

THE EFFECT OF ROOTSTOCK AND MATURITY ON BITTERNESS IN ORANGE JUICE*

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Abstract. The effect of rootstock and maturity on the bitterness, limonin content and other physicochemical characteristics of orange juice was determined. The rootstock trials showed that juice from Jaffa oranges grown on Jullundur khatti (rough lemon) and Jatti khatti (rough lemon) contained the highest amount of limonin followed by those grown on Kharna khatta and Seville kimb (sour orange), Mithi (sweet lemon) and Jamberi Lyallpur in decreasing order. Studies on the effect of maturity have shown that the limonin content of Valencia oranges decreased from a high level of 6.2 p.p.m. in December to about 0.9 p.p.m. in following March. Acidity, pH, ascorbic acid and the total soluble solids were also determined in both Jaffa and Valencia orange juices. Organoleptic evaluation confirmed the physicochemical results.

Citrus fruits occupy an important position in the fruit industry of Pakistan. According to a survey¹ conducted by the Ministry of Food and Agriculture, Government of Pakistan in 1969-70, the area under citrus fruits was 102,100 acres from which 406,500 tons of fresh citrus fruit were harvested. These fruits are consumed mainly as fresh fruit but a substantial quantity is also preserved in the form of squash, canned juice, orange juice concentrate and ready to drink beverages. These products have not been readily accepted by the consumers primarily due to the development of bitterness during storage. Bitterness is incorporated into these products from rag, peel and seeds of the fruits in the form of limonate—a ring lactone (precursor of the bitter principle, limonin).² The extent of limonin bitterness depends upon the variety of orange,³ rootstock,⁴⁻⁶ method of extraction,^{7,8} age of the plant⁹ and stage of maturity.^{3,4,6,10-13} The present study was undertaken to determine the effect of rootstocks and maturity on the bitterness in the orange juice.

Materials and Methods

The Effect of Rootstocks. Jaffa oranges grown on six different rootstocks, i.e. Jullundur khatti (rough lemon), Jatti khatti (rough lemon), Kharna khatta, Seville kimb (sour orange), Mithi (sweet lemon) and Jamberi Lyallpur, were collected from Horticulture Research Station, Sahiwal. The fruit was washed in cold running water, allowed to drain for 1 hr. The fruit was halved with stainless steel knife and the juice was extracted by manually operated reamer machine. The juice was strained through a pulper fitted with a screen having 0.06 in dia holes. Immediately after extraction, the juice was filled in bottles and stored in an ice box. Limonin was extracted and ascorbic acid, acidity, soluble solids and pH were also determined.

The Effect of Maturity. Valencia orange grown on rough lemon rootstocks were collected from a commercial garden. The fruit was collected on 10th December, 1972, and at two weeks intervals thereafter up to 10th March, 1973. The juice was extracted and stored in the same manner as described for the rootstocks effect. Rapid assay method¹⁴ was used for the preparation of extract and formation of TLC plates. The chemical assay of limonin, the bitter principle of oranges as described by Chandler and Kefford¹⁵ was used for the reaction of limonin through its ketone group to give a dinitrophenylhydrazone. The separation of this product from contaminants by TLC followed by the elution of appropriate spot and spectrophotometric examination of the resulting solution.¹⁴ Ascorbic acid was determined according to the method of A.O.A.C.,¹⁶ using 2,6-dichlorophenol indophenol. Total titratable acidity was determined according to A.O.A.C. method and pH of the juice was determined with a Pye 70-model pH meter. The juice samples were also evaluated organoleptically for the bitterness. Total soluble solids were determined with an Abbe's refractometer.¹⁵

Results and Discussion

In determining the limonin content it was observed that the development time of chromatograms was 2 hr instead of 1 hr as reported by Chandler and Kefford.¹⁵ Tariq *et al.*⁷ had also observed a development time of 2 hr. The study of thin layer chromatogram showed that the *R_f* value of limonin spot was 0.10 and spots of limonoids were detected in the range of 0.10-0.30. Chandler and Kefford¹⁵ and Chandler¹⁴ reported that the *R_f* value of limonoid spots was in the range of 0.10-0.55 and 0.05-0.70 respectively. The absorbance spectrum of limonin obtained with Unicam SP800 and Beckman DB-G spectrophotometers showed that the maximum peak absorbance of standard limonin in chloroform was at 365 μ and that in acetone at 363 μ .

The data on the limonin content and other physicochemical characteristics of juice obtained from Jaffa oranges grown on different rootstocks are

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given in Table 1. The average limonin content of the juice from Jaffa oranges grown on Jullundur (rough lemon), Jatti khatti (rough lemon), Seville kimb (sour orange), Kharna khatta, Mithi (sweet lemon) and Jambri Lyallpur was observed to be 6.5, 5.1, 3.7, 2.3, 1.6 and 1.4 p.p.m. respectively as is indicated in Table 1. The effect of rootstocks on limonin content was statistically significant except that the difference between Mithi and Jambri Lyallpur was insignificant. Marsh⁴ had also reported that juice obtained from oranges grown on rough lemon contained the highest amount of limonin as compared to other rootstocks which included trifoliolate orange, sweet orange, sour orange, navel cutting and grape fruit. Similarly Kefford and Chandler⁵ observed that navel oranges grown on rough lemon remained definitely more bitter even at the last picking of the fruit in mid September. Wilson and Crutchfield⁹ showed that there was definitely less bitterness in oranges grown on trifoliolate rootstock as compared to those grown on Troyer or Cleo rootstocks. The ascorbic acid content of the juice from Jaffa orange grown on

Mithi was lowest, i.e. 48 mg/100 ml and highest in case of those grown on rough lemon.

Per cent acidity and T.S.S. were also highest in the juice of orange grown on rough lemon rootstock and the pH was correspondingly lowest.

The data on the limonin content and other physicochemical characteristics of Valencia (grown on rough lemon rootstock) orange juice as affected by maturity are presented in Table 2. It has been observed that the limonin content of Valencia juice in mid December was 6.2 p.p.m. which decreased gradually with maturity. By 10th March, of the following year, the limonin content had decreased to only 0.9 p.p.m. (Fig. 1). The decrease in limonin content of Valencia orange juice with maturity was highly significant. It was reported by Kefford⁶ that fortunately the limonin disappeared from endocarp of most citrus fruits by the time they reached optimum maturity. Similar trends have also been reported by Marsh,⁴ Braverman,¹² Higby,¹⁷ Emerson,¹⁰ Samisch and Ganz³ and Kefford and Chandler.⁵ Flavian *et al.*¹³ observed that an enzymatic process occurred in the peel of Shamouti oranges

TABLE 1. EFFECT OF ROOTSTOCKS ON LIMONIN CONTENT AND OTHER PHYSICOCHEMICAL CHARACTERISTICS OF JAFFA ORANGE JUICE.

Name of the rootstock	Limonin (p.p.m.)	Vitamin C (mg/100)	Acidity (%)	pH	T.S.S. (%)
Jullundur khatti (rough lemon)	6.5	50.0	0.46	3.1	7.0
Jatti khatti (rough lemon)	5.1	68.0	0.65	2.8	9.5
Kharna khatta (sour orange)	3.7	50.0	0.53	3.0	7.0
Seville kimb (sour orange)	2.3	40.9	0.61	2.8	8.0
Mithi (sweet lemon)	1.6	48.0	0.55	3.9	7.5
Jambri Lyallpur (sweet lemon)	1.4	55.0	0.56	2.9	8.0
LSD 5%	0.27	2.18	0.38	0.78	0.62
1%	0.38	3.06	0.54	1.09	0.87

TABLE 2. THE EFFECT OF MATURITY ON LIMONIN CONTENT AND OTHER PHYSICOCHEMICAL CHARACTERISTICS OF VALENCIA ORANGE JUICE.

Date of picking	Limonin (p.p.m.)	Vitamin C (mg/100 ml)	Acidity (%)	pH	T.S.S. (%)
10.12.1972	6.2	63.0	1.38	3.5	7.0
25.12.1972	5.1	57.0	1.13	3.3	7.0
9.1.1973	5.4	50.9	1.05	3.4	7.0
24.1.1973	6.8	55.0	0.92	3.5	7.0
8.2.1973	3.1	57.0	0.88	3.6	7.5
23.2.1973	1.1	65.0	0.83	3.7	8.0
10.3.1973	0.9	70.0	0.69	4.2	8.5
LSD 5%	0.18	0.25	0.32	0.63	0.49
1%	0.26	0.35	0.45	0.88	0.68

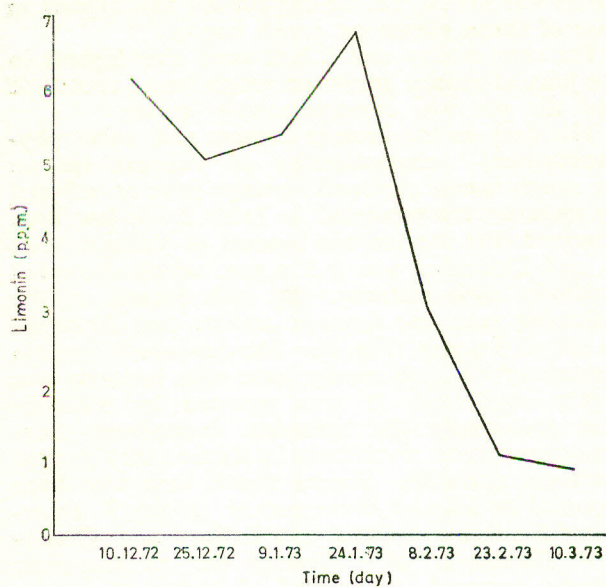


Fig. 1. Effect of season on limonin content (p.p.m.) of Valencia orange.

toward the optimum maturity stage, which could be responsible for the disappearance of limonic acid—a ring lactone, the nonbitter precursor of limonin. Valencia being late maturing orange, ripening in 12–15 months from blossoming would usually give juices free from bitterness at normal ripeness. Maier and Beverly¹⁰ reported that mid season to late season navel oranges frequently produced juice that did not become bitter. The limonin content decreased and almost disappeared from navel orange juice as the fruit ripened beyond commercial maturity. Tariq *et al.*⁷ had reported that the limonin content of Valencia oranges in February was 2 p.p.m. The ascorbic acid of Valencia orange juice in early December was 63 mg/100 ml and it decreased to 50 mg/100 ml by early January after which time it increased progressively reaching a maximum level of 70.0 mg/100 ml in March.

Acidity decreased continuously with maturity and the pH showed corresponding increase. The total soluble solids were 7.0% in early December and remained at that level until later half of January. Thereafter, this constituent showed a gradual increase reaching a maximum level of 8.5% in March.

The organoleptic evaluation of Jaffa orange juice and Valencia orange juice showed that the effect

of rootstock and maturity was highly significant. Juices with higher limonin content were rated lower in acceptability.

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