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QUINOA BEVERAGES: FORMULATION, PROCESSING AND POTENTIAL HEALTH BENEFITS

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Abstract

Background and Aims: *Research on innovative foods and beverages that serve well to the nutritional needs of individuals suffering from metabolic disorders like obesity, hypertension, diabetes, dyslipidemia is an urgent need for today. This study aims to describe a method for preparing gluten free quinoa beverages and to investigate their effects on human health.* ***Material and methods:*** *Quinoa beverages were prepared from raw, soaked, germinated and malted quinoa seeds. We investigated their antioxidant activity, antidiabetic and antihypertensive potential using in vitro models.* ***Results:*** *Among all beverages, malted quinoa beverage (MQB) showed higher protein content (2.9 g/100ml), total phenolic content (2.9 mg Gallic Acid Equivalents (GAE)/ g), antioxidant activity (92%) which was well correlated with higher antidiabetic potential (40% at 150µL) by α-glucosidase inhibition. Very low - α-amylase inhibition was exhibited by all the beverages (0.4-1.5 %). ACE inhibitory activity was almost negligible for raw quinoa beverage (RQB), soaked quinoa beverage (SQB), minor for germinated quinoa beverage (GQB) (0.2% at 300µL) and higher for MQB (0.9% at 300µL). Total phenolic content was found to be well correlated with DPPH (1,1-Diphenyl-2-picryl-hydrazyl), α-glucosidase and α- amylase inhibition activity in all beverages but poor correlation was found in case of ACE inhibition activity. Among all, GQB was highly acceptable with acceptability magnitude at par with commonly available commercial soya milk.* ***Conclusion:*** *We conclude that quinoa beverages, especially MQB, have antidiabetic and antihypertensive potential, and hence, can be effectively included among diet choices for the management of diabetes and hypertension. In the future, further studies are required in order to characterize phenolic constituents in quinoa beverages responsible for the antidiabetic and antihypertensive potential.*

key words: *Antioxidant activity, Chenopodium quinoa, Diabetes, Gluten free beverages, Hypertension*

Background and Aims

Increased rates of metabolic disorders like diabetes, hypertension, dyslipidemia, obesity and certain specific food intolerances like lactose or gluten intolerance leave individuals suffering from these disorders with limited choice among food items, sometimes not at par with the nutritional needs of the body. American Heart Association considers artificially flavored and

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sugar sweetened beverages as a root cause of such major metabolic disorders [1]. Understanding the need in question, this research is an effort to produce lactose free and gluten free beverages with different processing techniques from the underutilized pseudocereal - *Chenopodium quinoa*, and to study its antidiabetic and antihypertensive potential.

Since long, food products made from cereals and pulses have been known for their remarkable potential in food and beverage industry. Cereal and pulse beverages like those from oat, rice, soy and various vegetable milks have been gaining popularity for their specific health benefits. Recently, the researchers worldwide have been keen towards exploring underutilized grains and pseudocereals for their health potential and future in the food industry. Among them, Andean cereals, legumes and pseudocereals have proved their potential and could be included as grains for dietary approaches to control diabetes and hypertension [2].

Chenopodium quinoa, a pseudocereal from the Andes region of South America, mainly Peru and Bolivia, has been gaining attention worldwide due to its nutritional content and crop tolerance to extreme climatic conditions. It is a rich source of protein (12-16.5%) with protein quality equivalent to that of casein. In addition, this "wonder grain" is gluten free, rich in bioactive compounds like antioxidants, polyphenols, flavonoids, vitamins and minerals [3], which impart various health benefiting characteristics to this grain [4]. Quinoa functional products like quinoa cereal bar, quinoa flakes, quinoa pasta, have been known to have various health benefits and to be effective in cases of obesity, cardiovascular diseases, hypertension, and celiac disease [3]. Recently, Pineli et al. [5] prepared a novel quinoa milk and demonstrated its benefit for diabetic individuals due to lower glycemic index. Different grain processing techniques like soaking, germination and malting, are known to improve the nutrient content and decrease the antinutrients in grains [6]. In this present study, we aimed to prepare quinoa beverages from raw, soaked, germinated and malted quinoa seeds, and study their acceptance and potential health benefits.

Materials and method

Procurement of grain

White *Chenopodium quinoa* grains, imported from Bolivia were procured from Devshree grains and pulses, New Delhi, India for the study.

Basic grain treatment prior to beverage preparation

The seeds were then steeped in 0.03 mol/L sodium chloride at *p*H 5.0 for better protein yield, as described by Pineli et al. [5]. Further, raw seeds were used as such (raw), soaked, germinated and malted for production of their respective quinoa beverages.

Soaked quinoa seeds: Quinoa seeds were soaked in milli Q water for 24 hours at room temperature. Water was changed every 8 hours. Soaked seeds were further processed on the same day for beverage preparation.

Germinated quinoa seeds: Soaked seeds were spread onto Petri dishes layered with a filter paper dipped in 3ml distilled water and incubated at 20°C in an incubator (Biotechnics, India) for 72 hours [7]. Water was changed and checked for dryness every 6 hours. Germinated seeds were further processed on the same day for beverage preparation.

Malted quinoa seeds: The germinated quinoa was malted according to standard procedure given by Gokavi and Malleshi [8]. Germinated seeds were air dried to remove excess moisture and toasted at 60°C for 20 minutes to obtain malted quinoa seeds. Malted

quinoa seeds were further processed on the same day for beverage preparation.

Preparation method for raw, soaked and germinated quinoa beverages

Basic process for preparation of raw, soaked, germinated and malted quinoa beverages was followed according to [9] with some modifications. Flow chart representation of the preparation process has been described in Figure 1.

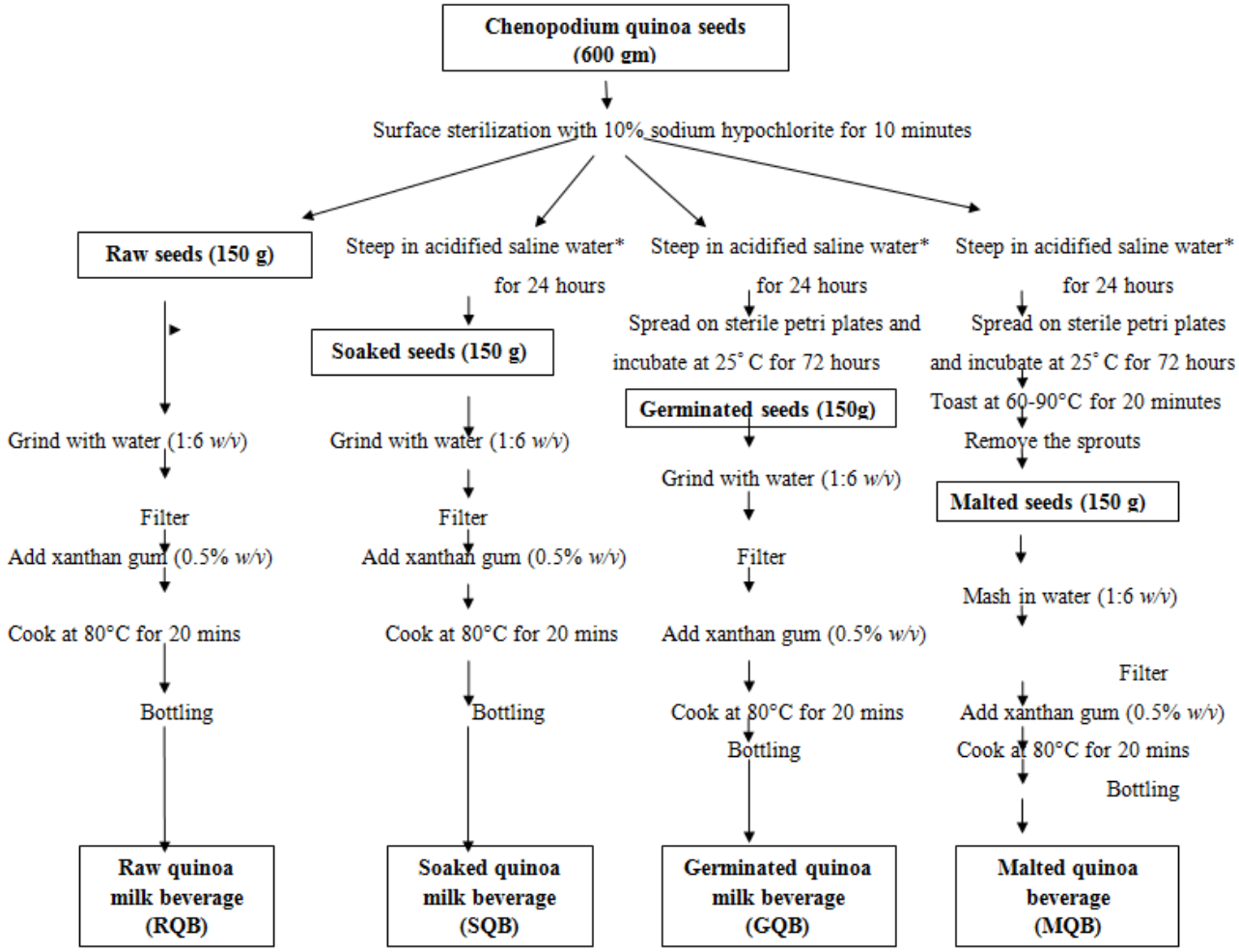

Figure 1. *Method of preparation of quinoa beverages.*
**Acidified saline solution: 0.03 mol/L NaCl at pH 5 (Pineli et al. 2015)*

All quinoa beverages were stored at 4°C for further analysis.

Physical and chemical composition

Carbohydrate, ash and fat content were determined by standard Association of Official Agricultural Chemists (AOAC) [10] methods. Protein content was determined through nitrogen content estimation by the method of Kjeldahl using a factor of 6.25. [11]. Total sugars were estimated by anthrone method [12]. *p*H was measured using a digital *p*H meter at 20°C. Total soluble solids were measured according to the method followed by Kim et al. [13]. Viscosity was measured using a rotational viscometer (Cole-Parmer Basic Viscometer, Cole-Parmer India Pvt. Ltd, India) and expressed in centipoise (cp). To determine the effectiveness of xanthum gum, serum separation was assessed using a graduated cylinder (with a

volume of 50 ml) according to the method used by Koksoy and Kilic [14].

Sensory evaluation

Quinoa beverages were assessed for organoleptic acceptance using a nine point hedonic scale, from extremely dislike to extremely like, according to the method followed by Welsh et al. [15]. The samples were randomly marked and served at room temperature in white paper cups to a semi-trained panel of 25 members. Commonly available commercial soya milk (Sofit natural unflavoured soya milk, Hershey India Pvt. Ltd.) was used as a reference beverage to evaluate acceptance. Panellists were asked about their favourite and least favourite beverage and also about positive and negative sensory aspects of each beverage.

In vitro assays

Quinoa beverages (5ml) were diluted with distilled water and volume was made up to 10 ml. It was then centrifuged at 11000 g for 25 minutes. The supernatant was then separated and used for *in vitro* assays. Extraction and all analyses were done in triplicates.

Total phenolics assay

The total phenolic content was assayed by the Folin-Ciocalteu method as described by Ranilla et al. [16]. The results were expressed as mg GAE/g of sample weight, where GAE stands for Gallic Acid Equivalents.

Antioxidant activity by DPPH (1,1-Diphenyl-2-picryl-hydrazyl) inhibition assay

The DPPH scavenging activity was determined using the method followed by Ranilla et al. [16] with slight modifications. Summarily, 250 µL of quinoa beverage was added to 4ml of 60 µM DPPH solution prepared in 95% ethanol. The reaction mixture was placed in a dark environment for about 20 minutes and absorbance was read at 517 nm. For comparison, 250 µL of 95% ethanol was used as control. Percentage inhibition was calculated according to the formula:

$$\%inhibition = \frac{DPPH_{\text{control}} - DPPH_{\text{test sample}}}{DPPH_{\text{control}}} \times 100$$

The α -amylase inhibitory activity was followed according to Ranilla et al [2]. The α -amylase inhibitory activity (%) was calculated according to the following equation below:

$$\%inhibition = \frac{I_{\text{control}} - I_{\text{test sample}}}{I_{\text{test sample}}} \times 100$$

α-Glucosidase inhibition assay

The α-glucosidase inhibition assay was followed according to Ranilla et al. [16]. The α-glucosidase inhibitory activity was expressed as percentage of inhibition and was calculated according to the following equation:

$$\%inhibition = \frac{I_{\text{control}} - I_{\text{test sample}}}{I_{\text{test sample}}} \times 100$$

Angiotensin I converting enzyme (ACE) inhibition assay

The ACE inhibition assay was assessed by the "bond cleavage method" and determined according to the procedure followed by Oboh et al [17]. ACE inhibition was calculated by the following equation:

$$\%inhibition = \frac{I_{\text{control}} - I_{\text{test sample}}}{I_{\text{test sample}}} \times 100$$

Statistical analysis All experimental analyses were performed in triplicates. The results are expressed as mean ± standard deviation. Statistical data was analysed using Graphpad Prism 6 Software (La Jolla, CA, USA). Nutritional, physical, chemical evaluation, and *in vitro* analysis data were compared using 2-way ANOVA and post hoc

Tukey's range test at $p<0.05$. Pearson product-moment correlation coefficient was used to test linear correlation between variables.

Results

Physicochemical composition

Physicochemical evaluation of quinoa beverages is shown in Table 1.

The ash content of quinoa beverages ranged from 0.11 to 0.28 g/100 ml. Fat content of beverages ranged from 0.23 to 0.93 g/100 ml. Protein content of quinoa beverages ranged from 0.68 to 2.91 g/100 ml. The carbohydrate content of quinoa beverages ranged from 11.12 to 17.08 g/100 ml with the trend in the increasing order being: *germinated quinoa beverage (GQB) < soaked quinoa beverage (SQB) < malted quinoa beverage (MQB) < raw quinoa beverage (RQB).* Sugar content of quinoa beverages ranged from 8.24 to 14.38 g/ 100 ml with the trend in the increasing order being: RQB<MQB< SQB<GQB.

Table 1. Nutritional and physico chemical evaluation of quinoa beverage.

Quinoa beverages	Protein (g/100ml)	Carbohydrate (g/100 ml)	Fat (g/100 ml)	Ash (g/100 ml)	Sugar (g/100 ml)	pH	Total soluble Solid (% Brix)	Viscocity (cp)	Serum Separation (%)	
									Without xanthan Gum	With Xanthan gum
RQB	0.68± 0.01^{a}	16.2 ± 0.02^{a}	0.93 ± 0.02^{a}	0.13± 0.04^{a}	8.24 ± 0.01^{a}	6.2± 0.01^{a}	9.08± 0.02 ^{a}	15.31 ± 0.04^{a}	15.3± 0.02	5.4±0.04
SQB	1.2 ± 0.10^{b}	15.5± 0.01^{b}	0.81 ± 0.01^{b}	0.11± 0.01^{b}	12.92 ± 0.04^{b}	6.5 ± 0.10^{b}	9.45 ± 0.01^{b}	15.12± 0.01^{b}	14.8± 0.13	4.2±0.11
GQB	1.5 ± 0.01^{c}	14.90± 0.1^{c}	0.23 ± 0.04^{c}	0.28 ± 0.01^{c}	14.38 ± 0.11^{c}	6.3 ± 0.03^{a,c}	9.39 ± 0.04^{c}	15.02 ± 0.01^{c}	14.5± 0.11	4.3±0.16
MQB	2.9 ± 0.03^{d}	14.70± 0.03^{cd}	0.69 ± 0.12^{d}	0.19± 0.02^{d}	9.48 ± 0.01^{d}	5.9 ± 0.01^{d}	9.69 ± 0.12^{d}	17.52± 0.02^{d}	14.01± 0.12	4.04±0.15

For n=3, values are mean± standard deviation. Data in column denoted by different letters are significantly different ($p<0.05$)

The *p*H, total soluble solids and viscosity of all quinoa beverages were almost similar. pH ranged from 6.1 to 6.5. Total soluble solid content (%) ranged from 9.08 to 9.69%. The addition of 0.5% (*w/v*) reduced serum separation in ranging from 82 to 88% with major reduction in MQB (88%). Viscosity of quinoa beverages ranged from 17.02 cp to 18.12 cp.

Sensory evaluation

Overall acceptability of quinoa beverages, as indicated in Table 2, ranged from extremely dislike to moderately like, with the acceptability trend being: RQB<SQB<MQB<GQB.

Table 2. Sensory evaluation of quinoa beverages.

Quinoa Beverage	Favourite (%)	Least favourite (%)	Overall acceptability
RQB	0	45.3	2.2 ± 2.1^{a}
SQB	16.7	26.5	3.9 ± 3.4^{b}
GQB	32.3	1.5	6.8 ± 2.5^{c}
MQB	17.4	25.4	5.4 ± 1.3^{d}
Commercial Soya Milk	33.2	1.4	6.9 ± 1.4^{c}

Data in column (n=25), denoted by different letters are significantly different ($p<0.05$)

GQB was most liked among all quinoa beverages with an overall acceptability score matching significantly ($P<0.05$) to that of commercial soya milk. Panellists opinion on the positive and negative aspect of quinoa beverages, as indicated in the spider diagram in Figure 2, indicated higher after taste i.e. negative aspect of RQB and least good mouth feel. GQB qualified all positive aspects and was rated similarly to the commercial soya milk.

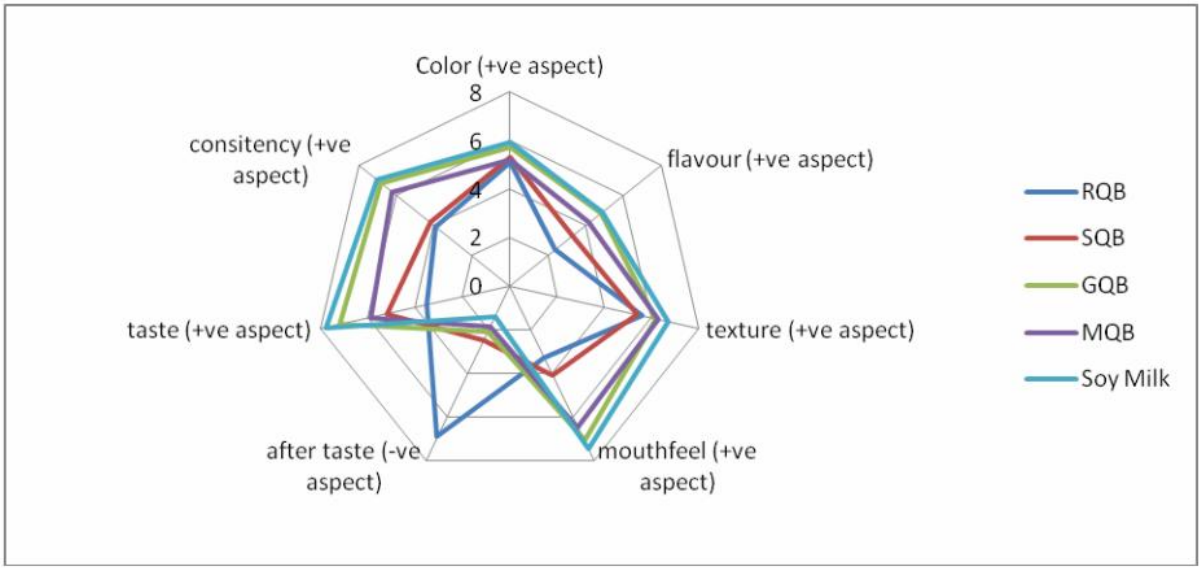

Figure 2. *Panelists opinion on positive and negative aspects of quinoa beverages with respect to commercial soy milk*

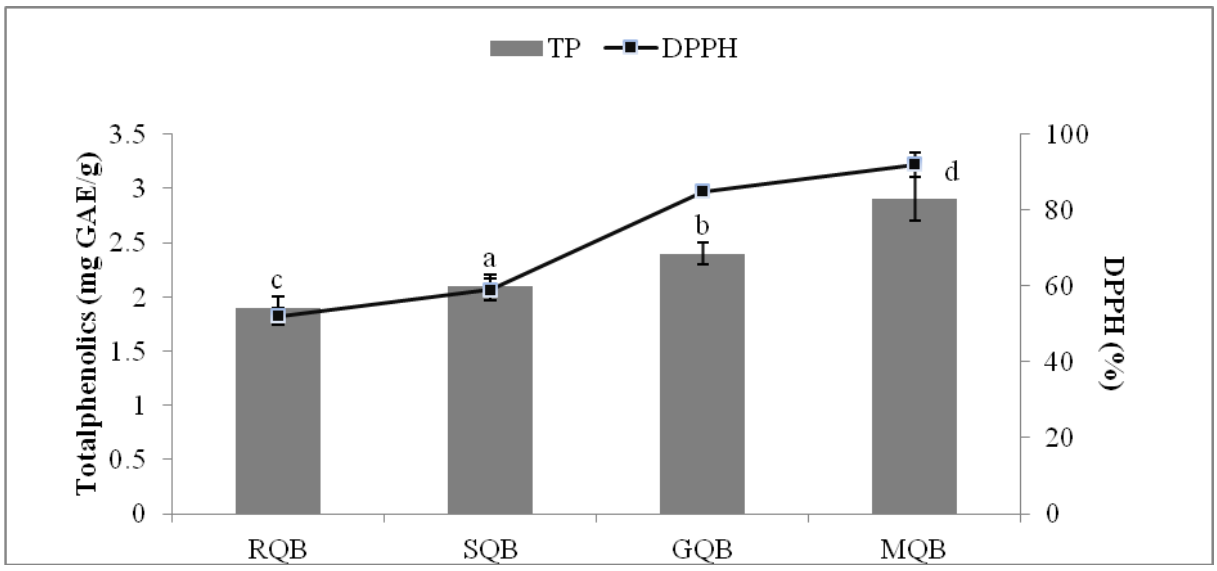

Figure 3. *DPPH and Total phenolic content of quinoa beverages. Bars with different letters are significantly different ($p<0.05$)*

Total phenolic content and antioxidant activity

As indicated in Figure 3, the total phenolic content of all quinoa beverages was well correlated with their antioxidant activity (r = 0.8845). Total phenolic content of quinoa beverages ranged from 1.9 to 2.9 mg GAE/g. Malted quinoa beverage (MQB) showed the highest phenolic content (2.9 ± 0.4 mg GAE/g) followed by GQB (2.4 ± 0.2 mg GAE/g), SQB (2.1 ± 0.2 mg GAE/g) and RQB (1.0 ± 0.3 mg GAE/g). The antioxidant activity of quinoa beverages, determined by DPPH method ranged from 52 to 92%.

In vitro α-glucosidase, α-amylase and angiotensin I-converting enzyme (ACE) inhibition

The α-glucosidase inhibition activity in quinoa beverages, indicated in Figure 4, was dose dependant and strongly correlated to their total phenolic contents (r= 0.9 at 300µL).

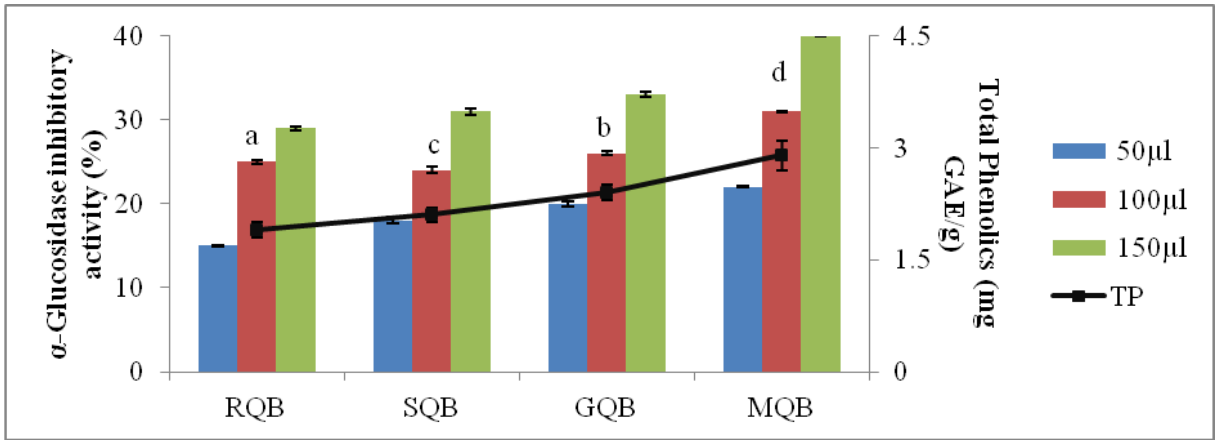

Figure 4. *α-glucosidase activity of quinoa beverages. Bars with different letters are significantly different (P< 0.05*

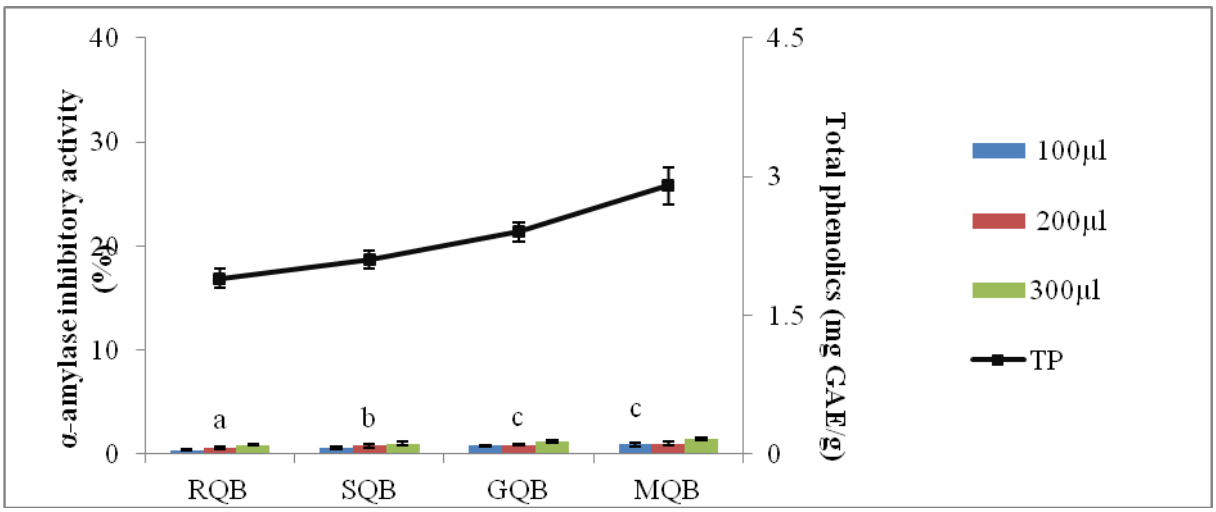

Figure 5. *α-amylase inhibition activity of quinoa beverages. Bars with different letters are significantly different (P<0.05)*

A very strong correlation (r = 0.9 at 300µL) between alpha amylase inhibition and total phenolic content, indicated in Figure 5, has been observed in our study. The relationship was dose-dependant, although a very low level of α-amylase inhibition ranging from 0.4 to 1.5% has been reported in quinoa beverages.

No correlation between total phenolic content and ACE inhibition (Figure 6) was observed in quinoa beverages as indicated in Figure 6. RQB and SQB showed negligible ACE inhibition while GQB showed minor inhibitory activity (0.2%) at a dose level of 300 µL.

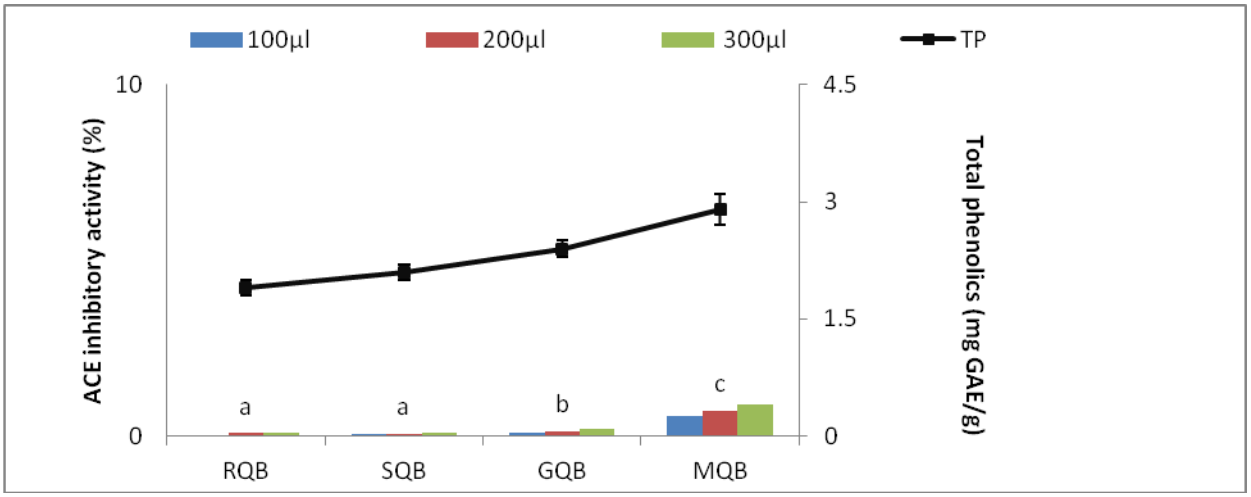

Figure 6. *ACE inhibition activity of quinoa beverages. Bars with different letters are significantly different (P<0.05.)*

Discussion

A significant increase in ash content (P<0.05) was reported in GQB as compared to RQB. Similarly, an increase in ash content upon germination has been reported by Echendu et al. [18]. Germinated quinoa beverage reported less fat content which may be due to use of fat as energy during grain sprouting. Decrease in fat content upon malting has also been reported by Choudhury *et al.* [19] and corresponds with the results reported in our study. Protein content in processed seeds was 1.7 to 4 folds higher due to steeping in acidified saline solution as stated by Pineli *et al.* [5]. The values of protein content in quinoa beverages are similar to the protein content in quinoa milk (0.49- 1.72 g/100ml) reported by Pineli *et al.* [5]. Significant increase in protein content (P<0.05) was reported in MQB as compared to RQB, this might be because of an increase in activity of proteolytic enzymes during the process of malting which results in degradation of proteins to peptides and amino acids [20]. The values of carbohydrate content was well correlated to the values reported by Pineli *et al* [5]. Carbohydrate content decreased in SQB (4%) and GQB (8%), and MQB (9%) with respect to RQB. Similar decreases in carbohydrate content upon soaking and germination has been reported by Uppal and Bains [21]. This decrease may be accounted to hydrolysis of starch during the process of soaking and germination [22]. The result agreed with the sugar content in quinoa milk (9.7g/ 100 ml) as reported by Pineli *et al.* [5]. The increase in sugar content was well correlated and was found inversely proportional to the decrease in carbohydrate content. Soaking and germination lead to increase in sugar content due to starch hydrolysis leading to breakdown of carbohydrates and release of sugars [23].

No significant difference was observed in the pH content of all quinoa beverages. Similar pH value has been reported by Kim *et al.* [13] in soy milk. No significant difference was observed in total solid of all quinoa beverages. Results reported in our study are quite similar to total solid content (%) in soy milk as reported by Kim *et al.* [13].

Xanthan gum increased the viscosity and helped in textural stabilization of the beverages

by reducing serum separation. Xanthan gum has been reported as successful hydrocolloid for textural stabilization of beverages [24].

Viscosity of quinoa beverages ranged from 17.02 cp to 18.12 cp, similar results have been reported by Terhaag et al [25].

In the case of the total phenolic content of beverages, similar results (2.8 mg GAE/ g) for total phenolic content have been reported by Miranda *et al* [26]. Higher phenolic content in malted quinoa beverage might be attributed to better liberation of bound phenolic contents from the cereal matrix during the process of malting and higher crude extractive yields of malted cereals as compared to the raw, soaked and germinated cereal samples [27].

Antioxidant activity had good correlation with the total phenolic content ($r = 0.8$, $p<0.05$). Potential to inhibit DPPH free radical i.e. the antioxidant activity followed same trend as their total phenolic content. As compared to RQB, antioxidant activity of SQB, GQB and MQB increased by 11, 1.6 and 1.7 fold. Similar increases in antioxidant activity of cereal malts has been reported by Ondrejovic *et al.* [27]

The inhibitory activity for quinoa beverages ranged from 29 to 40%. Similar results for α-glucosidase activity (30%) has been reported by Ranilla *et al.* [2] in quinoa seeds. The trend for α- glucosidase activity observed was MQB>GQB>SQB>RQB. Pineli *et al.* [5] have reported low glycemic index of quinoa milk, which further supports the anti diabetic potential of quinoa beverages.

Low or minor α-amylase inhibition activity of foods been reported to be beneficial because of less side effects like, flatulence and diarrhoea for control of postparandial hyperglycemia by Kwon et al. [28] rather than foods with high α-amylase inhibition activity.

Null ACE inhibition activity in quinoa seeds has also been reported by Ranilla *et al* [2]. Among all quinoa beverages MQB showed the highest (0.9 % at 300 µL) dose dependant ACE inhibition. Malting results in the liberation of proteases by protein hydrolysis at its step which involves roasting. Hence, emergence of ACE inhibition in malted quinoa beverages can be due to protein hydrolysis. [29]

Conclusion

The results infer that quinoa beverages could be included as a part of the diet in effective dietary management strategy for type 2 diabetes and hypertension. Higher inhibitory potential against α-glucosidase enzyme and lower inhibitory potential against α-amylase enzyme is an added benefit to health potential related to hyperglycemia. Although all quinoa beverages have potential health benefits, malted quinoa beverages (MQB) seem to be more effective in management of type 2 diabetes and hypertension.

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