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Studies on Debitteringof Sweet Orange Juice

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Abstract: Sweet orange is the important citrus fruit crop grown throughout the world; it contributes 71 per cent of the total citrus fruit production. Fresh juice of sweet orange is refreshing, thirst quenching and energizing drink that improves health and nutritional requirements. It provides 45 kcal, moderate quantity of vitamin C, potassium, bioflavonoids and folic acid. Cost of processing and development of bitterness in sweet orange juice shortly after extraction is the factor for low consumption of sweet orange juices. This bitterness is caused mainly by a compound limonin in sweet orange juice. The bitterness caused by limonin is referred as delayed bitterness since it is not detected in fresh juice but develops gradually and slowly during storage or with heat treatment. Hence an attempt is taken to develop an economic process to reduce the bitter component limonin from sweet orange juice.

Key words: Sweet orange · Juice · Beverage · Limonin

INTRODUCTION

Sweet orange is one of the important citrus fruit crop grown throughout the world and contributes 71 per cent of the total citrus fruit production [1]. Fresh juice of sweet orange is an important nutritious product providing 45 kcal, moderate quantity of vitamin C, potassium, bioflavonoids and folic acid and essential items of breakfast. It is a refreshing, thirst quenching and energizing drink that improves health and nutritional requirements. Despite of good nutritional value, it is considered as luxury product and hence unpopular in developing countries. Cost of processing may be a factor for low consumption of juices but most pronounced cause is the development of bitterness in sweet orange juice shortly after extraction, even though the fruit or freshly extracted juice is not bitter. This bitterness is caused mainly by a compound limonin in sweet orange juice and by naringin in grapefruit and sour orange juice [2]. The bitterness caused by limonin is referred as delayed bitterness since it is not detected in fresh juice but develops gradually and slowly during juice storage or with heat treatment [3]. Bitterness due to limonin in a variety of citrus juices is a major problem of the worldwide citrus industry and causes significant negative impact on juice processing. Excess bitterness affects the quality of many processed juice products and results in a significant loss of products due to rejection by consumers. The delayed bitterness cause storage problem and forces the processor to sale juices at reduced prices. The US public health services in 1969 classified bitterness in citrus juices as one of the 101 most interesting and complex problems in food science and technology. Even today, control of bitterness in citrus juices continues to remain as active area of research [4, 5].

Many attempts have been made and much research has been carried out to debitter the citrus juices involvingphysical separation using adsorbent resins. Johnson and Chandler [6] and Manlan *et al*. [7] studied different polyamide, polystyrene, ion exchange and adsorbent resins for their relative efficiency for adsorbing limonin and naringin from juices.These resins remove upto 85per cent limonin from grapefruit juice. Couture and Rouseff [8] concluded that polystyrene divinylbenzene XAD-16 and anionic resin IRA-93 can remove 100per cent and 30per centlimonin respectively from sour orange juice.

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Fayoux *et al*. [9] used low molecular weight polyvinyl chloride beads to successfully debitter navel orange juice. Kola *et al*. [10] successfully used Amberlite XAD-16HP and Dowex-L285 to reduce bitterness to acceptable levels in Washington navel orange juices. LalKaushal and Thakur [11] demonstrated use of adsorbent Amberlite XAD-16 packed in glass column to debitter the kinnow orange juice. The reduction in limonin and naringin contents in the first lot (0-10 L) was to an extent of 98.3 and 88.5 per cent, respectively which gradually decreased.Debittering of processed juices seems to be the most promising approach and some citrus industries are already equipped with debittering devices [5, 6].

Most of these methods constitute less economic feasibility or violate the Federal Standards of identity and quality prescribed for orange juices. Further, question arises concerning the nutritional and constitutional changes that take place during debittering. At present limited commercial processes are being used to debitter the orange juices and other processed products. Therefore, an improved economic process is needed to be developed to reduce the bitter components from orange juices, which do not introduce any new substance or remove desirable juice components while maintaining the expected flavour and nutrition of the product. Hence this study is carried out to develop an economic process to reduce limonin content from orange juice.

MATERIALS AND METHODS

Sweet orange fruits at definite maturity level wereselected and distribution of Limonin compound in different parts of the fruit tissue viz. flavedo, albedo and segment walliswas determined as per method described by Maier and Grant [12]. Limonin content in seeds was quantified as per the method of Emerson [13]. Fresh sweet orange juice was extracted, filtered and used for the experiment. Three different adsorbent resins wereselected for debittering of juice as,R1(strongly cationic resin), R2(strongly anionic resin) and R3(weekly cationic resin). The juice was treated into different samples as fresh (F), pasteurized (P) and juice after 6 hrs (A) of extraction. All the samples of juices were passed through columns of these three resins [14]. Adoptedan assay for extraction and determination of limonin from sweet orange juice. The Limonin content in all the samples prior to and postdebittering treatment wasdetermined by HPLC and the chromatogram obtained was referred for calculation of ppm levels of Limonin using the following formula.

$$C_j = \left(\frac{1000 \text{ X } V_a}{V_j \text{ X } V_s} \right) W$$

where,

Cj= concentration of limonin in juice (mg/l)
Va= Volume of the solvent (ml)
Vj= volume of aliquot juice (ml)
Vs= volume of sample injected (µl)
W= weight of limonin in sample injected (µg)

RESULT AND DISCUSSION

Bitter Limonin Compound in Fruit Tissues and Seeds: It was stated earlier that limonin or its precursor is mainly present in peel portion including flavedo, albedo and segment wall and in seeds of orange fruit [11]. The limonin content of flavedo, albedo and segment wall was estimated through HPLC- analysis. Table 1 shows the distribution of limonin in different fruit parts.

The seeds of sweet orange fruit contained about 480 mg per 100 g limonini.e. 0.48 per cent limonin and a single seed weighing on an average 0.8 to 1.2 g contained approximately about 3.8 to 4.0 mg of limonin. Fig. 1 illustrates the HPLC – chromatogram of limonin found in flavedo, albedo, segment wall and juice of fruit. The retention time ofLimonin wasat 9 min. Flavedo contained high amount of limonin about 38.8-mg/100gm as potential limonin. The area of limonin peak obtained from chromatogram data was referred for quantitative estimation of limonin.The peak of limonin in albedo was well separated and sharp as compared to peak of limonin in flavedo. The height of peak was also small. The albedo tissue contained about 13.8 mg/100g of limonin or potential limonin. Albedo wasthe main tissue, which incorporated bitter compound in juice due to maceration during juice extraction. No clear peak of limonin from segment wall was separated. A very negligible amount of limonin was eluted which was inseparable from interfering compounds. It was found from Table 1 that the segment wall contains less amount of limonin about 0.95 mg/100 g of the tissue. It was stated earlier that limonin or

Table 1: Limonin content of fruit tissue

No.	Fruit tissues	Limonin (mg/100gm)[a]
1	Seeds	480.00±0.03
2	Flavedo	38.80 ±0.02
3	Albedo	13.80 ±0.03
4	Segment wall	0.95 ±0.01
5	Juice	0.015 ±0.002

[a]Values are mean ± SD of three or more determinations.

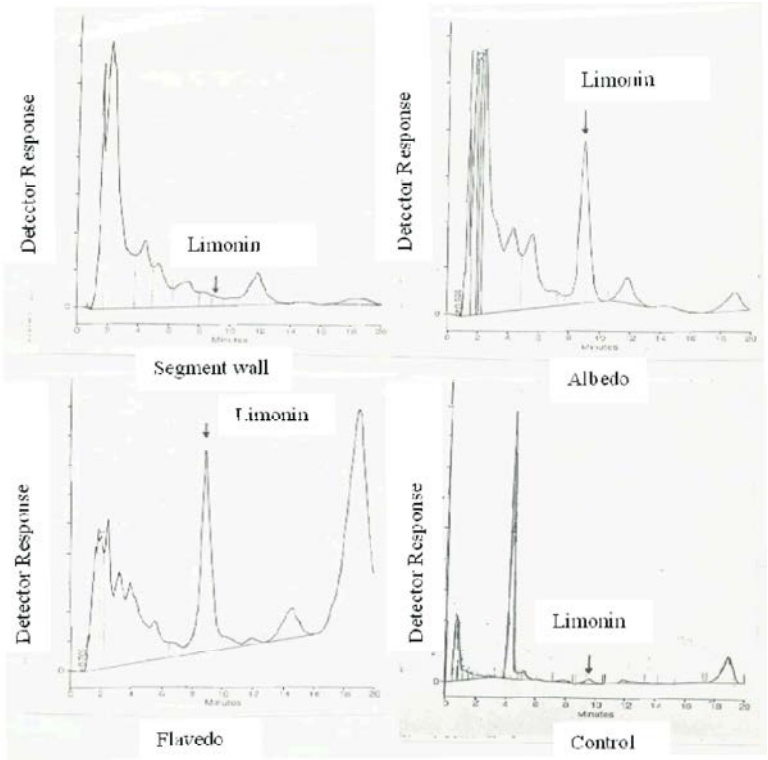

Fig. 1: HPLC-chromatogram of Limonin in Juice held for 6 hrs

Table 2: Effect of debittering resin treatment on limonin content of sweet orange juice

Samples	Limonin (mg/l)[a]
Control	0.15
F_1	ND
F_2	ND
F_3	ND
**P	15.2
P_1	2.3
P_2	0.53
P_3	14.7
**A	10.2
A_1	1.58
A_2	0.50
A_3	7.68

[a]Values are mean ± SD of three or more determinations.

ND = Not detected, F_1 = fresh juice treated with resin-1

F_2 = fresh juice treated with resin-2, F_3 = fresh juice treated with resin-3

**P = Pasteurized untreated juice, P_1 = pasteurized juice treated with resin-1

P_2 = pasteurized juice treated with resin-2,P_3 = pasteurized juice treated with resin-3

**A = Juice after 6 h of extraction, A_1 = juice held for 6 h treated with resin-1

A_2 = juice held for 6 h treated with resin-2, A_3 =juice held for 6 h treated with resin-3

its precursor are mainly present in peel portion and in seeds of orange fruit. However, the information on their total contents and distributions in different parts of the fruit is not available. Maier and Grant [12] reported that mature Navel orange fruit membranes contain 50ppm limonin while lemon peel contains 219ppm limonin. Premi *et al*. [4] reported that the peel of Kinnow Mandarin contains 4.69 mg per g of limonin and seeds containthe highest limonin about 9.50 mg per g.

It was stated earlier that fresh juice did not contain significant amount of limonin but a non-bitter precursor, which slowly is converted to bitter limonin under acidic conditions of juice. It was evident from Fig. 1 that a minute quantity of limonin (potential limonin) was present initially in fresh juice, which was eluted at retention time of 9.6 minutes, slightly more than that of peel and segment wall. It was found from Table-1 that freshly extracted sweet orange juice contain 0.15mg/l limonin. Since this quantity of limonin was far below the threshold value 6ppm [16] the fresh juice appeared non-bitter in taste.

Effect of Debittering Resin Treatment on Limonin Content of Fresh Sweet Orange Juice: It was observed from Table 2 that freshly extracted juice contains 0.15 mg per l of limonin. When the juice was subjected to debittering columns of three different resins and subsequently analyzed, a clear chromatogram was not obtained for these samples. It is suggested thatthe resins adsorb all of the limonin present in fresh juice and hence, it was not detected in HPLC- analysis.

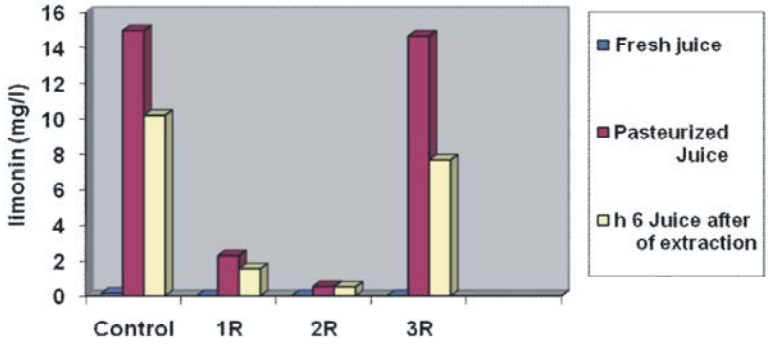

Fig. 2: Effect of debittering resin treatment on limonin content of sweet orange juice.

Effect of Debittering Resin Treatment on Limonin Content of Pasteurized Sweet Orange Juice: One lot of freshly extracted juice was pasteurized at 80°C for 3 minutes and was cooled to room temperature. It was found that pasteurized juice become bitter in taste. Table 2 clearly revealed that limonin content in pasteurized juice issignificantly increased to 15.2mg/l. On treatment with resins the pasteurized juice showed a significant reduction in limonin content. Juice treated with R1 and R2 i.e. P_1 and P_2 was found to contain low amount of limonin about 2.3mg per l and 0.53mg per l respectively. R3 was not that much effective as that of R1 and R2 and adsorbed only slight amount of limonin. Sample P3 contained about 14.7mg per l limonin. The results are in good accordance with Kimball [17] who studied the nutritional and compositional changes (via adulteration screenings) in California navel orange juice during commercial debittering using a hydrophilic absorbent. Both reconstituted and freshly extracted juices were analyzed. There were significant reductions in limonin, citrus oils and pulp.

Limonin Content of Sweet Orange Juiceheld for 6 H after Extraction: The sweet orange juice after extraction was held for 6 h at room temperature with prime objective to observe the extent of delayed bitterness with respect to increase in limonin content. As limonin slowly and gradually develops in juice, it takes at least 6 hours to be detected on taste receptor of an individual [2]. Table 2 revealed that limonin content isreached to 10.2 mg/l in 6 hours from an initial content of 0.15mg/l in freshly extracted juice. When the juice was treated with resin columns a significant reduction in limonin content of sample A_1 and A_2 treated with R1 and R2 respectively was observed. Sample A_1 contained 1.58mg/l and sample A_2 contained 0.50mg/l limonin after treatment. Only a limited quantity of limonin was reduced in treated sample A_3 and it contained about 7.68mg/l limonin. The results of present study are comparable with the results of Chandler and Kefford [14] who reported that the limonin content in pasteurized sweet orange juice is about 22 ppm and about 10 ppm in juice after 6 h of extraction.

Fig. 2 summarized the above findings of present investigation. It couldbe observed from the figure that freshly extracted juice which was used as control, contain very low amount of limonin i.e. about 0.15mg/l, which was far below the threshold level (6ppm). Therefore bitterness was not detected if the juice is consumed as fresh or immediately after extraction.

The bitter compound limonin was found to be distributed in different parts of fruit. The seeds contained high amount of limonin about 480 mg/100 g. The freshly extracted juice contained 0.15 mg/L limonin which is below threshold level and therefore fresh juice feels non bitter, during consumption. The limonin was not detected when fresh juice was treated with adsorbent resins. The limonin content was increased during pasteurization i. e. up to 15.2 mg/L. It was also found that limonin slowly and gradually is developed in juice if the juice is held up to 6 h at room temperature. After 6 h limonin content was increased up to 10.2 mg/L. The adsorbent resins R1 and R2 were found effective in removing bitter compound limonin from juice. The resin R3 adsorbed negligible amount of limonin, hence was not much effective. Sweet orange juice can be successfullydebittered by using adsorbent resin and the treatment can be scaled up to industries.

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