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Comparison of juice extraction methods, determination of bittering principles and standardization of debittering of lime juice

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In spite of good yields, high vitamin C content, nutritional and medicinal values, lime (*Citrus aurantifolia* Swingle is not commercially exploited due to the presence of bittering compounds. The present investigations were therefore, aimed at reducing the bitterness of juice for preparation of concentrate. Out of the different methods of juice extraction tried, screw type juice extractor was found to be the most effective with respect to high juice yield (57.75 %) and quality. The lime juice extracted through screw type juice extractor contained 0.50 % recoverable oil, 23.24 ug/mL limonin and 47.21 ug/mL naringin. Among the various treatments tried for debittering of lime juice, use of absorbent XAD-16 resulted in a considerable reduction in bitterness. The XAD-16 resulted in 70.45 % reduction of limonin content and 72.07 % reduction in naringin content, thereby, lowering them to 5.91 and 11.71 ug/mL, respectively. Nutritionally, maximum ascorbic acid retention (25.0 mg/100 mL) was observed in XAD-16 treated juice, while non-significant change occurred in total soluble solids and acidity of treated juice. On the basis of sensory evaluation, the juice treated with XAD-16 was adjudged the best in extent of debitterness, taste, body, and flavour among all the treatments tried. However, a slight decrease in colour score was obtained that could be adjusted easily during processing.

Keywords: Absorbent XAD-16, Debittering, Lime juice, Limonin, Naringin.

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Introduction

Citriculture is the third largest fruit industry in India next to mango and banana. It accounts for 10.8 % of total fruit production and occupies 9.4 % of total area under fruits in country[1]. Among the citrus fruits, Kagzi lime (Citrus *aurantifolia* Swingle) is popular for its attractive colour, distinctive flavour and vitamin C content. In India, lime is grown in tropical and subtropical regions occupying an area of 31,6,050 ha with an annual production of 2.57 lakh MT[2]. The kagzi lime fruit has a great variety of uses like culinary, beverages, pickles, and medicinal. Like any other fruits, kagzi lime is perishable in nature where about around 25-40 % harvested fruits are lost before consumption due to faulty postharvest handling and microbial attack after harvest[3]. Due to the limited shelf-life at room temperature[4], it has to be processed to assure availability of its product and also to minimize the glut in the market[5], but, its wide spread use in citrus industry is limited by the development of bitterness in juice[6].

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Consequently, the juice cannot be converted into concentrate for further use as a juice. Therefore, the present study was undertaken to develop suitable technology for debittering of the kagzi lime juice and to conduct its quality evaluation.

Materials and Methods

Raw materials

Fully mature, well developed, and uniform sized fruits of kagzi lime (*Citrus aurantifolia* Swingle) were purchased from Dhaulakuan region in Sirmour district of Himachal Pradesh situated at an elevation of 500 m above mean sea level and brought to the Department of Postharvest Technology, College of Horticulture, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan.

Extraction of juice

Selected fruits were washed in running tap water to remove dirt, dust particles, and insecticidal residues. Different methods like manual extraction (M_1); milling, grating, and basket pressing (M_2); milling, grating, enzyme treatment, and basket pressing (M_3); and screw type juice extractor (M_4) were used for extraction of juice from lime fruits and the best method was selected on the basis of juice yield, waste generated, time taken for juice extraction, and presence of bittering principles. The juice was then, strained through the muslin cloth and preserved by heating at 90 ºC for 10 seconds[7].

Methods of debittering

Different methods were used for debittering of lime juice wherein treatment T_1 and T_2 juice was extracted in syrup of 82 ºB to bring down the TSS to 65 and 45 ºB, respectively. In the treatment T_3, T_4, T_5, and T_6, lime juice after extraction was treated with 2N $NaHCO_3$ to raise its pH from 2.40 to 2.50, 3.00, 3.50, and 4.00, respectively. Treatments T_7 and T_8 involved the use of adsorbents Amberlite XAD-16 and β cyclodextrin monomer, respectively. All the treatments were compared with the control. Amberlite XAD-16 was supplied by ROHM and HAAS France Sa and used for debittering of lime juice. Stored lime juice was then passed with a definite flow rate and retention time (retention time 0, 5, 10, 15, 20, and 25 min with a flow rate of 10 mL/min) through the glass column (internal diameter 3.0 cm) packed with XAD-16 polymer using glass wool as a polymer support[8]. For the standardization of fractions, the fractions of 25 mL were withdrawn from the column after different retention time 0, 5, 10, 15, 20, and 25 min[9] with a flow rate of 10 mL/min. For the standardization of fractions, the lime juice samples were collected at selected retention time in ten fractions (25 mL each) with a flow rate of 10 mL/min. The best retention time and fraction was standardized on the basis of reduction in bittering compound. The column was regenerated with 0.50 % NaOH followed by washing with distilled water properly to remove left-over alkali and sediment residues[10]. Efficacy of β cyclodextrin monomer was also tested for debittering of lime juice in different concentrations of 0.5, 1.0, 1.5, and 2.0 % and compared with the control. Samples were drawn after a retention time of 5, 10, and 15 min for estimating limonin and naringin. The steps undertaken for the standardization procedure is given in Fig. 1.

Physico-chemical analysis

The physico-chemical analysis of the fresh lime fruits and juice were conducted for different parameters like fruit size, weight, and specific gravity. The total soluble solids, titratable acidity, and pH were measured by methods recommended by AOAC[11]. Sugars were estimated by calorimetric method of Ting[12], while ascorbic acid content was determined as per the standard method ofAOAC[11]. Viscosity was measured as relative flow compared to water in Ostwald viscometer. Pectin was estimated by colorimetric method[13] while, naringin and limonin were determined by using standard analytical procedure of Davis [14] and Vaks and Lifshitz[15], respectively.

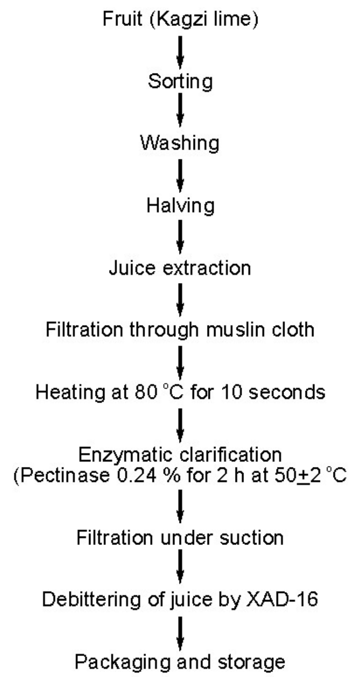


Fig. 1 — Flow sheet for the extraction and debittering of lime juice

Sensory Evaluation

Sensory evaluation was carried out by a semi-trained panel of 7-9 judges for bitterness evaluation[16] on 9 point Hedonic Scale. The judges were given the coded samples in separate booths. The response was recorded in the prescribed format.

Statistical analysis

The data pertaining to physico-chemical and sensory characteristics were statistically analysed by CRD and RBD factorial. Statistical analysis of data pertaining to physico-chemical was compared at 5 % level of significance as per the standard statistical method given by Cochran and Cox[17].

Results and Discussion

Physico-chemical characteristics of Kagzi lime

Table 1 pertaining to the physico-chemical characteristics of kagzi lime shows that the mean fruit size with respect to its length and diameter was

3.87±0.25 and 3.49±0.30 cm, respectively with the mean fruit weight of 35.60±5.48 g. These values are in conformity with those reported by other researchers[18,19]. Among the different parts of kagzi lime fruit (Table 1), limonin content was found to be maximum in seed (117.59±7.20 μg/g), followed by in peel (114.62±6.20 μg/g) and minimum in juice (23.24±2.0 μg/g) due to the presence of maximum phenolic content in seeds, followed by seed and juice. However, naringin content ranged within 46.0±4.28 to 85.0±5.25 μg/g with the highest in peel, followed by juice (47.21±3.50) and seed (Table 1). Similar results in other citrus fruits have been observed in earlier studies by other researchers[20,21].

Table 1 — Physico-chemical characteristics of kagzi lime fruits

Parameters	Mean±SD
Fruit size	
Length (cm)	3.87±0.25
Diameter (cm)	3.49±0.30
Fruit weight	35.60±5.48
Juice yield (% w/w)	57.75±2.68
Limonin content(μg/g)	
Juice	23.24±2.0
Seed	117.59±7.20
Peel	114.62±6.20
Naringin (μg/g)	
Juice	47.21±3.50
Seed	46.0±4.28
Peel	85.0±5.25

Method of juice extraction

Out of the different methods of juice extraction studied, maximum recovery of juice was obtained by using screw type juice extraction (57.75 %) followed by manual extraction (53.56 %), while milling and basket pressing (P_3) yielded the minimum (45.32 %) recovery. The lime juice extracted by using screw type juice extractor had a significantly higher values of limonin and naringin (23.24 and 47.21 μg/mL) followed by juice obtained by milling/grating, enzymatic treatment and basket pressing (Table 2). It is therefore concluded that the juice extracted by screw type juice extractor gave higher juice yield in minimum time period as compared to other methods, and therefore, standardized for further juice extractions. Comparatively, high degree of bitterness was observed in lime juice extracted by grating and pressing[22].

Evaluation of debittering treatments of bittering principles

Among the different treatments tried for debittering of lime juice (Table 3), use of adsorbent XAD-16 (T_7) resulted in a considerable reduction in bitterness and the juice extracted in syrup (T_1 and T_2) was found to have favourable sensory acceptance due to its use in

Table 2 — Standardization of method (M) of juice extraction for kagzi lime fruit

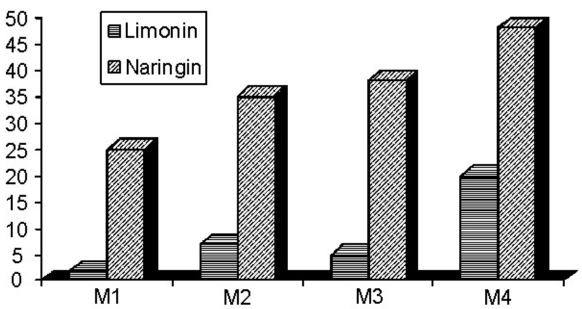
Parameters methods	Manual (P_1)	Milling/grating and basket pressing (P_2)	Milling/grating enzymatic treat and basket pressing (P_3)	Screw Extractor (P_4)	CD $_{p0.05}$
Juice yield (% w/w)	53.56	45.32	46.34	57.75	0.01
Time taken*	16.00	13.33	13.33	42.25	0.005
Bitterness					
Limonin (μg/mL)	1.00	6.00	6.50	23.24	0.43
Naringin (μg/mL)	26.00	33.00	34.50	47.21	0.090

*includes time taken for halving of fruit and juice extraction from one kg fruits

Table 3 — Effect of various debittering treatments on bitering principles of Kagzi lime juice

Treatments	Limonin (μg/mL)	Reduction (%)	Naringin (μg/mL)	Reduction (%)
T1 (Final juice TSS 65 °B)	9.20	60.38	17.30	63.35
T2 (Final juice TSS 45 °B)	14.00	39.76	24.00	49.16
T3 (Final juice pH 2.5)	21.93[b]	5.62	47.21[a]	0.00
T4 (Final juice pH 3.00)	21.87[b]	5.89	47.21[a]	0.00
T5 (Final juice pH 3.50)	21.50[b]	7.48	47.21[a]	0.00
T6 (Final juice pH 4.00)	20.67[ab]	10.64	47.21[a]	0.00
T7 (Use of XAD-16)	5.91	74.56	11.71	75.19
T8 (Use of β-cyclodextrin monomer)	19.93[a]	14.24	41.92	11.20
T9 (Control)	23.24	-------	47.21[a]	------
CD (*p*0.05)	1.269	-------	0.667	------

*Figures with similar superscript are non-significantly different preparation of squash and other finished products. In treatments T_3 and T_4, only 5.62 and 5.89 % of reduction in limonin content was observed respectively, while no reduction in the concentration of narignin content was evident. Similarly, in treatment T_4 (final juice pH 3.0) and T_5 (final juice pH 4.0), 7.48 and 10.64 % of reduction in limonin content was observed respectively; while no reduction in the concentration of naringin content was evident. Out of the two adsorbents, XAD-16 (T_7) and β cyclo-dextrin monomer (T_8), the former gave the highest reduction in limonin and naringin contents of the treated juices (Fig. 2). Adsorbent β Cyclodextrin monomer and raising pH of the juice was not much useful for debittering of lime juice although all the treatments reduced the limonin content as compared to the control. The minimum limonin content of 5.91 µg/mL (74.56 % reduction) was recorded in the treatment T_7 (XAD-16) followed by 9.20 µg/mL (60.38 % reduction) in the treatment with extraction of juice of syrup to final TSS of 65 °B (T_1). The lowest naringin content of 11.71 µg/mL (75.19 % reduction) was obtained by the treatment of the juice with adsorbent XAD-16 (T_7), which was markedly superior to all other treatments.



M_1-Manual extraction, M_2-Milling/grating and basket pressing, M_3-Milling/grating enzymatic treatment and basket processing, M_4-Screw type juice extractor

Fig. 2 — Effect of methods of juice extraction on bittering principles of kagzi lime fruit juice

Standardization of retention time in XAD-16 debittering

Limonin reduction

The limonin content of kagzi lime juice observed after treatment with XAD-16 ranged between 2.04 and 8.41 µg/mL, where maximum content was observed in 5 min retention time and minimum in 25 min, thereby suggesting that the maximum reduction took place in 25 min retention time showing a reduction of 83.70 to 89.80 % (Table 4).

Naringin reduction

Table 5 shows that naringin content of kagzi lime juice observed after treatment with XAD-16 ranged from 1.50 to 11.00 µg/mL for different retention times. Similar to limonin content, naringin content decreased with increasing retention time. Maximum naringin reduction of 96.42 % took place in 25 min retention time. With the increasing retention time, reduction in the bittering principles also increased but, increased salt concentration in the juice was also observed thereby changing the taste of the juice. Thus, in order to remove the salt accumulation in the juice, the bed height of the resin was reduced from 6.0 to 3.0 cm and no retention time was given to the juice, which reduced the limonin and narignin content to a tune of 74.56 and 75.19 %, respectively and also making it free from salty taste. Apart from this, debittering was also more easy and efficient. However, Premi *et al*[8] optimized a retention time of 15 min for debittering of kinnow juice by using Amberlite XAD-16 resin.

Table 4 — Standardization of retention time in XAD-16 column debittering of kagzi lime juice (limonin µg/mL)

Fractions**	Time (min)				
	5	10	15	20	25
1	8.41 (57.95)	7.54 (62.30)	5.56 (72.20)	4.49 (77.55)	3.26 (83.70)
2	8.41 (57.95)	7.54 (62.30)	5.54 (72.30)	4.49 (77.55)	3.10 (84.50)
3	8.41 (57.95)	7.52 (62.40)	5.54 (72.30)	4.41 (77.95)	3.02 (84.90)
4	7.67 (61.65)	7.42 (62.90)	5.42 (72.90)	4.41 (77.95)	2.85 (85.75)
5	7.60 (62.00)	7.42 (62.90)	5.39 (73.05)	4.41 (77.95)	2.77 (86.15)
6	7.60 (62.00)	6.70 (66.50)	4.98 (75.10)	4.24 (78.80)	2.45 (87.75)
7	7.58 (62.10)	6.53 (67.35)	4.96 (75.20)	4.00 (80.00)	2.28 (88.60)
8	7.58 (62.10)	6.53 (67.35)	4.96 (75.20)	3.75 (81.25)	2.20 (89.00)
9	7.57 (62.15)	6.53 (67.35)	4.88 (75.60)	3.59 (82.05)	2.12 (89.40)
10	7.57 (62.15)	6.53 (67.53)	4.88 (75.60)	3.51 (82.45)	2.04 (89.80)
Control	20.00				

Figures in parentheses indicates per cent reduction in limonin; *Quantity of XAD-16 used= 6 g; **Volume of fraction collected= 25 mL; Juice was continuously passed and fraction collected

Table 5 — Standardization of retention time in XAD-16* column debittering of Kagzi lime juice (naringin μg/mL)

Fractions**	Time (min)				
	5	10	15	20	25
1	11.00 (73.76)	10.10 (75.91)	8.06 (80.78)	5.80 (86.17)	2.04 (95.13)
2	11.00 (73.76)	10.10 (75.91)	8.06 (80.78)	4.62 (88.98)	2.04 (95.13)
3	11.00 (73.76)	9.99 (76.71)	7.95 (81.04)	4.08 (90.27)	1.82 (95.66)
4	10.64 (74.62)	9.89 (76.41)	7.84 (81.30)	4.08 (90.27)	1.82 (95.66)
5	10.64 (74.62)	9.89 (76.41)	7.52 (82.06)	4.08 (90.27)	1.82 (95.66)
6	10.53 (74.89)	9.67 (76.94)	7.31 (82.57)	3.97 (90.53)	1.82 (95.66)
7	10.42 (75.15)	9.67 (76.94)	7.20 (82.83)	3.97 (90.53)	1.82 (95.66)
8	10.42 (75.15)	9.56 (77.20)	6.98 (83.35)	3.87 (90.77)	1.82 (95.66)
9	10.32 (75.39)	9.46 (77.44)	6.98 (83.35)	3.87 (90.77)	1.61 (95.16)
10	10.32 (75.39)	9.46 (77.44)	6.88 (83.59)	3.87 (90.77)	1.50 (96.42)
Control	41.93				

Figures in parentheses indicates per cent reduction in limonin; *Quantity of XAD-16 used= 6 g; **Volume of fraction collected = 25 mL; Juice was continuously passed and fraction collected

Table 6 — Standardization of concentration of β-cyclodextrin monomer and time in batch debittering of Kagzi time juice

Concentration (%)	Time (min)	Limonin (μg/mL)	Naringin (μg/mL)	Debitterness score (maximum 10.0)	Description
0.5	5	20.00	41.93	3.00	Moderately bitter
0.5	10	20.00	41.93	3.00	Moderately bitter
0.5	15	20.00	41.93	3.00	Moderately bitter
1.0	5	20.00	41.93	3.00	Moderately bitter
1.0	10	20.00	41.93	3.00	Moderately bitter
1.0	15	20.00	41.93	3.00	Moderately bitter
1.5	5	20.00	41.93	3.00	Moderately bitter
1.5	10	20.00	41.93	3.00	Moderately bitter
1.5	15	20.00	41.93	3.00	Moderately bitter
2.0	5	20.00	41.93	3.00	Moderately bitter
2.0	10	19.93	41.93	7.00	Moderately non-bitter
2.0	15	19.93	41.93	7.00	Moderately non-bitter
Control	-	20.00	41.43	2.00	Very much bitter
$CD_{0.05}$	-	NS	NS	0.242	

Batch debittering with β cyclodextrin monomer

It is evident from Table 6 that limonin and naringin contents ranged from 19.93 to 20.00 μg and 41.93 μg/mL, respectively. Bittering principles remained unaltered by the treatment with β-cyclodextrin monomer but sensory evaluation showed reduction in bitter taste in comparison to the untreated juice. Juice treated with 2 % β cyclodextrin monomer for 10 and 15 min scored maximum (7.0) score and ranked moderately non-bitter as compared to minimum score of 2.0 obtained by the control. Reduction in the bitter taste by treatment with β-cyclodextrin monomer may be due to the formation of inclusion compounds with naringin and limonin[23]. Thus, 2 % of β-cyclodextrin monomer can be used to moderately debitter lime juice, which was similar to the observations by Shaw and Wilson[10] while, Premi *et al*[8] optimized 4.0 % β-cyclodextrin for debittering of kinnow juice possibly due to the type of bittering principles that varies with the type of fruit.

Quality evaluation of debittered lime juice

Quality evaluation of debittered lime juice (Table 7) showed non-significant changes in total soluble solids in treatment T_3, T_4, T_5, and T_6 (raising juice pH) and the use of adsorbents (T_7 and T_8) except in the extraction of juice in syrup (T_1 and T_2), which was on expected lines. The titratable acidity differed significantly in all the treatments as compared to the control treatments using adsorbents (T_7 and T_8) where only a slight loss of titratable acidity was noted in the juice. Reducing and total sugars remained unaltered in treatments T_3, T_4, T_5, T_6, T_7, and T_8 (raising juice pH and use of adsorbents) except juice extracted in syrup (T_1 and T_2). Maximum ascorbic acid retention (25.0 mg/100 mL) was observed in XAD-16 treated juice (T_7) whereas, juice treated with adsorbent β-

Table 7 — Effect of various debittering treatments on quality characteristics of kagzi lime juice

Treatments	TSS (°B)	Acidity (% CA)	Reducing sugar (%)	Total sugars (%)	Ascorbic acid (mg/100 g)	Relative viscosity
T_1 (Final juice TSS 65 °B)	65.00	2.63	3.12	61.25	12.75	Did not pass
T_2 (Final juice TSS 45 °B)	45.00	3.05	4.01	41.17	15.80[b]	13.56
T_3 (Final juice pH 2.5)	6.60[a]	3.64	1.00[a]	1.20[a]	20.90	2.38[b]
T_4 (Final juice pH 3.00)	6.60[a]	3.24	1.00[a]	1.20[a]	18.33[c]	2.43[b]
T_5 (Final juice pH 3.50)	6.70[a]	2.84	1.00[a]	1.20[a]	17.60[c]	3.05[c]
T_6 (Final juice pH 4.00)	6.72[a]	2.44	1.00[a]	1.20[a]	15.40[b]	3.34[c]
T_7 (Use of XAD-16)	6.60[a]	4.90[a]	1.00[a]	1.20[a]	25.00[a]	1.11[a]
T_8 (Use of β-cyclodextrin monomer)	6.60[a]	4.90[a]	1.00[a]	1.20[a]	24.93[a]	1.15[a]
T_9 (Control)	6.50[a]	5.30	1.00[a]	1.20[a]	25.66[a]	1.61
$CD_{(0.05)}$	0.346	0.059	0.12	0.28	0.952	0.421

*Figures with similar superscript are non-significantly different

cyclodextrin monomer (T_8) also had appreciably high retention of ascorbic acid (24.93 mg/100 mL). The relative viscosity was consistently increased in juice extracted in syrup (T_1 and T_2) while, non significant differences in relative viscosity were obtained in other treatments tried to debitter the lime juice.

In sensory quality evaluation among all the treatments, the juice treated with XAD-16 (T_7) was adjudged to be the best in extent of debitterness, taste, body and flavour, slight decrease in colour score was however obtained that could be adjusted during processing.

Conclusion

The present study indicates that the Kagzi lime juice can be debittered successfully using adsorbent XAD-16. The juice has the potential for successful utilization for preparation of various products including RTS juice and squash and finally, can be produced into a concentrate.

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