STUDIES ON PROCESSING OF KINNOW JUICE EXTRACTED VIA 'MODIFIED METHOD OF BITTERLESS KINNOW JUICE EXTRACTION'

A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF

MASTER OF SCIENCE (BIOTECHNOLOGY)

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CANDIDATE'S DECLARATION

I here by declare that the work that is being presented in dissertation entitled, **Studies on Processing of Kinnow Juice Extracted via ' Modified Method of bitterless Kinnow Juice Extraction'** in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE IN BIOTECHNOLOGY**, Department of Biotechnology and Environmental Sciences, Thapar Institute of Engineering and Technology, Patiala is an authentic record of my own work during the period of 5 months from January 2003 to May 2003, under the supervision of Dr. Pratima Khandelwal, Lecturer, Department of Biotechnology and Environmental Sciences, Thapar Institute Engineering and Technology, Patiala. The matter embodied in this dissertation has not submitted by me for the award of any other degree or diploma.

Place: Patiala **Himani Kansal**
Date: 15 May, 2003 Author

This is to certify that the above statement made by the candidate is correct and true to the best of our knowledge.

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ABSTRACT

The high production and productivity of kinnow, which is one of the major fruit crop of northern India especially of Punjab, has remained underutilized. The major reason has been the problem of (delayed) bitterness in the extracted juice along with short shelf life of the fruit. The bitterness is due to triterpenoid limonin, formed during and after juice extraction, thus rendering the juice unpalatable. Various debittering techniques (physicochemical & biotechnological) have been attempted in the country, which have met with variable and incomplete successes, thus processing of the juice is still a challenge. In the present study attempts were exclusively made on preventive approach for controlling the formation of limonin thus producing bitterless kinnow juice.

Kinnow juice was extracted by the 'Modified method of bitterless kinnow juice extraction' which has been developed in this institute and patent has been filed on it (January 2003). After giving certain pretreatments to the fruits, the juice was extracted by using only electric juicer and it was observed that only mid and late season kinnows (January to march) could be utilized for extracting desirable juice.

The bitterless kinnow juice thus obtained was monitored for period of 7 days and only after the limonin content in juice was found to be below threshold level (2- 4ppm), it was then processed into kinnow squash, kinnow RTS/RTD beverage, frozen kinnow juice concentrate and refrigerated kinnow juice concentrate. After optimizing the method of production of each product, shelf life studies were conducted for the period of 2 months by keeping kinnow squash at ambient temperature and refrigerated temperature (both in glass bottles), kinnow RTS/RTD beverage in PET and glass bottles (both at refrigerated temperature), and concentrates in frozen and refrigerated condition (both in glass bottles). The physiochemical studies reveal that limonin content of all the products slightly increased after 7^{th} day from 3.3 to 4.2 ppm in case of the concentrate; thereafter, limonin content in all products was found to remain stable and below threshold value till the end of storage period. Naringin content was estimated during the first and last day of the storage studies and was found to be quite below the threshold value (250-300ppm). Insignificant changes in pH and TSS were observed in all the products. The %TA was found to decrease from 0.44 to 0.30% in squash, from 0.38 to 0.28% in RTS and in kinnow concentrates it decreased from 0.56 to 0.52%. The ascorbic acid values also decreased from 14.20 to 11.22mg/100ml in squash, 17.34 to 10.71mg/100ml in RTS and from 28.8 to 16.3mg/100ml in concentrates. Microbiological evaluation revealed that no coliforms were obtained during the storage period in any of the products. Yeast and mold count and TPC count were found to be much below the permissible limits and were decided only towards the storage period. Sensory evaluation was indicative of high acceptance of products. Thus, it can be inferred that the preventive approach of extraction of bitterless kinnow juice led to elimination of debittering of kinnow juice in totality and such juice could be processed into acceptance and shelf stable products. The attempts for pilot scaling up of extraction and processing of bitterless kinnow juice are underway.

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INTRODUCTION

1. **INTRODUCTION**

India stands second in the production of fruits and which accounts for 8% of the world's total fruit production **(TIFAC, 2002).** India holds third rank in respect of production of citrus fruits in the world, accounting for 4MMT annual production. Of these, Kinnow, a hybrid of *citrus nobilis* and *citrus delicosa*, is an important citrus crop with the production of over 0.32MMT **(National Horticulture Board, 2000).**

Kinnow mandarin was introduced in India in the early 1940's. At present its cultivation has assumed great importance among north Indian growers and a large acreage of land is being brought under cultivation particularly in Punjab, Haryana, Rajasthan and Himachal Pradesh **(Kurdiya and Lotha, 1994).** Fully ripe fruits of kinnow have bright and deep attractive color, thin tight and compact skin. The fruits are juicy and the fresh juice extracted from the fruit harvested at appropriate stage of maturity, has refreshing flavor, characteristic pleasing aroma and thirst quenching properties, but also have the problem of short shelf life **(Kamaljeet, 2002 and Premi *et al*., 1996).**

Presently 95% of the production goes for fresh fruit market. It is notable that due to poor post harvest infrastructure wastage of kinnow is around 25-30% and that only 5% of the total production is processed presently **(Vijay, 2002; National Horticulture Board, 2000).** Thus, in order to fully utilize the high production of kinnow, the only alternative is to make it feasible to process it into juice and other juice based products. The processing of Kinnow juice, however, has faced formidable problems in terms of development of bitterness **(Puri *et al*., 1996; Premi *et al*., 1995; Sandhu *et al*., 1985)** due to the presence of two bittering components – limonin (a limonoid) and naringin (a flavanoid) therefore affecting its consumer acceptability **(Kamaljeet, 2002; Puri *et al*., 1996 and Premi *et al*., 1995).**

Limonin is an intensely bitter triterpenoid dilactone present mainly in citrus seeds **(Hasegawa *et al*., 1980).** Limonin develops gradually in juices after juice

extraction. The intact fruit do not normally contain limonin rather a non-bitter precursor, a limonoate-A-ring lactone **(Hasegawa, 1972; Maier and Beverly, 1968)**. The conversion is accelerated by the action of enzyme limonoate-D-ring lactone hydrolase that is present in citrus fruits **(Maier *et al.*, 1969).** Naringin, on the other hand is the primary bittering water-soluble component in the fruit membrane and albedo, which become extracted in fruit juices. Its concentration is a function of degree of fruit maturity, with the least amount in ripened fruits **(Premi *et al.*, 1995; Maier *et al.*, 1969).**

A number of physiochemical and biotechnological approaches have been attempted to reduce the developed bitterness in citrus juice below threshold level for acceptability. All these approaches have been focused on curative means to attack the problem of bitterness. These have met with variable and incomplete success. Among the physiochemical approaches, there has been use of various chemicals and adsorptive resins etc. Among the biotechnological approaches for debittering the juice, use of bacterial cells, immobilized enzymes and cells have been extensively made **(Puri *et al.*, 1996).** Survey of literature reveals lot of work has been and are being going on for debittering the juice. Yet no complete success has been achieved, especially from commercial point of view.

At Department of Biotechnology and Environmental Sciences in the Institute, efforts were concentrated on development of a preventive approach for control of bitterness due to limonin in kinnow juice. Thus controlling the formation of limonin in the kinnow juice was worked out and subsequently a 'Modified method of bitterless kinnow juice extraction' was developed**.** This could lead to utilization of kinnow juice in terms of its processing into various products such as kinnow squash, kinnow beverages and concentrates. Thus, the critical point in kinnow juice processing is attacking bitterness problem and once bitterless juice is obtained; its further processing can be easily achieved.

In view of the above-mentioned points, the present investigation was undertaken with the following **objectives-**

1. To optimize the method of extraction of *bitterless kinnow juice* at higher levels at lab scale.
2. *Processing of the bitterless kinnow juice* into kinnow squash, kinnow RTS/RTD beverage, frozen kinnow juice concentrate, refrigerated kinnow juice concentrate and kinnow juice powder (freeze dried).
3. To *undertake shelf life studies* of each of the processed products.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Globally India stands second in the production of fruits with its current production of around 32MMT and thus accounts for about 8%of the total world's total fruit production. The diverse agro climatic zones in the country makes it possible to grow almost all the varieties of fruits and vegetables in India. The major tropical fruits are mango, banana, citrus fruits, apple, guava, papaya, pineapple and grapes **(TIFAC, 2002)**. India holds third rank in respect of the production of citrus fruits in the world with the produce of around 4MMT annually. Of these kinnow, a hybrid of *citrus nobilis* and *citrus delicosa* has an annual production of over 0.32MMT **(Vijay, 2002; National Horticulture Board, 2000)**.

2.1 Kinnow

Kinnow a first generation hybrid of "King" and "Willow leaf" mandarins (*citrus nobilis and citrus delicosa*) **(Joshi *et al.*, 1997)**, was evolved by late Dr H.B.Frost at University of California, Regional fruit station USA **(Kurdiya and Lotha, 1994)**. It was first introduced in India during early 1940's at the fruit experiment station of Punjab Agriculture College and Research Institute Lyallpur by S.Bhadur Lal Singh **(Singh, 1978)**. Since then it has assumed great importance among north Indian growers and a large acreage is being brought under its cultivation particularly in Punjab, Harayana, Rajasthan and Himachal Pradesh **(Kurdiya and Lotha, 1994)**. Punjab tops amongst the states in terms of production with the annual production of over 0.175 MMT **(Hindustan Times, May 2003 and The Tribune, Chandigarh, 2002).**

The Northern region of India is considered to be as natural home of citrus **(http://ww.ipgr.org/region/apo/publication/tfasia/heptri.pdf)**. The reason behind this is the adaptability to the agro climatic conditions of the region **(Kurdriya and Lotha, 1994)**. Kinnow has shown to have superior characters such as heavy bearing, wide adaptability and fruit quality **(Jawanda, 1978)** but is also

reported to have short shelf life **(Jawanda and Singh, 1978)**. Presently 95% of the production goes for fresh fruit market. The availability of large quantities of fruit over a short period of time poses a problem for efficient marketing and utilization. Owing to the poor post harvest infrastructure, wastage of kinnow fruit is over 25% to 30% of the total national production amounting to 0.32MMT. This is mainly due to perishable nature of fruit along with the major hurdles like lack of precooling and cold storage infrastructure facilities, low price due to seasonal glut and stem puncturing. Therefore the only alternative is to process this fruit in the form of juice and other juice-based product. Presently only 5% of this is processed **(Vijay, 2002; National Horticulture Board, 2000)**. The single most hindrance in the popularity and processing of the juice is the development of bitterness, a phenomenon called delayed bitterness among the citrus fruits. This is of great importance in kinnow processing due to its high intensity **(Puri *et al.*, 1996; Premi *et al.*, 1994).**

2.2 Delayed Bitterness in Kinnow Juice

The bitterness in citrus juices is mainly due to limonin (a limonoid) and naringin (flavonoid) **(Hasegawa and Maier, 1983).** Kinnow juice processing and commercial utilization has faced a great hindrance due to the development of bitterness, primarily due to limonin, by the phenomenon known as delayed bitterness. The intense bitterness development within few hours of juice extraction renders the juice unpalatable and thus processing of juice in beverages, juice concentrate or juice powder is highly limited **(Puri et al., 1996).**

2.2.1 Limonoids

It has been reported that limonoids are chemically related triterpenes and found in the families of Rutaceae (citrus families) and Meliaceae (neem families) **(Hasegawa and Maier, 1983)**. Thirty-six limonoids aglycones and seventeen glucosides have been isolated from citrus and its hybrids **(Maier *et al.*, 1968)**. It

has also been found that four limonoids namely limonin, nomilin, ichangin, nomilinate are bitter **(Hasegawa and Maier, 1990).**

2.2.2 Limonin

Limonin, an intensely bitter triterpenoid dilactone present mainly in citrus seeds has been found to be largely responsible for bitterness in citrus juice **(Hasegawa *et al.*, 1980)**. Limonin was first isolated from Washington Naval Orange juice by **Higby (1938)** and its complete structure was reported by **Arigoni *et al.*, 1960 (Maeir and Beverly, 1968).** It has been reported that limonin develops gradually in juices after juice extraction **(Hasegawa *et al.*, 1972)** and the intact fruit do not normally contain limonin rather a non-bitter precursor, limonoate A-ring lactone **(Hasegawa *et al*, 1972; Maier and Beverly, 1968).** Researchers have also found that this precursor, as shown in the figure 2.1, gets converted to limonin under acidic condition **(Maier and Beverly, 1968)** and the conversion is accelerated by the action of enzyme limonoate-D-ring lactone hydrolase that has shown to be present in citrus fruits **(Maier *et al.*, 1969).**

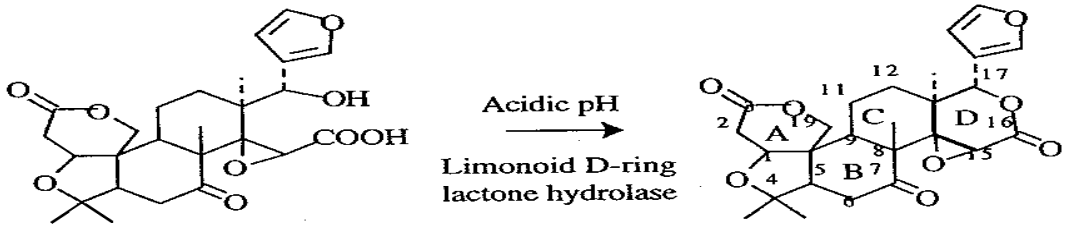


Figure 1. Mechanism of delayed bitterness

The threshold level of Limonin content in kinnow has been to be 6ppm **(Hasegawa and Maier, 1983).** It has also been found that the limonin content is highest in seeds, followed by peels and lowest in juice **(Premi *et al.*, 1998).**

2.3 Approaches used for Debittering Citrus Juices

2.3.1 Physico-chemical Approaches

2.3.1.1 Adsorbent Resins

- Amberlite: Researchers have been carried out with the use of XAD 1, 2, 4, 7, and 16 to debitter the citrus juice. It has been reported that XAD -16 was best in reduction of Limonin content (0.03g/ml) without affecting the physico-chemical characters **(Premi *et al.*, 1995).**
- ô-cyclodextrin: This has been found to form complexes with limonin and naringin and reduces the bitterness to below threshold in citrus juices **(Hasegawa and Maier, 1983).**
- Cellulose acetate beads: Selective adsorption process with the column packed with cellulose acetate beads have been found to remove 50% of the limonin for use. But this method was found to remove other constituents to some extent **(Johnson, 1981).**
- Florsil: Activated magnesium silicate has been shown to reduce limonin without adversely affecting its nutritive quality **(Barmore *et al.*, 1986).**

2.3.1.2 Chemical Methods

- Bitterness suppressing agents: Neodiosmin has been reported to reduce bitterness. Suppressor, which has been found to raise limonin bitterness threshold level in orange juice and it reduces very high levels of limonin bitterness in juice also. The use of 50 to 150ppm of neodiosmin has been recommended **(Hasegawa and Maeir, 1983; Guadagni *et al.*, 1977).**
- Ethylene gas**: Maeir *et al.*, 1972** reported the use of ethylene for 3 hours in fruits and then holding the fruits for several days in air was found to decrease the limonin content to almost 50%of the initial level.
- Use of CO_2: Use of CO_2 at the pressure of 21 t0 41 MPA at 30^0C to 60^0C for 1 hour caused reduction of limonin by around 25% **(Kimball, 1987).**

2.3.1.3 Drawbacks of Physicochemical Approaches (Puri *et al.*, 1996)

i) The methods have been reported to alter the chemical composition of juice.

ii) The methods have been found to be non-specific in nature.

iii) Methods also have non-reproducibility, lower yield and cause partial loss of desired nutrient.

2.3.2 Biotechnological Approaches

Various approaches have been studied by researchers that degrade limonin. Table 2.1 gives a mention of list of bacteria metabolizing limonoids and their metabolic pathways **(Puri *et al.*, 1996):**

Bacteria	Pathways	Reference
Arthrobacter globiformis	17-Dehydrolimonoids	Hasegawa *et al.*, 1972
Pseudomonas	Deoxylimonoids 17-Dehydrolimonoids	Hasegawa *et al.*, 1972
Bacterium	Deoxylimonoids 17-Deoxylimonoids	Hasegawa &Kim, 1975
Acinetobactor sp	Deoxylimonoids	Vaks & Lifshitz, 1981
Arthrobacter globiformis	17-Dehydrolimonoids	Hasegawa *et al.*, 1983
Cornybacterium fascians	17-Dehydrolimonoids trans 19-HBAb	Hasegawa & King, 1983 Hasegawa *et al.*, 1985 Mangon *et al.*, 1991
Rhodococcus fascians		Martinez Madrid *et al.*, 1989
Rhodococcus fascians		Marwaha *et al.*, 1994
Psedomonas putida		Kamaljeet, 2002

Table 2.1 List of bacteria employed for degrading limonin

2.3.3 Bittersweet citrus Limonoids

ARS scientists with collaborators have been developing knowledge to eliminate citrus bitterness that arises after juicing. They have identified and isolated a gene that can eliminate delayed bitterness caused by synthesis of a class of limonoid compounds and are working to create a transgenic nonbitter citrus. Further more, limonoid compounds in both bitter and nonbitter forms have demonstrated anticancer properties and insect antifeeding properties and may have substantial value **(USDA, 1998).**

2.4 Factors Affecting the Bitterness in Kinnow Juice

2.4.1 Method of Juice Extraction

(Khurdiya and Lotha (1994) studied the effect of blending kinnow juice on the quality of processed juice products. They reported that kinnow mandarin juice extracted by hydraulic pressing (HP) has been reported to be non-bitter but poor in other quality attributes and the extracted by crushing with peels (CP) was found to be excellent in quality as color, flavor except intense bitterness. Blending of the CP juice improved the color and flavor of HP juice and nectar. CP juice can be blended up to 30% with a tolerable bitterness.

The juice extracted by the FMC citrus juice extractor has also been found to be bitter **(Kamaljeet, 2002).**

2.4.2 Effect of the Storage Temperature

Kinnow juice stored at 25^0C has been shown to develop full bitterness in four hours as against seven hours for juice stored at 12.5^0C **(Premi *et al.*, 1995).**

2.5 Kinnow Juice Constituents

a) Juice Content: Juice content obtained from kinnow fruit ranged from 36% to 62% **(Jagjiwan, 2002).**

b) Ascorbic Acid: The principle vitamin in the citrus fruits is vitamin C, the amount of which varies with variety, maturity and other factors. Ascorbic acid is relatively stable in citrus products during processing and storage **(Vedhius, 1971)**. The ascorbic acid content in kinnow was reported to be in the range of 13.3 to 46.9 mg/100 ml **(Pruthi *et al.*, 1983; Singh *et al.*, 1978)**.

c) Acidity: Citric acid is the major organic acid in citrus fruits, accumulates in young fruits to 2.5% (w/v) and in the juice before decreasing to about 1% at commercial maturity **(Veldihus, 1971)**. In case of kinnow juice, the acidity has been found to range from 0.28%-0. 51% **(Kamaljeet, 2002).**

d) pH: Citrus juices are characterized by the pleasantly acid taste, which contribute to the flavor and the acid medium **(Veldihus, 1971)**. pH has been reported to range from 4.20-4.28 **(Kamaljeet, 2002).**

e) TSS: In the citrus fruits, total soluble solids consist of sugar and acids, where in the sugars contribute 75-85% **(Jawanda *et al.*, 1981)**. Kinnow mandarin has the TSS ranging from 8 –15.75% as affected by different storage conditions **(Kamaljeet, 2002).**

f) Sugars: The sugars in citrus are mainly glucose, laevulose and sucrose. The amount of reducing sugars has been found around 3.95% and that of non-reducing sugar is 3.65% **(Veldihus, 1971).**

2.6 Citrus Juice Processing

Citrus fruits are considered as one of the nutritious fruits because these are rich source of â-carotene, ascorbic acid and folic acid. All these vitamins provide protection against cancer, heart ailments etc. Commercially manufactured citrus products abroad are frozen citrus, juice squash, cordials, concentrates etc. However in India fresh juices are common **(Premi and Hedge, 1998).** Fruit juices are preserved in different forms such as juice, squash, cordials, fermented juice etc **(Girdharilal, 1998).**

2.6.1 Squashes

Squashes consist essentially of strained juice containing moderate quantities of fruit pulp to which cane sugar is added for sweetening. Comminuted squashes were accepted to the consumers and found to cost less than squashes prepared from juice alone **(Girdharilal, 1998).**

2.6.2 RTS (Ready-to-Serve) Beverage

RTS or fruit juice beverage is juice that is considerably altered in composition before consumption. The product is ready to drink **(Girdharilal, 1998).**

2.6.3 Juice Concentrate

Fruit juice concentrate is fruit juice, which has been concentrated by the removal of water either by heat or by freezing. Carbonated beverages and other products are made from this. It is reconstituted by adding back the amount of water originally removed **(Ghirdharilal, 1998).**

2.7 Packaging of Citrus Juice and Processed Products

2.7.1 Glass Bottles

Glass is a senior member of the family of packaging materials and glass bottles are widely used in packaging. Glass containers supplied for food and beverages are available with a number of different surface treatments designed to protect the container from abrasion and to increase lubricity, which facilitates automatic high speed handling of the container. The advantages of glass in food packaging are its transparency, strength, shape, chemical inertness, impermeability, color, recycleability etc. Its disadvantage include heavy weight, fragile, disposable problem etc **(Mahadeviah and Gowramma, 1996).**

2.7.2 Polyethylene tetra phthalate (PET) Bottles

The superb properties of PET make it ideal for packaging a whole host of products ranging from beverages and food products to pharmaceuticals and cosmetics. It has been found that PET bottles can withstand filling at 95^0C. The bottles are crystal clear, pure, safe, good barrier, light weight and found to have design flexibility and are recyclable **(www.bpchemicals.com/napthalate/pdf/N-13Ppackaging.pdf).**

2.7.3 Tetra packaging

Tetra packaging is an aseptic packaging, which refers to the independent sterilization of the food and packaging and subsequent filling and sealing in an absolutely sterile environment. The layers in the tetra pack are made up of the alternating polyethylene, paper and aluminium foil. The paper gives the packaging rigidity, while the aluminium foil serves as an effective gas and aroma barrier. The polyethylene layer serves mainly as bonding material when it occurs between layers of different material (aluminium foil and paper, and aluminium foil and

innermost polyethylene layer) and as a water barrier layer when it is the outermost and innermost layer of the package. Aseptic packaging is used for perishable foods, mainly for juice and soy beverages, but can be used for milk, edible oil and soaps. This packaging keeps the food fresh for months, eliminating the need for refrigeration and preservation **(Klebasz, 1997).**

2.8 Preservation of kinnow juice

The effect of temperature on the stability of processed Kinnow mandarin juice has been studied and was found that heat processing of juice (90^0C for 10min) was quite effective to inactivate the microorganisms. Kinnow juice preserved by SO_2 treatment (350ppm) was found to have superior flavor as compared to heat processed bottle juice **(Kurdiya and Lotha, 1994).**

2.9 Storage Studies

It has been shown that kinnow mandarin fruit stored at ambient temperature (9^0C-24^0C; RH 65-90%) have life of 22 days and the amount of acidity, ascorbic acid, viscosity decreased while the fruit stored at refrigerated temperature (3^0C; RH 70-80%) have shelf life of 60 days and TSS, ascorbic acid were found to increase. Although the juice yield for both was same **(Lotha *et al.*, 1994). Eggested (1998)** reported that the deterioration of the orange juice during storage comprises of organoleptic, quality, nutritional value and appearance, also reported that the main parameters that influence the deterioration of orange juice is the temperature and the presence of oxygen. The orange juice packed in paper cartons has been found to lose55% of vitamin C over the test period while the juice packed in cartons with a medium barrier lost 15% of vitamin C and the one packed with high oxygen barrier. Aluminium foil layer lost negligible amount of vitamin C during the same time period **(Raymond A *et al.*, 1998).** Packaging material has been found to play an important role on flavor during storage of 3 months. Glass, as a control, was reported to be the best material to keep fresh

orange juice flavor during refrigerated storage. Hydrocarbon such as limonene, myrecene and á-pinene were found to sorb in greater extend by polyethylene packages and PET were the best materials to keep fresh orange juice **(Ayhan *et al.*, 2000)**. It has been shown that the washing with sodium hypochlorite extend 2-3 days the storage life of the juice (time to reach the microbial count of 10^6 cfu/ml) in the packaging film while the use of the organic acid, potassium sorbate, sodium benzoate (1.66-6.94mM) led the storage life values of more than 11days and 20 days in the low gas permeability film maintaining good sanitary conditions **(Andres *et al.*, 2001).**

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Material Procurement

3.1.1 Kinnow

Fresh and fully ripened kinnows were procured from the local market in Patiala, in the months from October '02 to April '03.

3.1.2 Chemicals and Packaging Material

All the chemicals used in the present investigation were of analytical grade (AR). Limonin was procured from Sigma Aldrich Corp, MO, USA; and naringin from Acros Organics, New Jersey, USA. Autoclavable screw capped glass bottles were used for packaging kinnow squash, kinnow RTS/RTD beverage, frozen kinnow juice concentrate and refrigerated kinnow juice concentrate. PET bottles used for packaging of kinnow RTS/RTD beverage were a gift from Beverly Beverages, Patiala.

3.2 Juice Extraction

Kinnow juice was extracted using:

a) Electric Juicer (Philips India Limited, Kolkata)
b) FMC Citrus Juicer (FMC Corporation- Citrus Machinery and Services Division, Lakeland, Florida).

3.3 Production of Bitterless Kinnow Juice

The bitterless Kinnow juice was produced by the method as shown in Fig 3.1

3.3.1 Pretreatment of Fruits

Procured Kinnows were washed, wiped and then stored at 4^0C in the cold chamber.

3.3.2 Juice Extraction

Juice was extracted by the 'Modified method of bitterless kinnow juice extraction' developed in the Department of Biotechnology and Environmental Sciences on which a patent has been filed in January 2003, Vide Patent Application No. 60/Del/2003 dated 22.1.2003. The juice was extracted in ~ 250 ml lots.

3.3.3 Pasteurization

The 250 ml lots of extracted juice were then clubbed together to around 3 liter in autoclavable glass bottles and this was then pasteurized by the in-bottle method of pasteurization in boiling water bath (95^0-100^0C) for 3, 3.5, 4, 4.5 and 5 min with constant shaking.

3.3.4 Cooling and Storage

The pasteurized juice in the bottle was cooled under the running tap water and then stored under refrigeration (~4^0C) till further use.

3.4 Analyses of Kinnow Juice

The extracted (bitterless) kinnow was analyzed for various physico-chemical and sensory attributes. The main focus was to determine the limonin content that was monitored daily for a period of 7 days to confirm stability in its level so as to render its process ability to various products. The details of the analyses are given in section 3.6. Once the bitterless juice was obtained, it was processed into kinnow squash, kinnow RTS/RTD beverage, frozen kinnow juice concentrate and refrigerated kinnow juice concentrate.

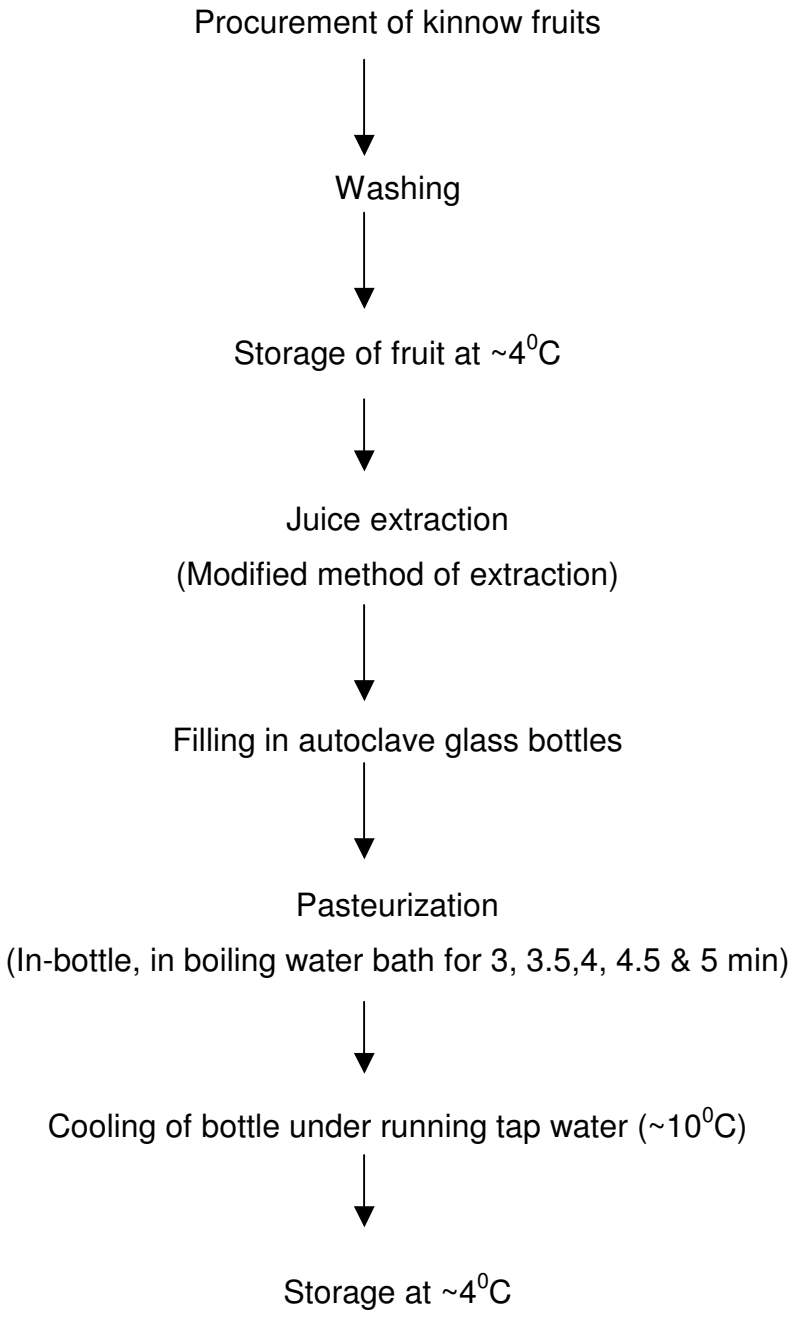


Fig 3.1 Flow chart showing methodology for producing 'bitterless kinnow juice'

3.5 Processing of Bitterless Kinnow Juice

3.5.1 Kinnow Squash

Fruit juice squash consists essentially of strained juice containing moderate quantities of fruit pulp to which cane sugar is added for sweetening. The kinnow juice was processed as Kinnow Squash according to the method for preparing orange squash as described by **Lal *et al.,* (1998)**. The flow chart for the preparation of kinnow squash is shown in Fig. 3.2

3.5.1.1 Bitterless Kinnow Juice

Bitterless kinnow juice was extracted as described in section 3.3 and was then pasteurized (in-bottle for 3 min) and juice was then used for preparing kinnow squash.

3.5.1.2 Addition of ingredients

660 ml of syrup (62^0 Brix), citric acid (0.6%) and KMS @ 350ppm were added in 1liter of bitterless kinnow juice as per FPO specifications. All the ingredients were then mixed well.

3.5.1.3 Storage

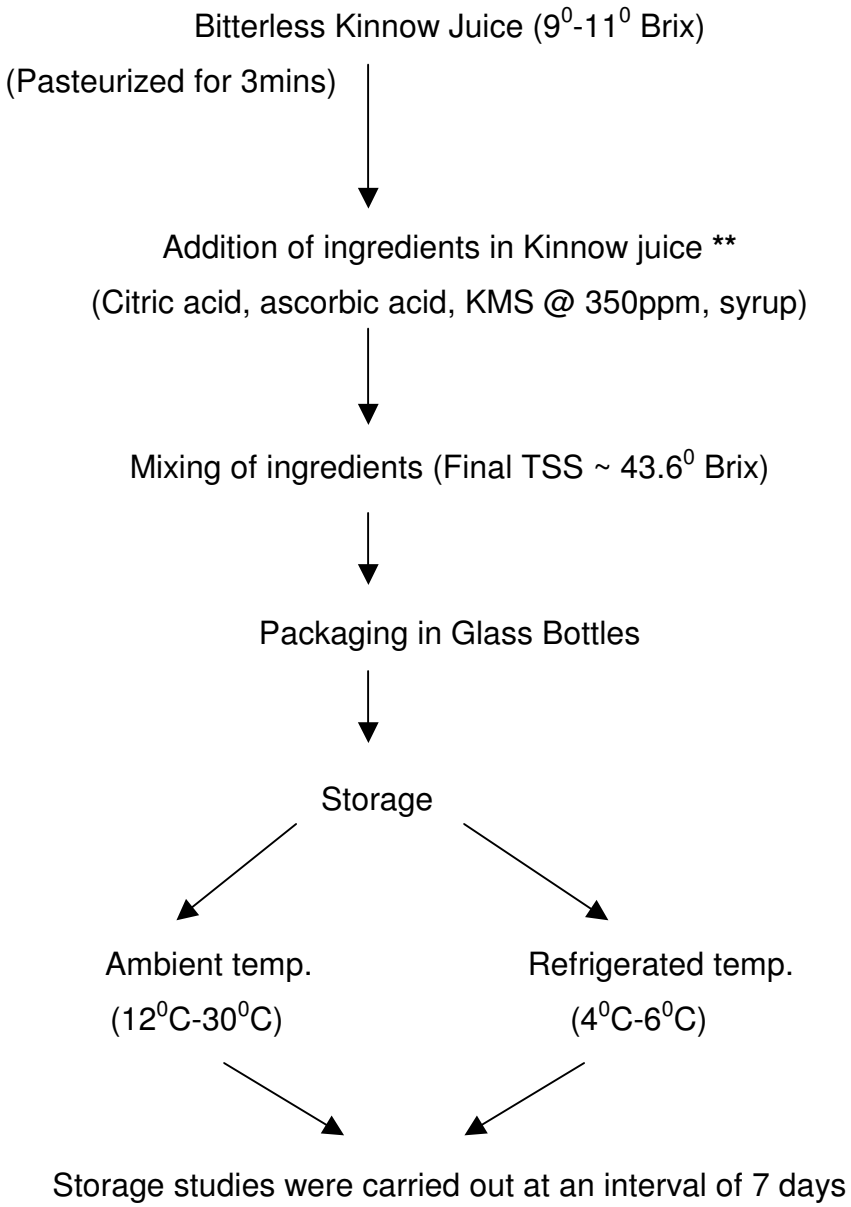
The prepared squash was packed in glass bottles and was stored at two different temperatures- ambient (12^0C-30^0C) and refrigeration temperature (4^0C-6^0C).

3.5.2 Preparation of Kinnow Squash by using Glucose Syrup

The details for preparing kinnow squash by glucose syrup are described in Fig 3.3.

3.5.2.1 Bitterless Kinnow Juice

Bitterless kinnow juice, extracted as described in section 3.3 was in-bottle pasteurized for 3 min and used for preparing of kinnow squash.



** No color & flavor added in the product externally

Fig.3.2 Flow chart showing methodology for producing kinnow squash

3.5.2.2 Addition of Ingredients

330ml of glucose syrup (91.6^0 Brix), citric acid (0.6%) and KMS @ 350ppm were added in 1liter of bitterless Kinnow juice. All the ingredients were then mixed well.

3.5.2.3 Storage

The prepared squash was packed in glass bottles and stored at refrigeration temperature (4^{0}C-6^{0}C).

3.5 3 Preparation of Kinnow RTS (Ready to Serve)/RTD (Ready to Serve) Beverage

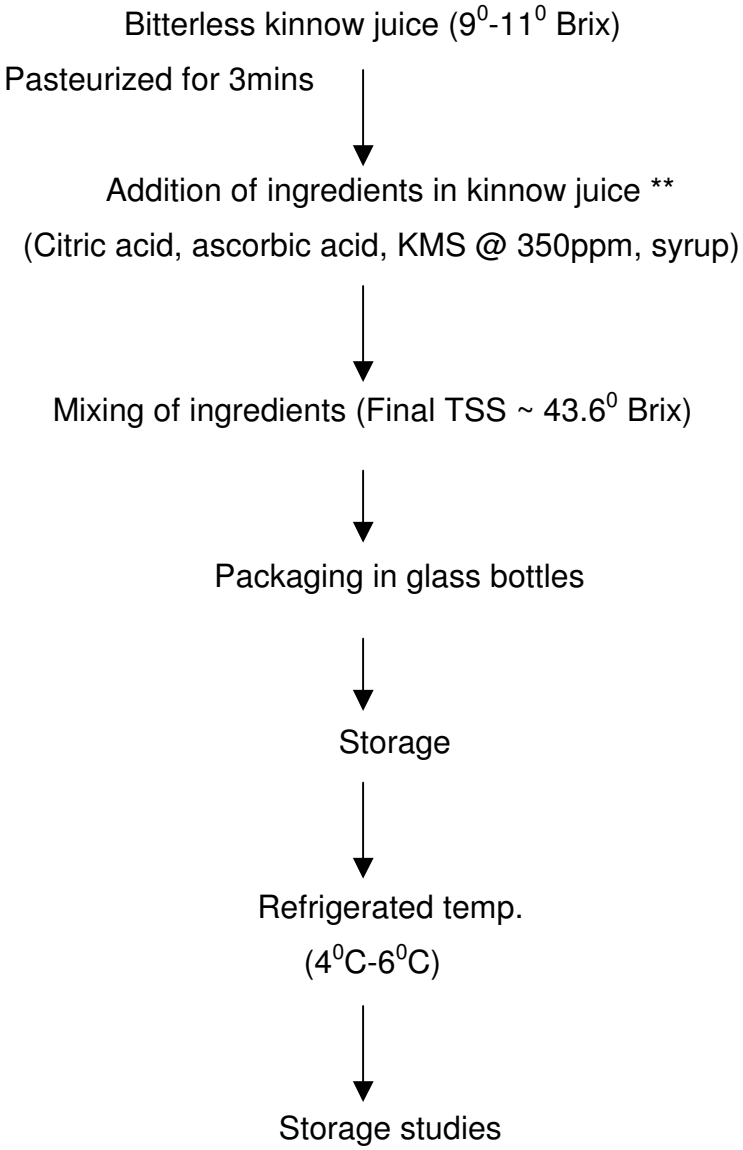
Fruit juice RTS is a fruit juice which is considerably altered in composition before packing. As the pack opened the juice is ready to drink. Kinnow juice was processed as Kinnow RTS (Ready to Serve) beverage according to the methodology described in Fig 3.4.

3.5.3.1 Bitterless Kinnow Juice

Juice used for processing was extracted as described in section 3.3.

3.5.3.2 Optimization of Kinnow RTS/RTD Beverage

Various trials for preparation of 100ml kinnow RTS/RTD beverage were conducted to make the desired final product. 10ml, 20ml and 30ml syrup (62^0 Brix) was taken separately in 3 bottles and 90ml, 80ml & 70ml of juice was respectively added to each bottles. The best product in the terms of taste, flavor, one having desired TSS (^{0}Brix) was taken and used in making of higher amounts (1000 ml) of the beverage.



** No color & flavor added in the product externally

Fig.3.3 Flow chart showing methodology for producing kinnow squash by using glucose syrup

3.5.3.3 Preparation of Kinnow RTS/RTD Beverage

To the bitterless kinnow juice (1000ml), was added 800ml of the syrup, 0.2% citric acid and KMS @ 350ppm. All the ingredients were then mixed well.

3.5.3.4 Packaging and Storage

The beverage thus prepared was packed in glass bottles as well as in PET bottles and was then stored under refrigeration (4^0C-5^0C).

3.5.4 Preparation of Kinnow RTS (Ready to Serve)/RTD (Ready to Serve) Beverage by using Glucose Syrup

The details of the preparation given in Fig 3.5

3.5.4.1 Bitterless Kinnow Juice

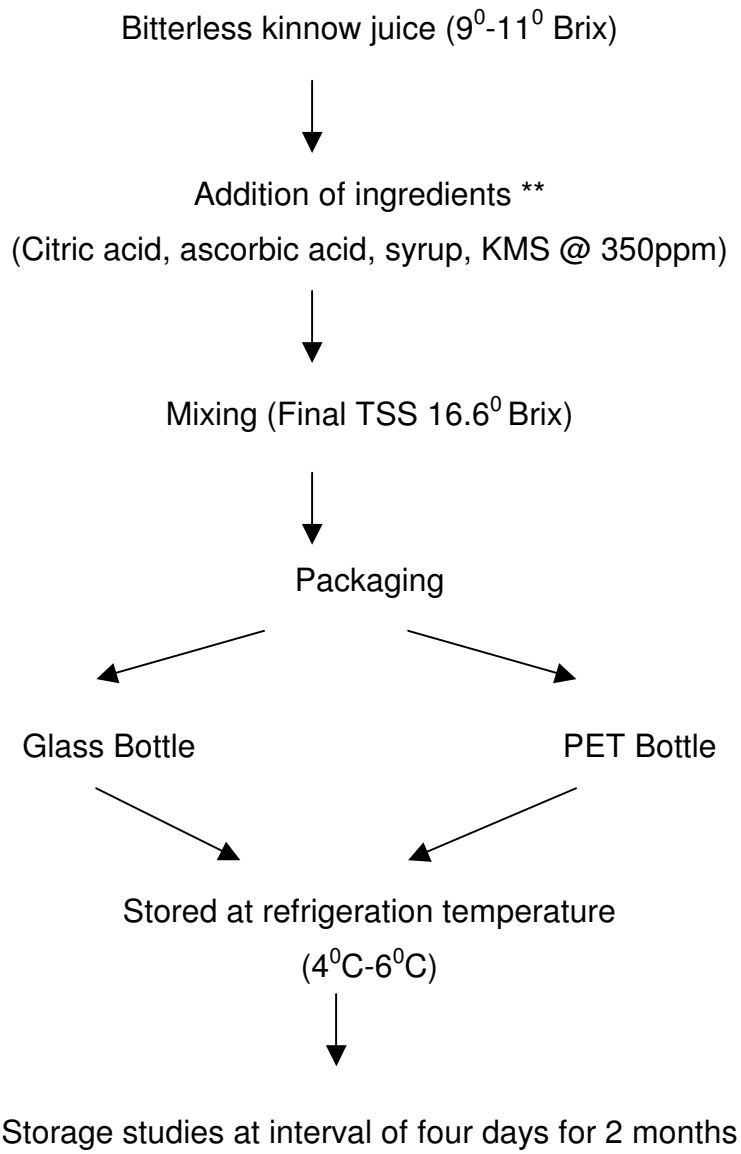
Juice used for processing was extracted as described in section 3.3.

3.5.4.2 Preparation of Kinnow RTS/RTD Beverage

To the bitterless kinnow juice (1000ml) was added 300ml of the glucose syrup, 0.2% citric acid and KMS @ 350ppm. All the ingredients were then mixed well.

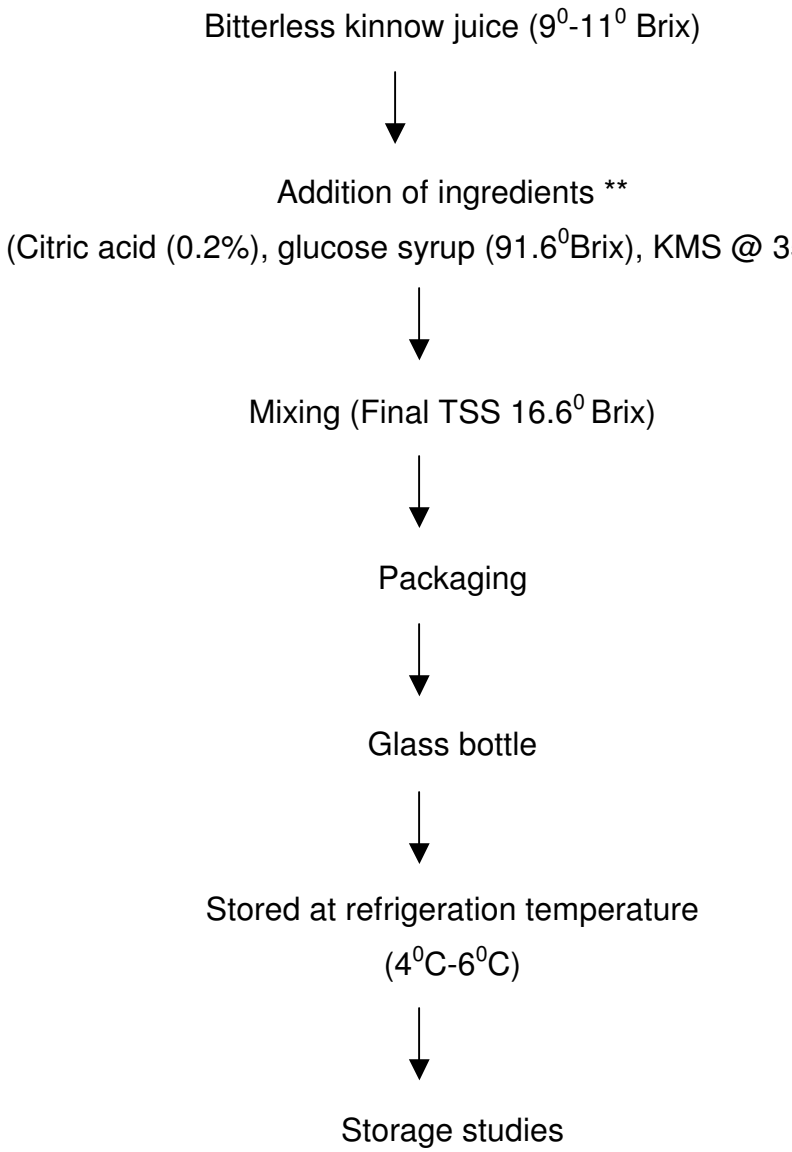
3.5.4.3 Packaging and Storage

The beverage thus prepared was packed in glass bottles and stored under refrigeration (4^0C-5^0C).



** No color & flavor added in the product externally

Fig 3.4 Flow chart showing methodology for the preparation of kinnow RTS (Ready to Serve) beverage



** No color & flavor added in the product externally

Fig 3.5 Flow chart showing methodology for the preparation of kinnow RTS (Ready to Serve) beverage by using glucose syrup

3.5.5 Preparation of Frozen Kinnow Juice Concentrate and Refrigerated Kinnow Juice Concentrate

Fruit juice concentrate is the fruit juice that has been concentrated by the removal of water either by heat or by freezing. Carbonated beverages and other products were made from this. The method used to prepare kinnow juice concentrate is described in Fig.3.6

3.5.5.1 Bitterless Kinnow Juice

Kinnow juice extracted by the method described in section 3.3 was used for the preparation of kinnow juice concentrate

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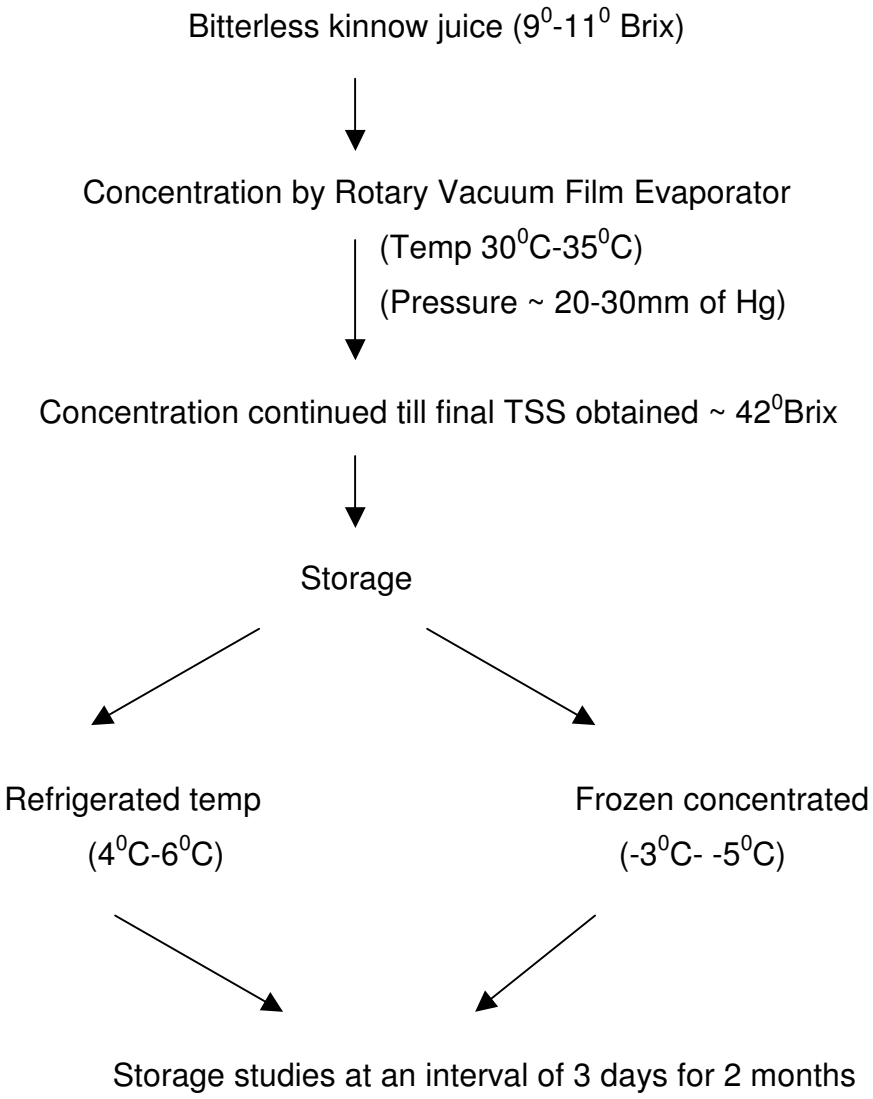
3.5.5.2 Concentration

The juice was concentrated to four folds in rotary vacuum film evaporator at the temperature of 30^0C-35^0C and pressure ~20-30mm of Hg.

3.5.5.3 Packaging and Storage

The concentrate was packed in glass bottles and then divided in two parts, one bottle was stored at refrigeration temperature (4^0C-6^0C) as Refrigerated Kinnow Juice Concentrate and the other was stored at –3^0C to –5^0C as Frozen Kinnow Juice Concentrate.



** No color & flavor added in the product externally

Fig 3.6 Flow chart for the preparation of kinnow juice concentrate

3.6 Storage Studies of Kinnow Juice, Kinnow Squash, Kinnow RTS/RTD Beverage and Frozen Kinnow Juice Concentrate and Refrigerated Kinnow Juice Concentrate

In order undertake storage studies of the processed products, samples were withdrawn from the three products kept at different temperature and/or packaging materials, and then were analyzed for various attributes. Kinnow squash, kinnow RTS/RTD beverage and frozen/refrigerated kinnow juice concentrate were analyzed for various attributes respectively on every 7^{th}, 4^{th}& 3^{rd} day, and shelf life studies were carried out for a period of 2 months.

3.6.1 Physico-chemical Analyses

3.6.1.1 pH: pH of the kinnow squash, kinnow RTS/RTD beverage, frozen kinnow juice concentrate and refrigerated kinnow juice concentrate was measured using electric/digital pH meter (Decibels Instruments India Limited).

3.6.1.2 Total Soluble Solids (TSS): TSS (^{0}Brix) of kinnow RTS/RTD beverage was measured by Hand Refractometer of range of 0-32^{0}Brix and for measuring TSS (^{0}Brix) of kinnow squash, frozen kinnow juice concentrate and refrigerated kinnow juice concentrate, 28-45 ^{0}Brix range refractometer was used.

3.6.1.3 % Titrable Acidity: the %titrable acidity of kinnow squash, kinnow RTS/RTD beverage and frozen kinnow juice concentrate and refrigerated kinnow juice concentrate was estimated by the method described by **AOAC (1984).** The kinnow squash, kinnow RTS/RTD beverage were diluted 5 times, and frozen and refrigerated kinnow juice concentrates were diluted 10 times with glass-distilled water. The known volume of the diluted sample was titrated with 0.1N NaOH with phenolphthalein as indicator to faint pink end point. The % titrable acidity is calculated by the following formula-

$$\% \text{ TA} = \frac{\text{Titre} \times \text{Normality of alkali} \times \text{volume made up} \times \text{Eq.wt of acid}}{\text{Volume of sample} \times \text{wt/vol taken for estimation} \times 1000}$$

3.6.1.4 Ascorbic Acid Estimation: Ascorbic acid content of the kinnow squash, kinnow RTS/RTD beverage, frozen kinnow juice concentrate and refrigerated kinnow juice concentrate was estimated by the 2,6 dichlorophenol indophenol visual titration method **(Ranganna, 1998).**

Standardization of Dye: 5ml of the standard ascorbic acid (0.1mg/ml) and 5ml of 3% meta phosphoric acid were taken. This was titrated with the dye solution to the pink color, which persisted for 15 sec. Dye factor is determined by the formula:

$$\text{Dye Factor} = 0.5 / \text{Titre}$$

The kinnow squash, kinnow RTS/RTD beverage and frozen kinnow juice concentrate and refrigerated kinnow juice concentrate sample were individually prepared in 3% meta phosphoric acid solution. To 10ml of each sample, 90ml of the acid was added. Out of this prepared sample, 10ml was taken and titrated against the 2, 6-dichlorophenol-indophenol dye solution till the pink end point obtained which persisted for at least 15sec. The ascorbic acid amount was estimated by the following formula:

$$\text{mg of ascorbic acid} = \frac{\text{Dye Factor} \times V_1 \times 100\text{ml} \times 100\text{ml}}{V_2 \times 5\text{ml} \times \text{Volume of Sample}}$$

Where V_1 and V_2 are the volume of the ascorbic acid used for standardization and volume of sample used for titration respectively.

3.6.2 Limonin Estimation

Standard curve preparation:

Limonin stock solution: To 0.2g of pure limonin Sigma Alrich Corp, MO, USA, was added 1ml of chloroform and 200ppm stock solution was prepared.

Limonin reagent: Limonin reagent was prepared with 100mg of dimethylbenzaldehyde and 3ml of glacial acetic acid +70% perchloric acid.

Method: 5 to 50µl pure limonin was drawn from stock solution in test tube and the volume was made to 1ml with chloroform. 3ml of the limonin reagent was added to each and mixed thoroughly using vortex. After the incubation period of 30mins at room temperature, the optical density of the upper red layer was noted at 503nm and the standard curve was drawn.

Limonin estimation of the samples kinnow Squash, kinnow RTS/RTD beverage, frozen kinnow juice concentrate and refrigerated kinnow juice concentrate were also done in similar manner, where instead of pure limonin, samples were taken. The limonin content was calculated from standard limonin curve.

3.6.3 Naringin Estimation

Standard curve preparation:
Stock solution: 50mg standard naringin was dissolved in 10ml of distilled water.
Reagents used: 90% diethylene glycol, 4N NaOH

Method: Different concentration of naringin stock were taken in a test tube (from 0.1 to 1ml), the volume in each was made upto 1ml with distilled water. To each of the test tube, 5ml of diethylene glycol and 100ì l of 4N NaOH was added. Optical density was measured at 420nm.

Naringin content of each sample was estimated in the same way by replacing the standard naringin with juice serum. The naringin content in the samples was estimated from the standard curve.

3.6.4 Microbial Analysis

The microbial analysis of all preserved products- kinnow Squash, kinnow RTS Beverage and frozen kinnow juice concentrate and refrigerated kinnow juice concentrate were carried out accordingly to **A.P.H.A (1992).** 1ml of each of the sample was taken and to this 9ml of 0.5% saline was added and then further diluted to four folds. 1ml of each from appropriate dilution was plated in required medium and then incubation was carried out. In each count, after incubation, the average count of colonies present on petriplates were multiplied by dilution factor and expressed as cfu (colony forming unit)/ml of sample.

3.6.3.1 Total Plate Count:

The total plate count was taken according to the method described in **A.P.H.A (1992)**. Appropriate dilutions of each of the samples were transferred aseptically to sterile petriplates. Pour plating was done using nutrient agar (Hi media Laboratories Pvt.Ltd, Mumbai) **(Appendix I).** Plates were then incubated at 37^0C for 24-48 hours respectively.

3.6.3.2 Yeast and Mold Count

Appropriate dilution of sample were pour plated in Potato Dextrose Agar (Hi Media Laboratories Pvt Ltd Mumbai) **(Appendix II)** for 3-5 days at 30^0C

3.6.3.3 Coliform Count

Appropriate dilution of samples were made and transferred to sterile pertiplates, pour plating was done using Mac conkey agar, pH 7.4 (Hi Media Laboratories, Pvt Ltd, Mumbai) **(Appendix III)**. Plates were incubated at 37^0C for 48hours.

3.6.4 Sensory Evaluation

Sensory evaluation was done on the 9 point Hedonic scale **(Ranganna, 1998)** by the group of semi trained panel from Department of Biotechnology and Environmental Sciences, TIET, Patiala and Center of Relevance and Excellence (CORE), TIET, Patiala which included faculty members and research scholars. The evaluation was done according to the following attributes- color, flavor, mouth feel and over all acceptability. The obtained results were recorded in sensory sheet **(Appendix IV).**

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

The current investigation was focused on controlling the development of bitterness due to limonin, the hitherto persistent problem in extracted kinnow mandarin juice. The attempts were navigated on exclusively preventive approach to curb the formation of limonin that is responsible for delayed bitterness. Such bitterless kinnow juice was then processed into products. Naringin content in kinnow juice and processed products was also monitored during the course of study. Thus the present investigation envisaged optimizing the method of extraction of *bitterless kinnow juice* (upto 3 liters at lab scale) and processing of such juice into kinnow squash, kinnow RTS/RTD beverage, frozen kinnow juice concentrate, refrigerated kinnow juice concentrate. All along with these, steps were undertaken to study the self-stability of processed products. The findings of the current investigation are presented here forth.

4.1 Optimizing of bitterless juice extraction method at the scaled up levels

In the present study, it was observed that only electric juicer could lead to production of bitterless juice. **Kamaljeet (2002)** also reported that only electric juicer could lead to production of bitterless juice and that the bitterness was found to appear in juice extracted by FMC and centrifugal juicer. In the present investigation the bitterless juice was extracted by 'Modified method of bitterless kinnow juice extraction' developed (lab scale) in Department of Biotechnology and Environmental Sciences, Thapar Institute of Engineering and Technology, Patiala and the patent has been filed on this process in January 2003, Vide Patent Application No. 60/Del/2003 dated 22.1.2003.

4.1.1 Extraction of Bitterless Kinnow juice

The modified method calls for certain pretreatments on the fruits before juice extraction, and subsequently pasteurization of the extracted juice by in-bottle

method of pasteurization using boiling water bath. 250ml lots of juice were extracted and all were then clubbed together to around 3 liter in autoclavable glass bottles, which were then pasteurized. Finally bitterless pasteurized juice thus obtained was cooled and was then preserved by the SO_2 treatment and stored at low temperature. Thus the combination method of preservation was employed.

4.1.1.1 Optimization of time interval for adequate pasteurization

In order to optimize the time period required for pasteurization of kinnow juice, trials for different time period – 3min, 3.5min, 4min, 4.5min & 5min were undertaken. The best combination of time period at the set temperature for pasteurization was selected based on organoleptic properties and limonin content of cooled pasteurized juice. The details of time period for pasteurization are shown in table 4.1

Sl.no	Pasteurization time (min)	Remarks
1	3	Bitterless & uncooked
2	3.5	Bitterless & uncooked
3	4	Bitterless & uncooked
4	4.5	Bitterless & cooked
5	5	Bitterless & cooked

Table 4.1 Effect of the time of pasteurization on the limonin content & organoleptic properties

The optimal time for pasteurization was found to be 3 min because there was no appearance of cooked flavor and the prime objective of controlling the development of limonin was met with.

4.1.1.2 Monitoring of Limonin Content in the Extracted (bitterless) Juice

The objective of extracting bitterless kinnow juice was to process it into various products - kinnow squash, kinnow RTS/RTD beverage, frozen kinnow juice concentrate and refrigerated kinnow juice concentrate. Then after the juice extraction, the limonin content was monitored on hourly basis at the frequency of 4hours, till a period of 7days. The values were found to be less than threshold (6 ppm) as shown in Table 4.2. Only after the amount of limonin was found between 2 to 4 ppm, such bitterless juice was then directed towards processing.

It is a worth mentioning observation that the early season kinnow fruits (early November) invariably posed difficulties in processing, as the bitterless was found to prevail in amounts higher than threshold levels (~9 to 21 ppm) in such juice. This is in consonance with the reports of various authors who also observed that bitterness was much higher in early season citrus fruits. Thus only mid and late season kinnow could be utilized in extraction of bitterless juice and its further processing in the present study.

Sl no.	**Hours**	**Limonin content (ppm)**				
Time (min)		**3.0**	**3.5**	**4.0**	**4.5**	**5.0**
1	0	1.0	1.0	1.0	1.0	1.0
2	4	1.0	1.0	1.0	1.0	1.0
3	8	1.0	1.0	1.0	1.0	1.0
4	12	1.0	1.0	1.0	1.0	1.0
5	24	1.6	1.6	1.6	1.6	1.6
6	28	1.6	1.6	1.6	1.6	1.6
7	32	1.6	1.6	1.6	1.6	1.6
8	36	1.6	1.6	1.6	1.6	1.6
9	48	1.6	1.6	1.6	1.6	1.6
10	52	1.6	1.6	1.6	1.6	1.6
11	56	1.6	1.6	1.6	1.6	1.6
12	60	1.6	1.6	1.6	1.6	1.6
13	72	1.6	1.6	1.6	1.6	1.6
14	96	1.6	1.6	1.6	1.6	1.6
15	120	1.6	1.6	1.6	1.6	1.6
16	144	1.6	1.6	1.6	1.6	1.6
17	168	1.6	1.6	1.6	1.6	1.6

Table 4.2 Limonin content of juice on four hourly basis for a period of 7 days

4.2 Processing of Bitterless Kinnow Juice and Storage Study of the Processed Products

After thorough examination of juice over the period of 7days from bitterness point of view, the bitterless juice was processed into kinnow squash, kinnow RTS/RTD beverage, frozen Kinnow juice concentrate and refrigerated Kinnow juice concentrate. The products thus processed were analyzed for physico-chemical attributes namely pH, TSS (^{0}Brix), %TA (as citric acid) and ascorbic acid (mg/100ml). Limonin content of each product was periodically monitored over the entire storage period and naringin content was analyzed on the zero and the last day of storage. Microbiological examination comprising of Total Plate Count, Yeast and Mold count and coliforms were examined periodically and subjective test (organoleptic test) was undertaken in order to check the acceptability of the products throughout the course of investigation. The storage study of the two-month period for each product is presented below, and storage study of each product is still in process.

4.2.1 Kinnow Squash

The squash produced by the methodology described in section 3.5.1 was kept at two different temperatures – ambient temperature (12^0C-30^0C) and refrigerated temperature (4^0C-6^0C). It was noted that the squash so prepared was found to be stable for a period of 2months, the period till which the investigation was carried out. The values obtained for various parameters as analyzed are shown in Table 4.3 & 4.4.

The pH of the squash kept at ambient temperature was found to range between 3.58-3.44 and between 3.58-3.48 for squash kept at refrigerated temperature. No significant difference was found in the pH of the squashes stored at different temperature. TSS (^{0}Brix) ranged between 38-42^{0}Brix and 43.6-43^{0}Brix respectively. Some lowering in TSS (^{0}Brix) values of squash stored at ambient

temperature was observed. %TA (as citric acid) in squash stored at ambient temperature was found to reduce from 0.49 to 0.35, while in the squash stored at refrigeration temperature no change in %TA (0.44%) was observed during the entire storage period. As expected the ascorbic acid content was found to slightly decrease during the storage in squashes at two different temperatures (14.4-12mg/100ml at ambient storage condition and 14.4-13.2mg/100ml at refrigerated storage conditions). **Purthi et al (1983)** reported the ascorbic acid content as 13.3 – 6.9 mg/100ml of kinnow juice. Details are shown in Table 4.3 and Table 4.4.

So the changes in various parameters were found to be relatively higher in squashes stored at ambient temperature. Importantly it was noticed that the limonin content of the squash kept at two different remained below the threshold level (4ppm) during the entire storage period, thus confirming the organoleptic acceptability of the products stored at two different temperatures. Limonin content was found to be similar for both the storage conditions (Table 4.5). **Kamaljeet (2002**) reported level of limonin ranging from 5-6ppm in kinnow squash at the end of 3months of storage period. Thus the values obtained in present investigation are little lower than reported by the said author. It calls for attention that after the period of 2weeks slight increase in limonin of squash kept under two storage temperature was observed (2-3.4ppm) which remained stable thereafter. Naringin content was also estimated on first and the last day of storage period, the amount of which was found to be below the threshold value (500-600ppm) as shown in Fig 4.2.

Microbiological examination of the product revealed that coliforms were found to be absent in the squashes throughout the period of investigation. The yeast and mold colony appeared after 31days and TPC after 56 days of storage in case of squash stored at ambient temperature as shown in Table 4.6. It is worth mentioning that till 35days there was no colony observed under any microbiological examination in kinnow squash kept at refrigerated temperature

(Table 4.7). Thus showing the product was prepared and stored under hygienic conditions.

Sensory evaluation

Organoleptic evaluation carried out on every 7th day of storage showed that the kinnow squash thus prepared was quite acceptable till the end of the storage period. No observable differences in organoleptic properties were found among the kinnow squashes at two different temperatures. **Kamaljeet (2002)** reported a shelf life of kinnow squash of over 3months. Thus the storage period obtained in current investigation are in consonance with those obtained by **Kamaljeet (2002).**

S No	Days	pH	TSS (^{0}Brix)	%TA (citric acid)	AA (mg/100ml)
1	0	3.58	43.6	0.44	14.40
2	7	3.46	43.4	0.43	14.05
3	14	3.45	38.4	0.38	13.70
4	21	3.45	38.2	0.38	13.20
5	28	3.44	38.0	0.35	12.50
6	35	3.44	38.0	0.35	12.00
7	42	3.44	38.0	0.33	11.75
8	49	3.42	38.0	0.33	11.50
9	56	3.42	38.0	0.33	11.50
10	63	3.42	38.0	0.30	11.22

TSS Total soluble solids %TA %Titrable acidity AA Ascorbic acid

Table 4.3 Changes in physicochemical attributes of the kinnow squash stored at ambient temperature during the storage period

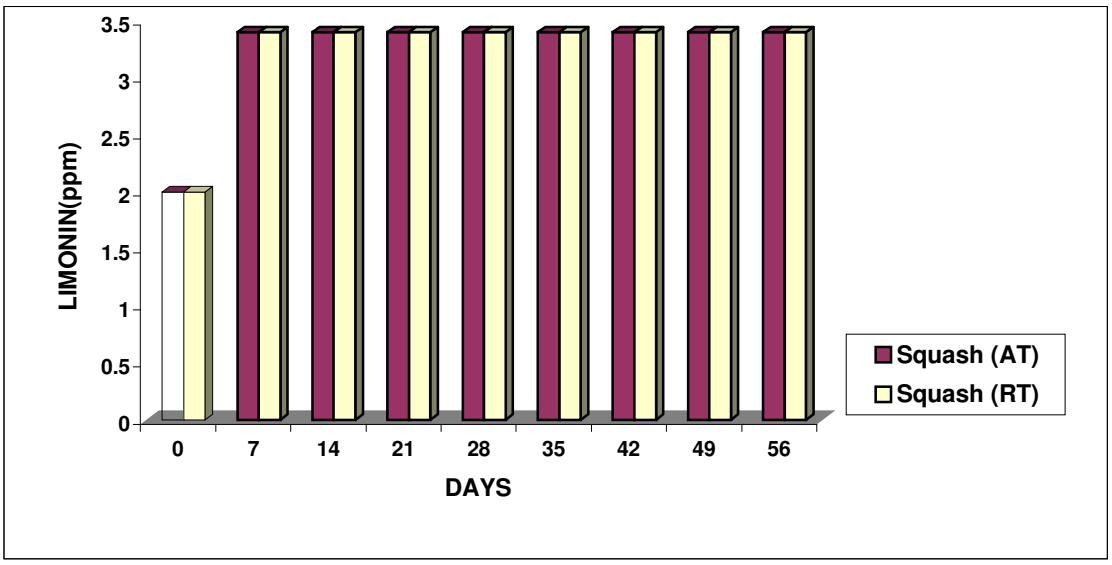
S No	Days	pH	TSS (^{0}Brix)	TA (%citric acid)	AA (mg/100ml)
1	0	3.58	43.6	0.44	14.4
2	7	3.57	43.4	0.44	14.2
3	14	3.48	43	0.44	14.2
4	21	3.48	43	0.44	13.8
5	28	3.48	43	0.44	13.8
6	35	3.48	43	0.44	13.2
7	42	3.47	43	0.44	12.5
8	49	3.47	43	0.44	12.5
9	56	3.47	43	0.44	12.00
10	63	3.47	43	0.44	11.75

TSS Total soluble solids %TA %Titrable acidity AA Ascorbic acid

Table 4.4 Changes in physicochemical attributes of the kinnow squash stored at refrigerated temperature during the storage period

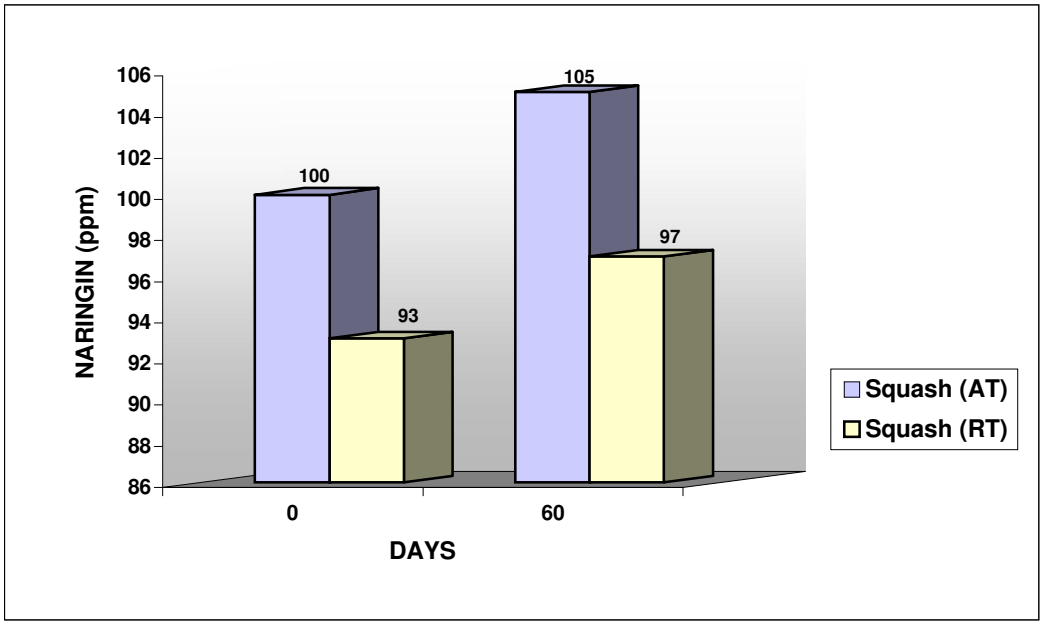
S No.	Days	Limonin content (ppm) in squash stored at AT	Limonin content (ppm) in squash stored at RT
1	0	2.0	2.0
2	7	2.0	2.0
3	14	3.4	3.4
4	21	3.4	3.4
5	28	3.4	3.4
6	35	3.4	3.4
7	42	3.4	3.4
8	49	3.4	3.4
9	56	3.4	3.4
10	63	3.4	3.4

Table 4.5 Limonin content in kinnow squash stored at ambient and refrigerated temperature



AT Ambient temperature RT Refrigeration temperature

Fig 4.1 Limonin content of kinnow squash stored at two different temperatures



AT Ambient temperature
RT Refrigeration temperature

Fig 4.2 Naringin content of the kinnow squash stored at two different temperatures

S No	Days	TPC (cfu/ml)	Y&M Count (cfu/ml)	Coliform Count
1	0	ND	ND	ND
2	7	ND	ND	ND
3	14	ND	ND	ND
4	21	ND	ND	ND
5	28	ND	ND	ND
6	35	ND	ND	ND
7	42	2×10^2	ND	ND
8	49	2×10^2	ND	ND
9	56	3×10^2	ND	ND
10	63	3×10^2	1×10^2	ND

ND Not detected

Table 4.6 Microbial analyses of the squash stored at ambient temperature

S No	Days	TPC (cfu/ml)	Y&M Count (cfu/ml)	Coliform Count
1	0	ND	ND	ND
2	7	ND	ND	ND
3	14	ND	ND	ND
4	21	ND	ND	ND
5	28	ND	ND	ND
6	35	ND	ND	ND
7	42	2×10^2	ND	ND
8	49	2×10^2	ND	ND
9	56	3×10^2	ND	ND
10	63	3×10^2	ND	ND

ND Not detected

Table 4.7 Microbial analysis of the Squash stored at refrigerated temperature

4.2.2 Kinnow RTS/RTD Beverage

The kinnow RTS/RTD beverage prepared according to the methodology described in section 3.5.2 was stored at the same temperature (4-6^{0}C) in two different packaging materials in glass bottles and polyethylene tetraphthate (PET) bottles. The analyses were made every fourth day of storage and the values obtained are presented in Table 4.8 & 4.9. It can be inferred that similar trend in values of pH, TSS (0Brix), %TA (as citric acid) and ascorbic acid (mg/100ml) were obtained for the beverage stored in the two types of packaging material. The readings for RTS stored in glass bottles and PET bottles respectively were as follows pH 4.07-4.05 and 4.07-4.02, TSS 16.6-16.2^{0}Brix and 16.5-16^{0}Brix, %TA (as citric acid) 0.38-0.28 and 0.38-0.28%, ascorbic acid (mg/100ml) 17.34-12 and 17.34-12.0 respectively.

Limonin content was found to remain unaffected by the usage of different packaging material used for kinnow RTS/RTD beverage. The amount of which

remained below threshold. As is seen in kinnow squash slight increase in the level of limonin was found initially in case of kinnow RTS/RTD beverage (2-4ppm) that remained stable thereafter (Table 4.10). Naringin content remained below threshold as shown in Fig 4.4.

The microbiological examination revealed that during the entire storage period no coliforms could be enumerated. Yeast and mold was also not found in RTS stored in both type of packaging material till days. Incidentally, TPC was found after 20 days of storage in case of beverage stored in glass bottles which could be attributed to some aerial contamination that might have been encountered while analyses were made as the bottles were opened and closed several times during the course of study; but no TPC found in beverage stored in PET bottles(Table 4.11 & 4.12).

S No	Days	pH	TSS (^{0}Brix)	%TA (citric acid)	AA (mg/100ml)
1	0	4.08	16.6	0.38	17.34
2	4	4.08	16.6	0.35	17.07
3	8	4.08	16.4	0.30	16.80
4	12	4.08	16.2	0.30	15.75
5	16	4.08	16.2	0.30	14.70
6	20	4.08	16.2	0.30	14.65
7	24	4.07	16.2	0.30	14.65
8	28	4.07	16.2	0.28	12.50
9	32	4.07	16.2	0.28	12.50
10	36	4.07	16.2	0.28	12.00
11	40	4.07	16.2	0.28	11.75
12	44	4.07	16.2	0.28	11.50
13	48	4.05	16.2	0.28	11.50
14	52	4.05	16.0	0.28	11.50
15	56	4.05	16.0	0.28	10.75
16	60	4.05	16.0	0.28	10.75

TSS Total soluble solids %TA %Titrable acidity AA Ascorbic acid

Table 4.8 Changes in physicochemical attributes of the kinnow RTS/RTD beverage stored in glass bottle

S No	Days	pH	TSS (^{0}Brix)	%TA (citric acid)	AA (mg/100ml)
1	0	4.07	16.5	0.38	17.34
2	4	4.07	16.4	0.33	16.47
3	8	4.07	16.2	0.30	15.60
4	12	4.06	16.0	0.30	14.65
5	16	4.06	16.0	0.30	13.80
6	20	4.06	16.0	0.30	13.20
7	24	4.04	16.0	0.30	13.20
8	28	4.04	16.0	0.30	12.50
9	32	4.02	16.0	0.30	12.00
10	36	4.02	16.0	0.28	12.00
11	40	4.02	16.0	0.28	11.75
12	44	4.01	16.0	0.28	11.50
13	48	4.01	16.0	0.28	11.22
14	52	4.01	16.0	0.28	11.22
15	56	4.00	16.0	0.28	11.22
16	60	4.00	16.0	0.28	10.71

TSS Total soluble solids %TA %Titrable acidity AA Ascorbic acid

Table 4.9 Changes in physicochemical attributes of the kinnow RTS/RTD beverage stored in PET bottle period

S No	Days	Limonin content (ppm) in RTS stored at glass bottle	Limonin content (ppm) in RTS stored at glass bottle
1	0	2	2
2	4	2	2
3	8	2	2
4	12	4	4
5	16	4	4
6	20	4	4
7	24	4	4
8	28	4	4
9	32	4	4
10	36	4	4
11	40	4	4
12	44	4	4
13	48	4	4
14	52	4	4
15	56	4	4
16	60	4	4

Table 4.10 Limonin content in kinnow RTS/RTD beverage stored in glass and PET bottle

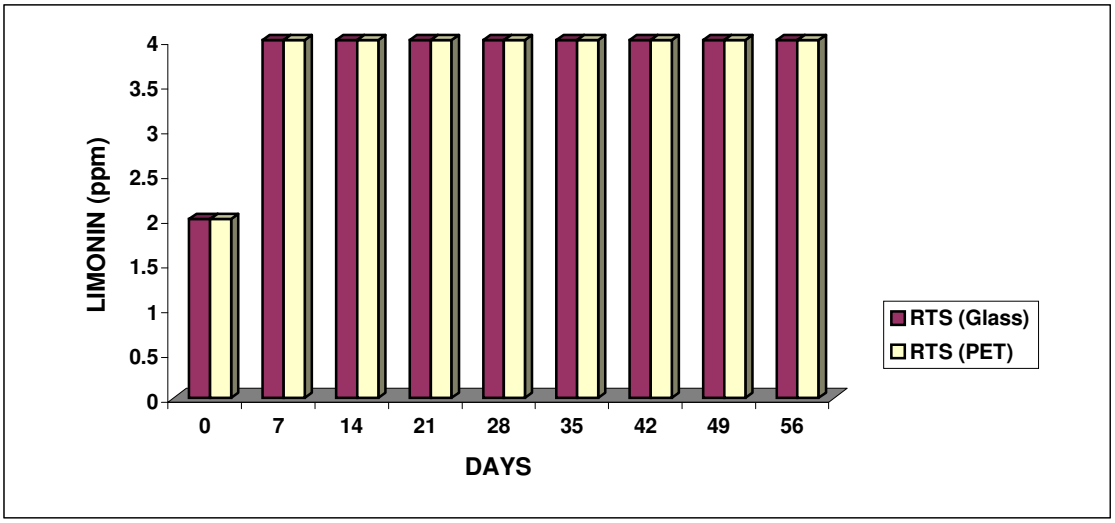


Fig 4.3 Limonin content of the kinnow RTS/RTD beverage stored in two different packaging materials.

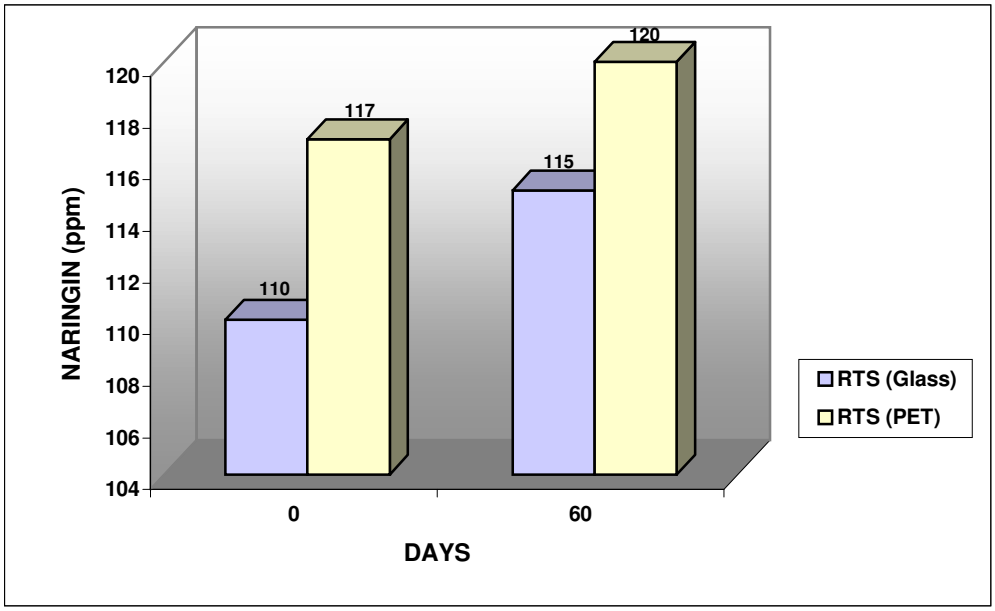


Fig 4.4 Naringin content of the kinnow RTS/RTD beverage stored in two different packaging materials

S No	Days	TPC (cfu/ml)	Y&M count (cfu/100ml)	Coliform Count
1	0	ND	ND	ND
2	4	ND	ND	ND
3	8	ND	ND	ND
4	12	ND	ND	ND
5	16	ND	ND	ND
6	20	ND	ND	ND
7	24	2×10^2	ND	ND
8	28	2×10^2	ND	ND
9	32	3×10^2	ND	ND
10	36	3×10^2	10	ND
11	40	3×10^2	ND	ND
12	44	5×10^2	ND	ND
13	48	5×10^2	ND	ND
14	52	3×10^2	10	ND
15	56	3×10^2	20	ND
16	60	4×10^2	20	ND

ND Not Detected

Table 4.11 Microbial analysis of the kinnow RTS/RTD beverage stored in glass bottle

S No	Days	TPC (cfu/ml)	Y&M count (cfu/100ml)	Coliform Count
1	0	ND	ND	ND
2	4	ND	ND	ND
3	8	ND	ND	ND
4	12	ND	ND	ND
5	16	ND	ND	ND
6	20	ND	ND	ND
7	24	2×10^2	ND	ND
8	28	2×10^2	ND	ND
9	32	3×10^2	ND	ND
10	36	3×10^2	ND	ND
11	40	3×10^2	ND	ND
12	44	4×10^2	20	ND
13	48	4×10^2	20	ND
14	52	4×10^2	20	ND
15	56	3×10^2	10	ND
16	60	6×10^2	20	ND

ND Not Detected

Table 4.12 Microbial analysis of the kinnow RTS/RTD beverage stored in PET bottle

4.3 Frozen Kinnow Juice Concentrate and Refrigerated Kinnow Juice Concentrate

After processing the bitterless kinnow juice into kinnow squash and kinnow RTS/RTD beverage, attempts were made to process the kinnow juice into kinnow concentrate of two types viz frozen kinnow juice concentrate (stored at -3^0Cto-8^0C) and refrigerated kinnow juice concentrate (stored at 4^0C-6^0C). Following is the analyses of the mentioned two concentrates.

Among the physico-chemical attributes pH, TSS (0Brix), %TA (as citric acid) and ascorbic acid (mg/100ml) content of the two frozen kinnow juice concentrate and refrigerated kinnow juice concentrate were found to be nearly same during the entire storage period of 2months. The values of said parameters were found to as

follows- pH 4.5-4.37, TSS 40-42^{0}Brix, %TA (as citric acid) 0.56-0.51% for frozen kinnow juice concentrate and pH 4.5-4.37 TSS 40-42^{0}Brix, %TA (as citric acid) 0.56-0.51%for refrigerated kinnow juice concentrate. Thus it was observed that in named parameters changes were found to be similar for both types of concentrates. The ascorbic acid content of the two products was found to decrease from 28.2-19.3 mg/100ml, thus confirming that certain significant loss of ascorbic acid occurred during storage (Table 4.13. & 4.14). Such observation of loss of ascorbic acid has also been reported by **Kamaljeet (2002)** in kinnow juice, which reduced from 46.9 to 13.3 mg/100ml.

The limonin content of the products were found to range between 3.3-4.2ppm as indicated by Fig 4.5. Thus confirming that with juice obtained by modified method of juice extraction, concentrate could be produced using the available varieties of kinnow fruits of mid and late season. Similar to the observations made in kinnow squash, kinnow RTS/RTD beverage, the values of limonin remained nearly same throughout the storage period. Naringin content, which was estimated on the first and last day of storage study, was found to be 179ppm for frozen kinnow juice concentrate and 182ppm for refrigerated kinnow juice concentrate (Fig 4.6).

The microbiological examination of the two concentrates produced from kinnow juice revealed that no observable growth of any form could be seen till 4 weeks of storage in frozen kinnow juice concentrate and 3 weeks of storage in refrigerated kinnow juice concentrate. No coliforms were found in either of the concentrates. In the total plate count one colony appeared in refrigerated kinnow juice concentrate after 1 month of storage and yeast and mold remained absent till 36 days (Table 4.16 & 4.17).

The condensate obtained during the course of concentration of kinnow juice was analyzed for pH, TSS (^{0}Brix), %TA (as citric acid) and ascorbic acid mg/100ml. pH was found to be 4.02; while TSS (^{0}Brix), %TA (as citric acid) &

ascorbic acid (mg/100ml) were almost negligible while limonin content estimation revealed the presence of limonin in the condensate as the amount obtained was ~ 2.3ppm.

S No	Days	pH	TSS (^{0}Brix)	%TA (citric acid)	AA (mg/100ml)
1	0	4.45	42.0	0.56	28.2
2	3	4.44	42.0	0.56	27.0
3	6	4.44	42.0	0.56	25.2
4	9	4.44	42.0	0.56	23.4
5	12	4.43	42.0	0.54	23.4
6	15	4.43	42.0	0.54	22.2
7	18	4.43	42.0	0.54	22.2
8	21	4.43	42.0	0.54	22.2
9	24	4.43	41.8	0.54	20.5
10	27	4.42	41.8	0.54	19.8
11	30	4.42	41.8	0.54	19.8
12	33	4.42	41.8	0.54	19.3
13	36	4.42	41.8	0.54	19.8
14	39	4.42	41.8	0.54	19.3
15	42	4.42	41.6	0.52	19.3
16	45	4.41	41.6	0.52	19.3
17	48	4.40	41.6	0.52	19.2
18	51	4.40	41.6	0.52	18.6
19	54	4.40	41.6	0.52	18.6
20	57	4.40	41.6	0.52	18.3
21	60	4.40	41.4	0.52	18.3

TSS Total soluble solids %TA %Titrable acidity AA Ascorbic acid

Table 4.13 Changes in physicochemical attributes of the frozen kinnow juice concentrate

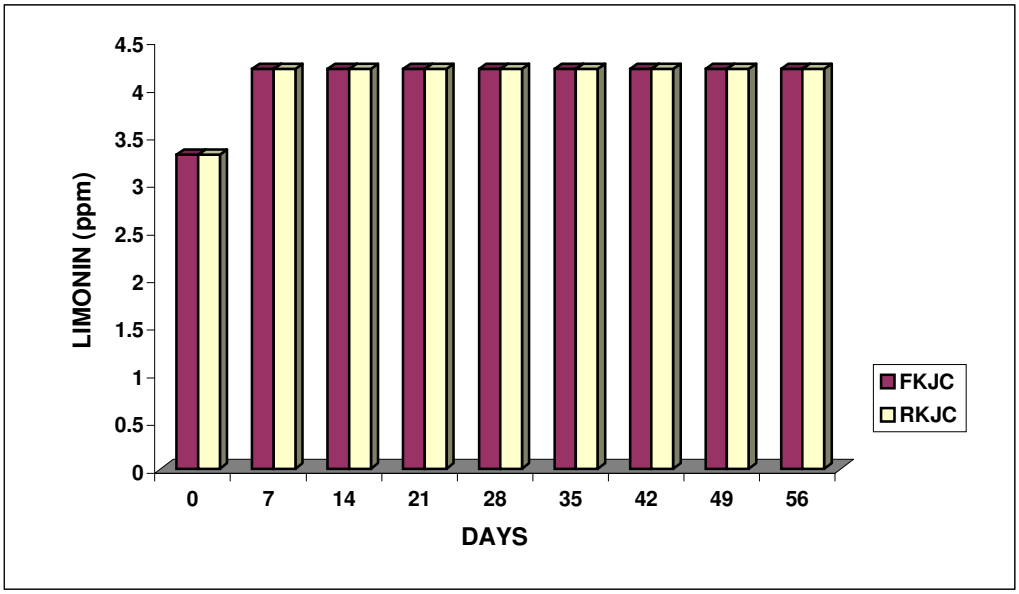
S No	Days	pH	TSS (^{0}Brix)	%TA (citric acid)	AA (mg/100ml)
1	0	4.45	42.0	0.54	28.8
2	3	4.50	42.0	0.54	26.1
3	6	4.42	41.2	0.54	23.4.
4	9	4.41	40.0	0.54	22.8
5	12	4.41	40.0	0.54	22.2
6	15	4.40	40.0	0.54	21.6
7	18	4.40	40.0	0.54	21.6
8	21	4.38	40.0	0.52	20.0
9	24	4.38	40.0	0.52	19.5
10	27	4.38	40.0	0.52	19.5
11	30	4.37	40.0	0.52	19.3
12	33	4.37	40.0	0.52	19.3
13	36	4.37	40.0	0.52	19.3
14	39	4.37	40.0	0.52	18.6
15	42	4.37	39.8	0.52	18.6
16	45	4.37	39.8	0.52	18.6
17	48	4.37	39.8	0.52	18.6
18	51	4.36	39.8	0.51	17.8
19	54	4.36	39.8	0.51	17.4
20	57	4.36	39.8	0.51	16.3
21	60	4.36	39.8	0.51	16.3

TSS Total soluble solids %TA %Titrable acidity AA Ascorbic acid

Table 4.14 Changes in physicochemical attributes of the refrigerated kinnow juice concentrate

S No	Days	Limonin content (ppm) in frozen kinnow juice concentrate	Limonin content (ppm) in refrigerated kinnow juice concentrate
1	0	3.3	3.3
2	3	3.3	3.3
3	6	3.3	3.3
4	9	4.2	4.2
5	12	4.2	4.2
6	15	4.2	4.2
7	18	4.2	4.2
8	21	4.2	4.2
9	24	4.2	4.2
10	27	4.2	4.2
11	30	4.2	4.2
12	33	4.2	4.2
13	36	4.2	4.2
14	39	4.2	4.2
15	42	4.2	4.2
16	45	4.2	4.2
17	48	4.2	4.2
18	51	4.2	4.2
19	54	4.2	4.2
20	57	4.2	4.2
21	60	4.2	4.2

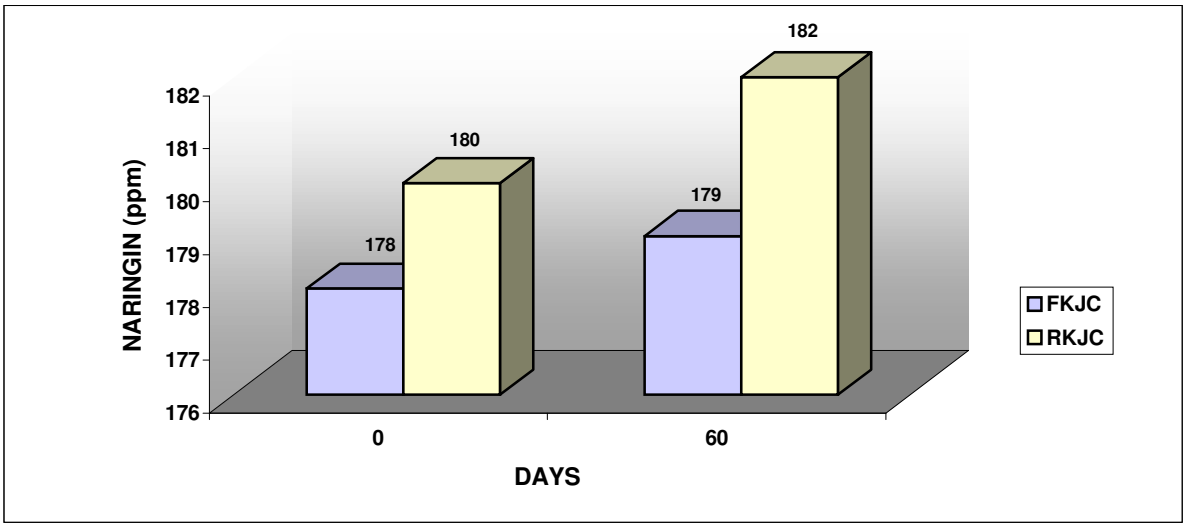
Table 4.15 Limonin content in frozen and refrigerated kinnow juice concentrate



FKJC Frozen kinnow juice concentrate

RKJC Refrigerated kinnow juice concentrate

Fig 4.5 Limonin content of the two types of concentrates



FKJC Frozen kinnow juice concentrate

RKJC Refrigerated kinnow juice concentrate

Fig 4.6 Naringin content of the two types of concentrates

S No	Days	TPC (cfu/ml)	Y&M count (cfu/100ml)	Coliform Count
1	0	ND	ND	ND
2	3	ND	ND	ND
3	6	ND	ND	ND
4	9	ND	ND	ND
5	12	ND	ND	ND
6	15	ND	ND	ND
7	18	$2x10^2$	ND	ND
8	21	$1x10^2$	ND	ND
9	24	$2x10^2$	ND	ND
10	27	$1x10^2$	ND	ND
11	30	$1x10^2$	ND	ND
12	33	$4x10^2$	ND	ND
13	36	$3x10^2$	ND	ND
14	39	$5x\ 10^2$	10	ND
15	42	$6x10^2$	10	ND
16	45	6x102	10	ND
17	48	6x102	30	ND
18	51	6x102	10	ND
19	54	8x102	30	ND
20	57	8x102	30	ND
21	60	8x102	30	ND

ND **Not detected**

Table 4.16 Microbial analysis of the frozen kinnow juice concentrate

S No	Days	TPC (cfu/ml)	Y&M count (cfu/100ml)	Coliform Count
1	0	ND	ND	ND
2	3	ND	ND	ND
3	6	ND	ND	ND
4	9	ND	ND	ND
5	12	ND	ND	ND
6	15	ND	ND	ND
7	18	ND	ND	ND
8	21	ND	ND	ND
9	24	ND	ND	ND
10	27	$1x10^2$	ND	ND
11	30	$1x10^2$	ND	ND
12	33	$1x10^2$	ND	ND
13	36	$1x10^2$	ND	ND
14	39	$1x10^2$	ND	ND
15	42	$4x10^2$	10	ND
16	45	$3x10^2$	10	ND
17	48	$3x\ 10^2$	30	ND
18	51	$3x10^2$	10	ND
19	54	3x102	10	ND
20	57	$4x10^2$	20	ND
21	60	$3x10^2$	20	ND

ND **Not detected**

Table 4.17 Microbial analysis of the refrigerated kinnow juice concentrate

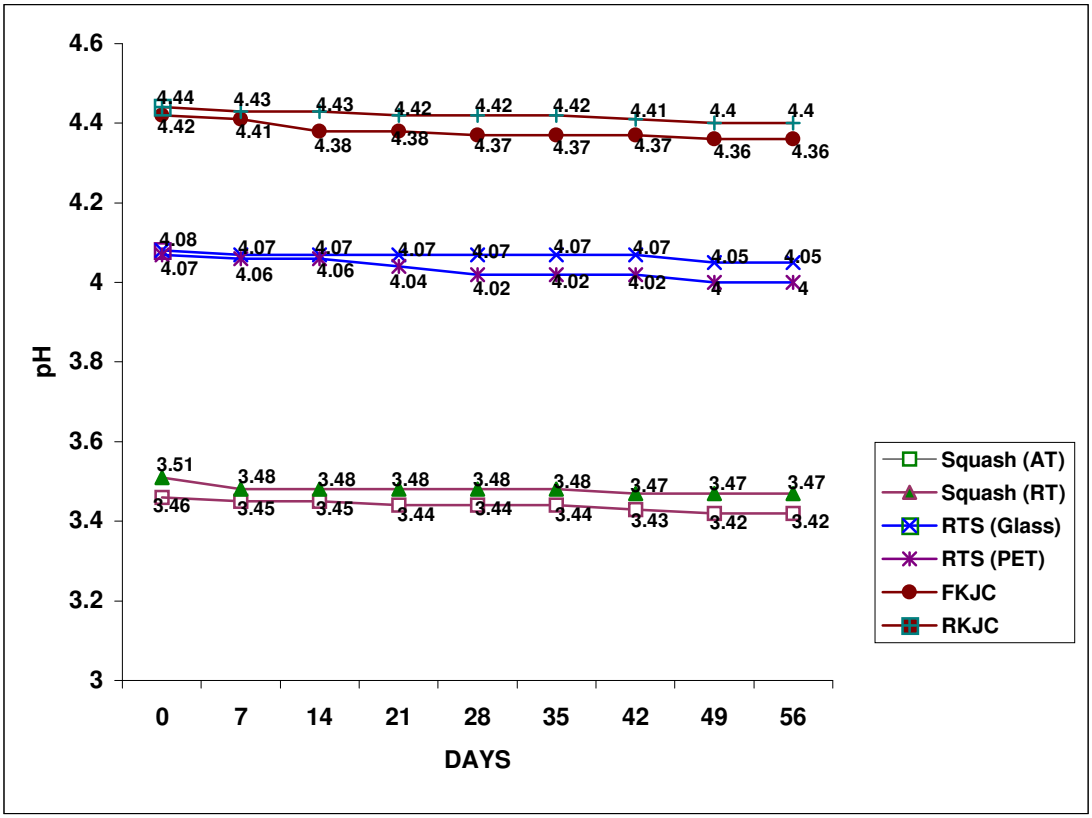
4.4 Comparison of the physico-chemical attributes of the various processed products

Comparison of the pH of all products were made and from Fig 4.7 it can be seen that the lowest pH was observed in kinnow squash followed by Kinnow RTS/RTD beverage while juice concentrate was found to have the highest pH. Thus it can be inferred that the pH of the concentrates were close to juice and addition of ingredients (citric acid and ascorbic acid) led to fall in pH of kinnow squash and kinnow RTS/RTD beverage.

Similarly comparing the total soluble solids of all products, it can be inferred from Fig 4.8 that, owing to addition of syrup in kinnow squash, the TSS (^{0}Brix) was found to be maximum amongst all the processed products.

Fig 4.9 reveals that differential variation in %TA (as citric acid) was observed amongst various products. Kinnow squash stored at ambient temperature showed maximum decrease in %TA (as citric acid) from 0.44% to 0.30% where as squash stored at refrigerated temperature had most constant %TA (as citric acid). Only slight variation was observed in %TA (as citric acid) values of juice concentrates. It can be seen from the mentioned figure, significant decrease in %TA (as citric acid) was observed in kinnow RTS/RTD beverage stored in both glass and PET bottles.

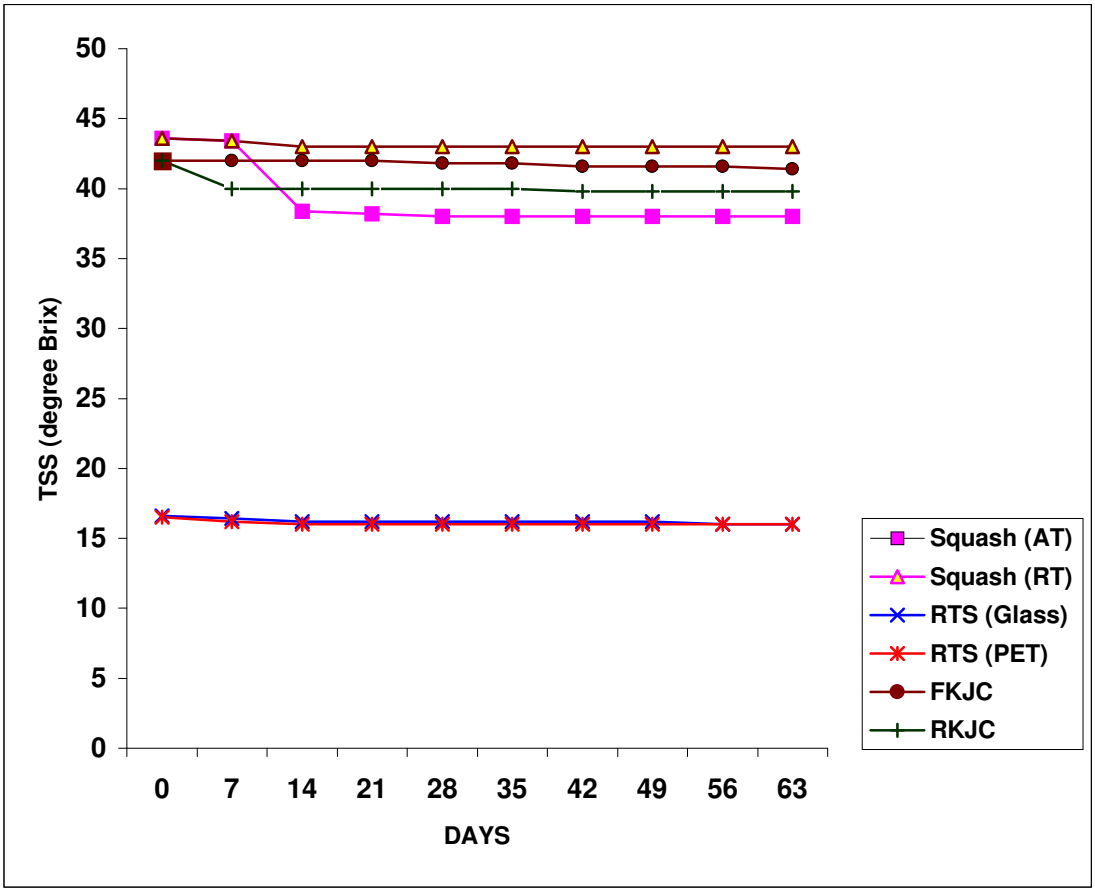
As expected, there was significant decrease in ascorbic acid (mg/100ml) amount in all the products. It can be inferred from the Fig 4.10 there was notable decrease in ascorbic acid (mg/100ml) in both the concentrates while there was slight decrease in the ascorbic acid (mg/100ml) amount in case of kinnow squash and kinnow RTS/RTD beverage.



AT Ambient temperature RT Refrigeration temperature

FKJC Frozen kinnow juice concentrate RKJC Refrigerated kinnow juice concentrate

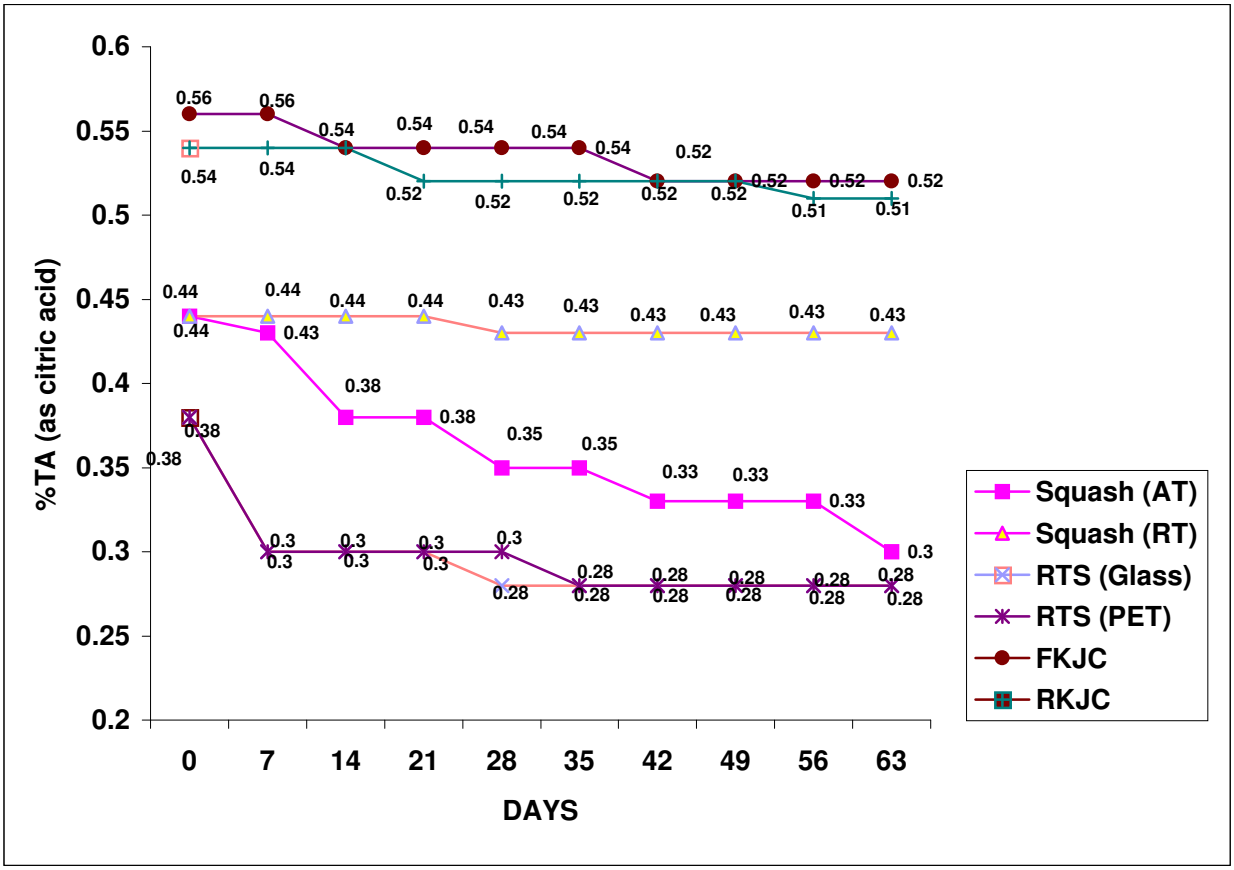
Fig 4.7 pH profile of the products obtained by processing of the bitterless kinnow juice



AT Ambient temperature RT Refrigeration temperature

FKJC Frozen kinnow juice concentrate RKJC Refrigerated kinnow juice concentrate

Fig 4.8 TSS (^{0}Brix) profile of the products obtained by processing of the bitterless kinnow juice



AT Ambient temperature RT Refrigeration temperature

FKJC Frozen kinnow juice concentrate RKJC Refrigerated kinnow juice concentrate

Fig 4.9 %TA (as citric acid) profile of the products obtained by processing of the bitterless kinnow juice

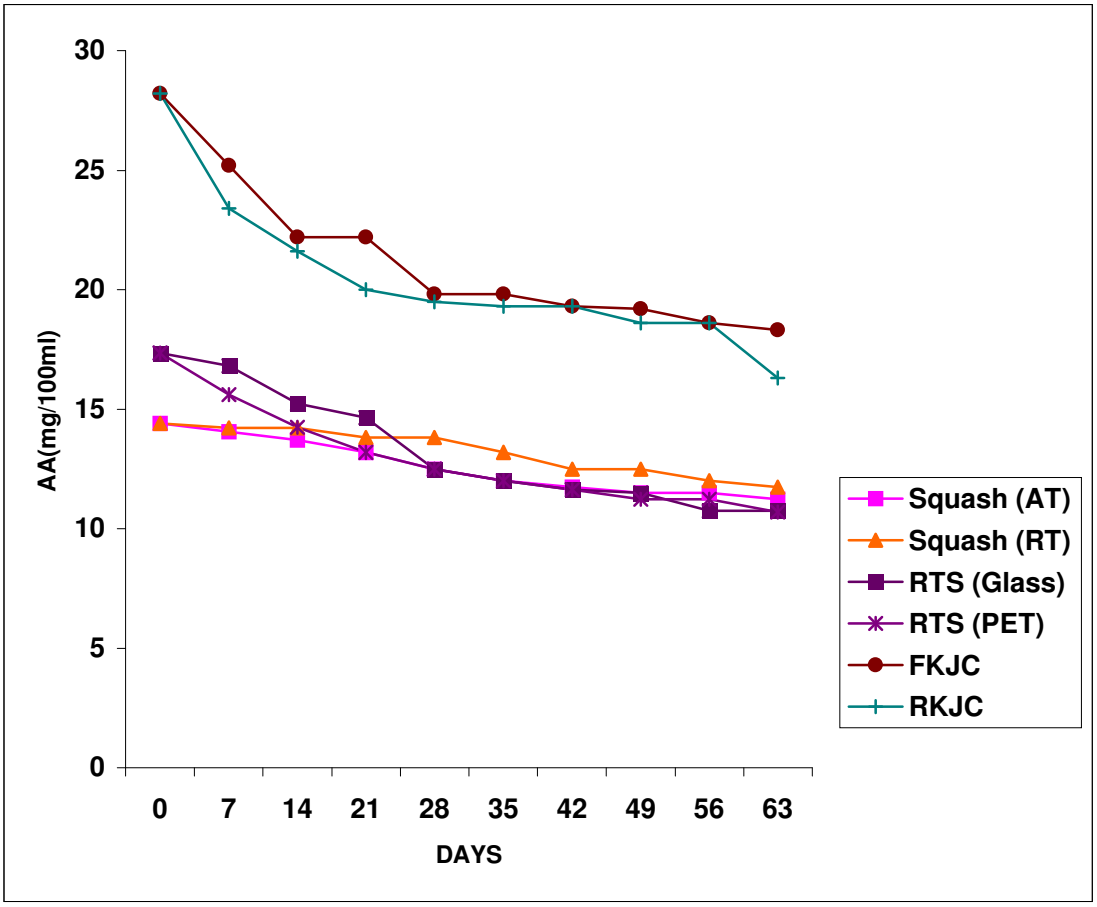


Fig 4.10 Ascorbic acid (mg/100ml) profile of the products obtained by processing of the bitterless kinnow juice

4.5 Kinnow Juice Powder

The attempts were also taken for preparation of kinnow juice powder by freeze-drying, but very little success was obtained. It could be due to technical problems associated with lyophilizer and thermoplastic nature of kinnow concentrate.

4.6.1 Path forward

Path forward is to under take pilot scale studies for bitterless kinnow juice extraction and subsequently it's processing to the products as kinnow squash, kinnow RTD/RTD beverage, kinnow juice concentrate & kinnow juice powder. Also attempts are underway for tetra packaging of kinnow juice and kinnow RTS/RTD beverage.

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

The present is an application of modified method of bitterless kinnow juice extraction and was attempted to follow preventive approach by controlling the development of bitterness due to limonin. The bitterless juice thus obtained was processed into products kinnow squash, kinnow RTS/RTD beverage, frozen kinnow juice concentrate and refrigerated kinnow juice concentrate, each of which was optimized. Thereafter, the storage studies were conducted for evaluation of the shelf stability of all the processed products for period of about 60 days. The summarized findings are presented here forth:

1. The juice was extracted by “Modified method of bitterless kinnow juice extraction” and only electric juice could aid in extraction of such juice.

2. Only mid and late season kinnow fruit could be utilized for the extraction of bitterless kinnow juice. The limonin content in such juice was found to range from (2 to 4ppm). Such juice had pH ranging from 3.84 to 4.20. TSS 9.2 to 11^0Brix, %TA (0.4 – 0.5%) and ascorbic acid 14 – 15 mg/100ml.

3. The best time interval for in bottle pasteurization of kinnow juice was found to be 3min.This was based on organoleptic acceptability as well as limonin content of cooled pasteurized juice.

4. Processing of the juice into various products was carried out only after monitoring the limonin content for period of 7 days.

5. Optimization of squash done as per FPO specification and the final TSS was maintained at 43.6^0Brix, packaging was done in glass bottles. Limonin content of both types of squash was found to be in the ranging of (2 to 3.4 ppm). During shelf life evaluation period, slight decrease in pH, %TA (as

citric acid) & ascorbic acid (mg/100ml) was observed. Significant decrease in TSS value (43.6 to 38^0Brix) of kinnow squash kept at ambient temperature was observed during 2 weeks of storage, thereafter it was stable till the end of storage.

6. The juice was then processed into RTS and optimization was done following FPO specifications. Final TSS was kept at ~16.6^0Brix and the beverage were packed in glass and PET bottles, both stored under refrigeration. Like in squash, the limonin content of beverages was noted below threshold level (2 to 4 ppm). During the course of investigation, slight decrease in pH, %TA (as citric acid), ascorbic acid (mg/100ml) in both RTS/RTD beverages was observed.

7. Finally kinnow juice concentrate was prepared by using Rotary flash film evaporator and the final TSS ~42^0Brix after it was stored in glass bottles under frozen and refrigeration after KMS treatment @350ppm.The initial and final limonin content of both the concentration was found to range from 3.3 to 4.2 respectively.

8. Naringin content of each product was estimated first and last day of the storage study and it was observed that in all products naringin content was much below the threshold level, (600ppm). The values of naringin obtained were 96.5ppm - 101ppm in kinnow squash, 113.5ppm - 117.5ppm in kinnow RTS, and 179ppm - 180.5ppm in kinnow concentrate.

9. The microbiological examination of products revealed that processing and storage study was under safe conditions. No coliforms were found in any of the products.

10. Sensory evaluation of all the products was suggestive of appreciable organoleptic appeal obtained in terms of taste and overall acceptability.

Little lower scores for appearance were obtained as no external addition of color and flavor was done. The approach was to process the sample products as natural, with out addition of external color and flavors.

11. The attempts were also taken for preparation of lyophilized kinnow juice powder by freeze-drying but very little success was obtained. It could be due to technical problems associated with lyophilizer and thermoplastic nature of kinnow concentrate.

Thus, it can be concluded from the current study that the modified method of extraction can be utilized for extracting bitterless juice, which can be processed into various consumers relished products. Thus attempting in the pilot scale extraction and processing of such juice would be have the ability of high production and steps are underway.

APPENDICES

APPENDICES

APPENDIX- I

Nutrient Agar

Beef extract	3g
Peptone	5g
Agar	15g
Water	1000ml
pH	7.0

APPENDIX- II

Potato Dextrose Agar

Potato infusion from	300g
Dextrose	20g
Agar	15g
Water	1000ml
pH	5.6

APPENDIX- III

Mac-conkey Agar

Peptone	2%
Lactose	1%
Bile salt	0.5%
NaCl	0.5%
Agar	1.5%
pH	7.4

APPENDIX- IV

Hedonic Scale

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

SENSORY EVALUATION SHEET

Kindly evaluate the given Kinnow Squash/Kinnow Ready-To-Drink Beverage on the Hedonic scale (1 to 9) according to the attributes mentioned below.

*Kindly indicate whether the provided sample is **bitter** or not.*

S.No.	Sample	Color	Flavor	Mouthfeel	Overall Acceptability
1.	Squash				
2.	Beverage				

Remarks:

Signatures

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