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RESEARCH PAPER

Use of clarification agents and methods on the development of pomegranate juice processing technology

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SUMMARY : Effects of methods and clarification agents on juice quality of pomegranate were studied. Juice extracted manually from arils of pomegranate cv. Bhagawa was clarified with gelatin, pectinase enzyme agents and centrifugation, homogenization and natural sedimentation methods with main purpose to reduce the amount of phenolic substances and to get clear sparkling, sweet juice. Different physicochemical properties *viz.*, pH, TSS, total phenolic substances, total anthocyanins, acidity, sugars and sensory attributes like colour, appearance, transparency, taste and flavour were recorded. In each clarification method, phenolic substances were controlled and clear, sparkling and most appealing pomegranate juice was obtained. Turbid but sweet juice was obtained in homogenization method. Use of gelatin @ 1 g/l as clarification agent gave clear, sparkling juice by removing considerable amounts of tannins which is considered to be the most important quality parameter of pomegranate juice.

KEY **W**ORDS : **:** Pomegranate juice, Clarification, Gelatin, Pectinase, Polyphenols, Anthocyanins

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Pomegranate (*Punica granatum* L.), a superfruit, belongs to the family Punicaceae and is one of the favourite table fruit of tropical, sub-tropical and sub-temperate regions of the world having great processing potential. The fresh fruit is of exquisite quality as medicinal values and high consumer appeal. The fruit is consumed directly as fresh seeds as well as fresh juice that can also be used in beverages, juice concentrate jellies and used as flavouring and colouring agents. Industrial processing of the pomegranate in India is scarce due to lack of technological development though there is great demand for the pomegranate derived products. Indian pomegranate cultivars are sweet acidic in taste having excellent flavour and nutraceutical value. The edible part of the pomegranate fruit contains phenolic constituents which give colour, astringency and bitterness to the juice (De Simon *et al.* 1992). If the pomegranate juice is not clarified, product has bitter, astringent taste with turbid, hazy appearance due to high tannin content and this limits the consumption of fresh pomegranate juice. The phenolic compounds cause formation of cloudy appearance of fruit juices in concentrates and storage (Spanos and Wrolstad, 1992). This deteriorates the product quality and shelf-life as well as consumer preference. Pomegranate juice contains only trace amount of pectin and juice can be filtered easily after pressing without clarification. But, clarification is necessary to prevent the formation of cloudy appearance during storage. The taste of the product is also improved by means of clarification (Bayinderli *et al.*, 1994). In pomegranate fruit, most of the tannins are present in rind and in seeds. During aril extraction they pass to the juice. Clarification or fining is one of the most important steps in processing fruit juice. Natural sedimentation is suitable for pomegranate juice. Effective use of clarification agents requires appropriate concentration.

The purpose of this study was to determine the effect of clarification agents and effective method on pomegranate juice.

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EXPERIMENTAL METHODS

Fruits :

The pomegranate cv. BHAGWA fruits of proper maturity and ripeness were brought from progressive farmer's field of Sangli area. The fruits were washed and surface sterilized with 100 ppm chlorine for 5 minutes. Then they were sectioned, peeled and arils were extracted manually.

Juice extraction :

Juice from arils was extracted with electrically operated hydraulic basket press machine. The pH and total soluble solids of the juice obtained were 3.67 and 14.80^0Brix. The experiment was conducted in completely randomized block design with five clarification treatments and three replications.

Clarification of pomegranate juice :

The extracted pomegranate juice was clarified by using different clarification methods as below :

– In natural sedimentation (C_1), juice was kept at refrigeration temperature (5 ± 1^0C) for 0-96 hours without adding any clarifying agent. The resultant clarified juice was passed through a clean muslin cloth.
– In homogenization (C_2), extracted raw juice was homogenized with a laboratory type homogenizer for 20 minutes.
– Addition of gelatin @1g/l (C_3) Gelatin was dissolved in water at about $40\text{-}50^0$C to produce 5 per cent solution. It wasn't added to the fruit juice directly since its solubility is low at the pH of fruit juice. After addition of gelatin solution, juice was kept at refrigeration temperature ($5\text{-}6^0$C) for 12 hours.
– Pectinase enzyme (0.10 %) (C_4) was added in pomegranate juice and mixture was kept for incubation at 30^0C for 20 minutes and filtered through muslin cloth.
– Centrifugation (C_5) Raw pomegranate juice was centrifuged at 6000 rpm for 10 minutes in Remi make laboratory type centrifuge machine. The clear supernatant juice was then filtered through muslin cloth.

The resulting clarified juices in all above treatments were passed through muslin cloth and stored at 5^0C for further analysis.

Physico-chemical analysis :

The clarified juice recovery percentage was worked out on non clarified juice weight basis (w/w) as well as on volume basis (v/v) and specific gravity of pomegranate juice was determined by dividing mass by volume. A unit was measured and weighed exactly. The pH of pomegranate juice was measured by using Perkin-Elmer pH meter. The total titratable acidity was determined as per AOAC (2005) method and expressed in percentage of citric acid. Total soluble solids was determined with hand refractometer and expressed in terms of ^{0}Brix. The reducing and total sugars were determined by the method of Lane and Eynon (1923) with slight modification suggested by Ranganna (1986). Total anthocyanin content was estimated by the procedure described by Ranganna (1986) and calculated in terms of molecular extinction coefficient values. The polyphenol content in the juice was determined by the colorimetric method using Folin Denis reagent (AOAC, 2005). Per cent total antioxidant activity was determined on the basis of scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical as described by Sandhu *et al.* (2003) and Bhalodia *et al.* (2011).

Colourimetric measurements :

Colour parameters L*, *a**, *b**, hue-angle and chroma of clarified pomegranate juice were measured with Premier scan colourimeter using a 1 cm quartz cuvette and expressed in diamensions of L*, *a**, *b**, C and H. These parameters were calculated according to the following equation: (180-H)/(L+C), to obtain colour index. In CIE, L* indicates lightness, *a** chromacity on a green (-) to red (+) axis and *b** chromacity on a blue (-) to yellow (+) axis. Before each measurement, the apparatus was standardized against black and white tile.

Sensory evaluation :

Clarified pomegranate juices were analyzed organoleptically by panel of seven judges in terms of colour, appearance, taste, flavour and overall acceptability using a 9-point hedonic scale (Amerine *et al.*, 1965).

Microbial analysis :

Microbial limit tests for clarified and filtered fresh unpasteurized pomegranate juice were carried out for the total viable bacterial and fungal count and for detection of specific indicator microbial species such as *E. coli*, *Staphylococcus aureus*, *Psuedomonas* sp., *Salmonella* sp. by following the standard operating procedure described in USFDA (BAM) I 5401: 2002, 5402: 2002 (Anonymous, 2011) and prescribed by Bureau of Indian Standards.

EXPERIMENTAL FINDINGS AND ANALYSIS

The results of the present study as well as relevant discussions have been presented under following heads:

Physical parameters :

The results of the physical parameters of pomegranate juice produced by five different clarification treatments are shown in Table 1. It is clear from the data that the specific gravity of pomegranate juice decreased significantly irrespective of the clarification treatment. This was due to either homogenization or removal of the colloidal substances. But specific gravity was minimum in natural sedimentation method due non settlement of colloidal particles. Similar results were reported by Neifar *et al*. (2009). The addition of gelatin @1g/l to pomegranate juice gave the highest recovery of clear sparking juice both on weight (94.259%) as well as on volume basis (93.423%). Surprisingly, it was observed that the addition of gelatin to pomegranate juice resulted in the flocculation and formation colloidal mass giving clear, bright red coloured sparkling juice. This treatment produced minimum sediment weight (40.743 g). A slight significant increase in the pH of juice was found after gelatin, pectinase and centrifuge clarification. Only the natural sedimentation (3.55) and homogenization (3.658) methods resulted decrease in pH at the end of clarification. The maximum increase in the pH value (3.818) was recorded in case of addition of gelatin. This might be due to alkaline property of gelatin as suggested by Bayinderli *et al.* (1994).

Chemical parameters :

The data in respect of changes in the chemical constituents of pomegranate juice due to the different clarification methods and treatments are presented in Table 2.

The initial TSS value of for unclarified fresh pomegranate juice was 14.80^{0}Brix. slight increase in total soluble solid content was noted in natural sedimentation and homogenization methods (14.95^{0}Brix) than the initial value. It decreased to 13.65 ^{0}Brix with the addition of gelatin. TSS of juice showed decreasing trend in rest of three clarification methods. The titratable acidity of pomegranate juice showed significant decreasing trend in all the methods of clarification. Addition of gelatin to the juice recorded maximum decrease in titratable acidity which might be due to alkaline property of gelatin. The sugar content in the pomegranate juice also showed decreasing trend over the initial value. Maximum sugar content (15.063%) was recorded in case of addition of pectinase enzyme.

The results showed that the anthocyanins in pomegranate juice were sensitive to the addition of clarifying agents and use clarification methods. Significant decrease in anthocyanin content of pomegranate juice as a function of addition of clarifying agents and use of different clarification methods (Table 2). The initial concentration of the anthocyanin in the juice was 85.86 mg/100g of juice. Addition of gelatin and

Table 1: Effect of clarification methods on the physical parameters of pomegranate juice

Treatments	Juice recovery		pH	Specific gravity	Wt. of sediment obtained after clarification of juice (g)
	on w/w basis (%)	on v/v basis (%)			
Initial value	-	-	3.67	1.10	-
C_1	84.480	87.692	3.550	1.059	160.175
C_2	87.044	85.962	3.658	1.047	129.531
C_3	94.259	93.423	3.818	1.040	40.743
C_4	83.327	83.077	3.748	1.042	163.022
C_5	90.791	90.385	3.703	1.045	99.710
G.M.	87.980	88.108	3.695	1.047	118.636
S.E.±	1.184	1.135	0.045	0.004	17.850
C.D. 5%	2.521	2.417	0.096	0.009	38.021
C.D. 1%	3.492051	3.348	0.133	0.012	52.65815
C.V.(%)	2.690929	2.576	2.436	0.769	30.09236

Table 2 : Effect of clarification methods on the chemical parameters of pomegranate juice

Treatments	TSS (^{0}Brix)	Acidity (%)	Reducing sugars (%)	Non-reducing sugars (%)	Total sugars (%)	Total polyphenols (mg/100g of fruit)	Total anthocyanins (mg/100g of fruit)	Antioxidant activity (%)
Initial value	14.80	0.46	14.70	1.41	16.11	276.75	85.86	45.62
C_1	14.950	0.443	12.498	1.290	13.788	225.500	82.170	42.388
C_2	14.950	0.418	13.035	1.258	14.293	249.375	80.645	45.983
C_3	13.650	0.345	12.823	1.140	13.963	176.000	75.370	40.008
C_4	14.400	0.373	14.018	1.045	15.063	202.250	76.495	41.025
C_5	14.250	0.383	13.693	1.135	14.828	208.500	77.588	41.625
G.M.	14.440	0.392	13.213	1.174	14.387	212.325	78.454	42.206
S.E.±	0.093	0.004	0.252	0.015	0.252	2.755	1.400	1.012
C.D. 5%	0.198	0.008	0.538	0.031	0.536	5.868	2.982	2.155
C.D. 1%	0.275	0.011	0.745	0.044	0.743	8.128	4.131	2.984
C.V. (%)	1.289	1.863	3.821	2.516	3.499	2.595	3.569	4.794

pectinase enzyme decreased the concentration of anthocyanin (Fig. 1). This may be due to the effects of the gelatin and pectinase enzyme on pH of the juice which in turn affects anthocyanin (Bayinderli *et al.*, 1994). The maximum anthocyanin concentration was obtained natural sedimentation (82.170 mg per 100 g of juice).

Total polyphenols content of pomegranate decreased in all the treatments irrespective of the clarification methods and agents used (Fig. 2). But the rate of decrease varied with treatment and agent used. Use of gelatin was better for clarification because it helped in decrease in polyphenol content, reducing the astringency and bitter taste of juice and gave clear, sparkling and sweet juice. The similar results were reported by Neifar *et al.* (2009) and Vardin and Fenercioglu (2009). The rate of decrease in the polyphenol content was slow in the homogenization method (249.395 mg per 100 g juice).

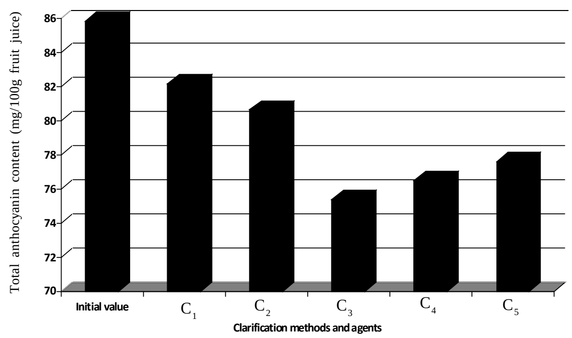

Fig. 1: Changes in total anthocyanin content (mg/100g fruit juice) of pomegranate juice in relation to different clarification methods and agents

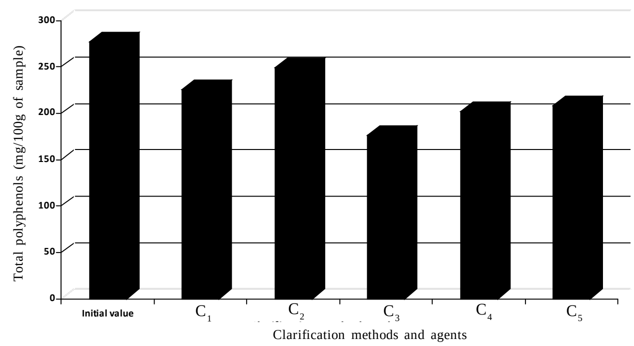

Fig. 2: Effect of clarification methods and agents on total polyphenol content of pomegranate juice

Table 3: Effect of clarification methods on the colour parameters L*, *a, *b**, hue-angle (h°) and chroma (C) of pomegranate juice**

Treatments	*L* *	*a**	*b**	*C* value	H°
C_1	23.320	12.342	1.207	12.404	5.511
C_2	23.513	14.471	1.720	14.588	6.495
C_3	25.741	20.225	3.786	20.586	10.775
C_4	26.125	16.953	2.719	17.181	9.171
C_5	25.589	20.102	4.184	20.536	11.792
G.M.	24.857	16.819	2.723	17.059	8.749
S.E.±	0.744	1.034	0.303	1.034	1.094
C.D. 5%	1.584	2.203	0.646	2.203	2.329
C.D. 1%	2.194	3.0516	0.895	3.051	3.226
C.V. (%)	5.985	12.301	22.274	12.127	25.000

Table 4: Effect of clarification methods on sensory evaluation and microbial population of pomegranate juice

Treatments	Colour	Appearance	Taste	Flavour	Overall acceptability	Total bacterial population (cfu/ml of juice)	Total yeast and mould population (cfu/ml of juice)	Total aerobic count (cfu/ml of juice)
Initial value	9.00	9.00	9.00	9.00	9.00	4.80×10^3	3.20×10^3	8.00×10^3
C_1	6.571	6.571	7.000	7.143	6.821	9.90×10^3	6.56×10^3	1.65×10^4
C_2	6.143	5.143	8.429	7.286	6.750	8.25×10^3	4.83×10^3	1.31×10^4
C_3	8.714	8.429	8.714	8.429	8.571	2.55×10^3	1.13×10^3	3.68×10^3
C_4	8.286	7.429	8.286	8.286	8.071	3.95×10^3	1.80×10^3	5.75×10^3
C_5	7.857	7.000	8.000	7.714	7.643	5.45×10^3	3.35×10^3	8.80×10^3
G.M.	7.600	7.400	8.600	8.200	7.950	6.02×10^3	3.53×10^3	9.55×10^3
S.E.±	0.326	0.655	0.313	0.315	0.264	0.249	0.134	0.236
C.D. 5%	0.665	1.335	0.638	0.643	0.538	0.530	0.286	0.502
C.D. 1%	0.896	1.800	0.861	0.867	0.725723	0.735	0.396	0.695
C.V. (%)	11.341	23.406	9.629	10.168	8.782541	8.272	7.603	4.934

Table 5 : Microbial limit test of pomegranate juice obtained after use of clarification methods and agents

Treatments	Indicator organism			
	Staphylococcus aureus	*Pseudomonas*	*E. coli*	*Salmonella* sp.
C_1	Absent	Absent	Absent	Absent
C_2	Absent	Absent	Absent	Absent
C_3	Absent	Absent	Absent	Absent
C_4	Absent	Absent	Absent	Absent
C_5	Absent	Absent	Absent	Absent

The use of different clarification methods and agents also recorded decrease in the antioxidant capacity of juice. Maximum antioxidant capacity (45.983 %) was recorded in the homogenized juice followed by natural sedimentation method of juice clarification (42.388%).

Colour :

Colour is one of the most important parameters when making a quality and sensorial evaluation of pigmentate fruits. The bright red colour of pomegranate juice is due to the anthocyanins content and their stability through processing and storage. The highest Hunter L value (26.125) was obtained when pectinase was added to pomegranate juice followed by addition of 1g/l gelatin (25.741). The highest *a** value (20.225) was recorded in the pomegranate juice treated with gelatin while highest H value (11.792) was obtained in centrifuged pomegranate juice (Table 3).

Sensorial analysis :

The data regarding the organoleptic score are given in Table 4. It is clear that addition of gelatin (C_3) to the pomegranate juice gave highest organoleptic score (8.571) for colour, appearance, taste and flavour as compared to the other treatments. Similar results were reported by Vardin and Fenercioglu (2009).

Microbial limit tests analysis :

The data pertaining to the microbial population is presented in the Table 4. Unpasteurized pomegranate juice after the use of clarification agents and methods was used for microbial limit tests analysis. Total microbial load in the pomegranate juice was found to be decreased over the initial count in treatment of gelatin (3.68 x 10^3 cfu per ml of juice) and pectinase enzyme (5.75 x 10^3 cfu per ml of juice). The indicator organisms were absent in all the juice samples obtained by using the different clarified methods and agents indicated that pomegranate juice was safe for the consumption (Table 5).

Conclusion :

Pomegranate juice contains high concentrations of phenolic substances and colloidal particles. The best method of clarification should be chosen to reduce astringent and acidic taste of juice and clear, bright coloured sparkling juice should be obtained. Clarity, turbidity, colour and anthocyanin content of juice also affects the consumer preference. Considering these points, the most effective method of clarification was the addition of 1g/l gelatin to juice before heat treatment. Natural sedimentation and homogenization methods were least effective with respect to astringency, clarity, turbidity, flavour, taste and appearance. Overall acceptance and organoleptic evaluation of pomegranate juice was excellent in gelatin clarified method. Advantages of the proposed gelatin clarification method is in reduction of phenolic substances and colloidal particles, decrease in turbidity and production of clear, bright red coloured and sparkling juice in pomegranate fruit juice industry.

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