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Studies on development of pomegranate beverage by incorporation of Quinoa extract

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Abstract
Pomegranateis an excellent source of anthocyanins (delphinidin, pelargonidin and cyanidin) and additional phenolic compounds (including hydrolyzable tannins such as punicalin, punicalagin, pedunculagin, gallicand ellagic acid), organic acids and antioxidant activity. Quinoa (*Chenopodium quinoa Wild*.) is a crop used by pre-Columbian cultures in South America for centuries. Quinoa contains higher protein content and incredible balance of essential amino acids, resulting in excellent protein value similar to casein in milk. Majority of the stored proteins in quinoa are composed of albumin (35%) and globulin (37%). Main carbohydrate in quinoa is starch (52% - 69%) and it is gluten free with low glycemic index. The main flavonoids in quinoa are kaempferol and quercetin both are strong antioxidants and free-radical scavengers. In the present study, synergetic effect of pomegranate beverage with the incorporation of quinoa extract showed noticeable differences among the treatments. Among different treatments, the highest phenol, antioxidant and flavonoid content were noticed in T_4 (80% Pomegranate juice+ 20% quinoa extract+ Sugar- 15˚Brix) with 463.33 mg GAE/100 mL, 463.56 mg AAE/100 mL and 54.00 mg QE/100 mL, respectively.

Keywords: Pomegranate juice, quinoa extract, antioxidant activity, phenolic compounds

Introduction
Pomegranate (*Punica granatum* L.) can be translated to “seeded apple” (*Punica*-apple; *granatum*-grainy). It is a chief fruit crop of arid and semiarid regions of the world. It is thought to be originated from Iran.It is a member of the Lythraceae family.It is considered to be a fortunate emblem for most aspects of life, including luck, abundance and fertility.It is a nutrient-dense fruit rich in phyto-chemical compounds (Miguel *et al.*, 2010) [14]. The antioxidant level in pomegranate juice was beyond that in other natural juices, green tea and red wine (Aviram *et al*., 2002) [4].

India is the second-largest producer of fruits next to Brazil. Karnataka is one of the major fruit growers in India. The total area under fruit cultivation in India is381.35 thousand hectares with the production of 7055.40 thousand tonnes and productivity of 18.50 MT/hectare (Anon., 2020) [2]. Maharashtra is the leading producer of pomegranate, followed by Karnataka, Andhra Pradesh, Gujarat and Tamil Nadu (Anon., 2019) [1]. In Karnataka, it is being cultivated in Vijayapura, Bagalakote, Koppala, Belagavi, Gadaga, Bellary, Raichuru, Bengaluru rural, Tumkuru, Chitradurga and Davanagere districts. The area under pomegranate cultivation in India is 283 thousandhectares with the production of 3186 thousandtonnes and productivity of 11.26 MT/hectare (Anon, 2020) [2].The wide range of utility of pomegranate in human health, nutrition and livelihood security has triggered its heavy demand in India and other countries (Chandra *et al*., 2013) [7].

Pomegranateis an excellent source of anthocyanins (delphinidin, pelargonidin and cyanidin) and additional phenolic compounds (including hydrolyzable tannins such as punicalin, punicalagin, pedunculagin, gallicand ellagic acid), organic acids and antioxidant activity (Gil *et al.*,2000; Noda *et al.*,2002) [12, 15]. It is one of the most nutritious grains used as human food and has been selected by Food and Agricultural Organization (FAO) of the United Nations as one of the crops destined to offer food security in this century and declared 2013 as the “International Year of Quinoa” (Anon., 2013) [1].

Quinoa (*Chenopodium quinoa Wild*.) is a crop used by pre-Columbian cultures in South America for centuries. There is a long history of safe use of the grain in South America.

Because it is not a member of the Gramineae family, it is commonly referred to as a pseudo-cereal, but seeds of quinoa can be milled into flour and used as a cereal crop.

Quinoa contains higher protein content and incredible balance of essential amino acids, resulting in excellent protein value similar to casein in milk. Majority of the stored proteins in quinoa are composed of albumin (35%) and globulin (37%). Main carbohydrate in quinoa is starch (52% - 69%) and it is gluten free with low glycemic index. Minerals such as calcium, iron, phosphorus, zinc, and copper are abundant in quinoa grains. It has a lipid-lowering action and comprises 4.4-8.8 per cent crude fat, with the important fatty acids linoleic and linolenic acid accounting for 55 to 63 per cent of total fatty acids. The main flavonoids in quinoa are kaempferol and quercetin both are strong antioxidants and free-radical scavengers.

Material and methods
The present investigation entitled “Studies on development of pomegranate beverage by incorporation of quinoa extract and

its hypocholesterolemic activity in rat model" was undertaken in the post graduate laboratory of Department of Post-Harvest Technology, College of Horticulture, Bagalkot, Karnataka during November 2020 to April 2021. The experiment was conducted in Completely Randomized Design with eight treatments and three replications.

Preparation of pomegranate juice: Fresh and disease-free pomegranate fruits (cv. Bhagwa) were 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.

Preparation of quinoa extract: Quinoa grains were cleanly washed and rubbed with water and water is added in the ratio of 18:1 (water/grain). Thermal treatment is done at 100 ℃ for 7 minutes and homogenization at room temperature. Filtration is done through muslin cloth and pasteurized the extract at 120 ℃ for 20 seconds.

Preparation of pomegranate beverage with the incorporation of quinoa extract: The extracted juice of pomegranate and quinoa were taken according to treatments requirement and sucrose was added to maintain TSS. Quinoa extract was added wherever needed in all the treatments with the different percentages (except T_1 and T_5). The prepared beverage was filled in clean, sterilized bottles and sealed with caps.

For the determination of the total phenol content of methanolic extracts (80%) of pomegranate beverage (PB) sample (1 ml PJC in 20 ml Methanol), the folin-ciocalteu reagent method was followed using gallic acid as a standard. The ferrous ion reducing antioxidant power (FRAP) of the samples was measured calorimetrically according to the method by Benzie and Strain (1996). Total flavonoids were determined using the aluminium chloride method using quercetin as a standard suggested by Maciel *et al*. (2011) [13]. Observations were recorded on Phyto-chemical parameters of pomegranate beverage by incorporation of quinoa extract like total phenol content (mg GAE/100 mL), antioxidants (mg AAE/100 mL), total flavonoids (mg QE/mL).

Results and Discussion

Total phenol content (mg GAE/100 mL)

Table 1 revealed the data regarding total phenol content of pomegranate beverage, which showed significant difference among the treatments by incorporation of quinoa extract. The total phenol content of beverage was ranged in between 431.67 and 463.33 mg/100 mL. The total phenol content was reported to be higher in T_4 (80% Pomegranate juice + 20% quinoa extract + Sugar 15º Brix: 463.33 mg GAE/100 mL) which was on par with T_8 (80% Pomegranate juice + 20% quinoa extract+ Sugar 18º Brix: 462.67 mg GAE/100 mL), followed by T_3 (85% Pomegranate juice + 15% quinoa extract+ Sugar 15º Brix: 455.00 mg GAE/100 mL) and T_7 (85% Pomegranate juice + 15% quinoa extract+ Sugar 18º Brix: 454.33 mg GAE/100 mL) which were on par with each other. The significantly minimum total phenol content was reported in control II T_5 (100% Pomegranate juice +Sugar 18º Brix: 431.67 mg GAE/100 mL) which was on par with control I T_1 (100% Pomegranate juice +Sugar 15º Brix: 432.00 mg GAE/100 mL).

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) [18]. The results are in conformity with the findings of Demir and Kilinc (2017) [9] who found 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) [8].

Antioxidant activity (mg AAE/100 mL)

Table 1 shows total antioxidants in FRAP method of pomegranate beverage by incorporation of quinoa extract has shown significant difference among the treatments.

The antioxidant activity increased in pomegranate beverage by incorporation of quinoa extract and it ranged between 342.20 mg AAE/100 mL and 463.56 mg AAE/100 mL. Numerically, highest value of antioxidant activity (463.56 mg AAE/100 mL) was identified in T_4 (80% Pomegranate juice + 20% quinoa extract+ Sugar 15º Brix) which was on par with T_8 (80% Pomegranate juice + 20% quinoa extract+ Sugar 18º Brix: 463.32 mg AAE/100 mL) followed by T_3 (85% Pomegranate juice + 15% quinoa extract+ Sugar 15º Brix: 441.24 mg AAE/100 mL) and T_7 (85% Pomegranate juice + 15% quinoa extract+ Sugar 18º Brix: 441.00 mg AAE/100 mL) and they showed no significant difference. Significantly minimum antioxidant activity was identified in control I T_1 (100% Pomegranate juice +Sugar 15º Brix: 342.48 mg AAE/100 mL) followed by control II T_5 (100% Pomegranate juice +Sugar 15º Brix: 343.20 mg AAE/100 mL) which were on par with each other.

Quinoa seeds are rich in high-quality protein, dietary fiber, and natural antioxidants. Quinoa seeds possess novel functional and bioactive properties. Appreciably higher concentrations of nutrients and bioactive components make them ideal effective functional grains against physiological disorders like diabetes, hypertension, cardiovascular diseases, and obesity. The present results are supported by the results of Goyat *et al*. (2018) [11] where in, the study indicated that an increase in the substitution of quinoa seed flour increases the antioxidant activity of cookies. The obtained results of the present study illustrated that there was a correlation between phenolic contents and ferric reducing antioxidant power. This finding declared the importance of phenolic acid content as a reducing agent in our study and this may be due to their potent electron donating abilities (Bilto *et al*., 2012) [6].

Total flavonoids (mg QE/100 mL)

Table 1 interprets the data regarding total flavonoids of pomegranate beverage, which has shown increased trend by incorporation of quinoa extract. The total flavonoids of beverage ranged in between 38.83 and 54.00 mg QE/100 mL.

Significant difference was acquired among the different treatments with regard to the total flavonoids of pomegranate beverage by incorporation of quinoa extract. Appreciably, highest value was reported in T_4 (80% Pomegranate juice + 20% quinoa extract+ Sugar 15º Brix: 54.00 mg QE/100 mL) which was on par with T_8 (80% Pomegranate juice + 20% quinoa extract+ Sugar 18º Brix: 53.92 mg QE/100 mL). No significant difference was observed for total flavonoids for treatments T_3 (85% Pomegranate juice + 15% quinoa extract+ Sugar 15º Brix) and T_7 (85% Pomegranate juice + Sugar 18º

Brix + 15% quinoa extract) having 50.50 mg QE/100 mL and 50.42 mg QE/100 mL flavonoid content respectively. The lowest value was reported in control II T_5 (100% Pomegranate juice +Sugar 18º Brix: 38.83 mg QE/100 mL) which was on level with control I T_1 (100% Pomegranate juice +Sugar 15º Brix: 39.00 mg QE/100 mL).

Total flavonoids contents are mostly used in plants to produce yellow and other pigments which play an important role in colour of plants and also flavonoids are the most common phytoconstituents of different fruits and vegetables which are responsible for antioxidant activities (Phuyal *et al.,* 2020) [16]. The main flavonoids in quinoa are kaempferol and quercetin both are strong antioxidants and free-radical scavengers and it is more abundant in quinoa than in other cereal crops (Bhathal and Kaur, 2018) [5]. In the study of Goyat *et al.* (2018) [11], they reported that the total flavonoid content of the control sample and 15 per cent substitution of quinoa seed flour cookies were 0.25µg QAE/g and 0.52 µg QAE/g, respectively. The results of the present study are in accordance with Shafie (2019) [17] who found that the total flavonoid content of quinoa flour incorporated bread was increased by 46 per cent (90% wheat flour+ 10% quinoa flour) as compared to 100 per cent wheat flour bread.

Table 1: Effect of incorporation of quinoa extract on total phenols, antioxidant activity and total flavonoids in pomegranate beverage

Treatments	Total phenols (mg GAE/100 mL)	Antioxidant activity (mg AAE/100 mL)	Total flavonoids (mg QE/100 mL)
T_1	432.00	342.48	39.00
T_2	447.33	417.60	46.50
T_3	455.00	441.24	50.50
T_4	463.33	463.56	54.00
T_5	431.67	343.20	38.83
T_6	447.00	417.24	46.45
T_7	454.33	441.00	50.42
T_8	462.67	463.32	53.92
Mean	449.17	416.21	47.45
S.Em±	0.73	0.55	0.24
CD @ 1%	3.00	2.26	0.99

T_1: 100% Pomegranate juice Sugar (15^0 brix) (Control-I)
T_2: 90% Pomegranate juice + 10% quinoa extract + Sugar (15^0 brix)
T_3: 85% Pomegranate juice + 15% quinoa extract+ Sugar (15^0 brix)
T_4: 80% Pomegranate juice + 20% quinoa extract+ Sugar (15^0 brix)
T_5: 100% Pomegranate juice Sugar (18^0 brix) (Control-II)
T_6: 90% Pomegranate juice + 10% quinoa extract + Sugar (18^0 brix)
T_7: 85% Pomegranate juice + 15% quinoa extract + Sugar (18^0 brix)
T_8: 80% Pomegranate juice + 20% quinoa extract + Sugar (18^0 brix)

Conclusion

Results revealed that among different treatments, the highest phenol, antioxidant and flavonoid content were noticed in Treatment T_4 (80% Pomegranate juice+ 20% quinoa extract+ Sugar- 15˚Brix) with 463.33 mg GAE/100 mL, 463.56 mg AAE/100 mL and 54.00 mg QE/100 mL, respectively and Treatment T_1 recorded lowest value in all the parameters *i.e.*, total phenol content, antioxidant activity and total flavonoids. From this, it may be concluded that pomegranate beverage with incorporation of 20 percent quinoa extract is considered best with respect to phyto chemical properties.

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