

11-12-2014

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Recommended Citation

Bhaduri, Sikha and Navder, Khursheed, "Freeze Dried Blueberry Powder Fortification Improves the Quality of Gluten Free Snacks" (2014). *CUNY Academic Works*.
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Freeze Dried Blueberry Powder Fortification Improves the Quality of Gluten Free Snacks

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Abstract

Since Quinoa flour is an excellent source of natural antioxidant compounds and blueberries are rich in polyphenolic anthocyanins, this study was undertaken to improve and evaluate the quality of two blueberry powder fortified gluten free products, muffins and cookies. Control products were made with 100% Rice flour and Rice flour was replaced by 50% and 100% Quinoa flour to prepare muffin and cookies. 10% freeze dried Blueberry powder was used for fortification. Effect of flour replacements and Blueberry fortification on moisture content, water activity, antioxidant value, shelf life, sensory quality and textural properties were studied. Blueberry fortification improved the shelf life of the baked products, since, no significant increase in water activity and moisture content and no microbial growth were observed during 3 months of storage. Fortified Quinoa muffins and cookies were significantly harder because of the high fiber content of the Quinoa flour found by a Textural profile analysis using TA.XT plus Texture Analyzer. Although, baking reduced the antioxidant content to some extent, the total antioxidant activity, phenolic content and anthocyanin content were significantly increased with Blueberry fortification. Freeze dried blueberry powder fortified 100% Rice flour muffins and 100% Quinoa flour cookies were considered as the most acceptable products by consumer Sensory analysis using a 9-point hedonic scale. The study suggests that Quinoa flour is a good gluten free alternative and Blueberry fortification to snack products is a simple way to increase phytochemical and antioxidant content in diet.

Keywords: Quinoa flour; Blueberry fortification; Antioxidant activity; Sensory evaluation; Texture profile analysis

Introduction

Natural compounds have long been used for the development of modern therapeutic drugs. There is a recent increase in the search for new phytochemicals from edible medicinal plants since their anti-inflammatory and anti-allergenic agents tend to reduce the risk of many diseases. Foods containing antioxidants are associated with reduced risk of chronic health disorders, including Cancer and Cardiovascular disease. Celiac Disease (CD), a gluten sensitive inflammatory disorder of the small intestine, also known as gluten intolerance, is a diet related autoimmune enteropathy characterized by chronic inflammation of intestinal villi and mucosa due to gluten intolerance. The only effective treatment [1] for CD is a life-long gluten-free diet. Gluten-free breads and cookies are typically made from rice or maize flours with low content and poor-quality proteins. Therefore, preparation of gluten-free bakery food product from high quality gluten free grains has become a growing challenge for food technologists and nutritionists.

Quinoa (*Chenopodium quinoa* Wild) has gained increasing interest in recent years due to its high nutritive value. It is a pseudo cereal grown mainly in the Andes and has become a popular gluten free flour because of its high nutritional value [2]. The World Health Organization has rated Quinoa to be a super grain and considers it to be equivalent to milk since it contains high levels of potassium, riboflavin, B6, niacin and thiamin along with magnesium, zinc, copper, manganese and some folate. The calcium, iron, phosphorus, magnesium and zinc contents are particularly higher in Quinoa compared to wheat, corn, rice, barley, oats, rye and triticale [3]. Quinoa is an excellent (6% of the total weight) source of easily digestible dietary fiber, and is rich in protein (13.81%), compared to other cereals. It is also a good source of vitamin E [4] and also it is an important source of antioxidants [5].

Fruits and vegetables are not only rich sources of vitamins, minerals, and fiber; they are also rich in bioactive compounds known as phytochemicals [6]. Much of the disease prevention potentials of fruits

and vegetables in human health attributed to these phytochemicals [7,8] are through their antioxidant properties. Although, the benefits of individual phytochemical supplements are largely unproven, laboratory studies have shown them to suppress tumor growth, interfere with sexual hormones, prevent blood clots, and have anti-inflammatory properties. Polyphenols are important phytochemicals and are probably the most investigated molecules of nutritional interest [6]. Polyphenols are found in celery, cranberries, onions, kale, dark chocolate, broccoli, apples, cherries, berries, tea, red wine or purple grape juice, parsley, soybeans, tomatoes, eggplant, and thyme. Most common berries contain polyphenolic compounds and are particularly rich in potent antioxidants. Phenolic antioxidants are believed to possess antioxidative, anti-inflammatory antimicrobial properties [9,10] and are associated with reduced lung cancer, cardiovascular disease and chronic obstructive pulmonary diseases [11-13].

Considering the benefits of Quinoa flour and dry blueberry powder, this study was undertaken to study gluten free snacks made from Quinoa flour fortified with freeze dried blueberry powder. Two popular snacks, muffins and cookies were prepared and both were fortified with 10% freeze dried Blueberry powder. It was of interest to study the effect of flour replacements and fortification on moisture content, water activity, antioxidant value, shelf life, sensory quality and textural properties.

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Received October 09, 2014; Accepted November 03, 2014; Published November 12, 2014

Citation: Bhaduri S, Navder KP (2014) Freeze Dried Blueberry Powder Fortification Improves the Quality of Gluten Free Snacks. J Food Process Technol 5: 396. doi:10.4172/2157-7110.1000396

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Materials

Modified Muffin [14] and Cookie [15] recipes were made with Rice and/or Quinoa flour (Bob's Red mill, Milwaukie, OR), granulated white sugar (Domino Foods, Inc., Yonkers, NY, USA), salt (IGA brand, IGA Inc., Chicago, IL, USA), double-acting baking powder (Clabber Girl, Co., Terre Haute, IN, USA), canola oil (Safeway brand, Safeway Inc., Pleasanton, CA, USA), 2% reduced fat milk (Safeway brand, Safeway Inc.), fresh large eggs, nonfat dry milk and unsalted butter as shown in Table 1a and 1b. Freeze-dried blueberry powder was purchased from Z Natural Foods (West Palm Beach, FL 33411 USA). Since fresh blueberry contains approximately 80-90% moisture and flour and fresh blueberries ratio in baked food products is usually 1:1 (www.blueberrycouncil.org/blueberry-recipes), 10% freeze dried blueberry powder was used in this study.

DPPH (2, 2-diphenyl-1-picrylhydrazyl), Trolox (6-hydroxy-2, 5, 7, 8 tetramethylchroman-2-carboxylic acid) and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals and solvents were of the highest commercial grade and were used without further purification.

Baking procedures

Muffin and cookie control recipes were made with Rice flour and the variations replaced the rice flour with 50% and 100% quinoa flour. For muffins, flour, sucrose, baking powder, and salt were mixed together in a separate bowl, and then were sifted into with the wet ingredients at speed 4 for 10 seconds. Muffin pans were filled with the batter (55-65g each) and were baked for 20 minutes or until done at 204°C in a preheated oven. For cookies, the ingredients were mixed to form a dough, which was rolled on a cookie sheet and cut with a cookie cutter. Cookies were baked at 218°C for 12-15 minutes until they reach a pleasing golden brown color. Following five-minutes setting period, muffins and cookies were removed from the pans and allowed to cool on wire racks for one hour after which analyses were performed.

Methods

Moisture determination: Changes in moisture content and water activity due to substitution of Rice flour with Quinoa with or without fortification with blueberry powder were measured at different time intervals starting from 0 days to 3 months. Moisture was determined by moisture analyzer (OHAUS Explorer, MB 45, Pinebrook, NJ). Water activity was determined by a water activity meter (Decagon, CX-1). Muffins and cookies were sealed in an air-tight Zip-lock bag and stored at a regular refrigeration temperature. Moisture content and water activity were determined for four time periods from 0 days to three months for all the products. All measurements were made in triplicate.

Microbiological analysis: Observations were made for microbial growth for all stored products. To determine the microbial count of the samples Aerobic Plate Count was performed on Plate Count Agar (Sigma-Aldrich). The samples were diluted in the Phosphate Buffered Saline (PBS) by adding one gram sample in 9 mL of sterile phosphate buffered saline. The samples were serially diluted in the phosphate buffered saline. One mL from the dilutions was plated on Aerobic Plate Count Agar by spread plate technique. The plates were incubated at 30-32°C for 24-72 hours. The colony count was performed with conventional plate count method [16]. All experiments were carried out in triplicates.

Preparation of extracts for antioxidant assays: Two solvent systems were used for extraction. Methanol was used for the

antioxidant capacity assay and methanol/HCl was used for the phenolic and anthocyanin content determinations, for extraction. 1 gram of freeze dried blueberry powder or 4 grams of batter for muffin and cookies were extracted with 20 mL of either methanol or methanol/HCl (99: 1 v/v) into a 50 mL polyethylene centrifuge tube for phenolic, anthocyanin and antioxidant capacity assays. The mixture was kept on a rotary shaker at 190-220 rpm for 24 hours at room temperature. After 24 hours, the filtrate was centrifuged at 5000 g for 10 minutes, the supernatant was collected. The extraction was done at least three times with the residue and all three collected supernatants were pulled out together and were filtered through Whatman No. 4 filter paper and were stored at 4°C in airtight bottles for analysis in the next step. Extracts were analyzed in triplicate.

Determination of antioxidant capacity: A modified DPPH free radical quenching method [17] was used to determine the antioxidant capacity of methanol extracts. In this method Trolox was used as the reference standard and Results were expressed in terms of Troloxequivalent Antioxidant Capacity (TEAC), i.e., $\mu\text{molTrolox/g dry mass (DM)}$. The methanol extract as sample and Trolox solution as standard were reacted with 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution and the absorbance changes were measured at 517 nm. Briefly, 100 μL of methanol extracts were put into the sample wells for samples and 100 μL of methanol were added to control well in a 96-well microplates. Then 100 μL of 0.208 mmol DPPH was added to all wells. 200 μL of methanol was added to blank wells. The decrease in absorbance was determined every minute for 2 h or until the absorbance became steady in a microplate spectrophotometer (Bio-Rad Laboratories, Inc., 2000 Alfred Nobel Drive, CA 94547, USA). Trolox solution was prepared in 50% methanol at concentrations of 10, 20, 40, 60, 80, and 100 μM . A standard curve was generated by plotting absorbance values for Trolox at selected concentrations. Standard curve for Trolox is linear between 25 and 800 μM Trolox [18]. Results were expressed in terms of Troloxequivalent antioxidant capacity (TEAC), i.e., $\mu\text{molTrolox/g dry mass (DM)}$.

Antioxidant capacity was calculated from the standard curve and

Ingredient (% w/w)	Control	Fortified
flour	40	36
Granulated sugar	4.5	4
Salt	0.5	0.5
Baking Powder (double action)	2	2
Vegetable oil	4.3	4
2 % reduced fat Milk	40	35.5
Raw Egg white	8.7	8
Freeze dried blueberry powder (BP)	X	10
Total	100	100

Table 1a: Muffin formulations

Ingredient (% w/w)	Control	Fortified
flour	30	26.23
Brown sugar	21	19.27
Salt	0.7	0.65
Baking Powder (double action)	0.3	0.26
Unsalted Butter	16	14.68
Nonfat Dry Milk	21	18.82
Whole Egg	11	10.09
Freeze dried blueberry powder (BP)	X	10
Total	100	100

Table 1b: Cookies formulations

expressed as $\mu\text{mol Trolox equivalent (TE) g}^{-1}$ DM according to the following equation:

$$\text{Antioxidant capacity } (\mu\text{mol TE g}^{-1}) = (\mu\text{mol L}^{-1}) \times \text{DF} \times (L_{\text{solvent}} / g_{\text{sample}})$$

Where DF is the dilution factor of methanol extract, L_{solvent} is the volume of solvent used for extraction of the experimental freeze dried blueberry powder, batter or muffin and g_{sample} is the mass of batter or muffin used for extraction.

Measurements were done thrice.

Determination of Total Phenolic content: Total phenolic content was determined using a modified [19] Folin-Ciocalteu procedure. Briefly a 0.5-mL of the sample extract or a series of gallic acid standards (0, 20, 40, 60, 80, and 100 mg/l) were mixed with 0.5 mL of the Folin-Ciocalteu reagent (Sigma Chemical Co., St. Louis, Mo., U.S.A.) and 7.5 mL deionized water. The mixture was held at room temperature for 10 min before adding 1.5 mL of 20% sodium carbonate (w/v). The mixtures were heated in a 40°C water bath for 20 min and then immediately cooled in an ice bath before measuring the absorbance at 750 nm. Results were expressed as milligrams of gallic acid equivalent per gram of dry mass (GAE/g DM). Absorbance was recorded in a Spectrophotometer (Spectronics 20, Spectronics, CA). All measurements were done in triplicate.

Determination of Total Anthocyanin Content: The total anthocyanin content of berries was determined using a modified pH differential method [20]. Briefly, 1 mL of clear extract was placed into a 25 ml volumetric flask, made up to a final volume with two different buffers at pH 1.00 and pH 4.5. Buffers were prepared in 0.025 M potassium chloride solution and in 0.4 M sodium acetate solution adjusted respectively to pH 1.0 and 4.5 with HCl. Mixture absorbance were recorded at 510 nm for pH 1.00 and at 700 nm for pH 4.5 in a Spectrophotometer (Spectronics 20, Spectronics, CA).

$$\text{Absorbance were calculated as } \Delta A = (A_{510\text{nm}} - A_{700\text{nm}}) \text{ pH}_{1.0} - (A_{510\text{nm}} - A_{700\text{nm}}) \text{ pH}_{4.5}$$

Results were calculated using the following equation and expressed as milligrams of cyanidin 3-glucoside equivalents per gram of dry basis weight:-

$$\text{Total anthocyanins (mg/g)} = (\Delta A / \epsilon L) \times \text{MW} \times D \times (V/G),$$

Where ΔA is absorbance, as calculated by the above relation, ϵ is the molar extinction coefficient for cyanidin 3-glucoside (which is 26,900), L is the cell path length (1cm), D is the dilution factor, V is the final volume of the mixture in ml and G is the sample weight in gm.

Extracts were analysed in triplicate.

Texture Profile Analysis (TPA): The textural properties of muffins and cookies were determined using a TA.XT Plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) (Stable Micro Systems Ltd.). Cubes of 2.5cm were gently cut out of the center of each muffin with a serrated bread knife to expose the crumb for texture measurement. Crumb texture measurement was performed by Texture Profile Analysis (TPA) using a TA-25 MUF1/P36R probe and a TA-90 platform, with pretest speed=5 mm/s, test speed=1 mm/s, post test speed=2 mm/s and distance=10 mm. Texture analysis program parameters were set as follows: pretest speed=5 mm/s; test speed=1 mm/s; post-test speed=2 mm/s; test distance of 5 mm; and distance of 10 mm. Textural variables from force and area measurements (6)

were: hardness=peak force (g) during the first compression cycle; cohesiveness=ratio of the positive force area during the second compression to that during the first compression; springiness=height that the sample recovers during the time that elapses between the end of the first bite and the start of the second bite (cm); and chewiness=hardness X cohesiveness X springiness (g cm). Three muffins from each formulation were used to evaluate textural parameters.

Cookie hardness and fracturability were measured using a three-point bending test, using a three point bending, HDP/3PB cookies probe. The hardness of cookies was indicated by the maximum peak force required to break them. The texture analyzer, was fitted with sharp-blade probe, 6 cm long and 1 mm thick, and was set to 'return to start' cycle, a pretest speed of 1.0 mm/s, test speed of 3.0 mm/s, post-test speed of 10 mm/s, and a distance of 5.0 mm. Textural variables measured were hardness (force in g) and fracturability (distance in mm). Three cookies from each formulation were used to evaluate textural parameters.

Sensory evaluation

A panel of 20 semi-trained judges of both genders, ages 18–50 years evaluated the muffins on a 9-point hedonic scale (1=dislike extremely, 5=neither like nor dislike, 9=like extremely) [14]. Muffins and cookies were sliced into half and identified by a three-digit random number. The samples were offered to the judges on a white plate at room temperature in individual booths under white light. Panelists were given room temperature water to cleanse their palate before tasting the samples from each formulation. All six samples were served, one at a time, to each panelist. Appearance, flavor, texture, sweetness and overall acceptability were evaluated using an attribute rating scorecard.

Statistical analysis

Three samples from each of the 4 batches for each treatment (control and variations) were used for all measurements. All data (n=12) were analyzed using Statistical Analysis System (SAS Institute, Inc, Version 9.1.3, Cary, NC) and were subjected to analysis of variance with Fisher's least significant test for mean separation at 0.05 level of probability.

Results and Discussions

Moisture properties of muffins and cookies

Moisture and water activity are two important physical properties of muffins and cookies and their qualities depend on these two physical properties. Table 2a and 2b represent moisture properties and Table 3a and 3b represent water activities for unfortified and blueberry powder fortified muffins and cookies after storage. Four different storage times were used: day 0, month 1, month 2 and month 3. One of its many novel functional properties of Quinoa is its Water Holding Capacity (WHC) specific for pseudo cereals [21]. Therefore, as expected, moisture content, and water activity increased with storage but fortification with blueberry powder prevented this increase in both muffins and cookies. When comparing flours with and without fortification, blueberry powder decreased the moisture content and water activities of muffins, but the reverse was seen in case of cookies. The reasons for these differences are not clear, but they might be due to the differences in their dough compositions, baking time and temperature conditions. Storage did not typically increase moisture content for unfortified products until 2 months. Most baked products experience a rapid increase of moisture content in the first 3 days and show smaller changes in their moisture content after that [22].

Flour formulation (for muffins)	Day 0	Month 1	Month 2	Month 3
100% Rice flour	24.54 ± 0.38 ^a	24.71 ± 0.41 ^a	25.04 ± 0.22 ^b	27.03 ± 0.19 ^c
100% Rice flour + 10% BP	22.55 ± 0.07 ^b	22.35 ± 0.05 ^b	21.79 ± 0.05 ^c	21.11 ± 0.06 ^d
50% Rice flour + 50% Quinoa flour	24.08 ± 0.04 ^c	24.24 ± 0.08 ^c	25.35 ± 0.07 ^d	26.47 ± 0.06 ^e
50% Rice flour + 50% Quinoa flour + 10% BP	23.41 ± 0.06 ^d	22.68 ± 0.09 ^b	21.57 ± 0.07 ^c	20.24 ± 0.08 ^f
100% Quinoa flour	26.61 ± 0.07 ^e	26.75 ± 0.08 ^e	28.29 ± 0.05 ^a	29.82 ± 0.06 ^a
100% Quinoa flour+ 10% BP	25.64 ± 0.08 ^f	25.22 ± 0.06 ^a	24.98 ± 0.06 ^b	24.85 ± 0.08 ^b

Results are expressed as Mean ± Standard deviation. Values with different superscripts (a, b, c, d, e, f, g, h) in a column differ significantly ($p < 0.05$).

Table 2a: Moisture content with time due to freeze-dried blueberry powder fortification in muffins

Flour formulation (for cookies)	Day 0	Month 1	Month 2	Month 3
100% Rice flour	12.92 ± 0.05 ^a	14.84 ± 0.03 ^c	15.27 ± 0.06 ^d	16.27 ± 0.07 ^e
100% Rice flour + 10% BP	15.33 ± 0.08 ^b	15.37 ± 0.07 ^b	15.56 ± 0.05 ^b	15.98 ± 0.05 ^c
50% Rice flour + 50% Quinoa flour	14.48 ± 0.05 ^c	14.51 ± 0.04 ^c	16.24 ± 0.04 ^a	18.61 ± 0.03 ^b
50% Rice flour + 50% Quinoa flour + 10% BP	21.26 ± 0.16 ^d	21.02 ± 0.12 ^d	20.82 ± 0.14 ^e	20.48 ± 0.14 ^a
100% Quinoa flour	12.45 ± 0.35 ^e	12.57 ± 0.33 ^e	14.01 ± 0.35 ^c	15.23 ± 0.18 ^d
100% Quinoa flour+ 10% BP	22.68 ± 0.44 ^f	21.86 ± 0.54 ^a	21.56 ± 0.24 ^b	21.25 ± 0.45 ^f

Results are expressed as Mean ± Standard deviation. Values with different superscripts (a, b, c, d, e, f, g, h, i) in a column differ significantly ($p < 0.05$).

Table 2b: Moisture content with time due to freeze-dried blueberry powder fortification in cookies

Flour formulation (for muffins)	Day 0	Month 1	Month 2	Month 3
100% Rice flour	0.835 ± 0.025 ^a	0.906 ± 0.007 ^c	0.927 ± 0.013 ^d	0.923 ± 0.018 ^e
100% Rice flour + 10% BP	0.839 ± 0.012 ^b	0.826 ± 0.011 ^b	0.824 ± 0.009 ^a	0.821 ± 0.008 ^f
50% Rice flour + 50% Quinoa flour	0.822 ± 0.011 ^a	0.937 ± 0.01 ^e	0.952 ± 0.011 ^b	0.956 ± 0.009 ^a
50% Rice flour + 50% Quinoa flour + 10% BP	0.823 ± 0.007 ^a	0.821 ± 0.008 ^a	0.815 ± 0.007 ^a	0.792 ± 0.008 ^b
100% Quinoa flour	0.816 ± 0.006 ^a	0.939 ± 0.004 ^d	0.939 ± 0.005 ^e	0.941 ± 0.005 ^b
100% Quinoa flour+ 10% BP	0.781 ± 0.006 ^c	0.778 ± 0.005 ^c	0.776 ± 0.006 ^c	0.774 ± 0.005 ^c

Results are expressed as Mean ± Standard deviation.. Values with different superscripts (a, b, c, d, e, f, g, h) in a column differ significantly ($p < 0.05$).

Table 3a: Water activities (A_w) with time due to freeze-dried blueberry powder fortification in muffins

Flour formulation (for cookies)	Day 0	Month 1	Month 2	Month 3
100% Rice flour	0.254 ± 0.004 ^a	0.301 ± 0.005 ^c	0.311 ± 0.006 ^b	0.321 ± 0.007 ^d
100% Rice flour + 10% BP	0.498 ± 0.001 ^b	0.503 ± 0.002 ^b	0.518 ± 0.005 ^a	0.523 ± 0.006 ^e
50% Rice flour + 50% Quinoa flour	0.425 ± 0.006 ^c	0.428 ± 0.003 ^a	0.438 ± 0.005 ^d	0.451 ± 0.005 ^e
50% Rice flour + 50% Quinoa flour + 10% BP	0.579 ± 0.005 ^d	0.569 ± 0.005 ^f	0.549 ± 0.006 ^a	0.521 ± 0.006 ^b
100% Quinoa flour	0.346 ± 0.003 ^e	0.348 ± 0.002 ^e	0.356 ± 0.004 ^b	0.361 ± 0.005 ^f
100% Quinoa flour+ 10% BP	0.599 ± 0.004 ^f	0.589 ± 0.004 ^a	0.571 ± 0.004 ^f	0.569 ± 0.005 ^f

Results are expressed as Mean ± Standard deviation.. Values with different superscripts (a, b, c, d, e, f, g, h, i, j) in a column differ significantly ($p < 0.05$).

Table 3b: Water activities (A_w) with time due to freeze-dried blueberry powder fortification in cookies

Microbiological analysis

No microbiological growth was observed up to three months of regular refrigeration storage for Quinoa flour and the blueberry powder fortified muffins and cookies. A few colonies were observed in unfortified Rice flour products after 2 months, but those were not analyzed in detail in this study. Reduced moisture content and water activities for the blueberry powder fortified products perhaps prevented microbiological growth. Additional work needs to be done to explore this.

Antioxidant capacity, total phenolic and total anthocyanin content assays

Table 4a and 4b represent Antioxidant activities of fortified and unfortified muffin and cookies. Blueberries, like all berries are believed to be a rich source of polyphenol and anthocyanin like antioxidants [23]. Blueberry fortification was found to bring about a significant increase in the antioxidant capacity, but baking was found to bring about a reduction. Quinoa flour is also an excellent source of antioxidant compounds [5], and Quinoa like pseudocereals have been shown to have a higher content of anthocyanin compounds [24]. Similar results

were seen in this study where muffins and cookies made with Quinoa flour had higher antioxidant and anthocyanin content than rice flour.

A 137% increase in antioxidant activity was observed in 100% Quinoa flour muffins compared to 100% Rice flour muffins (Table 4a). Total phenolic content and total anthocyanin contents were also the highest (0.185 GAE per g DM and 0.136 C3G per g DM) in 100% Quinoa flour muffins after freeze dried blueberry powder fortification. Blueberry powder fortified 100% Quinoa flour cookies showed highest total phenolic content (0.315 GAE per g DM), total anthocyanin content (0.204 C3G per g DM) and antioxidant activity (334.16 $\mu\text{mol TE per g DM}$) compared to other two flour formulated cookies after blueberry powder fortification. However, not a big difference in percentage increase of activity was observed for all three flour formulations, with fortification (Table 4b).

Texture properties

Tables 5a show the texture properties of fortified and unfortified muffins. Significant change in texture was observed in both muffins and cookies when rice flour was replaced by Quinoa flour. Quinoa

Flour formulations (for muffins)	Reaction gradient	Antioxidant activity (μ mol TE per g DM)	Total phenolic content (mg GAE per g DM)	Total anthocyanin content (mg C3G per g DM)
100% Rice flour	Before baking	149 \pm 3.61 ^h	0.028 \pm 0.004 ^g	0.038 \pm 0.008 ^b
	After baking	140 \pm 1.73 ^g	0.025 \pm 0.004 ^g	0.027 \pm 0.004 ^g
	% Loss in activity after baking	5.99 \pm 3.21 ^m	11.12 \pm 4.87 ⁱ	28.16 \pm 5.38 ^m
100% Rice flour + 10% BP	Before baking	255 \pm 5.56 ^j	0.201 \pm 0.011 ^f	0.1 \pm 0.005 ^f
	After baking	244 \pm 2.64 ^e	0.175 \pm 0.005 ^e	0.058 \pm 0.006 ^e
	% Loss in activity after baking	4.27 \pm 3.04 ^m	13.01 \pm 2.21 ^m	42.11 \pm 2.96 ⁿ
	% increase of activity after fortification in baked product	74.31 \pm 3.66 ⁿ	615.04 \pm 125.55 ⁿ	41.07 \pm 1.35 ^o
50% Rice flour + 50% Quinoa flour	Before baking	98.3 \pm 2.51 ^l	0.067 \pm 0.003 ^k	0.095 \pm 0.005 ^l
	After baking	92.5 \pm 1.51 ^k	0.06 \pm 0.004 ^k	0.067 \pm 0.004 ^k
	% Loss after baking	5.91 \pm 2.24 ^m	10.51 \pm 2.02 ^l	28.68 \pm 0.91 ^m
50% Rice flour + 50% Quinoa flour + 10% BP	Before baking	212.6 \pm 2.08 ^l	0.194 \pm 0.004 ^l	0.162 \pm 0.009 ^l
	After baking	204 \pm 3.61 ^l	0.17 \pm 0.002 ^l	0.094 \pm 0.005 ^l
	% Loss in activity after baking	4.08 \pm 0.75 ^m	12.34 \pm 1.81 ^m	41.96 \pm 1.04 ⁿ
	% increase of activity after fortification in baked product	120.54 \pm 0.96 ^o	184.28 \pm 18.68 ^o	40.30 \pm 1.29 ^o
100% Quinoa flour	Before baking	91.7 \pm 4.51 ^c	0.057 \pm 0.007 ^c	0.143 \pm 0.01 ^d
	After baking	86.6 \pm 1.52 ^c	0.05 \pm 0.005 ^c	0.102 \pm 0.004 ^c
	% Loss after baking	5.35 \pm 3.01 ^m	12.05 \pm 2.95 ^j	28.56 \pm 2.33 ^m
100% Quinoa flour + 10% BP	Before baking	214 \pm 1.52 ^b	0.211 \pm 0.01 ^b	0.234 \pm 0.007 ^b
	After baking	206 \pm 2.64 ^a	0.185 \pm 0.017 ^a	0.136 \pm 0.005 ^a
	% Loss after baking	4.03 \pm 1.41 ^m	12.44 \pm 4.51 ^l	41.88 \pm 0.59 ⁿ
	% increase of activity after fortification in baked product	137.73 \pm 4.14 ^p	270.94 \pm 24.76 ^o	66.01 \pm 8.33 ^o

Results are expressed as Mean \pm Standard deviation.. Values with different superscripts (a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p) in a column differ significantly ($p < 0.05$).

Table 4a: Antioxidant properties of muffins due to fortification with freeze-dried blueberry power

Flour formulations (for cookies)	Reaction gradient	Antioxidant activity (μ mol TE per g DM)	Total phenolic content (mg GAE per g DM)	Total anthocyanin content (mg C3G per g DM)
100% Rice flour	Before baking	128.8 \pm 6.48 ^f	0.196 \pm 0.009 ^f	0.164 \pm 0.012 ⁿ
	After baking	121 \pm 4.19 ^f	0.175 \pm 0.007 ^f	0.117 \pm 0.006 ^g
	% Loss in activity after baking	6.01 \pm 2.11 ^l	10.64 \pm 3.73 ^j	28.57 \pm 1.77 ^m
100% Rice flour + 10% BP	Before baking	179.26 \pm 6.54 ^e	0.297 \pm 0.038 ^e	0.284 \pm 0.013 ^f
	After baking	172.16 \pm 6.29 ^e	0.261 \pm 0.046 ^d	0.165 \pm 0.007 ^e
	% Loss in activity after baking	3.95 \pm 0.87 ^l	12.34 \pm 7.08 ^k	41.89 \pm 0.31 ⁿ
	% increase of activity after fortification in baked product	42.31 \pm 4.19 ^k	31.69 \pm 8.75 ^l	52.09 \pm 9.21 ^o
50% Rice flour + 50% Quinoa flour	Before baking	161.67 \pm 7.91 ^h	0.252 \pm 0.008 ^l	0.156 \pm 0.014 ^l
	After baking	152.06 \pm 3.49 ^h	0.225 \pm 0.004 ^l	0.111 \pm 0.006 ^k
	% Loss in activity after baking	5.86 \pm 2.48 ^l	10.68 \pm 1.72 ^j	28.71 \pm 2.38 ^m
50% Rice flour + 50% Quinoa flour + 10% BP	Before baking	208.42 \pm 7.22 ^g	0.325 \pm 0.007 ^h	0.251 \pm 0.013 ^l
	After baking	200.06 \pm 9.14 ^g	0.285 \pm 0.012 ^g	0.146 \pm 0.006 ^l
	% Loss in activity after baking	4.02 \pm 2.11 ^j	12.32 \pm 2.71 ^k	41.81 \pm 1.35 ⁿ
	% increase of activity after fortification in baked product	49.04 \pm 21.36 ^k	26.70 \pm 6.09 ^m	40.04 \pm 5.01 ^p
100% Quinoa flour	Before baking	233.83 \pm 10.71 ^d	0.252 \pm 0.013 ^c	0.173 \pm 0.026 ^d
	After baking	220.04 \pm 9.22 ^c	0.225 \pm 0.005 ^c	0.123 \pm 0.007 ^c
	% Loss in activity after baking	5.88 \pm 0.54 ^l	10.62 \pm 2.63 ^j	28.22 \pm 6.55 ^m
100% Quinoa flour + 10% BP	Before baking	347.67 \pm 6.02 ^b	0.361 \pm 0.009 ^b	0.352 \pm 0.011 ^b
	After baking	334.16 \pm 9.82 ^a	0.315 \pm 0.011 ^a	0.204 \pm 0.011 ^a
	% Loss in activity after baking	3.87 \pm 2.88 ^j	12.75 \pm 0.74 ^k	42.05 \pm 2.12 ⁿ
	% increase of activity after fortification in baked product	41.07 \pm 1.35 ^k	31.62 \pm 3.66 ^l	66.01 \pm 8.33 ^o

Results are expressed as Mean \pm Standard deviation.. Values with different superscripts (a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q) in a column differ significantly ($p < 0.05$).

Table 4b: Antioxidant properties of cookies due to fortification with freeze-dried blueberry power

flour products had increased hardness due to its high fiber content. The final volume of baked products is based on a two-step process. The first involves incorporation of air in the batter during mixing which depends on the viscosity of the batter and mixing conditions. The second expansion occurs during baking when the incorporated air and the carbon dioxide produced from leavening agents expand causing the elastic batter to further extend.

The 100% unfortified Rice flour muffins were the softest (868.27 g) while the 100% fortified Quinoa flour muffins were the hardest (1737.97 g). This was expected since Quinoa flour has a higher bulk volume compared to Rice flour which also reduces the moisture content. Increased hardness reduced the springiness in muffins. Quinoa flour fortification also increased the chewiness and gumminess in muffins. Blueberry powder fortification was found to increase hardness, and

Flour formulations (for muffins)	Hardness (g)	Springiness (cm)	Cohesiveness	Gumminess	Chewiness (g cm)
100% Rice flour	868.27 ± 20.33 ^a	0.906 ± 0.09 ^a	0.784 ± 0.02 ^a	229.52 ± 21.51 ^a	781.20 ± 33.69 ^a
100% Rice flour + 10% BP	1096.21 ± 21.09 ^b	0.657 ± 0.153 ^b	0.824 ± 0.19 ^a	272.06 ± 12.57 ^a	668.03 ± 43.64 ^a
50% Rice flour + 50% Quinoa flour	1079.12 ± 54.56 ^a	1.006 ± 0.06 ^a	0.842 ± 0.03 ^a	645.79 ± 43.27 ^b	1079.00 ± 33.04 ^b
50% Rice flour + 50% Quinoa flour + 10% BP	1137.82 ± 84.51 ^c	0.844 ± 0.05 ^c	0.861 ± 0.06 ^a	626.56 ± 45.44 ^b	1171.51 ± 88.93 ^b
100% Quinoa flour	1478.28 ± 30.46 ^a	1.040 ± 0.05 ^a	0.790 ± 0.04 ^a	1015.45 ± 23.43 ^c	1537.00 ± 24.24 ^c
100% Quinoa flour+ 10% BP	1737.97 ± 44.92 ^a	1.006 ± 0.01 ^a	0.834 ± 0.06 ^a	1007.51 ± 9.54 ^c	1232.22 ± 112.65 ^b

Results are expressed as Mean ± Standard deviation.. Values with different superscripts (a, b, c) in a column differ significantly (p < 0.05).

Table 5a: Texture properties of unfortified and freeze-dried blueberry powder fortified muffins

Flour formulations (for cookies)	Hardness (g)	Fracturability
100% Rice flour	343.81 ± 6.06 ^a	39.9 ± 1.69 ^a
100% Rice flour + 10% BP	568.86 ± 11.49 ^b	41.65 ± 2.45 ^a
50% Rice flour + 50% Quinoa flour	365.61 ± 10.03 ^a	38.34 ± 2.52 ^a
50% Rice flour + 50% Quinoa flour + 10% BP	725.41 ± 34.34 ^c	40.66 ± 7.21 ^a
100% Quinoa flour	503.79 ± 19.89 ^b	38.32 ± 6.85 ^a
100% Quinoa flour+ 10% BP	830.24 ± 19.96 ^a	38.11 ± 4.07 ^a

Results are expressed as Mean ± Standard deviation.. Values with different superscripts (a, b, c) in a column differ significantly (p < 0.05).

Table 5b: Texture properties of unfortified and freeze-dried blueberry powder fortified cookies

Flour formulations (for muffins)	Flavor	Texture	Sweetness	Appearance	Overall acceptance
100% Rice flour	7.38 ± 0.59 ^a	7.14 ± 0.35 ^a	6.04 ± 3.66 ^a	6.81 ± 0.61 ^a	7.04 ± 0.49 ^a
100% Rice flour + 10% BP	7.71 ± 0.46 ^a	7.23 ± 0.43 ^a	6.63 ± 3.98 ^b	6.57 ± 0.51 ^a	7.85 ± 0.79 ^b
50% Rice flour + 50% Quinoa flour	6.28 ± 0.56 ^c	6.52 ± 0.61 ^b	4.99 ± 3.06 ^d	6.14 ± 0.72 ^b	6.14 ± 0.85 ^c
50% Rice flour + 50% Quinoa flour + 10% BP	6.38 ± 0.49 ^c	6.23 ± 0.43 ^c	5.11 ± 3.09 ^d	6.52 ± 0.61 ^a	6.04 ± 0.21 ^c
100% Quinoa flour	6.52 ± 0.63 ^b	5.71 ± 0.46 ^d	5.13 ± 3.11 ^c	6.14 ± 0.79 ^b	6.23 ± 0.43 ^c
100% Quinoa flour+ 10% BP	6.04 ± 0.66 ^d	5.14 ± 0.38 ^d	4.25 ± 2.57 ^c	6.23 ± 1.17 ^b	5.28 ± 0.78 ^d

Results are expressed as Mean ± Standard deviation.. Values with different superscripts (a, b, c, d) in a column differ significantly (p < 0.05).

Table 6a: Sensory characteristics of unfortified and freeze-dried blueberry powder fortified muffins

Flour formulations (for cookies)	Flavor	Texture	Sweetness	Appearance	Overall acceptance
100% Rice flour	5.85 ± 0.79 ^a	6.38 ± 0.66 ^a	7.14 ± 0.79 ^a	6.81 ± 0.61 ^a	7.19 ± 0.41 ^a
100% Rice flour + 10% BP	6.47 ± 0.92 ^b	5.91 ± 0.76 ^b	6.28 ± 0.56 ^b	6.57 ± 0.51 ^a	7.61 ± 0.81 ^a
50% Rice flour + 50% Quinoa flour	6.38 ± 0.49 ^b	6.52 ± 0.61 ^a	6.23 ± 0.53 ^b	6.14 ± 0.72 ^a	6.19 ± 0.81 ^b
50% Rice flour + 50% Quinoa flour + 10% BP	6.52 ± 0.61 ^b	5.85 ± 0.72 ^b	6.19 ± 0.87 ^b	6.52 ± 0.61 ^a	7.23 ± 0.94 ^a
100% Quinoa flour	6.47 ± 0.63 ^b	6.47 ± 0.81 ^a	5.85 ± 0.85 ^b	6.14 ± 0.79 ^a	7.38 ± 0.81 ^a
100% Quinoa flour+ 10% BP	6.76 ± 0.83 ^b	5.52 ± 0.51 ^b	5.23 ± 0.62 ^c	6.23 ± 1.17 ^a	7.95 ± 0.86 ^a

Results are expressed as Mean ± Standard deviation.. Values with different superscripts (a, b, c) in a column differ significantly (p < 0.05).

Table 6b: Sensory characteristics of unfortified and freeze-dried blueberry powder fortified cookies

decrease springiness. Tables 5b represent the texture properties of fortified and unfortified cookies. Quinoa flour and blueberry powder fortification were found to increase the hardness in cookies. No significant effect of flour or fortification was seen on fracturability of cookies.

Sensory evaluation

Hedonic ratings for product attributes and overall likeability for unfortified and fortified muffins and cookies are presented in Table 6a and 6b. A Hedonic score of 5 was considered neutral, above 5 was considered to be in the desirable range, and a score below 3 was unacceptable.

Muffins are sweet, high-Calorie baked products, appreciated by consumers for their pleasant taste and soft texture. The Maillard reaction that occurs during baking produces slightly darker muffin crusts compared to its crumb [25]. As seen with textural measurements, both Quinoa flour and blueberry powder fortification increased hardness. Harder texture of fortified 100% Quinoa flour muffins showed lowest (5.14) hedonic rating for Texture. The lowest score of overall acceptance (5.28) was also associated with its hard texture. Quinoa flour also has

a natural bitter taste; which led to a lower value for sweetness (5.13 for 100% Quinoa flour and 4.25 for the blueberry fortified 100% Quinoa flour muffins), which also reduced the overall acceptability from 6.23 to 5.28. The values were still in the neither like nor dislike range. Both unfortified and fortified 100% Rice flour muffins show highest scores for flavor, texture, sweetness and overall acceptance.

Blueberry fortification reduced the hardness of all three flour formulated cookies. The Hedonic rating for overall acceptance was highest (7.95), for the blueberry fortified 100% Quinoa flour cookies. Sweetness score was highest (7.14), for unfortified 100% Rice flour cookies. The freeze dried blueberry powder also has a pleasant flavor, and Blueberry fortified 100% Quinoa flour cookies had the highest flavor score (6.76).

Blueberry powder fortification was found to improve the flavor and overall acceptability. Although, blueberry powder itself does not have any specific flavor, but its polyphenolic composition inhibits Maillard reaction during baking to produce a different flavor acceptable to the consumer [26]. Maillard reaction products have also been shown to possess antioxidant activity in food products in which they are formed [27]. Blueberry powder fortified 100% Rice flour muffin was observed

to have higher overall acceptance than unfortified 100% Rice flour muffin.

In summary, the best overall acceptable muffin was the 10% freeze-dried blueberry powder fortified 100% Rice muffin, while the most acceptable cookie was the one made with 100% Quinoa flour fortified with 10% freeze-dried powder.

Conclusions

Despite its slightly bitter taste, Quinoa flour produces acceptable gluten free baked products that are high in fiber and rich in antioxidants. In addition, dried blueberry powder is a simple way to improve the shelf life and increase phytochemical and antioxidant content in diet.

Acknowledgments

The work was supported by PSC-CUNY-TRADA-43-29 grant.

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