Indian Journal of Ecology (2023) 50(5) (SI): 1806-1811
DOI: https://doi.org/10.55362/IJE/2023/4141

Manuscript Number: 4141
NAAS Rating: 5.79

Evaluation of Biochemical and Sensory Parameters of Pomegranate Beverage by the Incorporation of Quinoa Extract

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Abstract: The present study was conducted on optimization and hypocholesterolemic activity of pomegranate beverage with the incorporation of quinoa extract in order to assess the biochemical and sensory evaluation of pomegranate beverage with the incorporation of quinoa extract. Eight treatments were formulated at different concentrations of pomegranate juice (80, 85 and 90%) and quinoa extract (10, 15 and 20%) by altering the sugar level (15 and 18° brix) and compared with control (100% pomegranate juice). Among the eight different treatments, there was no significant difference was observed for sensory quality Significantly maximum total phenols (463.33 mg GAE/100 mL), DPPH radical scavenging activity (98.05%), potassium (718.83 mg/100 mL), phosphorous (201.58 mg/100 mL), calcium (194 mg/100 mL), magnesium (66.80 mg/100 mL), iron (3.26 mg/100 mL) and zinc (0.43 mg/100 mL) were identified in T_4 (80% Pomegranate juice + 20% quinoa extract + Sugar 15º Brix).

Keywords: Pomegranate, Quinoa extract, Phenols, Sensory evaluation, TSS, pH, DPPH, Tannin, Minerals

Pomegranate (*Punica granatum* L.) is a fruit-bearing shrub in the family 'Lythraceae'. The edible part of the fruit (arils-pulp bearing seeds) contains considerable amounts of acids, sugars, vitamins (A, C and E), polysaccharides, polyphenols and important minerals (Vardin and Fenercioglu 2003). Punicalagin has anti-inflammatory, anti-cancer and anti-atherosclerotic effects, according to studies. Pomegranate polyphenols are capable of limiting the body's reaction to reactive oxygen species (ROS) (Aloqbi et al 2016).

Quinoa (*Chenopodium quinoa Wild*.) is a crop used by pre-Columbian cultures in South America for centuries. It is one of the most nutritious grains used as human food. In recent years, research into nutritional strategies aiming to prevent excess energy intake has increased in response to rising levels of obesity and obesity-related diseases. Drug treatments or supplements of bioactive molecules are ineffective or exhibit adverse side effects, focus has shifted towards natural dietary components with potential impact upon appetite and satiety. Synergetic effect of pomegranate beverage with the incorporation of quinoa extract may be beneficial to improve the health. With this background, the present study was conducted on optimization and to assess the biochemical and sensory evaluation of pomegranate beverage with the incorporation of quinoa extract.

MATERIAL AND METHODS

The present investigation was undertaken at College of Horticulture, Bagalkot, Karnataka, India. The pomegranate fruits (cv. Bhagwa) were procured from Bellary district and were sorted, graded and washed thoroughly in running water and cut into halves with the help of sharp stainless steel knife. Fruits were placed in the hand operated pomegranate juice extractor and pressed to obtain juice. After extraction, juice was allowed to settle down and subsequently it was strained through clean muslin cloth to get a clear juice extract. Quinoa seeds were procured from Department of Vegetable Science, College of Horticulture, Bagalkot. Extract of quinoa was obtained by the procedure as presented in Figure 1. The extracted juice of pomegranate and quinoa were used as per treatments (Table 1) and sucrose was added to maintain TSS. Quinoa extract was added wherever needed in all the treatments at different percentages (except T_1 and T_5). The prepared beverage was filled in clean, sterilized bottles and sealed with caps and stored at 4° C. Experiment was conducted in three replications for sensory and biochemical analysis.

Sensory evaluation: The sensory evaluation of developed pomegranate beverage by incorporation of quinoa extract was evaluated by a panel of semi trained judges (n=10) on a nine point hedonic scale using sensory score sheet.

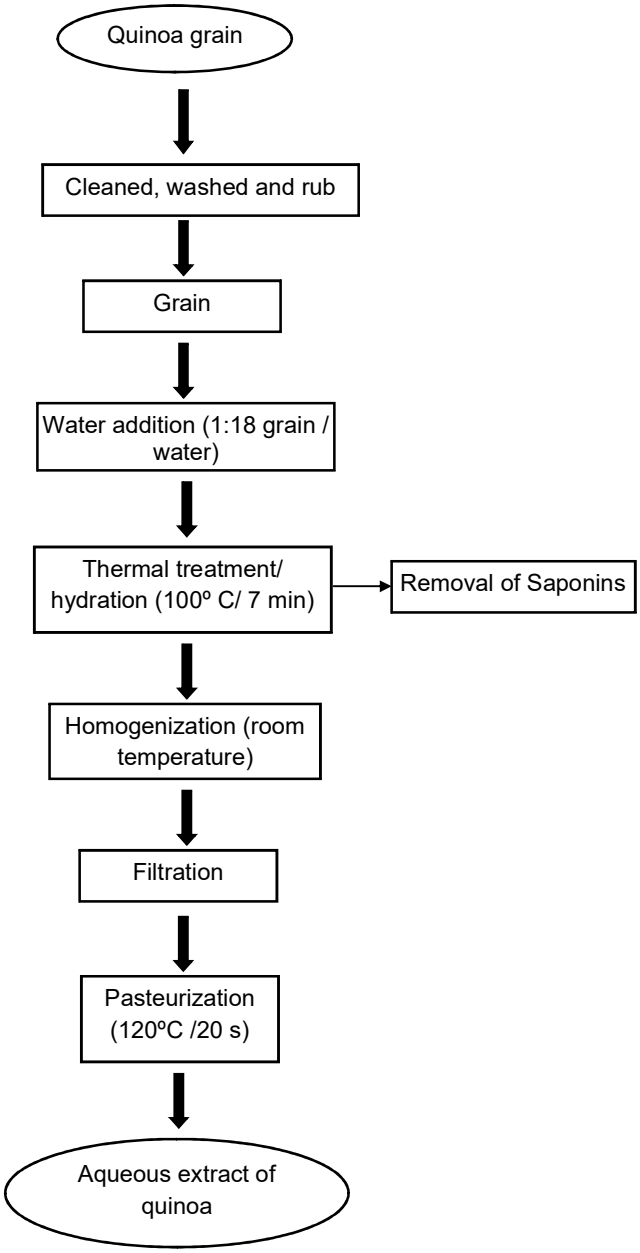

Fig. 1. Method of preparation of quinoa extract

Table 1. Treatments details of the experiment

T_1	100 % Pomegranate juice + Sugar (15° Brix) (Control-I)
T_2	90 % Pomegranate juice + 10% quinoa extract + Sugar (15° Brix)
T_3	85% Pomegranate juice + 15% quinoa extract+ Sugar (15° Brix)
T_4	80% Pomegranate juice + 20% quinoa extract + Sugar (15° Brix)
T_5	100 % Pomegranate juice + Sugar (18° Brix) (Control-II)
T_6	90 % Pomegranate juice + 10% quinoa extract + Sugar (18° Brix)
T_7	85% Pomegranate juice + 15% quinoa extract+ Sugar (18° Brix)
T_8	80% Pomegranate juice + 20% quinoa extract + Sugar (18° Brix)

Biochemical parameters: At room temperature, total soluble solids content of pomegranate beverage incorporated with quinoa extract was measured using a hand refractometer (Erma, Japan) in °Brix. The titratable acidity of pomegranate beverage incorporated with quinoa extract was estimated by the titration method. A known quantity of sample (5 mL) was taken and titrated against standard 0.1N NaOH using phenolphthalein indicator (Srivastava and Sanjeevkumar 1998). pH of the pomegranate beverage incorporated with quinoa extract was determined by using pH meter. To calibrate the instrument, standard buffer solutions of pH 4.0, 7.0 and 9.2 were used (Jackson 1969). The brix to acid ratio was estimated using the formula by taking the ratio of total soluble solids to titratable acidity.

***L*, a*,* and *b**colour values:** The colour of the pomegranate beverage incorporated with quinoa extract was assessed using a Hunter Lab colorimeter with a 45 mm (diameter) measurement tube. These three colour readings per sample were recorded using the *L*, a**, and *b** lightness (white-black), red-green and yellow-blue scales, respectively.

Phenols, DPPH free radical scavenging activity and tannin: For the determination of total phenols, methanol extracts (80 %) of pomegranate beverage incorporated with quinoa extract, folin-ciocalteu reagent method was followed using gallic acid as a standard (Madaan et al 2011). The ability of pomegranate beverage incorporated with quinoa extract to scavenge the stable 2, 2'-diphenyl-2-picrylhydrazyl (DPPH) free radical was assessed according to the method of Eghdami and Asli (2010). Tannin content of pomegranate beverage incorporated with quinoa extract was estimated by vanillin hydrochloride method using tannic acid as a standard (Sadasivam and Manickam 2015).

Minerals estimation: Potassium content in juice samples were estimated by using flame photometric method. Feed the di-acid or tri-acid digested sample to the flame photometer through a capillary tube and record reading (Jackson 1973).The phosphorous content present in the di-acid or tri-acid digested pomegranate beverage incorporated with quinoa extract reacts with vanadium and molybdenum in vanodomolybdate reagents to form 12 fold heteropoly phosphor-vanadomolybdate complex (Arora et al 2002). The filtrate after wet digestion by di-acid mixture was used for iron and zinc estimation by "Micro-wave plasma atomic emission spectrometer" instrument from Agilent Technologies. Calcium and magnesium was determined by complexo-metric titration method involving standard EDTA (Piper 1966).

The data was analyzed as applicable to completely randomized design (CRD). Statistical analyses of experiments were performed using Web Agri Stat Package (WASP) Version 2 (Jangam and Thali 2010).

RESULTS AND DISCUSSION

Sensory evaluation: Colour is the most important factor in determining consumer preference (Table 2). The maximum score was in control II T_5 (100 % pomegranate juice + sugar 18º Brix: 8.42) which was on par with control I T_1 (100 % pomegranate juice + sugar 15º Brix: 8.39). The control sample got the highest score because of its natural bright red colour and the quinoa extract incorporated sample scored less upon concentration however, they did not vary significantly. Incorporation of quinoa extract (10, 15%and 20%) to the pomegranate beverage did not vary consistency significantly. The addition of quinoa extracts does not change the taste and flavored pomegranate beverage significantly. Overall acceptability of pomegranate beverage incorporated with quinoa extract does not make any significant change. The overall acceptability of pomegranate beverage by incorporation of quinoa extract recorded highest score in T_1 which was at par with T_5 (100 % Pomegranate juice + Sugar 18º Brix: 8.07). Based on the above sensory information with all the quality parameters, there was no significant difference between the control and quinoa extract incorporated pomegranate beverage. It indicates that quinoa extract can be used for substitution of pomegranate beverage.

Biochemical parameters: There was a significant difference in TSS between the treatments (Table 3). The four treatments (T_1, T_2, T_3 and T_4) were maintained TSS of 15º Brix and (T_6, T_7 and T_8) 18º Brix. The maximum TSS (18.20^{0}Brix) content was in the treatment T_8 (80% Pomegranate juice + 15 % quinoa extract+ Sugar 15º Brix) and minimum (15.04 ºBrix) was in T_4 (80 % Pomegranate juice + 20% quinoa extract+ Sugar 15º Brix). The pH in T_8 was highest (3.66) and the lowest 3.54 was in control I. As the per cent incorporation of quinoa extract increased in the treatments, the pH also increased proportionately both in 15^o brix and 18^o brix. This may be due to more pH value of quinoa extract than pomegranate juice. Bianchi et al. (2015) also reported that

Table 2. Sensory evaluation of pomegranate beverage by incorporation of quinoa extract

Treatments	Colour and appearance	Consistency	Taste	Flavour	Overall acceptability
T_1	8.39	8.05	7.89	8.22	8.14
T_2	8.25	7.80	8.03	7.91	7.99
T_3	8.08	7.80	7.78	7.89	7.88
T_4	7.86	7.78	7.64	7.61	7.73
T_5	8.42	7.83	7.89	8.11	8.07
T_6	8.20	7.83	7.89	7.89	7.96
T_7	8.11	8.05	7.83	7.89	7.98
T_8	7.91	7.89	8.17	7.94	7.98
Mean	8.15	7.88	7.89	7.93	7.96
CD (p=0.01)	NS	NS	NS	NS	NS

See Table 1 for treatment detail

Table 3. Effect of incorporation of quinoa extract on total soluble solids, pH, titratable acidity, brix to acid ratio and colour values in pomegranate beverage

Treatments	TSS (°B)	pH	TA (%)	Brix: Acid	Colour values		
					*L**	*a**	*b**
T_1	15.04	3.54	0.85	17.81	15.72	26.47	1.05
T_2	15.16	3.58	0.77	19.75	18.90	24.85	1.28
T_3	15.14	3.62	0.70	21.37	21.05	23.53	1.78
T_4	15.15	3.64	0.65	23.07	24.40	21.82	2.07
T_5	18.10	3.58	0.83	21.87	16.31	26.14	1.16
T_6	18.15	3.60	0.74	24.74	19.00	24.08	1.35
T_7	18.18	3.63	0.69	26.32	21.53	23.14	1.83
T_8	18.20	3.66	0.63	28.84	24.69	21.27	2.19
Mean	16.64	3.60	0.73	22.97	20.20	23.91	1.59
CD (p=0.01)	0.17	0.03	0.05	1.43	1.49	1.28	0.18

See Table 1 for treatment detail

100 per cent quinoa extract has a pH of 6.35. Acid gives the characteristic sourness to the product. Effect of quinoa extract on the titratable acidity of pomegranate beverages ranged between 0.63 and 0.85 per cent. Both in sugar 15º Brix and 18º Brix, titratable acidity decreased as the concentration of quinoa extract increased. This may be due to less acidity and more pH value of quinoa extract. The negative association with pH and acidity was observed. Islam et al (2014) also inferred that acidity and pH are inversely proportional to each other, as pH increases the concentration of hydrogen ion decreases. The maximum pH sample has minimum titratable acidity. Brix to acid ratio is often an indicator of acceptability than either sugar or acid alone (Jayasena and Cameron 2008). Among the different treatments, T_8 recorded significantly highest value for Brix to acid ratio (28.84).

***L*, a*,* and *b*colour values:** The Significant difference was recorded among the different treatments concerning the *L** value of pomegranate beverage by incorporation of quinoa extract (Table 3). *L** value was increased by incorporation of quinoa extract both in sugar 15º Brix and 18º Brix and ranged between 15.72 to 24.69. There was a decreased trend in *a** value of pomegranate beverage by incorporation of quinoa extract both in sugar 15º Brix and 18º Brix. Numerically, the highest *a** value was recorded in control I T_1 (Brix: 26.47). There were difference related to the *b** among the different treatments in pomegranate beverage by incorporation of quinoa extract. Significantly, the highest *b** value (2.19) was in T_8. The reduction of the redness value of beverages occur due to the natural pigment content of the quinoa, especially because has high phenolic components (Demir and Bilgicli 2021). The results are in line with Bhalerao et al (2020), where pomegranate juice was blended with different concentrations of muskmelon and amla juice, and found that, the decrease in the concentration of pomegranate juice increases the *l**, *b** values and decreases *a** values. Gonzalez-Molina et al (2009) reported that an increase in the concentration of lemon juice (25, 50 and 75%) reduced *a** values and increased the *b** values. Anthocyanins are responsible for the red colour of pomegranate juice. Anthocyanin is pH-dependent (the red flavylium is stable at low pH) and as the pH changes, the colour of the juice also changes (Choi et al 2002). The lighter colours of pomegranate beverage with quinoa extract were due to the higher pH which inhibits the coloured flavylium form of anthocyanins.

Phenols, DPPH free radical scavenging activity and tannin: The total phenol content of pomegranate beverages incorporated with quinoa extract ranged between 431.67 and 463.33 mg GAE/100 mL. The total phenol of pomegranate beverage has shown an increasing trend as the percentage of quinoa extract increased both in 15° brix and 18°brix beverages (Fig. 2). Compared to control II T_5 (100% Pomegranate juice + Sugar 18º Brix) there was increased in 31.66 mg GAE/100 mL of total phenols in T_4. The quinoa extract (20%) incorporated pomegranate beverage showed higher phenolic content because, quinoa is the major source of phenolic acids *viz*., vanillic (523.92 $\mu g g^{-1}$), coumaric (275 $\mu g\ g^{-1}$), 3, 4-Dihydroxybenzoic acid (275 $\mu g\ g^{-1}$), p-hydroxybenzoic acid (97 $\mu g\ g^{-1}$), gallic acid (320 $\mu g\ g^{-1}$) and caffic acid (6.31 $\mu g\ g^{-1}$) (Tang and Rong 2017). The results are in conformity with the findings of Demir and Kilinc (2017) who observed that the phenol content was increased significantly by the substitution of quinoa flour in cookies and also utilization of quinoa flour in gluten-free pasta formulation at a 30 per cent ratio increased the total phenol content compared to control (Demir and Bilgicli 2021).

Appropriate diets that include fruits, vegetables, whole grains and pseudo cereals may contribute to good health due to rich source of anti-oxidants. Among these foods, cereals and pseudo cereals play an important role (Shela et al 2008, Calderelli et al 2016). The antioxidant activity of pomegranate beverages by incorporation of quinoa extract ranged between 95.38 and 98.05 per cent (Table 4). Control II T_5 showed 2.67 per cent less antioxidant activity than the treatment T_4. The antioxidant activity of fruits and vegetables largely depends upon an abundance of individual antioxidants or the combined effect of antioxidants like phenolic compounds, ascorbic acid and anthocyanins (Rai et al 2011). Quinoa extract incorporated pomegranate

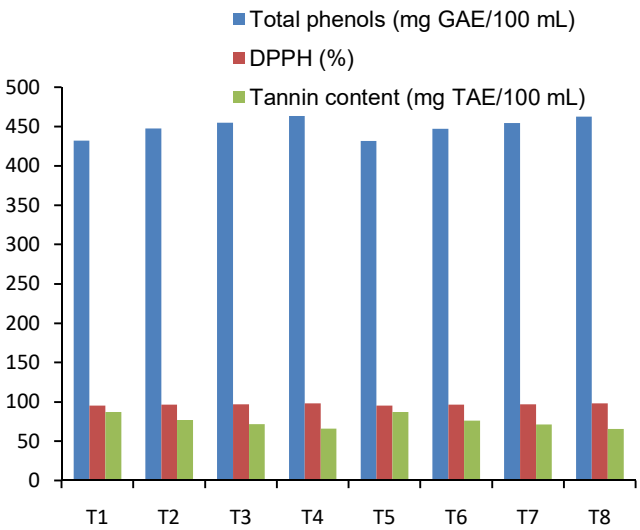

Fig. 2. Effect of incorporation of quinoa extract on total phenols antioxidant activity and tannin content in pomegranate beverage

Table 4. Effect of incorporation of quinoa extract on mineral contents in pomegranate beverage

Treatments	Potassium (mg/100 mL)	Phosphorous (mg/100 mL)	Calcium (mg/100 mL)	Magnesium (mg/100 mL)	Iron (mg/100 mL)	Zinc (mg/100 mL)
T_1	638.33	146.04	160.00	44.00	2.86	0.26
T_2	683.83	173.42	175.33	55.20	3.06	0.32
T_3	697.33	187.31	187.33	60.80	3.16	0.37
T_4	718.83	201.58	194.00	66.80	3.26	0.43
T_5	638.17	145.64	159.33	43.60	2.85	0.26
T_6	683.67	173.02	174.67	54.80	3.05	0.31
T_7	697.17	186.91	186.67	60.40	3.15	0.36
T_8	718.67	201.19	193.00	66.60	3.25	0.42
Mean	684.50	176.89	178.79	56.53	3.08	0.34
CD (p=0.01)	3.23	1.42	5.31	2.01	0.01	0.02

See Table 1 for treatment detail

beverages exhibited maximum antioxidant activity because quinoa contain compounds like polyphenols, phytosterols and flavonoids with possible nutraceutical benefits (Abugoch James 2009). In the same way, the addition of quinoa flour increases antioxidant activity in the bread as reported by Ballester-Sanchez et al (2019) and remarkable improvement in scavenging capacity was observed with increasing addition of quinoa flour *i.e.* 1.38-fold increase in DPPH radical scavenging capacity in wheat bread (Xu et al 2019). The total tannin content of pomegranate beverages by incorporation of quinoa extract showed a significant difference between the treatments (Fig. 2) and between 65.64 mg TAE/100 mL and 87.44 mg TAE/100 mL Treatment T_8 (80 % pomegranate juice + Sugar 15º Brix +20% quinoa extract) showed 21.80 mg TAE/100 mL less tannin content than the control I T_1 (100% ppomegranate juice + sugar 15º Brix). Pomegranate is also a rich source of hydrolyzable tannins. Upon adding different concentrations of quinoa extract to pomegranate juice, the tannin content of pomegranate beverage decreases with an increase in the percentage of quinoa extract. The quinoa seeds contain a less amount of tannins and adequate processing further reduce its content (Filho et al 2017). Same trend was observed in the Deepika and Panja (2017) where enrichment of aonla pulp with fruit pulp of mango, papaya, and jackfruit reduced the tannin content of the resultant fruit bars compared to that from pure aonla pulp, which is an indication in the reduction of astringency.

Minerals: Significant differences were observed among the treatments for potassium, phosphorous, calcium, magnesium, iron and zinc contents in pomegranate beverages incorporated with quinoa extract (Table 4). The highest amount of potassium (718.83 mg/100 mL), phosphorous (201.58 mg/100 mL), calcium (194 mg/100 mL), magnesium (66.80 mg/100 mL) content was in T_4(80% pomegranate juice + 20 % quinoa extract+ Sugar 15º Brix) and lowest amount of potassium (638.17 mg/100 mL), phosphorous (145.64 mg/100 mL), calcium (159.33 mg/100 mL), magnesium (43.60 mg/100 mL) content was in control II T_5 (100% ppomegranate juice +sugar 18º Brix). Highest content of minerals in quinoa incorporated pomegranate beverage may be due to quinoa is abundant in minerals such as calcium, iron, phosphorus, zinc and potassium and are reported to have more concentrations than most grain crops. Iron, calcium, and phosphorus levels are higher in quinoa than those of maize and barley (Arneja et al 2015). The present study is supported by Demir and Bilgicli (2021) where quinoa flours were replaced with rice: corn semolina blend at different (0–30%) ratios in gluten-free pasta formulation. The significant increment was observed in calcium, iron, potassium, magnesium, phosphorous and zinc content of the gluten-free pasta in addition to quinoa flour. Demir and Kilinc (2017) also observed that the potassium, magnesium, calcium, iron and zinc contents were increased as the concentration level of quinoa flour increased in the preparation of cookies.

CONCLUSION

Quinoa extract incorporated pomegranate beverage with combination of 80 % pomegranate juice + 20% quinoa extract+ sugar (15º Brix)had highest antioxidant activity, total phenols, total flavonoids and also addition of quinoa extract to pomegranate juice increased the mineral content such as potassium, phosphorous, calcium, magnesium, iron and zinc.

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Received 13 September, 2023; Accepted 01 November, 2023