

Antioxidant Activities of Selective Gluten Free Ancient Grains

George E. Inglett, Diejun Chen, Sean X. Liu

Functional Foods Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, Peoria, IL, USA

Email: George.Inglett@ars.usda.gov

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Abstract

Ancient grains were known for special nutritional values along with gluten free qualities. Amaranth, quinoa, teff, and buckwheat flours were evaluated for pasting properties, water holding capacities, phenolic contents, and antioxidant activities (free and bound). They all had higher water holding capacities than wheat flour. Amaranth, quinoa, and teff showed higher pasting viscosities than wheat flour. Buckwheat flour had the highest free, bound and total phenolic contents among the flours in all aqueous extracts. The bound phenolic contents were higher than the free phenolic contents regardless of the solvents with the exception of water extraction of quinoa and buckwheat. The free phenolic compounds for all four flours were highest in water extract, and least in 100% ethanol. Bound antioxidant activities were much higher than the free antioxidant activities regardless of solvents for all products. The free antioxidant activities from water and 50% ethanol were higher than 100% ethanol. In contrast, more bound phenolics were extracted with 100% ethanol than water and 50% ethanol. Our study suggested that the total phenolic contents and antioxidant activities of grains could be underestimated in the literature without considering the bound phenolic compounds. These ancient grains have nutrition, antioxidants, and textural qualities suitable for functional foods.

Keywords

Amaranth, Quinoa, Teff, Buckwheat, Gluten-Free, Antioxidant, Pasting

1. Introduction

Amaranth, quinoa, teff and buckwheat have received considerable interest because of their gluten-free uniqueness. Gluten is found in grains such as wheat, barley and rye. Gluten causes inflammation in the small intestines

of people with celiac disease. A gluten-free diet helps people with celiac disease control their signs and symptoms and prevent complications [1].

Amaranth (*Amaranthus caudatus*), an ancient grain, contains about thirty percent more protein than cereals such as rice, sorghum and rye [2]. Amaranth is a source of thiamine, niacin, riboflavin, folate, and dietary minerals including calcium, iron, magnesium, phosphorus, zinc, and manganese that are comparable to grain products such as wheat germ, oats, and others [3]. Amaranth flour particularly has an unusually rich source of the essential amino acid, lysine, which is low in other grains [4]. Regular consumption of amaranth oil reduces blood pressure and cholesterol levels while improving antioxidant status and some immune parameters because amaranth seed oil may be benefit for those with hypertension and cardiovascular disease [5].

Quinoa (*Chenopodium quinoa*) is a pseudocereal or pseudograin rather than a cereal or grain that has been called a superfood because of its remarkable nutritional value. Quinoa seeds contain large amounts of vitamins; protein (13% - 14%) with a good digestibility; a balanced amino acid with high lysine and methionine; and a good source of dietary fiber, K, Ca, Mg, P, and Fe that are much higher than conventional cereals [6] [7]. Quinoa contains antioxidant phytonutrients (polyphenols and phytosterols) and flavonoids (quercetin and kaempferol) in concentrated amounts with possible nutraceutical benefits [7] [8]. Also, quinoa can be considered as an oil crop containing omega-6 with notable vitamin E content. Moreover, quinoa starch has useful physicochemical properties, such as viscosity and freeze stability [8].

Another ancient crop, teff (*Eragrostis tef*), also has an attractive nutrition profile, being high in dietary fiber with significant levels of the minerals phosphorus, magnesium, aluminum, iron, copper, zinc, boron and barium, and thiamin [3]. Teff is high in proteins with an excellent amino acid composition including all 8 essential amino acids for humans. It is also higher in lysine than wheat or barley along with its high carbohydrates and fiber contents [9].

Buckwheat (*Fagopyrum esculentum* Moench), a well known pseudocereal, possesses good sources of manganese, magnesium and dietary fiber as well as its gluten free quality. Many of the health benefits of buckwheat have been attributed to its high levels of phenolic compounds and antioxidant activity [10]. Whole buckwheat contains 2 - 5 times more phenolic compounds than oats or barley, while buckwheat bran and hulls have 2 - 7 times higher antioxidant activity than barley, triticale, and oats [11] [12]. The primary antioxidants in buckwheat are rutin, quercetin, hyperin, and catechins [13].

Recently, amaranth, quinoa, buckwheat, and teff flour have been used in food products to replace wheat flour. The different levels of amaranth flour also have been mixed with the wheat flour and baking ingredients that were fermented, molded, pan-proved and baked. The loaf volume decreased with increasing amounts of amaranth grain flour. There were significant differences in using 15% amaranth flour in sensory evaluation [14]. Quinoa flour can substitute or completely replace wheat flour in many recipes. Studies on food products containing quinoa flour, such as noodles, cookies, and breads, have been reported [15]. Buckwheat flour was used for many food products, such as *gluten-free bread using tartary buckwheat and chia flour rich in flavonoids and omega-3 fatty acids as ingredients* [16]. The wheat flour bread containing 22.5% to 45% blends of teff, green pea and buckwheat (BW) flours provided superior nutritional composition with acceptable sensory properties compared with the 100% wheat flour bread [17].

Antioxidant-rich diets have been associated with a lower incidence of cardiovascular disease, cancers, and age-related degenerative processes [18]. Determination of total antioxidant activity including bound and free compounds provided a tool for investigating the protective role of antioxidant-rich products in degenerative diseases and potentially positive health benefits [19] [20]. A direct method was developed for total antioxidant activities by mixing solid samples with free radicals followed by a subsequent spectrometric measurement that gave total antioxidant activities higher than those by the traditional procedures [21].

The objective of this research was to compare the nutrition of amaranth, quinoa, teff and buckwheat, and study their physical and chemical properties including water holding capacity, pasting properties, phenolic content, and antioxidant activities. This study will provide information for developing new functional gluten free products for health concerned consumers.

2. Materials and Methods

2.1. The Source of Ingredients

Buckwheat flour (Farinetta) were supplied by Minn-Dak Growers Ltd. (Grand Forks, ND). Organic Gluten free

amaranth flour certified by international certification services Inc. was purchased from Dakota Prairie Organic Flour Co. Harvey, ND, USA. Gluten free quinoa and teff flour was purchased from Bob's Red Mill, Milwaukie, OR, USA.

2.2. Water-Holding Capacity

The water-holding capacity (WHC) of the samples was determined according to a previous procedure with minor modifications [22]. Each sample (2 g, dry weight) was mixed with 25 g of distilled water and vigorously mixed for 1 min to a homogenous suspension using a Vortex stirrer, held for 2 h, and centrifuged at 1590 g for 10 min. Each treatment was replicated twice. Water-holding capacity was calculated on dry basis by the difference between the weight of water added and decanted.

2.3. Pasting Property Measurement

The pasting properties of samples were evaluated using a Rapid Visco Analyzer (RVA-4, Perten Scientific, Springfield, IL). Samples (2.24 g, dry basis) were made up to a total weight of 28 g with distilled water in a RVA canister (8% solids, w/w). The viscosity of the suspensions was monitored during the following heating and cooling stages. Suspensions were equilibrated at 50°C for 1 min, heated to 95°C at a rate of 6.0°C/min, maintained at 95°C for 5 min, and cooled to 50°C at rate of 6.0°C/min, and held at 50°C for 2 min. For all test measurements, a constant paddle rotating speed (160 rpm) was used throughout the entire analysis except for 920 rpm in the first 10 s to disperse sample. Each sample was analyzed in duplicate. The results were expressed in Rapid Visco Analyser units (RVU, 1 RVU = 12 centipoises).

2.4. Sequential Alkaline Extraction

Step 1: double extraction for free compounds.

The extraction conditions were modified based on the research by Serpen (2008) [21]. Samples (1 g) were extracted with 10 ml of 50% ethanol twice under N₂ by shaking in a water bath for 20 min. Each extraction step was followed by centrifugation at 3000 rpm (1462 g) for 10 min. Combined supernatants were used for free phenolic and antioxidant activity measurements.

Step 2: alkaline extraction for bound compounds.

The solid residue from double extraction was hydrolyzed with 20 mL of 2 N sodium hydroxide for 2 h under N₂ by shaking in the dark at room temperature [23]. The alkaline extracts were acidified using about 20 mL of 2 N HCl and centrifuged at 3000 rpm (1462 g) for 10 min. The supernatants were used for bound phenolic and antioxidant activity measurements.

2.5. Total Phenolic Content

Phenolic content was determined by the Folin-Ciocalteu colourimetric method with minor modifications [24] [25]. To 100 µL of extract, 7.9 mL of deionized water and 0.5 mL of Folin-Ciocalteu reagent (F9252, Sigma Aldrich, St Louis, MO) were added, mixed on a vortex mixer, and 1.5 mL of 1.85 M Na₂CO₃ was added after 15 min. Absorbance of samples was measured at 765 nm after 2 h. Gallic acid was used as a standard and results were expressed as mg of gallic acid equivalents per g (d.m.).

2.6. Antioxidant Activity

Antioxidant activity determination was modified using a previous method by reacting 0.5 mL of extract with 0.5 mL of 200 µM 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) in a cuvette for 40 minutes in dark [26]. Convert the cuvettes after adding reagent and prior to reading the absorbance at 515 nm. Results were expressed as 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) equivalents per g (d.m.).

2.7. Statistical Analysis

Data from replicated samples were analyzed using SAS software using analysis of variance with Duncan's multiple comparison adjustment to determine significant differences ($p < 0.05$) between treatments [27].

3. Results and Discussion

3.1. Nutrition Composition

The ancient grain flours in **Table 1** contain more proteins and minerals including calcium, iron, magnesium, phosphorus, potassium, and zinc than wheat flour. Amaranth contains the highest magnesium and phosphorus;

Table 1. Composition of ancient grains and wheat.

Proximates (per 100 g)	Unit	Amaranth	Quinoa	Teff	Buckwheat	Wheat
Water	g	11.29	13.28	8.82	9.75	10.74
Energy	kcal	371	368	367	343	340
Protein	g	13.56	14.12	13.3	13.25	13.21
Total lipid (fat)	g	7.02	6.07	2.38	3.4	2.50
Carbohydrate, by difference	g	65.25	64.16	73.13	71.5	71.97
Fiber, total dietary	g	6.7	7	8	10	10.7
Sugars, total	g	1.69	-*	1.84	-	0.41
Minerals						
Calcium, Ca	mg	159	47	180	18	34
Iron, Fe	mg	7.61	4.57	7.63	2.2	3.6
Magnesium, Mg	mg	248	197	184	231	137
Phosphorus, P	mg	557	457	429	347	357
Potassium, K	mg	508	563	427	460	363
Sodium, Na	mg	4	5	12	1	2
Zinc, Zn	mg	2.87	3.1	3.63	2.4	2.6
Vitamins						
Vitamin C, total ascorbic acid	mg	4.2	-	-	-	-
Thiamin (B1)	mg	0.116	0.36	0.39	0.101	0.502
Riboflavin	mg	0.2	0.318	0.27	0.425	0.165
Niacin	mg	0.923	1.52	3.363	7.02	4.957
Vitamin B-6	mg	0.591	0.487	0.482	0.21	0.407
Folate, DFE	Âµg	82	184	-	30	44
Vitamin B-12	Âµg	-	-	-	-	-
Vitamin A, RAE	Âµg	-	1	-	-	-
Vitamin A, IU	IU	2	14	9	-	9
Vitamin E (alpha-tocopherol)	mg	1.19	2.44	0.08	-	0.71
Vitamin D (D2 + D3)	Âµg	-	-	-	-	-
Vitamin D	IU	-	-	-	-	-
Vitamin K (phylloquinone)	Âµg	-	-	1.9	-	-
Lipids						
Fatty acids, total saturated	g	1.459	0.706	0.449	0.741	0.43
Fatty acids, total monounsaturated	g	1.685	1.613	0.589	1.04	0.283
Fatty acids, total polyunsaturated	g	2.778	3.292	1.071	1.039	1.167
Cholesterol	g	-	-	-	-	-

*Under detection limit.

quinoa has the highest potassium; and teff has the highest calcium, iron, and zinc of all products. Also they are all rich in vitamins. Amaranth has the highest vitamin C, and vitamin B-6, quinoa has highest folate, vitamin E and D; buckwheat has highest riboflavin and niacin contents. Quinoa and teff contain higher thiamin (B1) than the other two ancient grain flours. Vitamin B1 helps the body metabolizes fats and protein, and is required for healthy skin, hair, eyes, and liver. It also helps the nervous system function properly and good brain functions [28]. Particularly, teff is the only one containing Vitamin K among the products in the **Table 1**. Vitamin K is a fat-soluble vitamin that the body stores it in fat tissue and the liver. It is best known for its role in helping blood clot and bone health [28]. Both quinoa and teff have high quality lipids including monounsaturated and polyunsaturated fatty acid contents. These ancient grains have endured the ages from early civilizations as an important food source to its current resurgence as a highly nutritious gluten-free source. Therefore, the nutritional value of gluten free baked products could be improved by adding ancient grain products to recipes.

3.2. Water-Holding Capacity

The WHC of the ancient grains and wheat flours are shown in **Figure 1**. Among the four gluten free flours, buckwheat had the highest WHC (164%), the teff ranked in second (144%), and WHC of amaranth was the least (125%), that was slightly lower than WHC of quinoa (130%). Overall, all ancient grain flours in this study had higher WHC than that of wheat flour (92%). These gluten free ancient gain flours could be widely used in different food applications because of better WHC for improved texture along with nutrition compared to wheat flour.

3.3. RVA Pasting Properties

The pasting curves of all products were obtained by RVA expressed as RVU (1 RVU = 12 centipoises). As shown in **Figure 2**, the pasting curves for all samples had dissimilar patterns. The Amaranth pasting viscosity exhibited a significantly high (~52 RVU) and sharp viscosity peak (~5 RVU/min) during the initial 11 min

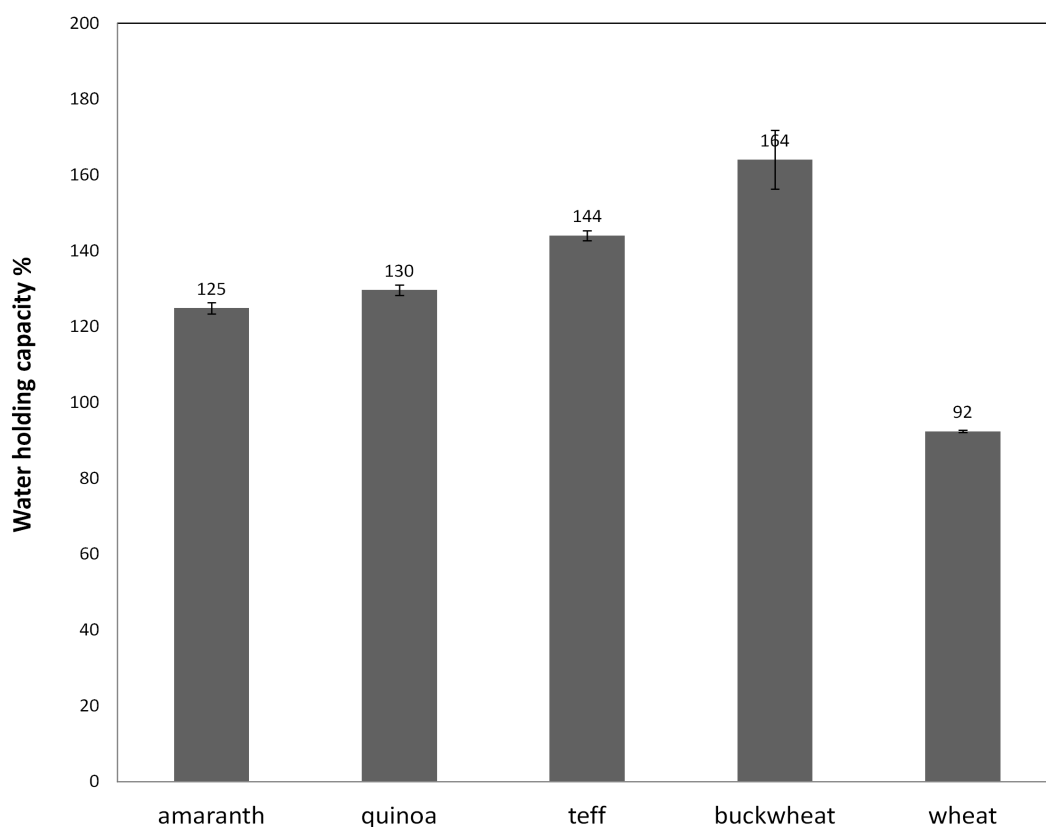


Figure 1. Water holding capacities ancient grain and wheat flours.

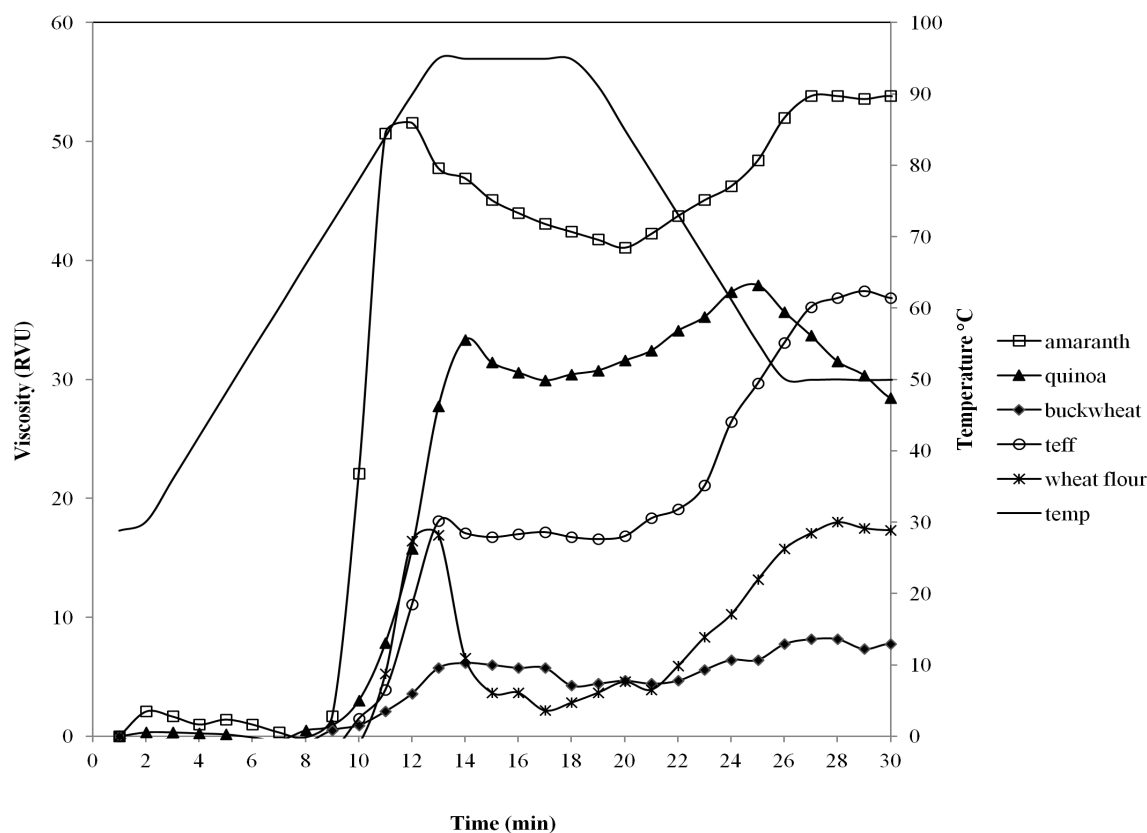


Figure 2. Pasting curves of ancient grain and wheat flours.

heating period at $\sim 90^{\circ}\text{C}$ followed by a gradually decrease in viscosity (1 RVU/min) to ~ 41 RVU during continued heating and cooling at 18 min, and increase to the final viscosity (53 RVU) during cooling down showing a break down (peak viscosity minus the lowest point of viscosity after peak). The viscosity of quinoa showed the initial peak (~ 33 RVU) at 95°C , and then the viscosity showed a shallow break down and then decreased to a final viscosity (~ 28 RVU) that was lower than that of amaranth (~ 53 RVU). The viscosity of teff increased to the initial peak (~ 18 RVU) after temperature reaching 95°C at 12 min, keeping a near constant viscosity during heating, and then increased sharply (~ 2 RVU/min) during cooling resulting in a final viscosity (37 RVU) that was higher than the final viscosity of quinoa (~ 28 RVU). Perhaps, it was due to highest calcium content that reacted with protein in teff. It could be a similar mechanism to make tofu by coagulating proteins in soymilk with calcium or magnesium sulfate. The proteins coagulate when bonding occurs between the positively charged calcium ions and negatively charged anionic groups of the protein molecules. The calcium and magnesium in teff could be reacted with protein causing the increased viscosity during cooling.

Buckwheat flour had the lowest initial viscosity peak (~ 6 RVU) than other samples at 95°C , and then kept nearly constant value to the final viscosity (~ 7 RVU) that was lowest among all samples. The initial peak (~ 17 RVU) for wheat was similar to teff but it decreased sharply on 12 min at 95°C . It is known that the viscosity of a completely gelatinized starch slurry decreases during heating [29].

The RVA data could provide useful information for food processing and product development. The product with low viscosity may be suitable for products such as cookies, muffin, or nutritional bars. The product with high viscosities could be used for products such as breads for improved the texture quality and health benefits.

3.4. Free, Bound and Total Phenolic Contents

Buckwheat flour had the highest free, bound and total phenolic contents among the four flours when extracted with water, 50% ethanol and 100% ethanol (Table 2). Buckwheat (Farinetta) flour contains a considerable amount of aleurone layer along with embryo. Cereals are rich in substituted cinnamic acids such as ferulic acid that are

Table 2. Free, bound and total phenolic contents of ancient grain products in aqueous extracts using water, 50% ethanol, and 100% ethanol.

Samples	Ethanol (%)	Free mg/g	% of total	Bound mg/g	% of total	Total mg/g
Amaranth	0	1.76 ± 0.09 e	38.10	2.86 ± 0.22 e	61.90	4.62 ± 0.03 d
Amaranth	50	0.6 ± 0.01 g	14.22	3.62 ± 0.13 d	85.78	4.22 ± 0.14 ef
Amaranth	100	0.53 ± 0.01 g	11.91	3.92 ± 0.13 d	88.09	4.45 ± 0.14 de
Quinoa	0	3.03 ± 0.16 d	77.30	0.89 ± 0.07 h	22.70	3.92 ± 0.13 fg
Quinoa	50	1.65 ± 0.02 e	44.72	2.04 ± 0.10 g	55.28	3.69 ± 0.12 g
Quinoa	100	0.88 ± 0.06 f	22.51	3.03 ± 0.11 e	77.49	3.91 ± 0.05 fg
Teff	0	1.82 ± 0.07 e	48.66	1.92 ± 0.10 g	51.34	3.74 ± 0.17 g
Teff	50	1.74 ± 0.03 e	41.93	2.41 ± 0.15 f	58.07	4.15 ± 0.17 ef
Teff	100	0.96 ± 0.09 f	20.38	3.75 ± 0.12 d	79.62	4.71 ± 0.06 d
Buckwheat	0	9.44 ± 0.25 a	60.05	6.28 ± 0.30 c	39.95	15.72 ± 0.27 c
Buckwheat	50	7.20 ± 0.25 b	40.47	10.59 ± 0.40 b	59.53	17.79 ± 0.17 a
Buckwheat	100	3.78 ± 0.04 c	22.03	13.38 ± 0.24 a	77.97	17.16 ± 0.22 b

esterified to cell walls, to arabinoxylan and arabinogalactan in the aleurone layer, and pericarp [30]. The free phenolic contents were ~20% - 60% of total phenolic contents while the bound phenolic contents were ~22% - 79% of total phenolic contents. Overall, the bound phenolic contents were higher than free phenolic contents regardless of the ethanol concentrations with exception of water extraction of quinoa and buckwheat. Studies have reported that the phenolic compounds are primarily bound to cell walls for most cereal grains [23]. The bound phenolic compounds probably are released under alkaline extraction conditions. Amaranth, quinoa, and teff flour were all significantly lower in the free, bound, and total phenolic contents than that of buckwheat regardless of the ethanol concentrations.

Interestingly, the free phenolic compounds for all four products were significant highest in water extract, secondly in 50% ethanol, and were least in (the) 100% ethanol. Cinnamic and benzoic acid derivatives are generally water soluble and universally present in plant foods [31]. It may be the explanation for the more free phenolic compounds when water or 50% ethanol was used for all four flours, respectively (Table 2). In contrast to the free phenolic contents, more bound phenolics were extracted with 100% ethanol than water and 50% ethanol. It implied that bound phenolics are more soluble in 100% ethanol.

The different letters indicated the significances between treatments in column ($p < 0.05$).

3.5. Free, Bound and Total Antioxidant Activities

The similar trends were observed for antioxidant activities as phenolic contents (Table 3). In general, the bound antioxidant activities are all much higher statistically than the free antioxidant activities for all products as phenolic content in spite of solvents. However, the differences of antioxidant activities bound among products seem not as large as bound phenolic contents. The free antioxidant activities ranged from 1.3% to 33.55% of total antioxidant activities whereas the bound antioxidant activities were ranged from 67.40% to 98.7% of total antioxidant activities. Our study suggests that the total phenolic contents and antioxidant activities of grains could be underestimated in the literature if not including the bound phenolic compounds.

The free antioxidant activities from water and 50% ethanol were higher than those from absolute alcohol. The free antioxidant activities from water were higher than those from 50% ethanol for amaranth and quinoa. In contrast, the free antioxidant activities from water were lower than those from and 50% ethanol for teff and buckwheat. Therefore, the conclusion of solvent selection for free antioxidant activities regarding water and 50% ethanol are inclusive.

Table 3. Free bound and total antioxidant activities of ancient grain products in aqueous extracts using water 50% ethanol and 100% ethanol.

Product	Ethanol (%)	Free $\mu\text{mol/g}$	% of total	Bound $\mu\text{mol/g}$	% of total	Total $\mu\text{mol/g}$
Amaranth	0	0.97 \pm 0.04 i	8.90	9.92 \pm 0.04 d	91.01	10.90 \pm 0.04 h
Amaranth	50	0.58 \pm 0.02 j	5.54	9.88 \pm 0.02 d	94.46	10.46 \pm 0.04 j
Amaranth	100	0.13 \pm 0.02 l	1.30	9.90 \pm 0.02 d	98.70	10.03 \pm 0.03 k
Quinoa	0	5.08 \pm 0.02 a	33.55	10.06 \pm 0.02 c	66.45	15.14 \pm 0.06 b
Quinoa	50	3.94 \pm 0.03 f	29.01	9.63 \pm 0.03 e	70.91	13.58 \pm 0.10 e
Quinoa	100	0.49 \pm 0.05 k	4.63	10.09 \pm 0.05 c	95.37	10.58 \pm 0.10 i
Teff	0	4.21 \pm 0.03 e	29.03	10.28 \pm 0.03 b	70.90	14.50 \pm 0.03 d
Teff	50	4.88 \pm 0.00 c	32.13	10.31 \pm 0.00 b	67.87	15.19 \pm 0.09 b
Teff	100	2.37 \pm 0.02 h	18.53	10.42 \pm 0.02 a	81.47	12.79 \pm 0.02 g
Buckwheat	0	4.50 \pm 0.03 d	30.32	10.25 \pm 0.03 ab	69.07	14.84 \pm 0.03 c
Buckwheat	50	5.02 \pm 0.01 b	32.53	10.40 \pm 0.01 a	67.40	15.43 \pm 0.02 a
Buckwheat	100	2.60 \pm 0.00 g	20.09	10.33 \pm 0.00 ab	79.83	12.94 \pm 0.02 f

The different letters indicated the significances between treatments in column ($p < 0.05$).

4. Conclusion

Amaranth, quinoa, teff and buckwheat all have special nutrients. Higher water holding capacities were found for the ancient grain flours than wheat flour. Buckwheat flour had the highest free, bound and total phenolic contents among the four products. In general, the bound phenolic contents and antioxidant properties were higher than the free phenolic contents and antioxidant activities regardless of the ethanol concentrations. The free phenolic compounds and antioxidant activities for all four products were significantly higher in extracts using water and 50% ethanol than 100% ethanol. In contrast, more bound phenolics were in 100% ethanol extracts than water and 50% ethanol extracts. Our study suggested that the total phenolic contents and antioxidant activities of grains could be underestimated in the literature without considering the bound phenolic compounds. These ancient grains have nutritional value, antioxidants, and texture qualities suitable for functional food applications.

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