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# Use of Pre and Post Harvest Low Cost Techniques to Control/Minimize Citrus Juice Bitterness

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**Abstract:** The processing of citrus juice industry faced formidable problems of excessive bitter taste in terms of "bitterness" and "delayed bitterness" of juice worldwide because it reduces the quality and commercial value of the product. The two main compounds which impart the bitterness in citrus fruits are naringin and limonin. Efforts have been made to reduce the accumulation of bitter principles during the development and maturing of citrus fruit through chemical sprays, agronomic practices and post-harvest treatment of fruit. Many debittering technologies have been developed based on chemical, physical and microbial processes e.g. raising pH of the juice, addition of β-cyclodextrin monomer, use of adsorbent XAD-16, suppression of bitterness by addition of sweetening agents and conversion of bitter principles to non-bitter components by the action of immobilized bacteria. Few of the above techniques *viz*., addition of β-cyclodextrin monomer, use of adsorbent XAD-16 and conversion of bitter principles to non-bitter components by the action of immobilized bacteria which have some limitations/drawbacks along with large cost warranted the necessity to look for innovative technique(s). Using low cost approaches to debitter the fruit juices have been discussed in this review article.

**Keywords:** Citrus juice; Bitterness causing compounds; Debittering techniques; low cost techniques

## Introduction

Excessive bitter taste in citrus juice is a major problem in the citrus industry worldwide because it reduces the quality and commercial value of the product (Mongkolkul et al., 2006). The problem occurs in a variety of oranges (including mandarins), grapefruit and lemons. Washington navel, Satsuma, Natsudaidai, and Shamouti oranges are particularly prone to this problem (Fayoux et al., 2007). Citrus fruits belong to the family Rutaceae, which has been cultivated for over 4000 years and are the most produced fruit crop in the world (Davies and Albrigo 1994; Khan 2007) originated from south eastern Asia (Ruberto, 2001; Calabrese, 2002). Citrus fruits are a rich source of vitamin C, also containing vitamin P, which keeps the small blood vessels in our bodies in a healthy condition and helps in the assimilation of vitamin C (Singh and Saxena, 2008).

The citrus processing industry did not progress due to lack of consumer awareness, uncertainty of market response, problems in debittering technology and entrepreneurial/financial hazards arising from a variety of sources, including the extension of farm services and marketing of the fruit juice. The processing of citrus juice faced formidable problems in terms of "bitterness" and "delayed bitterness", thereby affecting its consumer's 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).

## Bitterness Causing Compounds

Naringin (flavanone glycoside) and limonin (highly oxygenated triterpene) are the two principal compounds which impart the bitterness in citrus. Flavonoids in citrus include flavanones (naringin), flavones (nobiletin), flavonols (quercetin) and anthocyanins (Ranganna et al., 1986). Polymethoxylated flavones (tangeretin and nobiletin) are concentrated in the skin of unriped fruit and are the constituents of bitter citrus oils (Benavente-Garcia et al., 1997). Among the flavonoids, naringin is the widely occurring bitter principle. Generally flavanones are mainly found in higher amounts while flavones and anthocyanins are relatively present in small amounts.

### *Naringin*

Naringin, a flavanone neohesperidoside and neohesperidin are very bitter, whereas hesperidin is tasteless. On the other hand, neohesperidin dihydrochalcone is intensely sweet. Naringin concentrations are the highest in young leaves and in the pulp (albedo) of immature fruit (Del Rio et al., 1998). Naringinase itself has a mix of activities – a rhamnosidase and γ-glucosidase activity and acts to hydrolyse naringin to the non-bitter aglycone naringenin. The enzyme itself is produced by *Aspergillus niger* fermentation (Puri et al., 2005, Shanmugaprakash et al., 2011), from *Penicillium spp.* (Norouzian et al., 2000), and *Staphylococcus xylosus* MAK2 (Puri et al., 2012). The processes including the purification have been widely reported (Puri and Banerjee, 2000). The enzyme has been immobilised to a variety of supports for use in column reactors: ionic binding to DEAE-Sephadex (Ono et al., 1977), tannin-aminohexyl linked cellulose (Ono et al., 1978), glycophase coated glass (Ono et al., 1978), chitin (Tsen and Tsai, 1988), alginate beads (Puri et al., 1996), to packaging film (Soares and Hotchkiss 1988) and even covalently bound to woodchips (Puri et al., 2005).

### *Hesperidin*

Hesperidin is the main flavonoid found in sweet oranges and lemon, while naringin is the flavonoid responsible for the bitter flavour in grapefruit (Nagy and Shaw, 1990). The fruits containing high flavonoids are bitter even when consumed as fresh. The peel (rind) of the citrus fruit contains very high amounts of flavonoids like naringin, and neohesperidin which make them highly bitter. Naringin is common in bitter citrus species such as pummelo, grapefruit, sour orange, and pumello hybrid natsudaidai (Hasegawa et al., 1996). The major flavonoids present in orange are hesperidin, rutinoside of naringenin, isosakuranetin, 4-β-D-glucoside of naringenin, 4-β-D-glucoside of naringenin rutinoside and neohesperidin. The bitter principle of bitter (sour) oranges is naringenin in place of hesperidin of sweet oranges.

### *Limonin*

Limonin is the major cause of citrus juice bitterness and is widely distributed. Limonin first isolated from ‘Washington Naval Orange’ juice by Higby (1938) is the primary cause of ‘delayed bitterness’ in which the fruit or its juice is not bitter if consumed fresh but gradually becomes bitter upon storage, even when refrigerated or frozen. This phenomenon is due to the presence of non-bitter precursor of limonin – limonoate A-ring lactone (LARL) in the segment and juice sack membrane. When the membrane is ruptured during juice extraction, LARL comes in contact with the acidic juice medium and is converted to limonin by limonin D-ring lactone hydrolase (Maier et al., 1969; Mongkolkul et al., 2006). It has also been found that the limonin content is the highest in seeds, followed by peels and lowest in juice (Premi et al., 1995). Limonin concentrations as low as 6mg $L^{-1}$ render citrus products unacceptable to consumers (Guadagni et al., 1993). As many as 37 limonoids have been identified in citrus and their hybrids. Among the 37 limonoids four limonoids namely, limonin, nomilin, ichangin and nomilic acid are bitter (Maier et al., 1977).

Several methods have been tested to reduce bitterness in juices from citrus like raising pH of the juice (Ranote and Bains 1982), suppression of bitterness by addition of sweetening agents (Guadagni et al., 1974), 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). But a few of these techniques are very expensive and time consuming viz, use of enzymes, and use of adsorbent XAD-16 etc. The above limitations/ drawbacks warranted the necessity to look for innovative technique(s) using low cost approaches to debitter the fruit juices.

## Techniques Used for Debittering Citrus Juices

So many efforts have been made to reduce the accumulation of bitter principles during the development and maturing of orange fruit through chemical sprays, agronomic practices, and post-harvest treatment of fruit. Many debittering technologies have been developed based on chemical, physical and microbial processes. These debittering techniques are mainly divided into two group's viz. pre-harvest and post-harvest approaches.

## Pre-Harvest Approaches

### *Tree Treatment*

Treating Washington Navel orange trees after bloom with 200 or 300 ppm of 2-(3, 4- dimethyl phenoxy) triethylamine or 200 ppm 2-(4-cholorophenyl thio) tri-ethylamine is reported to reduce the concentration of limonin in the juice of oranges at the beginning of maturity, compared with untreated control, with a concomitant small decrease in soluble solids and acidity (Casas et al., 1979). However, these differences in limonin contents decreased with advancing maturity.

### *Rootstocks*

Marsh and Cameron (1950) reported the effect of rootstocks on the overall quality of navel orange juice bitterness. The fruit produced on trifoliate orange rootstock yielded juice of superior in an overall quality standpoint of tests whereas juice produced from fruit grown on the sour orange and sweet orange rootstocks were only of fair quality initially and developed the stale flavor of preserved juice very rapidly.

### *Plant Growth Regulators*

Application of plant growth regulators (PGRs), especially but not exclusively gibberellic acid, to citrus trees to enhance fruit yield and the quality has been studied by various scientists on commercial crops for a number of years (Wilson, 1984; Coggins and Henning, 1988). Berhow and Vander cook (1992) and Shaw et al. (1991) reported that application of $GA_3$ can lower the levels of the bitter flavonoid naringin in grapefruit. When $GA_3$ is applied on larger mature fruit, it slightly reduces the acid percentages and concentrations of naringin (Berhow, 2000). ABA treatment is effective in reducing the naringin levels slightly in juice when applied at the highest concentration of 50 ppm but had little effect on juice soluble solids, acid content (Berhow, 2000).

Application of PGRs can have an effect on the accumulation of flavonoids, especially if applied to young fruits during the period of intense flavonoids biosynthesis. Higher hesperidin levels can be maintained for a longer period of time in Tangelo (*C. reticulata* × *C. paradisi*) fruit treated with BA (Del Rio et al., 1995).

## Post Harvest Approaches

### *Harvesting Time*

The extent of bitterness and acidity are the highest in the early season fruit and declines as the season progresses in orange (Maier et al., 1973), navel oranges (Guadagni et al., 1977), grapefruit (Atta way, 1977), Kin now (Sandhu et al., 1990), Satsuma mandarin () Ishikawa et al., 1993) and Thai tangerine (Noomhorm and Kasemsuksakul, 1992). Mature Satsuma mandarin contains a relatively low concentration of bitter limonoids aglycons (Ozaki et al., 2000).

Unfortunately, the low bitterness levels were reached very late in the harvest season, after most of the crop had been harvested. Other investigators attempted to simulate this natural debittering process by storing early-season navel oranges in warm, moist rooms (Guadagni et al., 1977). Although this approach had a number of serious drawbacks that prevented its commercialization. These disadvantages included the growth of molds and other microorganisms, the large amount of time required the development of off-flavors, and the necessity for special storage rooms.

### Method of Juice Extraction

The method of juice extraction plays an important role on the contents of bitter principles in citrus juices (Mohsen et al., 1986; Lotha et al., 1994). The juice is obtained by squeezing or peeling the fruits and then the extraction of juice is done in a way that seeds are not crushed, with a soft press was found to be better than other methods, but the only disadvantage a small quantity of obtained juice. This is because the peel and seeds have a higher amount of limonin compared to juice as mentioned by Premi et al., 1994. Lotha and Khurdiya (1994) observed that the juice obtained from CP (crushing with peel) method was not acceptable due to its intense bitterness, while the juices obtained by hydraulic pressing and hand reaming were not bitter. The juice obtained by screw type extractor was found less bitter as compared to superfine pulper and also has a high preference due to instantaneous pressing of segments directly with minimum crushing of tissues and without much oxidation of juice (Sandhu and Singh, 2001).

The bitterness causing compounds viz., limonin and naringin in kinnow fruits were lower in the juice extracted to a level of 15% with highest TSS, sugars and titratable acidity and low in ascorbic acid content (Thakur and Lal Kaushal, 2000). Gaonkar and Bamzi (1989) conducted an experiment on juice extraction of grapefruit, orange, lime and lemon, and they found that the juice extracted with soft press was better than the one obtained with other methods.

Pareek et al. (2011) also suggested that the naringin and limonin contents were minimum along with better qualitative characteristics like total soluble solids, acidity, ascorbic acid, sugars and non-enzymatic browning during after a 6 months storage when the juice was extracted with screw type extractor and 65 °C processing temperature for 15 min.

### Lye Peeling

Anand et al. (2012) have tried several treatments e.g. lye, flurosil, naringinase, and combination of 'lye and flurosil' and 'flurosil and naringinase' to debitter kinnow juice. They pasteurized the debitter juice and stored it for six months. Among the different debittering techniques, they 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.

Lye peeling of the segments reduces the bitterness of juice of Kinnow which is due to the removal of white papery segment walls which contain a high amount of bitter compounds, and are incorporated to juice during juice extraction (Sandhu et al., 1990, Sandhu and Singh, 2001). But Sogi and Singh (2001) reported the negative effect of peeling of pummelo using lye at high concentration in kinnow mandarin.

### pH Effect

The citrus fruit juice with raised pH has a considerable lower bitterness. Kinnow juice (Ranote and Bains, 1982), Thai-tangerine juice (Chaiswadi et al., 1998) with raised pH had a considerable low content of limonin. This may be due to unfavorable conditions for conversion A-ring lactone to limonin (Maier et al., 1969). The changing the pH of pummelo fruit from 3.5 to 4.5 reduced the naringin content from 31.8% at pH 3.5 to 40.9% at pH 4.5 (Ghosh and Gangopadhyay, 2003).

### *Juice Blending*

A gradual increase of limonin in juice blends with an increased in storage period might be due to conversion of a chemical compound limonate-A-ring lactone (non-bitter) into limonin (bitter) in the juice (Premi et al, 1994). Among the juice blend prepared with ginger, aonla and pomegranate juice exhibited significantly lesse limonin as compared to pure kinnow juice because blending of non-bitter juice with bitter juice in proper ratio reduced the quantity of limonin in juice. Kinnow juice blended with pomegranate juice (10%) and ginger juice (3%) shows the minimum limonin (0.250 mg/ml juice) at the end of storage (Bhardwaj and Mukherjee, 2011). Guadagni et al. (1993) and Berry (2001) reported that blending of citrus juice with sugar in proper ratio also reduced bitterness.

## Use of Packaging Films

### *Cellulose Acetate*

The immobilization of enzymes in packaging materials has been proposed as a form of "active packaging" (Brody and Budny, 1995). Cellulose acetate films containing immobilized naringinase reduced naringin level in grapefruit juice at common refrigeration temperature i.e. up to 23% efficiency at 7$^{o}$C (Soares and Hotchkiss, 1998b). The cellulose acetate films showed a smoother surface which may have contributed to a lower desorption of naringinase from the film matrix and a higher film activity. Limonin is reported to be strongly absorbed by cellulose acetate film (Johnson et al., 1982).

The enzyme activity in cellulose films is less affected by lower temperature than free enzyme and did not lose activity during dry storage. Therefore, the cellulose acetate immobilized film showed potential to be used as an inner layer in citrus juice packages to reduce naringin.

### *Whey Protein Films*

Whey protein films show a great potential as supplemental aroma barriers for packaging of low moisture foods. Temperature and relative humidity have exponential effects on *d*-limonene permeability, interacting synergistically to influence aroma transport in whey protein films. Permeability of *d*-limonene in whey protein isolate polymer films is not influenced by concentration in the range 62-226 ppm (mol/mol) (Miller et al., 1998).

### *Ethylene Effect*

The intact fruits are exposed to 20 ppm ethylene gas for 3 h and then kept for several days in air. For instance, after 5 days at room temperature, the limonin content of juice prepared from the ethylene triggered stored fruit decreased almost 50% from the critical level of 24.3 ppm (Maier et al., 1973).

Treatment of citrus fruits like, navel oranges, lemons, grapefruits with 20 ppm of ethylene for 3 hrs induces accelerated limonoate A-ring lactone metabolism continued after ethylene exposure ceases and results in a substantial loss of limonoate A-ring lactone in several days. Juice from treated fruits has a lower limonin content, less bitter and is more preferred than juice from untreated fruits. The use of $CO_2$ at the pressure of 21 to 41 MPA at 30$^{o}$C to 60$^{o}$C for 1 hour caused the reduction of limonin by around 25% (Kimball, 1987).

## Biotechnological Approaches

### *Microbial Debittering*

Several investigators have reported studies directed at using biotransformation as a way to avert bitterness by converting bitter limonoids in citrus juice to non-bitter metabolites. Microorganisms and their enzymes have proven to be versatile biocatalysts and are extensively used for bio-transformations of various terpenoids (Chatterjee and Bhattacharya, 2001). A large variety of enzymes occur in several microorganisms (such as bacteria, yeast and fungi), which are effective in bio-transformations of various terpenoids and can be used in vivo. The microbial transformation processes have therefore been explored for terpenoids with a view to achieving desired conversions, optical resolutions of products, as well as understanding of the metabolic pathway for the biodegradation of terpenoids.

The use of 200 m-units of the limonite dehydrogenase of *Pseudomonas sp*. 321-18 per mm of navel orange juice reduced the eventual limonin content of 21 ppm to 3 ppm, a level below the general bitterness threshold (Brewster et al., 1976). Treatment of 30 mL of serum (10-27 ppm of limonin), on a 1.5 cm diameter column packed with 1.6 g of immobilized cells (16-mL bed volume) reduced limonin content by 70% or more (Hasegawa et al., 1982).

### Effect of Bitterness Suppressing Agents

Addition of sugar and dilution with water also mask bitterness in juice but it is an adulteration. Addition of sugar, salt and chaatmasala (a mixture of different condiments) was found to enhance acceptability of Kinnow juice (Sandhu and Singh, 2001).

The organoleptic evaluation of the juice extracted from the segments steamed after dipping in brine revealed the superiority of application of brine treatment. The dipping of the pummelo peels in 6% salt and boiling for 30 minutes reduced bitterness in pectin extracted from the peel (Madhav, 2001).

### Biological Processes

A biological process was developed which used immobilized bacterial cells for limonoids in citrus juice sera (Hasegawa et al., 1982). Two species of bacteria viz. *A. Globiformis* and *A. Globiformis* II had been used for the purpose (Hasegawa et al., 1982; Hasegawa et al., 1983), *A. Globiformis* cells immobilized in acrylamide gel converted limonin and nomilin of citrus juice to non bitter 17- dehydrolimonoate- A ring lactone, respectively. Immobilized cells of *A. Globiformis* II, on the other hand, converted limonin and nomilin to non bitter limonol and nomilol, respectively. Among the bacteria capable to metabolize limonoids, *Corynebacterium fascians* is the only one that produces constitutive enzymes for metabolism of limonoids. The others require the presence of limonoid inducer in their growth media to produce cells capable of metabolizing limonoids (Hasegawa and King, 1983). Immobilized *Acinebactor sp*. metabolized limonin to deoxylimonoic acid (Vaks and Lifshitz, 1981). Immobilized *Corynebacterium fascians* (Hasegawa et al., 1985), and *Rhodococcus fascians* (Iborra et al., 1994; Bianchi et al., 1995), were used to debitter the citrus juices.

### Chemical Processes

Citrus fruit juice is placed in alcohol with <1 vol% ethanol to fix complex compounds of limonoids such as primary limonin, flavonoids, and /or naringin and to remove the bitter taste in short time (Ogawa and Tezuka, 1988). Sucrose and pectin addition to juice reduces solubility properties of limonin; also some enzymes may affect the solubility leading to the precipitation of limonin and naringin and hence reduction in bitterness (Chandler, 1971).

Neodiosmin has been reported to reduce bitterness. Suppressor, which has been found to raise limonin bitterness threshold level in orange juice, reduces the very high levels of limonin bitterness in juice also. The use of 50 to 150 ppm of neodiosmin has been recommended (Hasegawa and Maier 1983; Guadagni et al., 1977).

## Future Needs of Research

Nowadays, the low cost debittering techniques have more attention because of high cost of enzymes, resins etc. The low cost techniques have ample scope of cost effectiveness and easy availability. The extraction processes should be developed so that the extraction of the juice is done with a minimal incorporation of pulp particles, but still keep a highly acceptable quality of juice. There is an ample scope for the development of cultivars through genetic engineering or other ways in which bitter principles or their precursors are not present.

It would be desirable to propagate the citrus trees with a genetic diversity of both scion and rootstock, to minimize the universal problem of post-processing bitterness. The extraction machinery has a greater chance for improvement. The methods to improve organoleptic acceptability need to be developed. Good production practices of raw material and product manufacturing technology should be developed throughout the world to meet the quality requirements of unprocessed juice.

## Conclusion

From the present study, it was concluded that the juice extracted with the screw type juice extractor gave less bitter juice than the one from other methods. $GA_3$ and 2-(4-cholorophenyl thio) tri-ethylamine also gave good results. Late harvested fruits have less bitterness. Lye treatment and addition of sugar, salt and *chaatmasala* improved the organoleptic qualities of juice. Blending is also very important to reduce the bitterness from the citrus juice. Biological and chemical debittering methods are also very useful. Packaging films were also very helpful in reducing the bitterness.

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