Deng and others 2011). In the human body, polyphenols are absorbed into the intestine along with dietary fiber but are released from the fiber matrix into the colon because of the hospitable antioxidant environment provided by bacterial microbiota (Saura-Calixto 2010). As an antioxidant, polyphenols contribute to the prevention of cancers, cardiovascular disease and may be linked to neurodegenerative disease prevention as well (Scalbert and others 2005).

Dietary fiber, the second component of ADF, refers to carbohydrates and lignin that are intact and natural in plants but are nondigestible by human digestive enzymes and are found in high amounts in plant products including fruits, vegetable, and grains (Deng and others 2011; Gropper and others). Dietary fiber can further be classified as soluble or insoluble fiber. Both types of fibers provide health benefits when consumed in the diet. Soluble fiber contributes to reduced serum cholesterol level in addition to decreasing the rate of carbohydrate absorption, an extremely beneficial action for people with diabetes mellitus (Gropper and others). Insoluble fiber is not fermentable in the human colon and can aid in the treatment of many gastrointestinal diseases and disorders. Populations with higher fiber consumption have a decreased occurrence of gastrointestinal disorders, colon cancer, and heart disease.

This study investigated the feasibility of incorporating wine grape pomace into bread and muffins by partially replacing the flour (w/w) with red or white WGP in order to create functional food items. This application not only utilizes a waste product from the wine industry but also allows for the introduction of the natural functional food ingredients (dietary fibers and polyphenols) in commonly consumed baked items. A majority of the polyphenols from red wine grapes remain in the skins and seeds after pressing lending it as a reasonable source for the compounds. In addition, by using the pomace in its natural form instead of an extract, there is a higher retention of polyphenols and their synergistic effects (Saura-Calixto 1998). All samples were evaluated for both physiochemical, bioactive, and sensory characteristics in order to determine the optimal formulation for WGP fortified bread and muffins based on important quality attributors and consumer acceptance of the products.

2. Materials and Methods

2.1 Sample preparation

2.1.1 Pomace preparation

One red wine grape pomace (RWGP) and one white wine grape pomace (WWGP) were used in this study, *Vitis vinifera* L. cv. Pinot Noir and cv. Pinot Grigio, respectively. Both were acquired from Oregon State University Research Winery (Corvallis, OR, USA). The fresh pomace was stored at -24 °C until use. The frozen product was thawed at room temperature and the stems were manually separated from grape skins and seeds and discarded. In this study, the combination of wine grape skins and seeds were classified as pomace. The skins and seeds were dried in a 40 °C dehydrator (Model MP-2000, Enviro-Pak division of Tech-Mark Inc., USA) until weight stabilized. The final moisture contents for the RWGP and WWGP after dehydration were 11.14% and 19.57%, respectively. In comparison, the moisture content of flour used in the baked items was 11.40%. Dried samples were ground using a disc mill (Glen Mills Inc., NJ, USA) and sifted using a number 0.589 mm mesh. The powders were then vacuum packaged (FoodSaver Vacuum Sealer 1075, Tilia Inc., USA) (FoodSaver Roll, Tilia Inc., USA) and stored at – 24 °C until further use or analysis.

2.1.2 Bread preparation

The procedure and a modified formulation from AACC Method 10-10.3 (AACC International 2010) were used to prepare the bread samples. Flour WGP blends of 5, 10, and 15% pomace replacement of flour (w/w) for both the RWGP and WWPG were used in the bread samples based on the result from our preliminary studies. Three replicates of each sample were prepared. The modified formulation of the control bread is listed in Table 1. The barley malt was omitted from the recipe because it was unnecessary given the flour used. The sugar and NaCl were made into a concentrated solution of 545.5 sugar, 136.4 g NaCl, and 605 g water per liter; 11 mL of this solution was dispensed per bread loaf. The ascorbic acid was also put into solution using 0.25 g ascorbic acid in 250 mL water; 5 mL of solution was dispensed per bread loaf. Optimum water and optimum mix time were determined by preliminary mixograph tests of the control and WGP flour blends (Table 2).

To prepare the bread, all ingredients were mixed with a pin mixer (National Manufacturing Co., Lincoln, NE, USA) for the appropriate time for each sample forming a dough. The dough was fermented for a total of 120 min at 30 °C with 90% humidity (Doyon Inc., Québec, Canada), receiving punches after 69 and 34 min (Somerset Industries Inc., Billerica, MA, USA). The dough was molded (Mono Equipment Limited, Swansea, United Kingdom), panned, and allowed to rise for 58 min before baking at 196 °C for 18 min (Baxter, Orting, WA, USA). The loaves were immediately removed from their pans and cooled on a wire rack for 60 min before further analysis.

Table 1. Formulation for control pup bread loaf.

Flour[1]	100 g
Shortening[2]	3 g
Instant yeast [3]	1 g
Sugar[4]	6 g
NaCl[5]	1.5 g
Ascorbic acid [6]	5 mg

[1] Harvest King Winter Wheat Enriched Flour Unbleached, General Mills LLCC, Minneapolis, MN
[2] Crisco, The J.M. Smucker Company, Orrville, OH, USA
[3] SAF-Instant Red, SAF, Milwaukee, WI, USA),
[4] C & H Baker's Sugar, C&H Sugar Company, Inc., Crockett, CA, USA
[5] Kosher salt, Morton Salt, Inc., Chicago, IL, USA
[6] Sigma-Aldrich,St. Louis, MO, USA

Table 2. Optimum water and optimum mix time for each bread formulation based on preliminary mixograph data.

Sample	Water (%)	Mix Time (minutes)
Control	64.0	5.0
RWGP 5%	63.0	4.5
RWGP 10%	64.9	4.5
RWGP 15%	64.3	5.0
WWGP 5%	61.8	5.0
WWGP 10%	61.2	5.5
WWGP 15%	61.8	5.5

2.1.3 Muffin preparation

Flour blends of 5, 10, and 15% RWGP and 10, 15, and 20% WWGP (w/w) pomace replacement of flour were used to prepare muffin samples, again based on the results from preliminary studies. Eleven muffins of each sample were made. The muffin formulation is illustrated in Table 3 (Better Homes and Gardens 1997). The oven (GE double gas oven, General Electric Company, Fairfield, CT, USA) was conditioned with a pan of water and heated to 177.7 °C. The flour, WGP, sugar, baking powder, and salt were combined in the bowl of a stand mixer (KitchenAid Heavy Duty, Max watts 325, St. Joseph, MI, USA) and mixed with the paddle attachment until combined. The egg, milk, and oil were combined in a separate bowl. The wet ingredients were added to the dry and mixed on speed 1 for 20 s then the bowl was scraped. The batter was mixed again on speed 1 for 10 s. The batter was portioned at 62 g into muffin liners (Betty Crocker Baking Cups, Signature Brands, LLC, Ocala, FL, USA) and baked at 177.7 °C for 13 min, rotated, and baked for an additional 13 min. The muffins cooled in the pan for 10 min before being transferred to a wire rack to cool for another 60 min before analysis.

Table 3. Formulation for control muffin.

Ingredient	**(% w/w)**
Flour[1]	40.41
Sugar[2]	11.57
Baking Powder [3]	1.56
Salt[4]	0.22
Egg	9.35
Milk[5]	28.40
Vegetable Oil[6]	8.48

[1] All Purpose Gold Medal Flour, General Mills, Minneapolis, MN, USA
[2] C&H granulated sugar, C&H Sugar Company, Inc. Crockett, CA, USA
[3] Clabber Girl Double Acting Baking Powder, Clabber Girl Corporation, Terre Haute, IN, USA
[4] Kosher salt, Morton Salt, Inc., Chicago, IL, USA
[5] 2% milk fat
[6] Fred Meyer, Portland, OR, USA

2.2 Solvent retention capacity

Solvent Retention Capacity (SRC) is a common test used to evaluate the functionality of soft and hard wheat flours (Kweon and others 2011). This test evaluates the exaggerated swelling of polymer networks as a result of different solvents. The WGP flour blends were tested in order to gain a better understanding of the effect of the WGP on the polymer network in the bread dough system. The SRC looks at the thermodynamics of the polymer-solvent compatibility and their relation to solubility (Kweon and others 2011). Each solvent evaluates a different characteristic of the flour.

The SRCs were performed on flour (Harvest King Winter Wheat Enriched Flour Unbleached, General Mills LLCC, Minneapolis, MN) and flour blends of 5, 10, and 15% (w/w) RWGP and WWGP according to the AACC approved method 56-11 (AACC International 2010). Solvents used were deionized water, 50% (w/w) sucrose in water, 5% (w/w) lactic acid in water, and 5% (w/w) sodium carbonate in water. For each solvent separately, 50 mL screw cap tubes were weighed and the weight recorded. Flour or flour blends of 5.000 ± 0.050 g were weighed into each tube and 25 mL of the appropriate solvent was added. The mixture was shaken vigorously by hand for 5 seconds to suspend the flour or blend. The mixture was then allowed to hydrate for 20 minutes. The mixture was shaken by hand for approximately 5 s at 5 min intervals during hydration. Tubes were immediately transferred to a Beckman GS-15R centrifuge (Beckman Coulter, Inc., Brea, CA) and centrifuged at 1,000g for 15 min. The supernatant was decanted and the tubes drained at a 90° angle for 10 min on a paper towel. Total weight of tube, cap, and pellet was measured.

2.3 Physicochemical properties of baked good items

2.3.1 Moisture content

AACC Methods 44-40 and 62-05.01 (AACC International 2010) were used to determine the moisture content of all three types of baked goods. Samples were sliced into 2-3 mm thick cross sections and allowed to dry on a large sheet of paper overnight at room temperature. The samples were then ground using a disk mill grinder and duplicates of 2 g of that powder were dried in a vacuum oven at 100 °C with 25 mm Hg overnight in order to obtain a constant weight. The moisture content of samples was calculated based on the weight changes before and after the vacuum oven and recorded on the wet base. Each sample was tested in triplicate.

2.3.2 Water activity

Water activity was measured using the AquaLab water activity meter (Decagon Device, Inc., USA) using samples from the gravimetric center of samples from each baked good item. Each sample was tested in triplicate.

2.3.3 Color

The lightness, chroma, and hue of the crust of each baked good were determined by a colorimeter (Minolta, Konica Minolta Holdings, Tokyo, Japan) the day of baking. Measurements were taken on the top crust in the center of three loaves of bread and across the top crust of three random muffins with their muffin liners still attached. The measured L* (lightness), a* (redness), and b*(yellowness) values were further calculated as hue angle (arctan(b*/a*)) and chroma values ($\sqrt{a^2+b^2}$).

2.3.4 Volume

The volume of each bread loaf was measured immediately after the loaf was removed from the oven using AACC Method 10-05, the rapeseed displacement method (AACC International 2010). Muffin volume index was determined using a modified AACC Method 10-91.01 (AACC International 2010) on three muffin samples. Muffins were sliced vertically in half and digitally scanned with a 100 mm ruler. The images were used to measure the height at the midpoint and 1.5 ± 0.03 cm from the midpoint along the base of the muffin. The sum of the three lengths determined the volume index in centimeters.

2.3.5 Texture

All texture analyses were conducted on a TA-XT2 Texture Analyzer (Stable Micro Systems, Texture Technologies Corp., Scarsdale, NY, USA). All of the data was expressed as force versus time.

Muffin samples were analyzed using Method CAK1/P36R (2013a). Samples were prepared by cutting cross sections of three muffins of each sample from the center of each muffin at 2.0 cm. The muffin cross sections were measured for firmness and springiness using a modified compression test with a 25 mm diameter cylindrical probe (Acosta and others 2011). The forces were measured by compressing the samples by 25% of their initial height at a probe speed of 1.0 mm/s. The distance was held for 60 s.

Firmness was equal to the maximum force achieved at the first compression in grams. Springiness was determined as the ratio of the force at 60 s compared to the maximum force.

The bread was evaluated for firmness, springiness, and gumminess. Bread loaves were cut into 25 mm thick slices from the center and 29.5 mm diameter circles were cut from the center of those slices for a standard TPA test (Bourne 2002) using a 50 mm diameter cylindrical probe. Firmness was measured as the maximum force at the first compression in grams. Springiness was calculated as the distance of the height after the second compression divided by the distance of the height after the first compression. Chewiness was calculated as the area under the peak of the second compression divided by the area under the peak of the first compression, multiplied by firmness and springiness and was reported in grams (Bourne 2002).

2.4 Bioactive compound analysis

2.4.1 Sample extract

Samples were extracted for the antioxidant assays by the following methods detailed by Wu, Frei, Kennedy, & Zhao (2010) for the bread and Tseng, Zhao (2012) for the muffins. All samples were freeze dried (The Virtis Company Inc., Gardiner, NY, USA) and ground into powders with a disk mill and pass through a 0.589 mesh sieve. The bread sample powders were extracted by using 100% aqueous acetone (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA) with 0.01% HCl (v/v) then sonicated at 50/60 Hz, 117 V, and 185 W (Branson B-220H, SmithKline Co., Shelton, CT, USA) for 0.5 min followed by centrifugation at 4000xG for 5 min (CL International Clinical Centrifuge, International Equipment Co., Boston, MA, USA). The supernatant was decanted to a 200 mL centrifuge bottle. This process was repeated with 70% aqueous acetone with 0.01% HCl (v/v). The supertant was mixed with 50 mL chloroform (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA) in the centrifuge bottle and centrifuged for 30 min at 3000xG in a refrigerated centrifuge at 4 °C (Sorvall RC-5C Plus Centrifuge, DuPont, Wilmington, DE). The supernatant was decanted in a separator funnel, purified using a rotating vacuum evaporator at 40 °C, and then diluted in a 50 mL volumetric flask. The muffin powders were extracted by 70% acetone /0.1% HCl /29.9% water (v/v/v) at a solvent to powder ratio of 4:1 (v/w). The mixture was sonicated for 60 min, and centrifuged at 10,000xG for 15 min. This procedure was repeated three times. All supernatants were then combined and concentrated using a rotation evaporator (Brinkmann Instruments, U.S.A.) at 40 °C. The extracts were then transferred to 2.0 mL microtubes and stored at -17 °C until further use.

2.4.2 Total phenolic content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu assay as outlined using the diluted extracts made from the samples in triplicate (Singleton and others 1999). The extracts reacted with the Folin-Ciocalteu (Sigma-Aldrich, St. Louis, MO, USA) reagent for 10 min then were heated at 40 °C with 20% $NaCO_3$ (Macron Fine Chemical, Avantor Performance Materials, Center Valley, PA, USA) for 15 min. The absorbance was measured at 765 nm using a spectrophotometer (UV160U, Shimadzu, Japan). A gallic acid (Sigma-Aldrich, St. Louis, MO, USA) standard curve was used to evaluate the data. The data was reported as mg gallic acid equivalents (GAE)/serving, where a serving of bread is 50 g and a serving of muffin is 55 g, as defined in the Code of Federal Regulations (1977b).

2.4.3 Radical scavenging activity

The radical scavenging activity (RSA) was determined by the 1,1-diphenyl-2-picryhyrazyl (DPPH) assay (Sharma and Bhat 2009) using the diluted sample extract in triplicate, which reacted with the DPPH (Sigma-Aldrich, St. Louis, MO, USA) in a solution of 9 mg DPPH/ 100 mL methanol (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA). The absorbance was measured by spectroscopy (UV160U, Shimadzu, Japan) at 517 nm and a standard of ascorbic acid was used to determine the results as mg ascorbic acid equivalents (AAE)/serving (Sharma and Bhat 2009).

2.4.4 Dietary fiber

Dietary fiber was determined using the freeze dried powders from each sample according an alternate enzymatic-gravimetric AOAC Method 985.29 (AOAC International 2012). Samples were treated with α-amylase 80mM (Sigma-Aldrich, St. Louis, MO, USA) and pH 6.0 phosphate buffer solution at 95-100 °C for 30 min. The pH of the solution was adjusted to 7.5 ± 0.2 with 0.275N NaOH (Macron Fine Chemical, Avantor Performance Materials, Center Valley, PA, USA) followed by a protease (Sigma-Aldrich, St. Louis, MO, USA) treatment at 60 °C for 30 min. The pH of the solution was adjusted to 4.2-4.4 with 0.325M HCl. The solution was treated with amyloglucosidase (Sigma-Aldrich, St. Louis, MO, USA) and heated at 60 °C for 30 min. The samples were filter over diatomaceous earth with 80 and 95% ethanol. One replicate of each sample was incinerated in a muffle furnace at 525 °C for 5 h to determine ash content. The results were expressed as grams dietary fiber per serving of bread or muffin.

2.5 Sensory evaluation

Approval to conduct a sensory panel evaluation was received from the Institute Review Board at Oregon State University. Panelists were recruited by e-mail to the graduate students and staff in the Department of Food Science & Technology and members of the Food and Fermentation Science Club at Oregon State University. Twelve panelists (between the ages of 18-30, 4 males and 8 females) participated in the sensory evaluation of both the products. Muffin samples were prepared in small muffin tins then massed out at 22 g of batter and baked for 17 minutes. Bread samples were prepared by cutting even thickness slices of the pup loaves. Panelists evaluated a control and two samples of both baked good items with 5 and 10% RWGP flour blends. Samples were randomly assigned a 3-digital number for each baked item and panelists were given distilled water and unsalted saltine crackers (Fred Meyer, Portland, OR, USA) as palate cleansers. Panelists rated the likeness of overall liking, color, aroma, flavor, sweetness, texture, mouth feel, moistness, and aftertaste on a 9-point hedonic scale (9= like extremely, 5= neither like nor dislike, 1= dislike extremely). A 5-point 'Just About Right' (JAR) scale was also used to evaluate color (5= much too dark, 3= just about right, 1= much too light), sweetness (5= much too sweet, 3=just about right, 1= not at all sweet enough), mouth feel (5=much too sticky, 3= just about right, 1= much too dry), and moistness (5= much too stick, 3= just about right, 1= much too dry). Two open-ended questions were asked after each sample to allow the panelists to describe reasons for liking or disliking the sample.

2.6 Statistical analysis

All data excluding the sensory panel was analyzed by analysis of variance (ANOVA) with StatGraphics Centurion XVI.I software (StatPoint Technologies Inc., Warrenton, Virginia). Variables were considered significant with ($P \leq 0.05$) and differences were determined based on LSD test. Data from the sensory panel was analyzed using the Compusense LAB software (Compusense Inc., Guelph, Ontario, Canada). Variables were considered significant with ($P \leq 0.05$) and differenced were determined based on Tukey's HSD test.

3. Results and Discussion

3.1 Solvent retention capacity

Table 4 reports the results of each SRC test for the different WGP blends. The first solvent was water, which was also the reference and describes the water holding capacity (WHC) of the water. The control flour had the highest WHC compared to all of the WGP flour blends. The WWGP flour blends had smaller WHC values than the RWGP flour blends; this may be attributed to the already high moisture content of the WWGP.

The second solution of sucrose was used to hydrate the solvent-accessible pentosans. Pentosans make up about 1-2% (w/w) of wheat flour but can have a large impact on the gluten properties by interacting with the proteins, causing a negative influence on the gluten yield and extension capabilities (Wang and others 2004). The sucrose SRC value exaggerates the swelling of the pentosan network. The control had significantly lower sucrose SRC value than all WGP flour blends. As the percent of WGP increased in the flour blends, the sucrose SRC increased in both the RWGP and WWPG.

Lactic acid (LC) was the third solution and it exaggerates the swelling of the glutenin network. Glutenins are responsible for the elasticity and strength of the bread (Sivam and others 2011) and the LC SRC value can be used to evaluate the gluten quality and functionality (Kweon and others 2011). This value has also been shown to correlate with the loaf volume of hard winter wheat bread. The control, 5 and 10% RWGP had significantly lower LC SRC values than the RWGP 15% flour blend and all WWGP flour blends.

Lastly, the Na_2CO_3 hydrates the solvent-accessible amylopectin in damaged starches. The Na_2CO_3 SRC value allows for the damaged or pregelatinized starches to be discriminated from the undamaged, native starch. Starches are damaged during the milling process and as a result, the damaged starches have more free volume around the granules to allow for more water absorption and greater amylase access to the granule compared to undamaged starches (Barrera and others 2007). Amylase breaks down the starch granule to produce a source of sugar needed by the yeast for fermentation (Barrera and others 2007). The RWGP 15% flour blend had the highest Na_2CO_3 SRC value (77.8%) followed by the RWGP 10% and WWGP 15% flour blends (76.1 and 76.0%, respectively). The control, RWGP 5%, and WWGP 5% flour blends had significantly lower Na_2CO_3 SRC values compared to the other flour blends. The lactic acid and Na_2CO_3 SRC values did not conform to expectations suggesting that the flour blends exceed the scope of those methods.

Table 4. SRC results of different WGP flour blends with water, sucrose, lactic acid, and Na_2Co_3 solvents.

	Water (%)		Sucrose (%)		Lactic Acid (%)		Na_2Co_3 (%)	
Control	72.24 ± 1.45	a	106.32 ± 1.08	a	97.49 ± 4.97	a	72.00 ± 0.72	a
RWGP 5%	69.36 ± 0.65	bc	114.78 ± 1.00	b	94.08 ± 3.83	a	72.41 ± 0.30	a
RWGP 10%	70.14 ± 0.34	b	122.97 ± 1.23	c	92.57 ± 2.10	a	76.14 ± 1.04	b
RWGP 15%	68.74 ± 0.66	bcd	136.16 ± 0.94	d	109.29 ± 1.77	b	77.78 ± 1.36	c
WWGP 5%	68.05 ± 0.77	cd	113.94 ± 0.59	b	113.12 ± 13.25	b	72.40 ± 0.53	a
WWGP 10%	67.64 ± 0.64	d	124.47 ± 2.37	c	116.38 ± 7.24	b	74.10 ± 0.86	d
WWGP 15%	68.04 ± 1.10	cd	136.24 ± 3.25	d	108.97 ± 4.45	b	75.97 ± 1.07	b

*Means and standard deviations (SD) followed by same letter in the same column are not significantly different ($P<0.005$, $n=21$).

3.2 Physicochemical properties of baked good items

3.2.1 Moisture properties

There was no significant difference between the bread samples for both moisture content (26.88 - 29.80%) and water activity (0.93-0.95) (Table 5). This may be attributed to the use of the optimal hydration (Table 2) for each loaf, instead of using the same hydration across all of the loaves. The optimal hydration accounted for the different characteristics of the RWGP and WWGP and their effect on the moisture in the sample dough with the purpose of creating similar loaves of bread.

The muffin samples however did see a difference in moisture content and water activity between different treatments. All WWGP samples and the RWGP 10% sample had significantly higher moisture contents than that of the control and RWGP 5 and 10% samples. The WWGP 20% sample had the highest moisture content out of all the samples (33%) (Table 5). These differences might be explained by the WWGP having a higher moisture content than the control flour and RWGP, most likely caused by the high concentration of sugar in the WWGP, which acts as a humectant and retains moisture in the WGP.

When looking at the water activity of the muffin samples, there was no significant difference between the control and all RWGP samples (Table 5). There was however a significant decrease in the water activity of the WWGP 15 and 20% samples compared to the control. Again, the humectant characteristic of the sugar in the WWGP might be the cause of the sugar to trap available water, decreasing the amount of available water to participate in reactions.

Table 5. Effect of different percentages of RWGP and WWGP flour replacement (w/w) in bread and muffins on the moisture content and water activity of samples.

		Moisture Content (%)	Water Activity (Aw)
Bread	Control	28.61 ± 0.0	0.94 ± 0.00
	RWGP 5%	28.82 ± 0.1	0.95 ± 0.00
	RWGP 10%	29.80 ± 0.2	0.94 ± 0.01
	RWGP 15%	26.88 ± 0.6	0.94 ± 0.01
	WWGP 5%	29.18 ± 0.0	0.94 ± 0.01
	WWGP 10%	26.40 ± 3.6	0.94 ± 0.00
	WWGP 15%	29.17 ± 0.6	0.93 ± 0.01
Muffins	Control	28.38 ± 0.09 a	0.94 ± 0.01 ab
	RWGP 5%	29.07 ± 0.21 ab	0.94 ± 0.00 b
	RWGP 10%	30.29 ± 1.59 abc	0.93 ± 0.01 acd
	RWGP 15%	29.88 ± 0.72 bcd	0.92 ± 0.00 ace
	WWGP 10%	31.73 ± 0.38 d	0.93 ± 0.01 ac
	WWGP 15%	31.10 ± 0.12 cd	0.91 ± 0.01 de
	WWGP 20%	33.00 ± 0.16 e	0.91 ± 0.01 e

*Means and standard deviations (SD) followed by same letter in the same column are not significantly different ($P<0.005$, $n=14$ for moisture content measurement, $n=21$ for water activity measurement).

3.2.2 Color

The RWGP used in this study was a dark purple to red color. Color values of WGP were found in a previous study in our laboratory by Tseng & Zhao (2012). The WWGP has a yellow brown color. The color of the control bread was compared to the WGP blend products (Table 6). All WGP fortified breads were significantly darker than the control. For both the RWGP and the WWGP fortified breads, the color became darker as the percentage of WGP increased, which was to be expected with the concentration of pigments from the WGP. The WWGP 15% bread was significantly darker than all other samples. This may be attributed to the Maillard reaction in the bread because of the higher sugar content of the WWGP. Maillard browning occurs when an amino acid and reducing sugar are heated, the conditions of which were provided in both the bread and muffin baked goods.

All WGP fortified breads had significantly less saturated color (lower chroma value) than the control (Table 6). For both the RWGP and the WWGP fortified bread, the color saturation decreased (decreasing chroma values) as the amount of WGP increased. All RWGP and WWGP samples had significantly different chroma values. All WWGP fortified breads had significantly lower hue angles than the control and RWGP samples. For the WWGP fortified breads, as the percentage of WGP increased, the hue angle decreased, indicating a more red color.

The color of the control muffin was compared to all WGP samples in Table 6. All WGP muffin samples were significantly darker than the control. For both the RWGP and the WWGP fortified muffins, the products got darker as the percent WGP increased. In addition, all WGP fortified muffins had significantly lower hue angle than that of the control. As the percent WGP increased, the hue angle for both the RWGP and the WWGP muffins decreased significantly, indicating a more red color.

Table 6. Effect of different percentages of RWGP and WWGP flour replacement (w/w) on the lightness, chroma, and hue angle of bread and muffin crusts.

		Lightness	Chroma	Hue Angle
Bread	Control	48.69 ± 0.91 a	30.14 ± 0.83 a	1.06 ± 0.01 a
	RWGP 5%	45.17 ± 0.51 b	24.12 ± 0.46 b	1.06 ± 0.00 a
	RWGP 10%	42.65 ± 0.66 c	20.24 ± 0.71 c	1.05 ± 0.01 a
	RWGP 15%	38.86 ± 1.43 d	17.68 ± 1.03 d	0.99 ± 0.03 b
	WWGP 5%	42.04 ± 2.17 c	23.56 ± 1.03 b	1.00 ± 0.00 b
	WWGP 10%	38.51 ± 0.80 d	21.63 ± 1.01 c	0.92 ± 0.01 c
	WWGP 15%	35.63 ± 0.28 e	17.33 ± 0.97 d	0.86 ± 0.01 d
Muffins	Control	82.47 ± 0.93 a	32.56 ± 0.27 a	1.53 ± 0.00 a
	RWGP 5%	56.94 ± 0.83 b	15.87 ± 0.80 b	1.32 ± 0.01 b
	RWGP 10%	49.39 ± 0.23 c	12.00 ± 0.47 c	1.16 ± 0.00 c
	RWGP 15%	43.07 ± 1.01 d	12.05 ± 0.11 c	1.09 ± 0.01 d
	WWGP 10%	62.26 ± 0.31 e	23.27 ± 0.46 d	1.33 ± 0.01 e
	WWGP 15%	57.07 ± 1.60 b	21.60 ± 0.54 e	1.27 ± 0.00 f
	WWGP 20%	51.47 ± 0.94 f	22.38 ± 0.47 f	1.22 ± 0.01 g

*Means and standard deviations (SD) followed by same letter in the same column are not significantly different ($P<0.0005$, n=21 bread, n=30 muffins).

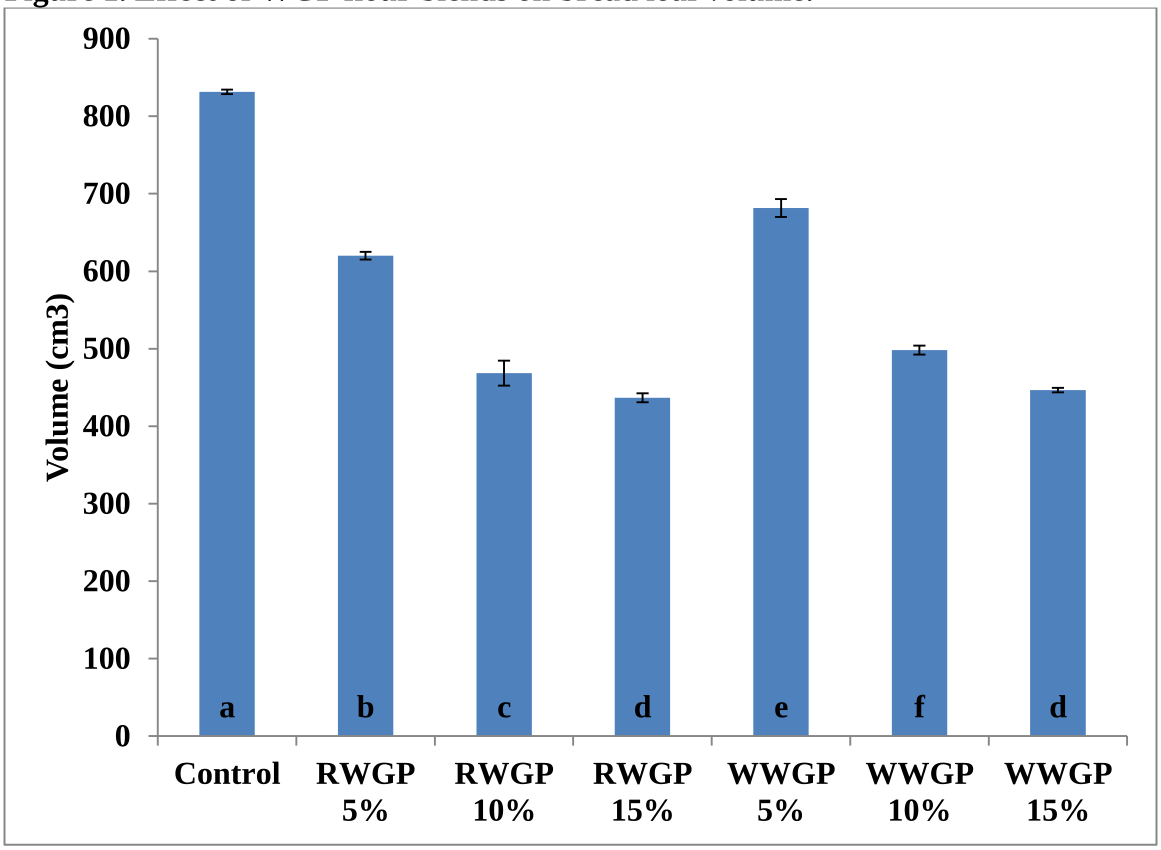
3.2.3 Volume

Loaf volume is the most important characteristic of baked goods for indicating the baking performance (Kohajdová and others 2012). All WGP fortified breads had significantly lower loaf volumes than the control (Figure 1), in which the control had the highest volume of all bread loaves of 832 cm^3 while the RWGP 15% and WWGP 15% samples had loaf volumes of 437 and 447 cm^3, respectively. For both the RWGP and WWGP fortified breads, loaf volume decreased along with the increase in WGP replacement of flour. This decrease in loaf volume was also observed in carrot pomace fortified wheat rolls (Kohajdová and others 2012) and grape byproduct fortified sourdough mixed rye bread loaves (Mildner-Szkudlarz and others 2011). This occurrence may be explained by the fibers from the WGP damaging the dough structure resulting in a decrease in CO_2 gas retention in the dough matrix (Kohajdová and others 2012). Kohajdová and others (2012) also discussed the possibility of increased binding of the fiber to water in the system that could result in less available water to develop the starch- gluten network and a less developed gluten network would cause a decrease in loaf volume.

Muffins fortified with WGP had significantly lower volume indexes than the control (Figure 2). As seen with the WGP fortified bread, both RWGP and WWGP fortified muffins had a decrease in volume index as the WGP percentage increased. The RWGP 15% and WWGP 20% muffins had significantly lower volume indexes than the RWGP 5% and WWGP 10% ones, respectively. The RWGP 15% muffin had a volume index of 12.8 cm compared to RWGP 5% sample with a volume index of 13.5 cm. The WWGP 20% muffin had a volume index of 12.4 cm compared to the 13.8 cm volume index of

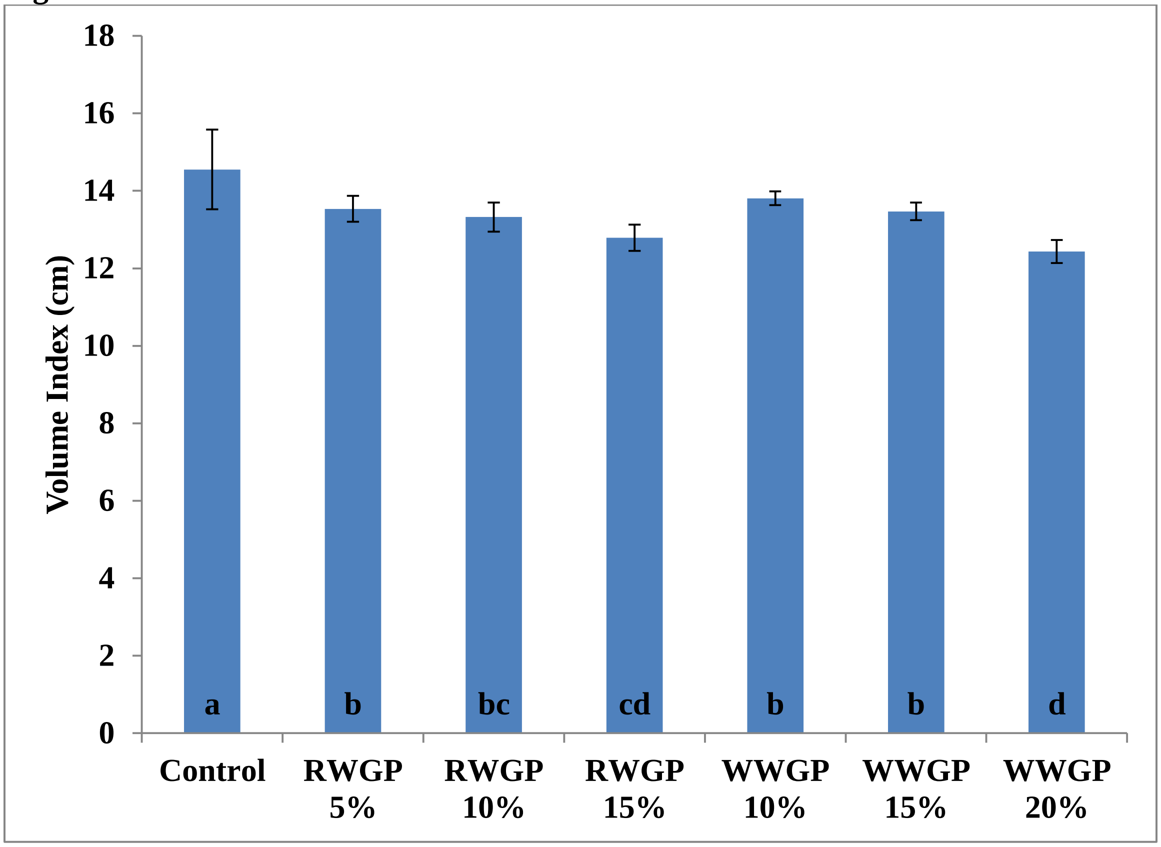
WWGP 5% muffin. This trend was also seen in a study of the addition of apple pomace to muffins (Vasantha Rupasinghe and others 2009). The destruction of CO_2 gas bubbles previously mentioned may explain this phenomenon of decreased muffin volume as well.

Figure 1. Effect of WGP flour blends on bread loaf volume.



*Columns with different letters at their base are not significantly different (P<0.0005, n=21).

Figure 2. Effect of WGP flour blends on muffin volume index.



*Columns with different letters at their base are not significantly different (P<0.0005, n=21).

3.2.4 Texture

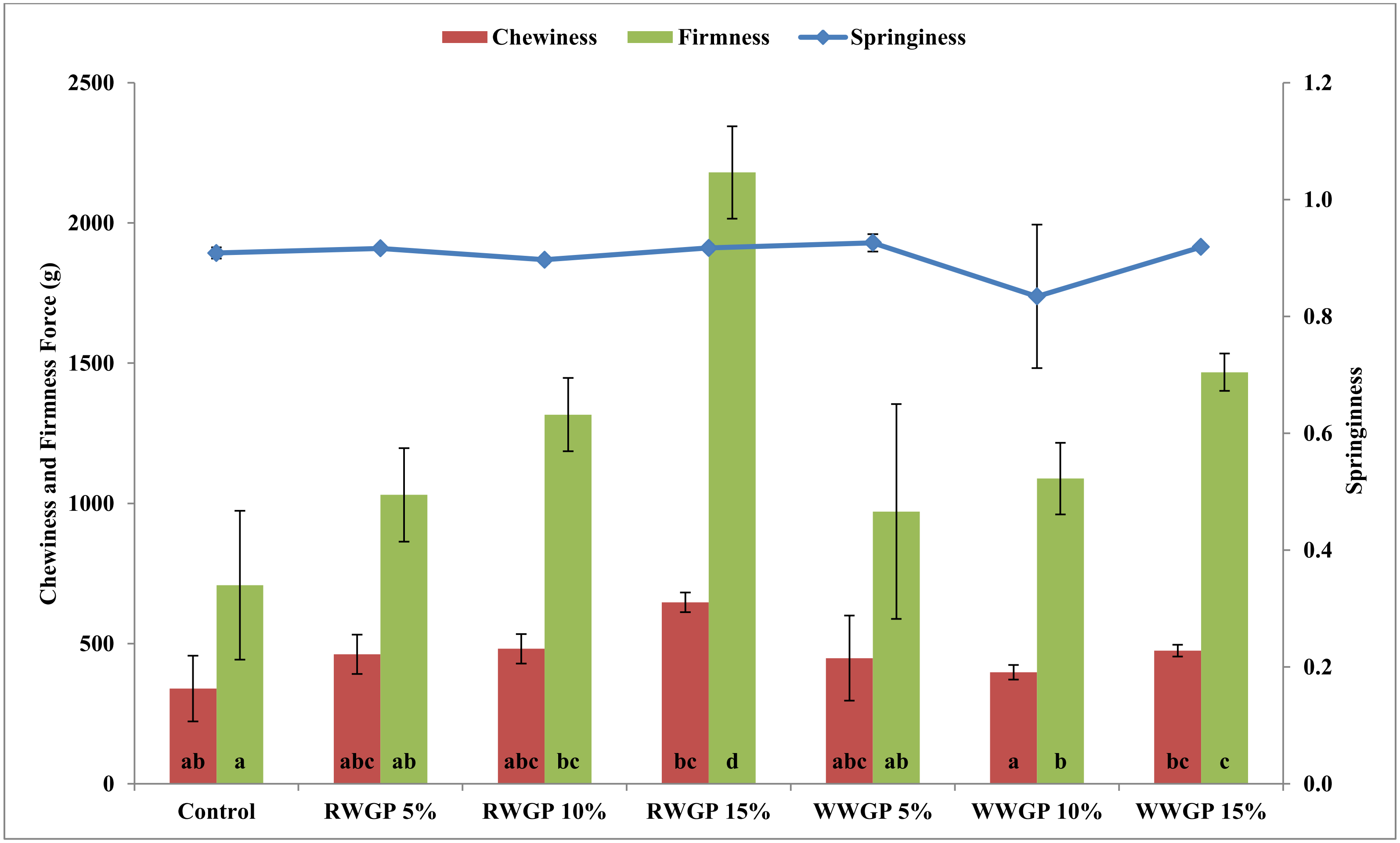
The WGP fortified breads were evaluated for springiness, chewiness, and firmness. Springiness is described as ability of a product to physically spring back after deformation from the first compression (Bourne 2002). There was no significant difference in springiness of bread among all samples (Figure 3). Chewiness describes the amount of time needed to chew a food in order to reduce it to a suitable consistency for swallowing (Bourne 2002). The RWGP 15% bread had a significantly higher level of chewiness compared to the WWGP 10% bread (Figure 3). Firmness is a measurement of the force required to compress the sample. The RWGP and WWGP 15% breads were significantly firmer than the control and RWGP 5% and WWGP 5 and 10% samples

(Figure 3). The RWGP 15% bread was the firmest (2180 g) and the control was the least firm (708 g). This increase in firmness along with WGP increase is consistent with the findings of Mildner-Szkudlarz and others (2011) who evaluated the effect of grape byproducts on sourdough rye bread. The increase in firmness may be a result of the increased fiber absorption of water in the system. It has also been suggested that the phenols in the WGP may affect the yeast as a result in changed enzyme activity in the system of the bread dough (Mildner-Szkudlarz and others 2011). The phenols may decrease the amylase activity resulting in decreased maltose availability for the yeast during the proofing process (Mildner-Szkudlarz and others 2011). Catechins have also exhibited behavior that inhibits yeast activity leading to decreased gas production (Mildner-Szkudlarz and others 2011).

The muffin samples were evaluated for springiness and firmness (Figure 4). The control sample had a significantly higher springiness than all other WGP fortified samples. The RWGP 15% and all WWGP fortified muffins were significantly less springy than the control and RWGP 5 and 10% samples. For both the RWGP and the WWGP fortified muffins, the springiness decreased as the WGP percent increased.

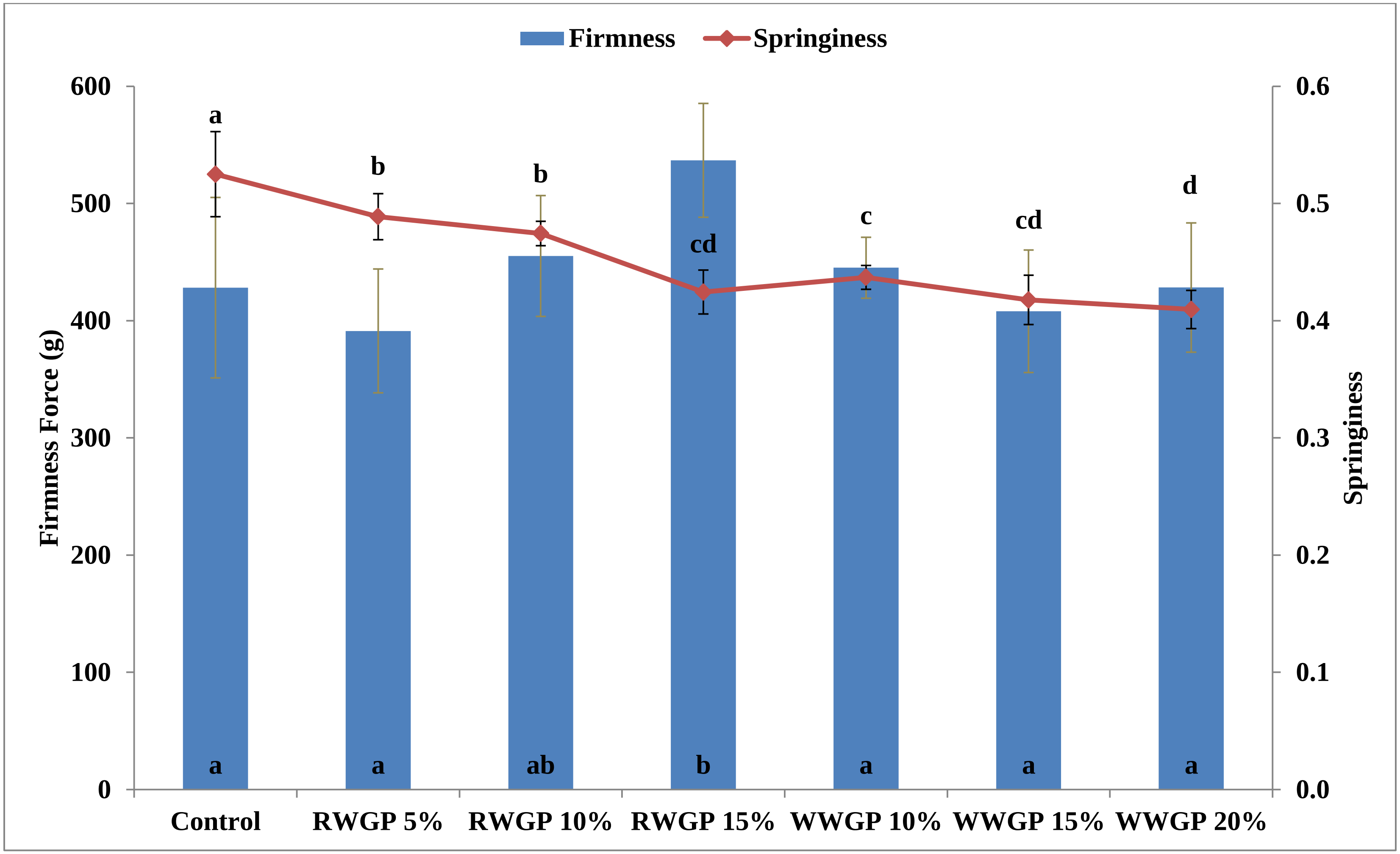
The RWGP 15% muffin was significantly firmer than all other samples except for the RWGP10% sample. This trend was also seen in apple pomace fortified muffins (Vasantha Rupasinghe and others 2009). The RWGP 15% muffin had the highest firmness of 537 g compared to the RWGP 5% one that had the lowest firmness value of 391 g. Again, the increase in firmness with increased WGP could be attributed to the high capacity of water absorption of the fiber in the WGP.

Figure 3. Effects of different WGP flour blends on the texture properties of bread.



*Columns with different letters at their base are not significantly different (P<0.05, n=21).

Figure 4. Effects of different WGP flour blends on the texture properties of muffins.



*Columns and points with different letters at their base are not significantly different (P<0.05, n=21).

3.3 Bioactive compound analysis

3.3.1 Total phenolic content (TPC) and radical scavenging activity (RSA)

All WGP fortified bread had significantly different RSA values per 50 g serving from the control (Figure 5). As the percent of WGP increased for both the RWGP and WWGP fortified breads, the RSA amount per serving increased. The RWGP fortified bread had higher levels of RSA than the WWGP fortified bread for corresponding flour replacement values. The bread fortified with 15% RWGP had the highest amount of RSA (80.70 mg AAE/serving), a 332% increase from the control. The RSA values are more specific than the TPC values in describing an antioxidant's ability to prevent the a reactive oxygen species from attacking lipoproteins, polyunsaturated fatty acids, DNA, amino acids, and sugars in both biological and food systems because it only reports free antioxidants in the extract (Sagdic and others 2011).

The bread TPC values followed a similar trend as the RSA values (Figure 5). As the WGP percent increased, the TPC value per serving increased as well. Again, the RWGP 15% bread had the highest TPC value of 68.32 mg GAE/serving, a 22% increase from the control. These values determined by the Folin-Ciocalteu represent both free and bound phenolics, a broader range of compounds compared to the RSA (Sagdic and others 2011).

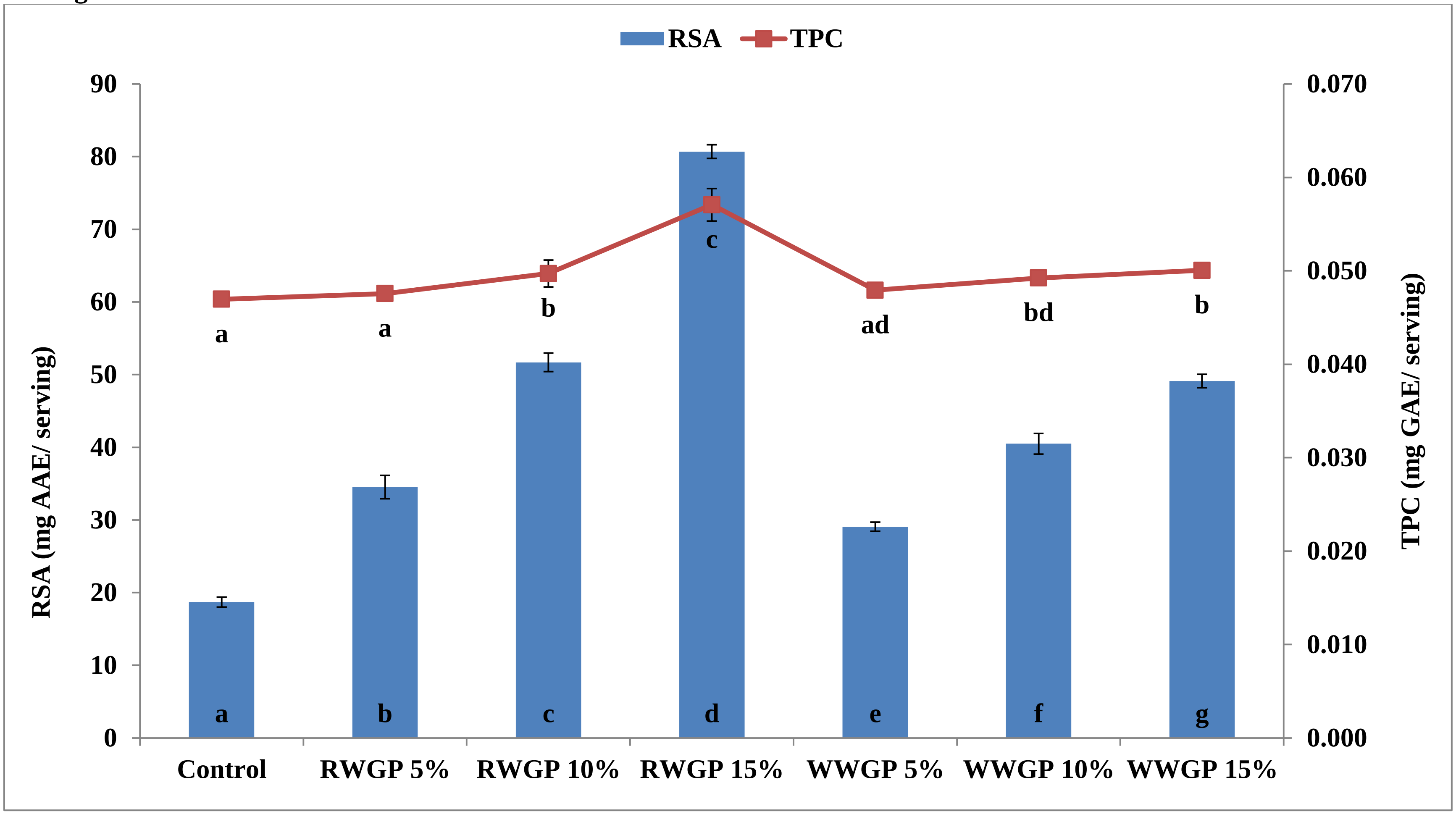
The WWGP fortified muffins were not significantly different from each other in terms of RSA values (Figure 6). However, all of the WWGP samples had significantly lower RSA values compared to the RWGP fortified muffins. The muffin fortified with 15% RWGP had the highest RSA and TPC value per 50 g serving (1526 mg AAE/serving and 2164 mg GAE/serving, respectively). These values are 1450% greater than the control RSA and 512% greater than the control TPC. The TPC values of the

WWGP fortified muffins were significantly different from each other and followed the trend of increasing values per serving as the percent WGP increased.

One thing to note is the magnitude difference in RSA and TPC values between bread and the muffins. Muffin RSA and TPC values were over 20 times higher than that of the bread (Figures 5 and 6). While the muffin and bread have different serving sizes (55g and 50g, respectively), those sizes only differ by 10% and may not be the only explanation for this difference in RSA and TPC values. The TPC values of the bread were the same magnitude of values determined in another study that evaluated grape byproduct fortified sourdough rye bread (Mildner-Szkudlarz and others 2011), however, the bread TPC values in this experiment did not increase as steeply as those found in study.

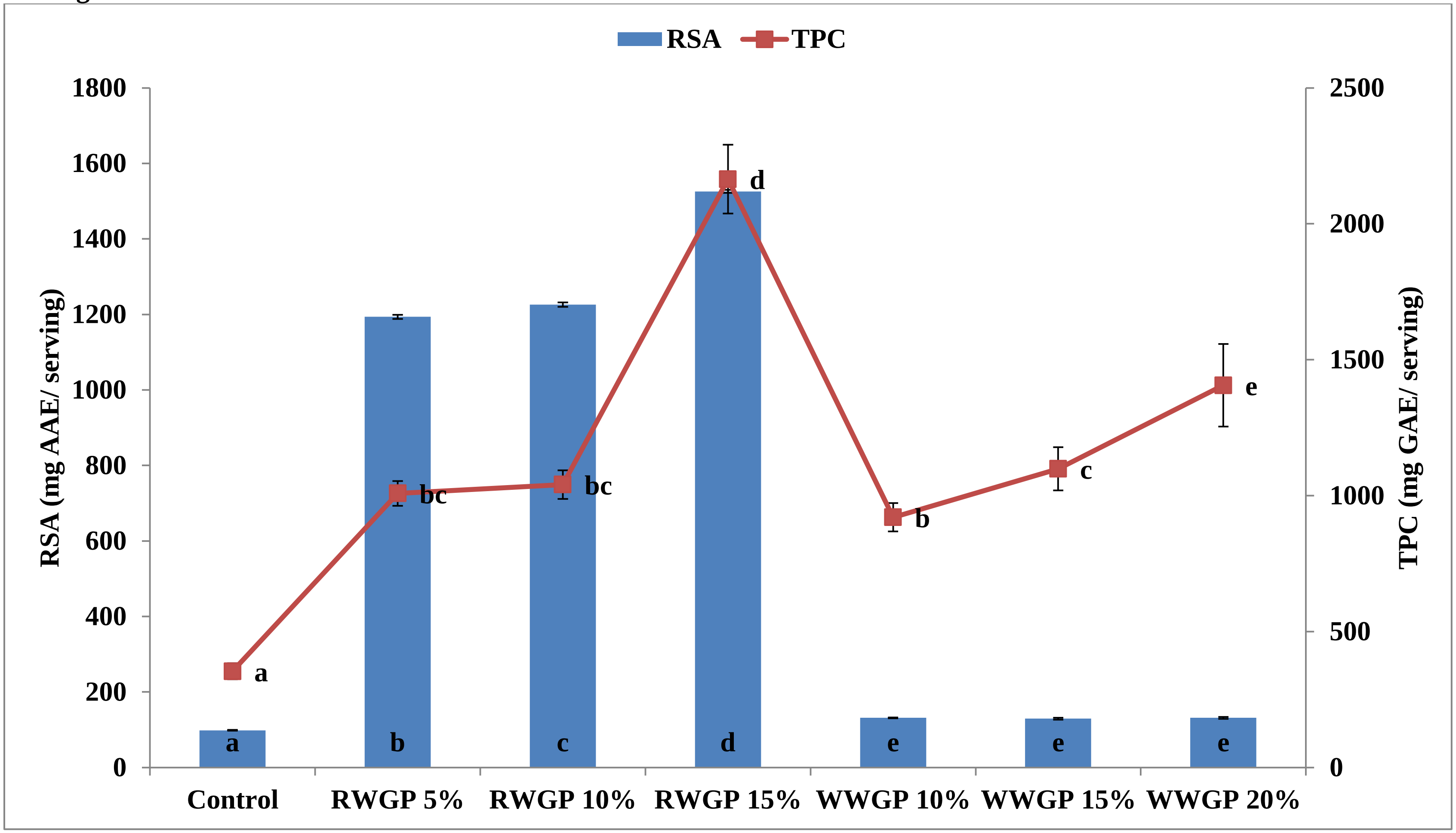
The small RSA and TPC values in the bread may be attributed to the different formulations and preparation procedures. The bread undergoes a long fermentation and proofing process, giving it more exposure to air and heat during that time, which may have accelerated the oxidation, leading to a decreased amount of RSA (Tseng and Zhao 2013) in the final bread product. Further testing would need to be done to evaluate the RSA and TPC values of the dough after the fermentation and proofing process but before it is baked to determine if this extended processing time and heat could be the cause for such a large decrease in RSA and TPC values.

Figure 5. Effect of different WGP flour blends on the total phenolic content (TPC) and radical scavenging activity (RSA) per serving of bread.



*Columns and points with different letters at their base are not significantly different (P<0.0005, n=21). Serving size of bread is calculated as 50 g.

Figure 6. Effect of different WGP flour blends on the total phenolic content (TPC) and radical scavenging activity (RSA) per serving of muffin.



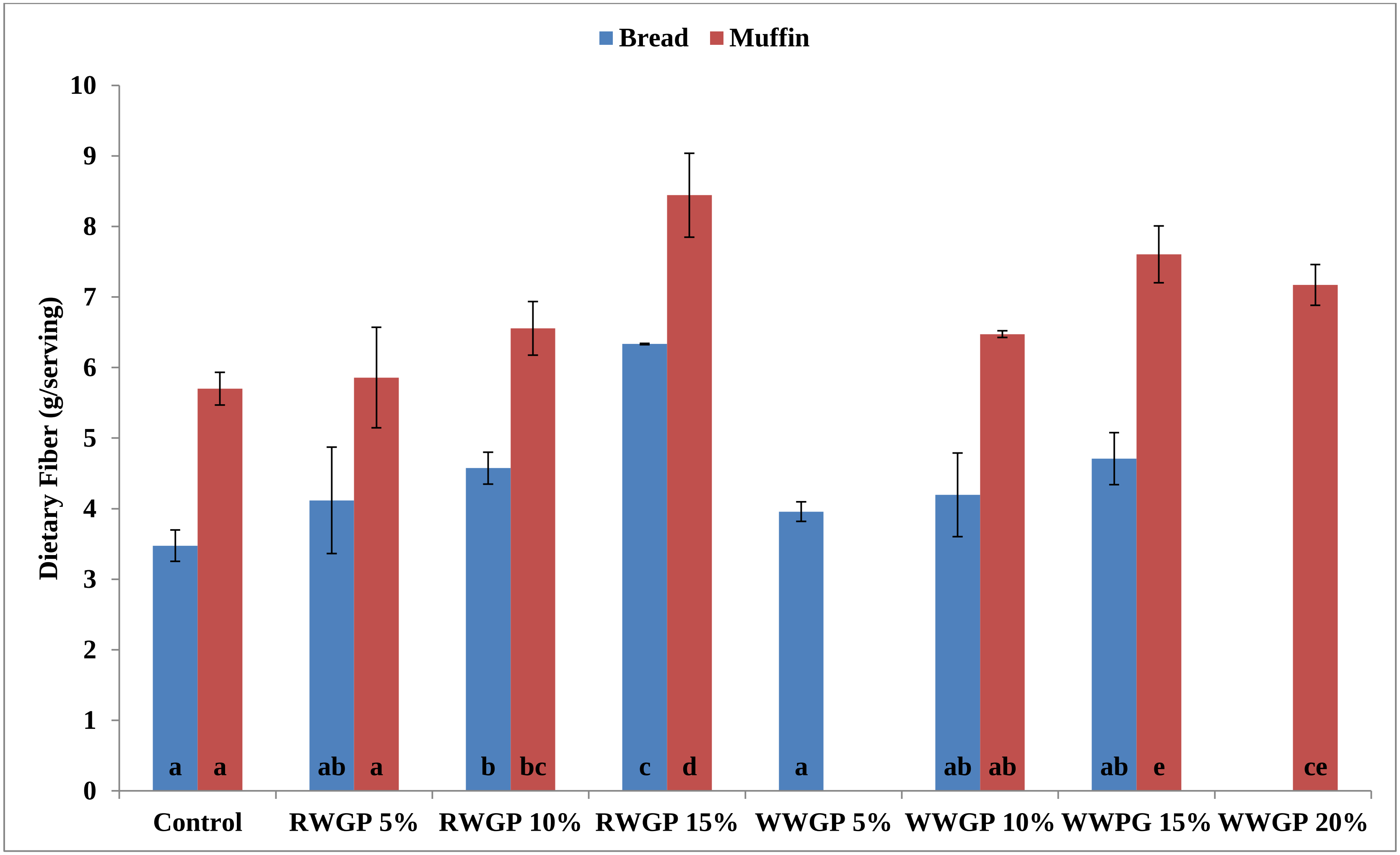
*Columns and points with different letters at their base are not significantly different (P<0.0005, n=21). Serving size of muffin is calculated as 55 g.

3.3.2 Dietary fiber

The total dietary fiber (DF) for each product was expressed as grams per serving of product as defined by Code of Federal Regulation (2013). Bread samples fortified with 10 and 15% RWGP had significantly higher DF per serving compared to the control (Figure 7). As the WGP concentration increased in the fortified bread, the amount of DF increased. The RWGP bread had higher amounts of DF than the control and WWGP at respective fortification with the highest amount of dietary fiber in RWGP 15% bread, which contains 6.33 g DF per serving. The control had the least amount of DF per serving (3.48 g).

The same trend of increasing dietary fiber with an increase in WGP was seen in the muffins fortified with WGP. RWGP 15% and WWGP 15 and 20% fortified muffins had significantly larger amounts of dietary fiber compared to the control, RWGP 5%, and WWGP 10%. As seen in the bread, the control had the lowest DF (5.70 g/serving) and the RWGP 15% muffin had the highest amount of DF (8.44 g/serving). These results were to be expected because of the high amount of dietary fiber in the WGP and it was also seen in studies using apple pomace and grape byproducts (Mildner-Szkudlarz and others 2011; Vasantha Rupasinghe and others 2009).

Figure 7. Effect of different WGP flour blends on the total dietary fiber per serving of bread or muffin.



*For each product, means and standard deviations (SD) followed by same letter in the same column are not significantly different (P<0.0005, n=21 bread, n=7 muffins). Serving size of bread is 50 g and serving size of muffin is 55 g.

3.4 Consumer acceptance of RWGP fortified bread and muffins

There was no significant difference between the Overall Liking of the bread products. Furthermore, there was no significant difference between the bread products for all attributes excluding Color JAR and Mouth Feel JAR (Table 7). The control bread had a higher Mouth Feel rating (3.83) on the JAR scale (5=much too stick, 3= just about right, 1=much too dry). Both RWGP products had an equally significantly lower Mouth Feel JAR rating (2.92) compared to the control suggesting that RWGP fortified bread was a little dry.

The control had a significantly lower rating for Color JAR (5=much too dark, 3= just about right, 1= much too light) compared to both RWGP products. Panelists commented that the control bread was too light with an average Color JAR rating of 2.08. In contrast, the RWGP 5% bread and RWGP 10% bread had average Color JAR ratings of 3.33 and 3.92, respectively, suggesting that RWGP 10% bread may have been too dark for consumer preference. When looking at the average ratings for all questions, the RWGP 5% bread received consistently higher ratings than the RWGP 10% one, even though they were not significantly larger.

Both the 5 and 10% RWGP fortified breads were well received by panelists based on statistical results on the sensory scores. The acceptability of the 10% RWGP was not expected since previous studies had shown a consumer threshold of about 5% pomace in fortified products (Hoye and Ross 2011; Kohajdová and others 2012). Kohajdová and others (2012) concluded that a maximum of 3% carrot pomace could be added to wheat rolls in order to maintain consumer acceptance. In a separate study, Hoye & Ross determined that sourdough bread made with grape seed flour could replace up to 5%

(w/w) of flour with grape seed flour in order for the product to remain acceptable to consumers (2011).

Again, there was no significant difference between the Overall Liking scores of all three muffin products (Table 8). There was no significant difference in other sensory attributes between the muffin products except for the Color JAR and Aroma Liking ratings. The RWGP 10% muffin had a significantly higher rating (3.58) for Color JAR (5=much too dark, 3= just about right, 1=much too light) than the control (1.75). These values suggested that the control was too light and the muffin fortified with RWGP 10% was too dark in color. The RWGP 5% muffin, however, received an average Color JAR rating of 2.92, making it the most acceptable sample based on color by the panelists. The control received the highest Aroma Liking rating of 6.92 that was significantly different than the RWGP 10% muffin with an Aroma Liking rating of 5.17, which suggests that the 5% RWGP fortified muffins were still acceptable for that quality with a rating of 5.75.

Based on the sensory findings, it would be suggested that the muffins fortified with 5 and 10% RWGP would both be accepted by consumers. A similar study determined that the maximum consumer acceptance of muffins with dietary fiber-rich orange bagasse product was 10% (Romero-Lopez and others 2011). While the 10% RWGP fortified muffins did receive consistently lower liking scores compared to 5% RWGP, the scores were not significantly different.

Table 7. Consumer acceptance of WGP fortified bread.

	Control	RWGP 5%	RWGP 10%
Overall Liking	6.83 ± 1.47 a	7.00 ± 0.95 a	6.58 ± 1.16 a
Appearance Liking	7.42 ± 1.38 a	6.75 ± 2.05 a	6.33 ± 1.67 a
Color JAR	2.08 ± 0.79 b	3.33 ± 0.78 a	3.92 ± 0.79 a
Aroma Liking	7.00 ± 1.35 a	6.58 ± 1.73 a	6.33 ± 1.92 a
Overall Flavor Liking	7.00 ± 1.76 a	7.33 ± 0.65 a	7.00 ± 1.21 a
Sweetness Liking	6.58 ± 1.62 a	6.83 ± 1.40 a	6.58 ± 1.73 a
Sweetness JAR	2.75 ± 1.14 a	2.83 ± 0.72 a	3.00 ± 0.95 a
Texture Liking	6.17 ± 1.80 a	6.67 ± 2.10 a	6.08 ± 1.62 a
Mouth feel liking	6.58 ± 1.62 a	6.25 ± 1.82 a	6.42 ± 1.31 a
Mouth feel JAR	3.83 ± 0.58 a	2.92 ± 0.67 b	2.92 ± 0.51 b
Moistness Liking	7.50 ± 1.17 a	6.83 ± 1.95 a	6.75 ± 1.29 a
Moistness JAR	3.25 ± 0.45 a	2.92 ± 0.67 a	2.75 ± 0.62 a
Aftertaste Liking	6.67 ± 1.61 a	6.67 ± 1.50 a	5.50 ± 1.83 a
Overall Liking #2	6.75 ± 1.71 a	7.08 ± 1.38 a	6.58 ± 1.24 a

*Means and standard deviations (SD) followed by same letter in the same row are not significantly different ($P<0.05$, $n=12$)

Table 8. Consumer acceptance of WGP fortified muffins.

	Control	RWGP 5%	RWGP 10%
Overall Liking	6.25 ± 1.29 a	6.83 ± 1.19 a	6.42 ± 1.56 a
Appearance Liking	6.08 ± 1.88 a	6.50 ± 2.24 a	5.92 ± 2.35 a
Color JAR	1.75 ± 0.75 b	2.92 ± 0.51 b	3.58 ± 0.79 a
Aroma Liking	6.92 ± 1.16 a	5.75 ± 1.76 ab	5.17 ± 1.27 b
Overall Flavor Liking	6.67 ± 1.83 a	7.75 ± 0.75 a	6.83 ± 1.70 a
Sweetness Liking	6.75 ± 1.42 a	7.75 ± 0.97 a	6.92 ± 1.68 a
Sweetness JAR	2.75 ± 0.62 a	2.83 ± 0.58 a	2.83 ± 0.83 a
Texture Liking	6.83 ± 1.19 a	6.92 ± 1.31 a	6.00 ± 1.76 a
Mouth feel liking	6.42 ± 1.68 a	6.58 ± 1.88 a	6.42 ± 1.78 a
Mouth feel JAR	2.50 ± 0.90 a	3.00 ± 0.74 a	2.67 ± 0.65 a
Moistness Liking	6.58 ± 1.56 a	7.08 ± 1.68 a	6.17 ± 1.70 a
Moistness JAR	2.50 ± 0.67 a	3.00 ± 0.74 a	2.42 ± 0.67 a
Aftertaste Liking	7.08 ± 1.56 a	7.08 ± 1.31 a	6.17 ± 1.95 a
Overall Liking #2	6.92 ± 1.31 a	7.42 ± 0.67 a	6.17 ± 1.95 a

*Means and standard deviations (SD) followed by same letter in the same row are not significantly different ($P<0.05$, $n=12$)

4. Conclusion

This study validated Pinot Noir and Pinot Grigio wine grape pomace as a source of antioxidant dietary fiber to be fortified in bread and muffin baked goods for promoting human health. Both breads and muffins were found to be acceptable with a 5 or 10% RWGP replacement of flour (w/w) based on physicochemical and sensory characteristics compared to the control. While sensory testing was not conducted on the WWGP products, it is not unreasonable to anticipate a consumer acceptance of up 15% WWGP fortified bread and muffin products given the physicochemical results and natural characteristics of the WWGP, including a lighter color and a more subtle, less fruity flavor. These characteristics might make the WWGP bake goods more easily accepted by consumers but this can only be confirmed by another sensory study. It would also be beneficial to conduct a larger sensory study to generate more statistically viable results.

During the sensory testing, panelists also commented on the grainy texture of the RWGP fortified products. It would be beneficial to produce the baked goods using a smaller particle size in hopes of the pomace being less noticeable when chewed. The smaller WGP particle size may also have an effect on the volume and texture of the baked items as well.

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