

Quality and Consumer Acceptance of Berry Fruit Pomace–Fortified Specialty Mustard

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Abstract: Blueberry pomace (BP) and cranberry pomace (CP) are good sources of dietary fiber and phenolics. This study aimed to develop berry fruit pomace (FP)–fortified specialty mustard with elevated bioactive compounds and ascertain consumer acceptance of a new product. Wet BP and CP were ground and incorporated into Dijon-style mustard at concentrations of 15%, 20%, and 25% (w/w). Total dietary fiber (TDF), total phenolic content (TPC), and radical scavenging activity (RSA) were evaluated for samples obtained from both chemical extraction (CE) and simulated gastrointestinal digestion (SGD). Physicochemical properties and consumer acceptance were also examined. Increasing concentrations of BP or CP significantly increased TDF of mustards from both CE (AOAC methods) and SGD, with the highest values from 25% fortifications. TDF from AOAC ranged from 26.86% to 40.16% for BP and from 26.86% to 38.42% for CP, while TDF from SGD ranged from 31.02% to 42.68% for BP and 31.02% to 63.65% for CP. From CE, no significant variation of TPC was found, but RSA significantly increased with increasing concentration of BP and CP. TPC from SGD was higher than that from CE, where TPC decreased with increasing concentration of BP or CP. RSA from SGD was lower than that from CE. Sensory scores of pomace-fortified samples were significantly lower than the control; however, informed panelists scored BP-fortified mustard significantly higher on appearance and color liking than uninformed panelists. This study demonstrated that with proper marketing, the utilization of FP in condiments is a viable option for potential health benefits.

Keywords: berry fruit pomace, bioactive compounds, consumer acceptance, simulated gastrointestinal digestion, specialty mustard

Practical Application: This research is applicable to multiple areas of the food industry. Juice manufacturers or other companies that process raw agricultural produce can use this research as another way to repurpose biowaste, and companies making specialty condiments can use this research to inform future product development. General considerations discussed regarding the use of berry fruit pomace can be applied by any company interested in pomace reuse.

Introduction

Berry fruit pomace (FP) consists of the skins, seeds, and pulp leftover from the juice production process. It generally composes 20% to 30% of the volume of fruit, generating a nontrivial amount of biowaste (Struck, Plaza, Turner, & Rohm, 2016). This biowaste is particularly difficult to dispose of due to the nature of berry fruit (high acidity, sugars, and other organic content), and its low-protein content makes usage in animal feed limited. Additionally, berry FP has a high antimicrobial activity, hindering its use in compost (Rohm et al., 2015). However, berry FP, particularly from cranberry (*Vaccinium macrocarpon*) and blueberry (*Vaccinium corymbosum*), has high levels of phenolics (Li, Feng, Huang, & An, 2013; Mildner-Szkudlarz et al., 2016) and dietary fibers (DFs) (Gouw, Jung, & Zhao, 2017). Several studies have reported the potential anticancer, antioxidant, and antimicrobial properties of blueberry pomace (BP) and cranberry pomace (CP) (Das, Islam, Marcone, Warriner, & Diarra, 2017; Huang, Zhang, Liu, & Li, 2012; Rupasinghe, Neir, & Parmar, 2016; Shi, Loftus, McAinch,

& Su, 2017). Berry FPs also retain a significant amount of color, aroma, and flavor compounds, and can therefore provide desirable sensory attributes to food products. These properties of berry FPs make them promising functional food ingredients.

Many previous studies have evaluated food applications of fruit and vegetable pomace, such as in baked goods (brownies, muffins, and cake) (Kamiloglu et al., 2017; Walker, Tseng, Cavender, Ross, & Zhao, 2014); in dairy products (Marchiani, Bertolino, Ghirardello, McSweeney, & Zeppa, 2016); and in cereal bars or meat products (Jung, Cavender, & Zhao, 2015). However, the reservoir of research involving BP and CP is much more limited, and primarily in baked goods such as cookies and muffins (Mildner-Szkudlarz et al., 2016; Šarić et al., 2016). Considering increasing demand for sustainability of food processing and concern for the environment, as well as consumers' consciousness of functional and healthy foods (Williams, Stewart-Knox, & Rowland, 2005), it is important and necessary to develop more novel and value-added utilizations of FP in the food segment.

One of the categories of food products that has been seldom explored, especially concerning the incorporation of FP, is condiments. Traditionally low in fiber and other health-promoting compounds, condiments are respectable targets for fortification, particularly considering their popularity as sources of specialty products. These products often have interesting and unique flavors and ingredients, all of which could aid in the market success of pomace-fortified condiments. This study has thus chosen to

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Table 1—Formulation of blueberry and cranberry pomace-fortified mustards.

	Ratio of pomace (%)	Mustard powder	Water	Ingredients (g)					Wine solution ^a	Total weight
				Wine	Sugar	Salt	Pomace			
Control	0	26.8	23.4	46.1	1.7	2.0	0	0	100	
Blueberry pomace	15	20.4	17.8	35.0	1.3	1.5	15	9.0 ^b	100	
	20	18.2	15.9	31.3	1.2	1.4	20	12.0	100	
	25	16.1	14.0	27.7	1.0	1.2	25	15.0	100	
Cranberry pomace	15	16.9	14.7	29.0	1.1	1.3	15	22.0 ^c	100	
	20	13.6	11.8	23.4	0.9	1.0	20	29.3	100	
	25	10.3	9.0	17.7	0.6	0.7	25	36.7	100	

^aWine solution was prepared by boiling 465 g of white wine (Chardonnay) with 8.3 g salt and 5 g of sugar for 15 to 20 min (the total amount was reduced by half).

^bAmount of additional wine solution added was governed by water holding capacity (WHC) data of FP. WHC of BP = 0.60 mL/g.

^cWHC of CP = 1.47 mL/g.

develop BP- and CP-fortified mustard because of its simplicity of formulation (ingredients) and lack of a standard of identity. Mustard is still widely consumed as a condiment only slightly behind ketchup in sale volume with almost 250 million units sold in 2017 (Tax, 2017). However, to develop high-quality pomace-fortified mustard with good consumer acceptance, several key factors should be considered. These include: desired ingredient characteristics and potential interactions within the fortified food; additional sensory properties, such as color, flavor, and aroma; and factors that dictate product safety, such as pH (Sun-Waterhouse, 2011). To manage these factors, several steps were taken in this study. First, the water absorption properties of the pomace in the mustard system were counteracted since they affect the viscosity of the final product. Second, different concentrations of pomace fortified in mustard were evaluated to obtain the most desirable organoleptic product qualities and potential consumer acceptance, while at the same time maximizing potential nutritional benefit. Finally, the products were formulated to maintain ingredient simplicity, reducing possible effects from additional components other than the target constituents of the product.

Therefore, the overall goal of this study was to develop formulations of BP- and CP-fortified specialty mustard products with elevated bioactive compounds, and to evaluate consumer acceptance. Specific objectives were to investigate the effect of BP and CP fortification in mustard with increasing levels of pomace incorporation (0%, 15%, 20%, and 25% (w/w)) on (1) physicochemical properties impacting flavor and appearance characteristics and (2) bioactive compounds including phenolics and DF as compared through chemical analysis and simulated gastrointestinal digestion (SGD). Furthermore, this study aimed to introduce a new type of product to consumers and obtain feedback about its acceptance in a potential commercial setting, providing guidance on how to reformulate similar products in the future for maximum approval. This study employed novel use of berry FP and expanded the breadth of research on its incorporation into food systems.

Materials and Methods

Materials

The BP and CP used in this study were provided by a juice concentrate processor in Oregon, U.S.A. Fruit were subject to pectinase and/or cellulase treatments prior to juicing. Fresh (wet) pomaces were packaged into plastic buckets and frozen at -18°C until further use. Yellow mustard powder was purchased from a local grocery store (Corvallis, OR, U.S.A.).

Folin-Ciocalteu's phenol (FC) reagent, gallic acid, 3,5-dimethylphenol, D-galacturonic acid monohydrate, α -amylase from *Aspergillus oryzae*, and protease from *Bacillus licheniformis* were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, U.S.A.).

L-ascorbic acid and D-glucose anhydrous were from Amresco (Solon, OH, U.S.A.); 2,2-diphenyl-1-picrylhydrazyl (DPPH) and anthrone were from Alfa Aesar (Ward Hill, MA, U.S.A.); and pepsin (1:3000), pancreatin, and bile salts were from Ward's Science (Rochester, NY, U.S.A.). All other solvents and reagents were analytical grade and used without further purification.

Preparation of fruit pomace powders and pomace-fortified mustards

Frozen BP and CP were thawed overnight at ambient temperature, ground cryogenically using liquid nitrogen, and then stored in Ziploc[®] freezer bags at -18°C until incorporation into the mustard product.

To prepare the pomace-fortified mustards, both BP and CP were incorporated at levels of 0%, 15%, 20%, and 25% (w/w wet basis) into a control (Dijon-style mustard, recipe from [Williams, 2010]). A smooth Dijon-style mustard was chosen due to its compatibility with fruit flavors, and its popularity in the commercial market. Ingredients and formulations are summarized in Table 1. The control (nonfortified) mustard was made as follows: the mustard powder and water were stirred together to form a paste and set aside. The white wine (Barefoot Chardonnay, Santa Rosa, Calif., U.S.A.) was brought to a boil, followed by addition of sugar and salt, mixing, and then simmering over medium heat to reduce the liquid by half. Mustard paste and white wine solution were then combined and cooked briefly over low heat. Considering the water holding capacities of BP and CP, the amount of wine solution was adjusted to produce mustards with similar viscosities. Each fortified product was made in triplicate, resulting in 12 prepared samples for each type of pomace.

Physicochemical properties of pomace-fortified mustards

Moisture content (MC) and water activity (A_w). MC of the sample (5 g) was gravimetrically measured by drying samples in a forced-air oven (Isotemp[®] Oven Forced Draft, Fisher Scientific, Waltham, Mass.) at 105°C for 24 hr to a constant final weight. A_w was determined using a water activity meter (AquaLab[®], Model Series 3, Pullman, WA, U.S.A.).

pH, titratable acidity (TA), and total soluble solids (TSS). A 5 g of mustard sample was blended (Osterizer[®], Jarden Corporation, Mexico) with 45 g of water and the resulting mixture was filtered through Whatman #1 paper (Whatman[™], Buckinghamshire, UK) to obtain the clear filtrate for analysis. pH was measured using an electrolytic pH meter (Orion 9102BNWP, Thermo Scientific, Waltham), and TA was determined via titration with 0.1N sodium hydroxide to an endpoint of pH 8.2 and calculated using citric acid as the predominant organic acid. TSS was evaluated using an electronic refractometer (Model

Table 2—Physicochemical properties of blueberry and cranberry pomace-fortified mustards.

Blueberry pomace	MC ^A (%)	<i>A_w</i>	TSS (%)	pH	TA (%)	Color values ^B			
						<i>L</i>	Hue	Chroma	ΔE ^C
0%	67.71 ^c	0.968 ^{ab}	16.83 ^a	4.10 ^a	5.07 ^a	64.64 ^a	1.38 ^a	26.62 ^a	—
15%	68.81 ^b	0.963 ^c	13.33 ^b	3.89 ^b	4.68 ^b	28.95 ^b	0.05 ^b	11.58 ^d	44.39 ^c
20%	69.42 ^a	0.966 ^b	13.00 ^b	3.82 ^c	4.23 ^c	25.21 ^c	0.01 ^c	12.84 ^c	47.88 ^b
25%	69.70 ^a	0.969 ^a	12.33 ^b	3.76 ^d	4.11 ^d	21.20 ^d	0.00 ^c	14.56 ^b	51.58 ^a

Cranberry pomace	MC (%)	<i>A_w</i>	TSS (%)	pH	TA (%)	Color values			
						<i>L</i>	Hue	Chroma	ΔE
0%	67.71 ^d	0.968 ^d	16.83 ^a	4.10 ^a	5.07 ^a	64.64 ^a	1.38 ^a	26.62 ^a	—
15%	71.93 ^c	0.973 ^c	14.33 ^b	3.89 ^b	4.99 ^a	46.43 ^b	0.66 ^b	16.23 ^c	25.59 ^b
20%	73.07 ^b	0.978 ^b	14.33 ^b	3.77 ^c	4.88 ^a	41.55 ^c	0.51 ^c	17.08 ^{bc}	30.75 ^a
25%	74.82 ^a	0.981 ^a	12.00 ^c	3.65 ^d	4.97 ^a	37.88 ^c	0.41 ^d	18.47 ^b	34.70 ^a

^AMC = Moisture content; *A_w* = water activity; TSS = total soluble solid; TA = titratable acidity. Means with different lowercase letters in the same row indicate significant difference ($P < 0.05$) among fruit pomace-fortified mustard formulations.

^BColor values: Hue = $\tan^{-1}(b/a)$; Chroma = $\sqrt{a^2 + b^2}$.

^CΔE = $\sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$, where L_1 , a_1 , and b_1 are control values, and L_2 , a_2 , and b_2 are FP-fortified mustard values.

RA-250HE, Kyoto Electronics Manufacturing Co., Ltd., Japan) (Tseng & Zhao, 2013).

Color measurement. Mustard samples were placed into a standardized 64 mm glass sample cup and analyzed in five different sections using a colorimeter (LabScan XE, Hunterlab, Reston, VA, U.S.A.). L^* (lightness), a^* (redness), and b^* (yellowness) values were obtained as mean values of the five replications, and hue, chroma, and color change (ΔE) were calculated using these values (Table 2).

DF profiling of pomace-fortified mustards

Soluble dietary fiber (SDF), insoluble dietary fiber (IDF), and Klason-lignin (KL) were measured using a slightly modified method from our previous study (Jung et al., 2015). For SDF, 0.5 g of mustard sample was defatted twice with 25 mL of chloroform in an ultrasonic water bath (Branson B-220H, SmithKline Co., Shelton, Conn., U.S.A.) for 10 min, and then filtered using Whatman #1 filter paper. The residue was washed 3 times using 80% ethanol (20 mL each) to remove lower molecular weight saccharides and dried overnight in a fume hood. The dried residue was enzymatically treated using 30 μL of protease (91 mg protein/mL) in 25 mL of 0.05M phosphate buffer (pH 7.5) and placed in a water bath (Precision, Model Shallow Form Bath, LabCare America, Winchester, Va.) at 60 °C for 30 min. The resultant suspension was filtered using Whatman #1 filter paper and the supernatant was collected and saved for SDF analysis. The residue was washed twice with 10 mL of deionized (DI) water and those subsequent supernatants were combined with the original SDF supernatant. The final residue was dried at 40 °C for 16 hr and used for IDF analysis.

SDF fraction was dialyzed using dialysis membranes (Spectrum Laboratories, Inc., Rancho Dominguez, Calif.) with a molecular weight cutoff of 12000 to 14000 kD in 1.5 L of water for a total of 48 hr. Water was changed once after 12 hr. The dialyzed samples were then freeze-dried (Consol 4.5, The Virtis Co., Inc., Gardiner, N.Y.), hydrolyzed in 36 mL of 6% sulfuric acid solution, and autoclaved at 121 °C for 1 hr. The resulting solutions were used for uronic acid (UA) and neutral sugar (NS) determination (Jung et al., 2015).

IDF samples were first hydrolyzed with 3 mL of 72% sulfuric acid and incubated at 30 °C for 1 hr. After adding 86 mL of DI

water, samples were autoclaved at 121 °C for 1 hr. The hydrolyzed mixture was then filtered using a crucible (Pyrex[®] 30 mL M, Corning, Inc. Corning, NY, U.S.A.). The filtrate was saved for UA and NS analyses, while the residue was dried and used for KL analysis. IDF value was the combined result of UA, NS, and KL.

To analyze UA, 250 μL aliquots of hydrolyzed samples were vortexed with 250 μL of boric acid–sodium chloride solution and 4 mL of 96% sulfuric acid and incubated in a 70 °C water bath for 40 min. The test tubes were cooled to ambient temperature, 200 μL of dimethylphenol solution was added, and the tubes were vortexed again. The absorbance of the solutions was measured at 400 and 450 nm using a spectrophotometer (Model UV-3100PC, VWR Intl., LLC, Radnor, PA, U.S.A.), and values were converted and quantified as galacturonic acid equivalents expressed as percentage of dry weight (DW) (Jung et al., 2015).

For NS analysis, 1 mL of hydrolyzed sample was vortexed with 2 mL of 75% sulfuric acid and 4 mL of anthrone solution, and placed in the 100 °C water bath for 15 min. After cooling the samples to ambient temperature, absorbance was measured at 578 nm using the same spectrophotometer. That absorbance was quantified as glucose equivalent and expressed as percentage of DW (Jung et al., 2015).

KL was gravimetrically determined by drying the residue from IDF in a medium porosity glass fritted Gooch crucible at 105 °C for 24 hr and recording its weight. The Gooch crucible was then subjected to the ashing process in a furnace (Thermolyne, Model F-A1730, Sybron Corp. Dubuque, IA, U.S.A.) at 525 °C for 5 hr and reweighed. The weight of the oven-dried sample was adjusted by subtracting the ash weight to get KL values, and expressed as percentage of DW (Gouw et al., 2017; Jung et al., 2015).

Extraction and analysis of phenolic compounds

To extract phenolics, 12 mL of 70% acetone solution acidified with 0.1% hydrochloric acid was added to 3 g of sample and sonicated in an ultrasonic water bath for 20 min. The extract was evaporated in a vacuum rotary evaporator (Brinkmann Instruments, Westbury, NY, U.S.A.) at 40 °C to remove the volatile acetone, diluted to 25 mL with DI water, and filtered using vacuum filtration with cellulose nitrate membrane filters (Whatman[™], Buckinghamshire, UK). The resulting extract was used for total

phenolic content (TPC) and radical scavenging activity (RSA) assays.

TPC was determined following the FC colorimetric method (Jung et al., 2015). A 0.5 mL of sample extract was vortexed with 7.5 mL of DI water and 0.5 mL of FC reagent, and then stored at ambient temperature for 10 min. Subsequently, 3 mL of 20% sodium carbonate solution was added and the samples were incubated at 40 °C for 20 min. Samples were cooled down to ambient temperature and the absorbance was measured at 765 nm using a spectrometer. Gallic acid was used as a standard to produce the calibration curve, and results were expressed as mg gallic acid equivalents (GAE)/g DW.

To determine RSA, 9 mg of DPPH was dissolved in 100 mL of methanol (Brand-Williams, Cuvelier, & Berset, 1995). A 0.5 mL of sample extract was mixed with 2 mL of DPPH reagent and incubated for 15 min in the absence of light. The absorbance was measured at 517 nm. Ascorbic acid was used as a standard to produce the calibration curve, and results were expressed as mg ascorbic acid equivalent (AAE)/g DW.

In vitro SGD study for phenolics and DF

SGD was evaluated following the method outlined by Minekus et al. (2014), which consisted of oral, gastric, and small intestinal phases of digestion. For the oral and gastric digestion phases, 1 g of sample was vortexed with 8.5 mL of 0.05M phosphate buffer, 0.5 mL of α -amylase (20 FAU/g), and 150 μ L of 50mM CaCl₂ solution. This mixture was placed in a 37 °C water bath and shaken at 50 rpm for 2 min to simulate oral mastication. Next, 5 mL of DI water was added, along with 1 mL of 0.2% pepsin solution and 30 μ L of 50mM CaCl₂ solution. The pH of this mixture was adjusted to 3 using 1M HCl, diluted to a total volume of 20 mL with DI water, and then incubated at 37 °C for 2 h shaken at 200 rpm. For the intestinal phase, 10 mL of 0.05M phosphate buffer, 3.0 mL of duodenal juice (12.5 g of bile salts and 2 g of pancreatin in 60 mL of 0.1M NaHCO₃), and 240 μ L of 50mM CaCl₂ solution were added to the sample. The pH of the sample mixture was adjusted to 7 with 0.1M NaOH, diluted to a total volume of 40 mL with 0.05M phosphate buffer, and incubated at 37 °C for 2 h at 200 rpm. The samples were centrifuged at 9200 rpm for 15 min at 4 °C and the supernatant and residue were separated. The supernatant was used to measure TPC and RSA as per the procedures outlined above, and the remaining supernatant was dialyzed. The dialyzed supernatant and the SGD residue were both freeze-dried and subjected to DF analysis as outlined above.

Sensory evaluation

Sensory evaluation of the pomace-fortified mustard samples was conducted at the Oregon State Univ.'s (OSU) Food Innovation Center in Portland, Oregon, wherein permission for the study was granted through the OSU Institutional Review Board. Ninety-two consumer panelists (ages 18 and older in a normal distribution with the largest group [31.5%] between 36 and 45 years, 30% men and 70% women) were recruited from the Portland metropolitan area via Facebook advertisements and email through the Oregon State Univ. Food Innovation Center database of consumers interested in participating in sensory testing. A screening survey was deployed using the Qualtrics survey tool, which probed among other things, hot dog and hamburger usage, demographics (age, gender, race, income, education, employment status, occupation), and panelist availability. Consumers who selected that they eat hot dogs regularly were selected. Two-thirds of the selected panelists

eat hamburgers once a week or more, while the remaining third eat burgers two to three times per month. Preference was given to consumers who when asked which toppings they prefer, listed mustard in their response. Three mustard samples were given to each panelist: one control mustard sample (0% pomace added), one 25% CP-fortified mustard sample, and one 25% BP-fortified mustard sample. Commercially made Dijon-style mustard (Grey Poupon Dijon Mustard, Kraft Heinz, IL, U.S.A.) was used as a control. CP- and BP-fortified samples were made following the same formulation quantities as shown in Table 1, but the wine solution was substituted with white wine vinegar to ensure consistency and safety of the product. In other words, commercial mustard took the place of the control mustard and white wine vinegar took the place of wine solution, but the amount of pomace and extra liquid added to the base mustard remained identical to each original formulation. Each individual sample was placed in plastic condiment cups containing 5 to 10 g of mustard. Samples were presented to the panelists along with pieces of cooked hot dog and a sample spoon (Figure 1). Panelists were instructed to rinse with water before and between samples and consume mustard samples with the given hot dog pieces in order to provide feedback based on the criterion outlined below. The hot dog pieces were provided to give consumers a more realistic experience with consuming mustard, as most consumers would not choose to eat mustard on its own. However, this may have negatively affected consumer perception due to the fruit-flavored nature of the fortified mustards.

Half of the panelists received an informational card on the mustard samples before beginning the evaluation. They were informed about the product information, health benefits of FP, and other reasoning behind the sensory evaluation (Figure 1). All panelists evaluated the samples for likeness of appearance, color, aroma, texture, flavor, and the overall product following a 9-point hedonic scale (9 = like extremely, 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); visual consistency (5 = much too thick, 1 = much too thin); flavor strength (5 = much too strong, 1 = much too weak); sweetness (5 = much too sweet, 1 = not at all sweet enough); acidity (5 = much too acidic, 1 = not at all acidic enough); and texture (5 = much too thick, 1 = much too thin). Two open-ended questions were asked after all other questions to allow the panelists to describe reasons for liking and/or disliking the evaluated samples.

The sensory study was designed with the intent of introducing the fortified mustards as commercial products, to gauge public opinion of these novel products, and to familiarize consumers to new forms of specialty condiments. The sensory samples were also analyzed for physicochemical properties including pH, TA, TSS, and color using the same methods described above to aid in the characterization of these samples.

Experimental design and statistical analysis

A completely randomized design was applied for investigating the effect of pomace concentrations (0%, 15%, 20%, and 25%) on the quality characteristics of pomace (BP and CP) fortified mustard. All experiments excluding the sensory study were conducted in triplicate, mean values and standard deviations were reported, and the resultant data were analyzed for statistical significance via one-way ANOVA and the *post hoc* least significant difference (LSD) testing using statistical software (SAS v9.4, The SAS Inst., Cary, N.C., U.S.A.). Results were considered to be significantly different if $P < 0.05$. Data from the sensory study were analyzed using Compusense LAB software

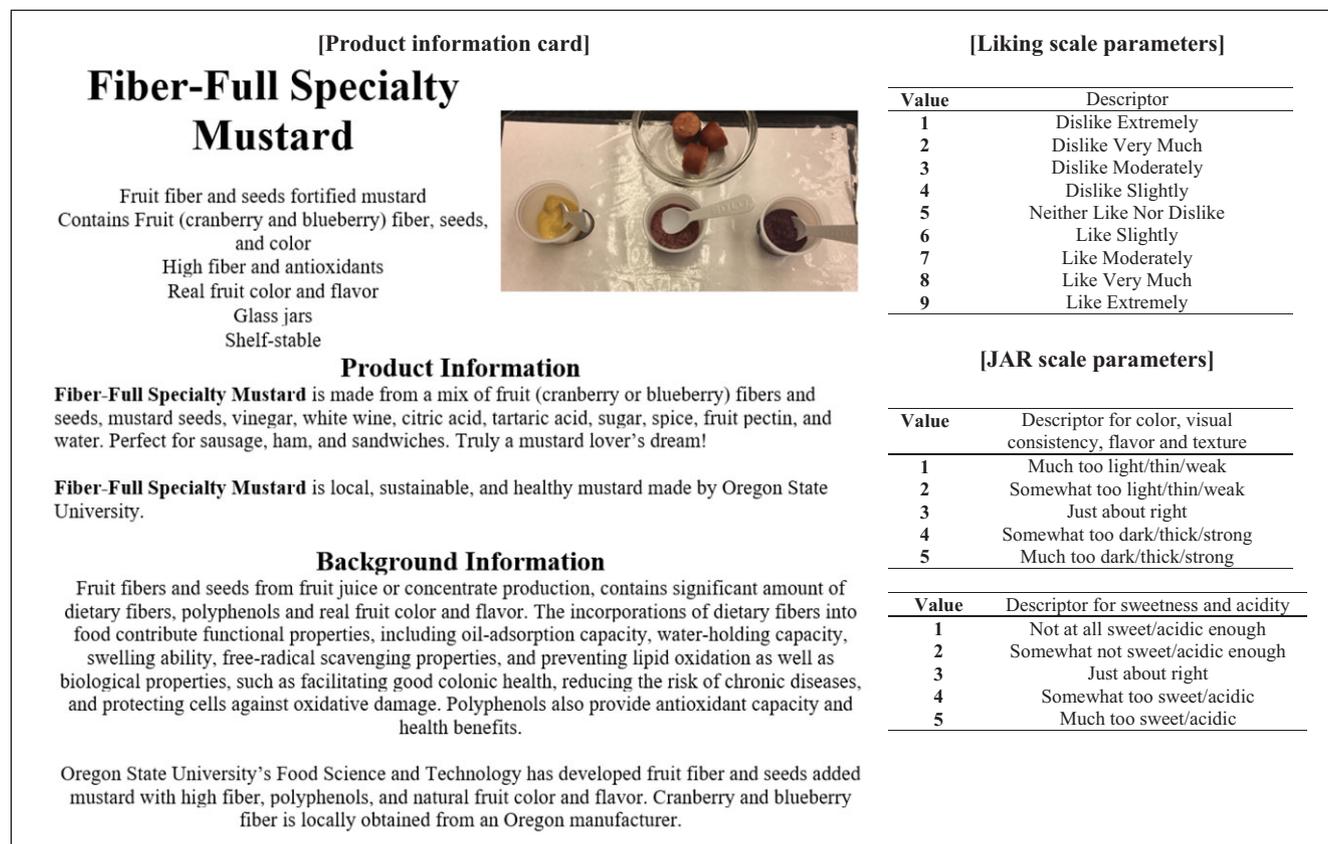


Figure 1—Product information card presented to consumers ($n = 45$) and scale parameters for liking (1 to 9) and just about right (JAR, 1 to 5) ranking of mustard samples.

(CompuSense Inc., Guelph, ON, Canada) and XLSTAT software (Addinsoft, Paris, France). JAR data were analyzed using penalty analysis to identify which attributes influenced the overall mean liking scores for each pomace-fortified mustard sample. The keywords were visualized by using the Word Cloud function in XLSTAT. Variables were considered significant with $P < 0.05$ and differences were determined based on Tukey's HSD test.

Results and Discussion

Physicochemical properties of pomace-fortified mustards

Table 2 reports the physicochemical properties of the BP- and CP-fortified mustards. In general, MC was significantly different ($P < 0.05$) across all concentrations and types of pomace except for 20% and 25% BP-fortified samples, increasing with higher levels of pomace addition for both BP and CP mustard. MC of mustard ranged from 67.7% (control) to 69.7% for BP (25% fortification), and to 74.8% for CP (25% fortification). This variation could be attributed to the different water holding capacities of the pomace fibers (0.60 mL/g BP and 1.47 mL/g CP). While the products were formulated to consider these differences, it is possible that different amounts of fiber degradation and lengths of fiber were present in each sample when grinding the pomace, thus impacting actual MC of pomace-fortified mustards. Moreover, the damaged insoluble fibers could decrease water holding capacity (Rodríguez, Jiménez, Fernández-Bolaños, Guillén, & Heredia, 2006; Thebaudin, Lefebvre, Harrington, & Bourgeois, 1997), and the higher pomace concentration most likely led to more variations in fiber size. All these could further explain the increase in overall MC of the samples, especially for CP-fortified ones.

Water activity (A_w) showed a similar trend to MC, ranging from 0.968 to 0.969 (BP) and to 0.981 (CP). A_w values were not significantly different ($P > 0.05$) for BP samples, but did increase significantly ($P < 0.05$) as CP concentration increased. This trend could be explained by the variation in fiber sizes as explained above, as well as the decreased amount of control mustard in formulations with more FP (Table 1). Incomplete hydration or increased degradation of the pomace fibers could lead to more free water in the system, corresponding to higher A_w as the FP concentration increased.

The pH of pomace-fortified samples showed an inverse relationship with the level of pomace incorporation. Values ranged from 4.10 (control) to 3.76 (25% BP) and 3.65 (25% CP). All values were below pH 4.2, the target pH considering potential *Clostridium botulinum* growth of a product. However, due to the high A_w of all samples, they could be susceptible to the growth of pathogenic organisms, and heat treatment (pasteurization) is necessary for ensuring food safety of the products (Sales & Daeschel, 2012). This inverse relationship can be explained by the addition of the extra acidic wine solution to counteract the water holding capacity of the pomace. The wine used in the formulations has a pH of 3.4, more acidic than the control mustard (Walman, 2010). The amount of wine solution added was proportional to the amount of pomace, thus leading to a decrease in pH as pomace concentration increased.

TA of BP-fortified samples showed a similar trend to pH, with significantly different ($P < 0.05$) values ranging from 2.53% (control) to 2.05% (25% BP), while no significant difference was observed between CP-fortified samples. These trends could be explained by the TA values of the wet pomace itself, 0.50% and

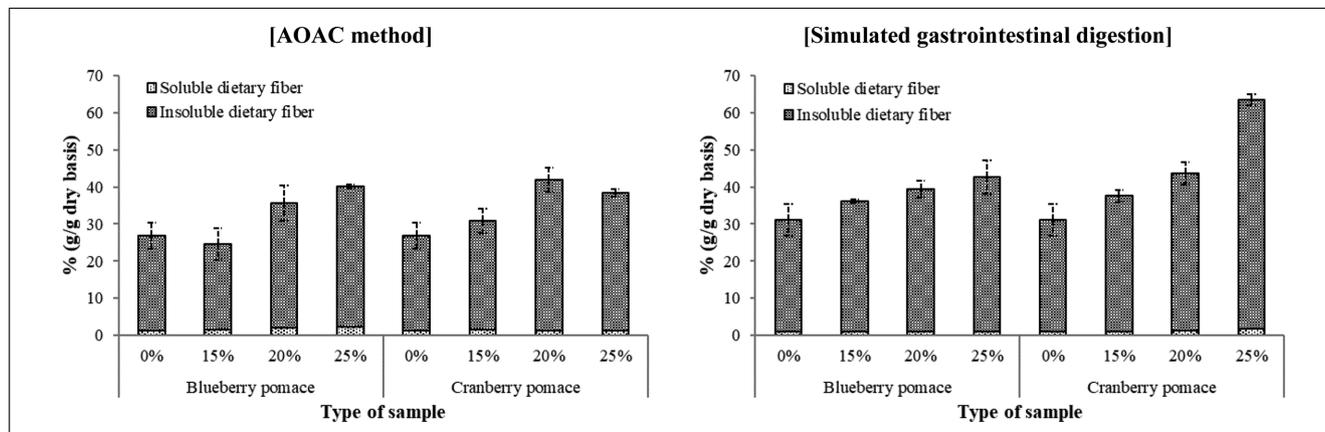


Figure 2—Dietary fiber profiles in control and fruit pomace-fortified mustards obtained from AOAC method and *in vitro* simulated gastrointestinal digestion.

0.92% for BP and CP, respectively (Gouw et al., 2017). CP had higher TA than that of BP, so that the larger decline in TA values of BP-fortified samples was expected. The control mustard had a higher TA value than any of the fortified samples because mustard seeds contain substantial phenolic acids (Kozłowska, Rotkiewicz, Zadernowski, & Sosulski, 1983).

Consistent with pH, TSS values also decreased with increasing pomace concentration. The control mustard had the highest value (16.8%), while 25% CP-fortified mustard had the lowest value (12.0%). The wine used in the formulations had TSS value of 0.57% (Walman, 2010), meaning that adding more wine solution could decrease the TSS value of pomace-fortified mustard because this value was comparatively much lower than that of the control. The control value could be significantly ($P < 0.05$) higher due to the compositional characteristics of the mustard powders, which had a polysaccharide-rich seed coat made up of glucose, mannose, xylose, and other polysaccharides (Cserhalmi, Márkus, Czukor, Baráth, & Tóth, 2000). These components could have easily contributed to TSS after the grinding and hydration process.

The BP used in this study had a dark purple color due to the high concentration of skins in the pomace, indicating the presence of anthocyanin pigments. The CP was redder, with purple undertones, also indicative of anthocyanins. The mustard powder used in this study was a yellow mustard variety; therefore, the control mustard had a muted yellow color. L^* values (Table 2) decreased as amount of pomace increased, indicating that all pomace-fortified mustards were significantly ($P < 0.05$) darker than the control. Hue angle, or the standard classification of color (red, blue, green, and so on) of mustard decreased significantly ($P < 0.05$) along with more addition of pomace, confirming the visual appearance of increased red color and decreased yellow color. Chroma value decreased significantly ($P < 0.05$) from the control to the pomace-fortified ones, but within the treatments (15%, 20%, and 25% pomace incorporation), chroma values increased, corresponding to the increased amount of pomace. The largest color change (ΔE) was observed in 25% BP and CP-fortified mustards, and the values were much higher for the 25% BP mustard (51.58) than that of 25% CP mustard (34.70).

Comparison of DF profiles in pomace-fortified mustards from modified AOAC and SGD methods

DF profiles are shown in Figure 2. IDF was the major fraction for both the control and pomace-fortified mustards, and SDF contributed a small fraction. From the AOAC determina-

tion, SDF values for 20% and 25% BP mustard were significantly ($P < 0.05$) higher (1.99% and 2.19%, respectively) than that for 0% and 15% BP samples (1.34% and 1.63%, respectively), while there was no significant difference ($P > 0.05$) in SDF values between CP-fortified samples (ranging from 1.27% to 1.43%). IDF values showed the same trend across both types of pomace-fortified mustard, with 20% and 25% pomace fortification giving significantly higher ($P < 0.05$) values (33.55% and 37.97% for BP, respectively, and 40.51% and 37.02% for CP, respectively). Total dietary fiber (TDF) values showed the same trend as the IDF values, explained by the high DF level (specifically IDF) in BP and CP, due to their large proportion of cell wall components.

Results from SGD also showed an increasing trend in SDF, IDF, and TDF along with increased pomace concentration for both types of products, with 25% fortification significantly higher ($P < 0.05$) than the control (except BP SDF, which showed no significant difference). CP SDF values ranged from 1.07% to 1.81%, BP IDF values ranged from 29.95% to 41.71%, and CP IDF values ranged from 29.95% to 61.58%. TDF values ranged from 31.02% to 42.68% for BP and to 63.65% for CP. Interestingly enough, the TDF value between AOAC and SGD methods at each level of fortification for both pomace types was only significantly different ($P < 0.05$) in the 15% BP, 15% CP, and 25% CP-fortified mustard, with higher TDF from SGD analysis than that from AOAC. This could be because the SGD process liberated bound DFs in the CP previously not available via other methods of analysis (Grundy et al., 2016). The SGD process involved mastication, which could reduce fiber particle size and cause cell rupture, thus allowing enzymes and other digestive mechanisms to act and increasing the bioavailability (Grundy et al., 2016). The significant difference ($P < 0.05$) in insoluble dietary fiber concentration of 15% BP- and CP-fortified mustards between AOAC and SGD methods could be because the ratio of pomace to mustard in the formulation increased over the control, and more bound components were released through the digestion process as compared with the control.

A comparison between BP- and CP-fortified mustards at each treatment level showed that CP fortification generally resulted in higher DF values, with the most significant difference ($P < 0.05$) through SGD for 25% fortification (42.68% for BP and 63.65% for CP). This indicated that CP contains more DF, which was consistent with our previous study (Gouw et al., 2017).

These results demonstrated that BP and CP are viable functional ingredients to use as a means of increasing DF content in specialty condiments.

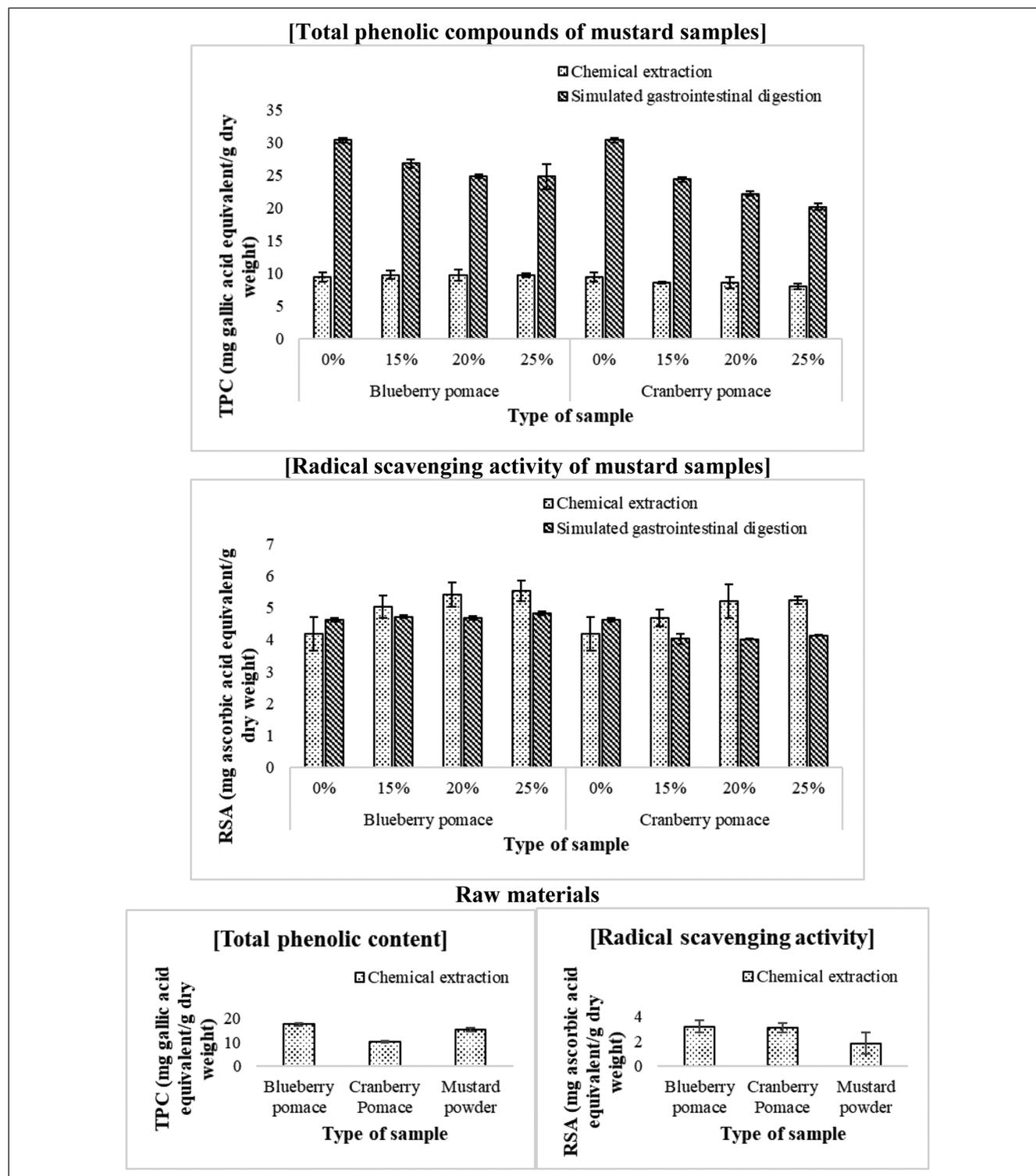
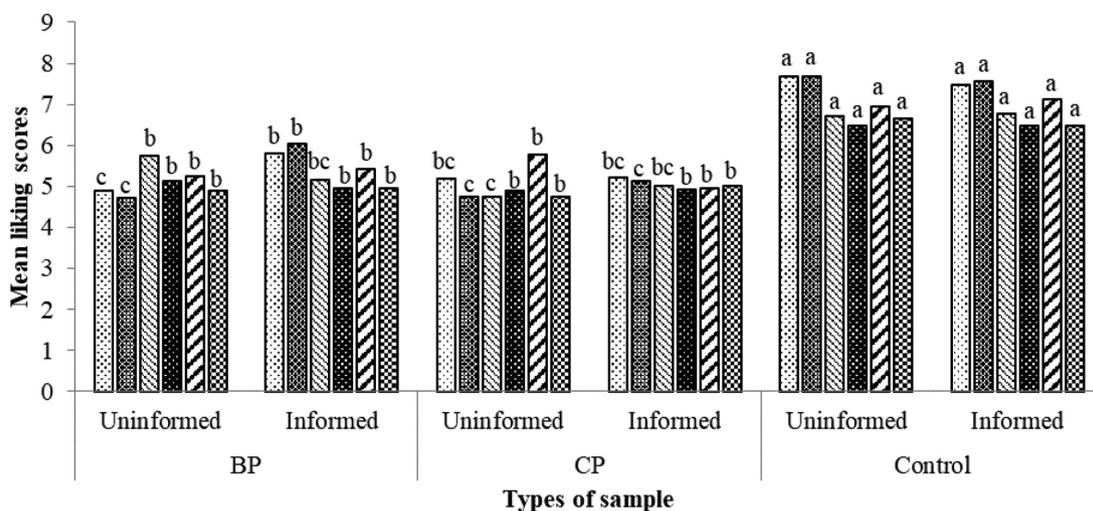


Figure 3–Total phenolic content (TPC) and radical scavenging activity (RSA) of raw materials (mustard powder and fruit pomace [FP]) and mustard samples obtained from chemical extraction and *in vitro* simulated gastrointestinal digestion (SGD). Control was prepared at 0% of FP.

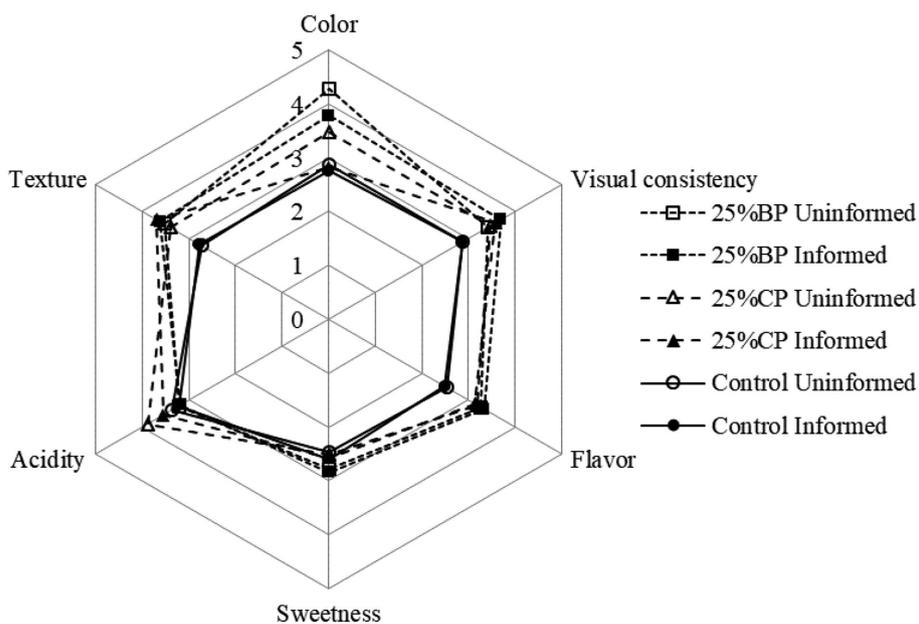
Characterization of phenolics in pomace-fortified mustards from chemical extraction (CE) and SGD

TPC values of pomace-fortified mustards from both CE and SGD are reported in Figure 3. TPC from CE showed no significant difference ($P > 0.05$) across the formulations, but TPC from SGD exhibited a decreasing trend, with values ranging from 30.32 (control) to 24.75 for 25% BP and to 20.19 for 25% CP.

Phenolics in foods can be free or bound depending on the food matrix in which they are present, and these forms determine their ease of release and absorption. Bound phenolics in foods are covalently bonded to cell wall structural components, such as cellulose, hemicellulose, lignin, and pectin (Acosta-Estrada, Gutiérrez-Urbe, & Serna-Saldívar, 2014). These bound forms need to be released through certain processes (such as SGD)



A □ Appearance ■ Color ▨ Aroma ■ Flavor ▩ Texture ▩ Overall



B

Figure 4—Means of consumers' hedonic rating [A] and intensity of attributes [B] using the just about right (JAR) scale on sensory attributes of blueberry (BP) or cranberry (CP) pomace-fortified mustard. Each 25% (w/w wet basis) pomace was fortified into Dijon-style mustard. Control was measured for a commercial product. Means with the same letter within each parameter are not significantly different at $\alpha = 0.05$ as determined by Tukey's test ($n = 90, 45$ for uninformed and 45 for informed panelists).

before they can be quantified. The gastrointestinal lumen replicated in SGD is the appropriate environment for partial release due to digestive enzymes; however, insoluble bound fibers often reach the colon before becoming fully released, as passage through the large intestine is the most significant step for release of phenolics (Acosta-Estrada et al., 2014; Capuano, 2017). Mustard has a relatively low amount of insoluble bound phenolics (<2% of total), while fruits like cranberry have a relatively high insoluble bound fraction (76% of total) (Acosta-Estrada et al., 2014). Based on our previous study, BP had less IDF than CP, and a much greater portion of IDF than SDF (Gouw et al., 2017). This explained the trend in phenolics content for both the pomace-fortified mustards and the raw materials (Figure 3). SGD liberated free phenolics

in the control, but was less successful at liberating the phenolics bound to the high fiber pomace, more so for CP than for BP. As the pomace concentration increased, the mustard concentration decreased with reduced amount of free phenolics, thus led to a decrease in TPC of pomace-fortified mustard.

It is worthwhile to mention that there was potential additional error in the TPC analysis due to the nature of the analytical assay. The FC method for TPC determination is prone to overestimate values due to several interferences, notably sugars, and proteins (Escarpa & González, 2001). In an interference study, glucose was found to appear as a low sensitive polyphenolic, while albumin appeared as a "high sensitivity polyphenolic with characteristics similar to those found in gallic and caffeic acids" (Escarpa & González,

Table 3—Physicochemical properties of blueberry and cranberry pomace-fortified mustards for sensory study.

Sample type	pH	TA ^A (%)	TSS (%)	Color values ^B			ΔE^C
				L	Hue	Chroma	
Control (0% pomace)	4.02 ^a	9.39 ^b	22.00 ^a	66.20 ^a	1.46 ^a	33.33 ^a	—
25% BP	3.77 ^b	9.61 ^b	14.00 ^b	26.03 ^c	0.19 ^c	13.63 ^c	41.39 ^a
25% CP	3.61 ^c	15.90 ^a	13.67 ^b	40.25 ^b	0.50 ^b	20.47 ^b	30.97 ^b

^ATA = Titratable acidity; TSS = total soluble solid. Means with different lowercase letters in the same row indicate significant difference ($P < 0.05$) among fruit pomace-fortified mustard formulations.

^BColor values: Hue = $\tan^{-1}(b/a)$; Chroma = $\sqrt{a^2 + b^2}$.

^C $\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$, where L_1 , a_1 , and b_1 are control values, and L_2 , a_2 , and b_2 are FP-fortified mustard values.

2001). Both BP and CP had low-protein contents, and relatively low-sugar contents (Gouw et al., 2017), so these interferences should not be a problem with the FC method. However, mustard meal is relatively high in protein as compared to BP and CP, and could therefore have had a significant effect on the TPC analysis (Sarwar, Bell, Sharby, & Jones, 1981).

Figure 3 also shows RSA of mustards from both CE and SGD analysis. RSA values obtained via CE increased significantly ($P < 0.05$) over the control for all BP and CP samples (ranging from 4.18 [control] to 5.52 for 25% BP and to 5.24 for 25% CP) with the exception of 15% CP, although between treatments within each type of pomace (15%, 20%, and 25%), there was no significant difference ($P > 0.05$). Both mustard seed and FP have high antioxidant capacity (Li et al., 2013; Vattem & Shetty, 2003; Wu, Hui, Eskin, & Cui, 2016), but the DPPH assay used to quantify RSA can react differently to each type of antioxidant (Brand-Williams et al., 1995). Therefore, it was possible that the antioxidant compounds in FP reacted more favorably with the DPPH radicals in the assay, leading to increased RSA.

For BP, RSA from SGD showed significant ($P < 0.05$) increase over the control (4.60) in 25% BP (4.83), while for CP, only the control had a significantly high value (4.60). These differences were most likely due to the compositional differences in the pomace itself. CP had more seeds than that of BP, which were mostly intact in the fortified mustard. Therefore, it is likely that the digestion process was not able to effectively release the full range of phenolics present in CP, while the fully ground mustard powder was more available for SGD. BP is composed of more skins and pulp than CP, which could be more thoroughly acted upon by the digestive enzymes in SGD and result in a slightly higher RSA value.

Consumer acceptance on sensory quality of pomace-fortified mustards

The sensory study was conducted to introduce the idea of this type of specialty condiment to consumers. The products tasted by consumers were not intended market-ready products, but simply ways to get a sense of the interest and flexibility of consumer perception. Therefore, the formulations used in the sensory evaluation were created for analytical simplicity, with as few added ingredients as possible. With this evaluation, we determined that correctly marketing this product is extremely necessary, as well as the addition of supplementary ingredients to enhance the pomace flavor.

Hedonic rating scores (Figure 4) for pomace-fortified mustards were all significantly lower ($P < 0.05$) than the control, while there was no significant difference ($P > 0.05$) in any sensory data between scores from informed panelists and uninformed panelists for the control. However, given the stark contrast of appearance and flavor between the control and the fortified mustards, this was

to be expected, especially considering that half of the panelists (uninformed) had very little knowledge about the product.

Between 25% BP-fortified mustard (BP) and 25% CP-fortified mustard (CP) from both uninformed and informed panelists, there was no significant difference ($P > 0.05$) among the ratings of flavor, texture, and overall liking (Figure 4A). For flavor, the control was rated with a mean score of 6.47, while the BP- and CP-fortified ones were rated from 4.89 to 5.13; for texture, the control rating mean was 6.96 and the BP and CP ratings ranged from 4.96 to 5.78; and for overall liking, the control mean score was 6.64, while the BP and CP ratings ranged from 4.73 to 5.02. Aroma liking of all fortified samples was significantly lower ($P < 0.05$) than the control (6.71); however, uninformed panelists rated BP significantly higher (5.76) ($P < 0.05$) than CP (4.76). No significant difference ($P > 0.05$) in aroma was found between BP and CP ratings from informed panelists.

Appearance and color hedonic ratings for CP showed no significant differences ($P > 0.05$) between uninformed and informed panelists, but for BP, ratings from informed panelists were significantly higher ($P < 0.05$) than ratings from uninformed panelists. For appearance liking, the mean informed rating was 5.80, while the uninformed rating was 4.89, with a control value of 7.69. For color liking, the mean informed rating was 6.04 while the uninformed rating was 4.71, with a control value of 7.67. These results indicated that given background knowledge, consumers can be receptive to this new product if it is marketed properly and the potential benefits are clearly stated. A potential explanation for the significance of BP but not CP could be that the color of BP was clearly purple (a color associated with blueberries), while CP was more pink/yellow (a color less strongly associated with cranberries). The color of BP would be more appealing to consumers after knowing the identity of the colorant. The parameters of appearance and color are purely perception-based, illustrating that even if the formulation was not up to market-product standards because of its simplicity, the concept behind the formulation has merit on a commercial level.

Visual consistency and texture JAR ratings for pomace-fortified mustards (Figure 4B) were significantly higher ($P < 0.05$) than the control for both uninformed and informed panelists, but between BP and CP, no significant difference was shown ($P > 0.05$). These parameters were both rated on the JAR scale (5 = much too thick, 3 = just about right, 1 = much too thin), where the control mean value (2.87 for visual consistency and 2.73 for texture) was closer to ideal (ratings closer to 3, or “just about right”) than both BP and CP values (ranging from 3.42 to 3.69 for visual consistency and ranging from 3.40 to 3.69 for texture). The sweetness JAR scale ratings (5 = much too sweet, 3 = just about right, 1 = not at all sweet enough) showed no significant difference ($P > 0.05$) among all samples, with values ranging from 2.47 to 2.84. These results indicated that sweetness is highly

Table 4—Penalty table of blueberry (BP) or cranberry (CP) pomace-fortified mustard.

Variable	Level	Blueberry pomace-fortified mustard ^a					Cranberry pomace-fortified mustard ^a				
		%	Mean drops	P-value for the endpoint	Penalties	P-value for the attribute	%	Mean drops	P-value for the endpoint	Penalties	P-value for the attribute
Color	Too light	0.00					31.52	0.594	0.641		
	JAR	32.61					25.00			1.304	<i>0.027</i>
	Too dark	67.39	0.608	0.240		43.48	1.820	0.011			
Visual Consistency	Too thin	3.26	-0.500			4.35	1.628				
	JAR	52.17			0.508	0.295	46.74			1.383	<i>0.006</i>
	Too thick	44.57	0.581	0.234		48.91	1.361	0.010			
Fruit Flavor Strength	Too weak	21.74	2.621	<i><0.0001^b</i>		25.00	1.951	0.008			
	JAR	38.04			2.659	<i><0.0001</i>	34.78			1.892	<i>0.000</i>
	Too strong	40.22	2.680	<i><0.0001</i>		40.22	1.855	0.004			
Sweetness	Not sweet enough	33.70	3.284	<i><0.0001</i>		41.30	2.957	<i><0.0001</i>			
	JAR	39.13			2.817	<i><0.0001</i>	40.22			2.442	<i><0.0001</i>
	Too sweet	27.17	2.239	<i><0.0001</i>		18.48	1.293				
Acidity	Not acidic enough	18.48	1.107			13.04	1.814				
	JAR	52.17			1.858	<i><0.0001</i>	28.26			1.867	<i>0.001</i>
	Too acidic	29.35	2.331	<i><0.0001</i>		58.70	1.879	0.001			
Texture	Too thin	3.26	1.795			6.52	1.020				
	JAR	47.83			1.670	<i>0.000</i>	44.57			1.736	<i>0.001</i>
	Too thick	48.91	1.662	<i>0.001</i>		48.91	1.831	0.001			

^aEach 25% (w/w wet basis) pomace was fortified into Dijon-style mustard.

^bSignificant *P*-values (*P* < 0.05) are highlighted with bold and italic.

dependent on consumer expectation of the product, as well as personal preference.

For flavor strength JAR ratings (5 = much too strong, 3 = just about right, 1 = much too weak), BP and CP were significantly higher (*P* < 0.05) than the control (2.53), with no significant difference (*P* > 0.05) between BP and CP ratings from both uninformed and informed panelists. However, the range of BP and CP ratings (from 3.13 to 3.33) was actually more ideal (closer to 3) than the control rating, which was favorable for their acceptance.

The acidity JAR rating (5 = much too acidic, 3 = just about right, 1 = not at all acidic enough) showed no significant difference (*P* > 0.05) between uninformed and informed panelists for BP and CP, as well as no significant difference (*P* > 0.05) between the control mean score (3.22) and informed mean score for both BP and CP (3.17 and 3.55, respectively), as well as the uninformed score for BP (3.22). The uninformed mean score for CP was significantly higher (*P* < 0.05) than the rest, indicating that the CP mustard was too acidic, which was corroborated by received written comments and by pH and TA values. As shown in Table 3, pH of the CP mustard was significantly lower (3.61) than both BP (3.77) and the control (4.02), and TA of the CP mustard was significantly higher (15.90%) than either BP (9.61%) or the control (9.39%). Correspondingly, most disliking comments stated that the product had a vinegar taste that was too strong.

The color JAR ratings (5 = much too dark, 3 = just about right, 1 = much too light) for both BP and CP were significantly different (*P* < 0.05) between uninformed and informed panelists, with the informed mean scores closer to ideal, and closer to the control value (2.87) (Figure 4B). The BP-informed mean score was 3.78, while the uninformed mean score was 4.27; and the CP informed mean score was 2.82, while the uninformed mean score was 3.47. These scores could be explained by the stark contrast between control samples and fortified samples, shown by the color data in Table 3. These results further supported the need for proper marketing of these products.

The JAR scale is inherently subjective based on the consumers' expectation of the product, which is why significant differences were identified in scores for certain parameters between the informed and uninformed panelists. Product labeling and marketing are therefore essential in the success of this and most other pomace-fortified products. Specialty fruit mustards are currently on the market, but often have substantial amounts of added sugar, so with some reformulation, our product could have market acceptability and be a healthier alternative to other fruit-based mustards commercially available.

Penalty analysis was conducted to determine how color, visual consistency, flavor strength, sweetness, acidity, and texture impacted mean overall liking of the pomace-fortified mustard samples in order to guide future product development (Table 4). For the pomace-fortified mustards' JAR appearance attributes, color, and visual consistency, it was observed that "too dark" and "too thick" was significantly related to the decreasing mean overall liking of CP samples. Conversely, BP's appearance attributes had no significant impact on the sample's mean overall liking. For flavor strength, CP and BP were penalized when the flavor was "too weak" and "too strong," and both had significant impacts on the mean overall liking. For the sweetness attribute, CP and BP were both penalized when they were "not sweet enough," which significantly decreased the mean overall liking of each sample. For BP, similar results were found when consumers considered it "too sweet." CP and BP were both penalized when they were considered "too acidic," and the mean drop for each was significantly different from zero indicating a negative impact on overall liking. For the texture attribute, similar results were seen for CP and BP when consumers considered the samples "too thick," which significantly impacted mean overall liking.

Consumer comments for the dislike open-response question were also sorted and grouped to generate a list of keywords (Figure 5). For CP, the keywords most frequently used in the dislike comments were color (*n* = 29 or 31%), acidity (*n* = 21 or 22%), and vinegar flavor (*n* = 17 or 18%). For BP, color (*n* = 18

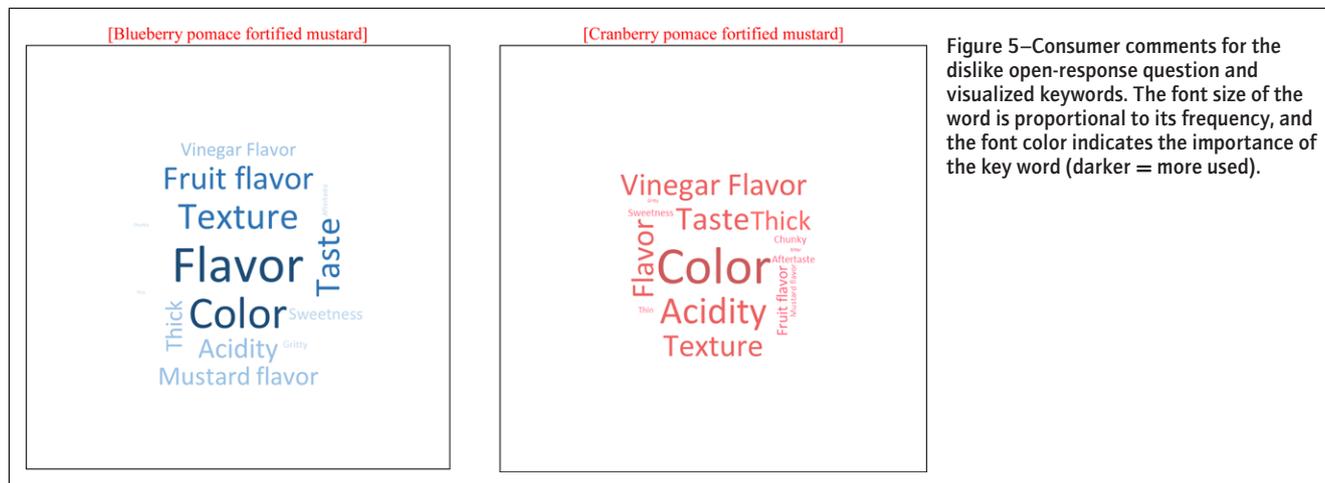


Figure 5—Consumer comments for the dislike open-response question and visualized keywords. The font size of the word is proportional to its frequency, and the font color indicates the importance of the key word (darker = more used).

or 19%) and texture ($n = 15$ or 16%) were most frequently used. Overall, a limited number of consumers explicitly compared the attributes of pomace-fortified mustard samples to traditional mustards for the dislike open-response question (CP: $n = 4$ or 4%; BP: $n = 10$ or 11%).

Beneficial consumer feedback was obtained from this sensory study to further improve the quality and sensory attributes of the fortified mustards, with changes such as acidity reduction and supplementary ingredient addition for pomace flavor enhancement. Additionally, the particle size of the pomace should be decreased, and perhaps incorporated into a mustard with more texture to increase acceptance. Above all, consumers choosing this product need to be informed of the health benefits, and product labeling should accurately describe what to expect with a product that is not necessarily usual or conventional.

Conclusion

This study confirmed BP and CP as potential functional ingredients for use in specialty mustard to increase DF content, add fruit flavor and color, and provide a healthier alternative to specialty fruit mustards. Significant increase in DF content was seen with both 20% and 25% fortification for both pomace types, and therefore either of these concentrations could be used for the product. From CE, no change for total phenolics content (TPC) was found, but RSA increased with increasing concentration of BP or CP. TPC from *in vitro* SGD was higher than that from CE. SGD showed a decreasing value for TPC with increasing concentration of BP or CP. RSA from SGD was slightly lower than that from CE but had no variation between control and fortification levels. More formulation development is needed to make the product market-ready, specifically improving mouthfeel by standardizing pomace particle size and exploring other varieties of mustard to use as the base of the formulation. Additionally, results from the consumer sensory study indicated that informing consumers about the potential benefits is key to improving acceptance of the products. Future studies may investigate the nutritional and physiological impacts of consuming pomace-fortified mustards in place of traditional mustard, and the stability of these products.

Acknowledgments

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Author Contributions

L. Davis developed the product formulations, performed the analyses and collected data, and drafted the manuscript. J. Jung assisted with data analysis and design of tables and figures, as well as overall study design. Y. Zhao provided guidance and oversight on the project direction and edited the manuscript. V. Gouw assisted with analysis and method development. A. Colonna and A. Hasenbeck designed and helped execute the sensory study.

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