Efficacy of various techniques on biochemical characteristics and bitterness of pummelo juice

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Revised: 30 September 2014 / Accepted: 27 October 2014 / Published online: 5 November 2014
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Abstract The consumer acceptability of pummelo juice is affected badly due to the presence of bitter principles in it. Therefore in order to avoid such bitterness development, the extracted juice from pummelo was subjected to five different treatments like juice diffused into syrup (70°Brix), lye peeling of segments in boiling NaOH for 2–3 min, increasing the pH of juice, hot water treatment (50 °C) prior to peeling for 20 min and without any treatment (control) for suppressing the development of bitterness in the juice. Based on biochemical analysis, diffusion of juice into syrup (70°Brix) showed better result as compared to other treatments. The maximum amount of TSS was found in juice diffused into syrup (i. e. 45, 30 and 15°Brix) along with highest TSS/acid ratio (92.21, 49.87 and 17.53). Higher amount of acidity was observed in pH adjusted samples with 4.25, 4.50 and 4.75 respectively. However, control samples showed higher amount of ascorbic acid (73.97 mg/100 ml juice) content followed by pH adjusted samples. The highest organoleptic score for taste (8.00), colour (8.83), aroma (8.66), overall acceptability (7.88) and extent of debittering (7.50) were recorded in juice diffused into syrup 70°Brix and achieved final TSS of juice at 45, 30 and 15°Brix respectively. Moreover, the above treatment (juice diffused into syrup 70°Brix) showed promising low cost and easy to adopt technique of debittering in respect of extent of debittering and maintaining sensory quality during storage of pummel juice.

Keywords Pummelo (*Citrus grandis* L. Osbeck) · Debittering techniques · Physico-chemical analysis · Lye peeling · Sugar syrup · Organoleptic quality · Bitterness

Introduction

Excessive bitter taste in citrus juice is a major problem in citrus industry worldwide because it reduces the quality and commercial value of the product (Mongkolkul et al. 2006). The processing of citrus juice faced formidable problems in terms of “bitterness” and “delayed bitterness”, thereby affecting its consumer acceptability. Bitterness due to flavonoids and limonoids poses a major problem for the citrus industry. Without proper de-bittering technology, the profitable citrus industry cannot flourish (Singh et al. 2003).

The bitterness in citrus fruit is affected by limonin and naringin, which are generally recognized as the major two bitter compounds. Limonin and naringin co-exist in most citrus cultivars, but in different amounts, and their threshold levels are different. Premi et al. (1995) reported that limonin and naringin are distributed in various parts of the kinnow fruit and each part of the fruit contains different amount of limonin i.e. seeds contained the highest limonin (9.50 mg/g), followed by peel (4.69 mg/g) and juice (0.128 mg/ml). As many as 37 limonoids have been identified in citrus and their hybrids. Among them four limonoids namely, limonin, nomilin, ichangin and nomilic acid are bitter (Maier et al. 1977). The cultivar type had an effect on the amount of limonin and naringin (Pichaiyongvongdee and Haruenkit 2001).

Limonin is synthesized as a non bitter form of limonoate A-ring lactone (LARL) in leaves and transported to fruit and seeds. Hasegawa et al. (1991) reported that the acidity of citrus plays a great role in terms of bitterness, because under low pH conditions, the A-ring lactone (LARL) can be converted to limonin. When the membrane is ruptured during juice

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extraction, LARL comes in contact with the acidic juice medium and is converted to limonin by limonin D-ring lactone hydrolase (Mongkolkul et al. 2006).

Naringin is by far the most dominant flavonoid bitter principle in grapefruit (Hagen et al. 1966) and neohesperidin is slightly more predominant in sour orange (Castillo et al. 1992). Neohesperidin and poncirin are also bitter and occur in relatively low amounts in citrus juices. Naringin is not transported after being synthesized in the fruit or leaves. Naringin is abundant in immature fruit but its concentration decreases as fruit ripens.

The major flavanones of pummelo are neohesperidin and naringin, which are higher in the seed than in unripe fruits (Chung et al. 2005), and its extract exhibited antioxidant activity through free radical-scavenging in vitro and reduced reactive oxygen species (ROS) in H₂O₂ treated HepG₂ cells (Lim et al. 2006). Hesperidin, naringin, caffeic, p-coumaric, ferulic and vanillic acid are present in the fruit juice (Jang et al. 2010). The polyphenols and vitamin C, present in fruit, are well known for their beneficial biological activity, mainly in the decreasing the risk of cancer and cardiovascular diseases (Fiorentino et al. 2009).

Several methods have been tried to reduce bitterness in citrus juice other than pummelo like raising pH of the juice (Ranote and Bains 1982; Chaisawadi et al. 1998), suppression of bitterness by addition of sweetening agents (Guadagni et al. 1974), lye peeling of segments (Sandhu et al. 1990; Sandhu and Singh 2001), addition of β -cyclodextrin monomer for forming inclusion complexes of limonin (Konno et al. 1981), use of adsorbent XAD-16 (Wilson et al. 1989) and conversion of bitter principles to non-bitter components in the juice by the action of immobilized bacteria (Hasegawa et al. 1983). However, till date, there is no information on suitability of debittering techniques in pummelo juice. That's why, in the present experiment various low cost and easy to adopt techniques has been used to remove bitter principles from pummelo juice and salient findings are presented in this communication.

Materials and methods

Plant materials The experiment was performed over various local cultivars of pummelo during the years 2010–2012 at Laboratory of Post Harvest Technology of Horticultural Crops, Directorate of Research Building, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, India. The well developed and mature fruits were collected from the different places of Nadia District like, Kalyani, Madanpur, Mohanpur, Kathhaltala, Chasrathi, Allaiapur, Birpara, Iswaripur and Natunpalli etc. and immediately brought to the laboratory for further investigation. Maximum efforts were made to

select the fruits that were uniform in size, good in quality and free from injury or disease.

Treatments and methodology Fruits were divided into five groups; each group had four replications containing one fruit in each replication. These five groups were treated as; juice diffused into syrup (70°Brix) (T₁), lye peeling of segments in boiling NaOH for 2–3 min (T₂), increasing the pH of juice (T₃), hot water treatment (50 °C) prior to peeling for 20 min (T₄) and control (without any treatment) (T₅). The juice was extracted by using electric juicer and treated as per the following details:

T₁: Juice diffused into syrup (70°Brix) The juice from a lot of fruit was extracted by using electric juicer to diffuse in to syrup (70°Brix) in a stainless steel vessel. The samples of juice were mixture mixed with sugar syrup (70°Brix) were drawn to achieve final TSS of (a) 45°Brix (b) 30°Brix and (c) 15°Brix.

T₂: Lye peeling of segments in boiling NaOH for 2–3 min Another lot of fruit was peeled manually and divided into three sublots. The segments from each subplot were treated for 40 s at 82–83 °C with NaOH solution of different strengths (a) 1.25 % (b) 1.50 % and (c) 1.75 %. This was followed by rinsing the segments in 1 % citric acid, washing under running tap water and juice extraction was done by electric juicer after neutralization.

T₃: Increasing the pH of juice The extracted juice divided into three lots. The pH of these juices was raised from their initial pH 3.90 to (a) 4.25, (b) 4.50 and (c) 4.75 by using sodium bicarbonate.

T₄: Hot water treatment (50 °C) prior to peeling for 20 min The fruits of pummelo dip into 50 °C hot water prior to peeling for 20 min. After the hot water treatment, the fruits were subjected to hand peeling and juice extraction.

T₅: The untreated juice served as a control.

Laboratory analysis The physico-chemical parameters including Total soluble solids (TSS) of the fruit juice was determined by Hand Juice Brix Refractometer (Erma, Japan), values corrected to 20 °C and expressed as °Brix. Acidity (as citric acid) was determined by using standard N/10 NaOH solution in the presence of phenolphthalein as an indicator, AOAC (1984). The vitamin 'C' (ascorbic acid) content of the juice was estimated by visual titration method with 2, 6-dichlorophenol-indononol dye solution AOAC (1984). Total and reducing sugar content of fruit was estimated following the method of Lane and Eynon as described by Ranganna (2000) using Fehling's solution A and B and methylene blue as an indicator. pH of the fruits products were analyzed by using hand pH meter (eco Testr pH

2, Eutech Instruments, Oakton) ranging from 0.0 to 14.0 pH. TSS: Acid ratio was calculated by taking the value of TSS to that of the acidity.

Organoleptically the debittered samples were evaluated on the basis of colour, taste, aroma and overall acceptability score as per the standard methods (Ranganna 2000) and extent of debittering were evaluated based on the method of Amerine et al. (1980) on a 9-point hedonic scale by a taste panel consisting of ten judges. The score card that represented grading of samples by the judges for different characteristics was as follows: 9=extremely desirable (ED), 8=very much desirable (VMD), 7=moderately desirable (MD), 6=slightly desirable (SD), 5=neither desirable (ND) nor undesirable (UD), 4=slightly undesirable (SUD), 3=moderately undesirable (MUD), 2=very much undesirable (VMUD), 1=extremely undesirable (EUD)

Statistical analysis Determinations were made for each attribute and data pertaining to the physico-chemical and sensory quality was statistically analyzed by using completely randomized design (CRD). The data were analyzed by analysis of variance (ANOVA) and means were compared by Duncan's Multiple Range Test (DMRT) by using SAS Version 9.1 for windows, 2002–2003, SAS Institute Inc., Cary, NC, USA. Differences between the means at the 5 % level were considered significant.

Results and discussion

The effect of different debittering treatments on pummelo juice was shown in Table 1. The methods of debittering of

juice had significantly influenced the bio-chemical characteristics of pummelo juice. Reduction in TSS, acidity, and sugar was observed with lye peeled samples, pH adjusted juice and hot water treated samples. The highest value for TSS was recorded in diffusion of juice syrup to 45°Brix followed by diffusion of juice syrup to 30°Brix. However, the TSS content of control (T_5 ; 11.10°Brix) fruits was higher than T_2 , T_3 , and T_4 treatments.

The titratable acidity of T_5 (1.32 %) was significantly higher than other treatments whereas diffusion of juice syrup to 45°Brix had less acidity content (0.50 %). Brix:acid ratio is a best indicator for measuring relative sweeteners or tartness of the product. The higher the brix in relation to acid contents then it means higher will be the ratio of sugars and sweeter the taste. Increase in TSS:acid ratio was observed in each treatment, however, The highest TSS/acid ratio (92.21 %) was found in juice treated with diffused in to syrup 45°Brix followed by juice diffused in to syrup 30°Brix (49.87 %), while lowest ratio was found in T_5 (8.41 %). The total sugar content of diffusion of juice syrup to 45°Brix (36.98 %) was significantly higher than rest of the treatments, however, the total sugar content of control sample (6.06 %) was significantly higher than lye peeled and pH adjusted juice. The highest reducing sugar content was recorded in diffusion of juice syrup to 45°Brix (18.47 %) followed by 30°Brix. However, the reducing sugar content of T_5 (4.61 %) (control) was significantly higher than T_2 , T_3 and T_4 treatments. Loss of ascorbic acid content was observed in each treatment. Minimum loss was observed with pH adjusted juice. The significantly higher ascorbic acid content was recorded in T_5 (73.97 mg/100 ml) as compared to rest of the treatments. While, T_4 (hot water treatment) had least amount of ascorbic acid content (32.54 mg/100 ml).

Table 1 Effect of various debittering techniques on bio-chemical characteristics of pummelo juice

Treatments	TSS (°Brix)	Acidity (%)	TSS/Acid ratio	Total sugar (%)	Reducing sugar (%)	Ascorbic acid (mg/100 ml juice)	pH
T_1 45°Brix	45.00 ^a	0.50 ^g	92.21 ^a	36.98 ^a	18.47 ^a	42.35 ^{cd}	4.03 ^{de}
30°Brix	30.00 ^b	0.62 ^{fg}	49.87 ^b	24.63 ^b	15.16 ^b	52.75 ^{bc}	4.01 ^e
15°Brix	15.00 ^c	0.86 ^{cd}	17.53 ^c	13.87 ^c	12.18 ^c	59.95 ^{abc}	4.21 ^c
T_2 1.25 %	8.93 ^f	0.93 ^{bc}	9.66 ^{cd}	5.21 ^d	2.32 ^e	61.57 ^{ab}	4.10 ^{cde}
1.50 %	8.60 ^g	1.00 ^b	8.69 ^d	5.49 ^d	2.26 ^e	53.74 ^{bc}	4.05 ^{de}
1.75 %	9.33 ^e	0.70 ^{ef}	13.39 ^{cd}	6.09 ^d	2.28 ^e	62.69 ^{ab}	4.05 ^{de}
T_3 4.25	9.26 ^e	0.97 ^{bc}	9.50 ^{cd}	5.68 ^d	2.84 ^{de}	62.30 ^{ab}	4.21 ^c
4.50	8.70 ^{fg}	0.76 ^{de}	11.81 ^{cd}	5.78 ^d	2.69 ^{de}	69.25 ^{ab}	4.50 ^b
4.75	8.60 ^g	0.64 ^{ef}	13.35 ^{cd}	5.44 ^d	2.97 ^{de}	56.71 ^{abc}	4.77 ^a
T_4 –	9.40 ^e	0.94 ^{bc}	10.12 ^{cd}	6.84 ^d	2.99 ^{de}	32.54 ^d	4.05 ^{de}
T_5 –	11.10 ^d	1.32 ^a	8.41 ^d	6.06 ^d	4.61 ^d	73.97 ^a	4.15 ^{cd}

T_1 =Juice diffused into syrup (70°Brix), T_2 =Lye peeling of segments in boiling NaOH for 2–3 min, T_3 =Increasing the pH of juice, T_4 =Hot water treatment (50 °C) prior to peeling for 20 min, T_5 =Control (without any treatment)

Values followed by different superscript letters are significantly ($P<0.05$) different from each other

Highest pH content (4.77) was recorded in pH adjusted to 4.75 and was significantly higher than pH adjusted to 4.50 (4.50) and pH adjusted to 4.25 (4.21). It was found that the pH value of pH adjusted to 4.25, reduced from 4.25 to 4.21 while, pH value of pH adjusted to 4.75 increased from 4.75 to 4.77. The pH content of juice without any treatment (control) was recorded higher than T₁, T₂ and T₄.

Based on organoleptic evaluation, the result showed that there was an appreciable reduction in bitterness of the treated juice, as compared to control (Table 2). The treatment of pummelo fruit with juice diffused into syrup 45, 30 and 15°Brix showed significantly higher score for colour (7.00, 8.00, 8.83), taste (8.00, 7.00, 6.00), aroma (8.50, 8.66, 7.00), overall acceptability (7.83, 7.88, 7.27) and extent of debittering (7.50, 6.83, 6.16) respectively. The maximum reduction in bitterness was achieved in juice treated with diffused in to syrup 45°Brix (7.50), followed by treatment with diffused in to syrup 30°Brix (6.83), while minimum reduction of bitterness was observed in control sample (1.16). A further perusal of the data on sensory scores and contents of bitter principles indicated that the treatment of juice with juice diffused in to syrup reduced bitterness as tested.

The lowering of bitter principles in juices diffused into syrup is apparently due to the dilution effect (Premi et al. 1995). The reduction of bitter compounds in kinnow juice from lye-peeled segments might be due to removal of white pappy segment walls, which contain high amounts of bitter compounds, and are incorporated to juice during juice

extraction (Sandhu et al. 1990; Sandhu and Singh 2001). Anand et al. (2012) found that the 'lye' and 'naringinase' treatments proved beneficial for the removal of bitterness of kinnow juice. They also found that at the end of storage, the 'control' juice was rated moderately bitter whereas 'lye' treated was rated between 'neither bitter nor sweet' and 'not bitter' category. The same result was observed in kinnow mandarin fruit by Bhatia et al. (1958). They observed that the lye treated fruit slightly reduce the recovery of juice. Lye treatment considerably alters the composition of juice. From the table it was revealed that the all components of lye treated fruits such as total soluble solids, total sugar, reducing sugar and titratable acidity were found to decrease to varying extent as compared to control. This reduction may be attributed to the leaching loss of these components during the treatment of segments.

The citrus fruit juice with raised pH has considerably lower bitterness. The same result was observed by Ranote and Bains (1982) and Ghosh and Gangopadhyay (2003) in case of kinnow juice and Chaisawadi et al. (1998) in case of Thai-tangerine juice. The observation that The juice treated with raised pH, had significantly lower bitterness content than control may be due to unfavourable conditions for conversion of limonoate-A-ring lactone to limonin (Maier et al. 1969). The higher score for colour may be due to the effect of addition of higher amount of sugar in to the juice. The loss of aroma in the treated fruits may be due to the effect of loss of oil during the processing. This finding is corroborating with the finding of Premi et al. (1995).

Table 2 Sensory analysis (colour, aroma, taste and overall acceptability) and extent of debittering of pummelo juice obtained by different debittering treatments

Treatments	Colour	Aroma	Taste	Overall acceptability	Extent of debittering
T ₁ 45°Brix	7.00 ^c	8.50 ^a	8.00 ^a	7.83 ^a	7.50 ^a
30°Brix	8.00 ^b	8.66 ^a	7.00 ^b	7.88 ^a	6.83 ^b
15°Brix	8.83 ^a	7.00 ^b	6.00 ^c	7.27 ^b	6.16 ^c
T ₂ 1.25 %	7.66 ^b	4.83 ^c	4.00 ^d	5.50 ^c	4.83 ^d
1.50 %	7.83 ^b	4.66 ^c	3.66 ^c	5.38 ^c	4.50 ^d
1.75 %	7.83 ^b	5.00 ^c	3.83 ^{de}	5.55 ^c	4.66 ^d
T ₃ 4.25	7.00 ^c	3.00 ^e	2.83 ^f	4.27 ^d	3.83 ^e
4.50	7.00 ^c	3.00 ^e	2.83 ^f	4.27 ^d	3.00 ^f
4.75	7.00 ^c	3.00 ^e	3.00 ^f	4.33 ^d	2.83 ^f
T ₄ –	7.00 ^c	4.00 ^d	1.00 ^g	4.00 ^e	1.50 ^g
T ₅ –	8.00 ^b	1.00 ^f	1.00 ^g	3.33 ^f	1.16 ^g

T₁=Juice diffused into syrup (70°Brix), T₂=Lye peeling of segments in boiling NaOH for 2–3 min, T₃=Increasing the pH of juice, T₄=Hot water treatment (50 °C) prior to peeling for 20 min, T₅=Control (without any treatment)

Values followed by different superscript letters are significantly ($P<0.05$) different from each other

Conclusions

The study revealed that the treatment of diffusion of juice syrup to 45, 30 and 15°Brix had more effective in bitterness removal than other treatments based on organoleptic score. The treatment of diffusion of juice syrup to 45, 30 and 15°Brix (T₁) had highest organoleptic score (7.83, 7.88 and 7.27, respectively) and extent of debittering (7.50, 6.83 and 6.16, respectively) and also rich in other quality attributes like TSS, total sugar, reducing sugar and TSS/acid ratio. The control treatment was too bitter, but rich in TSS, acidity and colour than T₂, T₃ and T₄ treatments along with highest amount of ascorbic acid content (73.97 mg/100 ml juice). Lye treatment and raised pH also improved the organoleptic qualities. From the present study, it was concluded that the treatment of pummelo juice with diffusion of juice syrup (70°Brix) to 45, 30 and 15°Brix could be regarded as a promising treatment for removal of bitterness and making pummelo juice acceptable on the basis of organoleptic score.

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