DEVELOPMENT OF PROCESS TECHNOLOGY FOR ENHANCING SHELF-LIFE OF SWEET ORANGE JUICE

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CERTIFICATE

This is to certify that the thesis entitled **"DEVELOPMENT OF PROCESS TECHNOLOGY FOR ENHANCING SHELF-LIFE OF SWEET ORANGE JUICE"** submitted by **Er. LAVANYA, D** for the degree of **MASTER OF TECHNOLOGY (AGRICULTURAL ENGINEERING)** in **PROCESSING AND FOOD ENGINEERING** to the University of Agricultural Sciences, Raichur, is a record of research work carried out by her during the period of her study in this University, under my guidance and supervision, and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Place: RAICHUR

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Members: 1. **(UDAYKUMAR NIDONI)**

2. **(ABBAS HUSSAIN)**

3. **(NAGARAJ NAIK)**

Affectionately Dedicated

To

My beloved Parents

and Teachers

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Date:

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CONTENTS

LIST OF ABBREVATIONS

ANOVA	:	Analysis of variance
AOAC	:	Association of Official Analytical Chemists
AJC	:	Apple Juice Concentrate
BCC	:	Black Current Concentrate
CA	:	Citric Acid
CD	:	Critical Difference
CV	:	Coefficient of Variance
CCD	:	Centrifuged Juice
CMJ	:	Centrifuged Moosambi Juice
CRD	:	Completely Randomized Design
DC	:	Direct Current
DAD	:	Diode Array Detector
DNA	:	Deoxyribonucleic Acid
DPPH	:	2-2 Diphhenyl- 1 Picrylhydrazyl
DRBC	:	Dichloran Rose Bengal Chlromphenicol
ETCJ	:	Enzyme Treated Centrifuged Moosambi Juice
FAO	:	Food Act Order
FCR	:	Follin Ciocalteu Reagent
GC	:	Gas Chromatography
GAE	:	Gallic Acid Equivalents
HMF	:	Hydroxymethyl Furfural
HHP	:	High Pressure Processing
HPH	:	High Pressure Homogenization
HDPE	:	High Density Polyethylene
HPLC	:	High Performance Liquid Chromatography
LDPE	:	Low Density Poly Ethylene
MF	:	Microfiltration
MIC	:	Minimal Inhibitory Concentration
NIC	:	Non Inhibitory Concentration
OS	:	Oxygen Scavenger

OCC	:	Open Column Chromatography
PDI	:	Polydispersity Index
PEF	:	Pulsed Electric Field
PET	:	Polyethylene terephtalate
PME	:	Pectin methyl esterase
PVPP	:	Polyvinyl polypropylidone
TBC	:	Total Bacteria Count
TFC	:	Total Fungal Count
TSS	:	Total Soluble Solids
TVC	:	Total Viable Count
UF	:	Ultrafiltration
UV	:	Ultra Violet
USFDA	:	Unites States of Food and Drug Administration
WHO	:	World Health Organization

LIST OF SYMBOLS

a^*	:	Redness or greenness
a_w	:	water activity
b^*	:	Yellowness or blueness
C^*	:	Chroma of the colour
°Brix	:	degree Brix
cm	:	Centimetre
C	:	Centigrade
$cfu.g^{-1}$	:	Colony Forming Units per gram
$cfu.ml^{-1}$	:	Colony Forming Units per millimetre
et al.	:	Others
Fig	:	Figure
h^*	:	Hue chroma
H	:	Hour
hp	:	Horsepower
$IU.ml^{-1}$	:	International units per millilitre
kDa	:	Kilodaltons
kHz	:	Kilohertz
kW	:	Kilowatt
kWh	:	Kilowatt hour
$kJ.l^{-1}$	:	Kilojoules per litre
$kV.m^{-1}$	:	Kilovolts per metre
min	:	Minute
mm	:	Millimetre
MPa	:	Mega Pascal
$mg.g^{-1}$	:	Milligrams per gram
$mg.kg^{-1}$	:	Milligrams per Kilogram
$mg.l^{-1}$	:	Milligrams per litre
$mJ.cm^{-2}$	:	Milli joules per centimetre square
N	:	Newton
Nm	:	Newton metre

nm	:	nanometre
No.	:	Number
ppm	:	Parts per million
Psi		Pounds per square inch
t/h	:	Tons per Hour
V	:	Volt
g.ml^{-1}	:	Grams per millilitre
g.100g^{-1}	:	Gram per 100 gram
g.kg^{-1}	:	Grams per kilogram
gal	:	Gallons
Hz	:	Hertz
µs	:	Microseconds
s	:	Seconds
V/V	:	Volume/Volume
W	:	Watt
W/V	:	Weight/Volume
var	:	Variety
viz	:	Namely
<	:	Less than
>	:	Greater than
"	:	Inch
°	:	Degree
@	:	at the rate
C	:	Centigrade
%	:	Percent
±	:	Plus or minus

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INTRODUCTION

I INTRODUCTION

The oranges especially the sweet orange (*Citrus sinensis (L) Osbeck)* is the fruit of the citrus species, cultivated in China for many centuries before it was introduced into Europe, most likely during the early 15th century. Sweet oranges are also called as Moosambi in Kannada, Mousambi/Mosambi in Hindi or Urdu. The fruit of sweet orange is a fleshy indehiscent berry that ranges widely in diameter from 4 to12 cm. The botanical classification of sweet orange is, that it belongs to kingdom: plantae, division: Magnoliophyta, class: dicotyliophyta, subclass: sapindales, order: rosidae, family: rutaceae, sub family: aurantoideae, genera: *Citrus*, sub genera: Papeda, species: *sinensis* (Milinda and Dev, 2012).

Area, production and productivity of sweet oranges

India ranks 3rd in the production of sweet oranges after banana and mango next to China and ranks 6th in the production of citrus in the world followed by Brazil, which is the largest producer of citrus and China has the largest area under citrus production. The main citrus growing states in India are Andhra Pradesh, Maharashtra, Punjab, Haryana, Karnataka and Rajasthan. Sweet oranges are the second largest citrus fruits cultivated in the country and accounts for approximately 70% of the citrus production. The production of sweet oranges is largely favoured by dry, semi-arid to tropical conditions and plants grow well under subtropical climate and can withstand occasional light frosts. The main production of sweet oranges is found in United States of America followed by Brazil, Mexico, Argentina, the Mediterranean basin followed by China, India, Egypt and Turkey (Singh and Naqvi, 2001).

The total area for sweet orange production in India during 2012-13 was 164.66 million hectare, production: 1186.41 million tonnes and productivity: 7.21 million tonnes per hectare. Maharashtra stands first in area wise production of sweet oranges in India followed by Andhra Pradesh, Karnataka and Punjab (www.indiastat.com).

Sweet orange varieties

Many varieties of sweet orange have been introduced into India but only a few are prolific ones having good quality. Currently, exotic varieties like Jaffa, Hamlin and Pineapple are performing well in Punjab, Haryana and Rajasthan. However, main varieties of sweet orange in India being cultivated on commercial scale are Blood Red, Moosambi and Sathgudi. Different varieties and characteristics of sweet oranges are: Mosambi, fruit having light yellowish orange colour with rough surface, prominent streaks on the rind, oblate to spherical in shape with broad apex, thick rind with well defined segments numbering 9-12, difficult to peel, pulp is light yellowish colour with sweet juice.

Malta (common) is another variety of sweet orange with orange yellow colour having smooth surface with spherical shape. Fruits are medium to large in size; the thickness of the rind is medium with 10 segments which are well defined. The pulp is orange with abundant juice and good flavour. Malta (blood orange) is a sweet orange variety with yellow skin scarlet blush. The rind is relatively thin, tight and glossy. Pulp is corn coloured and red streaked; these are early ripening varieties with sweet pulp, abundant juice, red coloured and pleasant flavour. Sathgudi sweet orange fruits are smooth with attractive orange colour having spherical shape with variable size. The rind is medium thick having 10-12 segments; pulp is orange coloured with abundant juice and good flavour (Milinda and Dev, 2012).

Sweet oranges grow well at an average temperature of about 16 to 20 °C. Being evergreen, citrus requires good amount of water and at least a well distributed rainfall of 500-775 mm. Quality is very good under dry, semi arid conditions, while in humid condition fruits are insipid. Sweet oranges mature in 9-12 months and should be harvested when they are fully ripe and attain proper size, attractive colour and acceptable sugar: acid ratio, as these are non-climacteric fruits, there is no improvement in colour, taste and flavour after harvesting. Sweet oranges mature in twelve months in north India from December to February, while in south India it is October to March and fruits are harvested by clipping using secateurs (Bal, 2005).

Nutritional composition of sweet orange and its juice

Sweet oranges are rich source of vitamin A, C and potassium and supplies around 116.2% of daily value of vitamin C. It contains moisture of 86.0 g.100 g^{-1} followed by carbohydrates 12.0-12.69 g.100 g^{-1}, calcium 40-43 g.100 g^{-1}, protein 0.8-1.4 g.100 g^{-1}, fiber 0.8 g.100 g^{-1} and fat 0.2-0.4 g.100 g^{-1}. Sweet orange juice has pH 3.5, total soluble solids 10 °Brix, acidity 0.4%, moisture content 88.4%, protein 0.6%, fat 0.05%, carbohydrates 10.5%, fiber 0.12% and ash 0.3%. Sweet oranges are not available round the year so should be processed in the form of juice; concentrate, squash, etc., to minimize the post harvest losses due to spoilage (Syed *et al.*, 2012).

Health benefits of sweet orange juice

The beneficial effects of citrus fruit consumption on human health have long been known because of its anti-oxidant and anti-radical properties (Betoret *et al.*, 2009). Citrus fruit juices are rich source of flavonoids which have important health-related properties, such as anti-microbial, anti-carcinogenic, anti-aggregative and are known to protect against cardiovascular diseases. Narirutin, hesperidin and didymin belonging to the flavanone glycosides group are the most abundant flavonoids in mandarin orange juice which have antioxidant activity and appear to influence the lipid metabolism. Different studies have shown that hesperidin can inhibit chemically induced breast cancer, bladder cancer and colon cancer in humans (Miyagi *et al.*, 2000).

Among citrus juices, sweet orange juice or mosambi juice is the most globally accepted and consumed fruit juice product because of its pleasant aroma, taste and colour. Sweet orange juice is an excellent dietary source of bioactive compounds that posseses rich antioxidant properties and is a rich source of provitamin A carotenoids like β-carotene, $\propto$-carotene, β-cryptoxanthin and $\propto$-cryptoxanthin. Other non volatile free acids like oxalic, tartaric, galacturonic, quinic and many others are found in much lower quantities (Rai *et al.*, 2007). Increased consumption of these compounds has been related to a decreased risk of developing several types of cancer, age-related macular degeneration and cardiovascular diseases. Sweet orange juice contains citric acid in abundance, followed by mallic acid, both being present as free acids, although in limited quantities they are also combined as citrates and malates, which gives orange juice its buffer effect. Ascorbic acid, also called as vitamin C, in sweet orange juice plays an important role in prevention of scurvy, maintenance of healthy skin, gums and blood vessels. It reportedly reduces the risk of arthrosclerosis, cardio vascular diseases and some forms of cancer in human beings (Cvetkovic and Jokanovic, 2009).

Causes of spoilage of sweet orange juice and its preservation techniques

Spoilage of sweet orange juice is primarily due to the proliferation of its natural acid tolerant and osmophilic microflora. This microflora is composed of yeasts responsible for the fermented taste accompanying carbon-dioxide production, moulds which contribute to the deterioration of juice by their surface growth and lactic acid bacteria which can grow in acidic conditions to produce a buttermilk off-flavour. In addition, pathogenic bacteria can also proliferate in fresh untreated juice during fruit picking or juice processing, representing great risk of food-borne infection with significant economic repercussions (Krinsky and Johnson, 2005).

Now a days preservation of fruit juices has become the business activity of great significance and countries with abundant fruit resources, having short harvest season are emphasizing more for established storage to maintain quality of fruits, increase shelf-life and preserve fruit juices for off-season use (Tasnim *et al*., 2010). Fruit juices are thermally pasteurized at 63-65 °C for relatively long time and at 90-95 °C for 15 to 30s, which is based on 5-log reduction of the most resistant microorganisms of public health significance (USFDA, 2001).

Novel preservation technologies are adopted to enhance the shelf-life and to prevent the spoilage of fresh fruit juice products, where the processing temperature does not rise beyond 40 °C to maintain the sensory and nutritional characteristics similar to fresh fruit juice products like High hydrostatic pressure processing (HHP), where pressure of upto 1000 MPa with or without heat is used to inactivate microorganisms in juice products (Ramaswamy *et al*., 2005), pulsed electric field (PEF), applies short burst of high voltage electricity for microbial inactivation which causes no or minimum effect on juice quality attributes (Knorr *et al*., 1994), Power ultrasound technique for preservation of orange juice over a frequency range of 500 kHz for 15 min at 60 °C for inactivation of total mesophilic aerobes, ultraviolet light technology with electromagnetic spectrum in the range of 100-400 nanometers, known to have biocidal effects and to destroy microorganisms by degrading their cell walls and DNA (Ngadi *et al.*, 2003), membrane filtration such as ultrafiltration (UF) and microfiltration (MF) are the most common membrane filtration techniques with a membrane pore size of 15 kDa to filter carrot and citrus juices and high pressure homogenization (HPH) with a pressure of 340 MPa processing pressure was required to inactivate microorganisms or enzymes which may alter or damage fresh food products (Chanes *et al*., 2009).

Preservation of fruit juices with chemicals is mainly adopted to prevent microbial spoilage during storage, both in the retail stores and consumer homes. In Europe, benzoic acid (E210) and sodium benzoate (E211) are permitted food preservatives with an acceptable daily intake of 5 $mg.kg^{-1}$ of body weight by the FAO/WHO due to its long history of safe use. Benzoic acid occurs naturally in blueberries, cranberries, coffee beans, tea, cinnamon, clove and other foods at a level of 10-100 $mg.kg^{-1}$(Hussain *et al.,* 2009). Sodium benzoate with its broad anti-bacterial range, non-volatility and water solubility, is widely used as a fruit beverage preservative (Walker and Philips, 2008).

Nisin is an allowed preservative in a range of food products in more than 55 countries. Bacteriocin nisin, produced by *lactobacillus lactis* subsp *lactis*, exhibits anti-microbial activity against a broad range of Gram-negative and Gram-positive bacteria including *A. acidoterrestris*. In orange juice, spore outgrowth was shown to be inhibited by addition of 25-50 $IU.ml^{-1}$ of nisin (Yamazaki *et al.,* 2000).

Packaging and storage of sweet orange juice

In order to facilitate preservation and distribution, it is technological practice to pack juices in metal cans, glass bottles or plastic containers. As for orange juice bottling, polyethylene terephthalate (PET) has been proposed as a packaging material because of its excellent oxygen barrier properties, clearness and UV resistance. Traditional method for juice packaging aims to reduce the exposure of the juice to oxygen through the use of high barrier materials such as glass or aluminum foil laminates in brick packs, with or without nitrogen flushing or improving gas barrier of PET by blending with aromatic polyamides (Hu *et al.*, 2005).

Metal cans are expensive and require sophisticated machinery for container closure; they have been shown to affect ascorbic acid retention better than other packaging materials (Mathooko *et al.,* 2002). However, packaging alone cannot preserve the quality of juice. Therefore, juices are treated with chemical preservatives along with suitable storage temperature of 4 to 25 °C plays an important role in enhancing the shelf-life of sweet orange juice (Zerdin *et al.,* 2003).

Scope of the work

Sweet orange juice is one of the most perishable products with pH of 3.70 to 4.60 which is favourable for the growth and activity of spoilage microorganisms like yeast, mould, lactic acid bacteria, fungi and pectin methylesterase enzyme which degrades the pectin present in sweet orange juice. Keeping in view of the above important aspects, the present research has been undertaken to enhance the shelf-life of sweet orange juice with the following objectives:

Objectives

- To study the effect of different preservation methods, packaging materials and storage conditions on shelf-life of sweet orange juice
- To analyse the quality parameters of sweet orange juice during storage

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

A comprehensive review is mandatory in any research endeavour. This requires thorough efforts on the part of investigator to select relevant subject matter, to organize and to report it systematically. This chapter deals with a brief account of literature, which has direct and indirect bearing on the specific objectives of the investigation.

A brief account on enhancing the techniques of shelf-life of sweet orange juice by earlier researchers was reviewed and presented in this chapter. The literature pertaining to present investigations is reviewed under the following sub headings:-

- Different preservation methods for enhancing the shelf-life of sweet orange juice
- Effect of different packaging materials and storage conditions for enhancing the shelf-life of sweet orange juice
- Quality parameters of sweet orange juice during storage
- Economics for preservation of sweet orange juice

2.1 Different preservation methods for enhancing the shelf-life of sweet orange juice

Preservation plays an important role in enhancing the shelf-life of sweet orange juice by protecting it from microbial activity. A brief reviews on prolonging the shelf-life of sweet orange juice by various preservation methods like physical, chemical and biological methods adopted in present research work are listed below.

Akpan and Kovo (2005) examined the production and preservation of passion fruit juice to reduce the spoilage and to increase the shelf-life of juice by using chemical preservatives like sugar and a combination of citric and benzoic acid at room temperature. The results revealed that the juice maintained its colour, aroma and tastes for at least 1 month when 3% benzoic acid was used as a preservative, whereas the juice preserved with 4% sugar went bad after 3 days and the juice preserved with 4% citric acid maintained its qualities for one week and thereafter aroma started to fade. They concluded that the combination of 3% benzoic acid and 4% citric acid maintained the qualities of the juice fairly between 2-3 weeks. The juice containing sugar and citric acid retained the highest alcoholic percentage and also altered the pH, so that it was impossible for pathogens to exist at such a low pH environment.

Sarkar *et al.* (2008) investigated the effect of external electric field on the enhancement of permeate flux during clarification of mosambi juice by ultrafiltration (UF). A detailed study has been carried out using a 50 kDa molecular weight cut-off flat sheet polyethersulfone membrane in a cross-flow ultrafiltration under laminar flow conditions for a wide range of operating conditions. They found that use of d. c electric field resulted in a 32% enhancement of permeate flux and applied a steady state gel polarization model to evaluate the gel layer concentration, effective diffusivity and effective viscosity of the juice within the concentration boundary layer, by optimizing the experimental flux.

Walker and Philips (2008) investigated the effect of preservatives like sodium benzoate, potassium sorbate and also bacteriocin nisin on two spoilage bacteria *Propionibacterium cyclohexanicum* and *Alicyclobacillus acidoterrestris* in orange juice. The sodium benzoate (0.5 and 1.0 $mg.ml^{-1}$) and potassium sorbate (1.0 $mg.ml^{-1}$) both alone in combination with 2.5, 5 or 10 $IU.ml^{-1}$ of nisin, inhibited the growth of *P. cyclohexanicum* at 30 °C with no viable cells detected at 29 days and in apple juice at 30 °C, 0.1 $mg.ml^{-1}$ sodium benzoate or potassium sorbate inhibited the growth of 10^1 cells ml^{-1} *A. acidoterrestris* while 0.5 $mg.ml^{-1}$ inhibits the growth of 10^4 cells ml^{-1}. Nisin alone and in combination with either sodium benzoate or potassium sorbate was also effective in inhibiting the multiplication of *A. acidoterrestris.*

Barwal and Shrera (2009) standardized the extraction methods and preservation techniques of hill lemon juice. The hill lemons were harvested at proper maturity stage and juice was extracted using screw press juice extractor and juice was preserved with potassium metabisulphite (KMS) at 700 ppm and sodium benzoate of 0.05% to study its keeping quality at room temperature. They concluded that screw press juice extractor gave highest juice of 44.28% as compared to other methods. Among preservatives potassium metabisulphite showed lower losses in quality and was found effective in preservation of juice for 180 days with least browning and stable retention of other physico-chemical attributes of juice except ascorbic acid during storage.

Betoret *et al.* (2009) investigated the effect of homogenization pressures of 0, 5, 10, 15, 20, 25 and 30 MPa on the particle size distribution, colour, cloudiness and flavonoid content of fresh citrus juice to determine the ideal conditions of the juice to be used in the development of functional fresh fruit. The results showed that homogenization pressure affected the particle size distribution and colour of the citrus juice which made it possible to define different sample groups on the basis of applied pressure. In fresh juice, the contents of the flavonoids were not affected by homogenization pressure but after 5 months of storage flavonoid hesperidin was affected in the juice.

Chanes *et al.* (2009) conducted high pressure homogenization on orange juice at five pressures (0-250 MPa) at three initial temperatures (22, 35 and 45 °C). A maximum of five passes for the selected conditions were used to process orange juice and observed pectinmethylesterase (PME) activity, microbial load, cloudy appearance and vitamin C in just squeezed and homogenized orange juices. A reduction of 50.4, 49.4 and 37.8% of PME activity was found in juice homogenized by one pass at 250 MPa at the initial temperatures of 22, 35 and 45 °C, respectively. PME activity in orange juice was reduced as passes number increased. The final temperature of the five times homogenized orange juice was not beyond 28 and 37 °C after being treated at 100 and 250 MPa, respectively. More than 30 and 80% of enzyme activity was reduced after five passes at 100 and 250 MPa, respectively. Less than 8.7×10^2 and 1.85×10^3 cfu.ml^{-1} of mesophiles and yeast plus moulds, respectively were counted in orange juice treated five times at 100 MPa. The cloudy appearance of the homogenized orange juice was maintained for 12 days under low temperature conditions.

Gill *et al*. (2009) standardized the preservation technique of low alcoholic plum wine. The wine was blended with sand pear juice at the rate of 20 and 30% and preserved with heat treatments *i.e.*, by pasteurizing the wine at different temperatures at different time intervals and by chemical preservatives using sodium benzoate (NaB) and sulphur dioxide at 100 ppm each. In all the treatments, there was no change in TSS, acidity and alcohol concentration, whereas when heated either at 70 °C for 10 min or 80 °C for 2 min in comparison to the control (without heating or any preservative) showed the highest reduction in TSS, increase in alcohol concentration and acidity and also complete elimination of microorganisms hence could be used for preservation of low alcohol wine.

Nandi *et al*. (2009) studied microfiltration (MF) of mosambi juice using low cost ceramic membrane prepared from locally available inorganic precursors. Dead end MF experiments were performed for both centrifuged mosambi juice (CJ) and enzyme treated centrifuged mosambi juice (ETCJ). They observed that after MF, important properties like total soluble solids, pH, acidity and density of both CJ and ETCJ were almost unaffected with significant improvement in juice colour, clarity and the clarified juice could be stored in refrigerated condition for more than 30 days without significant change in juice quality. Different membrane pore blocking models were used to analyze the observed permeate flux decline.

Bharadwaj and Mukherjee (2010) studied the effect of different fruit juice blending ratios on kinnow juice preservation at ambient storage condition. They prepared different fruit juice blends from kinnow:anola:ginger juice in the ratio of 100:0:0, 95:5:0 and 92:5:3 followed by kinnow:pomegranate:ginger juice in the ratios of 90:10:0, 87:10:3 for improving juice flavour, palatability, nutritive and medicinal value. The juice blends were preserved by pasteurization (75°C for 15 min) and by addition of potassium meta-bisulphite at 750 ppm. These blends were stored in 200 ml colourless glass bottles at room temperature (28 ± 4°C) for six months and tested at two month interval for physico-chemical, sensory evaluation and microbial population. It was observed that the kinnow juice blend with pomegranate and ginger juice in the ratio of 87:10:3 was the most effective juice blend for minimum changes in TSS (12.00 to 14.13°Brix), acidity (0.720 to 0.510%), ascorbic acid (18.38 to 12.90 mg.100 ml^{-1} juice), and limonin (0.103 to 0.250). Sensory evaluation score was also higher in the same treatment due to better consistency and flavour upto end of storage. The juice blend ratios of kinnow juice: anola juice: ginger juice (92:5:3) was the best in view of non-enzymatic browning (0.081 to 0.104) and minimum population of bacteria (4.0×10^3), mould (1.5×10^3) and yeast (2.1×10^3) at the end of storage (six months). It contained fair amount of vitamin ‘C’ (38.95 mg.100 ml^{-1} juice) at the end of six month of storage. It was also observed that the addition of ginger juice in blends improves the quality and reduced the microbial growth. Further, the juice was found acceptable after six months of storage at room temperature.

Esteban and Palop (2010) evaluated the combined effect of heat pre-treatment with the use of antimicrobials, nisin and carvacrol, on the growth of *L. monocytogenes,* and their potential uses as food preservatives. Carvacrol showed a dose-dependent inhibitory effect, while nisin did not, it decreased the growth rate of *L. monocytogenes* upto 20%, and it increased lag time for approx. 25% at any of the concentrations tested (0.13–0.39 mm). When both antimicrobials were combined, a synergistic effect was observed. This effect was further increased when they were combined with a heat pretreatment for 15 min at 55 °C, where no growth was observed for at least 15 days, even at the lowest concentration tested. The effect was proved both in tryptic soy broth and in carrot juice. This indicated the potential use of carvacrol and nisin applied simultaneously for preservation of minimally processed foods.

Durrani *et al*. (2010) investigated the effect of chemical preservatives and antioxidants mixed in different concentrations on the overall quality of apple pulp packed in plastic containers and stored at ambient temperature at 25-35 °C for 90 days. The samples were studied for ascorbic acid, % acidity, pH, total soluble solids (TSS), sugar acid ratio, mineral content (Ca, Mg, Iron, Potassium and Sodium) and for organoleptic evaluation (colour, flavor and overall acceptability). A decrease was recorded in ascorbic acid content (25.98 to 21.45 mg.100 g^{-1}), sugar acid ratio (14.20 to 13.92), pH (3.62 to 3.28), calcium (12.12 to 4.74 mg.100 g^{-1}), magnesium (12.64 to 11.02), iron(31.22 to 15.65mg.100 g^{-1}) and sodium (0.57 to 0.45 mg.100 g^{-1}) while an increase was recorded in titratable acidity (0.31 to 0.40), TSS (9.71 °Brix to 11.36 °Brix) and potassium (140.00 to 176.22 mg.100 g^{-1}). Statistical analysis showed that storage intervals and treatments had a significant ($P<0.05$) effect on physico-chemical and sensory analysis of apple pulp.

Mathooko and Kiniiya (2002) studied the effect of two levels each of sodium metabisulphite and sodium benzoate on the shelf-life of canned lime juice stored at ambient temperature based on ascorbic acid degradation as an index. They found that sodium metabisulphite increased the shelf-life significantly ($P<0.05$) compared to sodium benzoate, whose effect was not significantly different from that of the control samples. Increased sodium metabisulphite concentration from 150 ppm to 300 ppm had a significant stabilizing effect ($P<0.05$) on ascorbic acid, although it did not prevent complete destruction during storage. Doubling sodium benzoate concentration from 150 ppm to 300 ppm had no significant increase in ascorbic acid stability. From linear regression calculations, 300 ppm sodium metabisulphite resulted in the longest shelf-life of 49 weeks, while the control samples had the shortest shelf-life of 22 weeks.

Hussain *et al.* (2011[a]) studied the effect of chemical additives on the shelf-life of tomato juice using different chemicals like sodium benzoate, potassium metabisulphite and sorbic acid. The shelf-life of these tomato juices were studied for 60 days of storage followed by chemical analysis and sensory tests at an interval of 15 days to assess the effect of chemical additives on the shelf-life of tomato juice. Tomato juice prepared with sodium metabisuphite was spoiled after 45 days, whereas tomato juice prepared with sorbic acid was spoiled after 30 days and tomato juice with sodium benzoate was more stable than others and spoiled at the end of 60th days of storage period. Total number of viable bacteria was highest in potassium metabisulphite and tomato juice whereas sodium benzoate contained least viable bacteria. There were no significant differences among the juices with three different additives with respect to colour and acceptability. By considering all the parameters, they concluded that sodium benzoate was found to be the better additive than potassium metabisulphite and sorbic acid for preservation.

Hussain *et al*. (2011[b]) investigated the effect of sodium benzoate on shelf-life of apple and apricot blended juices. The samples were stored at 4 °C for three months and stored juice samples were analyzed for parameters like ascorbic acid, acidity and pH, total soluble solids, reducing and non reducing sugars, overall acceptability and microbial study after the interval of 15 days for the period of three months. The results showed that ascorbic acid content was decreased during storage. Results regarding overall acceptability showed that maximum mean score observed for T_8 and T_{10} were found most acceptable in maintaining the sensory characteristics compared with others during storage. Minimum microbial load was observed in T_8 and maximum in T_1 to T_5 (uncountable). Among all the treatments T_8 and T_{10} were most effective in maintaining the sensory and nutritional quality during storage.

Maresca *et al.* (2011) evaluated the effects of a multiple-pass high-pressure homogenization treatment on the microbial inactivation of selected microbial strains (*Saccharomyces cerevisiae, Lactobacillus delbrueckii*, *Escherichia coli*) inoculated into commercial fruit juices (orange, red orange and pineapple) as well as the application of this non-thermal technology to the pasteurization of fresh juices (Annurca apple juice) with pressure levels ranging from 50 to 250 MPa, the number of passes from 1 to 5 and the inlet temperature from 2 to 20 °C. The subsequent extension of the multiple-pass treatment to the inactivation of *S. cerevisiae* inoculated into three different fruit juices highlighted that the inactivation induced by the high pressure treatment did not depend on the properties of the tested juices and was not statistically different from inactivation in water (p value < 0.05). These findings were supported by the comparison of two different mathematical models used to fit the inactivation kinetics, whose fitting parameters were not significantly different for water and the fruit juices for any pressure level applied. Three homogenization passes of 150 MPa at 25 °C, which resulted to be optimal for yeast inactivation in fruit juices, were effective for the stabilization of the endogenous microbial load of fresh Annurca apple juice. The treated apple juice showed a minimum shelf-life of

28 days under refrigerated conditions, during which the natural qualities of the fresh juice were completely preserved.

Pareek *et al.* (2011) investigated the influence of juice extraction methods, pasteurization temperature and time on quality of mandarin juice. The experiment consisted of 65 °C pasteurization temperature with 15, 25 and 35 min holding time; 75 °C with 10, 20 and 30 min and 85 °C with 5, 10 and 15 min holding times and two types of juice extraction methods and was laid out in factorial completely randomized design with three replications. Juice extracted with screw press juice extractor and processed at 65 °C for 15 min maintained better qualitative characteristics like total soluble solids, acidity, ascorbic acid, sugars and non-enzymatic browning during storage. Naringin and limonin contents were minimum with the screw extractor and 65 °C processing temperature for 15 m.

Talasila *et al*. (2011) investigated a combined method for preservation of cashew apple juice with an intention to reduce the astringency and microbial count, thereby prolonging shelf-life of the juice. The devised method combines the effects of clarification, sterile filtration and chemical preservation. The juice quality was analyzed for sensory attributes, physico-chemical parameters and microbial count at an interval of 15 days. The experimental data was statistically analyzed using STATISTICA 6.0. The results revealed that the juice can be preserved safely under refrigerated condition for three months.

Bevilacqua *et al.* (2012) reported the combined approach of high pressure homogenization (HPH), sodium benzoate and citrus extract to inactivate spores of *Fusarium oxysporum* in pineapple juice. In first step, HPH and antimicrobials were applied as single hurdles. Homogenization alone reduced spores at the undetectable level only through a 3-step treatment at 120/150 MPa; treatments at 1 or 2 steps reduced *F.oxysporum* pores by 1 and 2 cfu.ml^{-1}, respectively. Concerning the effectiveness of the antimicrobials, NIT (Non inhibitory concentration) and MIC values (minimal inhibitory concentration) of sodium benzoate and citrus extract were 181-289 mg.ml^{-1} and 1450-3700 mg.l^{-1}, respectively. In the last step of the research, benzoate (0-100 mg.l^{-1}) and citrus extract (0-200 mg.l^{-1}) were combined through a two variable-five levels CCD (Central Composite Design) and used in combination with a single step HPH treatment at 120 or 150 MPa. The use of sodium benzoate and citrus extract strengthened the effect of homogenization in pineapple juice and reduced the spores of *F. oxysporum* below the detection limit immediately after homogenization.

Adeola and Aworh (2013) investigated the effect of sodium benzoate on the quality attributes of improved tamarind beverage during storage. Tamarind beverages were produced with or without the addition of sodium benzoate at 100 $mg.ml^{-1}$ and the tamarind beverage produced by the traditional method were kept as control. The produced beverages were tested for chemical, sensory and microbiological attributes at regular intervals of time kept at both ambient 29±1 °C and refrigeration condition of 10 °C. They used overall acceptability score of 5.9 or the appearance of coliform as deterioration index and found that the control beverages deteriorated by 2^{nd} and 10^{th} days at room temperature, whereas the improved tamarind beverages produced without the inclusion of sodium benzoate was stable for 3 to 5 weeks both at room and refrigeration conditions respectively. Finally they showed that the sodium benzoate extended the shelf-life of the tamarind beverage upto 6 to 13 weeks at ambient and refrigeration conditions, respectively.

Shahnwaz *et al.* (2013) examined the effect of sodium benzoate with different concentrations on orange juice packed in various popular packing materials for various time intervals of storage. The effect of sodium-benzoate on chemical compositions of orange juice like total sugars, reducing sugars, TSS and pH was highly significant ($P<0.01$). In case of non-reducing sugars, acidity and vitamin C, the effect of sodium benzoate was non-significant ($P>0.05$). On the basis of higher total sugars, reducing sugars, total soluble solids and lower acidity, the quality of preserved orange juice was better when sodium benzoate was added at a level of 1.0 $g.1000\ ml^{-1}$. Total sugars, reducing sugars and total soluble solids increased during storage. The quality of preserved orange juice packed in amber bottles was relatively better than rest of the packing materials. Results revealed that fresh orange juice with sodium benzoate without the addition of sugar could be useable up to 30 days. Sensory analysis showed that colour, flavour, taste and appearance of preserved orange juice remained excellent up to 30 days. They concluded that amber bottles may be preferred for orange juice and sodium benzoate may be applied at the rate of 1.0 $g.l^{-1}$ for prolonging shelf-life of orange juice.

Kaur *et al.* (2014) studied the effect of chemical preservatives like sodium benzoate, potassium metabisulphite and their combination on shelf-life of cucumber juice.

The studies were done for 6 months at room temperature and analysed the physico-chemical properties like TSS, pH, vitamin C, colour, phenols and antioxidant activity of the preserved cucumber juice. They found significant decrease in these parameters and cucumber juice preserved with potassium metabisulphite maintained a nutrient stability than the combination of both sodium benzoate and potassium metabisulphite.

2.2 Effect of different packaging materials and storage conditions for enhancing the shelf-life of sweet orange juice

Plestenjak *et al*. (2001) determined the changes in sensorial properties of ice tea (peach and pear flavoured) filled in polyethylene terephthalate bottles (PET) and tin cans. Bottles and cans were stored at 2 °C and at 20 °C for 12 weeks. Analyses were carried out weekly on tin, iron, aluminum, 5-hydroxymethyl-2-furfuraldehyde (HMF), ascorbic acid and colour intensity. As expected, canned ice tea had higher concentration of tin, iron and aluminum as a result of migration from packaging material. Concentration of metal ions appeared not to be influenced by storage time but depended mainly on the quality of single can. Concentration of 2-hydroxymethyl-2-furfuraldehyde, which indicates intensity of heat treatment increased during storage. HMF and colour intensity (darker colour) were higher in canned ice tea and higher at higher storage temperature in all treatments. Retention of aroma compounds was better in pear flavoured ice tea and always higher in ice tea packed in PET bottles. The level of ascorbic acid decreased during storage with increased temperature. Faster decrease was always observed in ice tea packed in PET bottles. Sensorial properties that dictate the consumer acceptance classified ice tea packed in PET bottles superior compared to those packed in cans.

Choi *et al.* (2002) investigated the influence of ascorbic acid retention on colour stability of blood orange juice using CIE L^*, a^*, b^*, hue, chroma, polymeric colour and browning index during the storage period. Blood orange juice samples with two different levels of ascorbic acid content were placed in HDPE plastic bottles, pasteurized and stored at 4.5 °C, changes in monometric anthocyanin pigments, total carotenoid contents, cyanidin-3-glucoside and ascorbic acid were also measured. Ascorbic acid degradation was highly correlated (r >0.93) to anthocyanin pigment degradation. Ascorbic acid content also showed linear correlation with red colour intensity (CIE a^* and chroma) in the juice. Polymeric colour and browning index increased with storage time and were more pronounced for juice fortified with ascorbic acid.

Zerdin *et al.* (2003) conducted the storage study on the vitamin C content of orange juice packed in an oxygen scavenger (OS) film and oxygen barrier films as a function of time and temperature. The initial concentration of ascorbic acid in the orange juice was 374 $mg.l^{-1}$ and this was found to decrease by 74 and 104 $mg.l^{-1}$ after 3 days of storage at 25 °C in the OS and oxygen barrier film, respectively. This rapid loss in ascorbic acid correlated well with the amount of oxygen initially present in the headspace and that dissolved in the juice. The loss of ascorbic acid also correlated with an increase in the browning of the juice, where the extent of browning was found to be lower for the juice packed in the OS film than that packed in the oxygen barrier material. The rapid removal of oxygen was found to be an important factor in sustaining a higher concentration of ascorbic acid over long storage time.

Souza *et al*. (2004) assessed the stability of orange juice obtained from a small extractor, stored in a polyethylene bottle under isothermal and non-isothermal storage conditions at 4, 8 and 12 °C for 72 h. pH, titratable acidity and °Brix did not alter significantly during the 72 h of storage. Microbiological analysis showed high initial count for moulds and yeasts that increased in the juice stored for 72 h under the non-isothermal conditions with temperature abuse at 12 °C for 4 h. Date of the sensory evaluation showed a small reduction in product acceptance in this condition. The juice, in the recommended validity period of 48 h, presented losses of less than 20% of the initial ascorbic acid content regardless of the treatment. However, after this time, the degradation became accentuated reaching, at 72 h of storage, retentions of 72 to 85%.

Sheung *et al.* (2005) determined the diffusion coefficient for various flavour compounds in orange juice into packaging material as a function of time using a numerical approach and the experimental results showed that the D of ethyl butyrate and octane have significant variations during the diffusion process, while diffusion coefficients of d-limonene and ∝-pinene donot change significantly with respect to time. It thus showed that care must be taken when one makes the assumption that the diffusion coefficient is constant.

Chumillas *et al.* (2007) conducted a packaging study of orange juice aseptically packaged in bottles by using different materials and filling procedures to determine their influence on the evolution of juice quality and shelf-life using glass, multilayer PET (Poly ethylene terephtalate) and monolayer PET bottles. Monolayer PET bottle showed the lowest retention of ascorbic acid during storage and shelf-life compared with multi layer and glass bottles. They concluded that, if oxygen scavengers, liquid nitrogen drop addition in headspace during filling aluminum foil seal in screw cap and refrigeration temperature are combined with the monolayer PET bottles, orange juice shelf-life can be extended and values similar to glass and multilayer PET bottles can be obtained.

Hashmi *et al*. (2007) studied the effect of microbial and sensory quality of mango pulp along with preservatives like potassium metabisulphite, potassium sorbate individually and in combination packed in 1 kg glass and plastic containers stored at ambient temperature (30-36 °C) for 90 days with an interval of 15 days. Mean score of taste panel for colour, flavour and overall acceptability were significantly ($p<0.01$) decreased, while microbial growth was significantly ($p<0.01$) increased during storage. Results showed that samples with 0.2% potassium metabisulphite packed in plastic containers had negligible microbial growth, maintained maximum nutrients stability and best quality characteristics during storage.

Petrus *et al*. (2010) investigated the influence of storage temperature on the microbiological and sensory stability of homogenized whole pasteurized milk at 75 ºC for 15 s, packaged in high density polyethylene (HDPE) bottle and low density polyethylene (LDPE) pouch, both were monolayer materials pigmented with titanium dioxide (TiO_2). The storage temperatures investigated were 4, 9 and 14 ºC. The results confirmed that exposure to abusive storage temperature at 14 ºC dramatically reduced the shelf-life of pasteurized milk and that the temperature around 4 ºC could be considered ideal for maintaining the microbiological, sensory quality and integrity of the product. They also concluded that HDPE bottles were found to produce better results when compared to LDPE pouch, mainly for abuse storage temperature.

Emamifar *et al.* (2010) investigated the shelf-life of fresh orange juice using nanocomposite low density polyethylene (LDPE) films containing silver (Ag) and zinc oxide (Zno), nanoparticles were prepared by melt mixing in a twin screw extruder. The prepared packages were filled with fresh orange juice and stored at 4 °C and evaluated for microbial stability, ascorbic acid content, browning index, colour and sensory attributes after 7, 28 and 56 days of storage. They concluded that packages containing the nanoparticles, expect 1% nano Zno kept the microbial load of fresh juice below the limit of microbial shelf-life upto 28 days and least degradation of ascorbic acid, development of brown pigments and losing of colour were observed in pouches containing 0.25% nano-Zno. Sensory attributes and shelf-life of fresh juice packed in packages containing nano-silver ranked highest.

Elmoltrafy (2012) investigated the retention of vitamin C in orange juice by using two cans having three brands of canned orange juice and one brand of orange beverage reconstituted by using an electric blender, other by stirring and one can of imitation orange juice which was reconstituted by stirring only. The beverages were analyzed immediately after reconstitution at 3 and 7 days of storage in a refrigerator at 0±1 °C. They found that method of preparation did not have significant effect on vitamin C levels, both product and time affects of vitamin C levels. The main value for imitation orange juice and orange beverages were higher in vitamin C of 52 $mg.ml^{-1}$ as compared with the canned orange juice. All kinds of juice samples were slightly differ in retention of vitamin C, retaining 92% or more after 3 days and 88% or more after 7 days.

Kaushal and Sharma (2012) investigated the suitability of tin cans and glass jars for processing of peach fruit in juices, where the peach juice with or without ascorbic acid of 500 ppm was evaluated on the basis of storability, nutritional value and sensory attributes. On the basis of physico-chemical and sensory evaluation it was observed that higher retention of ascorbic acid and carotenoids were obtained in cans as compared to jars. Further vitamin C treatments had a higher retention to ascorbic acid both in cans at 27.7 $mg.ml^{-1}$ and glass jar at 18.54 $mg.ml^{-1}$ than that of unfortified treatment having mean values of 10.91 and 9.5 mg.100 g^{-1} for cans and jars, respectively. While the vitamin C in un-treated samples (4 °Brix sucrose syrup with 0.3 % citric acid) was recorded at 3.91 mg.100 g^{-1} after storage. No apparent spoilage was noticed in cans and jars at the period of 6 months. It was suggested that conventional processing of peach halves in sucrose syrup in tin cans and glass jars was successfully replaced with a covering medium containing peach pulp.

Bacigalupi *et al.* (2013) investigated the changes in nutritional and sensory properties of orange juice packed in PET bottles with oxygen scavenger bottles. They found that dissolved oxygen was similar in all bottles, whereas ascorbic acid degradation was related to the oxygen transfer with higher loss in standard PET bottles (53% against active PET) and also observed the changes in aromatic profile where juice was exposed to high intensity light in which degradation of limonene and formation of ∝-terpineol, an off flavour took place. They also developed mechanistic model to predict the shelf-life of orange juice coupled with oxygen transfer and ascorbic acid oxidation reaction in the bottled juice by neglecting oxygen permeation through packaging material.

Johnson *et al*. (2013) studied the effect of Nigerian market storage conditions on ascorbic acid, titratable acidity and pH values of selected tetra pack packaged citrus fruit juice with increase in shelf-life upon storage. They selected 6 different brands of commercially available tetra pack packaged citrus fruit juice in Nigerian markets. The juice samples were stored at 28±5 °C with a relative humidity of 75±5 °C with less ventilated rooms. The sample analyses were carried out from 3 to 10 months from the day of preparation. From the results, they concluded that there were gradual decrease in ascorbic acid content and pH with increase in storage period irrespective of the brands and increase in titratable acidity after storage period of 10 months at ambient temperature.

2.3 Quality parameters of sweet orange juice during storage

Gardner *et al*. (2000) assessed the relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. Vitamin C was found to account for 65±100% of the antioxidant potential of beverages derived from citrus fruit but less than 5% of apple and pineapple juice. The contribution of carotenoids to antioxidant potential was found to be negligible. They concluded that the phenolics appear to be major contributors to the antioxidant potential of the non-citrus juices, their identity and bio-availability requires further investigation.

Tfouni and Toledo (2002) analyzed different brands of soft drinks, fruit juice, margarine, yoghurt and cheese available on the Brazilian market for benzoic and sorbic acids by high performance liquid chromatogramphy (HPLC) with a photodiode array detector. The levels of benzoic and sorbic acids were respectively in the range of not detected to 804 $mg.l^{-1}$ and 1371 $mg.kg^{-1}$. According to the result, it was concluded that the real utilization of benzoates and sorbates is significantly lower than the maximum authorized levels.

Belajova and Suhaj (2003) studied some flavonoid compounds like naringin, hesperidin, neohesparedin and quercetin generally present in fruit juices and quantified by high performance liquid chromatography (HPLC) using C6- phase column and diode array detector (DAD). The method was partly validated with good results. Flavanones such as naringin, hesperidin and neohesparedin were identified as markers of 100% citrus juices and their contents in juice were compared with published values established by extensive research of citrus composition. Quercetin, as a constituent of grape fruit and some other fruit juices, was also studied.

Chia *et al*. (2003) evaluated the effect of storage time on the quality of ultraviolet-irradiated and thermally pasteurized pineapple juice They irradiated the juice with ultraviolet light (UV-C) at wavelength 254 nm (53.42 $mJ.cm^{-2}$, 4.918 s), thermally pasteurized at 80 °C for 10 minutes and stored at 4 °C for 13 weeks. They noted the significant changes in the total soluble solids, pH, titratable acidity and turbidity of UV-irradiated juice during storage, whereas for the same quality attributes of thermally pasteurized juice remained stable throughout the storage time. There were no significant changes in total phenolics for both treatments throughout the storage period. Other quality parameters (ascorbic acid, colour L^*, hue angle and chroma) were significantly affected by the storage time. Regarding the microbiological analysis, the total plate counts and yeast and mould counts of the UV-irradiated juice increased gradually throughout the 13 weeks of storage while these parameters remained unchanged in the thermally pasteurized juice with almost no microorganism growth. UV-irradiated pineapple juice preserved better quality attributes (TSS, pH, titratable acidity, ascorbic acid, turbidity, total phenolic, L^* (lightness), hue angle and chroma) than the thermal pasteurized juice during the storage time. Hence, UV irradiation has great potential as an alternative technology to thermal pasteurization in producing products of high nutritive values.

Lee and coates (2003) studied the changes in carotenoid pigment content and juice colour due to thermal pasteurization of Valencia orange juice. They noted that the total carotenoid content loss was significant ($P>0.05$) after thermal pasteurization at 90 °C for 30 s and also observed the thermal effects on carotenoid pigment contents especially on violaxanthin (-46.4%) and antheraxanthin (-24.8%). With the loss of violaxanthin and antheraxanthin, leutin became the major carotenoid followed by zeaxanthin, in pasteurized Valencia orange juice. There was perceptible colour change after orange juice pasteurization, which led to juice colour becoming light and more saturated. Decrease in CIE a^* value and increase in CIE L^*, a^*, h^* and C^* are the major colour changes after pasteurization. They also showed that overall increase in reflected light might also influence the colour perception to a great extent in orange juice pasteurization. Total colour difference of (ΔL^*) compared to the juice colour was 2.92±0.97 ($P<0.03$).

Koca *et al.* (2003) investigated the kinetics of non-enzymatic browning in citrus juice concentrates of orange, lemon, grapefruit and tangerine, during 8 weeks of storage at 28, 37 and 45 °C. Browning development was measured at absorbance of 420 nm (A_{420}) using CIE-Lab colour system. Analysis of kinetic data from A_{420} values suggested a zero-order reaction for non-enzymatic browning, while changes in L^* and b^* parameters followed a first-order reaction. Activation energy for non-enzymatic browning determined by A_{420} values ranged from 17.60 to 35.27 kcal mol^{-1}, while those for L^* and b^* parameters were 6.67-28.99 kcal mol^{-1} and 15.38-34.2 kcal mol^{-1}, respectively. Activation energies were higher in orange (28.99-35.27 kcal.mol^{-1}) and tangerine (27.84-33.1 kcal.mol^{-1}) juice concentrates than those in grapefruit (6.74-27.81 kcal mol^{-1}) and lemon (6.67-17.6 kcal.mol^{-1}) juice concentrates. The lower activation energies determined for grapefruit and lemon juice concentrates indicated that non-enzymatic browning reactions are favoured in these samples.

Alper *et al.* (2005) investigated the influence of processing and pasteurization of colour values and total phenolic compounds of pomegranate juice. The juices were prepared by different clarification techniques like conventional fining, conventional fining together with poly vinyl poly propylidone (PVPP), ultrafiltration by taking non-clarified juice as control. They found that conventional methods and heat treatments together at $p<0.05$ significantly affected the colour values of pomegranate juices and moreover, they concluded that conventional fining together with PVPP treatment was the most effective method to remove phenolic compounds.

Burdurlu *et al.* (2005) investigated the kinetics of ascorbic acid degradation in citrus juice concentrates of orange, lemon, grapefruit and tangerine during eight week storage at 28, 37 and 45 °C. The loss of ascorbic acid at each temperature followed a first-order kinetic model. Activation energy was determined in the range of 12.77±0.97 to 25.39±1.98 kcal.mol^{-1}. Ascorbic acid retention after storage at 28, 37 and 45 °C was about 54.5-83.7%, 23.6-27% and 15.1-20.0%, respectively. Since hydroxyl-methylfurfural (HMF) is one of the decomposition compounds of ascorbic acid degradation, which is formed. HMF accumulation fitted to a zero-order kinetic model and activation energy ranged from 43.41±0.67 to 80.02±0.07 kcal.mol^{-1}. Significant correlation was obtained between HMF accumulation and ascorbic acid loss at all storage temperatures in all citrus juice concentrates.

Esteve *et al.* (2005) investigated the effect of physico-chemical and quality characteristics of various minimally pasteurized refrigerated Spanish orange juices and their changes with storage time and temperature. Essential oils, acidity, conductivity, diacetyl index, hydroxyl-methyl furfural, formal index, viscosity and ascorbic acid were varied with storage time more significantly at 10 °C than at 4 °C. Density, colour and pectinmethylesterase did not vary at 4 °C. A period of at least 42 days at 4 °C and 35 days at 10 °C was established as the shelf-life of the juices.

Gama and Sylos (2005) determined the carotenoid composition of Brazilian Valencia orange juice by Open Column Chromatography (OCC) and High-Performance Liquid Chromatography (HPLC). Carotenoid pigments were extracted using acetone and saponified using 10% methanolic potassium hydroxide. Sixteen pigments were isolated by open column chromatography and identified as ∝-carotene, γ-carotene, ∝-carotene, β-cryptoxanthin, α-cryptoxanthin, lutein-5, 6-epoxide, violaxanthin, lutein, antheraxanthin, zeaxanthin, luteoxanthin A, luteoxanthin B, mutatoxanthin, auroxanthin B and trollichrome B. Thirteen carotenoid pigments were separated using a ternary gradient (acetonitrile–methanol–ethyl acetate) elution on a C18 reversed-phase column. Among these, violaxanthin, lutein, zeaxanthin, β-cryptoxanthin, β-carotene, α-carotene, and γ-carotene were quantified.

Gunes *et al.* (2005) investigated the effects of dense phase carbon dioxide processing parameters including temperatures at 25-35 °C, CO_2 concentration of 0, 85 and 170 $g.kg^{-1}$ and pressure of 6.9, 27.6 and 48.3 MPa on yeast survival and sensory properties of grape juice. The dense phase CO_2 process resulted in more than a 6 log reduction in yeast population. As the CO_2 juice concentration, temperature and pressure increased, the inactivation rate increased. CO_2 in the supercritical state was more effective in inactivating yeast than in the subcritical state. The process did not cause detectable flavour degradation. They concluded that the dense phase CO_2 processing can be an effective non-thermal alternative process for pasteurization of grape juice.

Komthong *et al*. (2005) investigated changes in the odours of apple juice during enzymatic browning after squeeze at different time points (0-6 h) by sensory evaluation and gas chromatography (GC). Trained panelists assessed a decrease in green odour with an increase in sweet odour during the browning. Fresh, fruity and apple-like odours temporarily increased in the first 2 h of browning and gradually decreased thereafter. In the GC determination, butyl-pentyl and hexyl acetate as well as *trans*-2-hexenal were used as the volatile representatives because of their highest aroma values. Volatile release which was estimated from the proportion of volatile concentration in the vapour phase to that in the juice phase was used to elucidate the changes in odours of apple juice. The decrease in volatile release of *trans*-2-hexenal and the increases in those of all acetate esters during the browning corresponded well with the low intensity of green odour and the high intensity of sweet odour, respectively.

Bayindirli *et al.* (2006) investigated the effect of high hydrostatic pressure treatment with a mild treatment on inactivation of pathogenic microorganisms like *Staphylococcus aureus* 485, *E. coli* 0157:H7 933 and *salmonella enteritidis*, polyphenol oxidase activity and enzymes in apple, orange, apricot and sour cherry juices. They found that there was complete inactivation of microorganisms at 350 MPa and 40 °C in 5 min. The residual polyphenyl and pectinesterase activity in apple and orange juice after treatment at 450 MPa at 50°C in 60 min and 450 MPa at 50 °C in 30 min were 9±2.2% and 7±1.6% approximately. They concluded that the inactivation and enzyme was not reactivated upon storage and high pressure processing constitutes an effective technology to inactivate the enzymes in fruit juices. Pressure higher than 400 MPa can be combined with mild heat of <50 °C to accelerate enzyme inactivation.

Torregrosa *et al.* (2006) determined the degradation kinetics of ascorbic acid in orange–carrot juice treated by pulsed electric field (PEF) in order to establish its shelf-life. Different electric field intensities (25, 30, 35, and 40 $kV.cm^{-1}$) and different treatment times (from 30 to 340 ls) were studied. The ascorbic acid degradation rate (k) obtained was −0.009±0.0008 μs^{-1}, −0.0140±0.0009 μs^{-1}, −0.0220±0.0023 μs^{-1} and −0.0187±0.0049 μs^{-1} for fields of 25, 30, 35, and 40 $kV.cm^{-1}$, respectively. The treatment selected was 25 $kV.cm^{-1}$. The shelf-life of the orange–carrot juice treated by pulses at 25 $kV.cm^{-1}$ for two times (280 µs and 330 µs) was compared with a heat treated juice (98 °C, 21 s) kept in refrigerated storage at 2 and 10 °C. The remaining concentration of ascorbic acid in the pasteurized orange-carrot juice was 83%, whereas in the PEF-treated juice it was 90%. The ascorbic acid degradation rate in the juice stored at 2 °C was less than in the juice stored at 10 °C and in the pasteurized juice it was greater. PEF treatment at 25 kV.cm^{-1} for 280-330 μs extended the half-life of the juice stored at 2 °C for 50 days.

Rein and Heinonen (2007) investigated the colour stability and co-pigmentation of four different berry juices enhanced by phenolic acids and commercial colour enhancers. Phenolic acid enrichment improved and stabilized the colour of the berry juices during storage. The commercial colour enhancers immediately produced an intensive colour to the juices. However, was not very stable. The colour enhancement was intensive in strawberry and raspberry juices and effective in lingonberry and cranberry juices. Sinapic acid induced the strongest colour in strawberry juice. Ferulic and sinapic acids improved raspberry juice colour equally. Rosmarinic acid enhanced the colour of lingonberry and cranberry juices the most. The addition of the simple cinnamic acids produced novel peaks to the end of the high-performance liquid chromatography chromatogram, indicating a formation of new compounds.

Kelebek *et al.* (2008) determined the phenolic composition and antioxidant capacity of blood orange juice obtained from *cvs* Moro and Sanguinello (*Citrus sinensis (L) Osbeck*) grown in Turkey. They identified and quantified a total of 18 phenolic compounds in Moro and Sanguinella orange juice including hydroxybenzoic acids (2), hydroxycinnamic acid (5), flavanones (5) and anthocyanins (6) by using high performance liquid chromatographic method (HPLC) coupled with diode array detector. They found that the total phenolic content of Moro juice was higher than that of Sanguinello juice. Ferulic acid was the most dominant hydroxycinnamic acid and cyaniding 3- (6''- malonyl glycoside) and cyanidin-3-glucoside were the most dominant anthocyanins in both cultivars. Antioxidant activities were measured by DPPH (2-2- diphenyl-1-picrylhydrazyl) method showed that Moro juice has higher antioxidant capacity than Sanguinella juice.

Cvetkovic and Jokavnovic (2009) determined the amount of ascorbic acid lost in beverages by applying different preservation methods and storage conditions. Beverage was made in laboratory condition with synthetic L-ascorbic acid added according to the National legislations. After 30 days of storage at 4-8 °C, ascorbic acid overall loss was from 81.01% to 90.27% in thermally pasteurized samples and from 97.83% to almost complete loss in samples preserved with sodium benzoate.

Mahmud *et al*. (2009) studied the varietal effect on the prepared tomato juice. Three varieties of tomatoes were selected for juice preparation like Manik, Ratan and Anupoma. The shelf-life studies were carried out for 60 days storage period followed by chemical analysis, microbial count and sensory tests during the 60 days at an interval of 15 days. They observed negligible change in chemical constituents except vitamin C throughout the 60 days storage period. The major loss was found in case of vitamin C, with gradual fading of colour and off-flavor during the storage time at both ambient and at refrigeration temperature. They concluded that the tomato juice prepared from Manik and Anupoma varieties spoiled after 30 days but Ratan variety was spoiled between 30-45 days, evidenced by the production of off-flavour and colour fading. Total number of viable bacteria was highest in tomato juice prepared from Anupoma and least in Ratan variety. There were no significant differences in colour and overall acceptability in three varieties of juices. However, Ratan variety showed best textural properties and shelf-life of these three varities of tomato juices can be enhanced under refrigeration conditions.

Kagtla *et al.* (2010) investigated the effect of processing and preservation on sensory qualities of prickly pear juice like colour, taste, aroma, flavour, astringency, visual browning and overall acceptability. Prickly pear pulp and juice had unique properties of low pH 3.38, soluble solids 3.68 °Brix and high titratable acidity of 0.47. Sensory profiling and descriptive analysis revealed that non-treated juice had a bitter taste with high astringency, whereas treated prickly pears were significantly sweeter. All treated juice had a good sensory acceptance with values approximately or exceeding 7. Regression analysis of the consumer sensory attributes for non-treated prickly pear juice indicated an overwhelming rejection, while treated prickly pear juice received overall acceptability.

Durrani *et al.* (2011) conducted sensory evaluation of mango pulp preserved with addition of selected chemical preservatives like potassium sorbate (PS), sodium benzoate and potassium metabisulphite (KMS) alone and PS in combination with sodium benzoate and citric acid (CA). and anti-oxidant during storage. All the samples were stored at room temperature and evaluated in sensory evaluation laboratory. They noted that samples preserved with KMS, PS in combination with KMS and PS in addition with CA retained their overall eatable quality for colour, flavour and odour during 45 and 60 days of storage.

Rahman *et al.* (2011) assessed the microbial quality of fresh and commercially packed available juices collected from different locations of Dhaka city. A total of six fresh juice and commercially packed juice samples were collected, standard culture techniques were followed to asses Total Viable Count (TVC), Total *Staphylococcus* Count (TSC) Total Bacillus Count (TBC) and Total Fungal Count (TFC) on different culture media. The total viable count was varied from the range of 10^2 to 10^5 cfu.ml^{-1} with the highest of 2.4×10^5 cfu.ml^{-1}. A large number of *Staphylococci* and *Bacillus* was found in several samples. Total *coliform* and fecal *coliform* was found in six and five out of fifteen samples. Among total *coliforms, Klebsella, Enterobactor* along with *E.coli* were detected. From all the assessments it was determined that microbial quality of commercially packed juice was fair than that of fresh juice collected from the local market.

Chukwumalume *et al*. (2012) conducted the microbiological assessment of preservation methods for African star apple juice samples, which were subjected to pasteurization, chemical treatment using 0.1% (v/v) sodium benzoate and a combined treatment of both or no treatment. All samples were stored at ambient temperature of 28±2 °C and refrigeration temperature at 5 °C for six weeks to determine the effect of treatments on growth count. They found that juice samples pasteurized and preserved with sodium benzoate stored longer than any other sample at both storage. The combination of pasteurization, use of sodium benzoate and juice stored at refrigeration temperature gave the best storage stability.

Mishra *et al*. (2012) developed a suitable formulation for preparation of mixed amla-grape juice, as amla is rich in vitamin C upto 950 mg. 100 g^{-1} and is astringent in taste; hence an effort was made to make it palatable by blending it with grape juice. The fruit juices were checked for its chemical composition and different formulations of mixed juices prepared by different proportions of amla and grape juices packed in glass bottles with cork cap and stored at room temperature for a period of 2 months and tested for its keeping quality, acceptability and sensory attributes by taste testing panels. It was shown that fruit mixed beverages having composition 50:50 juice, 0.4% acidity, 10% sugar and 15.25 °Brix was found to be optimum among other formulations.

Saddozoi *et al.* (2012) investigated the influence of microbiological activity on different quality factors of strawberry juice during storage which was purchased from local market and its juice was kept for two weeks at room temperature to check its shelf-life. Total plate count (TPC), pH, flavour, colour, yeast and moulds were checked weekly. Quality changes of strawberry juice during storage were caused both by microbiological and physiological processes. At day 0; pH 3.2, colour bright red, excellent flavour, TPC 2.7×10^{2} cfu.g^{-1} were recorded, while no yeast or mould were found. After one week, a pH 3.6, dull red colour, TPC 2.25×10^{2} cfu.g^{-1}, yeast 1.7×10^{3} cfu.g^{-1} and mould 1.6×10^{5} cfu.g^{-1} were observed. While after two weeks (day 14) colour was brown while pH 4.0, TPC 1.9×10^{2} cfu.g^{-1}, yeast 2.47×10^{6} cfu.g^{-1} and mould 1.6×10^{5} cfu.g^{-1} were noticed. Yeast, mould and pH were increased with the passage of time. They concluded that spoilage can be un-treated by keeping the juice in refrigerator and using preservatives. Thus with good storage conditions, the shelf-life of the strawberry juice can be increased satisfactorily.

Diamante *et al.* (2013) studied the effect of different levels of apple juice concentrate (AJC) and blackcurrant concentrate (BCC) and pectin on the moisture content, water activity, color, texture and ascorbic acid content of apple-blackcurrant fruit leather using the response surface methodology. The results showed the moisture content increased with increasing pectin level and with greater increases at higher AJC and BCC levels while the water activity increased with increasing pectin level and with increasing AJC level, at low pectin levels, but with decreasing AJC, at high pectin levels. The chroma decreased with increasing pectin level and with lower values at the middle AJC level. The puncturing force decreased with increasing AJC level but with a lower value at the middle pectin level. Lastly, the ascorbic acid content increased with increasing BCC level regardless of AJC and pectin levels. There is a need to reduce the drying temperature or time of apple-blackcurrant fruit leather just enough to bring the water activity closer to 0.60, thereby increasing the moisture content resulting in higher product yield.

Pala and Tokulucu (2013) investigated the microbial, physico-chemical and sensory properties of UV-C processed orange juice and its microbial stability during refrigerated storage at 4 °C and 10 °C, the results were compared with untreated and treated juice at 90 °C for 2 minutes. UV-C treated orange juice at 36.09 kJ.l^{-1} dose resulted in 2.8 log and 0.34 log reductions in aerobic plate count, yeast and mould count, respectively. Also, a 5.72 log reduction in *Escherichia coli* ATCC 25922 after UV-C treatment at 36.09 kJ.l^{-1} dose was achieved, which indicates an acceptable reduction of a potential pathogen in juice. Ascorbic acid content as a major quality parameter of orange juice did not change significantly after the UV-C treatment. Differences between untreated and UV-C treated orange juices at 48.12 $kJ.l^{-1}$ dose were small in terms of organic acids, antioxidant capacity and phenolics. Based on sensory analysis results, no significant differenced were detected between fresh and UV-C treated juices and UV-C treated orange juice was preferred more than the heat treated juice. UV-C treatment partially extended the shelf-life of fresh juice during storage at the refrigerated conditions.

Rustagi and Kumar (2013) conducted the storage studies of developed amla-mango blended juice. They showed that the value of acidity, reducing sugar and total soluble solids (TSS) increased while the value of pH and ascorbic acid decreased during the two month storage with different preservatives at different temperatures. The addition of preservatives increased the acidity by decreased the pH. It was observed that at freeze temperature oxidation of vitamin C was less and more acidic conditions are maintained. The control showed more number of microorganisms than the juice preserved with sodium benzoate and potassium metabisulphite. The results showed that the juice stored at refrigeration temperatures at 4-6 °C was ranked the best for colour, flavour, taste, texture, appearance and overall acceptability as compared to the others stored at room temperature at 30 °C.

Sarkar *et al*. (2014) studied the effect of storage and preservatives on antioxidant status of seven fruit juices available in the local market of Kolkata and they observed the deterioration of total phenols, ascorbic acid, total flavonoids after 10 days at refrigeration condition. However, upon addition of sodium benzoate to fruit juices, they observed the retention of all the bioactive molecules and less deterioration was observed in these juices even after 10 days of storage at refrigeration condition and finally they concluded that that sodium benzoate might not only inhibiting microbial growth, but also scavenged free radicals generated due to oxidation over time. The study indicated a plausible way of preserving commercial fruit drinks during refrigerated storage.

2.4 Economics on preservation of sweet orange juice

Dillon *et al*. (1993) conducted an economic analysis of grape juice production by wineries ranging in capacity from 5,000 to 100,000 gallons of wine annually. The results demonstrated that, for all but the 5,000-gal winery, grape juice production can be a positive supplement to income at the assumed 5% of capacity and also the desirability of hot press technology for juice volumes greater than 4,000 gal annually. For these volumes, the cost of a small heat exchanger was justified through increased juice extraction. Cold press juice technology with lower investment costs was more profitable for wineries that had juice production of 2,000 gallons or less.

Bulatovic *et al*. (2012) assessed the various possibilities of fruit processing regarding assortments of both semi-processed and finished products. Within a assortment of processed fruit products there were semi-processed fruit products, which can be directly marketed as raw materials in further stages of processing, thus causing different economic effects. They also indicated the economic effects like production value, production costs especially direct costs and difference between production value and total production costs in all stages of a certain type of fruit processing. The obtained results showed that advanced stages of fruit processing entail an increase in costs but these increased costs eventually enables higher revenue.

MATERIALS AND METHODS

III MATERIALS AND METHODS

This chapter deals with different preservation methods, packaging materials and storage conditions followed for the shelf-life enhancement of sweet orange juice. The present investigation was carried out in the Department of Processing and Food Engineering, College of Agricultural Engineering, University of Agricultural Sciences, Raichur during 2013-14.

3.1 Location

The experiment was conducted at the Department of Processing and Food Engineering, College of Agricultural Engineering, UAS Raichur, Karnataka.

3.2 Procurement of raw materials

Fresh sweet oranges (*Citrus sinensis (L) Osbeck*) *var*. Sathgudi for conducting experiments were procured from the Department of Horticulture, Main Agricultural Research Station, UAS Raichur. The sweet oranges were selected according to proper maturity stage, size, ripeness, colour and freshness as shown in Plate 1, which were then processed into juice and was used for all the experiments.

3.3 Equipment and instruments

The equipments used for extracting juice from sweet oranges were power operated screw press type juice extractor shown in (Plate 2), water bath for pasteurization of sweet orange juice and digital thermometer to note down the temperature, homogenizer for homogenizing the juice at 5000psi, packaging materials like PET (Polyethylene terephthalate) bottles, glass bottles and tin cans of 200 ml, glass bottle crown corking machine, tin can seaming machine, domestic refrigerator. Digital hand refractometer to determine the total soluble solids in sweet orange juice, pH meter to determine pH of the sample, water activity meter to determine water activity of the juice sample, Hunter’s lab colorimeter to determine the colour of the sample and spectrophotometer to determine total carotenoids and flavonoids were used for the experimentation and the general specifications are detailed in Appendix-A.

Plate 1. Sweet oranges (*var.* Sathgudi) used in the experiment

Plate 2. Power operated screw press type juice extractor

3.4 Extraction of juice

The sweet orange juice was extracted from peeled fruits by using power operated screw press juice extractor. The juice was strained through muslin cloth, followed by the addition of preservatives namely sodium benzoate, a chemical preservative at a level of 0.5 $mg.ml^{-1}$, nisin, a biopreservative at a level of 0.001$mg.ml^{-1}$ (Walker and Philiphs, 2008) and homogenization of sweet orange juice was done at 5000 psi, which was a method of physical preservation (Betoret *et al*., 2009). The juice was pasteurized at 80 °C for 20 s. The pasteurized juice was filled in glass bottles and sealed by using glass cap sealing machine shown in Plate 3, in monolayer PET bottles and in tin cans, where seaming of tin can was done by using can seaming machine shown in Plate 4, respectively (Chumillas *et al*., 2007). The prepared sweet orange juice with different preservation methods were filled in different packaging materials and stored at ambient and refrigerated condition (4±1°C) and storage studies were conducted upto 40 days and the observations were taken for all the dependent variable during 1st, 15, 30 and 40 days interval. The process flow chart for preparation of sweet orange juice is shown in Fig.1. The pictorial process flow chart for preparation of sweet orange juice is presented in Plate 5.

The treatments are listed below;

T1- Pasteurized juice +Glass bottle+ Ambient condition (30±2 °C) (Un-treated)

T2- Pasteurized juice +PET (polyethylene terephtalate) bottle + Ambient condition (30±2 °C) (Un-treated)

T3- pasteurized juice + Tin cans+ Ambient condition (30±2 °C) (Un-treated)

T4- Homogenized juice (5000 psi) + Glass bottle +Ambient condition (30±2 °C)

T5- Homogenized juice (5000 psi) + PET bottle+ Ambient condition (30±2 °C)

T6- Homogenized juice (5000 psi) + Tin cans+ Ambient condition (30±2 °C)

T7- Juice preserved with sodium benzoate (0.5 $mg.ml^{-1}$) + Glass bottle + Ambient condition (30±2 °C)

T8- Juice preserved with sodium benzoate (0.5 $mg.ml^{-1}$) + PET bottle + Ambient condition (30±2 °C)

T9- Juice preserved with sodium benzoate (0.5 $mg.ml^{-1}$) + Tin cans + Ambient condition (30±2 °C)

T10- Juice preserved with nisin (0.001 $mg.ml^{-1}$) + Glass bottle + Ambient condition (30±2 °C)

T11- Juice preserved with nisin (0.001 $mg.ml^{-1}$) + PET bottles + Ambient condition (30±2°C)

T12- Juice preserved with nisin (0.001 $mg.ml^{-1}$) + Tin cans + Ambient condition (30±2 °C)

T1- Pasteurized juice + Glass bottle+ Refrigerated condition (4 ±1 °C) (Un-treated)

T2- Pasteurized juice+ PET (polyethylene terephthalate) bottle + Refrigerated condition (4 ±1 °C) (Un-treated)

T3- Pasteurized juice+ Tin cans + Refrigerated condition (4 ±1 °C) (Un-treated)

T4- Homogenized juice (5000psi) + Glass bottle + Refrigerated condition (4 ±1 °C)

T5- Homogenized juice (5000psi) + PET bottle + Refrigerated condition (4 ±1 °C)

T6- Homogenized juice (5000psi) + Tin cans+ Refrigerated condition (4 ±1 °C)

T7- Juice preserved with sodium benzoate (0.5 $mg.ml^{-1}$) + Glass bottle+ Refrigerated condition (4 ±1 °C)

T8- Juice preserved with sodium benzoate (0.5 $mg.ml^{-1}$) + PET bottle+ Refrigerated condition (4 ±1 °C)

T9- Juice preserved with sodium benzoate (0.5 $mg.ml^{-1}$) +Tin cans + Refrigerated condition (4 ±1 °C)

T10- Juice preserved with nisin (0.001 $mg.ml^{-1}$) + Glass bottle + Refrigerated condition (4 ±1 °C)

T11- Juice preserved with nisin (0.001 $mg.ml^{-1}$) + PET bottle + Refrigerated condition (4 ±1 °C)

T12- Juice preserved with nisin (0.001 $mg.ml^{-1}$) + Tin cans + Refrigerated condition (4 ±1 °C)

Plate 3. Glass bottle capping machine

Plate 4. Can seaming machine

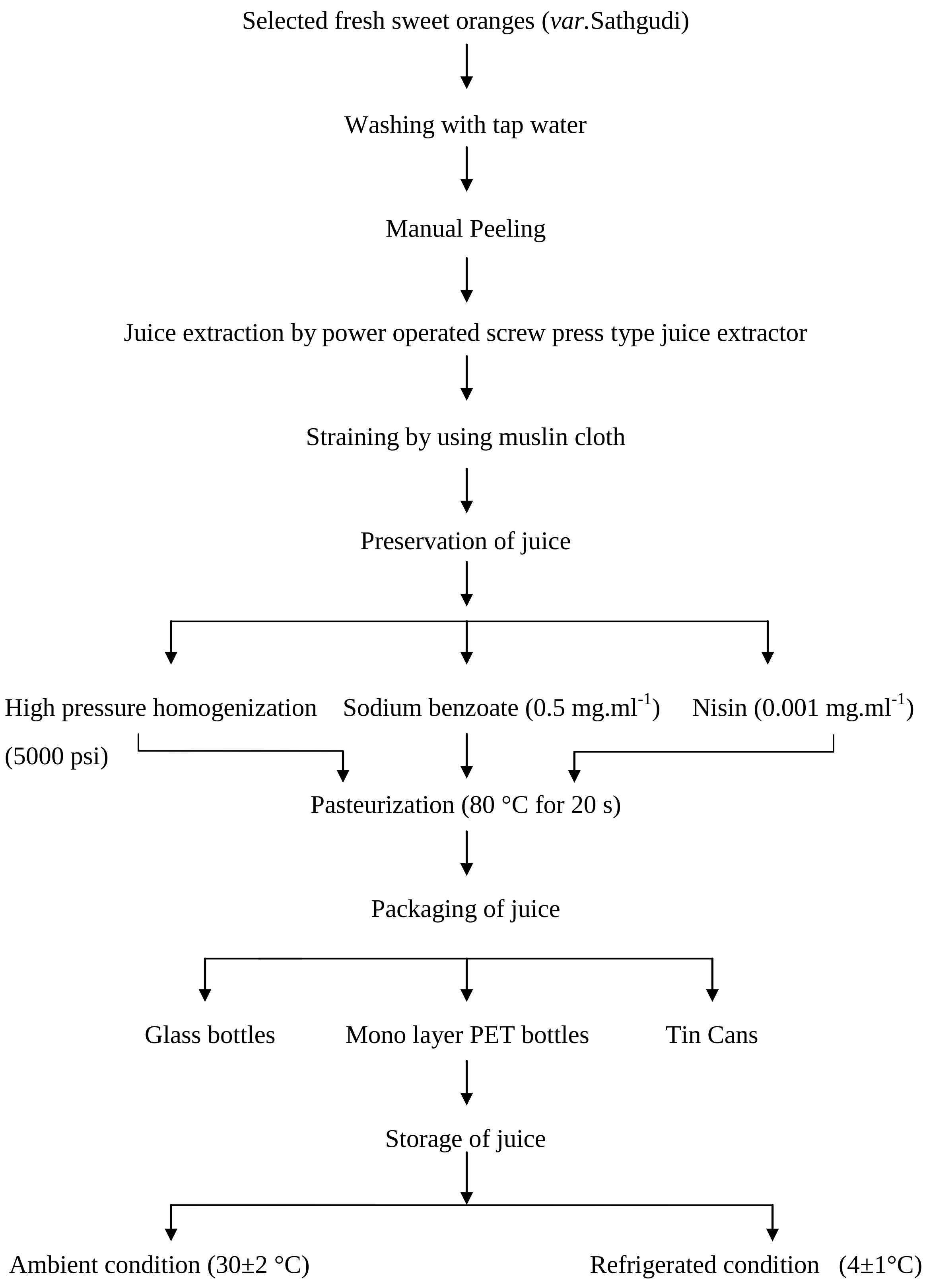

Fig. 1. Process flow chart for preparation of sweet orange juice

Sweet oranges of *var.* Sathgudi

Washing with tap water

Peeled sweet oranges

Juice extraction by power operated screw press type juice extractor

Straining of sweet orange juice using muslin cloth

Pasteurization of juice using water bath and digital thermometer at 80 °C for 20 seconds

Juice filled in glass bottle; PET bottles and tin cans stored at ambient condition (30±2 °C) and refrigerated condition (4±1 °C)

Plate 5. Pictorial process flow chart for preparation of sweet orange juice

3.4.1 Preparation of sodium benzoate solution

Sodium benzoate of 0.5 $mg.ml^{-1}$ was used as a chemical preservative for the preservation of sweet orange juice. Solution was prepared by weighing the required amount of sodium benzoate and mixing it with sweet orange juice as per the quantity required (Hussain *et al.*, 2011).

3.4.2 Preparation of nisin solution

Nisin solution of 1% (w/v) in 0.02N HCl was prepared by centrifuging at 2000 rpm for 20 min and the supernatant was passed through a 0.22 m pore size filter (Millex, Millipr corp., Bedford MA, USA) to give a stock solution of 10,000 $IU.ml^{-1}$. The solution was stored at 4 °C for further usage (Walker and Philips, 2008).

3.4.3 Homogenization of sweet orange juice

The sweet orange juice after extraction was subjected to homogenization, which was done at 5000 psi in a homogenizer to reduce the particle size (Betoret *et al.*, 2009). The homogenizer used in the present investigation is shown in plate 6 and the particle size was analyzed by using zetasizer (nano series) shown in Plate 7 and results are presented in Table 2 (Chapter IV).

3.5 Determination of proximate composition of sweet orange juice

The proximate composition *viz.*, moisture content, soluble protein, carbohydrates of sweet orange juice was estimated by following standard procedure.

3.5.1 Moisture content

The moisture content of sweet orange juice was determined by following (AOAC 1991) method. Five ml of sweet orange juice sample was kept in a pre-dried moisture box. The initial mass of the sample was recorded as W_1 and the box was placed in the hot air oven (Model: KOS.6FD Make:Kemi) maintained at 105 °C for 24 hours. The sample box was kept in the desiccators for cooling and then weighed. The mass of the dried sample was recorded as W_2. The moisture content of the sample was calculated by using the following equation. All the measurements were replicated thrice and the average moisture content was calculated Moisture content

$$(\%\ \mathrm{w.}\ .) = \frac{W_1 - W_2}{W_1} \times 100 \qquad \ldots (3.1)$$

Where,

W_1= initial weight of the sample, g

W_2= final weight of the sample, g

3.5.2 Soluble protein

The soluble protein in sweet orange juice was determined by Lowry's method (Rai *et al*., 2007). Half ml extract was mixed with 10 ml of distilled water in centrifuge tube. After vortex for 2 min, the tube was centrifuged for 10 min at 2700 rpm. Supernatant was used for analysis. A volume of 0.1 ml supernatant was taken out in a test tube and after making the volume 1ml with distilled water. Three ml of reagent C were added which was made by mixing 50 ml of reagent A (2% sodium carbonate in 0.1 N sodium hydroxide) and 1 ml of reagent B (0.5% copper sulphate, 1% potassium sodium tartarate).

After adding 0.2 ml of FCR reagent, tube was inverted for 30 min at room condition. Bovine albumin serum (V) was added as standard in a range of 12.50 to 100 $\mu g.ml^{-1}$. All the samples and standards were prepared in triplicate and absorbance was measured at 600 nm against a blank having all the reagents except the sample. Total soluble protein was calculated from linear regression equation obtained from the standard curve.

3.5.3 Carbohydrates

The total carbohydrates of sweet orange juice was determined using phenol sulphuric acid method (AOAC, 1999). The reagents used were 4% phenol and 96% sulphuric acid. One ml of sweet orange juice was hydrolyzed in water bath at 60 °C for 3 hours with 2.5 N-50 ml hydrochloric acid and then cooled to room condition. The sample was neutralized with solid sodium carbonate until effervescence ceased, volume make up of sample was made up to 100 ml. The sample was centrifuged at 9000 rpm at 4 °C for 10 min to collect the supernatant.

A portion of supernatant to be analyzed was transferred into 10 ml test tube and 1 ml of phenol was added followed by 5 ml of sulphuric acid then the test tube was placed in water bath at 25-30 °C for 20 min for cooling. When the samples were cooled, it was transferred into the cuvettes and absorbance was measured at 490 nm in pectrophotometer (make: Systronics; model: PC based double beam spectrophotometer 2202). To calculate the concentration of sugar present in the sample,

Plate 6. Homogenizer

Plate 7. Zetasizer (nano series)

a graph of absorbance versus concentration of sugar was plotted along with a standard curve generated from the analysis of dextrose.

3.6 Determination of quality parameters of sweet orange juice

The quality parameters such as pH, water activity, colour, total soluble solids, ascorbic acid, browning index, total carotenoids and flavonoids in sweet orange juice were determined by the following standard procedures, which are explained below

3.6.1 pH

The pH of sweet orange juice was measured by using digital pH meter (make: Systronics; model: 361) as shown in Plate 8. Accurately 5ml of juice sample was weighed and was placed in a beaker then the electrode of the pH meter was dipped in the juice sample under test. The enter key was pressed to show the pH value and temperature of sample simultaneously. For next sample measurement, electrode was removed and washed properly with distilled water, then the above procedure was followed and reading was taken as the pH of the next sample. All the readings were taken in triplicate (Fustier *et al*., 2011).

3.6.2 Water activity

The measurement of water activity is a key parameter in the quality control of any moisture sensitive product or material like sweet orange juice. Water activity is defined as the active part of products moisture content or free water. It indicates how tightly water is bound, structurally and chemically, within a substance. Water content implies a quantitative analysis to determine the total amount of water present in a sample. The concept of water activity is of particular importance in determining product quality and safety. Water activity influences colour, odour, flavour, texture and shelf-life of many products. It predicts safety and stability with respect to microbial growth, chemical and biological reaction rates and physical properties.

The water activity of sweet orange juice was measured by Rotronic hygrolab 3 water activity analyzer as shown in Plate 9. Before measuring the water activity of juice sample, the instrument was tested for its accuracy by measuring the water activity of the distilled water, if the water activity for distilled water recorded one, then the instrument was said to be calibrated. Two ml of sample under test was kept in sample cup provided with water activity meter. The sensor was placed on the sample cup by firmly closing in

Plate 8. pH meter

Plate 9. Water activity meter

such a way that the air should not enter into the sample cup. The reading was directly displayed on the water activity meter and was taken as water activity of the sweet orange juice (Fustier *et al.,* 2011).

3.6.3 Colour

Colour is an important attribute because it is usually the first property the consumer observes. It is one of the most important quality acceptances for products, which reflects sensation to the human eye. Hunter's lab colorimeter shown in Plate 10 was used for the measurement of colour of sweet orange juice. The colour was measured by using CIELAB scale at 10° observer at D_{65} illuminant. It works on the principle of focusing the light and measuring the energy reflected from the sample across the entire visible spectrum. It provides reading in terms of L^*, a^* and b^*. Where, luminance (L^*) forms the vertical axis, which indicates whiteness (+) to darkness (-). In the same way, a^* indicates redness (+) to greenness (-) and b^* indicates yellowness (+) to blueness (-).

The instrument was standardized before placing the sample, by placing black tile and white tile provided with the instrument. Once the instrument was standardized, it was ready to measure the colour. It can also be cross checked by placing the white tile which was provided by the L^*, a^* and b^* values. The juice sample was filled in the sample cup. There should not be any void space at the bottom while filling the juice. The deviation of the colour of the sample to standard were also observed and recorded in the computer interface (Timmermans *et al.,* 2009).

3.6.4 Total soluble solids

The total soluble solids (TSS) were determined according to the method described by Timmermans *et al.,* 2009, using Atago digital handheld refractometer as shown in Plate 11. Before use, the instrument was cleaned and adjusted to zero at 20 °C using distilled water. An appropriate quantity of sweet orange juice sample was placed on the prism of the refractometer with the help of a glass rod and press the start button to get the readings. For each sample, the instrument was calibrated by using distilled water. The reading appeared on the screen was directly recorded as total soluble solids (°Brix).

Plate 10. Colorimeter

Plate 11. Digital handheld refractometer

3.6.5 Ascorbic acid

Vitamin C (ascorbic acid) was described by titration method as described by Mazumdar and Majumder (2003). Ten grams, of sample was mixed with distilled water for 10 minutes and filtered through Whattman filter paper #4. The 10 ml sample was taken in 250 ml conical flask and 15 ml 21% oxalic acid was added. The sample was titrated with 2% dichlorophenol indophenol till pink colour appeared. The results were calculated using the following formula and expressed in mg.100 g^{-1} fresh weight.

$$\text{Ascorbic acid (mg/100ml)} = \frac{\text{Titre} \times \text{Dye factor} \times \text{volume}}{\text{Volume of filtration taken} \times \text{volume of sample}} \quad \ldots (3.2)$$

3.6.6 Browning index

The juice browning index was estimated as follows: orange juice (10 ml) was centrifuged for 10 min at 7800 rpm at 4 °C. Five ml of ethanol (95%) was added to 5ml of the supernatant and centrifugation was repeated. The absorbance of the supernatant was read at 420 nm using a UV-visible spectrophotometer with a cell path length of 1 cm by keeping 95% ethanol as a standard (Fustier *et al.*, 2011).

3.6.7 Total carotenoids

The total carotenoids content in sweet orange juice such as β-carotene was purchased from Sigma Aldrich. The method used by *Lee.*, 2001 was followed for total carotenoid quantification. In brief, 5 ml of juice sample and 50 ml of n-hexane-acetone-ethanol in v/v 50:25:25 was placed in a flask extracted on a shaker at 200 rpm for 10 min at room temperature, centrifuged at 2000 rpm for 10 min at room temperature again centrifuged at 6500 rpm for 5 min at 4 °C and the supernatant were collected and made upto 50 ml with extraction solvent. Absorbance was measured at 400 nm and the total carotenoid was expressed as β-carotene equivalants (mg.ml^{-1}).

3.6.8 Total flavonoids

Total flavonoids content in sweet orange juice was examined by the method followed by Jia, Tang and Wu (1999). Two and half ml of juice sample was placed in a soxhlet extractor and refluxed with methanol for more than 12 h at 85 °C. The extract was evaporated to dryness in a rotary vacuum evaporator at less than 40 °C and 1 ml of extract in a 10 ml volumetric flask was kept for 6 min at room temperature. Again 0.3 ml of 10%

Aluminium nitrate ($AlNO_3$) was added to the extract and was incubated at room temperature for 6 min, followed by addition of 4 ml of 1N sodium hydroxide and methanol and incubated for 15 min at room temperature for colour development and absorbance at 800 nm was measured, in spectrophotometer. Total flavonoids were expressed as rutin equivalents ($mg.ml^{-1}$).

3.6.9 Sensory evaluation of sweet orange juice.

Sensory evaluation is a multi-disciplinary approach that uses human panelists with their sense of sight, taste, feeling to measure the sensory characteristics and acceptability of food products as well as many other materials. There is no instrument that can replicate or replace the human response making the sensory evaluation. Freshly prepared sweet orange juice filled in different packaging materials like PET (polyethylene terephtalate) bottles, glass bottles and tin cans with preservation methods were used in the present study and a product oriented testing was conducted using a panel of 15 judges. Panelists were chosen from the institute where the research work was conducted. They were all requested to identify differences among the similar juice products to identify the difference in colour, appearance, flavour, aroma and overall acceptability of sweet orange juice. Each panelist was served with 15 ml of sweet orange juice in transparent cups and was asked to complete the questionnaire giving their choice from like extremely to dislike extremely. Panelists were provided with salted chips and water to rinse their mouth after tasting each sample. An average score of fifteen judgments were determined for each sample.

The juice samples were rated on a nine-point hedonic scale. Nine points were awarded as like extremely-9, like very much-8, like moderately-7, like slightly-6, neither like nor dislike-5, dislike slightly-4, dislike extremely-3, dislike very much-2, dislike extremely-1 (Shahnawaz *et al.*, 2013).

3.7 Enumeration of microbial population

3.7.1 Total plate count

On each sampling day, 10 ml portion of sweet orange juice was aseptically weighed into 90 ml of sterile water and blended for 15 min at room temperature. From this, 1 ml of the sweet orange juice sample was accurately pipette, using a micropipette into test tubes containing 9 ml of sterile distilled water (10^{-1}) and serially diluted until 10^{-6}

dilution was reached. One ml aliquot from 10^{-3}, 10^{-4} and 10^{-5} dilutions were transferred to the sterile petriplates for the enumeration of fungi, yeast and bacteria, respectively. Plates were duplicated for each dilution. Approximately 15 to 20 ml of molten and cooled media, (plate count agar at 45 °C for the respective organisms) were added to the petriplates and the plates were rotated clockwise and anti-clockwise directions on the flat surface to have a uniform distribution of colonies. After the solidification of agar, the plates were inverted and incubated at room temperature for 2-5 days (bacteria 2 days, yeast and fungi 5 days). Total plate counts were determined on plate count agar pour plates and enumerated after an incubation period of 48-72 h at 30 °C (Rahman *et al.*, 2011). The colonies were counted after the incubation period and the number of cfu per ml of sample were calculated by applying the following formula:

$$\text{No of cfu/ml of the sample} = \frac{\text{Mea n number of cfu} \times \text{Dilution factor}}{\text{Volume of the sample}} \quad \text{... (3.3)}$$

Where,

Dilution factor is the reciprocal of the dilution (e.g. $10^{-3} = 10^{3}$)

3.7.2 Total yeast and mould count

The total yeast and mould counts were enumerated as per Rahman *et al*. (2011). Homogenate juice sample was prepared and decimal dilutions were prepared as directed under determination of total plate count. All petriplates were labelled with sample number, dilution and date. Petriplates with the samples were poured with 15 to 20 ml of the medium dichloran rose bengal chloramphenicol (DRBC) solidified agar and spread over the entire surface using a sterile bear stored in the dark. Analyze 25-50 g from each sub sample and incubate 0.1 ml of appropriate decimal dilutions in triplicate on the solidified agar and spread over the entire surface using a sterile bent glass rod. Dichloran 18% was preferred when water activity of the analyzed sample was less than 0.95.

Petriplates with sample of 1ml from 10^{-3} dilution were poured with 15 to 20 ml of Martin's rose Bengal agar (MRBA) and yeast extract peptone dextrose (YEPD) agar for enumeration of fungi and yeast respectively.

3.7.3 Shelf-life assessment of sweet orange juice based on ascorbic acid content

The shelf-life studies of sweet orange juice was calculated by taking into account the ascorbic acid concentration changes upon storage and the prescribed ascorbic acid value of 40 $mg.ml^{-1}$ which was considered as the limit value to predict the shelf-life of citrus juices (Ribeiro *et al.*, 2009). The ascorbic acid concentration changes would follows the pseudo-first order kinetics rate constant and the required intercept and goodness of fit values were obtained from MATLAB software by plotting the values of ascorbic acid on Y-axis and storage period on X-axis and shelf-life of the sweet orange juice was calculated by using the formula

$$SL= \frac{ln(\mathrm{AA})-\mathrm{Intercept}}{\mathrm{Rate\ constant\ (K)}} \quad \ldots (3.4)$$

Where AA is the ascorbic acid concentration corresponding to the acceptability limit.

3.8 Statistical analysis

Statistical analysis was carried out to study the effect of different parameters on all the dependent variables by Completely Randomized Design (CRD) using the statistical software AGRES.

Analysis of variance (ANOVA) were conducted to determine whether significant effect of packaging and preservation methods used to enhance the shelf-life of sweet orange juice. Based on the objectives of the present investigation the details of statistical analysis are given below

- Treatments

  Main treatment: 3

  Subtreatment:1
- No of replications: 3
- Degrees of freedom: 36
- No of observations: 54

A. Preservatives

Level 1: Homogenization (5000 psi)

Level 2: sodium benzoate 0.5 $mg.ml^{-1}$

Level 3: Nisin 0.001 $mg.ml^{-1}$

B. Packaging

Level 1: Glass bottle

Level 2: PET bottle

Level 3: Tin cans

C. Storage conditions

Level 1: Ambient condition (30±2 °)

Level 2: Refrigerated condition (4 ± 1 °C)

3.9 Economics of sweet orange juice

A method of fruit processing value was applied in order to determine cost-effectiveness of using fresh fruits in production. The assessment of raw materials according to processing value is based on economic gains which are obtained as results of their utilization in production. This value indicates cost-effectiveness of fruit processing and demonstrates how raw materials purchased on the market gain money value during production.

The processing (yield) value of every agricultural product can be determined, and this value basically represents the economic valorization of products in technological processing.

The processing yield value is determined in the following manner

$$Y=\frac{TV - UTP}{X} \qquad \dots (3.5)$$

Where, Y= Processing yield value of agricultural product (Fresh fruits)

TV= Market value of obtained processed fruit products

UTP= Total processing costs reduced by raw materials cost of input agricultural products (Fresh fruits)

X= Amount of a used agricultural product in kilograms, which is valorized

RESULTS

IV EXPERIMENTAL RESULTS

This chapter deals with the results obtained from the research project entitled "Development of Process Technology for Enhancing Shelf-life of Sweet Orange Juice" conducted in the Department of Processing and Food Engineering, College of Agricultural Engineering, University of Agricultural Sciences, Raichur, Karnataka during the year 2013-14.

The present study was undertaken to standardize the process technology for enhancing shelf-life of sweet orange juice by using different preservation methods, packaging materials and storage conditions. The investigation includes the proximate composition, quality parameters, sensory properties and to assess the economics of the processed products.

4.1 Phyisco-chemical composition of fresh sweet orange juice

Physico-chemical properties *viz*., moisture content, protein, pH, colour, total soluble solids, water activity, ascorbic acid, browning index, total carotenoids and total flavonoids were determined using standard methods as explained in Chapter III. The physico-chemical properties of the fresh sweet orange juice were determined using standard methods with 3 replications and were presented in Table 1.

4.2 Effect of homogenization on particle size of sweet orange juice

The particle sizes of fresh sweet orange juice was analyzed in zetasizer (nano series) before and after subjecting to homogenization pressure of 5000 psi and the results were presented in Table 2. It was observed that the Z-average value, PDI (Polydispersity Index), intercept and standard deviation of fresh sweet orange juice was found to be 1236, 0.365, 0.943 and 278 at viscosity of 0.8872 and % intensity of 100, respectively before subjecting to homogenization. After subjecting the sweet orange juice to homogenization pressure of 5000 psi, the reduction in particle size was observed, where the Z-average value of 713.9 was noted, followed by PDI value of 0.565, intercept and standard deviation of 0.914, 1694, and 921.1, respectively, at viscosity of 0.8872. Since lower Z-average value was noted in homogenized juice sample than that in fresh juice sample due to particle size reduction, high standard deviation value was observed in homogenized juice sample compared to fresh juice sample when analyzed in zeta sizer.

Table 1. Physico-chemical properties of sweet orange juice

Sl.No.	Composition		Range	Mean Value
1	Moisture content (% w.b.)		89.0 to 89.04	89.02
2	Soluble protein (%)		0.49 to 0.60	0.54
3	Carbohydrates (%)		10.42 to10.50	10.46
4	pH		3.89 to 4.86	4.38
5	Water activity (a_w)		0.878 to 0.886	0.882
		L^*	56.26 to 56.92	56.59
6	Colour	a^*	(-5.54) to (-5.64)	-5.59
		b^*	18.12 to 19.12	18.62
7	Total soluble solids (°Brix)		10.96 to 11	10.98~11.0
8	Ascorbic acid (mg.100 ml^{-1})		62.2 to 62.9	62.5
9	Browning index		0.13 to 0.15	0.14
10	Total carotenoids (mg.ml^{-1})		35.0 to 40.0	37.5
11	Total flavonoids (mg.ml^{-1})		22.5 to 26.8	24.6

Table 2. Effect of homogenization on particle size of sweet orange juice

Sl.No.	Parameters	Fresh juice	Homogenized juice
1	Z- Average	1236	713.9
2	PDI	0.365	0.565
3	Intercept	0.943	0.914
4	Viscosity	0.8872	0.8872
5	Result	-	Good
6	Peak (d. nm)	1191	1694
7	Per cent intensity	100	79.4
8	Standard deviation	278	219.1

4.3 Effect of different preservation methods, packaging materials and storage conditions on proximate composition of sweet orange juice

The detailed procedure for enhancing the shelf-life of sweet orange juice with different preservation methods, packaging materials and storage conditions were briefed in chapter 3 (section 3.2 to 3.4). The statistical analysis was carried out by using completely randomized design (CRD).

4.3.1 Moisture Content

The effect of different preservation methods and packaging materials on moisture content of sweet orange juice stored at ambient and refrigerated condition was analyzed and presented in Table 3a and 3b. Table 3a details about the moisture content of sweet orange juice subjected to different preservation methods, packaging materials and stored at ambient condition. During the first day of storage period, the moisture content was found to be 88.92 % in T1, whereas in T4, T7 and T10, moisture contents were 88.85, 88.69, 88.78 (%w.b.), respectively. In T2, the moisture content was 88.95 (%w.b.) which was more compared to T5 (88.90), T8 (88.74), T11 (88.81 %w.b.). The moisture content in T3 was found to be 88.93 (%w.b.) and in all the treated samples *i.e.,* in T6, T9 and in T12, the moisture contents were 88.88, 88.71 and 88.83 (%w.b.), respectively.

On 15, 30 and 40th days of storage period, significant increase in moisture content was registered in all the samples stored at ambient condition. The moisture content was increased from 92.45 to 94.42% in T1, followed by 90.29 to 93.75 (%w.b.) in T4, 89.58 to 92.15 (%w.b.) and 89.90 to 93.26 (% w.b.) in T7 and T10, respectively. In T2, the moisture content was increased from 92.54 to 94.50 (%w.b.), 90.34 to 93.80 (%w. b.), 89.66 to 92.24 (%w.b.) and 89.85 to 93.32 (%w.b.) in T5, T8 and T11, respectively. In T3, the moisture content was increased from 92.52 to 94.48 (%w.b.), followed by 90.31 to 93.78 (%w.b.), 89.62 to 92.28 (%w.b.) and 89.82 to 93.29 (%w.b.) in T6, T9 and T12, respectively.

Table 3b shows the moisture content of sweet orange juice subjected to various preservation methods, packaging materials and stored at refrigerated condition at 4±1°C. The moisture content in T1 *i.e.,* in juice samples filled in glass bottle and stored at refrigerated condition was found to be 88.94 (%w.b.) and in juice treated with homogenization, sodium benzoate (0.5 $mg.ml^{-1}$) and nisin (0.001 $mg.ml^{-1}$), filled in glass bottles in T4, T7 and T10, the moisture content was 88.87, 88.72 and 88.80 (%w.b.) respectively and the moisture content of T2 *i.e.,* in juice sample filled in PET

Table 3a. Effect of different preservation methods and packaging materials on moisture content of sweet orange juice, stored at ambient condition (30±2 °C)

Moisture content (%w.b.)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	88.92	92.45	93.28	94.42
T2	88.95	92.54	93.32	94.50
T3	88.93	92.52	93.31	94.48
T4	88.85	90.29	92.54	93.75
T5	88.90	90.34	92.62	93.80
T6	88.88	90.31	92.52	93.78
T7	88.69	89.58	90.50	92.15
T8	88.74	89.66	90.58	92.24
T9	88.71	89.62	90.57	92.28
T10	88.78	89.90	91.79	93.26
T11	88.81	89.85	91.87	93.32
T12	88.83	89.82	91.84	93.29
S.Em±	0.035	0.041	0.017	0.016
CD @ (1%)	0.140	0.163	0.065	0.063
CV	0.069	0.079	0.031	0.030
Factor	NS	NS	S	S

Moisture content in fresh sweet orange juice -89.02 (%w.b.)

NS: Non significant

S: Significant

Table 3b. Effect of different preservation methods and packaging materials on moisture content of sweet orange juice, stored at refrigerated condition (4±1 °C).

Moisture (%w.b.)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	88.94	91.30	92.62	93.88
T2	88.97	91.45	92.71	93.94
T3	88.95	91.43	92.68	93.90
T4	88.87	89.90	91.51	93.35
T5	88.92	89.92	91.55	93.46
T6	88.90	89.91	91.53	93.42
T7	88.72	88.78	89.75	91.57
T8	88.76	88.83	89.77	91.62
T9	88.72	88.81	89.76	91.60
T10	88.80	88.95	90.23	92.24
T11	88.84	89.00	90.33	92.32
T12	88.84	88.98	90.26	92.29
S.Em±	0.077	0.030	0.868	0.042
CD @ (1%)	0.305	0.118	3.432	0.078
CV	0.150	0.058	1.655	0.164
Factor	NS	S	S	S

Moisture content in fresh sweet orange juice- 89.02 (%w.b.)

NS: Non significant

S: Significant

(polyethylene terephthalate) bottles and stored at refrigerated condition was found to be 88.97(%w.b.),whereas juice stored at refrigerated condition in T5, T8 and T11 was found to be less compared to un-treated samples and were 88.92, 88.76 and 88.84%, respectively. In case of T3 *i.e.*, juice sample filled in tin cans and stored at refrigerated condition, the moisture content was found to be 89.95 (%w.b.), whereas, increase in moisture content in the range of 88.90, 88.72 and 88.84 (%w.b.), was noticed in juice samples with different preservation methods, filled in tin cans and stored at refrigerated condition in treatments T6, T9 and T12, respectively.

During 15 to 40 days of storage, the moisture content increased from 91.30 to 93.88 (%w.b.) in T1, whereas in case of T4, T7 and T10 showed less increase in moisture content from 89.90 to 93.35 (%w.b.), 88.78 to 91.57% and 88.95 to 92.24 (%w.b.), compared to T1. In T3, the moisture content was found to increase from 91.45 to 93.94 (%w.b.) and in all treated samples of T5, T6, T11, it increased from 89.92 to 93.46%, 88.83 to 91.62 and 89.00 to 92.32 (%w.b.), respectively. In case of T3, the moisture content was from 91.43 to 93.90 (%w.b.), whereas less increase in moisture content from 89.91 to 93.42 (%w.b.), 88.81 to 91.60 (%w.b.) and 88.98 to 92.29 (%w.b.) was observed in the treatments T6, T9 and T12, respectively.

From the Tables 3a and 3b it was observed that during the storage period, gradual increase in moisture content was observed in all the samples. The maximum retention of moisture was found in T7 which was from 88.69 to 92.15 (%w.b.) *i.e.*, in juice samples treated with chemical preservative sodium benzoate (0.5 $mg.ml^{-1}$), filled in glass bottle, stored at ambient condition, whereas in T7, with same preservative, filled in glass bottle and stored at refrigerated condition was from 88.72 to 91.57 (%w.b.) recording moisture retention compared to all other samples, with different treatments during 40^{th} days of storage.

4.3.2 Soluble protein

The effect of different preservation methods and packaging materials on soluble protein content of sweet orange juice stored at ambient and refrigerated condition was analyzed and presented in Tables 4a and 4b. Table 4a shows the protein content of sweet orange juice with different preservation methods and packaging materials, stored at ambient condition. During the first day of storage, the soluble protein content in T1 *i.e.*, in juice sample filled in glass bottle and stored at ambient condition was found to be 0.35%, whereas in T4, T7 and T10, the soluble protein content of 0.39,

Table 4a. Effect of different preservation methods and packaging materials on soluble protein content of sweet orange juice, stored at ambient condition (30 ±2 °C)

Soluble protein (%)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	0.35	0.22	0.15	0.12
T2	0.30	0.18	0.10	0.07
T3	0.33	0.21	0.12	0.10
T4	0.39	0.30	0.24	0.18
T5	0.35	0.25	0.18	0.13
T6	0.37	0.24	0.20	0.17
T7	0.46	0.42	0.36	0.30
T8	0.44	0.38	0.33	0.26
T9	0.45	0.41	0.35	0.29
T10	0.43	0.40	0.32	0.28
T11	0.40	0.35	0.28	0.23
T12	0.41	0.37	0.31	0.25
S.Em ±	0.015	0.019	0.017	0.021
CD @ (1%)	0.060	0.076	0.069	0.082
CV	6.693	10.814	12.277	17.962
Factor	NS	S	S	S

Soluble protein in fresh sweet orange juice- 0.54%

NS: Non significant

S: Significant

0.46 and 0.43% was observed which was more compared to un-treated sample. In T2 *i.e.*, in juice samples filled in (PET) polyethylene terephthalate bottles and stored at ambient condition showed protein content of 0.30%, whereas in T5, T8 and T11 the soluble protein content of 0.36, 0.44 and 0.40%, respectively. In T3 *i.e.*, in juice samples filled in tin cans, the soluble protein content of 0.33% was recorded, whereas in T6, T9 and T12, the soluble protein was found to be 0.37, 0.45 and 0.41% respectively.

During 15, 30 and 40th days of storage, the soluble protein content in sweet orange juice decreased from 0.22 to 0.12 % in T1, followed by 0.30 to 0.18%, 0.42 to 0.30 % and 0.40 to 0.28% in T4, T7 and T10, respectively. In T2, the soluble protein content of 0.18 to 0.07% was recorded followed by 0.25 to 0.13 in T5, 0.38 to 0.26 in T8 and 0.35 to 0.23% in T11, respectively. In T3, the protein content of 0.21to 0.10% was recorded and in T6, T9 and T12, the protein content of 0.24 to 0.17%, 0.41 to 0.29% and 0.37 to 0.25%, respectively was recorded in juice samples with different treatments stored at ambient condition.

Table 4b explains the soluble protein content in sweet orange juice subjected to different treatments and stored at refrigerated condition. During the first day of storage, the protein content in T1, was found to be 0.36%, whereas in T4, T7 and T10, the protein content was found to be ranged from 0.41, 0.47 and 0.44% respectively, whereas, the soluble protein content of 0.32% was recorded in T2, followed by 0.35, 0.45 and 0.42% in T5, T8 and T11, respectively. In T3, the protein content was 0.34%, whereas in T6, T9 and T12, the soluble protein was 0.40, 0.46 and 0.43%, respectively.

During 15, 30 and 40th days of storage, the protein content in all the treated and un-treated samples were gradually decreased. In T1, the soluble protein was found to be decreased from 0.34 to 0.22%, in T4 0.35 to 0.30%, in T7 0.46 to 0.40% and in T10 0.42 to 0.33%. In T2, the protein content decreased from 0.28 to 0.19, 0.33 to 0.26, 0.43 to 0.32% and 0.39 to 0.27% in T5, T8 and T11, respectively. Whereas, in T3, the protein content was found to be decreased from 0.32 to 0.20%, followed by 0.37 to 0.28% in T6, 0.44 to 0.37% and 0.40 to 0.31% in T9 and T12, respectively.

From Tables 4a and 4b the soluble protein content with different treatments stored at ambient condition showed less retention of soluble protein compared to the samples from different treatments stored at refrigerated condition. This shows that, as storage

Table 4b. Effect of different preservation methods and packaging materials on soluble protein content of sweet orange juice, stored at refrigerated condition (4±1 °C)

Soluble protein (%)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	0.36	0.34	0.29	0.22
T2	0.32	0.28	0.25	0.19
T3	0.34	0.32	0.26	0.20
T4	0.41	0.35	0.32	0.30
T5	0.35	0.33	0.29	0.26
T6	0.40	0.37	0.30	0.28
T7	0.47	0.46	0.44	0.40
T8	0.45	0.43	0.40	0.32
T9	0.46	0.44	0.41	0.37
T10	0.44	0.42	0.39	0.33
T11	0.42	0.39	0.36	0.27
T12	0.43	0.40	0.37	0.31
S.Em±	0.020	0.023	0.026	0.018
CD @ (1%)	0.081	0.090	0.103	0.071
CV	8.608	10.487	13.041	10.539
Factor	NS	S	S	S

Soluble protein in fresh sweet orange juice- 0.54%

NS: Non significant

S: Significant

period proceeds the soluble protein content decreases due to denaturation of protein by microorganisms which further cause loss of nutrients and spoilage.

4.3.3 Carbohydrates

The effect of different preservation methods and packaging materials on carbohydrates of sweet orange juice stored at ambient and refrigerated condition was analyzed and presented in Table 5a and 5b. Table 5a shows the carbohydrates of sweet orange juice with different preservation methods and packaging materials stored at ambient condition. During first day of storage, the carbohydrates in T1 *i.e.,* in sweet orange juice filled in glass bottle and stored at ambient condition was found to be 9.92%, whereas in T4, T7 and T10, the carbohydrates of 10.10, 10.26 and 10.20% was observed which was more compared to un-treated sample. In T2 *i.e.,* in sweet orange juice filled in (PET) polyethylene terephthalate bottles and stored at ambient condition showed protein content of 9.88%, whereas in T5, T8 and T11 the carbohydrates was 10.06, 10.22 and 10.15%, respectively. In T3 *i.e.,* in sweet orange juice samples filled in tin cans, the carbohydrates of 9.91% was recorded, whereas in T6, T9 and T12, the carbohydrates was found to be 10.09, 10.25 and 10.19%, respectively.

During 15, 30 and 40 days of storage, the carbohydrates in sweet orange juice was from 6.65 to 4.72% in T1, followed by 8.79 to 5.39%, 9.40 to 7.30% and 8.77 to 5.94 % in T4, T7 and T10, respectively. In T2, the carbohydrates of 6.54 to 4.65% were recorded followed by 8.63 to 5.24% in T5, 9.35 to 7.20% in T8 and 8.80 to 5.72% in T11, respectively. In T3, the carbohydrates of 6.60 to 4.67% were recorded and in T6, T9 and T12, the carbohydrates of 8.72 to 5.35%, 9.37 to 7.26 and 8.85 to 5.88% were recorded in juice samples with different treatments stored at ambient condition.

Table 5b explains the carbohydrates of sweet orange juice subjected to different treatments and storage at refrigerated condition. During the first day of storage, the carbohydrate in T1 was found to be 10.00%, whereas in T4, T7 and T10, the carbohydrates of 10.15, 10.28 and 10.23% was determined, respectively. Likewise in T2, the carbohydrates of 9.94%, followed by 10.10, 10.25 and 10.20% in T5, T8 and T11, were determined and in T3, carbohydrates of 9.98, followed by 10.12, 10.27 and 10.21% in T6, T9 and T12, respectively.

Table 5a. Effect of different preservation methods and packaging materials on carbohydrates of sweet orange juice, stored at ambient condition (30±2 °C)

Carbohydrates (%)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	9.92	6.65	5.84	4.72
T2	9.88	6.54	5.81	4.65
T3	9.91	6.60	5.82	4.67
T4	10.10	8.79	6.42	5.39
T5	10.06	8.63	6.38	5.24
T6	10.09	8.72	6.40	5.35
T7	10.26	9.40	8.28	7.30
T8	10.22	9.35	8.19	7.20
T9	10.25	9.37	8.25	7.26
T10	10.20	8.77	7.83	5.94
T11	10.15	8.80	7.88	5.72
T12	10.19	8.85	7.92	5.88
S.Em±	0.029	0.123	0.109	0.086
CD @ (1%)	0.144	0.488	0.433	0.341
CV	0.493	2.553	2.677	2.588
Factor	NS	NS	S	S

Carbohydrates of fresh sweet orange juice- 10.46%

NS: Non significant

S: Significant

Table 5b. Effect of different preservation methods and packaging materials on carbohydrates of sweet orange juice, stored at refrigerated condition (4±1 °C)

Carbohydrates (%)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	10.00	7.52	6.91	5.44
T2	9.94	7.45	6.83	5.38
T3	9.98	7.48	6.86	5.41
T4	10.15	9.21	7.45	6.74
T5	10.10	9.15	7.41	6.65
T6	10.12	9.18	7.43	6.69
T7	10.28	10.20	9.65	8.44
T8	10.25	10.14	9.59	8.32
T9	10.27	10.18	9.62	8.35
T10	10.23	9.89	9.22	7.50
T11	10.20	10.01	9.13	7.30
T12	10.21	10.03	9.17	7.46
S.Em±	0.156	0.135	0.126	0.101
CD @ (1%)	0.615	0.536	0.499	0.400
CV	2.659	2.548	2.644	2.513
Factor	NS	NS	S	S

Carbohydrates of fresh sweet orange juice -10.46%

NS: Non significant

S: Significant

During 15, 30 and 40th days of storage, the decrease in carbohydrates in sweet orange juice was ranged from 7.52 to 5.44% in T1, followed by 9.21 to 6.74%, 10.20 to 8.44% and 9.89 to 7.45% in T4, T7 and T10 , respectively. In T2, the carbohydrates of 7.45 to 5.38% was recorded which was followed by 9.15 to 6.65% in T5, 10.14 to 8.32% in T8 and 10.01 to 7.30% in T11, respectively was observed. In T3, the carbohydrates of 7.48 to 5.41% were recorded and in T6, T9 and T12, the carbohydrates of 9.18 to 6.69%, 10.18 to 8.35% and 10.03 to 7.46%, respectively was recorded in juice samples with different treatments stored at refrigerated condition.

From Tables 5a and 5b the carbohydrates of sweet orange juice with different treatments stored at ambient condition showed less retention of carbohydrates compared to that of the samples with different treatments stored at refrigerated condition. The difference in values for same juice filled in different container for storage study of various parameters at the initial stage might be due to the influence of temperature fluctuations during processing and effect of preservation methods on juice in maintaining overall quality.

4.4 Effect of preservation methods and packaging materials on quality parameters of sweet orange juice during storage

4.4.1 pH

The effect of different preservation methods and packaging materials on quality parameters of sweet orange juice stored at ambient and refrigerated condition was analyzed and presented in Table 6(a) and 6(b). Table 6(a) shows the pH of sweet orange juice subjected to different treatments and stored at ambient condition. During first day of storage the pH content of sweet orange juice in T1 *i.e.,* juice sample filled in glass bottles and stored at ambient condition was found to be 3.74, whereas in T4, T7 and T10, the pH content was in the range of 4.09, 4.24 and 4.18, respectively in sweet orange juice filled in glass bottles subjected to homogenization (Physical), sodium benzoate (chemical) and nisin (biological) method of preservation. In T2 *i.e.,* in sweet orange juice filled in PET bottles and stored at ambient condition, the pH content was found to be 3.56 where less acidic pH in the range of 3.86, 4.15 and 4.06 was recorded in T5, T8 and T11. In T3 *i.e.,* in sweet orange juice filled in tin cans and stored at ambient condition was found to be 3.68 and in all the treatments the pH of 4.02, 4.21 and 4.12 in T6, T9 and T12, respectively.

Table 6a. Effect of different preservation methods and packaging materials on pH of sweet orange juice, stored at ambient condition (30±2 °C)

pH of juice				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	3.74	3.35	3.20	3.12
T2	3.56	3.24	3.15	3.09
T3	3.68	3.32	3.17	3.10
T4	4.09	3.94	3.38	3.20
T5	3.86	3.80	3.26	3.17
T6	4.02	3.91	3.34	3.19
T7	4.24	4.15	3.90	3.65
T8	4.15	4.08	3.84	3.59
T9	4.21	4.10	3.86	3.61
T10	4.18	4.12	3.74	3.45
T11	4.06	3.90	3.69	3.36
T12	4.12	4.09	3.72	3.40
S.Em ±	0.09	0.08	0.08	0.07
CD @ (1%)	0.34	0.33	0.30	0.28
CV	3.70	3.72	3.76	3.75
Factor	S	S	S	S

pH of fresh sweet orange juice- 4.38

NS: Non significant

S: Significant

During 15, 30 and 40 days of storage periods the gradual decrease in acidity was noticed in all the samples. In T1, the pH content decreased from 3.35 to 3.12 and in T4, T7 and T10, the pH content were decreased from 3.94 to 3.20, 4.15 to 3.65 and 4.12 to 3.45, where more acidic content was noticed in T4 compared to other two treatments. In T2, the pH content was decreased from 3.24 to 3.09 and in the pH content of 3.80 to 3.17, 4.08 to 3.59 and 3.90 to 3.36 in T5, T8 and T11 was recorded. In T3, the pH content of 3.32 to 3.10 was noticed which was followed by 3.91 to 3.19, 4.10 to 3.61 and 4.09 to 3.40 in all the treatments of T6, T9 and T12, respectively.

Table 6b shows the effect of different preservation methods and packaging materials on pH content of sweet orange juice stored at refrigerated condition. During the first day of storage, the pH content of 3.78 was noticed in T1, whereas less acidic content of 4.10, 4.26 and 4.21 was registered in T4, T7 and T10. In T2, the pH was 3.57 which were found to be more acidic in treated samples T5, T8 and T11 in the range of 3.95, 4.17 and 4.08, respectively. In T3, the pH was 3.71, whereas in T6, T9 and T12, the pH content of 4.04, 4.23 and 4.15 was noticed. During 15, 30 and 40 days of storage a decrease in pH from 3.46 to 3.27 was found in T1, whereas in T4, T7 and T10, a decrease in pH from 4.05 to 3.88, 4.20 to 4.10 and 4.19 to 3.90 was observed. In T2, the pH content of 3.38 to 3.20 was determined, where less acidic pH of 3.88 to 3.26, 4.11 to 3.92 and 4.02 to 3.83 was in treatments T5, T8 and T11. In T3, the decrease in pH from 3.40 to 3.25 was noted followed by decrease in pH from 3.98 to 3.32, 4.18 to 4.02 and 4.10 to 3.85 in treatments T6, T9 and T12, respectively.

From the above Tables, it was observed that the pH content in treated samples was found to be less under refrigerated condition compared to ambient storage condition. The decrease in pH in all the samples were observed, as the storage period increases, whereas un-treated (control) juice samples showed more acidic content under ambient condition compared samples stored at refrigerated condition. The decrease in pH in the first day itself was due to the effect of preservation methods and process conditions.

4.4.2 Water activity (a_w)

The effect of different preservation methods and packaging materials on water activity of sweet orange juice stored at ambient and refrigerated condition is presented in Table 7a and 7b. Table 7a shows the water activity (a_w) of sweet orange juice subjected to different treatments. During first day of storage the a_w in T1 *i.e.,* in juice sample filled in

Table 6b. Effect of different preservation methods and packaging materials on pH of sweet orange juice, stored at refrigerated condition (4±1 °C)

pH of juice				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	3.78	3.46	3.34	3.27
T2	3.57	3.38	3.26	3.20
T3	3.71	3.40	3.28	3.25
T4	4.10	4.05	3.56	3.38
T5	3.95	3.88	3.49	3.26
T6	4.04	3.98	3.50	3.32
T7	4.26	4.20	4.15	4.10
T8	4.17	4.11	4.08	3.92
T9	4.23	4.18	4.12	4.02
T10	4.21	4.19	4.06	3.90
T11	4.08	4.02	3.95	3.83
T12	4.15	4.10	4.04	3.85
S.Em±	0.086	0.084	0.081	0.079
CD @ (1%)	0.339	0.332	0.321	0.311
CV	3.709	3.714	3.771	3.780
Factor	S	S	S	S

pH of fresh sweet orange juice- 4.38

NS: Non significant

S: Significant

Table 7a. Effect of different preservation methods and packaging materials on water activity of sweet orange juice, stored at ambient condition (30±2 °C)

Water activity (a_w)				
Treatments	**DAY1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	0.894	0.910	0.926	0.942
T2	0.902	0.918	0.934	0.950
T3	0.896	0.912	0.928	0.944
T4	0.872	0.888	0.904	0.920
T5	0.878	0.894	0.910	0.926
T6	0.875	0.891	0.907	0.923
T7	0.860	0.862	0.874	0.886
T8	0.856	0.868	0.880	0.892
T9	0.852	0.864	0.876	0.888
T10	0.862	0.875	0.888	0.902
T11	0.868	0.881	0.893	0.905
T12	0.864	0.877	0.890	0.903
S.Em ±	0.008	0.009	0.013	0.014
CV	1.506	1.827	2.582	2.603
CD @ (1%)	0.030	0.037	0.053	0.054
Factor	S	S	S	S

Water activity of fresh sweet orange juice- 0.882

NS: Non significant

S: Significant

glass bottle and stored at ambient condition was found to be 0.894, whereas in T1, T4 and T10, the a_w values were 0.872, 0.860 and 0.862, respectively in juice samples filled in glass bottles subjected to homogenization (Physical), sodium benzoate (chemical) and nisin (biological) method of preservation. In T2 *i.e.,* in sweet orange juice samples filled in PET bottles and stored at ambient condition, the a_w was 0.902, which was found to be more compared to treatments T5, T8 and T11 *i.e.,* in the range of 0.878, 0.856 and 0.868. In T3 *i.e.,* juice sample filled in tin cans and stored at ambient condition, the a_w was found to be 0.896 and in all the treatments the a_w of 0.875, 0.852 and 0.864 was registered in T6, T9 and T12, respectively.

During 15, 30 and 40th days of storage periods the gradual increase in a_w was noticed in all the samples. In T1, the a_w was increased from 0.910 to 0.942 and in all the treatments the a_w was found to be increased from 0.888 to 0.920 in T1, 0.862 to 0.886 in T4 and 0.875 to 0.902 in T10, where more a_w was noticed in T1 compared to other two treatments. In T2, the a_w was increased from 0.918 to 0.950 and in all the treatments, the a_w of 0.894 to 0.926, 0.868 to 0.892 and 0.881 to 0.905 was recorded in T5, T8 and T11. In T3, the a_w of 0.912 to 0.944 was noticed, followed by 0.891 to 0.923, 0.864 to 0.888 and 0.877 to 0.903, was recorded in the treatments T6, T9 and T12, respectively.

Table 7b shows the effect of different preservation methods and packaging materials on a_w of sweet orange juice stored at refrigerated condition. During first day of storage, the a_w of 0.881 was noticed in T1, whereas less water activity of 0.854, 0.837 and 0.849 was recorded in T4, T7 and T10. In T2, the a_w was 0.889 was recorded and in treated samples T5, T8 and T11 the a_w in the range of 0.865, 0.843 and 0.855, respectively. In T3, the a_w was 0.883, whereas in T6, T9 and T12, the water activity of 0.862, 0.839 and 0.851 was noticed.

During 15, 30 and 40th days of storage, an increase in the a_w from 0.893 to 0.917, was observed in T1, whereas in T4, T7 and T10, the a_w of 0.866 to 0.890, 0.845 to 0.861 and 0.858 to 0.880 was recorded, whereas in T2, the water activity was increased from 0.901 to 0.925 and less water activity of 0.877 to 0.901, 0.851 to 0.867 and 0.864 to 0.886 was registered in treatments T5, T8 and T11. In T3, the a_w was increased from 0.895 to 0.919, followed by a_w of 0.874 to 0.898, 0.847 to 0.863 and 0.860 to 0.884 recorded in treatments T6, T9 and T12, respectively.

Table 7b. Effect of different preservation methods and packaging materials on water activity of sweet orange juice, stored at refrigerated condition (4±1 °C)

Water activity (a_w)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	0.881	0.893	0.905	0.917
T2	0.889	0.901	0.913	0.925
T3	0.883	0.895	0.907	0.919
T4	0.854	0.866	0.878	0.890
T5	0.865	0.877	0.889	0.901
T6	0.862	0.874	0.886	0.898
T7	0.837	0.845	0.853	0.861
T8	0.843	0.851	0.859	0.867
T9	0.839	0.847	0.855	0.863
T10	0.849	0.858	0.865	0.880
T11	0.855	0.864	0.877	0.886
T12	0.851	0.860	0.872	0.884
S.Em±	0.003	0.009	0.011	0.012
CV	0.696	1.865	2.124	2.277
CD @(1%)	0.014	0.037	0.043	0.046
Factor	S	S	S	S

Water activity of fresh sweet orange juice- 0.882

NS: Non significant
S: Significant

From the above Tables it was observed that the water activity in all the treatments stored at refrigerated condition was less, when compared to that of treatments kept at ambient condition. With increase in storage period, water activity was also increased in all the samples. More water activity was observed in un-treated samples *i.e.,* in T1, T2, T3 than that in treated samples which indicates the effect of preservation methods in making free water less available for microbial growth and multiplication. Different preservation methods and storage conditions shows different modes of action in controlling water activity to minimize juice spoilage.

4.4.3 Colour

L^* Values

Effect of different preservation methods and packaging materials on the colour of sweet orange juice stored at ambient and refrigerated condition was determined using Hunter's lab colorimeter and the values of L^* were presented in Table 8a and 8b, respectively. It was observed from the Table 8a. that the L^* values showed a gradual decreases with increase in storage period with respect to treatments, packaging materials and stored at ambient condition.

In T1, the decrease in L^* value from 54.69 to 37.68 was observed, followed by 60.46 to 47.65, 56.10 to 48.46 and 55.62 to 44.85 in T4, T7 and T10. Whereas in T2, the L^* value of 50.84 to 33.32, 58.92 to 46.54 in T5 and 54.36 to 46.75, 54.50 to 41.98 in T8 and T11, respectively. In T3, the decrease in L^* value ranged from 53.32 to 36.48, followed by 60.34 to 47.48 in T6, 54.44 to 47.56 in T9 and 55.41 to 42.70 in T12 from initial to 40th days of storage period at ambient condition.

From the Table 8b, it was observed that the less decrease in L^*value was noted in sweet orange juice with different preservation methods, packaging materials and stored at refrigerated condition. The decrease in L^*value from 54.62 to 44.36 was noted in T1, followed by 59.52 to 49.29 in T4, followed by 54.52 to 49.94 in T7 and 54.56 to 45.07 in T10, respectively. In T2, the L^*value of 52.70 to 41.29 was observed, followed by 50.34 to 47.48 in T5, 52.29 to 48.74 and 53.68 to 43.92 in T8 and T11, respectively. The decrease in L^* value from 53.68 to 42.84, was observed in T3, followed by 57.02 to 48.90, 53.88 to 49.42 and 52.96 to 44.57 in T6, T9 and T12, respectively during the 1 to 40 days of storage period at refrigerated condition.

Table 8a. Effect of different preservation methods and packaging materials on colour (L^* value) of sweet orange juice, stored at ambient condition (30±2 °C)

L^* values				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	54.69	52.84	48.52	37.68
T2	50.84	46.45	42.08	33.32
T3	53.32	47.40	45.92	36.48
T4	60.46	56.32	51.84	47.65
T5	58.92	54.56	50.90	46.54
T6	60.34	55.42	49.56	47.48
T7	56.10	54.64	52.32	48.46
T8	54.36	53.68	51.47	46.75
T9	54.44	54.52	51.64	47.56
T10	55.62	51.60	45.54	44.85
T11	54.50	49.64	42.19	41.98
T12	55.41	50.52	43.98	42.70
S.Em ±	1.173	1.114	1.029	0.930
CD @ (1%)	4.639	4.407	4.072	3.680
CV	3.649	3.695	3.720	3.714
Factor	S	S	S	S

L^* value of fresh sweet orange juice- 56.59

NS: Non significant

S: Significant

Table 8b. Effect of different preservation methods and packaging materials on colour (L^* value) of sweet orange juice stored at refrigerated condition (4±1 °C)

L^* value				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	54.62	52.54	45.07	44.36
T2	52.70	49.26	45.67	41.29
T3	53.68	50.13	44.69	42.84
T4	59.52	56.40	53.54	49.29
T5	50.34	55.42	49.56	47.48
T6	57.02	56.39	51.42	48.9
T7	54.52	53.66	50.48	49.94
T8	52.29	50.42	49.63	48.74
T9	53.88	52.14	50.86	49.42
T10	54.56	49.10	47.40	45.07
T11	53.68	48.44	46.50	43.92
T12	52.96	48.84	46.73	44.57
S.Em ±	1.138	1.095	1.025	0.984
CD @ (1%)	4.502	4.332	4.055	3.910
CV	3.646	3.661	3.669	3.702
Factor	S	S	S	S

L^* Value of fresh sweet orange juice- 56.59

NS: Non significant

S: Significant

a^* Values

The effect of different preservation methods and packaging materials on a^* value of sweet orange juice stored at ambient and refrigerated condition was analyzed and presented in Table 9a and 9b, respectively. It was observed that in all the treatments, the increase in a^* values were observed with respect to an increase in storage period. In T1, the increase in a^* values was observed from -3.29 to 2.62, followed by - 4.84 to 1.18, - 4.68 to 1.40 and -3.24 to 0.76 in T4, T7 and T10, respectively. In T2, the increase in a^* values from - 4.08 to 3.42 was noticed and -4.92 to 1.68, -4.26 to 0.14 and -3.14 to 2.36 in T5, T8 and T11, respectively. The a^* values of -3.25 to 2.66 was observed in T3, followed by -4.74 to 1.12 in T6, -4.62 to 0.14 and -3.18 to 2.06 in T9 and T12, respectively from initial to 40th days of storage of sweet orange juice at ambient condition.

It was observed from the Table 9b. that the increase in a^* values from -4.06 to -2.74 in T1, followed by -4.63 to 0.40 in T4, -4.36 to 2.54 in T7 and -4.29 to 0.85 in T10, respectively. Whereas in T2, the increase in a^* values from -4.24 to -1.28 was noted, followed by -4.42 to 0.72, -4.48 to 1.65 and -4.34 to 2.24 in T5, T8 and T11, respectively. In T3, the increase in a^* values from -3.25 to -2.66 was observed, followed by -4.39 to 1.40 in T6, -4.28 to -2.18 and -4.25 to 0.75 in T9 and T12, respectively from first to 40 days of storage period at refrigerated condition.

b^* Values.

The effect of different preservation methods and packaging materials on the colour value of sweet orange juice stored at ambient and refrigerated condition was analyzed and presented in Table. 10a and 10b From the Table 10a, it was observed that the decrease in b^*values in sweet orange juice with different preservation methods and packaging materials, stored at ambient condition. The decrease in b^* value from 18.64 to 10.78 was observed in T1, followed by 23.70 to 18.62 in T4, 24.68 to 20.80 in T7 and 21.84 to 18.82 in T10, respectively, followed by 17.85 to 5.46 in T2, 20.74 to 15.98 in T5, 23.82 to 18.48 in T8 and 20.72 to 17.68 in T11, respectively. Whereas in T3, the decrease in b^* value from 18.46 to 8.84 was observed, followed, by 22.82 to 17.64 in T6, 24.62 to 19.95 in T9 and 21.60 to 18.50 in T12, respectively.

Table 9a. Effect of different preservation methods and packaging materials on colour (a^* value) of sweet orange juice, stored at ambient condition (30±2 °C)

a^* value				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	-3.29	-1.30	0.71	2.62
T2	-4.08	-1.54	-0.91	3.42
T3	-3.25	-1.24	0.75	2.66
T4	-4.84	-2.72	-0.86	1.18
T5	-4.92	-2.56	-0.54	1.68
T6	-4.74	-2.62	-0.78	1.12
T7	-4.68	-3.72	-2.42	1.40
T8	-4.26	-3.42	-1.65	0.14
T9	-4.62	-3.51	-1.82	0.10
T10	-3.24	-2.68	-0.2	0.76
T11	-3.14	-2.49	-0.12	2.36
T12	-3.18	-2.42	-1.24	2.06
S.Em ±	0.086	0.059	0.028	0.032
CD @ (1%)	0.340	0.232	0.115	0.156
CV	-3.731	-4.035	-6.642	4.201
Factor	S	S	S	S

a^* value of sweet orange juice- -5.59

NS: Non significant

S: Significant

Table 9b. Effect of different preservation methods and packaging materials on the colour (a^* value) of sweet orange juice, stored at refrigerated condition (4±1 °C)

a^* value				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	-4.06	-0.28	-1.22	-2.74
T2	-4.24	-2.36	-0.54	-1.28
T3	-3.25	-1.24	0.75	-2.66
T4	-4.63	-3.22	-2.54	0.40
T5	-4.42	-3.55	-1.68	0.72
T6	-4.39	-3.48	-2.24	1.40
T7	-4.36	-4.21	-3.48	2.54
T8	-4.48	-3.92	-3.58	1.65
T9	-4.28	-4.19	-3.62	-2.18
T10	-4.29	-2.74	-1.26	0.85
T11	-4.34	-2.63	-0.18	2.24
T12	-4.25	-2.72	-1.15	0.75
S.Em ±	0.091	0.068	0.051	0.04
CD @ (1%)	0.358	0.269	0.202	0.159
CV	-3.697	-4.095	-5.817	5.564
Factor	S	S	S	S

a^* value of sweet orange juice- -5.59

NS: Non significant

S: Significant

Table 10a. Effect of different preservation methods and packaging materials on the Colour (b^* value) of sweet orange juice, stored at ambient condition (30±2 °C)

b* **value**				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	18.64	14.56	12.80	10.78
T2	17.85	12.62	7.14	5.46
T3	18.46	15.42	9.65	8.84
T4	23.70	29.65	19.48	18.62
T5	20.74	18.56	16.42	15.98
T6	22.82	19.54	18.70	17.46
T7	24.68	23.81	21.46	20.80
T8	23.82	30.58	19.69	18.48
T9	24.62	22.38	20.44	19.95
T10	21.84	20.56	19.70	18.82
T11	20.72	19.86	18.55	17.68
T12	21.60	20.24	19.16	18.50
S.Em ±	0.466	0.452	0.378	0.359
CD @ (1%)	1.842	1.787	1.493	1.419
CV	3.736	3.797	3.868	3.903
Factor	S	S	S	S

b^* value of fresh sweet orange juice- 18.62

NS: Non significant

S: Significant

Table 10b. Effect of different preservation methods and packaging materials on the colour (b^* value) of sweet orange juice, stored at refrigerated condition (4±1 °C)

Colour (b^* value)				
Treatment	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	18.52	17.84	15.46	12.70
T2	17.26	16.82	13.34	10.75
T3	18.43	17.58	14.06	12.62
T4	21.98	20.85	20.42	19.76
T5	21.30	20.48	19.62	17.54
T6	21.87	20.74	19.85	18.69
T7	24.48	23.36	22.74	21.62
T8	23.80	22.45	20.92	19.68
T9	24.36	23.52	21.68	20.81
T10	22.92	22.68	20.59	19.54
T11	21.66	21.80	19.35	18.74
T12	22.59	22.32	20.48	19.28
S.Em ±	0.468	0.452	0.415	0.387
CD @ (1%)	1.852	1.320	1.642	1.529
CV	3.760	3.758	3.781	3.795
Factor	S	S	S	S

b^* value of fresh sweet orange juice- 18.62

NS: Non significant

S: Significant

From Table 10b, the decrease in b^* value from 18.52 to 12.70 was observed in T1, followed by 21.98 to 19.76 in T4, 24.48 to 21.62 in T7 and 22.92 to 19.54 in T10, respectively. Whereas in T2, the decrease in the b^*value from 17.26 to 10.75 was observed followed by the decrease in b^*value from 21.30 to 10.75 in T5, 23.80 to 19.68 in T8 and 21.66 to 18.74 in T11, 18.43 to 12.62 in T3, 21.87 to 18.69 in T6, 24.36 to 20.81 and 22.59 to 19.28 in T9 and T12, respectively, during the first and 40 days of storage period at refrigerated condition.

4.4.4 Total soluble solids (TSS)

The effect of different preservation methods and packaging materials on total soluble solids content of sweet orange juice stored at ambient and refrigerated condition was represented in Table 11a and 11b.Table 11a details about the total soluble solids content of sweet orange juice subjected to different treatments and stored at ambient condition. During first day of storage the total soluble solids content of sweet orange juice in T1 *i.e.,* in sweet orange juice filled in glass bottles and stored at ambient condition was found to be 11.11°Brix, whereas in T4, T7 and T10, the total soluble solids content was in the range of 11.17, 11.36 and 11.24 °Brix, respectively in juice samples filled in glass bottles subjected to homogenization (Physical), sodium benzoate (chemical) and nisin (biological) method of preservation. In T2 *i.e.,* in sweet orange juice filled in PET bottles and stored at ambient condition, the TSS was 11.08 °Brix, which was found to be less compared to treatments T5, T8 and T11 in which TSS levels were 11.13, 11.30 and 11.20 °Brix, respectively. In T3 *i.e.,* sweet orange juice sample filled in tin cans and stored at ambient condition, the TSS was found to be 11.09 °Brix and in all the treatments the TSS of 11.15, 11.34 and 11.23 °Brix were registered in T6, T9 and T12, respectively.

During 15, 30 and 40 days of storage a gradual decrease in TSS were noticed in all the samples. In T1, the TSS was from 7.55 to 5.58 °Brix, whereas, in all the treatments the TSS of 9.71 to 6.25 °Brix in T4, 10.42 to 7.86 °Brix in T7 and 10.20 to 6.78 °Brix in T10, where more TSS was noticed in T4 compared to other two treatments. In T2, the TSS decreased from 7.46 to 5.50 °Brix and in all the treatments; the decrease in TSS from 9.66 to 6.20, 10.34 to 7.76 °Brix was noticed in T5, T8 and T11. In T3, the decrease in TSS from 7.48 to 5.52 °Brix was noticed, followed by 9.69 to 6.23, 10.38 to 7.72 and 10.15 to 6.74 °Brix, was registered in the treatments T6, T9 and T12, respectively.

Table 11a. Effect of different preservation methods and packaging materials on total soluble solids content (TSS) of sweet orange juice, stored at ambient condition (30±2 °C)

Total soluble solids (TSS °Brix)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	11.11	7.55	6.72	5.58
T2	11.08	7.46	6.68	5.50
T3	11.09	7.48	6.69	5.52
T4	11.17	9.71	7.46	6.25
T5	11.13	9.66	7.38	6.20
T6	11.15	9.69	7.48	6.23
T7	11.36	10.42	9.50	7.86
T8	11.30	10.34	9.42	7.76
T9	11.34	10.38	9.43	7.72
T10	11.24	10.20	8.21	6.78
T11	11.20	10.08	8.13	6.71
T12	11.23	10.15	8.16	6.74
S.Em±	1.890	0.153	0.127	0.106
CD @ (1%)	0.748	0.606	0.504	0.418
CV	2.936	2.818	2.782	2.790
Factor	S	S	S	S

Total soluble solids content in fresh sweet orange juice- 10.9 °Brix

NS: Non significant

S: Significant

Table 11b details about the total soluble solids content of sweet orange juice subjected to different treatments and stored at refrigerated condition. It was observed that the total soluble solids content of 11.08 °Brix was found in T1, followed by 11.15, 11.31 and 11.22 °Brix in T4, T7 and T10, respectively. Whereas, the total soluble solids contents of 11.05, 11.10, 11.26 and 11.17 °Brix were noticed in T2, T5, T8 and T11, respectively. Likewise, the total soluble solids content of 11.07 was observed in T3, followed by 11.12, 11.29 and 11.19 °Brix in T6, T9 and T12, respectively during the first day of storage.

During the 15, 30 and 40th days of storage periods, the decrease in TSS from 8.70 to 6.12 °Brix was observed in T1, followed by 10.10 to 7.60%, 11.22 to 10.05 and 11.05 to 8.76 °Brix in T4, T7 and T10, respectively. In T2, total soluble solids decreased from 8.55 to 6.07, followed by 10.08 to 7.54 in T5, 11.17 to 9.64 °Brix in T8 and 11 to 8.54 °Brix in T11, respectively. Likewise in T3, the decrease in the total soluble solid content was ranged from 8.57 to 6.09 °Brix, followed by 10.09 to 7.58, 11.18 to 10 and 11.01 to 8.69 °Brix in T6, T9 and T12, respectively.

From the above Tables, it was noted that the total soluble solids contents decreased gradually with respect to different treatments as storage period proceeds and were discussed in chapter V of section 5.2.4. It was observed that when sweet orange juice was subjected to chemical as well as pasteurization treatment due to loss of moisture, juice becomes concentrated. Hence T9 (11.34 °Brix) showed increase in TSS compared to that of fresh juice sample (10.9 °Brix), where it is not subjected to any treatments.

4.4.5 Ascorbic acid

Tables 12a and 12b represents the ascorbic acid content of sweet orange juice with respect to the different preservation methods, packaging materials and storage conditions. It was found that the ascorbic acid content in fresh sweet orange juice was found to be 62.5 mg.ml^{-1}. Table 12a reveals the ascorbic acid content in sweet orange juice stored at ambient condition. It was observed that the ascorbic acid content in T1 was found to be 54.60 mg.ml^{-1}, followed by 55.42, 55.96 and 55.59 mg.ml^{-1} in T4, T7 and T10, respectively followed by 48.14 mg.ml^{-1} in T2 and 49.90 mg.ml^{-1} in T5, 51.88 mg.ml^{-1} in T8 and 51.32 mg.ml^{-1} in T11, respectively. Similarly in T3, the ascorbic acid content was

Table 11b. Effect of different preservation methods and packaging materials on total soluble solids (TSS) of sweet orange juice, stored at refrigerated condition (4±1°C).

Total soluble solids (TSS °Brix)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	11.08	8.70	7.34	6.12
T2	11.05	8.55	7.29	6.07
T3	11.07	8.57	7.32	6.09
T4	11.15	10.10	8.49	7.60
T5	11.10	10.08	8.45	7.54
T6	11.12	10.09	8.47	7.58
T7	11.31	11.22	10.25	10.05
T8	11.26	11.17	10.00	9.64
T9	11.29	11.18	10.24	10.00
T10	11.22	11.05	9.70	8.76
T11	11.17	11.00	8.13	8.54
T12	11.19	11.01	9.66	8.69
S.Em±	0.148	0.221	0.194	0.160
CD @ (1%)	0.584	0.875	0.765	0.635
CV	2.283	3.750	3.823	3.780
Factor	S	S	S	S

Total soluble solids content in fresh sweet orange juice- 10.9 °Brix

NS: Non significant

S: Significant

Table 12a. Effect of different preservation methods and packaging materials on ascorbic acid content of sweet orange juice, stored at ambient condition (30±2 °C)

Ascorbic acid ($mg.ml^{-1}$)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	54.60	33.82	21.74	9.20
T2	48.14	29.32	18.83	7.54
T3	50.86	31.44	20.52	8.93
T4	55.42	45.78	30.36	21.84
T5	49.90	42.37	28.44	15.76
T6	52.36	43.40	29.68	18.84
T7	55.96	52.44	47.86	32.20
T8	51.88	50.16	45.52	30.48
T9	53.39	51.86	46.63	31.70
T10	55.59	48.95	41.84	28.50
T11	51.32	47.43	36.62	24.78
T12	52.95	48.82	37.58	25.32
S.Em ±	1.123	0.965	0.798	0.566
CD @ (1%)	4.430	3.817	3.158	2.238
CV	3.697	3.820	4.082	4.252
Factor	S	S	S	S

Ascorbic acid content in fresh sweet orange juice- 62.5 $mg.ml^{-1}$

NS: Non significant

S: Significant

found to be 50.86 mg.ml^{-1}, followed by 52.36, 53.39 and 52.95 mg.ml^{-1} in T6, T9 and T12, respectively during the initial day of storage period at ambient condition.

On the other hand, during 15, 30 and 40th days of storage, the degradation of ascorbic acid content was noticed in all the samples including the treated samples and was found to be ranged from 33.82 to 9.20 mg.ml^{-1} in T1, followed by 45.78 to 21.84 mg.ml^{-1} in T4, 52.44 to 32.20 mg.ml^{-1} in T7 and 48.95 to 28.50 mg.ml^{-1} in T10, respectively. Similarly in T2, the ascorbic acid content was found to be ranged from 29.32 to 7.54 mg.ml^{-1}, followed by 42.37 to 15.76, 50.16 to 30.48 and 47.43 to 24.78 mg.ml^{-1} in T5, T8 and T11, respectively and in T3, the ascorbic acid content was found to be decreased from 31.44 to 8.93 mg.ml^{-1}, followed by 43.40 to 18.84, 51.86 to 31.70 and 48.82 to 25.32 mg.ml^{-1} in T6, T9 and in T12, respectively.

Table 12b details about the ascorbic acid content in sweet orange juice with different preservation methods and packaging materials, stored at refrigerated condition. From Table 12b it was observed that the ascorbic acid content in T1 was 54.60 mg.ml^{-1}, followed by 55.42, 55.96 and 55.59 mg.ml^{-1} in T4, T7 and T10, respectively and in similar manner, the ascorbic acid content in T2 was 48.14 mg.ml^{-1}, where the more retention of ascorbic acid compared to T2 was noticed in treatments T5, T8 and T11 in the range of 49.90, 51.88 and 51.32 mg.ml^{-1} respectively. In T3, the ascorbic acid content of 50.86 mg.ml^{-1} was observed followed by 52.36, 53.39 and 52.95 mg.ml^{-1} in T6, T9 and in T12, respectively during the initial day of the storage period.

During the 15, 30 and 40th days of storage period, the ascorbic acid content was found to be decreased from 40.76 to 20.58 mg.ml^{-1}in T1, followed by 52.77 to 30.39 mg.ml^{-1} in T4, 55.30 to 49.43 mg.ml^{-1} in T7 and 54.36 to 42.99 mg.ml^{-1} in T10 treatment, respectively and in T2, the ascorbic acid content of 36.62 to 15.49 mg.ml^{-1} was observed followed by 48.81 to 26.66 mg.ml^{-1} in T5 and 50.94 to 44.79, 50.20 to 40.32 mg.ml^{-1} in T8 and T11, respectively. On the other hand ascorbic acid content range of 37.71 to 18.88 mg.ml^{-1} was noticed in T3, followed by 49.24 to 28.82 in T6, 52.73 to 46.52 and 51.68 to 41.86 mg.ml^{-1} in T9 and T12, respectively.

From the above Tables, it was observed that as the storage period proceeds, the degradation of ascorbic acid also increases in faster rate at ambient condition where less degradation of ascorbic acid with respect to increase in storage period was observed in the refrigerated condition.

Table 12b. Effect of different preservation methods and packaging materials on ascorbic acid content of sweet orange juice, stored at refrigerated condition (4±1 °C)

Ascorbic acid ($mg.ml^{-1}$)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	54.60	40.76	32.49	20.58
T2	48.14	36.62	28.33	15.49
T3	50.86	37.71	30.59	18.88
T4	55.42	52.77	38.33	30.39
T5	49.90	48.81	36.78	26.66
T6	52.36	49.24	35.53	28.82
T7	55.96	55.30	53.89	49.43
T8	51.88	50.94	49.63	44.79
T9	53.39	52.73	51.97	46.52
T10	55.59	54.36	46.63	42.99
T11	51.32	50.20	44.83	40.32
T12	52.95	51.68	45.42	41.86
S.Em ±	1.12	1.043	0.943	0.7984
CD @ (1%)	4.44	4.126	3.728	3.15803
CV	3.70	3.737	3.904	4.1161
Factor	S	S	S	S

Ascorbic acid content in fresh sweet orange juice- 62.5 $mg.ml^{-1}$

NS: Non significant

S: Significant

4.4.6 Browning index

Table 13a and 13b details about the browning index of sweet orange juice in response to different preservation methods and packaging materials, stored at ambient and refrigerated condition. It was noted that the browning index of freshly extracted sweet orange juice was found to be 0.14% presented in Table 13a. In T1, the browning index of 0.214% was observed followed by 0.208, 0.180 and 0.186% in T4, T7 and T10 respectively. In T2, the browning index of 0.220% was observed, followed by 0.212, 0.184% and 0.190% in T5, T8 and T11, respectively and in the similar way in T3, the juice browning index of 0.218% was observed, followed by 0.210, 0.182 and 0.187% in T6, T9 and in T12, respectively during the initial day of the storage period.

During the 15, 30 and 40th days of storage period the increase in browning index was observed from 0.263 to 0.412% in T1, followed by 0.246 to 0.390, 0.194 to 0.352% and 0.232 to 0.379 in T4, T7 and in T10, respectively. In T2, the browning index in the range from 0.278 to 0.439% was observed, similarly in T5, T8 and in T11, the browning index of 0.254 to 0.420, 0.218 to 0.372% and 0.243 to 0.391% was noticed and in T3, the browning index value of 0.272 to 0.419% was observed, followed by 0.249 to 0.415% in T6, 0.189 to 0.365% in T9 and 0.235 to 0.384% in T12, respectively.

On the other hand, the juice browning index of sweet orange juice with different preservation methods, packaging materials and stored at refrigerated conditions were analyzed and the values obtained as follows were detailed in Table 13b. It was observed during the initial day of storage period the juice browning index was found to be 0.212% in T1, followed by 0.206, 0.170 and 0.180% in T4, T7 and T10, respectively and in the similar way, the browning index of 0.218% was observed in T2, followed by 0.210, 0.182 and 0.189% in T5, T8 and in T11, respectively. In T3, the browning index of 0.215% was observed followed by 0.208, 0.177 and 0.186% in T6, T9 and T12, respectively.

During the15, 30 and 40 days of storage period, the increase in browning index from 0.240 to 0.372% was observed in T2, followed by 0.230 to 0.342% in T4, 0.180 to 0.247% in T7 and 0.210 to 0.321% in T10, respectively. In T2, the browning index value of 0.254 to 0.390% was observed, followed by 0.241 to 0.354% in T5 and 0.196 to 0.257%, 0.232 to 0.338% in T8 and T11, respectively. In similar way, the browning index of 0.248 to 0.385 was observed in T3, followed by 0.237 to 0.349% in T6, 0.189 to 0.251% in T9 and 0.219 to 0.325% in T12, respectively.

Table 13a. Effect of different preservation methods and packaging materials on browning index of sweet orange juice, stored at ambient condition (30±2 °C)

Browning index (%)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	0.214	0.263	0.389	0.412
T2	0.220	0.278	0.394	0.439
T3	0.218	0.272	0.391	0.419
T4	0.208	0.246	0.310	0.390
T5	0.212	0.254	0.315	0.420
T6	0.210	0.249	0.312	0.415
T7	0.180	0.194	0.230	0.352
T8	0.184	0.218	0.240	0.372
T9	0.182	0.189	0.237	0.365
T10	0.186	0.232	0.294	0.379
T11	0.190	0.243	0.306	0.391
T12	0.187	0.235	0.298	0.384
S.Em ±	0.004	0.005	0.007	0.008
CD @ (1%)	0.016	0.020	0.026	0.033
CV	3.629	3.613	3.643	3.646
Factor	S	S	S	S

Browning index of fresh sweet orange juice- 0.140%

NS: Non significant

S: Significant

Table 13b. Effect of different preservation methods and packaging materials conditions on browning index of sweet orange juice, stored at refrigerated condition (4±1 °C)

Browning index (%)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	0.212	0.240	0.318	0.372
T2	0.218	0.254	0.326	0.390
T3	0.215	0.248	0.320	0.385
T4	0.206	0.230	0.285	0.342
T5	0.210	0.241	0.292	0.354
T6	0.208	0.237	0.284	0.349
T7	0.170	0.180	0.192	0.247
T8	0.182	0.196	0.236	0.257
T9	0.177	0.189	0.230	0.251
T10	0.180	0.210	0.264	0.321
T11	0.189	0.232	0.274	0.338
T12	0.186	0.219	0.269	0.325
S.Em ±	0.004	0.005	0.006	0.007
CD @ (1%)	0.016	0.018	0.023	0.027
CV	3.626	3.633	3.621	3.593
Factor	S	S	S	S

Browning index of fresh sweet orange juice- 0.140%

NS: Non significant

S: Significant

From the above Tables 13a and 13b, it was noticed that as the storage period proceeds, the increase in browning index was observed in all the un-treated samples including the treated samples with respect to different preservation methods and packaging materials used.

4.4.7 Total carotenoids

Table 14a and 14b details about the total carotenoids content of sweet orange juice with different preservation methods and packaging materials, stored at ambient and refrigerated conditions. It was found that the total carotenoids content of 37.5 mg.ml^{-1} was observed in freshly squeezed sweet orange juice. From the Table 14a it was observed that during the initial day of storage period the total carotenoids content of 32.70 mg.ml^{-1} was determined in T1 followed by 34.89, 35.21 and 35.05 mg.l^{-1} in T4, T7 and T10, respectively. In T2, the total carotenoids content of 30.42 was observed, followed by 31.84, 33.88 and 33.45mg.ml^{-1} in T5, T8 and T11 and similarly in T3, the total carotenoids content of 32.51 mg.l^{-1} was observed, followed by 32.90, 34.80 and 33.90 mg.ml^{-1} in T6, T9 and T12, respectively.

On the other hand during the 15, 30 and 40 days of storage period, the decrease in total carotenoids content was ranged from 27.42 to 18.85 mg.ml^{-1} in T1, followed by 30.94 to 19.41, 34.74 to 26.40 and 33.69 to 24.58 mg.ml^{-1} in T4, T7 and T10, respectively. Likewise in T1, the total carotenoids content of 26.80 to 16.34 mg.ml^{-1} was observed, 29.32 to 17.58 mg.ml^{-1} in T5, 32.90 to 24.49 mg.l^{-1} in T8 and 31.74 to 22.56 mg.l^{-1} in T11. In T3, the decrease total carotenoids content from 26.58 to 17.42 mg.ml^{-1} was observed followed by 30.54 to 18.86 mg.ml^{-1} in T6, 34.62 to 25.76 mg.ml^{-1} in T9 and 32.82 to 23.45 mg.ml^{-1} in T12, respectively.

Table 14b details about the decrease in total carotenoids content in sweet orange juice with different preservation methods and packaging materials stored at refrigerated condition. During the first day of storage the total carotenoids content of 32.92 mg.ml^{-1} was observed in T1, followed by 34.89, 35.25 and 35.15 mg.ml^{-1} in T4, T7 and T10, respectively. In T2 the total carotenoids content of 31.54 mg.ml^{-1}was observed, followed by 34.42, 34.50 and 33.70 mg.ml^{-1} in T, T5, T8 and T11, whereas in T3, the total carotenoids content of 32.66 was observed, followed by 33.56, 34.86 and 34.18 mg.ml^{-1} in T6, T9 and T12, respectively.

Table 14a. Effect of different preservation methods and packaging materials on total carotenoids content of sweet orange juice, stored at ambient condition (30±2 °C)

Total carotenoids ($mg.ml^{-1}$)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	32.70	27.42	20.51	18.85
T2	30.42	26.80	19.65	16.34
T3	32.51	26.58	20.36	17.42
T4	34.89	30.94	27.88	19.41
T5	31.84	29.32	26.40	17.58
T6	32.90	30.54	27.72	18.86
T7	35.21	34.74	32.45	26.40
T8	33.88	32.90	30.76	24.49
T9	34.80	34.62	31.94	25.76
T10	35.05	33.69	30.32	24.58
T11	33.45	31.74	29.80	22.56
T12	33.90	32.82	30.21	23.45
S.Em ±	0.769	0.762	0.612	0.475
CD @ (1%)	3.043	2.775	2.422	3.860
CV	3.980	3.916	3.877	3.860
Factor	S	S	S	S

Total carotenoids content-37.5 $mg.ml^{-1}$

NS: Non significant

S: Significant

Table 14b. Effect of different preservation methods and packaging materials on total carotenoids content of sweet orange juice, stored at refrigerated condition (4±1 °C)

Total carotenoids ($mg.ml^{-1}$)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	32.92	29.85	24.44	20.70
T2	31.54	28.60	22.72	19.81
T3	32.66	29.72	24.35	20.50
T4	34.89	32.56	30.71	27.82
T5	34.42	30.51	28.42	25.48
T6	33.56	32.40	30.69	27.50
T7	35.25	34.80	33.72	31.34
T8	34.50	33.46	32.82	30.64
T9	34.86	34.29	33.14	31.20
T10	35.15	34.20	32.54	30.61
T11	33.70	32.62	30.85	28.44
T12	34.18	33.51	31.30	29.92
S.Em ±	0.456	0.735	0.670	0.607
CD @ (1%)	1.802	2.906	2.651	2.401
CV	2.326	3.948	3.913	3.892
Factor	S	S	S	S

Total carotenoids content-37.5 $mg.ml^{-1}$

NS: Non significant

S: Significant

During the 15, 30 and 40 days of storage period, the decrease in total carotenoids from 29.85 to 20.70 $mg.ml^{-1}$ in T1, followed by 32.56 to 27.82, 34.80 to 31.34 and 34.20 to 30.61 $mg.ml^{-1}$ was determined in T4, T7 and T10. In T2, the decrease in total carotenoids from 28.60 to 19.81 was observed, followed by 30.51 to 25.48 $mg.ml^{-1}$ in T5, 33.46 to 30.64 $mg.ml^{-1}$ in T8 and 32.62 to 28.44 $mg.ml^{-1}$ in T10. In T3, the decrease in total carotenoids content from 29.72 to 20.50 $mg.ml^{-1}$, followed by 32.40 to 27.50, 34.29 to 31.20 and 33.51 to 29.92 $mg.ml^{-1}$in T6, T9 and in T12. From the above Tables, it was observed that the degradation of total carotenoids content in sweet orange juice was found to be faster than that in the samples stored at a refrigerated condition.

4.4.8 Total flavonoids

The effect of different preservation methods, packaging materials and storage conditions on the total flavonoids content of sweet orange juice was analyzed and presented in Table 15a and Table 15b. From the Table 15a it was observed that the total flavonoids content in sweet orange juice was found to be 24.6 $mg.ml^{-1}$. In un-treated samples, it was observed that during the first day of storage, the total flavonoids content of 22.60 $mg.ml^{-1}$ was observed in T1, followed by 23.05, 23.60 and 23.45 $mg.ml^{-1}$ in T4, T7 and T10, respectively. In T2, the total flavonoids content of 20.43 $mg.ml^{-1}$ was observed and in T5, T8 and T11, the total flavonoids content of 21.86, 22.91 and 21.67 $mg.ml^{-1}$ was recorded. Similarly, in T3, the total flavonoids content of 21.74 was observed, followed by 22.71, 23.52 and 22.94 $mg.ml^{-1}$in T6, T9 and T12, respectively.

During the 15, 30 and 40 days of storage period, the decrease in total flavonoids content from 12.40 to 4.09 $mg.ml^{-1}$ was observed in T1, followed by 15.42 to 6.70 $mg.ml^{-1}$, 20.74 to 12.85 and 17.06 to 8.72 $mg.ml^{-1}$ in T4, T7 and T10, respectively. However, in T2, the decrease in total flavonoids content was ranged from 9.68 to 3.26 $mg.ml^{-1}$, followed by 11.48 to 4.66 $mg.ml^{-1}$ in T5, 16.80 to 10.51 $mg.ml^{-1}$ and 13.26 to 6.31 $mg.ml^{-1}$ in T8 and T11, respectively. In T3, the total flavonoids content of 10.37 to 3.92 $mg.ml^{-1}$ was observed, followed by 13.64 to 5.89 $mg.ml^{-1}$ in T6, 18.74 to 11.46 and 15.08 to 7.19 $mg.ml^{-1}$ in T9 and T12, respectively.

It was observed from the Table 15b that the total flavonoids content of 22.61$mg.ml^{-1}$ in T1, followed by 23.05 in T4, 23.62 and 23.40 $mg.ml^{-1}$ in T7, T10, respectively. In T2, it was observed that the total flavonoids content of 20.43 $mg.ml^{-1}$ was noted, followed by 21.87 $mg.ml^{-1}$ in T5, 22.90 and 21.68 $mg.ml^{-1}$ T9 and T11,

Table 15a. Effect of different preservation methods and packaging materials on total flavonoids content of sweet orange juice, stored at ambient condition (30±2 °C)

Total flavonoids ($mg.ml^{-1}$)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY40**
T1	22.60	12.40	7.21	4.09
T2	20.43	9.68	5.34	3.26
T3	21.74	10.37	6.50	3.92
T4	23.05	15.42	10.86	6.70
T5	21.86	11.48	7.30	4.66
T6	22.71	13.64	8.92	5.89
T7	23.60	20.74	15.41	12.85
T8	22.91	16.80	13.47	10.51
T9	23.52	18.74	15.89	11.46
T10	23.45	17.06	12.24	8.72
T11	21.67	13.26	8.90	6.31
T12	22.94	15.08	11.72	7.19
S.Em±	0.482	0.331	0.247	0.180
CD @ (1%)	1.902	1.308	0.976	0.711
CV	3.709	0.394	4.148	4.375
Factor	S	S	S	S

Total flavonoids content-24.6 $mg.ml^{-1}$

NS: Non significant

S: Significant

Table 15b. Effect of different preservation methods and packaging materials on total flavonoids content of sweet orange juice, stored at refrigerated condition (4±1 °C)

Total flavonoids (mg.ml^{-1})				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	22.61	18.05	10.76	7.40
T2	20.43	16.84	8.62	5.09
T3	21.74	17.90	9.61	6.42
T4	23.05	20.46	13.52	10.60
T5	21.87	18.69	11.32	7.51
T6	22.72	20.02	12.96	9.68
T7	23.62	23.03	21.48	17.50
T8	22.90	21.68	18.17	15.43
T9	23.51	21.9	19.26	15.80
T10	23.40	21.08	16.54	13.65
T11	21.68	19.05	13.2	8.42
T12	22.94	20.66	15.45	11.07
S.Em±	0.477	0.432	0.335	0.262
CD @(1%)	1.885	1.708	1.323	0.765
CV	3.695	3.755	4.052	4.227
Factor	S	S	S	S

Total flavonoids content-24.6 mg.ml^{-1}

NS: Non significant

S: Significant

respectively. In T3, the total flavonoids content of 21.74 $mg.ml^{-1}$ was observed, followed by 22.72, 23.51 and 22.94 $mg.ml^{-1}$ in T6, T9 and T12, respectively during first day of storage at refrigerated condition.

During 15, 30 and 40 days of storage period, the decrease in the total flavonoids content of 18.05 to 7.40 $mg.ml^{-1}$ in T1 was observed, followed by 20.46 to 10.60 $mg.ml^{-1}$ in T4, 23.03 to 17.50 $mg.ml^{-1}$ and 21.08 to 13.65 $mg.ml^{-1}$ in T7 and T10, respectively. However, in T2, the decrease in total flavonoids content from 16.84 to 5.09 $mg.ml^{-1}$ was observed, followed by 18.69 to 7.51 $mg.ml^{-1}$ in T5 and 21.68 to 15.43 $mg.ml^{-1}$ in T8 and 19.05 to 8.42 $mg.ml^{-1}$ in T11. In T3, the decrease in total flavonoids content from 17.90 to 6.42 $mg.ml^{-1}$ was observed, followed by 20.02 to 9.68 $mg.ml^{-1}$ in T6, 21.90 to 15.80 $mg.ml^{-1}$ and 20.66 to 11.07 $mg.ml^{-1}$ in T9 and T12, respectively.

4.5 Effect of preservation methods, packaging materials and storage conditions on sensory properties of sweet orange juice

Sensory properties of fresh sweet orange juice with different treatments were judged by the panelists, which includes staffs and students of the department and were presented in Table 16(a) and 16(b). The sensory score was judged with the help of 9 point hedonic scale and scores were given as 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.with random coding were given to the samples. The fresh samples of sweet orange juice with different treatments were served to the panelists immediately after processing and score to judge the colour, appearance, flavour, aroma and overall acceptability.

Sensory score for freshly extracted sweet orange juice subject to different treatments and stored at ambient condition were assessed and marks for colour and appearance, sweetness, sourness, aroma, flavour and overall acceptability was judged and the score obtained by the panelists were as follows. Table 16a shows that for T1 *i.e.*, juice sample filled in glass bottles and stored at ambient condition, the colour and appearance score was found to be 6 followed by 7, 5.5, 5, 4.5 and 6 for sweetness, sourness, aroma, flavor and overall acceptability. Similarly for T4, the sensory score of 7, 7.5, 7.5,7.5, 7 and 7.5 were obtained, followed by 8.5, 9, 8, 8, 8.5 and 8.5 for T7 and 8.0, 8.0, 7.0, 8.0, 7.0 and 8.0 for T10 for its colour and appearance, sweetness sourness, aroma, flavour and overall acceptability, where highest score was observed in treatment T7 compared to

Table 16a. Effect of different preservation methods and packaging materials on sensory properties of sweet orange juice, stored at ambient condition (30±2 °C)

Sensory score of fresh juice						
Treatments	**Colour and appearance**	**Sweetness**	**sourness**	**aroma**	**flavour**	**overall acceptability**
T1	6.0	7.0	5.5	5.0	4.5	6.0
T2	4.0	6.0	5.0	4.5	4.0	5.5
T3	5.0	7.0	5.0	4.0	4.0	6.0
T4	7.0	7.0	7.5	7.5	7.0	7.5
T5	6.5	6.5	6.0	6.0	6.0	6.5
T6	8.5	8.0	7.0	7.0	6.0	7.0
T7	8.5	9.0	8.0	8.0	8.5	8.5
T8	6.5	8.0	7.0	7.5	7.0	7.0
T9	7.0	8.0	7.5	7.0	8.0	7.5
T10	8.0	8.0	7.0	8.0	7.0	8.0
T11	7.5	7.5	7.0	6.5	6.0	7.0
T12	7.0	8.0	7.0	7.5	6.0	7.5

NS: Non significant

S: Significant

other samples. In T2 *i.e.*, juice filled in PET bottles and stored at ambient condition, the sensory score of 4, 6, 5, 4.5 4 and 5.5 was recorded for its colour and appearance, sweetness, sourness, aroma, flavour and overall acceptability, whereas in treatment T5, the sensory score of 6.5, 6.5, 6, 6, 6 and 6.5 was observed, followed by 7, 8.5, 7, 7.5, 7 and 7, was noted in T8 and in T11, the sensory score of 7.5, 7.5, 7, 6.5, 6 and 7.0 was registered for its colour and appearance, sweetness, sourness, aroma, flavor and overall acceptability. In T3, *i.e.*, juice sample filled in tin cans and stored at ambient condition, the sensory score of 5, 7, 5, 4, 4 and 6 was observed, whereas in T6, the sensory score of 8.5, 8, 7, 7, 6 and 7 was given followed by 7, 8.5, 7.5, 7, 8 and 7,5 in T9 and 7, 8, 7, 7.5, 6 and 7.5 in T12 was given for its colour and appearance, sweetness, sourness, aroma, flavor and overall acceptability.

From the Table 16b it was observed that in T1, T4, T7 and T10, the colour and appearance, sweetness, sourness, aroma, flavour and overall acceptability values were from 6.5 to 7.0 followed by 7.5 to 8.8, 9 to 9 and 8.5 to 8.5, respectively. Similarly in T2, the colour and appearance, sweetness, sourness, aroma, flavour and overall acceptability was found to be 5 to 6.5, followed by 7 to 7.5 in T5, 8 to 8 in T9 and 6 to 8 in T11, respectively. In T3, the overall values were found to be in the range of 5.5 to 7, followed by 9 to 8 in T6, 8.5 to 8 in T9 and 6.5 to 8 in T12, respectively.

4.6 Enumeration of microbial population

4.6.1 Total Plate Count (TPC)

From the Table 17a it was observed that no microbial load was detected in all the treatments during the initial day of storage period. During 15, 30 and 40th days of storage period the TPC of 39.2 to 71.4×10^3 cfu.ml^{-1} was detected in T1, followed by 20.2 to 62.2×10^3 cfu.ml^{-1} in T4, where no microbial load was observed in T7 during 15th day of storage and at 40th day 48.0×10^3 cfu.ml^{-1} was detected. In T2, the TPC of 44 to 76×10^3 cfu.ml^{-1} was detected, followed by 27.3 to 70×10^3 cfu.ml^{-1}, 21.5 to 54.1×10^3 cfu.ml^{-1} and 23.0 to 61×10^3 cfu.ml^{-1} in T5, T8 and T11 respectively. Similarly in T3the TPC of 42.1 to 73.2 was observed, where in T6, T9 and in T12, the TPC of 25.0 to 64.2×10^3 cfu.ml^{-1}, followed by 16.0 to 51×10^3 cfu.ml^{-1} and 19.2 to 54.0×10^3 cfu.ml^{-1} was detected at the end of storage period.

Table 16b. Effect of different preservation methods, packaging materials on sensory properties of sweet orange juice, stored at refrigerated condition (4±1 °C)

Sensory score of fresh juice						
Treatments	**Colour and appearance**	**sweetness**	**sourness**	**aroma**	**flavour**	**overall acceptability**
T1	6.5	8.0	6.0	6.0	6.0	7.0
T2	5.0	6.5	5.5	5.5	5.0	6.5
T3	5.5	7.5	6.0	5.0	5.5	7.0
T4	7.5	8.0	8.0	8.0	7.5	8.5
T5	7.0	7.0	6.5	6.5	6.5	7.5
T6	9.0	7.5	8.0	7.5	7.0	8.0
T7	9.0	8.5	8.5	9.0	9.0	9.0
T8	8.0	7.5	8.0	8.0	7.5	8.0
T9	8.5	8.5	8.0	7.5	8.5	8.0
T10	8.5	9.0	8.5	8.5	8.0	8.5
T11	6.0	8.0	8.0	7.0	7.0	8.0
T12	6.5	7.0	8.0	8.0	7.0	8.0

NS: Non significant

S: Significant

Table 17a. Effect of different preservation methods and packaging materials on total plate count of sweet orange juice, stored at ambient condition (30±2 °C)

Total plate count (cfu.ml^{-1}) (10^3)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	ND	39.2	57.1	71.4
T2	ND	44.0	68.2	76.0
T3	ND	42.1	64.5	73.2
T4	ND	20.2	46.6	62.0
T5	ND	27.3	59.0	70.0
T6	ND	25.0	51.4	64.2
T7	ND	ND	20.0	48.0
T8	ND	21.5	39.2	54.1
T9	ND	16.0	25.1	51.0
T10	ND	15.4	36.0	53.0
T11	ND	23.0	42.1	61.0
T12	ND	19.2	37.2	54.0
S.Em±	-	0.697	1.208	1.506
CD (%)	-	2.757	4.595	4.240
CV	-	4.945	4.778	5.956
F	-	S	S	S

ND- Negligible Detection

NS: Non significant

S: Significant

From the Table 17b it was observed that no microbial growth was detected in all the treatments during the first day of storage at refrigerated condition. During 15 to 40 days of storage the TPC of 20 to 54×10^3 $cfu.ml^{-1}$ was detected, where no microbial activity was detected in T4, T7 and T10 on 15th day and on 40th day, the TPC of 49, 5.5 and 30×10^3 $cfu.ml^{-1}$ was identified in the respective treatments. Similarly, in T2 the TPC of 28 to 61×10^3 $cfu.ml^{-1}$was detected, where no microbial growth was determined in T5, T8 and in T10 during 15th day and a TPC of 55, 13.2 and 37×10^3 $cfu.ml^{-1}$ was detected on 40th day of storage.

4.6.2 Yeast and mould counts

The yeast and mould counts for sweet orange juice with different preservation methods, packaging materials and storage conditions on the shelf-life of sweet orange juice was assessed and presented in Table 18a and Table 18b. From the Table 18a it was observed that no yeast and mould activity was predicted in all the treatments during the initial day of storage period. During 15, 30 and 40 days of storage period, the yeast and mould count of 2.2 to 4.6×10^3 $cfu.ml^{-1}$ was determined in T1, followed by 1.5 to 3.8×10^3 $cfu.ml^{-1}$ in T4, where no yeast and moulds were detected in T7 on 15th day and at 40th day, the yeast and mould count of 2.0×10^3 $cfu.ml^{-1}$ was detected and 0.8 to 3.4×10^3 $cfu.ml^{-1}$ in T10, respectively. In T2, the yeast and mould count of 3.1 to 6.1×10^3 $cfu.ml^{-1}$ was detected, followed by 2.1 to 4.9, 3.3 and 1.5 to 4.1×10^3 $cfu.ml^{-1}$ in T5, T8 and in T11, respectively. Similarly in T3, the yeast and mould count of 2.5 to 5.7×10^3 $cfu.ml^{-1}$ was detected and 1.9 to 4.4, 0 to 2.7, 1.2 to 3.8×10^3 $cfu.ml^{-1}$ in T6, T9 and T12, respectively.

From the Table 18b it was observed that the no yeast and mould activity was detected in T1, T4, T7 and T10, during the initial and 15 days of storage period and at the 40th day, the yeast and mould count of 3.9×10^3 $cfu.ml^{-1}$, 2.3 and 1.5×10^3 $cfu.ml^{-1}$, where no yeast and mould count were observed in T7 from 15 to 30 days of storage and on 40th day 0.9×10^3 $cfu.ml^{-1}$ was detected. In T2, the yeast and mould count of 2.6 to 5.9×10^3 $cfu.ml^{-1}$ was observed and no yeast and moulds were detected during the first day of storage followed 0 to 2.8×10^3 $cfu.ml^{-1}$ in T5, where no yeast and mould growth was observed in T8 and T11 during 15th day and at the end of the 40th day the yeast and mould count of 1.7 and 2.5×10^3 $cfu.ml^{-1}$ was determined and in T3 it was observed that increase in yeast and mould count was from 1.5 to 4.1×10^3 $cfu.ml^{-1}$, where no yeasts and

Table 17b. Effect of different preservation methods and packaging materials on total plate count of sweet orange juice, stored at refrigerated condition (4±1 °C)

Total plate count (cfu.ml^{-1}) (10^{3})				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	ND	20.0	36.0	54.0
T2	ND	28.0	45.0	61.0
T3	ND	23.0	40.0	58.0
T4	ND	ND	28.0	49.0
T5	ND	ND	34.0	55.0
T6	ND	ND	32.0	51.0
T7	ND	ND	ND	5.4
T8	ND	ND	ND	13.2
T9	ND	ND	ND	9.6
T10	ND	ND	19.0	30.0
T11	ND	ND	28.0	37.0
T12	ND	ND	26.0	33.0
S.Em ±	-	0.327	0.725	1.087
CD @(1%)	-	1.295	2.868	4.031
CV	-	9.586	5.328	4.959
Factor	-	S	S	S

ND- Negligible Detection

NS: Non significant

S: Significant

Table 18a. Effect of different preservation methods, packaging materials condition on yeast and mould counts of sweet orange juice, stored at ambient condition (30±2 °C)

Yeast and mould count (10^3) cfu.ml^{-1}				
Treatment	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	ND	2.2	3.4	4.6
T2	ND	3.1	5.0	6.1
T3	ND	2.5	4.6	5.7
T4	ND	1.5	2.5	3.8
T5	ND	2.1	3.4	4.9
T6	ND	1.9	3.1	4.4
T7	ND	ND	1.2	2.0
T8	ND	ND	2.4	3.3
T9	ND	ND	1.9	2.7
T10	ND	0.8	1.7	3.4
T11	ND	1.5	3.0	4.1
T12	ND	1.2	2.7	3.8
S.Em ±	-	0.034	0.062	0.085
CD @ (1%)	-	0.133	0.247	0.338
CV	-	4.168	3.725	3.64
Factor	-	S	S	S

ND- Negligible Detection

NS: Non significant

S: Significant

Table 18b. Effect of different preservation methods, packaging materials condition on yeast and mould counts of sweet orange juice, stored at refrigerated condition (4±1 °C)

Yeast and mould count (cfu.ml^{-1}) (10^3)				
Treatments	**DAY 1**	**DAY 15**	**DAY 30**	**DAY 40**
T1	ND	0.9	2.8	3.9
T2	ND	2.6	3.8	5.9
T3	ND	1.5	3.3	4.1
T4	ND	ND	1.5	2.3
T5	ND	ND	2.1	2.8
T6	ND	ND	1.9	2.4
T7	ND	ND	ND	0.9
T8	ND	ND	ND	1.7
T9	ND	ND	ND	1.2
T10	ND	ND	0.7	1.5
T11	ND	ND	1.6	2.5
T12	ND	ND	1.3	2.1
S. Em ±	-	0.017	0.042	0.061
CD @ (1%)	-	0.069	0.165	0.243
CV	-	7.367	4.005	7.367
Factor	-	S	S	S

ND- Negligible Detection

NS: Non significant

S: Significant

moulds were detected in T9 and T12 and at the end of 40th day, the yeast and mould count of 1.7 and 2.1×10^3 cfu.ml^{-1} were detected in sweet orange juice sample stored at refrigerated condition, respectively.

From the above Tables, it was observed that as the storage period proceeds, yeast and mould count also increases at ambient condition but at refrigerated condition, the shelf-life of juice can be enhanced by suppressing the multiplication of yeast and mould multiplication for a certain period.

4.7 Effect of different preservation methods, packaging materials and storage conditions on shelf-life prediction of sweet orange juice based on ascorbic acid limit.

The effect of different preservation methods, packaging materials and storage conditions on the shelf-life of sweet orange juice was assessed and presented in Table 19a and Table 19b. From the Tables 19a it was observed that T1 showed shelf-life of 10 days, followed by 16.5, 32 and 24 days in T4, T7 and T10, respectively. In T2, the shelf-life of 6 days was observed followed by 12, 27.9 and 19 days in T5, T8 and T11 and in T3, the shelf-life of 14 days was observed, followed by 8, 30.8 and 20.4 days in T6, T9 and T12, respectively. Among the treatments T7 showed highest shelf-life of 32.5 days at ambient condition.

From the Table 19b, it was observed that in T1 the sweet orange juice showed a shelf-life of 14.4 days followed by 25.5, 130 and 53.4 days in T4, T7 and T10. In T2, the shelf-life of 9.8 was recorded, followed by 20.0 days in T5, 87.7 days in T8 and 50.5 days in T11. Similarly, in T3, the shelf-life of 11.8 days was recorded followed by 21 days in T6, 104 days in T9 and 52.3 days in T12.

4.8. Economics of sweet orange juice

Economics of sweet orange juice was calculated at ambient condition and refrigerated condition to all the treated samples are presented in Table. 20a and 20b. The economics of sweet orange juice was estimated for 300 days.

4.8.1 Economics of sweet orange juice with different preservation methods and packaging materials, stored at ambient condition (30±2 °C)

Table 20a presents the cost economics calculation of sweet orange juice with different preservation methods and packaging materials, stored at ambient condition and the results obtained are explained as follows

Table 19a. Effect of different preservation methods and packaging materials on shelf-life prediction of sweet orange juice, based on ascorbic acid limit stored at ambient condition (30±2 °C)

Treatments	DAYS	K- value	C-value	SSE	R^2	Adjusted-R^2	RMSE
T1	10.0	-0.0421	4.095	0.1093	0.9372	0.9078	2.3370
T2	6.0	-0.0400	3.938	0.0674	0.9562	0.9343	0.1837
T3	8.0	-0.0408	4.007	0.1038	0.9365	0.9047	0.2278
T4	16.5	-0.0234	4.077	0.0182	0.9650	0.9474	0.0955
T5	12.0	-0.0279	-4.029	0.0794	0.9004	0.9506	0.1992
T6	14.0	-0.0249	4.040	0.0412	0.9327	0.899	0.1436
T7	32.5	-0.0122	4.086	0.0425	0.7649	0.6473	0.1458
T8	27.9	-0.0118	4.019	0.0506	0.7181	0.5771	0.1591
T9	30.8	-0.1160	4.047	0.0480	0.7220	0.583	0.1549
T10	24.0	-0.0158	4.071	0.0270	0.8939	0.8408	0.1168
T11	19.0	-0.0173	4.019	0.0417	0.8691	0.8037	0.1444
T12	20.4	-0.0175	4.046	0.0399	0.8768	0.8152	0.1413
S.Em±	0.480						
CD @ (1%)	1.880						
CV	4.460						
Factor	S						

NS: Non significant

S: Significant

Table 19b. Effect of different preservation methods and packaging materials on shelf-life prediction of sweet orange juice, based on ascorbic acid limit stored at refrigerated condition (4±1 °C)

Treatments	DAYS	K- value	C-value	SSE	R^2	Adjusted-R^2	RMSE
T1	14.4	-0.02408	4.0370	-0.0147	-0.9732	0.9597	0.0857
T2	9.8	-0.0264	3.9480	0.0684	0.9039	0.8559	0.1850
T3	11.8	-0.0229	3.9600	0.0362	0.9302	0.8954	0.1346
T4	25.5	-0.0154	4.0830	0.0206	0.9139	0.8709	0.1015
T5	20.0	-0.0156	3.9990	0.0349	0.8650	0.7974	0.1322
T6	21.0	-0.0152	4.0170	0.0172	0.9257	0.8885	0.0926
T7	130.0	-0.0026	4.0320	0.0016	0.8033	0.7049	0.0285
T8	87.7	-0.0031	3.9600	0.0030	0.7522	0.6282	0.0390
T9	104	-0.0029	3.9910	0.0047	0.6335	0.4503	0.0485
T10	53.4	-0.0066	4.0400	0.0035	0.9180	0.8769	0.0421
T11	50.5	-0.0051	3.9560	0.0105	0.6937	0.5406	0.0727
T12	52.3	-0.0059	3.9870	0.0028	0.9197	0.8795	0.0038
S.Em±	1.520						
CD @ (1%)	6.010						
CV	5.450						
Factor	S						

NS: Non significant

S: Significant

Table 20a. Economics of sweet orange juice with different preservation methods and packaging materials, stored at ambient condition (30±2 °C)

Treatments	B:C ratio
T1	₹ 1.01:1
T2	₹ 0.92:1
T3	₹ 1.00:1
T4	₹ 1.15:1
T5	₹ 1.08:1
T6	₹ 1.10:1
T7	₹ 1.42:1
T8	₹ 1.38:1
T9	₹ 1.41:1
T10	₹ 1.28:1
T11	₹ 1.21:1
T12	₹ 1.22:1

Pasteurized juice filled in glass bottles and stored at ambient condition (T1)

It was observed that the total quantity of sweet oranges handled per day was 100 kg and the total quantity of sweet oranges handled were 30,000 kg for 300 days, where the cost of sweet oranges per kg was assumed to be ₹ 25 and the total cost for 30,000 kg of sweet oranges was ₹ 75,000. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of sweet orange juice in market without addition of preservatives filled in glass bottles, stored at ambient condition was assumed to be ₹ 64 and the total selling price was found to be ₹ 9,60,000. The total number of labours required were 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was ₹1,05,000. The cost of screw press juice extractor was₹36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6000/5= ₹ 6,000. The total repair and maintenance cost was assumed to be₹ 1,000 followed by the total energy consumption charges for 300 days was ₹1,140 and the total electric charges were found to be ₹4,560.

The cost of glass bottle was found to be ₹5. The total number of glass bottles required for filling 15,000 liters of juice was found to be ₹75,000 and the total gross returns was found to be ₹9,60,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹19,01,560. The benefit cost ratio was found to be ₹ **1.01:1**

Pasteurized juice filled in PET bottles and stored at ambient condition (30±2 °C) (T2)

It was observed that the total quantity of sweet oranges handled per day was 100 kg and the total quantity of sweet oranges handled were 30,000 kg for 300 days, where the cost of sweet oranges per kg was assumed to be ₹ 25 and the total cost was ₹75,000 for 30,000 kg of sweet oranges. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of sweet orange juice in market without addition of preservatives filled in PET bottles, stored at ambient condition was assumed to be ₹56 and the total selling price was found to be ₹8,40,000. The total number of labours required were 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was ₹1,05,000. The cost of screw press juice extractor was ₹ 36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as

36,000-6000/5= ₹6,000. The total repair and maintenance cost was assumed to be ₹1,000 followed by the total energy consumption charges for 300 days was ₹1,140 and the total electric charges were found to be ₹ 4,560.

The cost of PET bottle was found to be ₹2.5. The total number of PET bottles required for filling 15,000 liters of juice was found to be ₹37,500 and the total gross returns was be ₹ 8,40,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹9,04,060. The benefit cost ratio was calculated by $\frac{\text{Gross returns}}{\text{Total cost}}$**=8,40,000/9,04,060=₹ 0.92:1.**

Pasteurized juice filled in tin cans and stored at ambient condition (T3)

It was observed that the total quantity of sweet oranges handled per day was 100 kg and the total quantity of sweet oranges handled were 30,000 kg for 300 days, where the cost of sweet oranges per kg was assumed to be ₹25 and the total cost was ₹ 75,000 for 30,000 kg of sweet oranges. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of sweet orange juice in market without addition of preservatives filled in tin cans, stored at ambient condition was assumed to be ₹62 and the total selling price was found to be ₹9,30,000. The total number of labours required were 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was ₹1,05,000. The cost of screw press juice extractor was ₹36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6000/5= ₹6000. The total repair and maintenance cost was assumed to be 1,000, followed by the total energy consumption charges for 300 days was ₹1,140 and the total electric charges were found to be ₹4,560.

The cost of tin cans was found to be ₹4. The total number of tin cans required for filling 15,000 liters of juice was found to be ₹60,000 and the total gross returns was found to be ₹9,30,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹9,26,560. The benefit cost ratio was calculated by $\frac{\text{Gross returns}}{\text{Total cost}}$ = **9,30,000/9,26,560= ₹1.00:1.**

Homogenized juice filled in glass bottles and stored at ambient condition (T4)

It was observed that the total quantity of sweet oranges handled per day was 100 kg and the total quantity of sweet oranges handled were 30,000 kg for 300 days, where the cost of sweet oranges per kg was assumed to be ₹ 25 and the total cost was ₹75,000 for 30,000 kg of sweet oranges. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of homogenized sweet orange juice in market without addition of preservatives filled in glass bottles, stored at ambient condition was assumed to be ₹74 and the total selling price was found to be ₹11,10,00. The total number of labours required were 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was ₹1,05,000. The cost of screw press type juice extractor was ₹36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6000/5=₹ 6,000. The total repair and maintenance cost for both screw press type juice extractor and homogenizer for 300 days was assumed to be ₹2,000, followed by the total energy consumption charges for 300 days was ₹1,191 and the total electric charges were found to be ₹4,764.

The cost of glass bottle was found to be ₹5. The total number of glass bottles required for filling 15,000 liters of juice was found to be ₹75,000 and the total gross returns was found to be ₹11,10,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹9,64,764. The benefit cost ratio was calculated by $\frac{\text{Gross returns}}{\text{Total cost}}$ = **11,10,000/9,64,764= ₹ 1.15:1.**

Homogenized juice filled in PET bottles and stored at ambient condition (T5)

It was observed that the total quantity of sweet oranges handled per day was 100 kg and the total quantity of sweet oranges handled were 30,000 kg for 300 days, where the cost of sweet oranges per kg was assumed to be ₹25 and the total cost was ₹ 75,000 for 30,000 kg of sweet oranges. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of homogenized sweet orange juice in market without addition of preservatives filled in PET bottles, stored at ambient condition was assumed to be ₹67 and the total selling price was found to be ₹10,50,000. The total number of labours required were 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was

₹1,05,000. The cost of screw press type juice extractor was ₹36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6000/5= ₹ 6,000. The total repair and maintenance cost for both screw press type juice extractor and homogenizer for 300 days was assumed to be ₹2,000, followed by the total energy consumption charges for 300 days was ₹1,191 and the total electric charges were found to be ₹4,764.

The cost of PET bottle was found to be ₹ 2.5. The total number of PET bottles required for filling 15,000 liters of juice was found to be ₹ 37,500 and the total gross returns was be ₹ 9,27,264 and the total cost incurred by selling 15,000 liters of juice was found to be ₹19,02,264. The benefit cost ratio was calculated by $\frac{\text{Gross returns}}{\text{Total cost}}$**=10,50,000/9,27,264= ₹1.08:1.**

Homogenized juice filled in tin cans and stored at ambient condition (T6)

It was observed that the total quantity of sweet oranges handled per day was 100 kg and the total quantity of sweet oranges handled were 30,000 kg for 300 days, where the cost of sweet oranges per kg was assumed to be ₹ 25 and the total cost was ₹ 75,000 for 30,000 kg of sweet oranges. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of homogenized sweet orange juice in market without addition of preservatives filled in tin cans, stored at ambient condition was assumed to be ₹ 70 and the total selling price was found to be ₹ 10,50,000. The total number of labours required were 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was ₹1,05,000. The cost of screw press juice extractor was ₹36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6000/5= ₹ 6,000. The total repair and maintenance cost for both screw press type juice extractor and homogenizer for 300 days was assumed to be ₹ 2,000, followed by the total energy consumption charges for 300 days was ₹1,191 and the total electric charges were found to be ₹4,764.

The cost of tin cans was found to be ₹4. The total number of tin cans required for filling 15,000 liters of juice was found to be ₹60,000 and the total gross returns was found to be ₹10,50,000 and the total cost incurred by selling

15,000 liters of juice was found to be ₹ 9,49,764. The benefit cost ratio was calculated by $\frac{\text{Gross returns}}{\text{Total cost}}$=**10,50,000/9,49,764= ₹ 1.10:1.**

Juice preserved with 0.5 mg.ml^{-1} of sodium benzoate filled in glass bottles and stored at ambient condition (T7).

It was observed that the total quantity of sweet oranges handled per day was 100 kg and the total quantity of sweet oranges handled were 30,000 kg for 300 days, where the cost of sweet oranges per kg was assumed to be ₹ 25 and the total cost for 30,000 kg of sweet oranges was ₹ 75,000. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of sweet orange juice in market preserved with sodium benzoate, filled in glass bottles, stored at ambient condition was assumed to be ₹ 90 and the total selling price was found to be ₹13,50,000. The total number of labours required were 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was ₹1,05,000. The cost of screw press juice extractor was ₹36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6000/5=₹6,000. The total repair and maintenance cost was assumed to be ₹1,000, followed by the total energy consumption charges for 300 days was ₹1,140 and the total electric charges were found to be ₹4,560.

The cost of 1kg of sodium benzoate was ₹500 and the total quantity of sodium benzoate required for preserving 15,000 liters of juice was 7.5 kg and the total cost of 7.5 kg of sodium benzoate for preserving 15,000 liters of juice= 15,000×7.5= ₹6,250.

The cost of glass bottle was found to be ₹5. The total number of glass bottles required for filling 15,000 liters of juice was found to be ₹75,000 and the total gross returns was found to be **₹13,50,000** and the total cost incurred by selling 15,000 liters of juice was found to be ₹ **9,47,810**. The benefit cost ratio was found to be **13,50,000/9,47,810= ₹ 1.42:1**.

Juice preserved with 0.5 mg.ml^{-1} of sodium benzoate filled in PET bottles and stored at ambient condition (T8).

It was observed that the total quantity of sweet oranges handled per day was 100 kg and the total quantity of sweet oranges handled were 30,000 kg for 300 days, where the cost of sweet oranges per kg was assumed to be ₹25 and the total cost was ₹ 75,000 for 30,000 kg of sweet oranges. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of sweet orange juice in market preserved with sodium benzoate, filled in PET bottles, stored at ambient condition was assumed to be ₹ 84 and the total selling price of 30,000 kgs of sweet oranges was found to be ₹ 12,60,000. The total number of labours required were 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was ₹1,05,000. The cost of screw press juice extractor was ₹ 36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6000/5= ₹ 6,000. The total repair and maintenance cost was assumed to be ₹1,000, followed by the total energy consumption charges for 300 days was ₹ 1,140 and the total electric charges were found to be ₹ 4,560.

The cost of 1kg of sodium benzoate was ₹ 500 and the total quantity of sodium benzoate required for preserving 15,000 liters of juice was 7.5 kg and the total cost of 7.5 kg of sodium benzoate for preserving 15,000 liters of juice= 15,000×7.5= ₹ 6,250.

The cost of PET bottle was found to be ₹ 2.5. The total number of PET bottles required for filling 15,000 liters of juice was found to be ₹37,500 and the total gross returns was be ₹12,60,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹ 9,10,310. The benefit cost ratio was calculated by $\frac{\text{Gross returns}}{\text{Total cost}}$**=12,60,000/9,10,310= ₹ 1.38:1.**

Juice preserved with 0.5 mg.ml^{-1} of sodium benzoate filled in tin cans and stored at ambient condition (T9).

It was observed that the total quantity of sweet oranges handled per day was 100 kg and the total quantity of sweet oranges handled were 30,000 kg for 300 days, where the cost of sweet oranges per kg was assumed to be ₹25 and the total cost was ₹ 75,000 for 30,000 kg of sweet oranges. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of sweet orange juice in market preserved with sodium benzoate, filled in tin cans, stored at ambient condition was assumed to be ₹ 88 and the total selling price of 30,000 kgs of sweet oranges was found to be ₹ 13,20,000. The total number of labours required were 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹ 175 per head and for 300 days it was ₹ 1,05,000. The cost of screw press juice extractor was ₹ 36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6000/5=₹ 6,000. The total repair and maintenance cost was assumed to be ₹1,000, followed by the total energy consumption charges for 300 days was ₹1,140 and the total electric charges were found to be ₹4,560.

The cost of 1kg of sodium benzoate was ₹ 500 and the total quantity of sodium benzoate required for preserving 15,000 liters of juice was 7.5 kg and the total cost of 7.5 kg of sodium benzoate for preserving 15,000 liters of juice= 15,000×7.5=₹ 6,250.

The cost of tin cans was found to be ₹4. The total number of tin cans required for filling 15,000 liters of juice was found to be ₹60,000 and the total gross returns was found to be ₹13,20,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹9,32,810. The benefit cost ratio was calculated by $\frac{\text{Gross returns}}{\text{Total cost}}$=13,20,000/9,32,810= ₹ **1.42:1.**

Juice preserved with 0.001 mg.ml^{-1} of nisin filled in glass bottles and stored at ambient condition (T10).

It was observed that the total quantity of sweet oranges handled per day was 100 kg and the total quantity of sweet oranges handled were 30,000 kg for 300 days, where the cost of sweet oranges per kg was assumed to be ₹ 25 and the total cost was ₹ 75,000 for 30,000 kg of sweet oranges. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of sweet orange juice in market preserved with nisin, filled in glass bottles, stored at ambient condition was assumed to be ₹82 and the total selling price of 30,000 kg of sweet oranges was found to be ₹ 12,30,000. The total number of labours required were 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was ₹1,05,000. The cost of screw press juice extractor was ₹36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6000/5=₹ 6,000. The total repair and maintenance cost was assumed to be ₹1,000, followed by the total energy consumption charges for 300 days was ₹1,140 and the total electric charges were found to be ₹4,560.

The cost of bio preservative nisin for 5g was ₹ 9,000 and the total quantity of nisin required for processing 15000 liters of sweet orange juice @ 0.001 mg.ml was 15g and the total price of 15g of nisin for preserving 15,000 liters of sweet orange juice was ₹ 27,000.

The cost of glass bottle was found to be ₹5. The total number of glass bottles required for filling 15,000 liters of juice was found to be ₹75,000 and the total gross returns was found to be ₹12,30,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹9,77,566. The benefit cost ratio was found to be **12,30,000/9,77,566= ₹1.28:1.**

Juice preserved with 0.001 $mg.ml^{-1}$ of nisin filled in PET bottles and stored at ambient condition (T11).

It was observed that the total quantity of sweet oranges handled per day was 100 kg and the total quantity of sweet oranges handled were 30,000 kg for 300 days, where the cost of sweet oranges per kg was assumed to be ₹25 and the total cost was ₹ 75,000 for 30,000 kg of sweet oranges. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of sweet orange juice in market preserved with nisin, filled in PET bottles, stored at ambient condition was assumed to be ₹75 and the total selling price of 30,000 kg of sweet oranges was found to be ₹11,25,000. The total number of labours required were 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was ₹1,05,000. The cost of screw press type juice extractor was ₹ 36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6000/5= ₹6,000. The total repair and maintenance cost was assumed to be ₹1,000, followed by the total energy consumption charges for 300 days was ₹1,140 and the total electric charges were found to be ₹ 4,560.

The cost of bio preservative nisin for 5g was ₹ 9,000 and the total quantity of nisin required for processing 15,000 liters of sweet orange juice @ 0.001 mg.ml was 15g and the total price of 15g of nisin for preserving 15,000 liters of sweet orange juice is ₹ 27,000.

The cost of PET bottle was found to be ₹ 2.5. The total number of PET bottles required for filling 15,000 liters of juice was found to be ₹37,500 and the total gross returns was be ₹11,25,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹9,31,060. The benefit cost ratio was calculated by $\frac{\text{Gross returns}}{\text{Total cost}}$**=11,25,000/9,31,060= ₹ 1.20:1.**

Juice preserved with 0.001 mg.ml^{-1} of nisin filled in tin cans and stored at ambient condition (T12).

It was observed that the total quantity of sweet oranges handled per day was 100 kg and the total quantity of sweet oranges handled were 30,000 kg for 300 days, where the cost of sweet oranges per kg was assumed to be ₹ 25 and the total cost was ₹75,000 for 30,000 kg of sweet oranges. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of sweet orange juice in market preserved with nisin, filled in tin cans, stored at ambient condition was assumed to be ₹78 and the total selling price of 30,000 kg of sweet oranges was found to be ₹11,70,000. The total number of labours required were 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was ₹1,05,000. The cost of screw press type juice extractor was ₹36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6,000/5=₹ 6,000. The total repair and maintenance cost was assumed to be ₹1,000, followed by the total energy consumption charges for 300 days was ₹1,140 and the total electric charges were found to be ₹4,560.

The cost of bio preservative nisin for 5g was 9,000 and the total quantity of nisin required for processing 15,000 liters of sweet orange juice @ 0.001 mg.ml^{-1} was 15g and the total price of 15g of nisin for preserving 15,000 liters of sweet orange juice was ₹ 27,000.

The cost of tin cans was found to be ₹ 4. The total number of tin cans required for filling 15,000 liters of juice was found to be ₹60,000 and the total gross returns was found to be ₹11,70,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹9,53,560. The benefit cost ratio was calculated by $\frac{\text{Gross returns}}{\text{Total cost}}$**=11,70,000/9,53,560= ₹ 1.22:1.**

4.8.2 Economics of sweet orange juice with different preservation methods and packaging materials, stored at refrigerated condition (4±1 °C)

Economics of sweet orange juice with different treatments stored at refrigerated condition was calculated and presented in Table 20b.

Pasteurized juice filled in glass bottles stored at refrigerated condition (T1)

It was observed that the total quantity of sweet oranges handled per day was 100 kg and the total quantity of sweet oranges handled were 30,000 kg for 300 days, where the cost of sweet oranges per kg was assumed to be ₹25 and the total cost was ₹75,000. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of pasteurized sweet orange juice in market was assumed to be ₹78 and the total selling price was found to be ₹11,70,000. The total number of labours required were 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was ₹1,05,000. The cost of screw press juice extractor was ₹ 36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6,000/5= ₹ 6,000. The actual cost of refrigerator was assumed to be ₹ 2,80,000 and the depreciated cost was found to be 2,80,00-30,000/6 =₹41,666. The total repair and maintenance cost was assumed to be ₹1,360, followed by the total energy consumption charges for 300 days was ₹4,140 and the total electric charges was found to be ₹16,560.

The cost of glass bottle was found to be ₹5. The total number of glass bottles required for filling 15,000 liters of juice was found to be ₹75,000 and the total gross returns was found to be ₹11,70,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹9,95,586. The benefit cost ratio was found to be **₹1.17:1.**

Pasteurized juice filled in PET bottles stored at refrigerated condition (T2)

It was observed that the total quantity of sweet oranges handled per day was 100 kg and the total quantity of sweet oranges handled were 30,000 kg for 300 days, where the cost of sweet oranges per kg was assumed to be ₹25 and the total cost was ₹75,000. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of pasteurized sweet orange juice, filled in PET bottles, stored at refrigerated condition in market was assumed to be ₹72 and the total selling price was found to be ₹10,80,000. The total number of labours required were 2 in nos. and for 300

Table 20b. Economics of sweet orange juice with different preservation methods and packaging materials, stored at refrigerated condition (4±1 °C)

Treatments	B:C ratio
T1	₹ 1.17:1
T2	₹ 1.12:1
T3	₹ 1.14:1
T4	₹ 1.25:1
T5	₹ 1.20:1
T6	₹ 1.22:1
T7	₹ 1.50:1
T8	₹ 1.46:1
T9	₹ 1.47:1
T10	₹ 1.35:1
T11	₹ 1.33:1
T12	₹ 1.34:1

days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was ₹1,05,000. The cost of screw press juice extractor was ₹36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6000/5=₹ 6,000. The actual cost of refrigerator was assumed to be ₹2,80,000 and the depreciated cost was found to be 2,80,00-30,000/6=₹41,666. The total repair and maintenance cost was assumed to be **₹1,360**, followed by the total energy consumption charges for 300 days was ₹4,140 and the total electric charges was found to be ₹16,560.

The cost of PET bottle was found to be ₹2.5. The total number of PET bottles required for filling 15,000 liters of juice was found to be ₹37,500 and the total gross returns was found to be ₹10,80,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹9,58,086. The benefit cost ratio was calculated by $\frac{\text{Gross returns}}{\text{Total cost}}$**=10,80,000/9,58,086= ₹1.12:1.**

Pasteurized juice filled in tin cans stored at refrigerated condition (T3)

It was observed that the total quantity of sweet oranges handled per day was 100 kg and the total quantity of sweet oranges handled were 30,000 kg for 300 days, where the cost of sweet oranges per kg was assumed to be ₹ 25 and the total cost was ₹ 75,000. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of pasteurized sweet orange juice, filled in tin cans, stored at refrigerated condition in market was assumed to be ₹75 and the total selling price was found to be ₹1,05,000. The total number of labours required were 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was ₹1,05,000. The cost of screw press juice extractor was ₹36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6000/5=₹6,000. The actual cost of refrigerator was assumed to be ₹2,80,000 and the depreciated cost was found to be 2,80,00-30,000/6=₹41,666. The total repair and maintenance cost was assumed to be **₹1,360**, followed by the total energy consumption charges for 300 days was ₹4,140 and the total electric charges was found to be ₹16,560.

The cost of tin cans was found to be ₹ 4. The total number of tin cans required for filling 15,000 liters of juice was found to be ₹60,000 and the total gross returns was found to be ₹1,05,000 and the total cost incurred by selling 15,000 liter of juice was found to be ₹9,80,586. The benefit cost ratio was calculated by $\frac{\text{Gross returns}}{\text{Total cost}}$**=10,50,000/9,80,586= ₹1.14:1.**

Homogenized juice filled in glass bottles and stored at refrigerated condition (T4)

It was observed that the total quantity of sweet oranges handled per day was 100 kg and the total quantity of sweet oranges handled were 30,000 kg for 300 days, where the cost of sweet oranges per kg was assumed to be ₹25 and the total cost was ₹75,000. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of homogenized sweet orange juice in market was assumed to be ₹85 and the total selling price was found to be ₹12,75,000. The total number of labours required were 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was ₹1,05,000. The cost of screw press juice extractor was ₹36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6000/5=₹6,000. The actual cost of homogenizer was found to be ₹1,25,000 and the depreciated cost was calculated by taking the junk value of ₹15,000 and life span of 5 years was found to be ₹22,000. The actual refrigerator was assumed to be ₹2,80,000 and the depreciated cost was found to be 2,80,00-30,000/6 =₹41,666. The total repair and maintenance cost was assumed to be ₹ 2,360, followed by the total energy consumption charges for 300 days was ₹ 4,191 and the total electric charges was found to be ₹16,764.

The cost of glass bottle was found to be ₹5. The total number of glass bottles required for filling 15,000 liters of juice was found to be ₹75,000 and the total gross returns was found to be ₹12,75,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹ 10,18,790. The benefit cost ratio was found to be ₹ **1.25:1.**

Homogenized juice filled in PET bottles and stored at refrigerated condition (T5)

It was observed that the total quantity of sweet oranges handled for 300 days was 100 kg and the total quantity of sweet oranges handled were 30,000 kg, where the cost of sweet oranges per kg was assumed to be ₹ 25 and the total cost was ₹ 75,000. The total quantity of juice obtained from 30,000 Kg of fruits was found to be 15,000 liters and selling price of homogenized sweet orange juice in market was assumed to be ₹79 and the total selling price was found to be ₹ 11,85,000. The total number of labours required was 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹ 175per head and for 300 days it was ₹ 1,05,000. The cost of screw press juice extractor was ₹ 36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press juice extractor as 36,000-6,000/5=₹ 6,000. The actual cost of homogenizer was found to be ₹1,25,000 and the depreciated cost was calculated by taking the junk value as 15,000 and life span of 5 yrs was found to be ₹ 22,000. The actual refrigerator was assumed to be ₹ 2,80,000 and the depreciated cost was found to be 2,80,000-30,000/6= ₹41,666. The total repair and maintenance cost was assumed to be ₹ 2,360, followed by the total energy consumption charges for 300 days was ₹ 4,191 and the total electric charges was found to be ₹16,764.

The cost of PET bottle was found to be ₹ 2.5. The total number of PET bottles required for filling 15,000 liters of juice was found to be ₹ 37,500 and the total gross returns was found to be ₹ 11,85,000and the total cost incurred by selling 15,000 liters of juice was found to be ₹ 9,81,290. The benefit cost ratio was calculated by $\frac{\text{Gross returns}}{\text{Total cost}}$**=11,85,000/9,81,280= ₹ 1.20:1.**

Homogenized juice filled in tin cans and stored at refrigerated condition (T6)

It was observed that the total quantity of sweet oranges handled for 300 days was 100 kg and the total quantity of sweet oranges handled were 30,000 kg, where the cost of sweet oranges per kg was assumed to be ₹ 25 and the total cost was ₹ 75,000. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of homogenized sweet orange juice in market was assumed to be ₹ 82 and the total selling price was found to be ₹ 12,30,000 for 300 days. The total number of labours required was 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹ 175 per head and for 300 days it was ₹1,05,000. The cost of screw press type juice extractor was ₹ 36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6000/5= ₹6,000. The actual cost of homogenizer was found to be ₹1,25,000 and the depreciated cost was calculated by taking the junk value as ₹15,000 and life span of 5years was found to be ₹22,000. The actual refrigerator was assumed to be ₹2,80,000 and the depreciated cost was found to be 2,80,00-30,000/6= ₹ 41,666. The total repair and maintenance cost was assumed to be ₹2,360, followed by the total energy consumption charges for 300 days was ₹4,191 and the total electric charges was found to be ₹16,764.

The cost of tin cans was found to be ₹4. The total number of tin cans required for filling 15,000 liters of juice was found to be ₹ 60,000 and the total gross returns was found to be ₹12,00,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹1,00,3790. The benefit cost ratio was calculated by $\frac{\text{Gross returns}}{\text{Total cost}}$=**12,00,000/1,00,3790= ₹ 1.22:1.**

Juice preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate filled in glass bottles and stored at refrigerated condition (T7).

It was observed that the total quantity of sweet oranges handled for 300 days was 100 kg and the total quantity of sweet oranges handled were 30,000 kg, where the cost of sweet oranges per kg was assumed to be ₹25 and the total cost was ₹ 75,000. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of sweet orange juice preserved with sodium benzoate (0.5 $mg.ml^{-1}$) in market was assumed to be ₹ 100 and the total selling price was found to be ₹15,00,000. The total number of labours required was 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹ 175 per head and for 300 days it was ₹ 1,05,000. The cost of screw press type juice extractor was ₹ 36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press juice extractor as $\frac{36,000-6000}{5}$=₹ 6,000. The repair and maintenance charges were assumed to be ₹ 1,360.The total energy consumption for 300 days (@ 10 kWh for refrigerator per day and 3.8 for screw press type juice extractor was ₹4,140.

The cost of 1kg of sodium benzoate was ₹500 and the total quantity of sodium benzoate required for preserving 15,000 liters of juice was 7.5 kg and the total cost of 7.5 kg of sodium benzoate for preserving 15,000 liters of juice= 15,000×7.5= ₹ 6,250.

The cost of glass bottle was found to be ₹ 5. The total number of glass bottles required for filling 15,000 liters of juice was found to be ₹ 75,000 and the total gross returns was found to be ₹ 15,00,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹10,01,836. The benefit cost ratio was found to be **15,00,000/10,01,836= ₹1.50** .

Juice preserved with 0.5 mg.ml^{-1} of sodium benzoate filled in PET bottles and stored at refrigerated condition (T8).

It was observed that the total quantity of sweet oranges handled for 300 days was 100 kg and the total quantity of sweet oranges handled were 30,000 kg, where the cost of sweet oranges per kg was assumed to be ₹ 25 and the total cost was ₹ 75,000. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of sweet orange juice preserved with sodium benzoate (0.5 mg.ml^{-1}) in market was assumed to be ₹ 94 and the total selling price was found to be ₹ 14,10,000. The total number of labours required was 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was ₹1,05,000. The cost of screw press type juice extractor was ₹36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press juice extractor as 36,000-6000/5= ₹ 6,000. The repair and maintenance charges were assumed to be ₹1,360.The total energy consumption for 300 days (@ 10 kWh for refrigerator per day and 3.8 for screw press type juice extractor was ₹ 4,140.

The cost of 1kg of sodium benzoate was ₹ 500 and the total quantity of sodium benzoate required for preserving 15,000 liters of juice was 7.5 kg and the total cost of 7.5 kg of sodium benzoate for preserving 15,000 liters of juice= 15,000×7.5= ₹ 6,250 .

The cost of PET bottle was found to be ₹ 2.5. The total number of PET bottles required for filling 15,000 liters of juice was found to be ₹ 37,500 and the total gross returns was found to be ₹ 14,10,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹ 9,64,336. The benefit cost ratio was found to be **14,10.000/9,64,336= ₹ 1.46:1** .

Juice preserved with 0.5 mg.ml^{-1} of sodium benzoate filled in tin cans and stored at refrigerated condition (T9).

It was observed that the total quantity of sweet oranges handled for 300 days was 100 kg and the total quantity of sweet oranges handled were 30,000 kg, where the cost of sweet oranges per kg was assumed to be ₹ 25 and the total cost was ₹ 75,000. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of sweet orange juice preserved with sodium benzoate(0.5 mg.ml^{-1}) in market was assumed to be ₹ 97 and the total selling price was found to be ₹14,55,000. The total number of labours required was 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175per head and for 300 days it was ₹ 1,05,000. The cost of screw press juice extractor was ₹ 36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6000/5= ₹ 6,000. The repair and maintenance charges were assumed to be ₹ 1,360.The total energy consumption for 300 days (@ 10 kWh for refrigerator per day and 3.8 for power operated screw press type juice extractor was ₹4,140.

The cost of 1kg of sodium benzoate was ₹ 500 and the total quantity of sodium benzoate required for preserving 15,000 liters of juice was 7.5 kg and the total cost of 7.5 kg of sodium benzoate for preserving 15,000 liters of juice= 15,000×7.5= ₹6,250 .

The cost of tin cans was found to be ₹4. The total number of tin cans required for filling 15,000 liters of juice was found to be ₹ 60,000 and the total gross returns was found to be ₹14,55,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹ 9,86,836. The benefit cost ratio was found to be **14,55,000/9,86,836=₹ 1.47:1**.

Juice preserved with 0.001 mg.ml^{-1} of nisin filled in glass bottles and stored at refrigerated condition (T10).

It was observed that the total quantity of sweet oranges handled per 300 days was 100 kg and the total quantity of sweet oranges handled were 30,000 kg, where the cost of sweet oranges per kg was assumed to be ₹ 25 and the total cost was ₹ 75,000. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of sweet orange juice preserved with nisin (0.001 mg.ml^{-1}) in market was assumed to be ₹ 92 and the total selling price was found to be ₹ 13,80,000. The total number of labours required was 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was ₹1,05,000. The cost of screw press type juice extractor was ₹36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press juice extractor as 36,000-6000/5= ₹ 6,000. The repair and maintenance charges were assumed to be ₹1,360.The total energy consumption for 300 days (@ 10 kWh for refrigerator per day and 3.8 for screw press type juice extractor) was ₹4,140.

The cost of bio preservative nisin for 5g was ₹ 9,000 and the total quantity of nisin required for processing 15,000 liters of sweet orange juice @ 0.001 mg.ml was 15g and the total price of 15g of nisin for preserving 15000 liters of sweet orange juice was ₹27,000.

The cost of glass bottle was found to be ₹ 5. The total number of glass bottles required for filling 15,000 liters of juice was found to be ₹ 75,000 and the total gross returns was found to be ₹13,80,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹ 10,22,586. The benefit cost ratio was found to be **13,80,000 /10,22,586= ₹1.35:1.**

Juice sample preserved with 0.001 mg.ml^{-1} of nisin filled in PET bottles and stored at refrigerated condition (4±1 °C) (T11).

It was observed that the total quantity of sweet oranges handled for 300 days was 100 kg and the total quantity of sweet oranges handled were 30,000 kg, where the cost of sweet oranges per kg was assumed to be ₹ 25 and the total cost was ₹ 75,000. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of sweet orange juice preserved with nisin (0.001 mg.ml^{-1}) in market was assumed to be ₹88and the total selling price was found to be ₹ 13,20,000. The total number of labours required was 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹ 175 per head and for 300 days it was ₹1,05,000. The cost of screw press juice extractor was ₹36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6000/5=₹6,000. The repair and maintenance charges were assumed to be ₹ 1,360.The total energy consumption for 300 days (@ 10 kWh for refrigerator per day and 3.8 for screw press juice type extractor was ₹ 4,140.

The cost of bio preservative nisin for 5g was ₹ 9,000 and the total quantity of nisin required for processing 15,000 liters of sweet orange juice @ 0.001 mg.ml was 15g and the total price of 15g of nisin for preserving 15000 liters of sweet orange juice was ₹ 27,000.

The cost of PET bottle was found to be ₹ 2.5. The total number of PET bottles required for filling 15,000 liters of juice was found to be ₹ 37,500 and the total gross returns was found to be ₹13,20,000 and the total cost incurred by selling

15,000 liters of juice was found to be ₹985086. The benefit cost ratio was found to be **13,20,000 /9,85,086= ₹1.33:1**

Juice preserved with 0.001 mg.ml^{-1} of nisin filled in tin cans and stored at refrigerated condition (4±1 °C) (T12).

It was observed that the total quantity of sweet oranges handled for 300 days was 100 kgs and the total quantity of sweet oranges handled were 30,000 kg, where the cost of sweet oranges per kg was assumed to be ₹ 25 and the total cost was ₹ 75,000. The total quantity of juice obtained from 30,000 kg of fruits was found to be 15,000 liters and selling price of sweet orange juice preserved with nisin (0.001 mg.ml^{-1}) in market was assumed to be ₹90 and the total selling price was found to be ₹13,50,000. The total number of labours required was 2 in nos. and for 300 days it was 600 labours, where the labour charges per head was assumed to be ₹175 per head and for 300 days it was ₹ 1,05,000. The cost of screw press juice extractor was ₹ 36,000 and the depreciated cost was calculated by taking the junk value and lifespan of screw press type juice extractor as 36,000-6000/5= ₹6,000. The repair and maintenance charges were assumed to be ₹1,360.The total energy consumption for 300 days (@ 10 kWh for refrigerator Per day and 3.8 for screw press type juice extractor was ₹4,140.

The cost of bio preservative nisin for 5g was ₹ 9,000 and the total quantity of nisin required for processing 15,000 liters of sweet orange juice @ 0.001 mg.ml was 15g and the total price of 15g of nisin for preserving 15,000 liters of sweet orange juice was ₹ 27,000.

The cost of tin cans was found to be ₹ 4. The total number of tin cans required for filling 15,000 liters of juice was found to be ₹60,000 and the total gross returns was found to be ₹13,50,000 and the total cost incurred by selling 15,000 liters of juice was found to be ₹10,07,586. The benefit cost ratio was found to be **13,50,000 /10,07,586= ₹1.34:1**

DISCUSSION

V DISCUSSION

Sweet orange (*Citrus sinensis* (*L*) *osbeck)* juice is known for its nutritive and therapeutic value, thirst quenching fruit beverage. It is known for its own aroma and flavour. The sweet orange juice is highly perishable in nature, after processing its shelf-life is very less when exposed to air due to the enzyme pectin methyl esterase and lactic acid bacteria activity. There is considerable loss of flavour, freshness and overall acceptability of sweet orange juice, when proper processing, preservation and storage methods are not followed. Hence, an appropriate processing technology and storage methods for enhancing shelf-life of this perishable fruit drink is essential to maintain its overall quality. In this context, the present investigation entitled "Development of Processing Technology for Enhancing Shelf-life of Sweet Orange Juice" was conducted in the Dept of Processing and Food Engineering, at College of Agricultural Engineering, UAS, Raichur and the results of the study were discussed in this chapter.

In the present investigation, it was observed that the sweet orange juice responded differentially to the different treatments used *viz*. preservation methods, packaging material and storage conditions. The preservation, packaging and storage methods involves the combination of physical, chemical and biological preservation methods *i.e.*, homogenization at 5000 psi, sodium benzoate 0.5 $mg.ml^{-1}$ and nisin 0.001 $mg.ml^{-1}$ followed by packing in different packaging materials like glass, PET (polyethylene terephthalate) and tin cans and stored at different temperatures like ambient and refrigeration temperature of 4±1 °C, respectively to study the response of sweet orange juice shelf-life upon different treatment conditions.

5.1 Effect of different preservation methods, packaging materials and storage conditions on proximate composition of sweet orange juice shelf-life

5.1.1 Moisture content

Moisture content expresses the amount of water present in a moist sample and it can be expressed in terms of % wet or dry basis depending upon the product type. The effect of different treatments on the moisture content of sweet orange juice was analyzed and presented in Table 3(a) and 3(b) in Chapter IV (Section 4.2.1). The moisture content of freshly squeezed sweet orange juice was found to be 89.09 (%w.b.) and reading was found to be similar with readings obtained by Syed *et al.*(2013) during the preparation of sweet orange squash from sweet orange. Durrani *et al.* (2010), analyzed the moisture content of apple fruit and found that it contains a moisture content of 84.70 (%w.b.).

The Fig. 2(a), 2(b) and 2(c), represents the moisture content of sweet orange juice subjected to different preservation methods, filled in glass, PET bottles and tin cans, stored at ambient condition. It was found that the more moisture content of 88.95 (%w.b.) in T2 was observed and among the different treatments (T5) presented moisture content of 88.90 (%w.b.) whereas, T7 and T10 presented minimum moisture content of 88.69 and 88.78 (%w.b.) respectively, which were found to be less when compared to the fresh juice samples which showed moisture content of 89.02 (%w.b.), this is because in un-treated juice samples due to the application of heat treatment at 80 °C for 20 s, some amount of moisture was found to be evaporated in T1, T2 and T3. Whereas, in T4, T5 and T6, less moisture content was noted. This is due to the combined effect of both pasteurization temperature as well as homogenization at a pressure of 5000 psi effected the moisture content of juice. On the other hand in T7, T8 and T9 in sweet orange juice preserved with sodium benzoate at a level of 0.5 $mg.ml^{-1}$ and pasteurization temperature, the moisture content was lessened, this is due to the desiccation effect of preservative in holding the moisture content to make it less available for microbial activity. In sweet orange juice samples preserved with 0.001 mg,ml^{-1} of nisin showed less moisture content similar to the chemical preservation method, this is due to the synergetic action of biopreservative and was found to be more compared to the chemical preservative. The similar results for decrease in moisture content in tomato pulp from 93.50 to 76.20 (%w.b.) after processing and addition of sodium benzoate and potassium sorbate was observed by Hossain *et al.* (2011). As the storage period increases, the increase in moisture content was observed in all the juice samples, this might be due to the fluctuations in temperature and variation in relative humidity and adsorption of moisture through the packaging materials from the outside environment to the inside product which leads to deterioration and the similar findings were observed by Idah *et al* .(2010) in orange and tomato fruits stored in “pot-in-pot” evaporator cooler, where increase in moisture was observed from 74.77 to 100 (%w.b.) in tomato fruit, where as in orange fruit the increase in moisture content from 85.32 to 89.91(%w.b.) was observed during 21 days of storage period.

Fig. 2(d), 2(e) and 2(f) shows the moisture content of the sweet orange juice with different treatment effects stored at refrigerated condition of 4±1 °C, where the moisture was increased in all the samples during the first day of storage, this is due to the

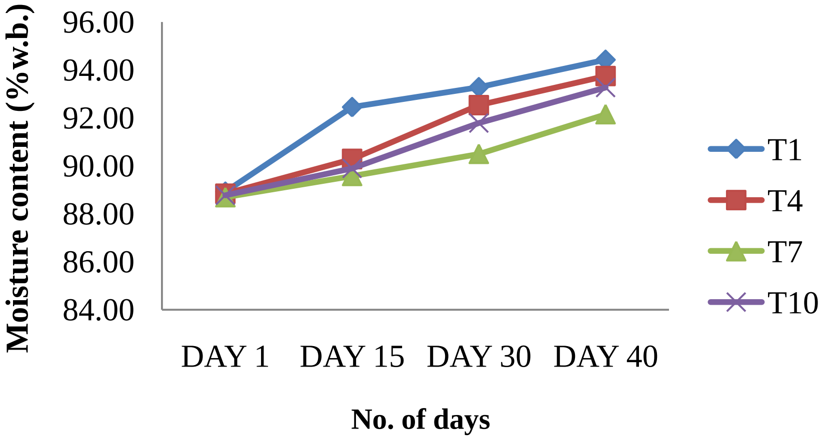

Fig. 2(a)

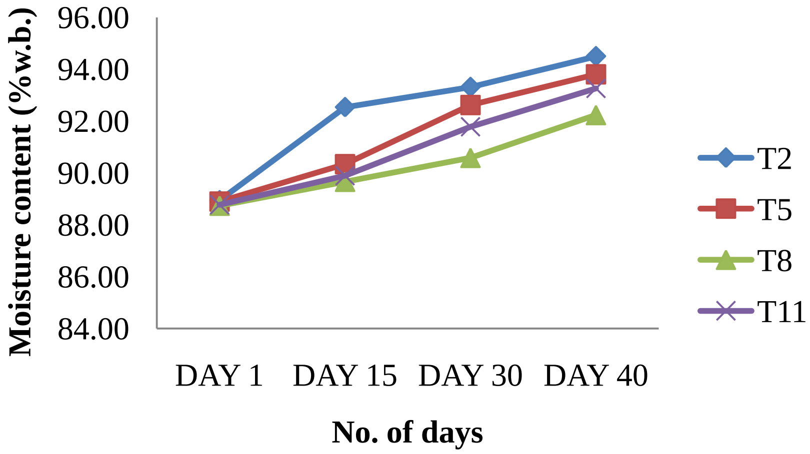

Fig. 2(b)

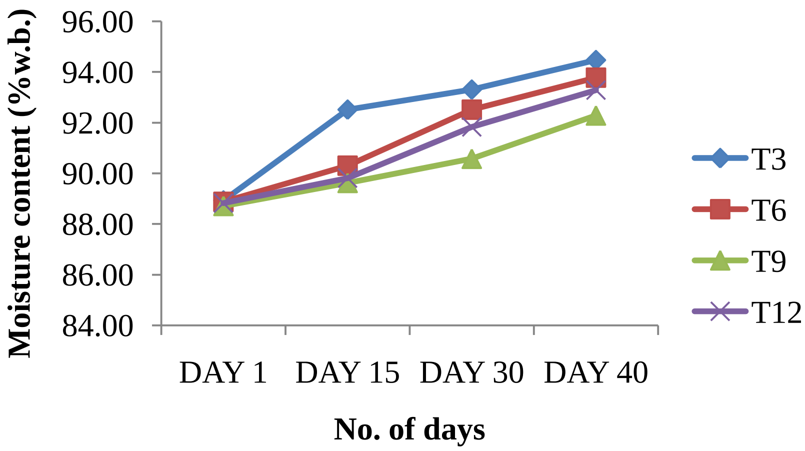

Fig. 2(c)

Fig. 2(c), Fig. 2(a), 2(b) and 2(c) Effect of different preservation methods on moisture content of sweet orange juice filled in glass, PET bottles and tin cans, stored at ambient condition (30±2 °C)

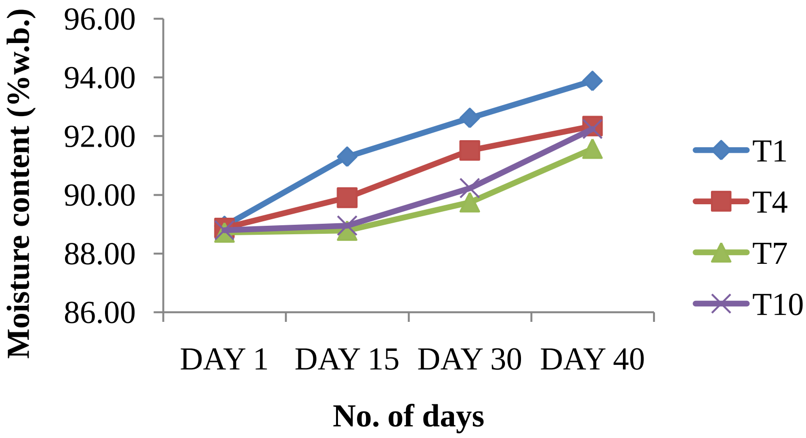

Fig. 2(d)

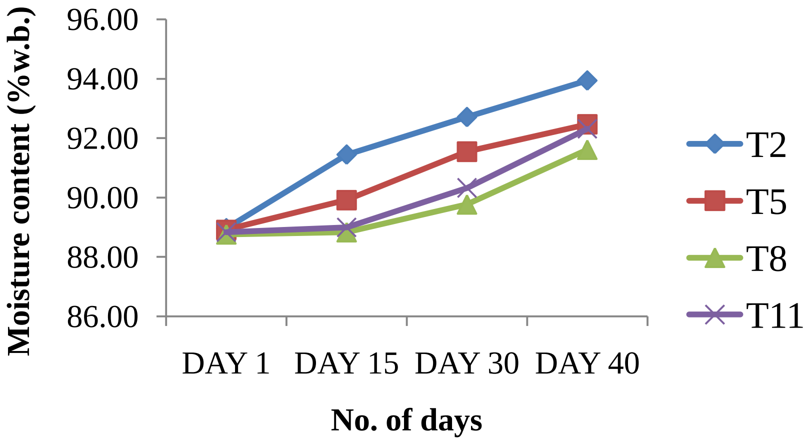

Fig. 2(e)

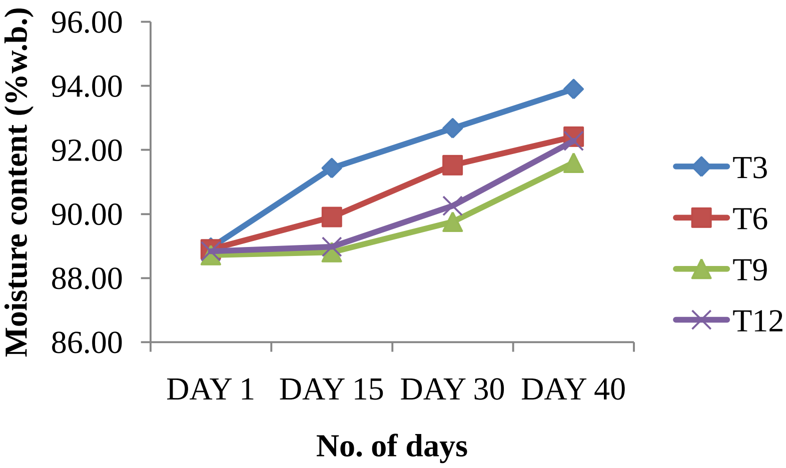

Fig. 2(f)

Fig. 2(f), Fig.2(d), 2(e) and 2(f) Effect of different preservation methods on moisture content of sweet orange juice filled in glass bottle, PET bottle and tin cans and stored at refrigerated condition (4±1 °C)

expansion of water molecules in the juice sample when heat is removed and stored. At refrigeration temperature where less increase in moisture was observed compared to the juice samples stored at ambient condition which was due to the lesser availability of water for microorganisms to grow and multiply at this condition. This helps to maintain the freshness of the sample as such as well as pH of the sweet orange juice. According to Diamante *et al.* (2013) the increase in moisture content is also due to the increase in the level of pectin in apple fruit juice that shows the interaction effect between the moisture and the level of pectin content in the apple fruit juice.

5.1.2 Soluble Protein

The effect of different preservation methods and packaging materials on soluble protein content of sweet orange juice stored at ambient condition was analyzed and presented in Fig 3(a), 3(b) and 3(c). From the analysis it was found that the soluble protein content of fresh sweet orange juice was 0.54% and the protein content of fresh moosambi juice analyzed by Syed *et al*. (2013) observed the protein content of 0.60% while, preparing the value added product from the moosambi juice. Similarly, Shahanwaz *et al.* (2009) analyzed the nutritive values of ready to drink juice filled in glass bottle and stored at refrigerated and ambient condition was found to be 0.41 and 0.21%, whereas the soluble protein content of juice filled in glass bottle, wrapped in aluminium foil and stored at refrigerated condition was found to be 0.44% and in room temperature it was 0.22%. The soluble protein content of 0.70g was found in orange juice (Shahanwaz *et al.*, 2013).

It was observed that the soluble protein content of processed sweet orange juice was less when compared to the soluble protein content of the fresh juice sample and the decrease upon storage condition was observed from 0.35 to 0.12% (T1), 0.30 to 0.07% (T2) and 0.33 to 0.10 (T3). This is because at pasteurization temperature of 80 °C for 20 s, denaturation of protein occurs, as it is sensitive to heat. Whereas in T4, T5 and T6, the losses are minimal in combination with homogenization pressure of 5000 psi and pasteurization temperature. On the other hand in T7, T8, T9 and in T10, T11 and T12, *i.e.*, in juice samples preserved with sodium benzoate at a level of 0.5 $mg.ml^{-1}$ and nisin at a level of 0.001 $mg.ml^{-1}$, were found to be effective in minimizing the nutrient losses by making less available moisture for the microbial activity and sodium benzoate being a chemical preservative was found to be more effective anti-microbial properties and thus minimizes the nutritive losses and preserves the juice upon storage compared to nisin.

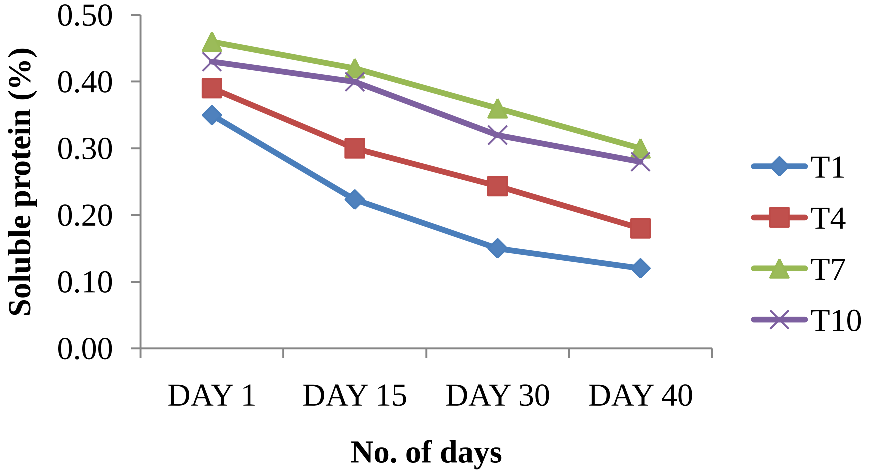

Fig. 3(a)

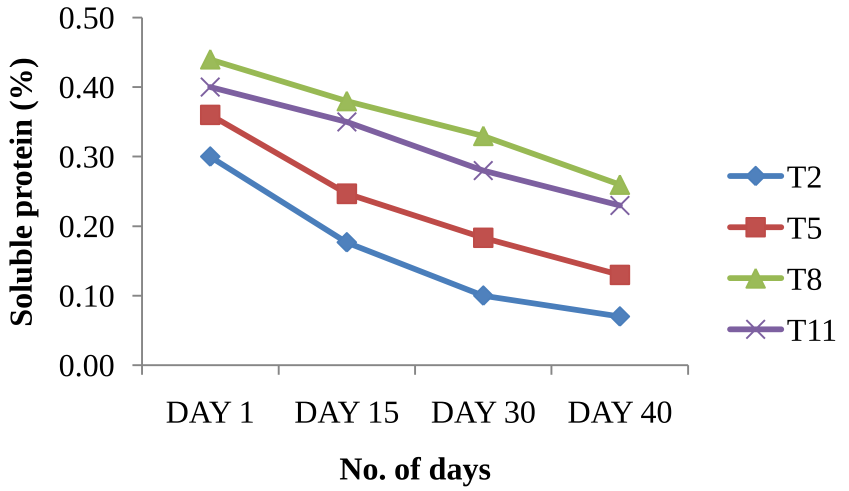

Fig. 3(b)

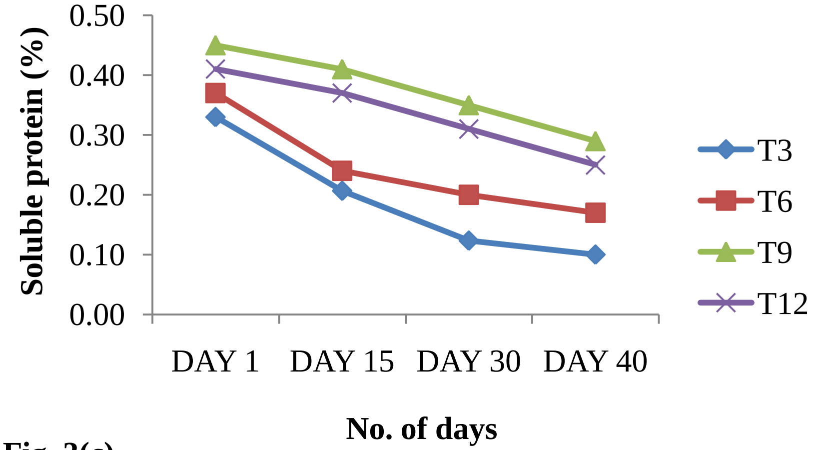

Fig. 3(c)

Fig. 3(a), 3(b) and 3(c) Effect of different preservation methods on soluble protein content of sweet orange juice filled in glass bottle, PET bottle and tin cans and stored at ambient condition (30±2 °C)

The less degradation of soluble protein upon storage at refrigerated condition was observed from Fig. 3(d), 3(e) and 3(f), even though some losses were observed during storage. By refrigerating the juice samples to a lower temperature of 4±1 °C, helps in maintaining the overall freshness of the juice by minimizing the nutrient degradation upon storage. In T7 at refrigerated condition the less degradation of protein was observed from 0.47 to 0.40% during the 40th days of storage period. The similar observation for loss of protein in tomato and orange fruit upon storage in 'pot-in –pot' evaporator cooler was observed by Idah *et al.* (2010) where the loss of protein from 0.05 to 0% was observed in tomato fruit, whereas in orange fruit the protein degradation was from 0.18 to 0.04% from 1st to 21st days of storage period. It was observed that sweet orange juice was highly perishable beverage and hence protein loss is high compared to tomato or orange.

It was observed that as the storage period proceeds, the soluble protein content was decreased, this is because microorganisms breaks down the available nutrients under unfavourable conditions for their growth and multiplication. Apart from that packaging material, processing as well as storage conditions, atmospheric condition influences the protein quality of sweet orange juice.

5.1.3 Carbohydrates

The effect of different preservation methods and packaging materials on carbohydrates of sweet orange juice stored at ambient and refrigerated condition was analyzed and found that carbohydrates of 10.46% and the findings were found similar to the results obtained by Syed *et al.* (2013). Shahanwaz *et al.* (2009) analyzed the carbohydrate content of fresh pulp of jam fruit and found that it contains carbohydrate of 17.37% and that in fresh jam juice and squash, they observed carbohydrate content of 61.43 and 2.31%.

From Fig. 4(a), 4(b) and 4(c), it was observed that at ambient condition the decrease in carbohydrates was observed from 9.88 to 4.65 (T2) and among the treatments T5 showed less carbohydrates of 10.06 to 5.24%, followed by 10.15 to 5.72 (T11), with a more retention of carbohydrates of 10.26 to 7.30 in (T7). This is due to the application of heat and at homogenization pressure of 5000psi, the carbohydrate was decreased and upon addition of preservatives like sodium benzoate and biopreservative nisin more

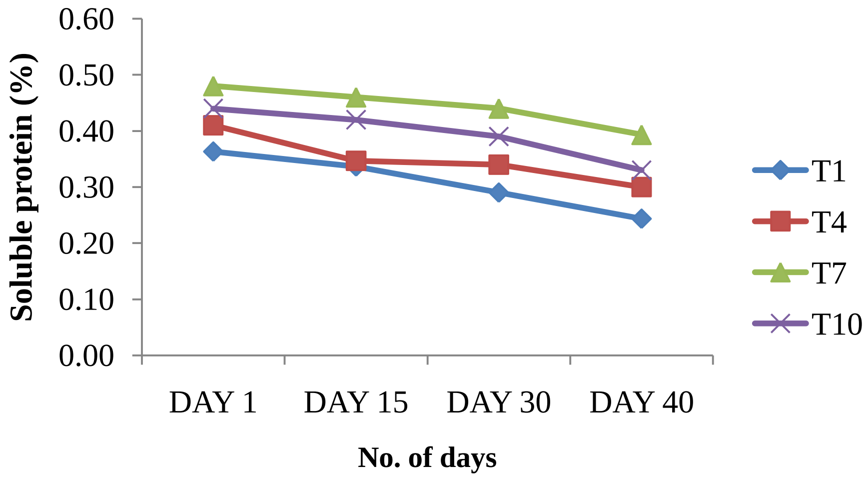

Fig. 3(d)

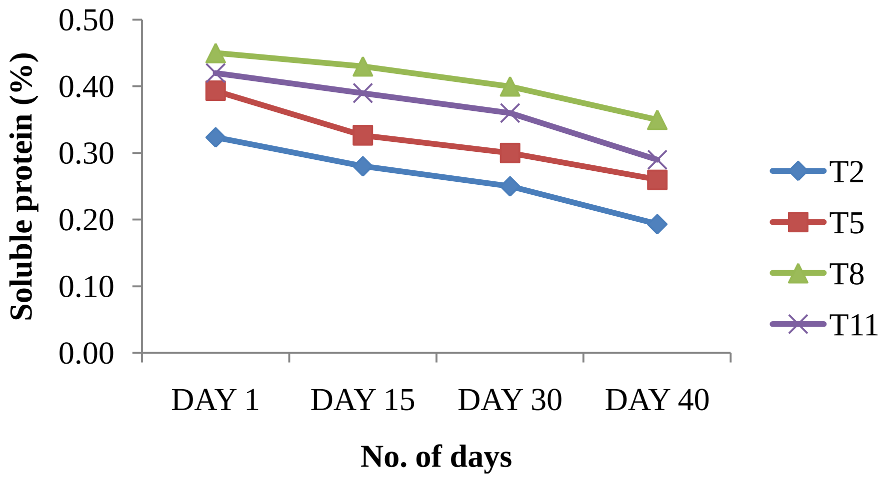

Fig. 3(e)

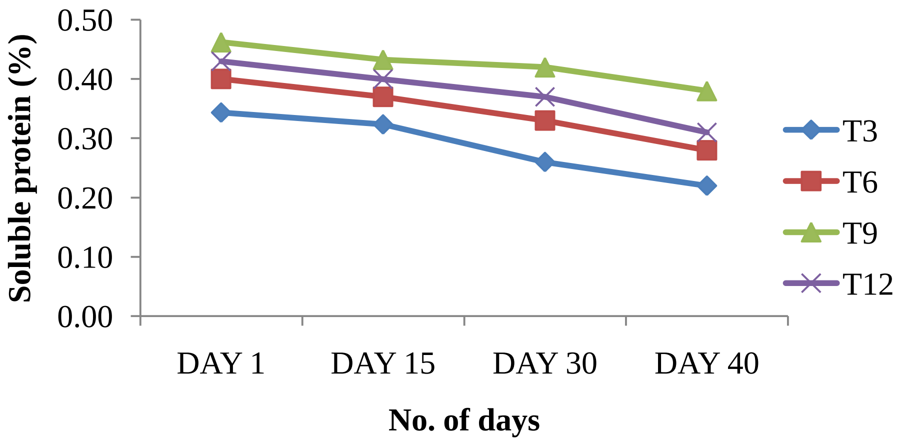

Fig. 3(f)

Fig. 3(d), 3(e) and 3(f) Effect of different preservation methods on soluble protein content of sweet orange juice filled in glass bottle, PET bottle and tin cans and stored at refrigerated condition(4±1 °C)

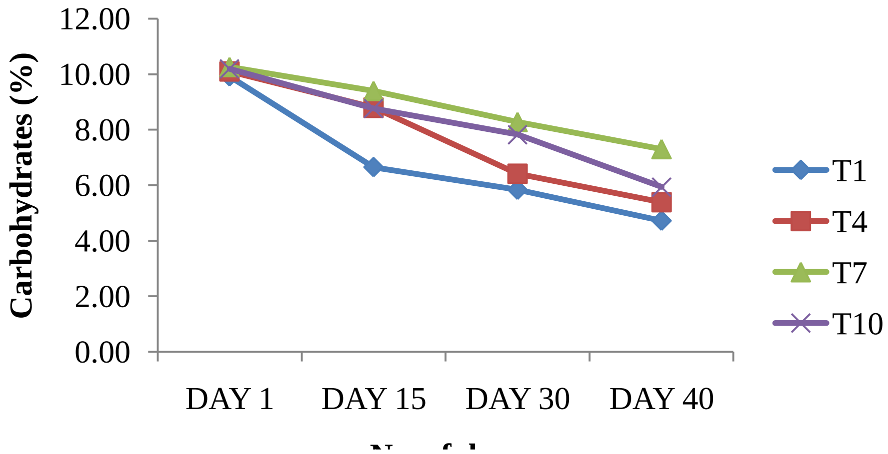

Fig. 4(a)

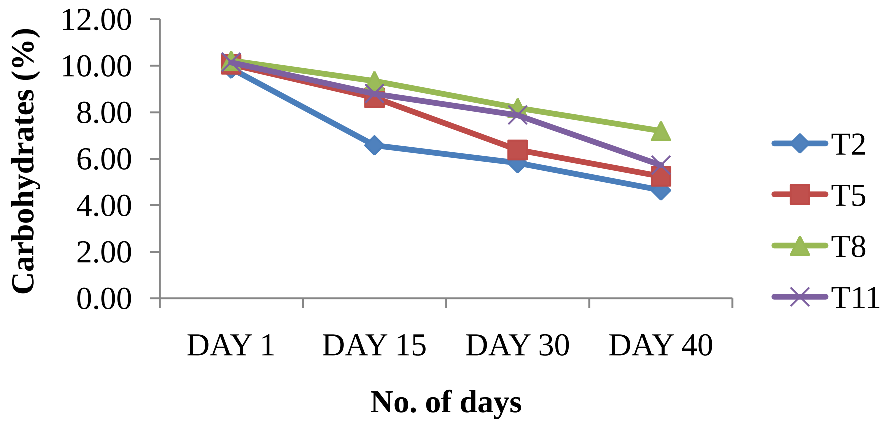

Fig. 4(b)

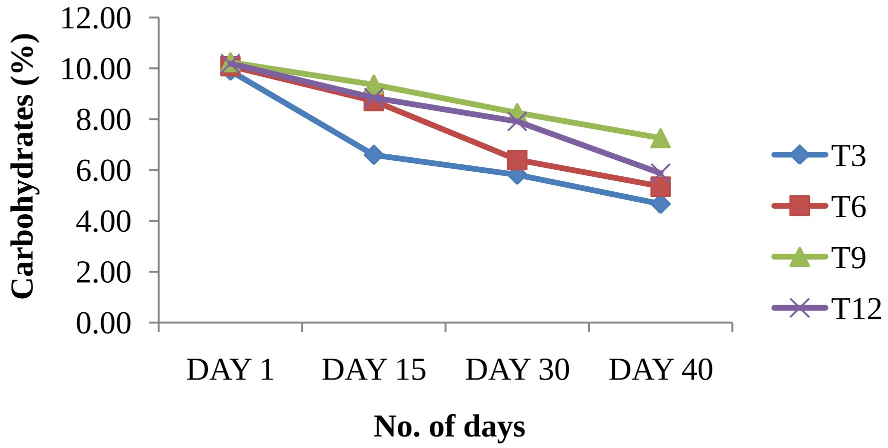

Fig. 4(c)

Fig. 4(a), 4(b) and 4(c) Effect of different preservation methods on carbohydrates of sweet orange juice filled in glass bottle, PET bottle and tin cans and stored at ambient condition (30±2 °C)

retention of carbohydrate was observed, this is due to the effect of preservative in retaining the nutritive value of sweet orange juice. On the other hand the degradation of carbohydrate was occurred in all the un-treated (control) as well as in treated samples due to the breakdown of carbohydrates by microorganisms and converting them into fermenting sugars, which leads to faster deterioration and spoilage of juice sample at ambient condition.

PET bottles because of its excellent mechanical properties, its clearness, UV resistance and good oxygen barrier properties is increasingly used in food packaging in liquid foods such as milk or oil (Moyssiadi *et al.*, 2004). Although materials with a low permeability to oxygen such as glass or multilayer PET bottles have been proposed by many authors as the best to preserve nutritive and sensory quality in liquid foods (Siegmud *et al.*, 2004). Idah *et al*. (2010) find the similar results in loss of carbohydrates in orange and tomato fruit as storage period increases, they found that in orange the carbohydrate loss ranged from 12.23 to 9.53%, similarly in tomato fruit the loss ranged from 23.47 to 0%, respectively during 1^{st},7^{th}, 14^{th} and 21^{st} days of storage period in pot in pot evaporator cooler. Shahanwaz *et al.* (2009) observed that juice beverages stored in glass bottles and stored at refrigerated condition can retain good quality nutritive values and leading to more extensive shelf-life of the products. They found that this loss is due to microbial activity as storage period increases.

From Fig 4(d), 4(e) and 4(f), the carbohydrates degradation was found to be very less when sweet orange juice stored at refrigerated condition of 4±1 °C, which provides unfavourable condition for the growth and multiplication of microorganisms to convert carbohydrates in to fermentable sugar, which was found to be one of the most suitable hurdle technology in inhibiting the microbial load and maintaining the overall quality of the juice (Meyer, 2004).

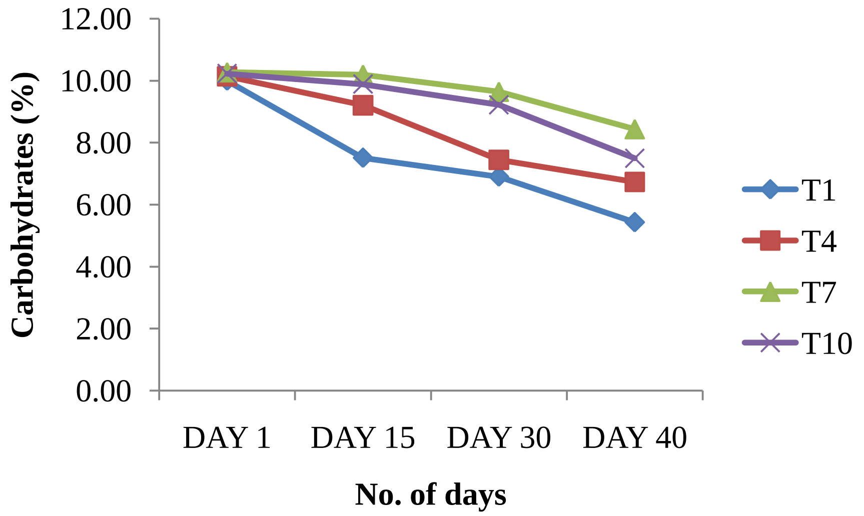

Fig. 4(d)

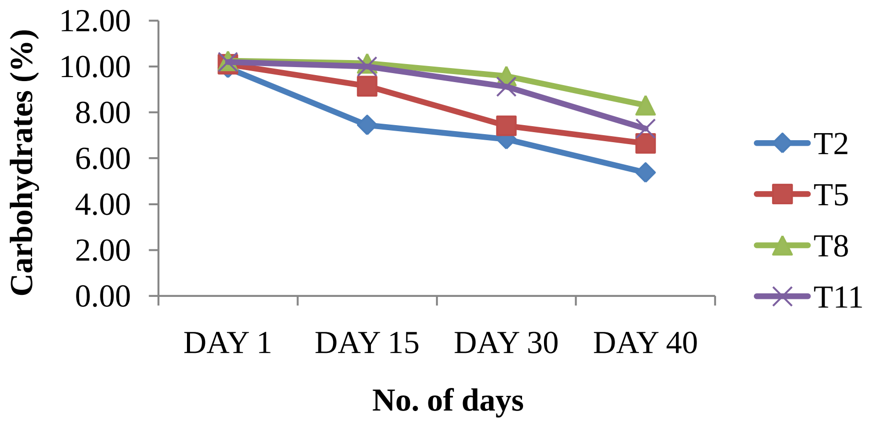

Fig. 4(e)

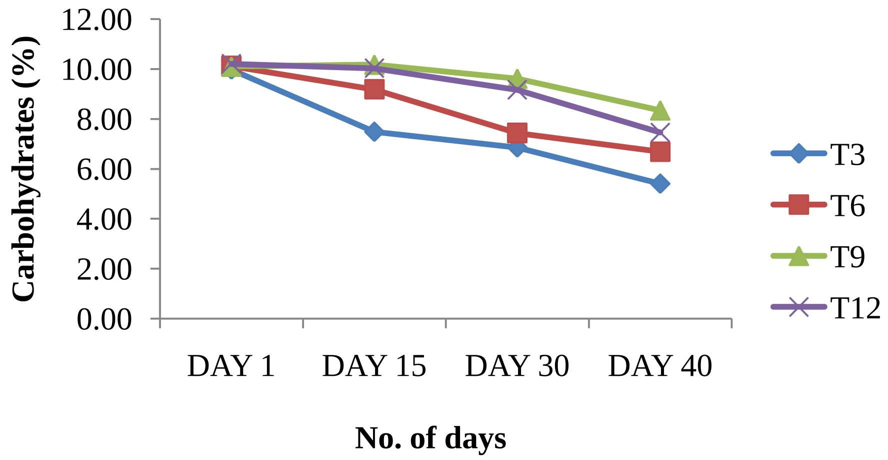

Fig. 4(f)

Fig. 4(d), 4(e) and 4(f) Effect of different preservation methods on carbohydrates of sweet orange juice filled in glass bottle, PET bottle and tin cans and stored at refrigerated condition(4±1 °C)

5.2 Effect of preservation methods, packaging materials and storage conditions on quality parameters of sweet orange juice shelf-life.

5.2.1 pH

The effect of different preservation methods and packaging materials stored at ambient and refrigerated condition on pH of juice were revealed and presented in Fig 5(a) and 5(b). From the Fig.5(a), it was observed that as the storage period increases, the decrease in pH was observed in all the samples. From the findings, it was noted that the pH of fresh orange juice was 4.38, where as in un-treated samples the pH of 3.74 to 3.12, 3.56 to 3.09 and 3.68 to 3.10, was observed in T1, T2 and T3 and these findings were found to be nearer to the readings of 3.72 to 3.75 in orange juice filled in glass bottles, followed by 3.59 to 3.45 in orange juice sample filled in PET bottle and 3.63 to 3.58 in orange juice sample filled in amber bottles by Shahanwaz *et al.* (2013). Similarly in T4, T5 and T6, slight increase in pH was observed compared to that of the un-treated samples, this is due to the synergetic effect of homogenization at 5000 psi on the particle size of sweet orange juice in stabilizing its pH and the similar result of 4±0.27 was obtained by Chanes *et al*. (2009) for high pressure homogenized orange juice to inactivate pectinmethylesterase.

It was observed in T7, T8 and T10, the pH of 4.24 to 3.65, 4.15 to 3.59 and 4.21 to 3.61 in sweet orange juice preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate filled in glass, PET bottles and tin cans, stored at ambient condition from 0^{th} to 40^{th} days and these findings were found to be true with the results obtained by shahanwaz *et al.* (2013) for orange juice sample preserved with sodium benzoate at different levels of 0.6g, 0.8g and 1.0g , filled in glass bottles was found to be 4.19 to 4.17, followed by 4.13 to 3.39 and 4.24 to 3.89 and in the similar way the pH of orange juice filled in PET bottles were found to be 4.34 to 4.19, 4.12 to 3.65 and 4.13 to 4.15 in orange juice filled in amber bottle with different concentration of sodium benzoate at 0.6, 0.8 and 0.1 were found to be 4.45 to 4.25, 3.87 to 4.16 and 4.10 to 4.22, respectively. This is due to the effect of sodium benzoate in neutralizing the acidity level compared to other biopreservative like nisin, thus stabilizes the pH and on other hand sodium benzoate being an effective antimicrobial agent when added to fruit juices along with the glass as a packaging material does not reacts with the acid content of juice and thus helps to maintain pH followed by tin cans and PET bottles (Mathooko and Nijru, 1994). They suggested that orange juice preserved with 1.0 g.$l^{-1}$of sodium benzoate filled in amber bottle can be used upto 30 days after storage at ambient condition. Pasteurization at 60 °C for 15 min inhibits the decrease of pH better than sodium benzoate or potassium sorbate and along with both gives the best effect in low alcoholic plum wine (Gill and Neeraj, 2009). The decrease in pH from 3.9 to 3.4 in amla-mango blended juice with no preservatives was observed by Rustagi *et al*. (2013) during 0^{th} to 60^{th} days of storage period and in amla-mango blended juice sample preserved with sodium benzoate at ambient condition was found to be 3.6 to 3.1, whereas, the pH of 3.4 to 3.1 was recorded in juice sample preserved with 100 mg of potassium metabisulphite and stored at ambient condition during 0^{th} to 60^{th} days of storage. During 0 to 90^{th} days of storage period the decrease in pH from 3.4 to 2.93 was observed in cashew apple juice preserved with 1ppm of potassium benzoate, this decrease in pH is due to increase in acidity upon the addition of preservative and most of the bacteria will not grow at low pH and hence keeping quality of juice is maintained (Ranganna, 1986). During initial to 90^{th} days of storage period the gradual decrease in pH from 3.63 to 3.10 was noticed by Durrani *et al.* (2011) in physico-chemical response of apple pulp to different preservatives upon storage and found that decrease in pH is due to the formation of free radicals and pectin hydrolysis as the storage period increases (Imran *et al.*, 2000).

From the Fig.(5)b it was observed that the pH of 3.78 to 3.27 in T1 and among the treatments, the pH value of 4.10 to 3.38 in T4, followed by 4.21 to 3.90 in T10 and 4.26 to 4.10 was observed in T7 and these values were found true with the values obtained by Rustagi *et al*. (2013) for amla-mango blended fruit juice beverage and the results of 3.8 to 3.4 was observed in amla-mango blended juice preserved with 100 mg of sodium benzoate, stored at refrigeration temperature of 4 to 6°C. This is because at lower temperature in combination with preservatives were found to be effective in lowering the acidity by stabilizing the moderate pH that are unfavourable for growth and multiplication of microorganisms as well the formation of free acids and pectin hydrolysis were found to be less in the juice samples due to low temperature available for pectinmethylesterase enzymes to form free acid radicals (Cecilia and Maia. 2002).

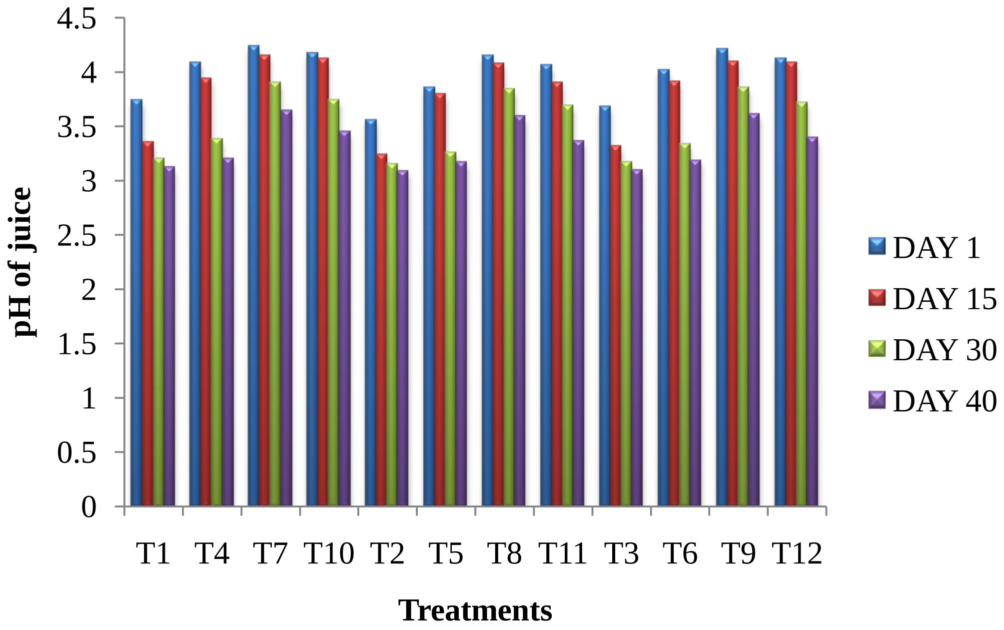

Fig. 5(a) Effect of different preservation methods and packaging materials on pH of sweet orange juice stored at ambient condition (30±2 °C)

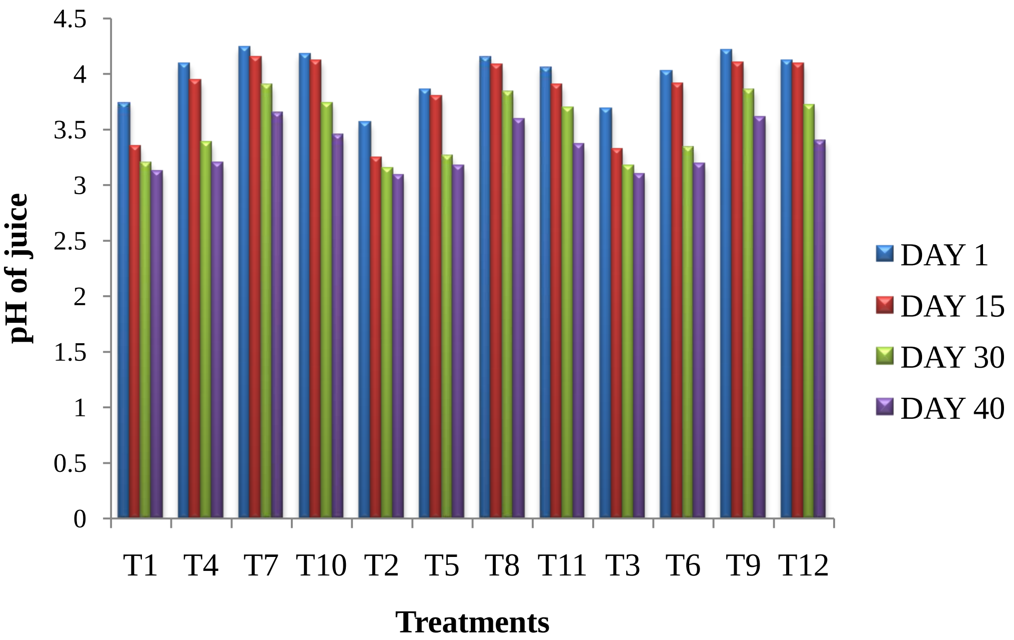

Fig. 5(b) Effect of different preservation methods and packaging materials on pH of sweet orange juice stored at refrigerated condition (4±1 °C)

5.2.2 Water activity (a_w)

Fig.6 (a) and 6(b) reveals the water activity of sweet orange juice with different preservation methods and packaging materials, stored at ambient and refrigerated condition. In T1 to T12, the water activity from 0.894 to 0.864 was observed, which depends upon the different preservation methods adopted with respect to the packaging materials used and the minimum water activity of 0.860 was revealed in T7 and maximum water activity of 0.878 was observed in T5. As storage period increases from day 1 to day 40, the increase in water activity from 0.942 to 0.903 was observed in all the un-treated samples including the treatments. This increase in water activity indicates the amount of water available for the activity of microorganisms to cause spoilage of foods or any perishable product like fruit juices. By the application of pasteurization temperature of 80 °C for 20 s, homogenization at 5000 psi, sodium benzoate and nisin as preservation methods the less moisture is available for the microbes to carry out the spoilage activity and sodium benzoate at 0.5 $mg.ml^{-1}$ being an effective antimicrobial agent stabilizes the pH, causes damage to the microbial membrane and thus makes less water available for the microorganisms to cause spoilage and nisin on the other hand at the level of 0.001 found to be less effective in controlling the water activity although with an increase in nisin level upto 1000 $IU.ml^{-1}$ was found to be effective in controlling microbial activity and similarly water activity also depends on the type of the packaging material used (Walker *et al.*, 2008) and among packaging materials glass bottle was found to be best in minimizing the water activity, followed by tin cans and PET bottles, along with preservatives was found to be best in controlling water activity (Chumillas *et al.*, 2007).

Fig. 6(b) shows the water activity of sweet orange juice stored at refrigerated condition of 4±1 °C, where less increase in water activity compared to that in the ambient condition was noted during initial day of storage, the water activity was found to be 0.881 and 0.851 in T1 and T12, respectively. This shows that at refrigerated condition along with the preservation methods and packaging materials, the microbial activity can be retarded due to less water availability by applying hurdle techniques. The change in water activity also depends on the temperature, moisture content and variety of fruit used for juice preparation. Diamante *et al* .(2013) observed the increase in water activity in apple juice concentrate with increase in pectin level and found it due to quadratic interaction and interaction effects of both apple juice concentrate and pectin levels. These results contrasted to those of Phimpharian *et al.* (2011) for the effect of pectin level on water

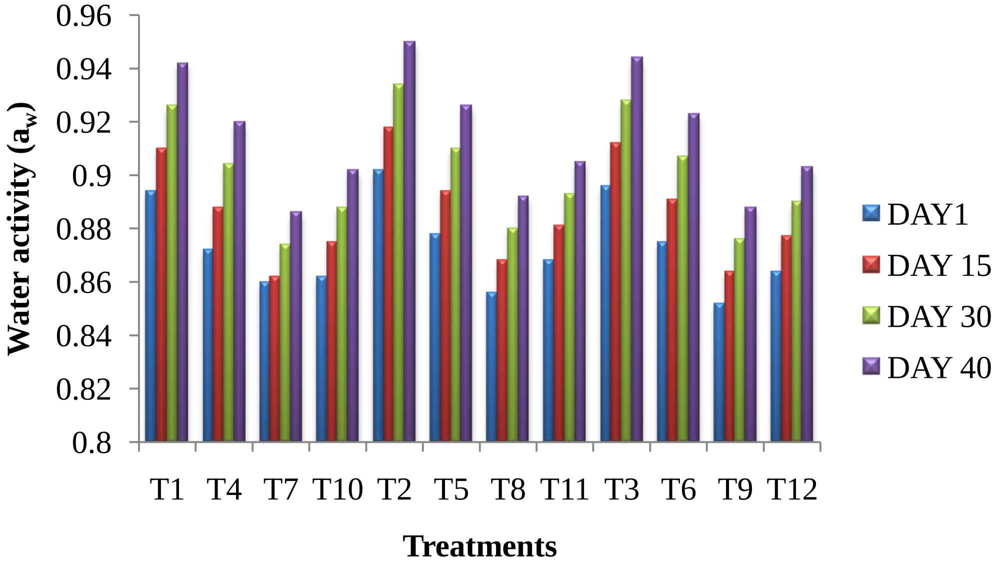

Fig. 6(a) Effect of different preservation methods and packaging materials on water activity (a_w) of sweet orange juice stored at ambient condition (30±2 °C)

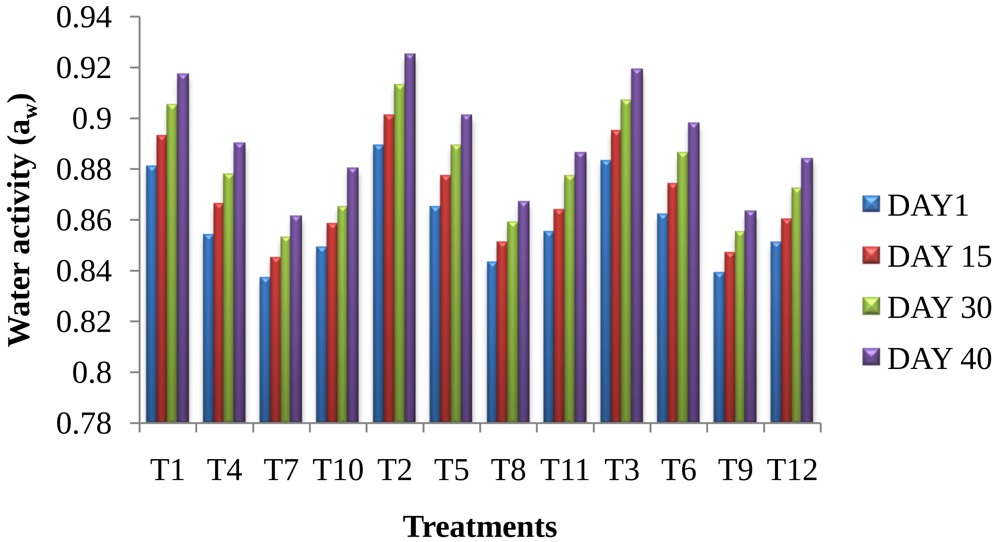

Fig. 6(b) Effect of different preservation methods and packaging materials on water activity (a_w) of sweet orange juice stored at refrigerated condition (4±1 °C)

activity of pineapple leather. This difference might be due to the heating of the pineapple fruit puree mixture, the lower pectin level used (0.5% to 1.5%) or the type of fruit used.

When there is no pectin in the fruit leather, the water activity of the samples increased with AJC level due to the presence of higher amounts of sugar thereby bound more water to the food matrix but when the pectin level increased to about 4% the water binding property of the product may have likely changed.

5.2.3 Colour

Effect of different preservation methods, packaging materials and storage conditions helps in maintaining the overall quality and acceptability of sweet orange juice to the consumer. However, colour is one of the most important factor in sweet orange juice that indicates its overall appearance and acceptance to the final consumer usage. All the colour data were expressed by Hunter L^*, a^* and b^* values corresponding to lightness, redness and yellowness.

From the Fig. 7(a) and Fig. 7(b). It was observed that as the storage period proceeds, the decrease in L^* value was observed in all the samples, with respect to different treatments, ackaging materials and stored at ambient and refrigerated condition. It was observed that the L^* values of pasteurized sweet orange juice was found to be in the decreasing range from 54.69 to 37.68 (T1), 50.84 to 33.32 (T2) and 53.32 to 36.48 (T3) at ambient condition, followed by 54.62 to 44.36 (T1), 52.70 to 49.29 (T2) and 53.68 to 42.84 (T3) in pasteurized sweet orange juice stored at refrigerated condition and these readings were found to be nearer with the readings obtained by Timmermans *et al.* (2011), where they obtained L^*values of 57.60 for pasteurized juice and the slight variation was observed in L^*values might be due to the pasteurization temperature and time on the colour of sweet orange juice, effect of packaging materials in retaining the colour and also decrease in L^*values may be due to the non-enzymatic browning by pectinmethyl esterase activity upon storage period increases,

On the other hand, the L^*values of 60.46 was noted in T4, followed by 56.10 in T7 and 55.62 in T10 and the L^*values of 54.62 to 44.36 was observed in T1, followed by 59.52 to 49.29, 54.52 to 49.94 and 54.56 to 45.07 in T4, T7 and T10, in sweet orange juice stored at refrigerated condition of 4±1 °C and these readings were neared to the reading of 57.10±3 and 56.31±0.02 for homogenized and pulsed electric field orange juice sample by Timmermans *et al.* (2011) for homogenized juice, whereas lesser

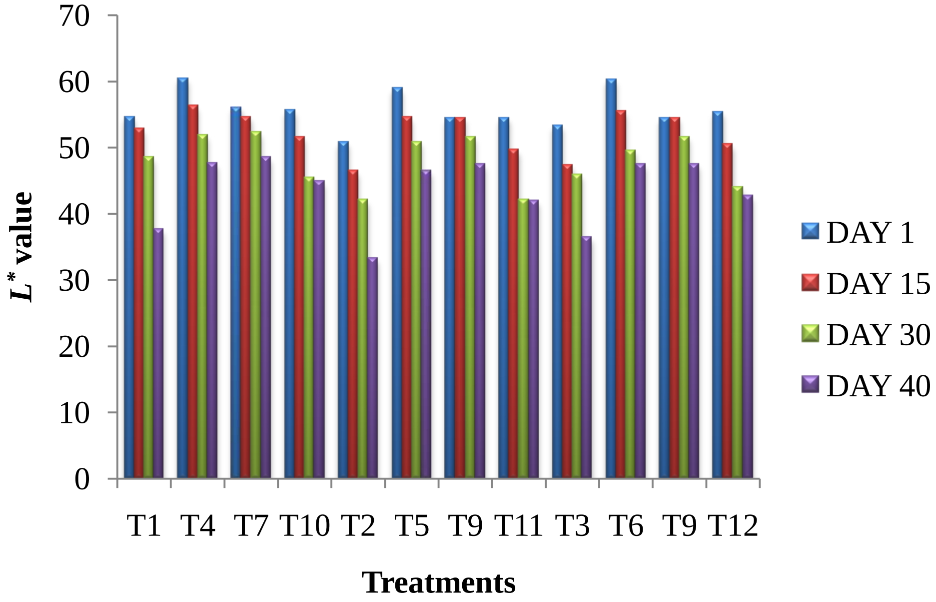

Fig 7(a). Effect of different preservation methods and packaging materials on the colour (L^* value) of sweet orange juice stored at ambient condition (30±2 °C)

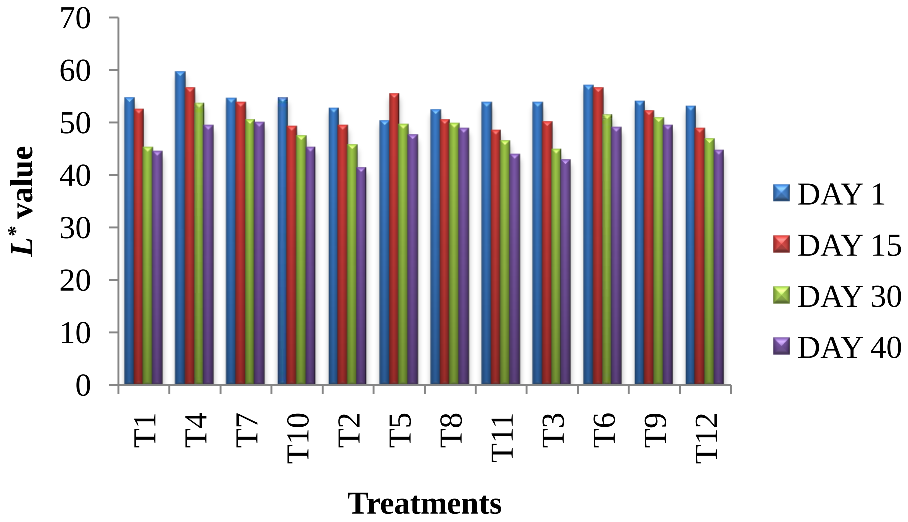

Fig. 7(b) Effect of different preservation methods and packaging materials on the colour (L^* value) of sweet orange juice stored at refrigerated condition (4±1 °C)

lightness values were observed for juice samples preserved with sodium benzoate and nisin is due to their effect in stabilizing the colour of the juice sample and decrease in juice L^*values may also due to the microbial activity and development of cloudiness in juice samples which are stored at different storage conditions.

a^*^ Values

The effect of different preservation methods, packaging materials and storage conditions on a^* Value of sweet orange juice was determined and presented in Fig. 8(a) and Fig. 8(b). It was observed that as the storage period proceeds, the redness value was also increased. In the un-treated samples the a^* Values of -4.08 to 3.42 was observed in pasteurized juice samples filled in PET bottles (T2), and a maximum a^* Values -4.92 t0 1.68 in T5, followed by -4.68 to 1.40 in juice sample preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate in T7, followed by -3.14 to 2.36 in juice samples preserved with 0.001 $mg.ml^{-1}$ of nisin and on the other hand, the samples stored at refrigeration condition of 4±1 °C, showed redness value of -4.24 to -1.28 in T2, -4.63 to 0.40 in T4, -4.48 to 1.65 in T8 and -4.35 to 2.24 in T11 and these values were found to be nearer to the values obtained by chumillas *et al.* (2007) in orange juice filled in PET bottles, glass bottles and stored at refrigerated conditions of 4 and ambient condition of 25 °C. It was observed that redness value was found to be stable in glass bottles stored at refrigerated condition of 4±1 °C, where less enzymatic activity was observed at these temperatures.

b^* values

From the Fig. 9(a) and Fig. 9(b), the decrease in b^* value was observed in all the samples as the storage period increases. The juice samples with different treatments stored at ambient condition showed more decrease in b^* values when compared to that of the juice samples stored at the refrigerated condition. Among the un-treated samples the maximum b^* value of 18.64 to 10.78 was observed in T2 at ambient condition and minimum b^* values of 18.52 to 12.70 was observed in sweet orange juice stored at refrigerated temperature of 4±1 °C and these readings were found to be true with the readings obtained by Chumillas *et al.*(2007), where they observed b^* values of 18.55 to 20.76 in orange juice sample filled in PET bottles with oxygen scavenger and stored at 4 °C and 17.92 to 19.62 in orange juice filled in PET bottles with oxygen scavenger and stored at 25 °C. Among the treatments T9 noted the maximum b^* values of 24.36 to 20.81.

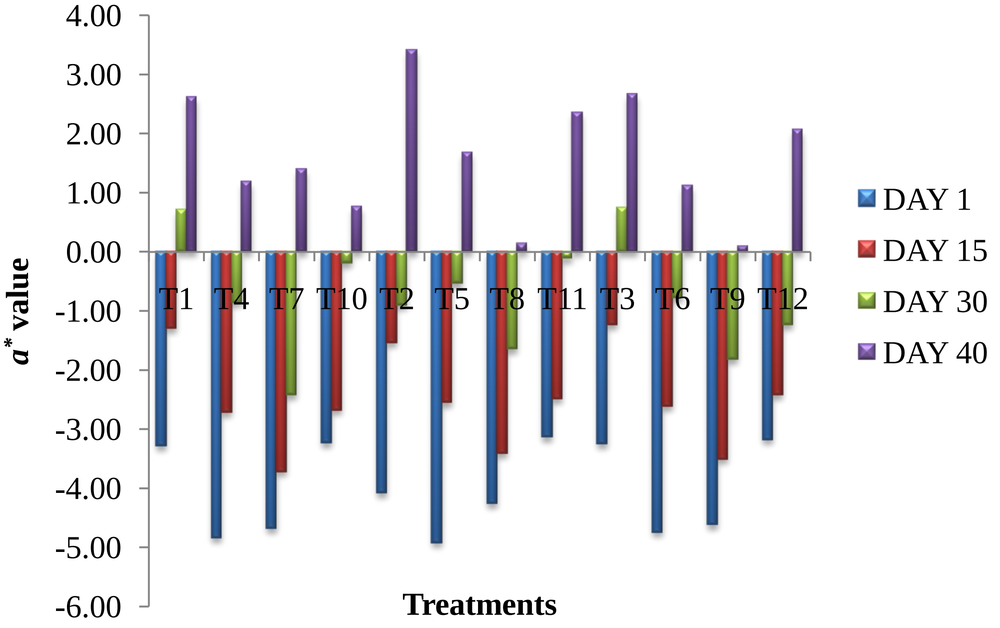

Fig 8(a) Effect of different preservation methods and packaging materials on the colour (a^* value) of sweet orange juice stored at ambient condition (30±2 °C)

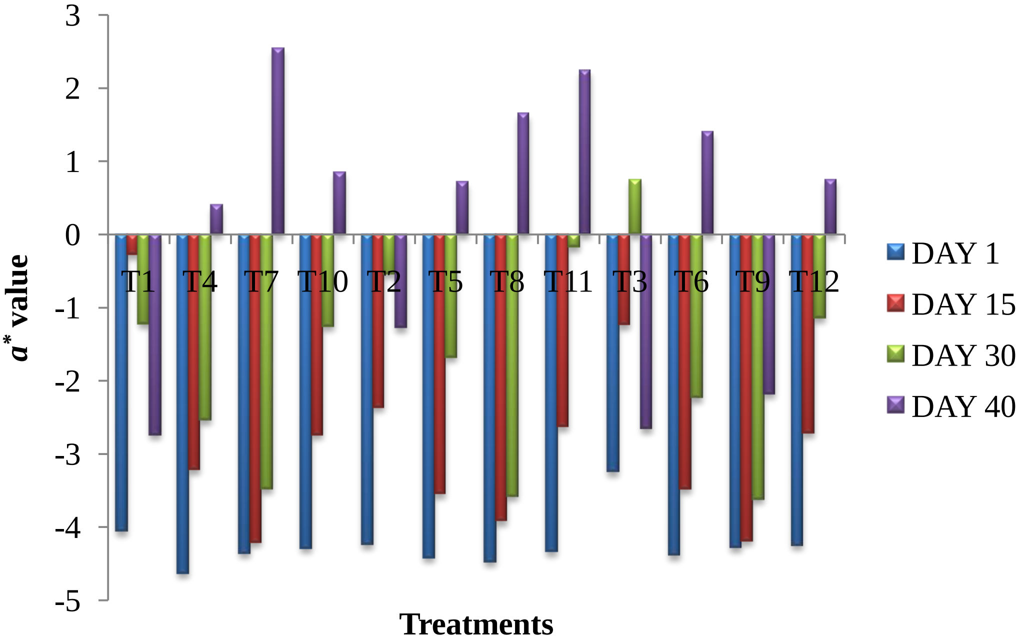

Fig 8(b) Effect of different preservation methods and packaging materials on the colour (a^* value) of sweet orange juice stored at refrigerated condition (4±1 °C)

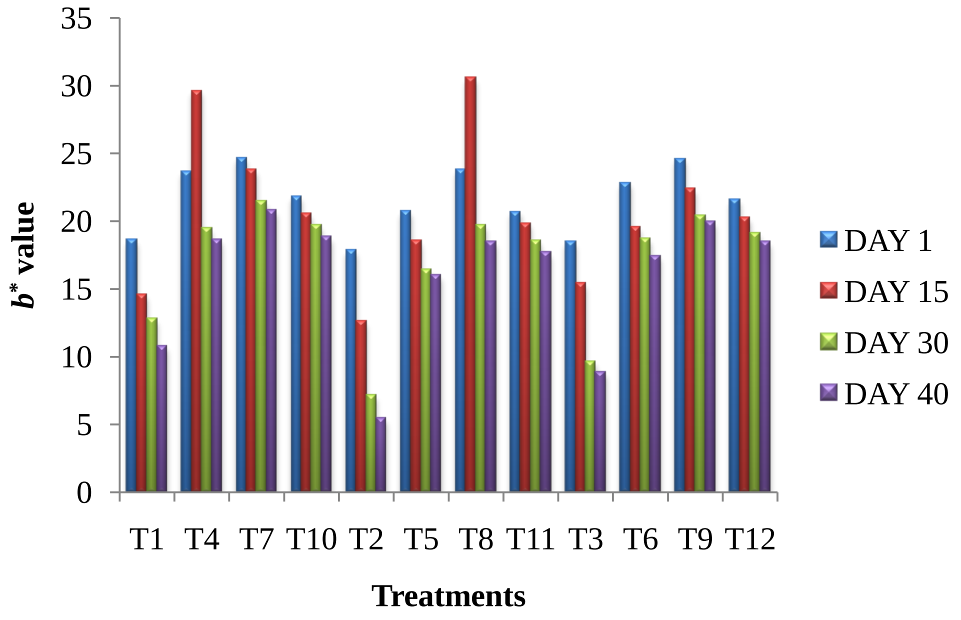

Fig. 9(a) Effect of different preservation methods and packaging materials on the colour (b^* value) of sweet orange juice stored at ambient condition (30±2 °C)

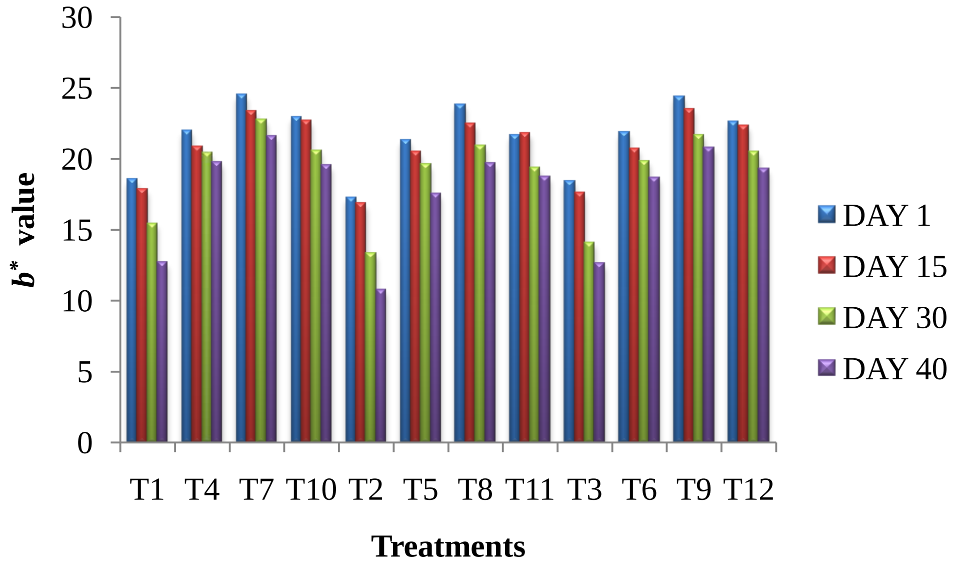

Fig 9(b). Effect of different preservation methods and packaging materials condition on the colour (b^* value) of sweet orange juice stored at refrigerated condition (4±1 °C)

The overall trend for the colour of orange juices, during the storage time showed that lightness (L^*values) decreased, redness (a^* Values) increased and yellowness (b^*values) decreased (Nienaber and Shellhammer, 2011) and there were no significant differences in colour between the high pressure processed (HP) and untreated juice were noticed, whereas mild heat pasteurized juice was significantly lighter than the HP and untreated orange juice, having less red colour and more yellow colour and the PFE juice samples showed more darker colour than the untreated, heat treated and HP juice with significantly more red and less yellowness.

Moreover, packaging materials, refrigerated condition and the preservatives used plays an important role in maintaining the colour of the sweet orange juice. With this regard, Sobek *et al.* (2003) observed the colour parameters of orange juice packed in monolayer PET bottles with and without oxygen scavenger stored at 4 and 25 °C and found no significant variations in colour till 60 days of storage at both 4 and 25 °C and after this time, they observed the decrease in L^* and °*H* values, which indicates that juice is losing its brightness and lightness and changes its colour from initial yellow to redness and found that this change might be due to non- enzymatic browning, degradation of ascorbic acid upon storage which leads to colour and overall change in quality as storage period increases. Apart from that, surrounding temperature, lack of oxygen barrier properties of packaging materials and storage conditions may leads to change in colour, which indicates the spoilage of juice due to microbial activity.

5.2.4 Total soluble solids

The total soluble solids of fruit juices indicate the maturity of fruits being processed for juice extraction. Figure 10(a) and 10(b) shows the total soluble solid content of sweet orange juice with different preservation methods, packaging materials and storage conditions. At the initial stage of storage period the total soluble solid content in fresh sweet orange juice was found to be 10.9 and the similar findings of 10.10 was found by Shahanwaz *et al*. (2013). The TSS of 10.20 and 10.0 °Brix in orange juice containing pulp and without pulp was observed by Chanes *et al.* (2009). It was found that as the storage period increases the decrease in total soluble solids was observed in all the juice samples with respect to preservation methods. The total soluble solid content in T1 to T12 was found to be ranged from 11.11 to 11.23 °Brix respectively and during the end of the storage, the decrease in TSS from 5.58 to 6.74 °Brix in T1 to T12 was observed in

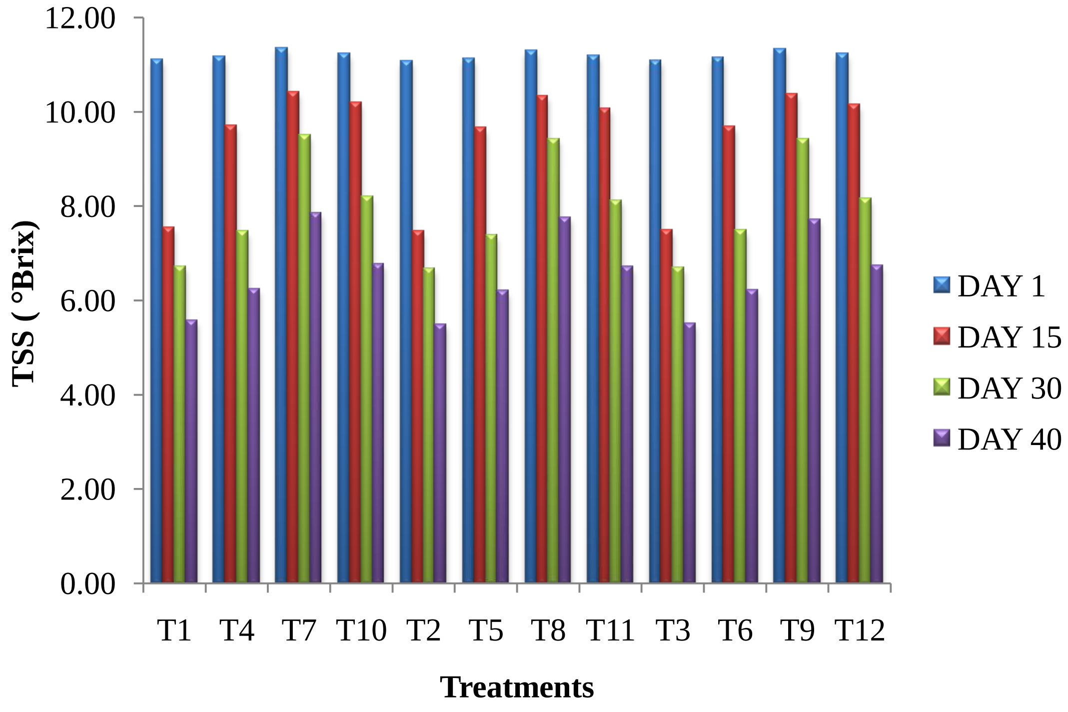

Fig. 10(a) Effect of different preservation methods and packaging materials on total soluble solids content of sweet orange juice stored at ambient condition (30±2 °C)

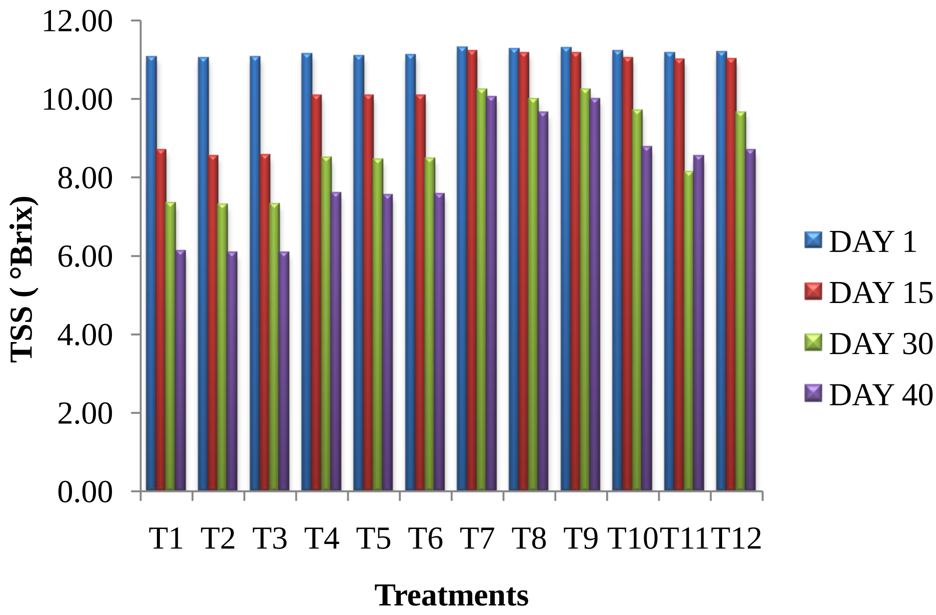

Fig. 10(b) Effect of different preservation methods and packaging materials on total soluble solids content of sweet orange juice stored at refrigerated condition (4±1 °C)

all the samples with respect to different preservation methods and packaging materials and the maximum retainment of TSS was found to be noticed in T7 from 11.36 to 7.86 °Brix followed by 11.24 to 6.78 °Brix in T10 and 11.11 to 6.25 °Brix in T4 with a minimum TSS of 11.13 to 6.20 °Brix in T5, 11.36 to 7.86 °Brix in T7 and 11.20 to 6.71 °Brix in T11 followed by 11.12 to 6.23 °Brix in T6, 11.34 to 7.72 °Brix in T9 and 11.23 to 6.74 %Brix in T12, respectively. These results are confirmed by the findings of polydera *et al.* (2003) who observed the decrease in sugars and total soluble solids as storage period increases with respect to the preservation methods of orange juice whereas decrease in ascorbic acid content was observed.

Shah *et al.* (1975) mentioned that the increase in soluble solid content in the juice product upon the addition of preservatives may be due to the solubilization of fruit constituents during storage. Talasila *et al.* (2011) studied the preservation and shelf-life extension of cashew apple juice and found out the decrease in total soluble solid content of cashew apple juice might be due to the utilization of sugars by fermenting organisms leading to degradation of sugars and moreover, decrease could also be attributed to the precipitation of tannins and colloidal particles in the juice by sago. The results were in conformity with costa *et al.* (2003) who stored the juice by hot fill aseptic methods.

On the other hand from the Fig 10(b), it was observed that the total soluble solid content of 11.08 to 11.19 °Brix in T1 to T12 was observed in juice samples with different treatments, filled in packaging materials and stored at refrigeration condition of 4±1 °C .The maximum TSS content of 11.31 to 10.05 °Brix was observed in T7 followed by 11.22 to 8.76 °Brix in T10 and 11.15 to 7.60 °Brix in T2 and a minimum of 11.10 to 7.54 °Brix in T4, 11.26 to 9.64 °Brix in T8, 11.17 to 8.54 °Brix in T11, followed by 11.12 to 7.58 °Brix in T6, 11.29 to 10.00 °Brix in T9 and 11.19 to 8.69 °Brix in T12 at refrigerated condition. It was observed that less decrease in total soluble solids at lower temperature is due to the lesser activity of fermenting organisms to degrade sugars. The similar observation was found by De souza *et al*. (2004) while analyzing the stability of unpasteurized and refrigerated orange juice and found that TSS at 4 °C was 10.43 °Brix which was less compared to TSS of 10.55 and 10.54 °Brix in unpasteurized orange juice stored at 8 and 12 °C. Moreover packaging materials plays an important role in retaining the TSS of sweet orange juice and glass bottles are found to be most effective followed by tin cans and PET bottles. The variation in TSS of juice depends upon the processing conditions, temperature, pH and the mode of storage conditions adopted.

5.2.5 Ascorbic acid

Ascorbic acid also called as vitamin C is found to be an essential nutrient for humans because of its high antioxidant power which provides protection against the presence of free radicals participating and prevention of many diseases. Quality and shelf-life determination of an orange juice is strongly based on the vitamin C evolution during storage although there are other quality parameters such as colour and flavour characteristics that are also very important (Zerdin, Rooney and Vermue, 2003). The ascorbic acid content of sweet orange juice was analyzed found to be 62.5 $mg.ml^{-1}$ and the obtained value was nearer to the ascorbic acid value of fresh sweet orange juice *i.e.,* 67.75 $mg.ml^{-1}$ analyzed by Shahanwaz *et al.* (2013). This variation in ascorbic acid content in sweet orange juice may be due to be the type of extraction method adopted to extract juice, processing temperature as well as the variety of sweet oranges selected for juice extraction. In similar way Pareek *et al* .(2011) obtained the ascorbic acid content of 31.6 $mg.ml^{-1}$ in hand operated screw press juice extractor and 31.0 $mg.ml^{-1}$ in Nagpur mandarin orange juice by using power operated screw press juice extractor. During the initial day of storage period at ambient as well in refrigerated condition, the ascorbic acid in all un-treated and treated samples were found to be same from 52.60 to 52.95 $mg.ml^{-1}$ in T1 to T12, respectively and as the storage period increases the decrease in ascorbic acid level in all the samples were observed.

Fig. 11(a), 11(b) and 11(c) represents the ascorbic acid content of sweet orange juice subjected to different levels of treatments, packaging materials used as well as storage conditions of ambient and refrigeration. It was found that in un-treated juice samples, the ascorbic acid level was found to be decreased in all the samples with respect to storage period in T1 and T8 from 9.20 to 8.93 $mg.ml^{-1}$ in pasteurized sweet orange juice at 85 °C for 20 s and the values nearer to 61.50, 43.467 and 52.637 $mg.ml^{-1}$ of ascorbic acid content in glass bottle, PET bottles and in amber bottles were observed by shahanwaz *el al.* (2013) and at the end of the storage period *i.e.,* at the 45th day of storage 11.22, 11.29 and 7.30 $mg.ml^{-1}$ of ascorbic acid content were observed in all the un-treated pasteurized sweet orange juice samples filled in glass, PET and amber bottles. This loss in ascorbic acid upon application of heat treatment is due to the sensitivity of vitamin C when exposed to pasteurization temperature.

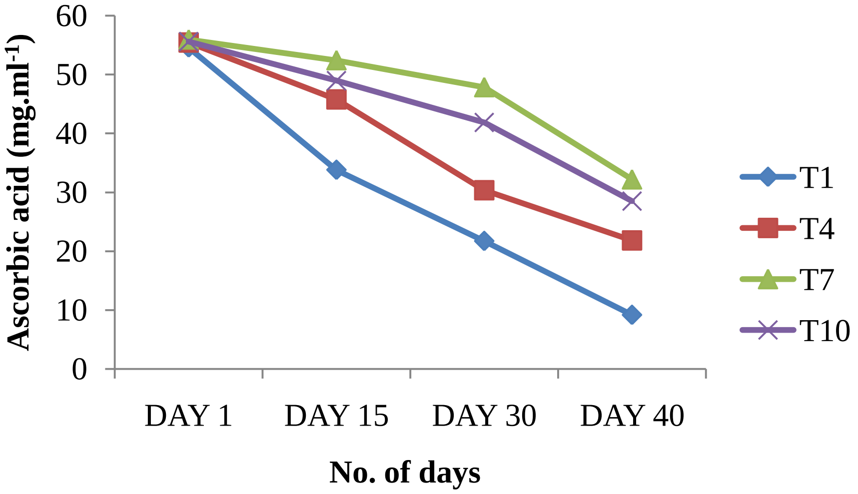

Fig. 11(a)

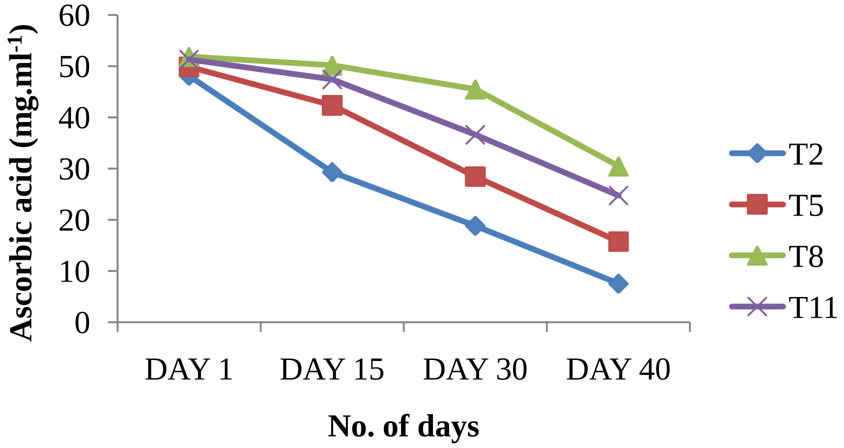

Fig. 11(b)

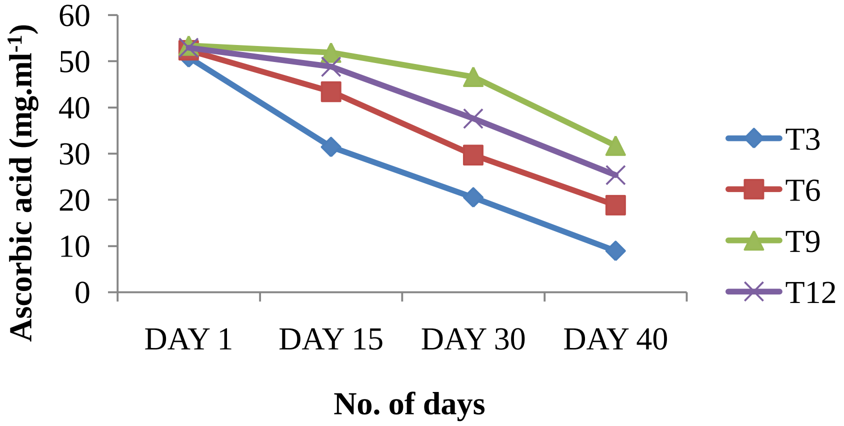

Fig. 11(c)

Fig. 11(a), 11(b) and 11(c) Effect of different preservation method on ascorbic acid content of sweet orange juice filled in glass bottle, PET bottles and tin cans and stored at ambient condition (30±2 °C)

Timmermans *et al.* (2011) analyzed that the thermal pasteurization temperature of 90 °C for 1 minute is needed for the inactivation of heat-stable pectinmethylesterase enzyme activity for preventing cloud loss and on the other hand thermal processing have negative impact on the quality of orange juice such as loss of freshness, flavor, degradation of ascorbic acid and discolouration. To avoid such quality loss of orange juice by adopting advanced technologies like thermal processing, non thermal processing techniques such as high pressure and pulsed electric field processing etc. Tannenbuam *et al.* (1985) found that as the storage period increases the decomposition of ascorbic acid together with non enzymatic browning are the main causative factors for deteriorative reactions and moreover the factors like pH, oxygen, enzymes, light, metal catalysts, initial ion of ascorbic acid, the ratio of ascorbic acid to dehydroascorbic acid, microbial load and protection provided by the container are also responsible in loss of ascorbic acid.

In homogenized juice samples at 5000 psi, the ascorbic acid content of 55.42 to 21.84 $mg.ml^{-1}$ was observed in T4 followed by 49.90 to 15.76 $mg.ml^{-1}$ and 52.36 to 18.84 in T5, T6 at ambient condition, however the same amount of ascorbic acid was observed in T4, T5 and T7 at refrigerated condition (4±1 °C), during initial storage period presented in 11(d), 11(e) and 11(f), but less degradation and more retention of ascorbic acid content was observed compared to that in samples stored at ambient condition. However, Maresca *et al.* (2011) observed that as the number of passes from 1 to 5 and inlet temperature of 2 to 20 °C with a homogenization pressure of 50 to 250 MPa was found to be effective in retaining all the organoleptic characteristics of orange juice by inactivating the pectinmethylesterase enzyme activity along with suitable refrigeration condition of 8 to 4 °C was sufficient enough to prolong the shelf-life of orange juice by reducing the microbial load.

On the other hand sweet orange juice preserved with sodium benzoate in treatments T7, T8 and T9, the less degradation of ascorbic acid was found compared to that in T10, T11 and T12 both at ambient and refrigerated condition, although bacteriocin nisin, produced by lactic acid bacteria was found to be more effective in retaining ascorbic acid content but less effective when compared to that of sodium benzoate at 0.5 $mg.ml^{-1}$, this is because sodium benzoate upon addition to the sweet orange juice showed broad antimicrobial activity followed by wide non-volatility and water solubility in preserving fruit beverages. This antimicrobial activity of preservatives is due to the undissociated acid ion which declines as the pH level increases (Walker and Philiphs,

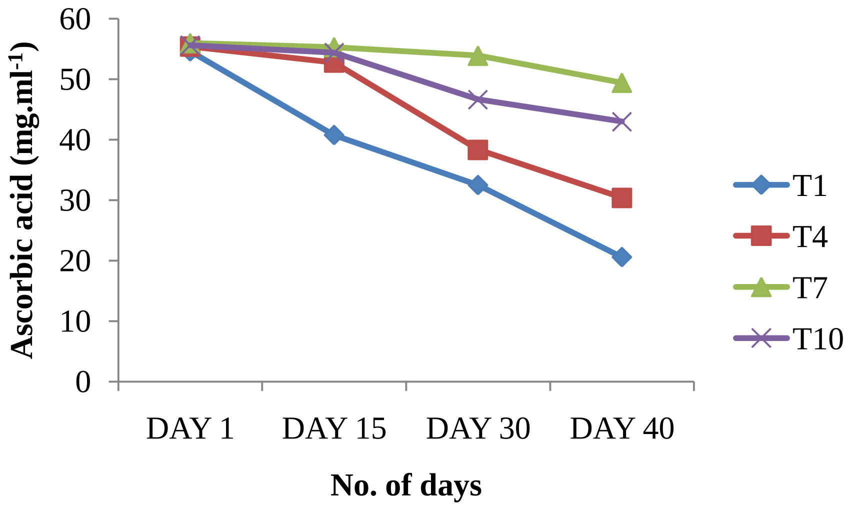

Fig. 11(d)

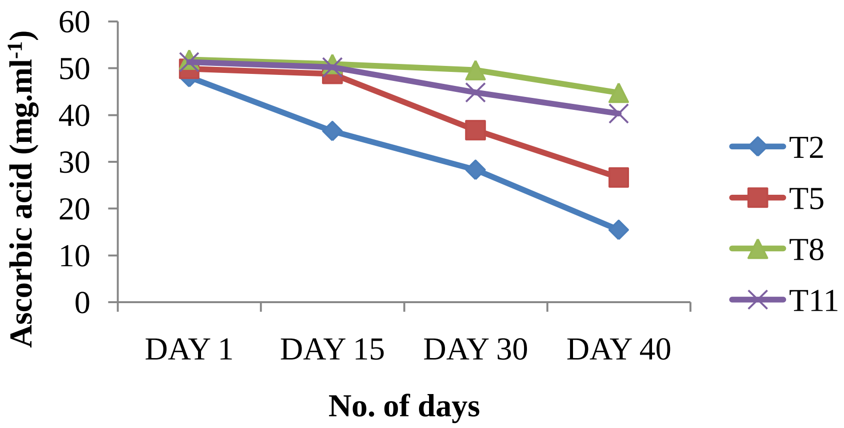

Fig. 11(e)

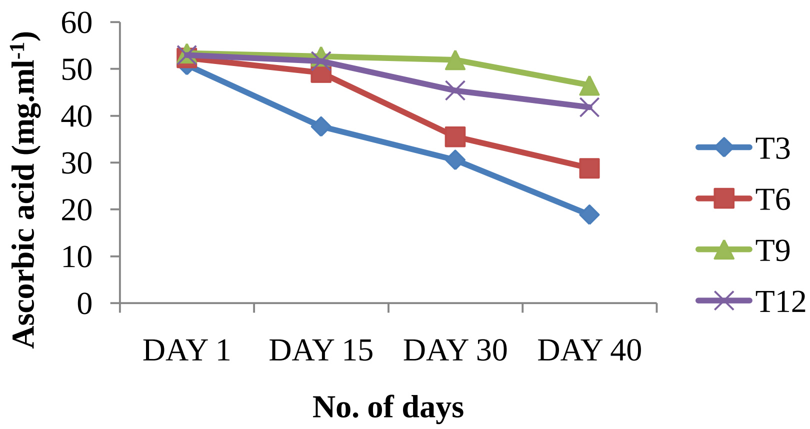

Fig. 11(f)

Fig 11(d), 11(e) and 11(f) Effect of different preservation methods on ascorbic acid content of sweet orange juice filled in glass bottles, PET bottles and tin cans at refrigerated condition (4±1 °C)

2008). Chumillas *et al*. (2007) observed that the orange juice aseptically packaged in monolayer PET bottles presented a poor retention of ascorbic acid as well as shorter shelf-life than juice bottled in glass or multilayer PET bottles. However, the PET bottling had an additive effect on ascorbic acid retention and can extend juice shelf-life to that provided by glass and multilayer PET bottles. Similarly, Matooko *et al*. (2002) suggested to pack juices in metal cans, glass or plastic containers as a technological practice of preservation and distribution. Although metal cans are expensive and required sophisticated machinery for container closure was found to be effective in preserving ascorbic acid in canned lime juice and Shahanwaz *et al*. (2013) observed good retention of ascorbic acid in amber bottles followed by glass and PET bottles for sweet orange juice. Esteve *et al*. (2005) studied the chemical, physical composition and colour in Spanish refrigerated orange juices and noted that at 4 °C the ascorbic acid can be retained and shelf-life of Spanish orange juice up to 42 days can be extended followed by 35 days at 10 °C, as ascorbic acid content is the major indicator in predicting the orange juice shelf-life.

5.2.6 Browning index

The effect of different preservation methods and packaging materials on browning index of juice stored at ambient and refrigerated condition was analyzed and found the browning index of freshly extracted sweet orange juice was 0.140%. Pareek *et al*. (2010) determined the juice browning index of Nagpur mandarin juice with different extraction methods and processing conditions during storage and observed the browning index of 0.13% in Nagpur orange juice and they found out different browning index values of 0.13% in both manual and power operated screw press juice extraction and they also observed as the storage period increase, the simultaneous increase in browning index value. Similarly, the juice browning index of 0.15% was observed by Emamifar *et al.* (2010). From Fig. 12(a), 12(b) and 12(c) the browning reactions in un-treated samples and as well in treated samples stored at ambient condition with respect to different packaging materials and preservation methods were observed. In T1, T2 and T3, the browning index values of 0.214, 0.220 and 0.218% was observed and found to be more compared to that in the fresh juice samples and the results similar with the findings of Pareek *et al.* (2011), where the browning index of 0.15 to 0.32% was observed with respect to the pasteurization temperature of 85 °C for 5 min, followed by 0.15 to 0.33% at 85 °C for 15 minutes during 1 to 6 months of storage period. This increase in browning

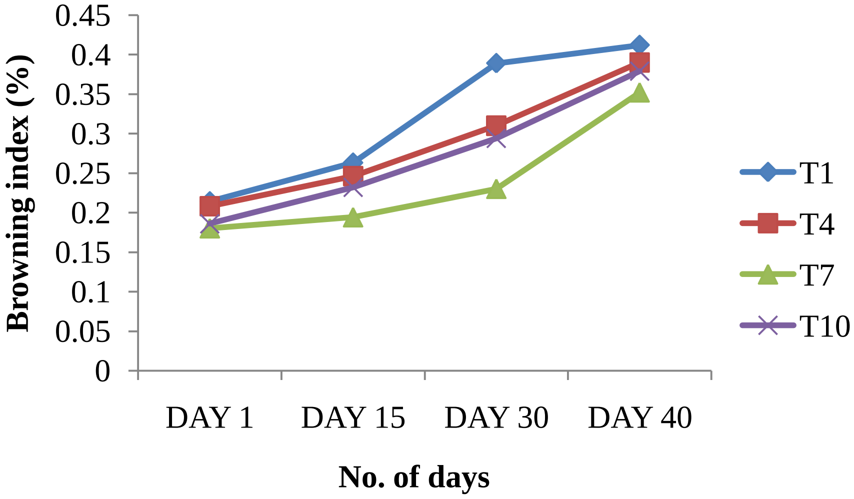

Fig. 12(a)

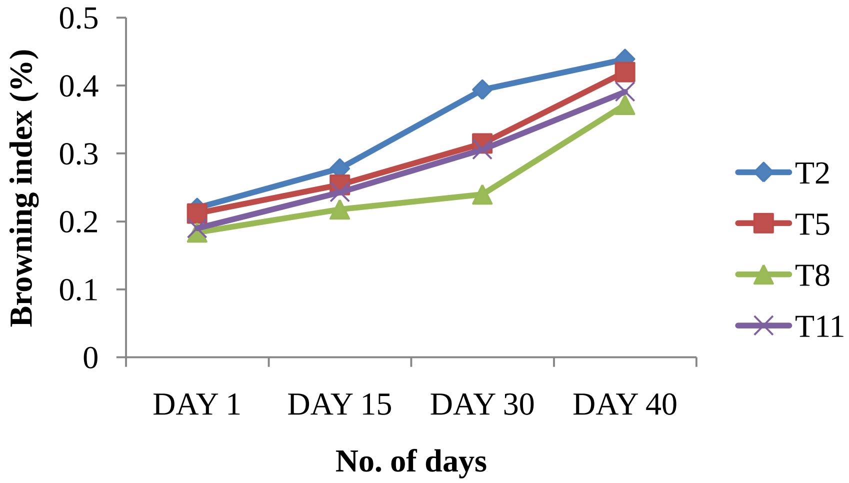

Fig. 12(b)

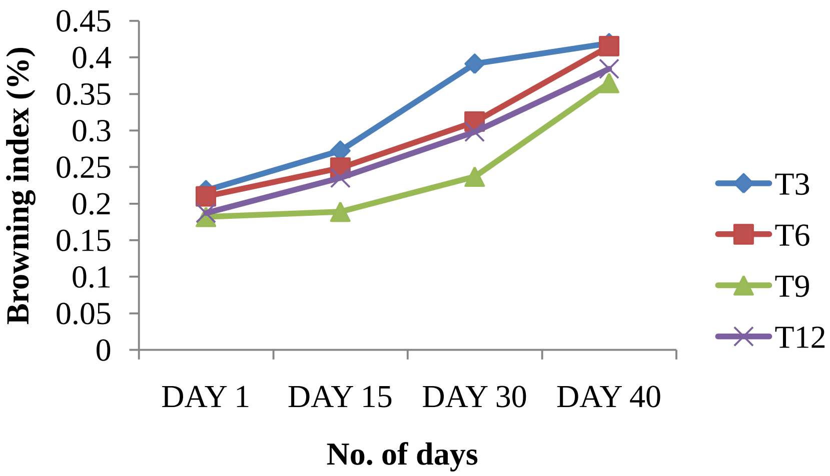

Fig. 12(c)

Fig. 12(a), 12(b) and 12(c) Effect of different preservation methods on browning index of sweet orange juice filled in glass bottles, PET bottles and tin cans and stored at ambient condition(30±2 °C)

index upon storage is due to the reactions involved between ∝-amino groups and reducing sugars which lead to caramelization or Millard reaction at high temperature treatments.

The aerobic and anaerobic degradeation of the ascorbic acid and further reactions of the carbonyl compounds via aldol condensation or reactions with the amino acids to yield brown pigments (Fustier *et al.,* 2011). In T4, T5 and T6 in homogenized juice samples at 5000 psi pressure showed decrease in browning index from 0.208 to0.390% followed by 0.212 to 0.420% and 0.210 to 0.415% was observed. This is because at high pressure of homogenization, the pectinmethyl esterase activity was reduced and thus lower enzymatic browning was observed in sweet orange juice along with pasteurization temperatures (Chanes *et al.,* 2009) and also causes minimal quality losses in orange juice compared to that of thermal treatment.

Burdur and Karadeniz (2003) noted the juice browning index of 0.278 to 0.321% in golden delicious apple variety during 16 weeks of storage period. In juice samples preserved with sodium benzoate in T7, T8 and T9 the browning index increased from 0.180 to 0.352% followed by 0.184 to 0.372% and 0.182 to 0.365%, respectively as storage period increases. Adeola *et al.* (2013) observed the browning index of tamarind beverage where at the initial stage the browning index was 0.190 to 0.20% and was reduced to 0.142 to 0.162 % upon the addition of sodium benzoate 100 $mg.ml^{-1}$. It was found to be more effective in reducing the microbial activity and also less amino acids and reducing sugars were available for the enzymes and microorganisms to multiply and to bring about undesirable colour changes up to storage period. On the other hand juice samples preserved with nisin showed more or less effective in inhibiting the pectin methylesterase enzyme activity that brought browning reaction when added at a level of $0.001mg.ml^{-1}$, however sodium benzoate because of its antimicrobial activity stabilizes the browning reaction in juices upon storage. The browning index of juice may also due to the formation of hydroxymethyl furfural and other browning pigments. They also observed that the effect of amino acids on browning of single strength orange juice was linear with concentration and found to be more pronounced in the presence of high levels of ascorbic acid. Jain and Khurdiya (2009) noticed the non-enzymatic browning in anola juice during the 6 months storage and also reported that it was minimized with sulphitation and low temperature storage. Zhang *et al.* (2008) observed the non-enzymatic browning in apple juice stored for 30 days was due to the total phenolic contents and oxidation reactions.

Fig. 12(d), 12(e) and 12(f) shows the browning reaction in sweet orange juice samples with different preservation methods, packaging materials and stored

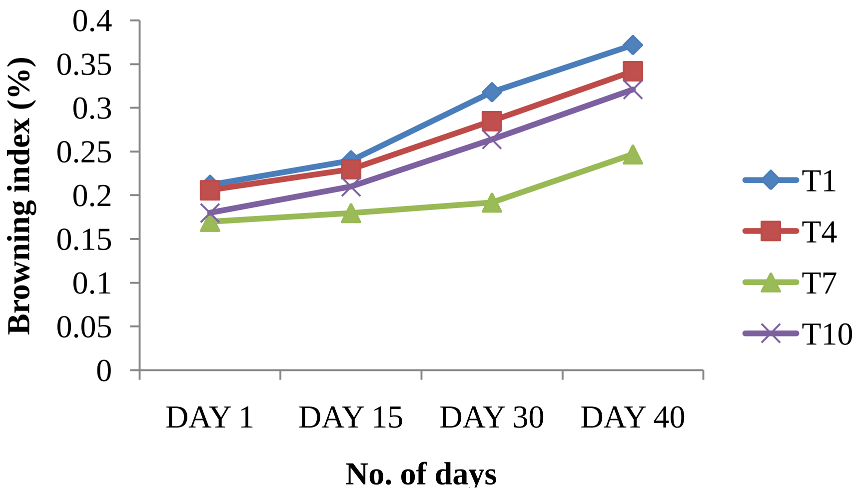

Fig. 12(d)

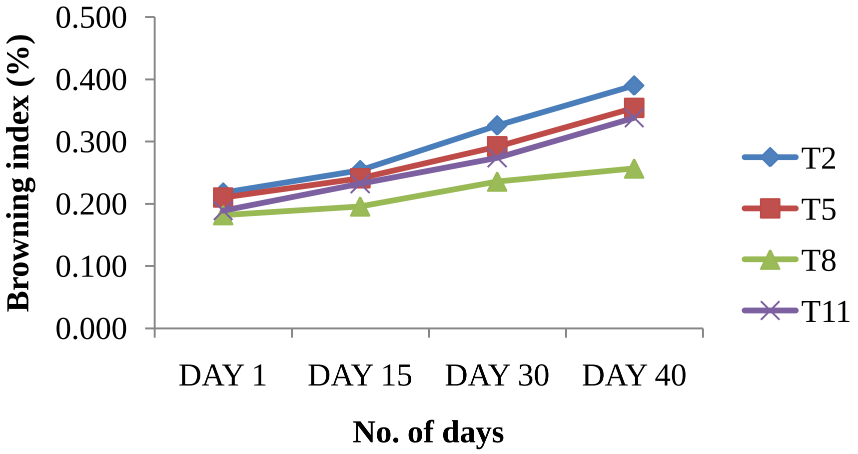

Fig. 12(e)

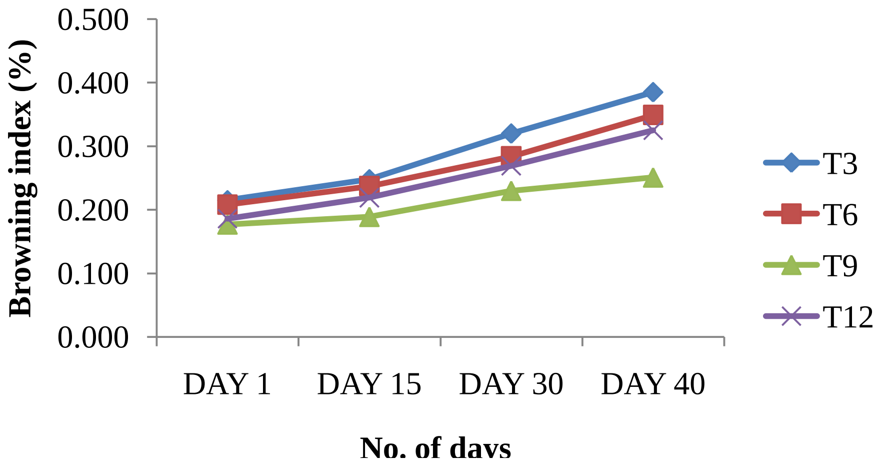

Fig. 12(f)

Fig. 12(d), 12(e) and 12(f) Effect of different preservation methods on browning index of sweet orange juice filled in glass bottles, PET bottles and tin cans and stored at refrigerated condition (4±1 °C)

at refrigerated condition. It was observed that in all the samples the browning index was found to be less compared to the samples stored at ambient condition. Fustier *et al.* (2011) investigated the non-enzymatic browning and ascorbic acid degradation of orange juice subjected to electro-reduction and electro oxidation treatments stored at 4, 21 and 37 °C and found that both the electro-reduced and control juice variants exhibited little but evident changes was observed during the 4 months storage at 21 °C. By increasing the levels of dissolved oxygen in the juice by electrolysis caused a substantial increase in the development of browning reactions, due to additional incorporation of dissolved oxygen in the juice and these reactions were temperature dependent.

The juice samples filled in glass bottles showed less browning reaction followed by tin cans and PET bottles. This is due to the faster degradation of browning pigments in packaging materials and also due to lack of oxygen barrier properties. Emamifar *et al.(*2011) observed the values of browning index in fresh orange juice immediately after packaging were measured at 0.15%. However, Leizerson and Shimoni (2005) reported that increased values of browning index by up to 0.367% are still invisible. Increasing temperature has a major effect on the increased rate of browning reaction in fruit juice. A significant decrease is observed in the ascorbic acid content of all the experimental packages during storage at 4 °C while the browning index increased significantly in all the test packages. This overall ascorbic acid reduction might be due to the non-barrier properties of packaging against oxygen and the duration of storage.

Berlinet *et al* (2003) used glass packages and PET bottles for storing orange juice and found that browning index in these samples were influenced by storage conditions, light and oxygen and showed permeability of PET bottles to oxygen and is the factor that detrimentally affects the vitamin C, colour and browning index of orange juice. In comparison with standard PET, multilayer or internal carbon coating PETs with good oxygen barrier properties showed better vitamin C contents after 3 months of storage. Flavour compounds, as well as vitamin C, play a major role on quality of orange juice. Future studies will investigate the influence of plastic packaging on the preservation of organoleptic quality during shelf-life.

5.2.7 Total carotenoids

The total carotenoids content of sweet orange juice was analyzed and found to be 37.5 mg.ml^{-1} and the observation similar to the readings of 34.25±5.09 mg.ml^{-1} found by

Plaza *et al.* (2011). The citrus fruits and their products in general are a complex source of carotenoid pigments, with the largest number of them reported for any fruit (Martinez *et al.*, 2007). Fig. 13(a), 13(b) and 13(c) details about the carotenoids content in sweet orange juice and its degradation as the storage period increases. In T1, the degradation of carotenoids content in sweet orange juice filled in glass bottles, PET bottles and tin cans were observed and found to be in the range of 32.70 to 18.85 $mg.ml^{-1}$ followed by 34.89 to 19.41 $mg.ml^{-1}$, 35.21 to 26.40 $mg.ml^{-1}$ in T4, T7 and T10, respectively. In T2, the carotenoid degradation was from 30.42 to 16.30 $mg.ml^{-1}$followed by 31.84 to 17.58 $mg.l^{-1}$, 33.88 to 24.49 $mg.l^{-1}$ and 33.88 to 24.49 $mg.l^{-1}$ and on the other hand in T3, the carotenoids of 32.51 to 17.42 was observed followed by 32.90 to 18.86, 34.80 to 25.76 and 33.90 to 23.14 in T6, T9 and T12, respectively. This fluctuation in increase and decrease in carotenoid content was due to the different treatments adopted as well as storage conditions. The faster degradation of carotenoids content was observed in all the samples is due to geometric isomerization of these compounds promoted by light, heat, and acids and oxidation stimulated by light, heat, metals, enzymes, peroxides and inhibited by antioxidants and different packaging materials responds to one of these oxidation process in degradation of carotenoids in sweet orange juice. On the other hand sweet orange juice with different treatments, filled in glass PET and tin cans stored at refrigerated condition in 13(d), 13(e) and 13(f) showed lesser degradation of carotenoids compared to that of sweet orange juice samples stored at refrigeration condition. Choi, Kim and Lee (2000) reported orange juice fortified with ascorbic acid showed lower lossess of total carotenoids than in un-treated juice samples after 7 weeks of storage at 4.5 °C in treatments like low pressure temperature (LPT), High pressure processing (HP) and pulsed electric field (PEF) and the carotenoid content of 16.90, 21.45 and 26.46% were observed in these samples, after 40 days of storage at 4 °C, being the processes. The obtained results are in agreement with those reported by Bull *et al.* (2004) for beta carotene content which did not significantly decrease during storage at 4 °C in high pressure homogenization at 600 MPa at 20 °C or thermal pasteurization of 85 °C at 25s in treated orange juice.

Sheikha *et al.*(2009) analyzed the quality of physalis juice packed in glass bottles and flexible laminated packs during the storage at 5 °C and observed the significant decrease in carotenoid composition in juice samples filled in flexible laminated packs and

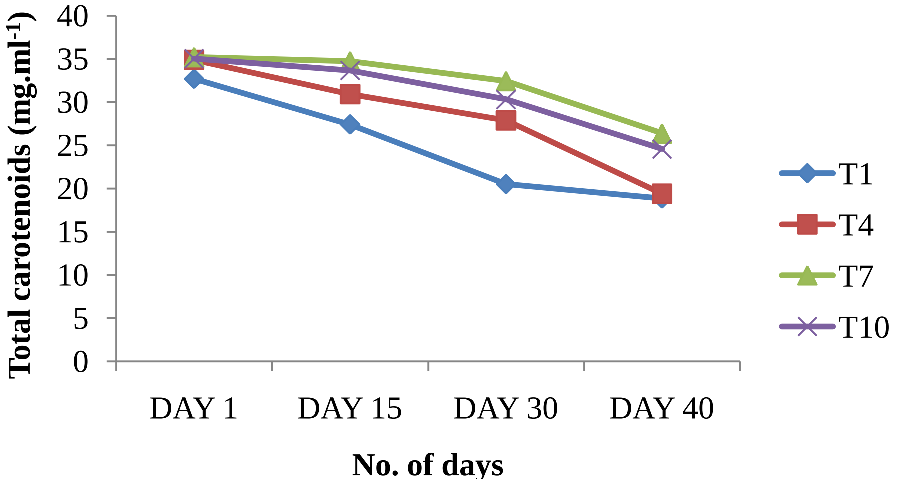

Fig. 13(a)

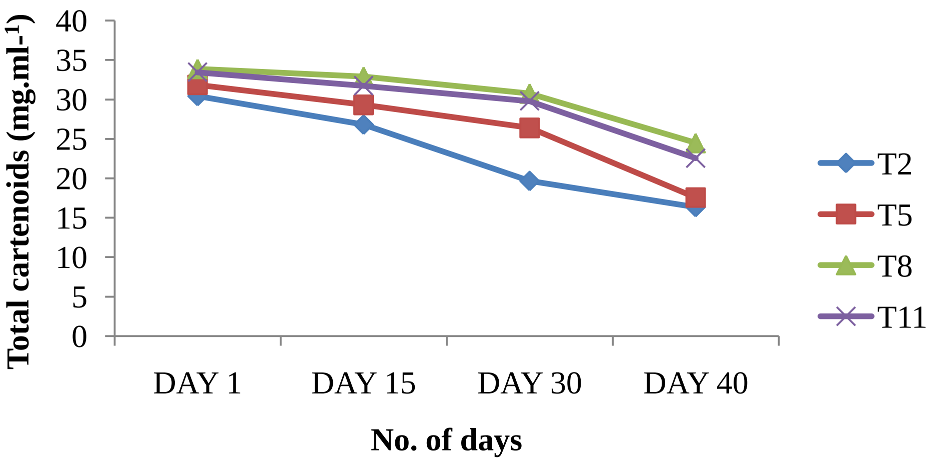

Fig. 13(b)

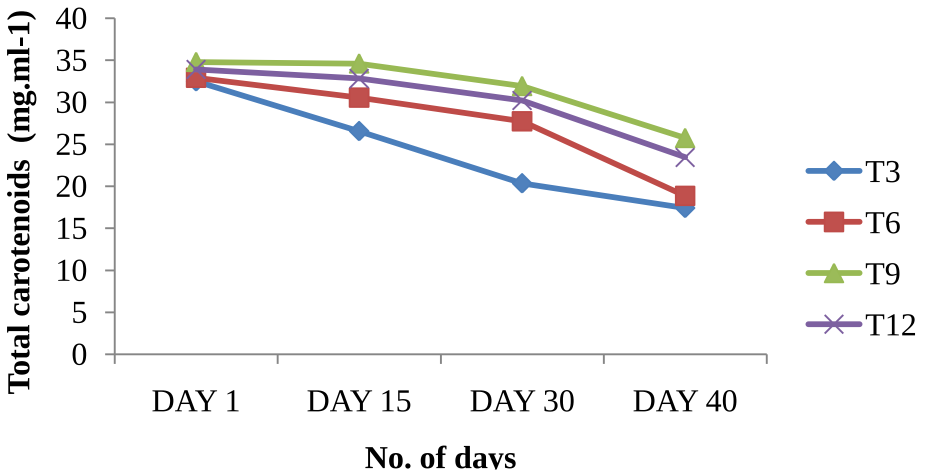

Fig. 13(c)

Fig. 13(a), 13(b) and 13(c) Effect of different preservation methods on total carotenoids of sweet orange juice filled in glass bottles, PET bottles and tin cans and stored at ambient condition (30±2 °C)

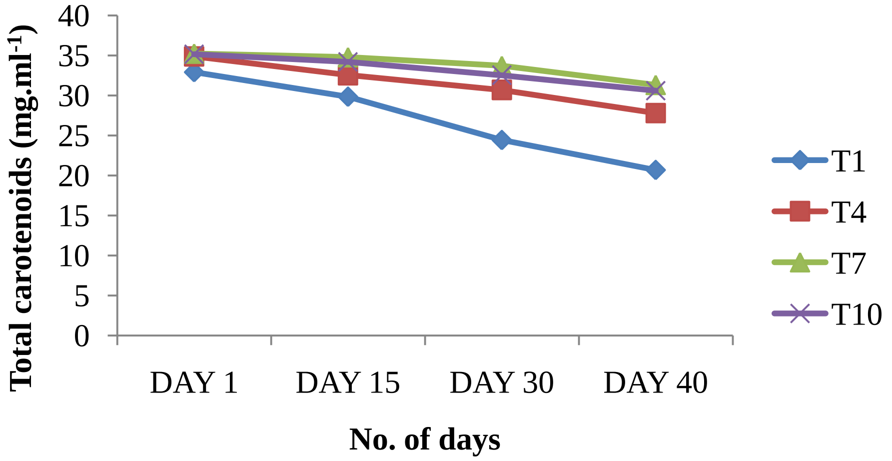

Fig. 13(d)

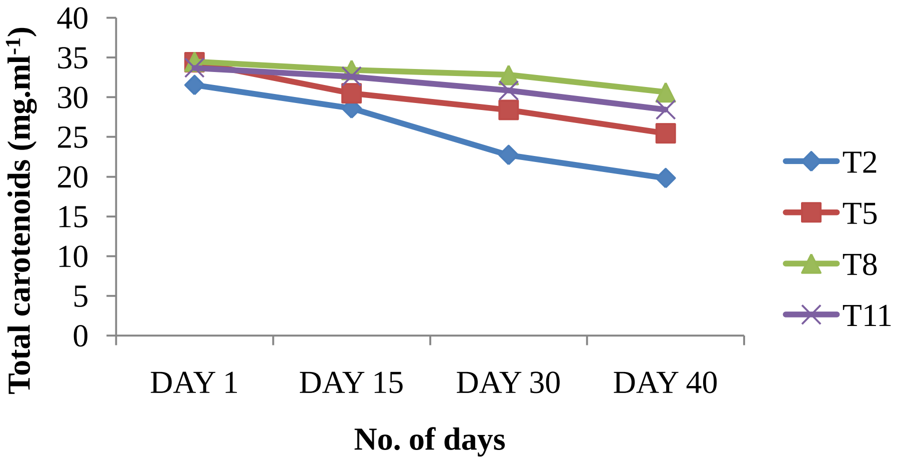

Fig. 13(e)

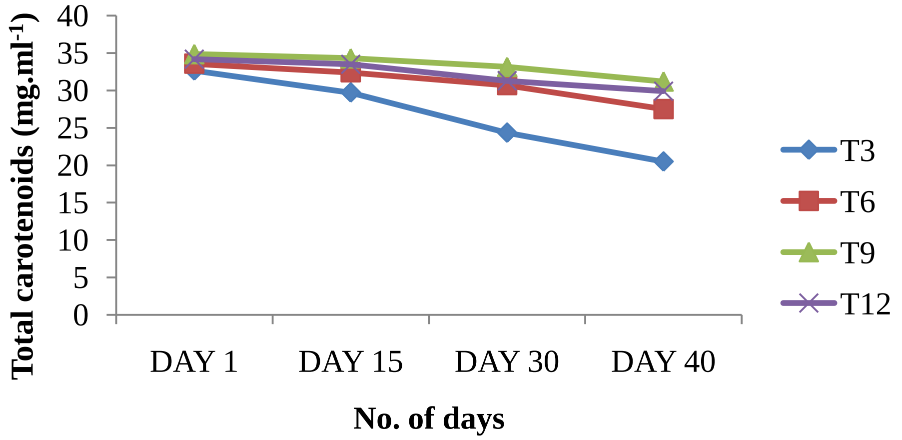

Fig. 13(f)

Fig. 13(d), 13(e) and 13(f) Effect of different preservation methods on total carotenoids content of sweet orange juice filled in glass bottles, PET bottles and tin cans and stored at refrigerated condition (4±1 °C)

retention of carotenoids content and over all nutrient values in glass bottles over a period of 6 months storage at 5±1 °C at a relative humidity of 85-90%.

5.2.8 Total Flavonoids

Flavanones are the secondary plant metabolites, mainly present in tissues as glycosidesin. The effect of different preservation methods and packaging materials on total flavonoids of sweet orange juice was analyzed at ambient and refrigerated condition and in fresh sweet orange juice, the total flavonoids of 24.6 $mg.ml^{-1}$ was recorded. It was observed from the Fig. 14(a), Fig 14(b) and Fig. 14(c) in sweet orange juice with different preservation methods, filled in glass bottles, PET bottles and in tin cans, stored at ambient condition, the decrease in total flavonoids content was noted from initial to 40^{th} days of storage period. In T1, T2 and T3 in un-treated juice samples, the decrease in total flavonoids from 22.60 to 3.92 $mg.ml^{-1}$ was observed, this is due to the pasteurization temperature effect on total flavonoids of sweet orange juice, where as in homogenized sweet orange juice samples at 5000 psi in T4, T5 and T6, the flavonoid content of 23.05, 21.86 and 22.71$mg.ml^{-1}$ was observed and the similar findings of increase in total flavonoids upon high pressure processing of orange juice at 400 MPa at 40 °C for 1 m was observed by Plaza *et al.*, (2011) which is due to the extraction stability of flavonones at High pressure temperature treatment retained similar levels of flavonoids than those of untreated orange juice. On the other hand, the sweet orange juice preserved with sodium benzoate at 0.5 $mg.ml^{-1}$ showed the total flavonoids of 23.60 to 23.52 during the initial storage period and as the storage period it decreased from 12.85 to 11.46 $mg.ml^{-1}$ and in T10, 11 and T12 and in sweet orange juice preserved with nisin showed total flavonoids of 23.45 to 22.94 $mg.ml^{-1}$ during initial period and gradually decreased from 8.72 to 7.19 at 40 days of storage. This shows that sodium benzoate has potent free radical scavenging activity especially potent hydroxyl radicals and can scavenge noxious radicals generated during storage (Sarkar *et al.*, 2014) which was found to be less in biopreservatives. It is known that packaging materials influences the quality of liquid foods during storage, due to the absorption of flavour compounds or permeation through packaging materials. In addition, degradation of flavour, colour, and nutrients also occurs by oxygen transmission through packages (Ayhan *et al.* 2001) and among packaging materials glass bottles retained total flavonoids is due to the better retention capacity of flavour components and less oxygen transmission rate in to the package that cause quality losses, than other packaging materials like Pet bottles and tin cans.

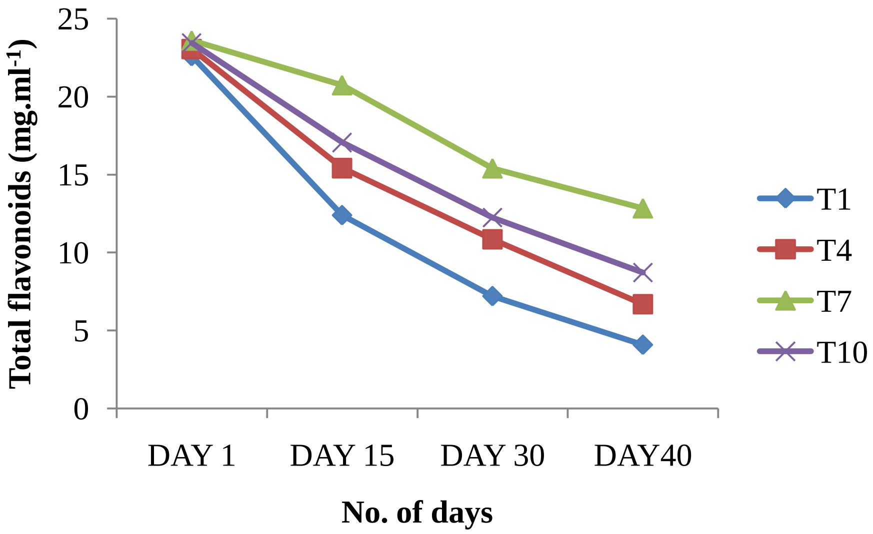

Fig. 14(a)

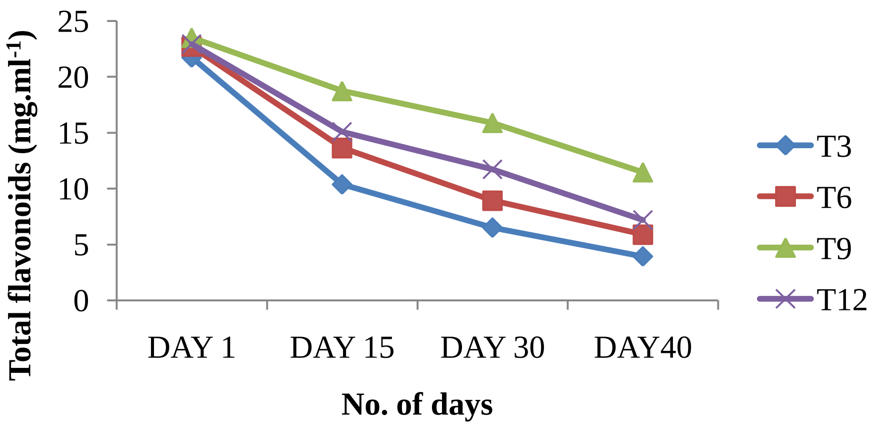

Fig. 14(b)

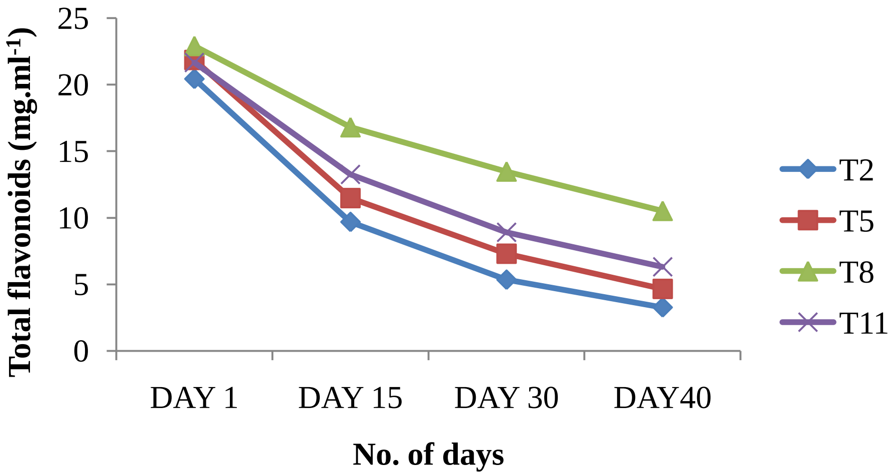

Fig. 14(c)

Fig. 14(a), 14(b) and 14(c) E[illegible]t preservation methods on total flavonoids content of sweet orange juice filled in glass bottles, PET bottles and tin cans and stored at ambient condition (30±2 °C)

From Fig 14(d), Fig 14(e) and Fig 14(f), the less decrease in total flavonoids were observed in all the samples filled in glass, PET bottles and tin cans, stored at refrigerated condition (4±1 °C) was found to be effective in retaining the total flavonoids content at lower temperature, but as the storage period increases the decrease in total flavonoids content was noticed and this observation was found true with Yeom *et al.* (2000) who reported that the decrease in total flavonone content in HP juice and at the end of the 40th day the decrease in total flavonoid was found similar to those of low pressure treatment, PFE and untreated juice might be due to the degradation of phenolic compounds during storage which is mainly related to the residual activity of polyphenol oxidase and peroxidase.

5.3 Effect of preservation methods, packaging materials and storage conditions on sensory properties of freshly processed sweet orange juice

The freshly processed sweet orange juice with different preservation methods, filled in different packaging materials and stored both at ambient and refrigerated condition were analyzed for organoleptic properties like colour and appearance, sweetness, sourness, aroma, flavor and overall acceptability and the results were discussed in Chapter III (Sec 4.4) and represent in Fig. 15 (a) and Fig. 15(b). From the results it was observed that the sweet orange juice stored at refrigerated condition (4±1 °C) maintained the overall quality after processing and the score of 9 was obtained for T7 *i.e.,* in sweet orange juice sample filled in glass bottles and stored at refrigerated condition when compared to that of the samples stored at ambient condition with different treatments. Through sensory properties it was observed that the sweet orange juice preserved with sodium benzoate, filled in glass bottles were found to be best at refrigerated condition and the similar observation were obtained by Shahanwaz *et al.* (2013) for sensory properties of orange juice filled in glass bottles followed by PET and amber bottles were best when preserved with sodium benzoate. This is because sodium benzoate being a chemical preservative helps in suppressing the enzymatic activity upon the overall quality of sweet orange juice, by preserving its overall organoleptic properties and due to its antimicrobial properties it prevents the action of spoilage causing enzymes to grow and degrade the over properties of sweet orange juice kept over a period of time. On the other hand homogenized sweet orange juice samples at 5000 psi in T4 to T6 and sweet orange juice preserved with nisin in the treatments T9 to T12 represents the same

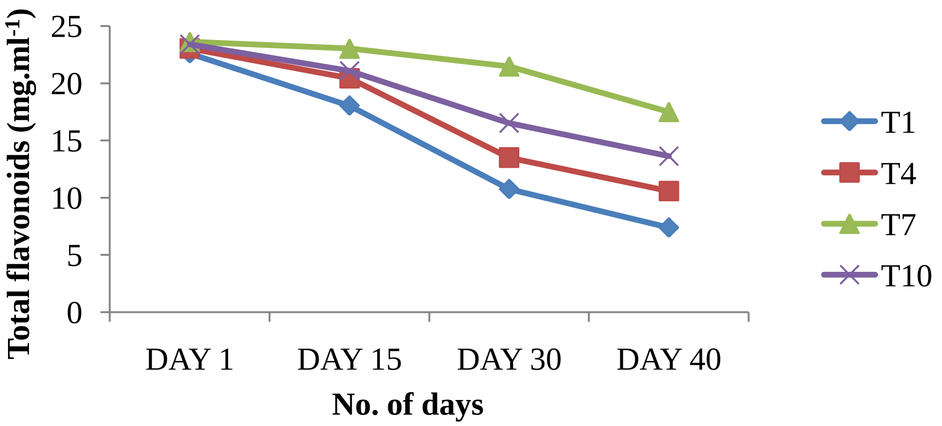

Fig. 14(d)

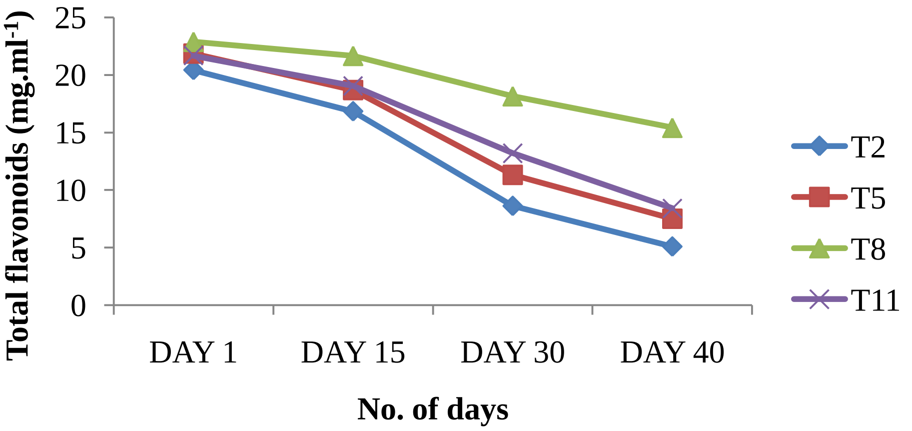

Fig. 14(e)

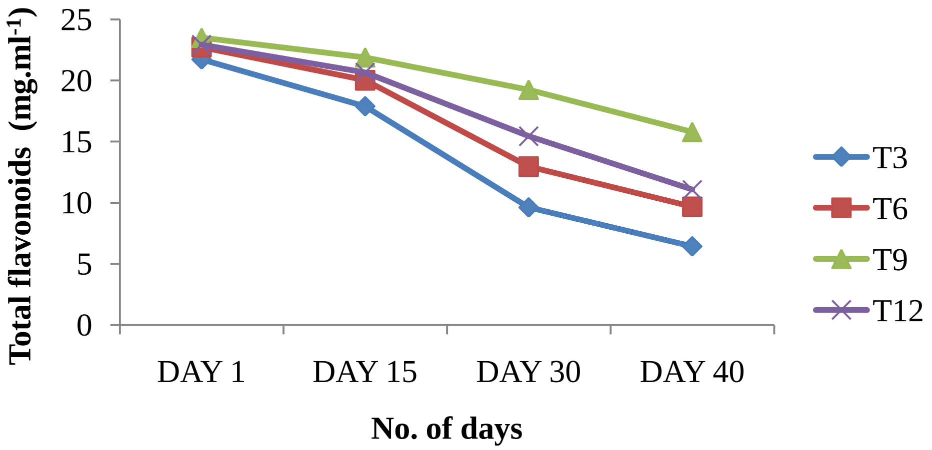

Fig. 14(f)

Fig. 14(d), 14(e) and 14(f) Effect of different preservation methods on total flavonoids content of sweet orange juice filled in glass bottles, PET bottles and tin cans and stored at refrigerated condition (4±1 °C)

organoleptic properties over chemical preservatives and through this it was observed that juice preserved with nisin and homogenized juice samples also more or less retains the overall nutritional properties to some extent but not as much effective compared to the chemical preservative. Sensory evaluation of orange juice after exposure to thermosonication and pulsed electric field was done by Ribeiro *et al.* (2009) and they found that pulsed electric field processed orange juice were not rated in terms of overall acceptability, colour, appearance, texture and flavour to that of the freshly squeezed orange juice and found to differ significantly from juice exposed to thermal treatments, which showed better texture, flavour and overall acceptability. These results were found to be similar with the score obtained by Fustier *et al*. (2011), while analyzing the sensory properties of orange juice subjected to electro reduction and electro-oxidation treatments and they found that juice samples stored at refrigerated condition of 4 °C retained all the sensory properties over a period of 6 months of storage compared to that of the samples stored at ambient condition and they suggested that through electro reduction favours in prolongation of orange juice shelf-life. Fabroni *et al* .(2010) analyzed the sensory properties of blood orange juice during 0, 5, 10, 15, 20, 25 and 30^{th} days of refrigeration storage subjected to supercritical carbon dioxide treatment, as a new product in the fresh fruit juice market and found that the freshness, flavour, intensity of taste and intensity of scent decreased, while off-flavour increased significantly after 25 days, showing sensory decay of ($TJ_{co2)}$ supercritical carbon dioxide treated orange juice and observed that high pressure supercritical carbon dioxide treated blood orange juice processed at 130 bar, 36±1 °C, 0.385 g_{co2}/g_{juice} is best before 20 days of refrigeration storage.

The sensory evaluation and shelf-life assessment of fresh orange juice packed in nano composite packaging containing Ag and ZnO was done by Emamifar *et al.* (2010). Through sensory panelists, they recognized low density polyethylene (LDPE) + 1% nano-ZnO film followed by LDPE + 5% P105 and LDPE + 1.5% P105 as the best packaging materials in terms of overall acceptability and they found that the change in flavour during storage is not only due to the growth of microorganisms but also due to heating, storage time and the common chemical interactions that occur in stored juices. On the other hand Chumillas *et al.* (2007) evaluated that there were no significant differences observed in sensory properties of orange juice samples filled in glass, monolayer and

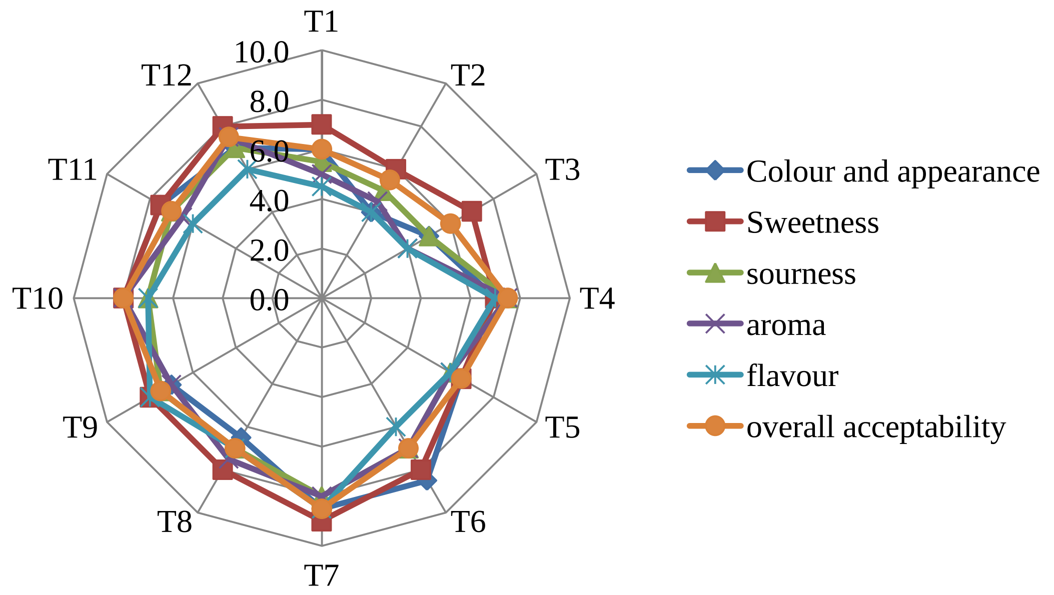

Fig 15(a). Effect of different preservation methods and packaging materials on sensory score of sweet orange juice stored at ambient condition (30±2 °C)

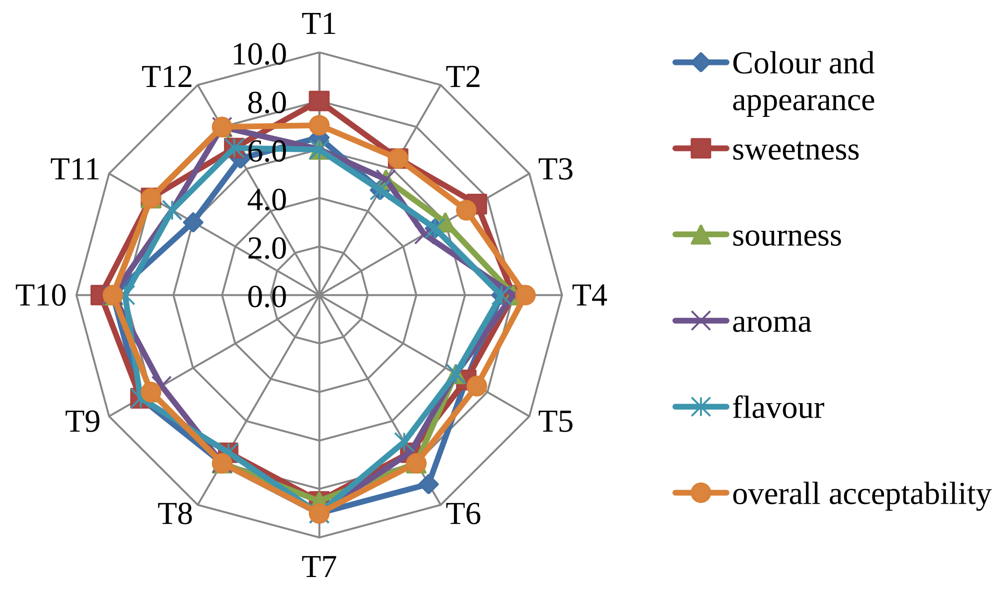

Fig. 15(a) Effect of different preservation methods and packaging materials on sensory score of sweet orange juice stored at refrigerated condition (4±1 °C)

multi-layer PET bottles with or without oxygen scavenger and liquid nitrogen drop during the initial period of storage at 4 and 25°C.

5.4 Enumeration of microbial population in sweet orange juice with different treatments and storage conditions

5.4.1 Total Plate Count (TPC)

The effect of different preservation methods, packaging materials and storage conditions on the microbial shelf-life of sweet orange juice was assessed at ambient condition and presented in Fig 16(a), Fig 16(b) and Fig 16(c). It was observed that as the storage period proceeds, the activity of microorganisms will also increases and the maximum anticipated and permitted count of TPC for juices according to European Union Standards (European Union Standards, 2005) should not be more than 100 $cfu.ml^{-1}$ .The maximum colony forming unit of 76×10^{3} $cfu.ml^{-1}$ was observed in (T1) and among the treatments, the total plate count of 0 to $70\times10^{3}cfu.ml^{-1}$ was observed in (T5), followed by 0 to 61×10^{3} $cfu.ml^{-1}$ in (T11) and 0 to 54 $\times10^{3}$ $cfu.ml^{-1}$ in (T8), with a least colony forming units of 48×10^{3} $cfu.ml^{-1}$was observed in T7, with no signs of microbial activity during the initial day of the storage period, followed by 0 to 53×10^{3} $cfu.ml^{-1}$ in (T10), from initial to 40^{th} days of the storage period.

On the other hand, the samples stored at refrigeration condition at 4±1 °C, presented in Fig. 16(d), Fig. 16(e) and Fig. 16(f), the least or minimal growth of microorganisms was observed in (T8), where there was no microbial activity was noticed at initial, 15^{th} and 30^{th} days of storage period and at the end of the 40^{th} day, the total plate count of 13.2×10^{3} $cfu.ml^{-1}$ was noticed. At refrigeration condition T2 showed the TPC of 0 to 61 $\times10^{3}$ $cfu.ml^{-1}$ at the initial to 40^{th} days of storage periods. Among the treatments (T5) showed 0 to 55 $\times10^{3}$ $cfu.ml^{-1}$, followed by least colony forming units of 0 to 5.6×10^{4} $cfu.ml^{-1}$ in (T7). This is because lower the temperature, lesser will be the microbial activity, which is one of the hurdle technology in inactivating or multiplication of microbial load with respect to storage in combination with different preservation methods. It plays a key role in inactivating the microbial activity, thus helps in extending the shelf-life of the product. The increase in microbial activity is due to the change in acidity level and the results are in confirmation with increase in storage period in plum wine preserved with sodium benzoate and sulphur dioxide (Gill *et al.*, 2009) and through

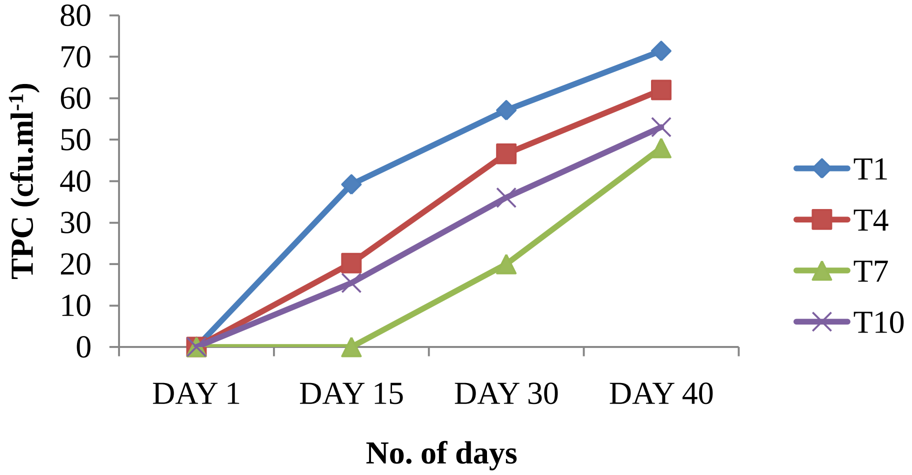

Fig. 16(a)

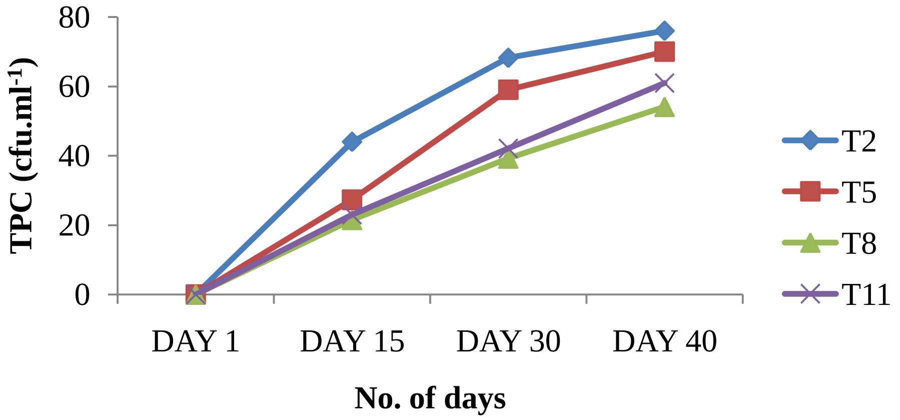

Fig. 16(b)

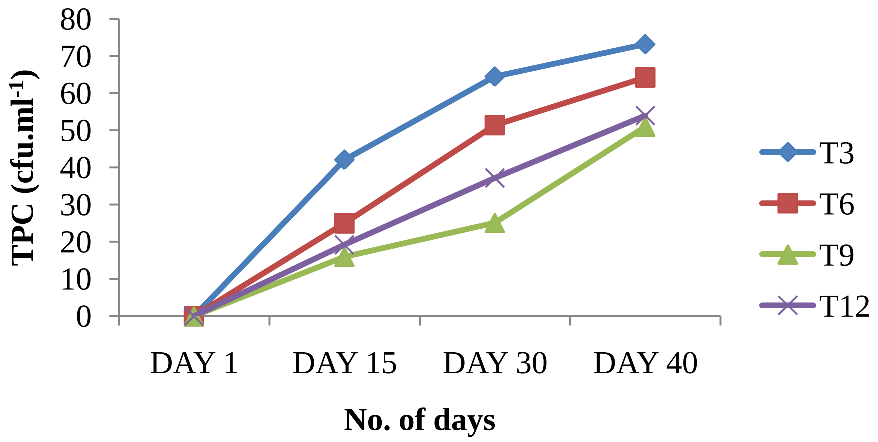

Fig. 16(c)

Fig. 16(a), 16(b) and 16(c) Effect of different preservation methods on total plate count of sweet orange juice filled in glass bottles, PET bottles and tin cans stored at ambient condition (30±2 °C)

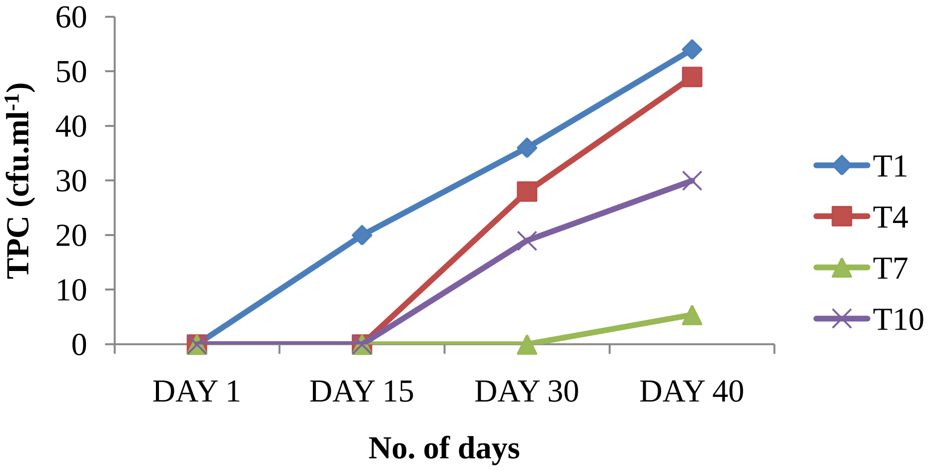

Fig. 16(d)

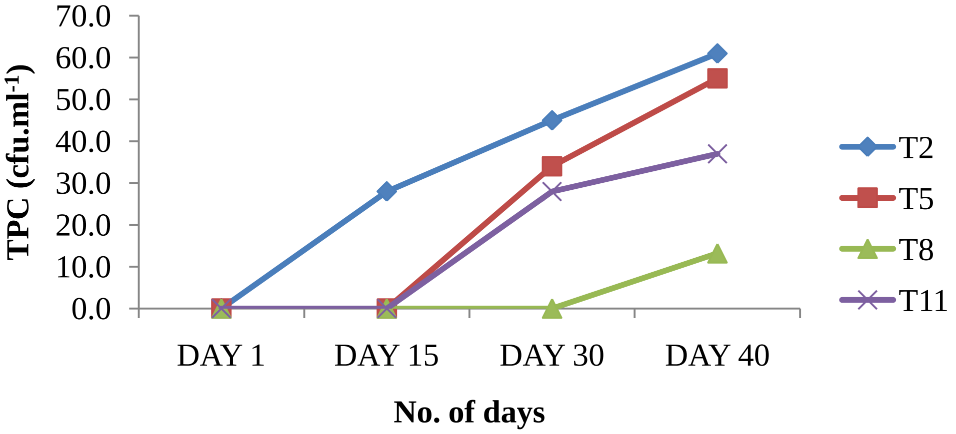

Fig. 16(e)

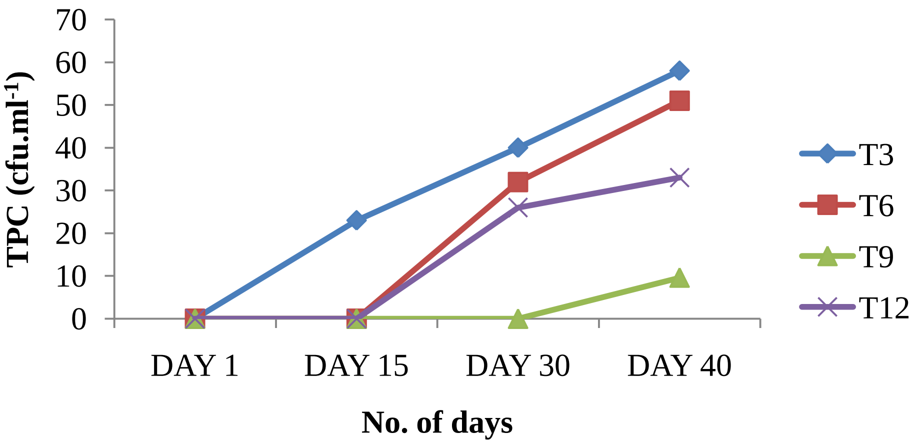

Fig. 16(f)

Fig. 16(d), 16(e) and 16(f) Effect of different preservation methods on total plate count of sweet orange juice filled in glass bottles, PET bottles and tin cans stored at refrigerated condition (4±1 °C)

pasteurization at 60 °C for 15 minutes that inhibits the increase of microbial load in plum wine. Microbial count and shelf-life studies of strawberry juice was conducted by Saddozai *et al*. (2012) and found the TPC of 2.7×10^{2} cfu.ml^{-1} in strawberry juice with pH of 3.2 at 1st day of storage followed by 2.25×10^{6} cfu.ml^{-1} at 6 weeks of storage period. Similarly, the microbial load of 10 cfu.ml^{-1} was observed by Maresca *et al*. (2011) in homogenized apple juice, In fact after 14 days of storage period >100 cfu.ml^{-1} was observed in untreated apple juice and found that this juice was not microbiologically stable due to the growth and reproduction of spoilage microbial cells at refrigerated condition.

The above observations were found to be similar with the readings obtained by Talasila *et al*. (2011), who studied the effect of preservation on shelf-life extension of cashew apple juice, where they found no activity of bacteria from 0 to 90 days and at 105 to 120 days <5 cfu.ml^{-1} was observed. Rustagi *et al*. (2013) conducted the storage studies on development of amla mango blended juice and found the total plate count of 5, 7, 9, 14, 18, 22 and 45 cfu.g^{-1}, during the storage studies of 0 to 45 days in P0T1 *i.e.*, in control samples with no preservatives stored at ambient condition on the other hand, the TPC of 3 to 30 cfu.g^{-1}in juice samples without preservatives at refrigeration condition of 4-6 °C, followed by 3 to 24 cfu.g^{-1}in juice samples preserved with 100 g of sodium benzoate, stored at ambient condition, 1 to 18 cfu.g^{-1}in P1T2 with 100g sodium benzoate at refrigeration temperature of 4-6 °C, 1 to 20 cfu.g^{-1} in juice samples preserved with 100 g potassium metabisulphite stored at ambient condition, 0 to 8 cfu.g^{-1}, in juice samples with 100 g potassium metabisulphite stored at refrigeration temperature, 1 to 21 cfu.g^{-1}, in 50 mg of potassium metabisulphite + 50 mg sodium benzoate+ambient condition and 0 to 10 cfu.g^{-1}in juice samples with 50mg potassium metabisulphite+50mg sodium benzoate at refrigeration temperature. These reading s were more or less supports the obtained values 20, 25 and 19 cfu.g^{-1}in sweet orange juice samples preserved with 0.5 mg of sodium benzoate and stored at refrigeration temperature in T8, T10 and T12.

The juice preserved with nisin at 0.001 mg.ml^{-1} filled in glass, PET bottles and tin cans, stored at ambient condition showed a colony forming units of 0 to 53, 0 to 61 cfu.ml^{-1} and 0 to 54 cfu.ml^{-1} in T10, T11 and T12, followed by 0 to 30, 0 to 37 and 0 to 33 cfu.ml^{-1} in T10, T11 and T12 *i.e.,* juice filled in glass, PET and tin cans preserved with nisin were found to be less effective compared to the chemical preservative but more effective at refrigerated condition of 4±1 °C, because nisin at 100 IU.ml^{-1} was required to prevent the growth of vegetative spores and microbial cells, where concentration of 5 to 10 IU.ml^{-1} alone was found to be less effective in inactivating the microbial spores (Komitopoulou *et al.,* 1999).

Increase in microbial load in all the control and treated juice samples may depends on the effect of preservation methods, packaging materials used, storage and processing conditions and more over sweet orange juice is a highly perishable product and contamination may occur while handling the juice leads to contamination and thus more number of microbial load was observed than in refrigeration condition.

5.4.2 Yeast and mould counts

The effect of different preservation methods, packaging materials and storage conditions in inhibiting the growth and multiplication of yeasts and moulds in sweet orange juice were assessed and presented in Fig. 17(a), Fig. 17(b), Fig. 17(c) and Fig. 17(d), Fig. 17(e), Fig. 17(f). From the Fig. 17(a), Fig. 17(b) and Fig. 17(c), the growth of yeast and moulds in sweet orange juice filled in glass bottles, PET and tin cans and stored at ambient condition showed increase in yeast and mould growth as the storage period increases. It was observed that there were no yeast and mould activities in T4, T7, T8, T9, T10, T11 and T12, where the multiplication of yeast and mould populations during the 15th days of storage in T4 and in 30th day in T7, T8, T10 and T12. In un-treated samples even though subjected to the pasteurization temperatures of 80 °C for 20 s, the yeast and mould growth of 0.9 to 3.9×10^{3} cfu.ml^{-1} were observed during to 15th to 40 days of storage period in T1. This indicates that pasteurization alone cannot inhibit the yeast and mould growth upon the storage period. Among the treatments T5 showed 0 to 4.9×10^{3}cfu.ml^{-1}, which was found to be less in comparison with the homogenized juice sample. This is because when sweet orange juice is subjected to high homogenization pressure of 5000 psi and at a pasteurization temperature of 80 °C for 20 s, helps in the inactivation of the microbial flora naturally or artificially in fruit juices (Betoret *et al.*, 2009). For instance the application of high pressure homogenization treatments to orange juice at 300 MPa in a two stage homogenization system significantly decreases *E.coli* as well as *Listeria innocua* and *Staphylococcus carnosus* (Brinez *et al.*, 2007).

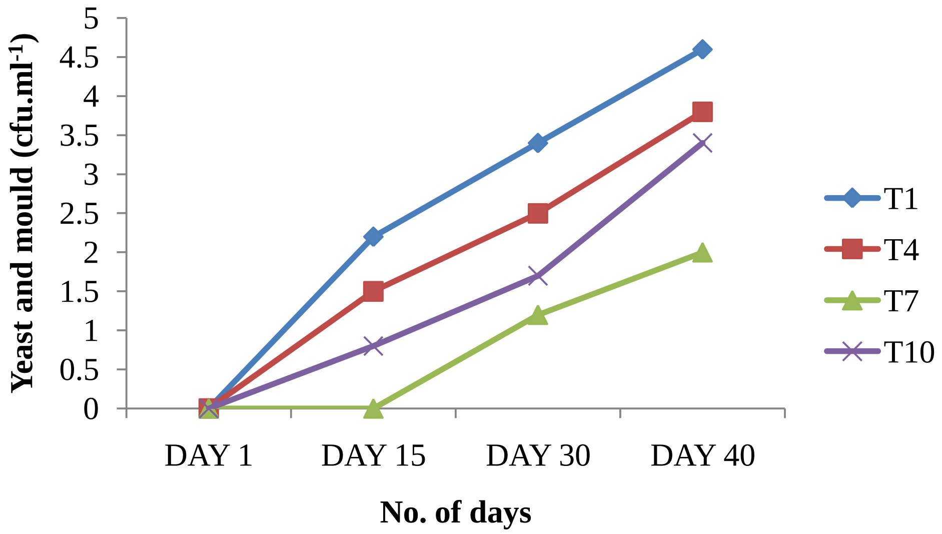

Fig. 17(a)

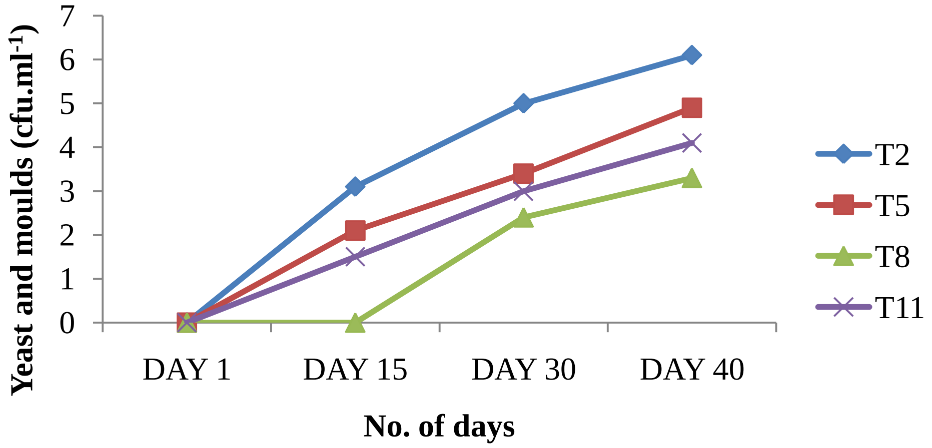

Fig. 17(b)

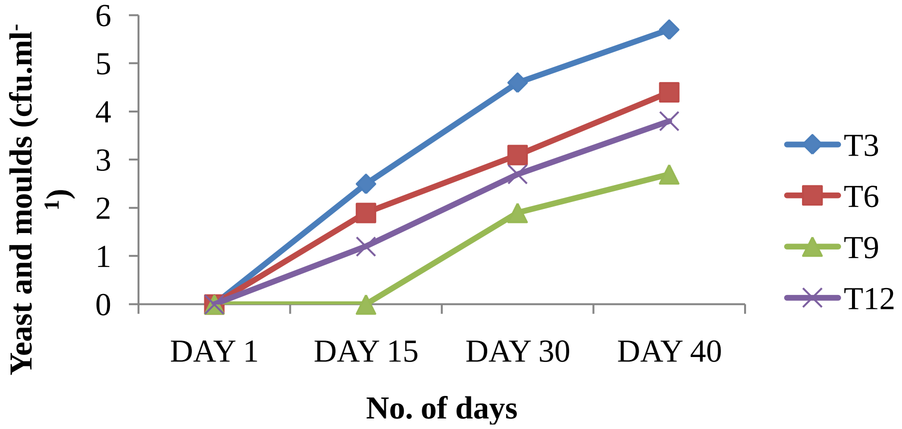

Fig. 17(c)

Fig. 17(a), 17(b) and 17(c) Effect of different preservation methods on yeast and mould count of sweet orange juice filled in glass bottles, PET bottles and tin cans stored at ambient condition (30±2 °C)

In treatments T7, T8 and T10, there were no yeast and mould growth were observed till 15th day of storage period and at 30th to 40th days, the yeast and mould counts were found to be increased from 1.2 to 2.0×10^{3} cfu.ml^{-1}, 2.4 to 3.3×10^{3} cfu.ml^{-1} and 1.9 to 2.7×10^{3} cfu.ml^{-1} in the respective treatments and these findings were found to be similar with the readings obtained by Rustagi *et al.* (2013), who observed that there were no yeast and mould growth till 0th to 10th days of storage period in amla-mango blended juice beverage and the total yeast and mould count of 5 to 10 cfu.g^{-1}were observed during 20th to 60th days of storage in amla-mango blended juice sample without preservatives stored at the ambient condition, whereas in amla-blended juice samples preserved with 100mg of sodium benzoate stored at ambient condition there were no yeast and mould growths were observed till 20th days of storage and at the end of 60th day the yeast and mould count of 8 cfu.ml^{-1} was identified and in amla-blended juice sample with 50mg of sodium benzoate along with 50 mg of potassium meta bisulphate no yeast and mould counts were identified till 20th days of storage and at the end of 60th day the yeast and mould count of 4 cfu.ml^{-1} was determined, which shows the combined effect of sodium benzoate along with potassium meta bisulphate in inhibiting the yeast and mould population in amla and mango blended juice. Talasila *et al.* (2011) determined the yeast and mould population in cashew apple juice preserved with 1 ppm of potassium mea bisulphate and observed there were no yeast and mould count till 0th to 105 days of storage period at ambient condition and at the 120th day $<$1 cfu.ml^{-1}was detected. The above observations were found to be supportive with the Saddozi *et al*. (2012) who noted nil growth of yeast and moulds during 1st day, whereas in 7th day, the yeast and mould count of 1.4×10^{3} cfu.g^{-1} and 1.7×10^{3} cfu.g^{-1} was observed in strawberry juice, followed by an increase in yeast and mould count upto 1.6×10^{5} cfu.g^{-1} to 2.47×10^{6} cfu.g^{-1} in strawberry juice. In treatments T11, T12 and T13, sweet orange juice preserved with nisin filled in glass, PET bottles and tin cans showed yeast and mould population of 0.8 to 3.4×10^{3} cfu.ml^{-1}, followed by 1.5 to 4.1×10^{3} cfu.ml^{-1} and 01.2 to 3.8×10^{3} cfu.ml^{-1} was found to be more compared to the juice sample preserved with sodium benzoate. This is because, even though nisin is an effective biopreservative at 0.001 mg.ml^{-1}alone was found to be less effective in inhibiting the vegetative spores of *Alicyclobacillus acidoterrestris* and *P. cyclohexanicum* and it was found to be more effective in inhibiting the vegetative cells of microorganisms in combination with either sodium benzoate or with potassium sorbate was found to be effective in inhibiting the multiplication of *A.acidoterristries.*

From Fig. 17(d), Fig. 17(e) and Fig. 17(f), less yeast and mould growths were observed at refrigerated condition, in all the treatments it was found to be one of the most effective hurdle technique in inhibiting the multiplication of yeast and mould population along with physical (homogenization), chemical (sodium benzoate) and biological(nisin) preservation methods. Among the treatments T7, T8 and T9 showed less increase in yeast and mould activity, this is due to the antimicrobial activity of sodium benzoate along with refrigeration temperature. Un-treated samples showed more yeast and mould population compared to the treated samples as storage period increases. The increase in yeast and mould counts were observed at the end of the storage period might be due to internal and external factors that favour the yeast and other microorganisms to grow and multiply to cause spoilage.

The increase in microbial load as storage period increases is influenced by storage conditions and also might be due to contamination that occur during processing of sweet orange juice because it is highly perishable, surrounding temperature and the stability of the packaging material used, method of preservation and its level sufficient enough to control yeast and mould population as well as sterilization of the packaging materials.

5.5 Effect of different preservation methods packaging materials and storage conditions on shelf-life prediction of sweet orange juice based on ascorbic acid limit

The effect of different preservation methods and packaging materials on shelf-life of sweet orange juice based on ascorbic acid limit of 40 $mg.ml^{-1}$ stored at ambient and refrigerated condition was studied and presented in Fig. 18(a) and Fig. 18(b). From the Fig. 18(a) it was observed that T7 showed the shelf-life of 32.5 days in sweet orange juice preserved with sodium benzoate filled in glass bottles and stored at ambient condition, followed by T9 and T10 *i.e.,* in sweet orange juice samples preserved with sodium benzoate filled in tin cans and juice preserved with nisin filled in glass bottle and stored at ambient condition. The findings were found to be true with that of Shahanwaz *et al.* (2013) who showed that optimum storage period for orange juice remained 30 days after preservation and after 45 days of storage bacterial growth was observed. However, orange juice preserved with 0.6, 0.8 and 1.0g of sodium benzoate had higher sugars, higher total soluble sugars, while vitamin C and pH was highest in the fresh orange juice. The interaction effect showed that he total sugars were higher in percent in amber bottles

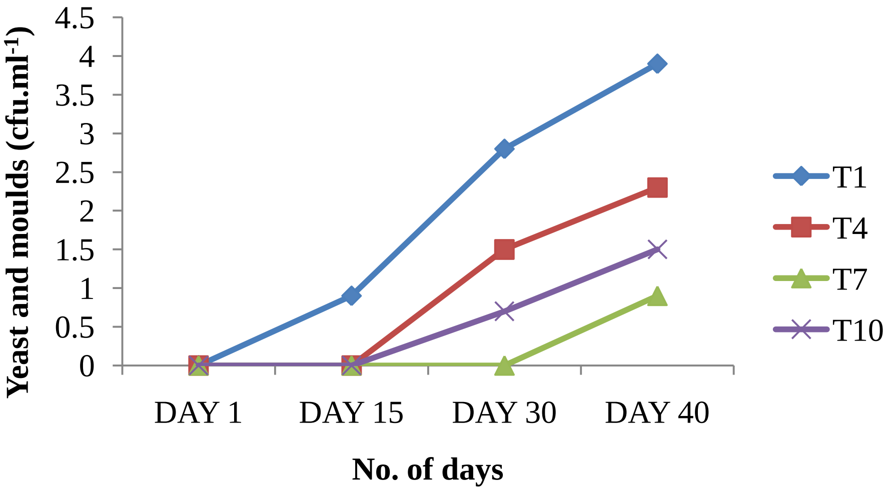

Fig. 17(d)

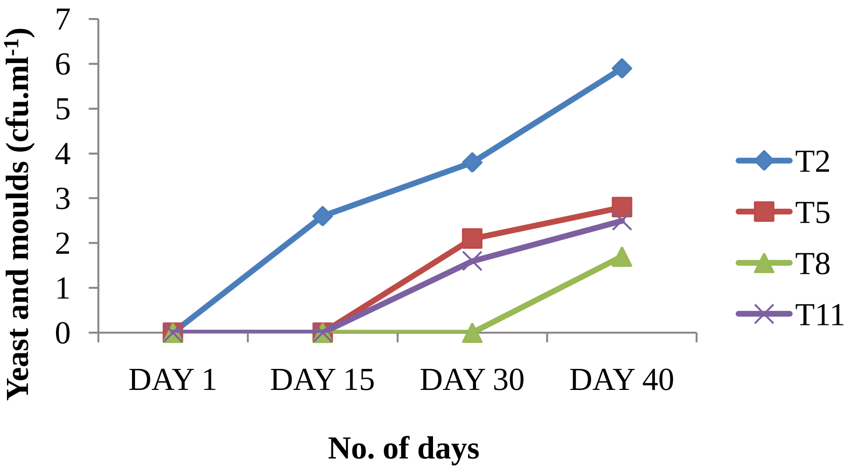

Fig. 17(e)

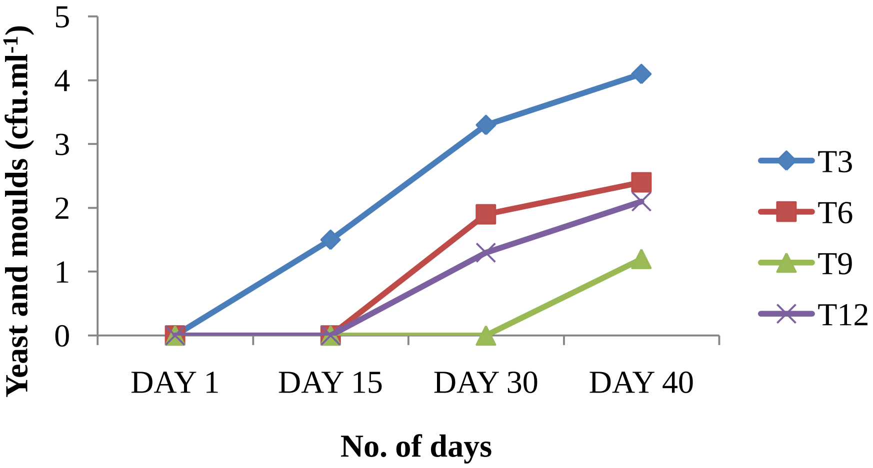

Fig. 17(f)

Fig. 17(d), 17(e) and 17(f) Effect of different preservation methods on yeast and mould count of sweet orange juice filled in glass bottles, PET bottles and tin cans stored at refrigerated condition (4±1 °C)

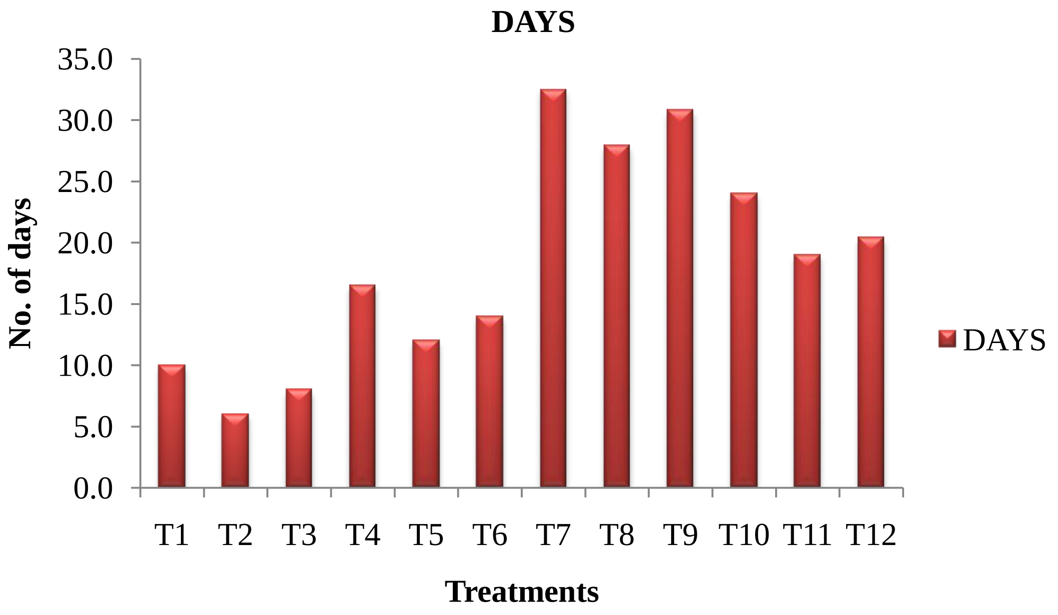

Fig. 18(a) Effect of different preservation methods and packaging materials on shelf-life prediction of sweet orange juice based on ascorbic acid limit stored at ambient condition (30±2 °C)

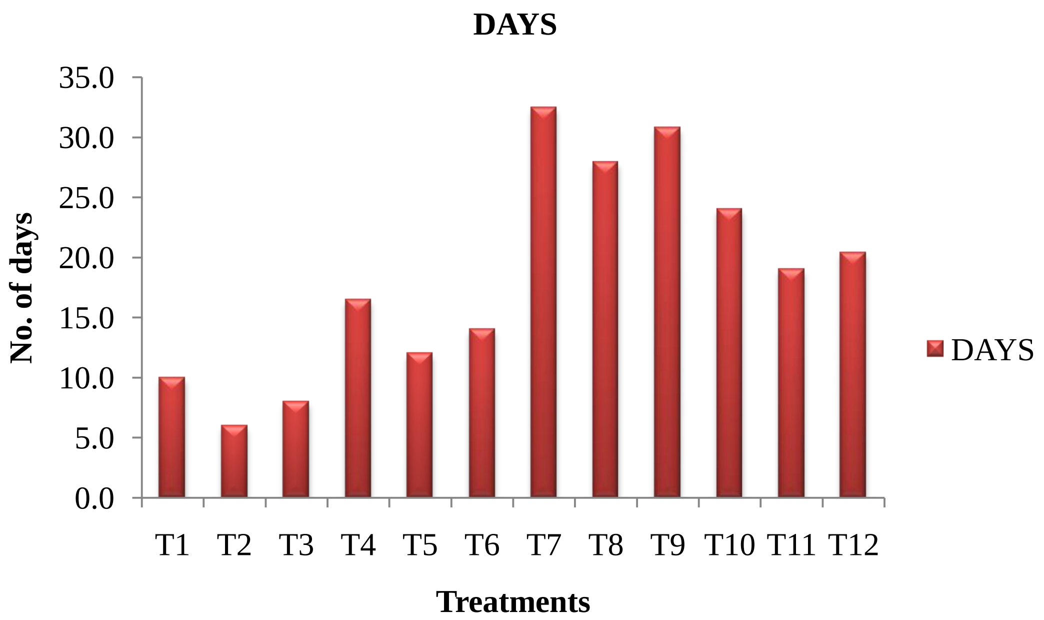

Fig. 18(b) Effect of different preservation methods and packaging materials on shelf-life prediction of sweet orange juice based on ascorbic acid limit stored at refrigerated condition (4±1°C)

after 30 days of storage at 0, 0.6 and 0.8 g of sodium benzoate concentrations respectively and lower total sugars percent was observed in PET bottles after 45 days of storage of storage with 0.8 g of sodium benzoate concentration and results showed that amber bottles could retain good quality composition during storage. However, Ayhan *et al.* (2001) evaluated orange juice aroma, colour, vitamin C in glass, PET, high density polyethylene and low density polyethylene and found that packaging material had a significant effect on the retention of orange juice aroma, flavour, colour and vitamin C and suggested PET bottle packaging. At the present study it was observed that the overall retention of nutrients, vitamin C, total soluble solids, aroma, flavour were found to be best in glass bottles followed by PET bottles and tin cans, where glass bottles along with sodium benzoates interacts better in preventing the microbial activity upto 40 days of storage period. Through sensory analysis it was revealed that glass bottles were found to be best in retaining all the organoleptic properties upto 32.5 days of storage period and the later showed the deterioration in these quality traits. Berlinet *et al.* (2003) packed orange juice in three different PET and glass packaging and indicated that PET packaging is the major factor detrimentally affecting the juice quality parameters. Chumillas *et al.* (2003) preferred PET and Amber packing for orange juice storage to increase the shelf life. The literature reviewed from the studies carried out in different parts of the world are well in agreement with the findings of the present research regarding the effect of packing materials on the chemical and organoleptic parameters of processed orange juice. However, some variation in these results might be due to change in climatic conditions. It was observed that in the un-treated sweet orange juice samples showed less shelf life of 10, 6 and 14 days in T1, T2 and T3, tells that pasteurization temperature of 80 °C for 20 s was not sufficient enough in destroying the vegetative microbial spores and enzymes present which become activated during the later stages and in homogenized juice sample with a homogenization pressure of 5000 psi along with the pasteurization temperature was found to be more effective than the un-treated samples in inhabiting the microbial activity, pectin methyl esterase activity as well, which are the major causative factors for sweet orange juice spoilage (Taylor, 2007) and the shelf-life can be extended from 12 to 16.5 days at ambient condition in T4, T5 and T6.

It was observed from the Fig 18(b) the shelf-life of un-treated samples were found to be 14.4 days, 9.8 and 11.8 days in T1, T2 and T3, which shows that upon pasteurization temperature at 80 °C for 20 s along with refrigeration temperature was found to be more effective in extending the shelf-life of sweet orange juice than that of sweet orange juice samples stored at ambient condition. In T4, T5 and T6, the shelf-life of 25.5, 20 and 21 days were noticed in homogenized juice samples at 5000 psi upon 40^{th} days of storage. The shelf-life of apple juice treated by a multi-pass homogenization treatment at 150 MPa was evaluated by Maresca *et al.* (2011) and observed that the shelf-life can be extended upto 28 days at refrigerated condition since there were no microbiological activity observed in these samples at low temperature and the physico-chemical properties were best retained at lower temperature. Jacob *et al.* (2010) observed a single pass high pressure homogenization treatment above 200 MPa was able to produce an apple juice with a microbiological activity over 60 days, while raw and 100 MPa treated juices showed significant microbial growth already after 1 day. Moreover more intense HPH treatment of 300 MPa ensured microbiological stability and preserved the organoleptic attributes of the juice over 28 days of storage at 4 °C. From T7, T8 and T9, the shelf-life of 130, 87.7 and 104 days were observed; this is due to the combined preservation effect of sodium benzoate at 0.5 $mg.ml^{-1}$ was found to be sufficient enough to inhibit the microbial activity when packed in glass bottles followed by PET bottles and tin cans. However, glass bottles showed more retention of organoleptic and sensorial properties followed by tin cans and PET bottles. On the other hand in treatments T10, T11 and T12, in juice samples preserved with 0.001 $mg.ml^{-1}$ of nisin was sufficient enough to stop the yeast, moulds and microbial activity at lower temperature of 4 °C but not as effective like chemical preservative in inhibiting the heat resistant, acid tolerant spore forming vegetative microorganisms Esteban and palop (2011). Chumillas *et al.* (2007) observed that orange juice sample filled in monolayer PET bottles stored at 4 °C provide longer shelf-life of 259 days and juice 213 days in juice sample stored at 25 °C. This result indicates the interest of maintaining juice monolayer PET bottles juice at refrigeration condition, whereas juice samples bottled in monolayer PET with oxygen scavenge reached a longer shelf-life of 213 to 258 days and in bottles packed with liquid nitrogen drop in head space presented slightly longer shelf-life of 245 days and without liquid nitrogen drop shelf-life of 226 days. The longest shelf-life of 293 days were obtained in oxygen scavenger, liquid nitrogen addition and aluminium foil seal on the bottle caps at 4 °C. Polydera *et al*. (2003) observed glass and multilayer PET bottles can reach more than 300 days at 4 °C and 250 days at 25 °C.

In the present investigation the lesser shelf-life values were obtained for all the samples with different preservation is due to the high perishable nature of the sweet orange juice and its interaction with different preservation methods, packaging materials used, temperature variations, contamination that occurred while handling and other environmental factors, lack of adoption of well advanced technologies might be responsible for lesser shelf-life of sweet orange juice.

5.6 Economics of sweet orange juice

The cost returns on processing of sweet orange juice with different preservation methods and packaging materials, stored at ambient condition (30±2 °C) was computed in terms of B:C ratio. The economics of sweet orange juice is discussed below.

The shelf-life of pasteurized sweet orange juice filled in glass bottles and stored at ambient condition (30±2 °C) in T1 was found to be 10 days. The benefit cost ratio of ₹ 1.01:1 was obtained, might be due to the effect of glass bottles on shelf-life when stored at ambient condition.

The shelf-life of pasteurized sweet orange juice filled in PET bottle stored at ambient condition was found to be 6 days (T2) and the B:C ratio of ₹ 0.92:1 was obtained which is less compared to T1 is due to the less effectiveness of PET bottles in maintaining the shelf-life of juice as storage period proceeds.

The shelf-life of pasteurized sweet orange juice filled in tin cans stored at refrigerated condition was found to be 8 days (T3) and the B:C ratio of ₹1.00:1 was obtained which is less compared to T1 and more compared to T2 is due to the less effectiveness of tin cans compared to glass bottle but more compared to that of PET bottles in maintaining the shelf-life of juice as storage period proceeds.

The shelf-life of homogenized juice sample at 5000 psi, filled in glass bottles and stored at ambient condition (T4) was found to be 16.5 days. The benefit of ₹1.15:1 was obtained. This might be due to higher shelf-life of the homogenized juice sample when filled in glass bottles and stored at room temperature.

In homogenized juice sample at 5000 psi, filled in PET bottles and stored at ambient condition, the B:C ratio of ₹1.08:1 (T5) was obtained. This is due to the lower shelf-life of 12 days, which indicates the less effectiveness of PET bottles in preserving the overall quality of sweet orange juice.

The shelf-life of 14 days was obtained in sweet orange juice sample filled in tin cans stored at ambient condition (T6) and the B:C ratio was found to be ₹1.10:1. This might be due to the effectiveness of tin cans was found to be more in retaining the overall quality of juice, when compared to that of the PET bottles.

In sweet orange juice samples preserved with sodium benzoate filled in glass bottles and stored at ambient condition in T7, the shelf-life of 32.5 days was recorded and the B:C ratio of ₹1.42:1 was obtained. This might be due to the effectiveness of sodium benzoate in preserving the overall quality of sweet orange juice, when compared to other preservation methods.

The shelf-life of (T8) was found to be 27.9 days in sweet orange juice samples filled in PET bottled and stored at ambient condition and the B:C ratio of ₹1.38:1 was obtained. This indicates the less efficiency of PET bottles in maintaining the sweet orange juice quality as storage period proceeds when preserved with sodium benzoate (0.5 $mg.ml^{-1}$).

The shelf-life of 30.8 days was observed in sweet orange juice filled in tin cans and stored at ambient condition (T9) was found to more compared to the PET bottles and the B:C ratio of ₹1.41:1 was obtained. This might be due to the more efficiency of tin cans in prolonging the shelf-life of sweet orange juice than that in PET bottles, where the juice spoiled early.

The shelf-life of sweet orange juice preserved with bio-preservative nisin at 0.001 $mg.ml^{-1}$ (T10) was found to be 24 days when filled in glass bottles and stored at ambient condition and the B:C ratio of ₹ 1.28:1 was obtained which was found to be lesser when compared to sodium benzoate preserved and homogenized juice samples, which indicates that even though nisin prolongs the shelf-life sweet orange juice but found to be less efficient than sodium benzoate, which fetches more returns upon investment.

The shelf-life of 19 days was recorded in (T11) sweet orange juice samples filled in PET bottles and stored at ambient condition, where the B:C ratio of ₹ 1.21:1 was obtained. This might be due to the usage of PET bottles and nisin in preserving the sweet orange juice samples which fetches lower returns in the market.

In sweet orange juice samples preserved with 0.001 $mg.ml^{-1}$ of nisin, filled in tin cans and stored at ambient condition (T12), recorded the shelf-life of 20.4 days and the B:C which was found to be more than sweet orange juice filled in PET bottles but comparatively lesser than T1 and B:C ratio of ₹ 1.22:1 was obtained upon investment.

The cost and returns on processing of sweet orange juice with different preservation methods and packaging materials, stored at refrigeration condition at 4±1 °C was computed in terms of B:C ratio. The economics of sweet orange juice are discussed below.

The shelf-life of pasteurized sweet orange juice filled in glass bottles and stored at refrigerated condition (4±1 °C) in T1 was found to be 14.4 days. The benefit cost ratio of ₹1.17:1 was obtained, might be due to the effect of glass bottles on shelf-life when stored at refrigerated condition.

The shelf-life of pasteurized sweet orange juice filled in PET bottle stored at refrigerated condition was found to be 9.8 days (T2) and the B:C ratio of ₹ 1.12:1 was obtained which is less compared to T1 is due to the less effectiveness of PET bottles in maintaining the shelf-life of juice as storage period proceeds.

The shelf-life of pasteurized sweet orange juice filled in tin cans stored at refrigerated condition was found to be 11.8 days (T3) and the B:C ratio of ₹ 1.14:1 was obtained which is less compared to T1 and more compared to T2 is due to the less effectiveness of tin cans compared to glass bottle but more compared to that of PET bottles in maintaining the shelf-life of juice as storage period proceeds.

The shelf-life of homogenized juice sample at 5000 psi, filled in glass bottles and stored at refrigeration condition at 4±1 °C (T4) was found to be 25.5 days. The benefit of ₹ 1.25 was obtained. This might be due to higher shelf-life of the homogenized juice sample when filled in glass bottles and stored at lower temperature.

In homogenized juice sample at 5000 psi, filled in PET bottles and stored at refrigeration condition, the B:C ratio of ₹ 1.20:1 (T5) was obtained. This is due to the lower shelf-life of 20.0 days in the sweet orange juice samples filled in PET bottles, which indicates the less effectiveness of PET bottles in preserving the overall quality of sweet orange juice.

The shelf-life of 21.0 days was obtained in sweet orange juice sample filled in tin cans stored at refrigeration condition of 4±1 °C (T6) and the B:C ratio was found to be ₹1.22:1. This might be due to the effectiveness of tin cans was found to be more in retaining the overall quality of juice, when compared to that of the PET bottles.

In sweet orange juice samples preserved with sodium benzoate filled in glass bottles and stored at refrigeration condition (4±1 °C) in T7, the shelf-life of 130 days was predicted based on ascorbic acid limit and the B:C ratio of ₹1.50:1 was obtained. This might be due to the effectiveness of sodium benzoate in preserving the overall quality of sweet orange juice, when compared to other preservation methods.

The shelf-life of (T8) was found to be 87.7 days in sweet orange juice samples filled in PET bottled and stored at refrigeration condition (4±1 °C).and the B:C ratio of ₹ 1.46:1 was obtained. This indicates the less efficiency of PET bottles in maintaining the sweet orange juice quality as storage period proceeds when preserved with sodium benzoate (0.5 $mg.ml^{-1}$).

The shelf-life of 104.5 days was observed in sweet orange juice filled in tin cans and stored at refrigeration condition (T9) was found to more compared to the PET bottles and the B:C ratio of ₹ 1.47:1 was obtained. This might be due to the more efficiency of tin cans in prolonging the shelf-life of sweet orange juice than that in PET bottles, where the juice spoiled early.

The shelf-life of sweet orange juice preserved with bio-preservative nisin at 0.001 $mg.ml^{-1}$ (T10) was found to be 53 days when filled in glass bottles and stored at refrigeration condition and the B:C ratio of ₹ 1.35:1 was obtained which was found to be lesser when compared to sodium benzoate preserved and homogenized juice samples, which indicates that even though nisin prolongs the shelf-life sweet orange juice but found to be less efficient than sodium benzoate, which fetches more returns upon investment.

The shelf-life of 50.5 days was recorded in (T11) sweet orange juice samples filled in PET bottles and stored at refrigeration condition (4±1 °C), where the B:C ratio of

₹1.33:1 was obtained. This might be due to the usage of PET bottles and nisin in preserving the sweet orange juice samples which fetches lower returns in the market.

In sweet orange juice samples preserved with 0.001 $mg.ml^{-1}$ of nisin, filled in tin cans and stored at refrigeration condition at 4±1 °C (T12), recorded the shelf-life of 52.3 days and the B:C which was found to be more than sweet orange juice filled in PET bottles but comparatively lesser than T1 and B:C ratio of ₹1.34:1 was obtained upon investment.

SUMMARY AND CONCLUSION

VI SUMMARY AND CONCLUSION

The present investigation on Development of Process Technology for Enhancing Shelf-life of Sweet Orange Juice (*Citrus sinensis (L) Osbeck*) was undertaken in the Department of Processing and Food Engineering, College of Agricultural Engineering, UAS, Raichur, Karnataka during 2013-14. The results are summarized and the conclusions drawn are presented here under.

There is an increase in demand for processed fruit juices with better retention of nutritional and organoleptic properties that provide good health benefits to the consumer upon consumption. Sweet orange juice is good source of vitamin C, an excellent source of bioactive components that promotes detoxification in the human body. Since it is highly perishable in nature and spoils within less interval of time as soon as extraction due to the action of pectinmethylesterase enzyme and lactic acid bacteria. Hence, the focus on the present study was to enhance the shelf-life of sweet orange juice to minimize the spoilage by inhibiting the microbial activities and make it available for the consumer usage.

The results of the above experiments conducted under this research work are summarized below.

The proximate composition of sweet orange juice was analyzed at both ambient and refrigerated condition at (4±1 °C), with different preservation methods, filled in glass, PET bottles and tin cans. At ambient condition, it was noted that as the storage period proceeds, the moisture content also increases with respect to storage period of 40 days. The increase in moisture content from (88.69 to 92.15 %w.b.) was observed in T7, followed by (88.85 to 93.75 %w.b.) T10 and (88.85 to 93.75 %w.b.) T4 and among the un-treated sample, the moisture content of (88.92 to 94.42 %w.b.) was observed in T1. Whereas, in sweet orange juice sample stored at refrigerated condition (4±1 °C), the moisture content of (88.72 to 91.54 %w.b.) was observed in T7, followed by 88.80 to 92.24 %w.b. in T10 and 88.87 to 93.45 %w.b. in T4. Among un-treated samples, the moisture content of (88.94 to 93.88 %w.b.) was observed in T1, which indicates that more loss of moisture content in sweet orange juice preserved with different preservation and packaging materials stored at ambient condition, than that in refrigerated condition, where glass bottles showed good moisture barrier properties than that of PET bottles and tin cans.

The decrease in soluble protein content from (0.46-0.30%) was observed in T7, followed by 0.43 to 0.28% (T10), 0.39 to 0.18% in (T4) and 0.35 to 0,12% (T1), where more depletion was observed at ambient condition. Whereas, the soluble protein content was found to be retained better in T7 (0.47 to 0.40%) in sweet orange juice sample preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate, filled in glass bottles and stored at refrigerated condition (4±1 °C), followed by 0.44 to 0.33% in biological method of preservation using 0.001 $mg.ml^{-1}$ of nisin (T11), followed by (T4) physical method of preservation by homogenization at 5000 psi, where soluble protein content of (0.41-0.30%) was recorded and among un-treated samples (T1), the protein content of (0.36 to 0.22%) was observed, where less retention of soluble protein content was recorded in all the un-treated as well as treated samples stored at ambient condition during the 40 days of storage period.

The decrease in carbohydrate content (10.26 to 7.30%) was recorded in (T7), 10.20 to 5.94% in (T10), followed by 10.10 to 5.39% (T4) and among un-treated samples (T1) recorded the carbohydrate content of (9.92 to 4.72%) in sweet orange juice samples filled in glass bottles and stored at ambient condition, where more decrease in carbohydrate content was noticed, and decrease in carbohydrate content from 10.28 to 8.44% was observed in (T7), followed by 10.23 to 7.50% (T11), 10.15 to 6.74% in T4, where in un-treated samples the less carbohydrate content of 10.5-5.44% was observed in (T1) at refrigerated condition. From the above it was noticed that the sweet orange juice sample filled in glass bottle preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate, stored at refrigerated condition retains the better nutritional properties than PET bottles and tin cans followed by biological and physical method of preservation when stored at ambient condition from initial to 40 days of storage period.

pH was found to be decreased in all the sweet orange juice samples as the storage period increases, with respect to different preservation methods packaging materials and storage conditions. It was observed that more decrease in pH was observed in juice samples stored at ambient condition than at refrigerated condition. The minimum decrease in pH ranging from 4.26 to 4.10 was observed in T7 in sweet orange juice filled in glass bottle, preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate stored at refrigerated condition, where more decrease of pH from 3.56 to 3.09 was observed in T2 (un-treated) juice sample stored in PET bottles and stored at ambient condition.

It was found that the water activity also increased in all the sweet orange juice samples from initial to 40 days of storage period. Among the treatments T7 showed the minimum increase in water activity from 0.860 to 0.886 with a maximum water activity of 0.878 to 0.926 in T5 and among un-treated samples T2 recorded a maximum water activity of 0.902 to 0.950 at ambient condition. At refrigerated condition, the water activity of 0.837 to 0.861 was observed in (T7) from initial to 40^{th} days of storage period, with a maximum pH of 0.865 to 0.901 in T5 and among un-treated samples, the pH of 0.889 to 0.925 was recorded in T3.

Colour values of L^{*}, a^{*}, and b^{*} values of sweet orange juice with different preservation methods and packaging materials and storage conditions were determined using Hunter lab colour flex. L^{*} values indicates the whiteness (+) to darkness (-). The L^{*} values showed a gradual decrease in all the samples as the storage period increases both at ambient as well as in refrigerated condition. The maximum retention of L^{*} values were observed in T7 (56.10 to 48.46) in sweet orange juice sample preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate filled in glass bottles and stored at ambient condition and (T5) homogenized juice sample at 5000 psi filled in PET bottles showed L^{*} value of 58.92 but gradual decrease was observed to 46.54 as the storage period increases at ambient condition and among un-treated samples (T2) showed L^{*} values of 50.84 to 33.32. At refrigerated condition, (T7) sodium benzoate preserved sweet orange juice sample filled in glass bottle stored at refrigerated condition (4±1 °C) showed L^{*} values of (54.52 to 49.94) and T6 showed the L^{*} values of (50.34 to 47.48) and among un-treated samples T2, showed the minimum L^{*} values of 52.70 to 41.29 from initial to 40^{th} days of storage period.

a^{*} value gives an indication of the redness (+) to greenness(-). Initially the a^{*} values for all the samples were in the range of -3.29 to -3.18 in all the control and treated samples and on the 40^{th} day of storage the increase in a^{*} values were observed in all the samples., whereas (T7), sweet orange juice preserved with sodium benzoate and stored at ambient condition showed a^{*} values of -4.68 to 1.40 from initial to 40^{th} days of storage period and among un-treated samples T2, juice samples filled in PET bottles stored at ambient condition registered, the a^{*} values of -4.08 to 3.42 from initial to 40^{th} days of storage period. In sweet orange juice samples stored at refrigerated condition (4±1 °C) T7 showed the a^{*} values of -4.36 to 2.54 in sweet orange juice sample preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate and stored at refrigerated condition with a maximum of -4.24

to -1.28 in (T2) sweet orange juice samples filled in glass bottles and stored at refrigerated condition (4±1 °C).

The b^* value is a measure of the yellowness of the sweet orange juice with different preservation methods, packaging materials and stored at both ambient and refrigerated condition. It was observed that as the storage period increases the decrease in b^* value was observed in all the sweet orange juice samples. At the initial storage period, the b^* value of 24.68 to 20.8 was observed in (T7) sweet orange juice sample preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate filled in glass bottles and stored at ambient condition, with a maximum b^* value of 20.74 to 15.98 (T5) and among un-treated samples (T2) registered b^* value of (17.85 to 5.46) Among refrigerated samples T7 (24.48-21.62) showed better retention of b^* value with respect to storage period, with a minimum of (22.92-19.54) in T11 and (21.98-19.76) in T4 and among the un-treated samples T1 showed minimum of (18.52 to 12.70) was registered.

It was observed that as the storage period increases the decrease in total soluble solids were observed in all the samples. At ambient condition, it was noticed that the maximum total soluble solids of 11.36 to 7.86 °Brix was registered in T7, followed by 11.24 to 6.78 °Brix in (T10), 11.17 to 6.27 °Brix (T4) and among the un-treated samples sweet orange juice filled in glass bottles (T1) showed maximum total soluble solid content of (11.11 to 5.58 °Brix). Among the refrigerated samples, sweet orange juice filled in glass bottles, preserved with 0.5 $mg.ml^{-1}$(T7) showed maximum retention of total soluble solids of (11.31 to 10.05 °Brix) followed by (11.22 to 8.76 °Brix) in T10 and (11.15 to 7.60 °Brix) in T4 and a maximum retention of TSS in control samples (T1) of (11.08 to 6.12 °Brix) was noted.

Ascorbic acid content of sweet orange juice was analyzed and found that as the storage period increases the ascorbic acid gradually decreases in all the control and treated samples. Among treated samples sweet orange juice preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate showed maximum retention of ascorbic acid, followed by biological and physical methods of preservation and among packaging materials glass bottles showed maximum ascorbic acid content followed by PET bottles and tin cans stored at refrigerated condition (4±1 °C), than that in ambient condition. The maximum ascorbic acid content of (55.96 to 32.20 $mg.ml^{-1}$) was observed in juice samples stored at ambient condition was recorded in (T7) followed by 55.42 to 21.84 $mg.ml^{-1}$) in (T4), with a minimum of (48.14 to 7.54 $mg.ml^{-1}$) ascorbic acid in T2, with a minimum of (49.90 to 15.76 $mg.ml^{-1}$) in (T5) in homogenized juice sample filled in PET bottle at ambient condition. Among refrigerated samples (T7) showed maximum ascorbic acid content of (55.96 to 49.43 $mg.ml^{-1}$) followed by (55.42 to 30.39 $mg.ml^{-1}$) in (T4) and 55.59 to 42.99 $mg.ml^{-1}$ in T11 and among un-treated samples T1 recorded ascorbic acid content of (54.60 to 20.58 $mg.ml^{-1}$) at refrigerated condition and minimum ascorbic acid content of (44.90 to 26.66 $mg.ml^{-1}$) in T5 among un-treated samples and 48.14 to 15.49 $mg.ml^{-1}$ in (T2) was registered.

Browning index was also found to be increased from 1 to 40 days of storage period. Among treatments T7 registered the minimum browning index of 0.180 to 0.352% with a maximum of 0.212 to 0.420% in (T5) and a maximum of 0.220 to 0.439% in (T2) was registered among un-treated samples at ambient condition. At refrigerated condition (4±1°C), it was observed that in T7, the juice browning index of (0.170 to 0.247%) was noted, with a maximum juice browning index of 0.210 to 0.354% and among un-treated samples T4 registered the juice browning index of (0.218 to 0.390%).

The total carotenoids content were found to be decreased with respect to the storage, both at ambient and refrigerated condition (4±1 °C). The maximum total carotenoids of (35.21 to 26.40 $mg.ml^{-1}$) was observed in T7, with a minimum total carotenoids of (31.84 to 17.58 $mg.ml^{-1}$) in T4 and a minimum total carotenoids of 30.42 to 16.34 $mg.ml^{-1}$ in T2 among un-treated samples at ambient condition during the initial to 40 days of storage period. Among refrigerated samples, the maximum carotenoids content of 35.25 to 31.34 $mg.ml^{-1}$ was observed in T7, followed by T5 with a minimum carotenoids content of (34.42 to 25.48 $mg.ml^{-1}$) and among un-treated samples T2 recorded, the minimum total carotenoids of (31.54 to 19.81 $mg.ml^{-1}$) during the initial and 40 days of storage period.

The maximum total flavonoids content of 23.60 $mg.ml^{-1}$ was observed in (T7) sweet orange juice preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate, filled in glass bottles and stored at ambient condition and at 40th day of storage, the total flavonoid content was reduced to 12.85, where minimum total flavonoids of 21.86 $mg.ml^{-1}$ was observed in T5 and at the end it reduced to 4.66 $mg.ml^{-1}$ and among un-treated samples (T2) recorded the minimum total flavonoids of 20.43 to 3.26 $mg.ml^{-1}$ during 1st to 40th days of storage period. However, in sweet orange juice preserved with 0.5 $mg.ml^{-1}$ sodium benzoate and stored at refrigerated condition (4±1 °C) in T7, recorded the total flavonoids content of 23.62 and at the end of 40^{th} day, the total flavonoids of 17.50 $mg.ml^{-1}$ was observed, with a minimum total flavonoids of 21.86 to 4.66 (T5) and among un-treated samples, the total flavonoids of 20.34 to 3.25 $mg.ml^{-1}$ was recorded.

The sensory analysis was done for the freshly processed sweet orange juice with different preservation methods, packaging materials and stored at refrigerated and ambient condition. It was observed that there were no significant difference was noticed in all the samples with respect to colour, aroma, flavour and overall acceptability. Sweet orange juice stored at refrigerated condition (4±1 °C), with different treatments and packaging was scored more than that of the juice samples stored at ambient condition during the initial to 40 days of storage period.

Total plate count (TPC) was done for the sweet orange juice samples with different preservation methods, packaging materials and stored at both ambient and refrigerated condition (4±1 °C) and it was observed that juice samples preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate recorded less microbial count of 0 to 48×10^3 $cfu.ml^{-1}$ in T7 with a more microbial population of 0 to 70×10^3 $cfu.ml^{-1}$ in (T5) and among un-treated samples T2 registered more total plate count of (0 to 76×10^3 $cfu.ml^{-1}$) at ambient condition. At refrigerated condition (4±1 °C), the minimum total plate count of 20×10^3 $cfu.ml^{-1}$ was recorded where no microbial counts were observed at an interval of 0^{th} to 30 days of storage period, with a maximum TPC of 0 to 55 $\times10^3$ $cfu.ml^{-1}$ in T5 and among the un-treated samples T2 recorded maximum TPC of 0 to 61 $\times10^3$ $cfu.ml^{-1}$ during 1^{st} to 40 days of storage period.

Total yeast and mould counts were analyzed for sweet orange juice samples stored at both ambient and refrigerated condition (4±1 °C), with different preservation methods and packaging materials. The total yeast and mould counts were found to be increased at ambient condition when compared to that at refrigerated condition (4±1 °C). The minimum yeast and moulds wet not detected in T7 at ambient condition where no yeast and mould counts were detected during the initial and 15^{th} days of storage period at the 30 and 40 days, the yeast and mould count of $1.2\times10^3$$cfu.ml^{-1}$ to 2.0 $\times10^3$ $cfu.ml^{-1}$ were detected, where the maximum increase in yeast and mould count of 2.1 was detected on 30^{th} day and 4.9 $\times10^3$ $cfu.ml^{-1}$ was recorded in T5 on 40^{th} day In T2, the maximum yeast and mould count of 3.1 to 6.1×10^3 $cfu.ml^{-1}$ was recorded during the 15, 30 to 40 days of storage period. However, at refrigerated condition minimum yeast and mould count was observed in T7 of 0.9×10^3 $cfu.ml^{-1}$ at 40^{th} day of storage, where maximum increase in yeast and mould count was noted in T5 (2.1 to 2.8×10^3 $cfu.ml^{-1}$) during 30 and 40 days of storage period. Among un-treated samples T2 recorded the maximum yeast and mould count of (2.6 to 5.9 $\times10^3$ $cfu.ml^{-1}$) during the 15, 30 and 40 days of storage at refrigerated condition (4±1 °C).

The effect of different preservation method, packaging materials and storage conditions on shelf-life of sweet orange juice was studied and observed that juice sample preserved with sodium benzoate at 0.5 $mg.ml^{-1}$ showed higher shelf-life of 130 days (T7) and less shelf-life of 9.82 (T2) days was observed in sweet orange juice samples stored at refrigerated condition (4±1 °C). At ambient condition (T7) in sweet orange juice preserved with 0.5 $mg.ml^{-1}$of sodium benzoate, recorded higher shelf-life of 32.5 days with a less shelf-life of 6 days was recorded in T2.

Cost estimation of sweet orange juice preserved with sodium benzoate of 0.5 $mg.ml^{-1}$ stored ambient condition showed B:C ratio of ₹1.42:1 upon investment with a less B:C ratio of ₹0.82:1 (T2) upon investment. At refrigerated condition (4±1 °C) T7, showed cost returns of ₹1.50:1 with a less B:C ratio of 1.12:1 (T2) upon investment.

From the above it was observed that juice preserved with sodium benzoate showed higher shelf-life followed by other preservation methods like biopreservation (nisin) and homogenization (physical). Among packaging materials glass bottles showed more retention of overall quality followed by tin can and PET bottles, when stored at refrigerated condition (4±1 °C) and more returns were also obtained from juice preserved with different preservation methods and packaging material, stored at refrigerated condition. Whereas, juice samples stored at ambient condition showed losses upon investment due to its lower shelf-life.

VI CONCLUSIONS

The major conclusions drawn from the present investigation are as follows

i. The sweet orange juice samples preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate, filled in glass bottles and stored at refrigerated condition (4±1 °C) was found to be more effective in preserving and retaining all the physico-chemical properties like moisture content, protein, pH, colour, total soluble solids, water activity, ascorbic acid, juice browning index, total carotenoids and total flavonoids

ii. Maximum moisture content (88.72 to 91.57 %w.b.) losses was observed in sweet orange juice samples preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate, filled in glass bottles and stored at refrigerated condition (4±1 °C) in T7, followed by biological preservative (0.001 $mg.ml^{-1}$ nisin) and homogenized sweet orange juice sample filled in tin cans and PET bottles

iii. Maximum level of protein (0.47 to 0.44%) and carbohydrate (10.28 to 8.44%) was observed in sweet orange juice samples preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate filled in glass bottles and stored at refrigerated condition (4±1 °C) during initial to 40^{th} days of storage period

iv. The less decrease in pH from 4.26 to 4.10 and less increase in water activity from (0.837 to 0.861) was observed in sweet orange juice samples preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate and stored at refrigerated condition (4±1 °C) in T7 from initial to 40 days of storage period

v. The maximum retention of L^*, a^*, b^* values were observed in T7, in sweet orange juice filled in glass bottles with 0.5 $mg.ml^{-1}$ of sodium benzoate and stored at refrigerated condition (4±1 °C) in T7, followed by other preservation methods and packaging materials during 40 days of storage period

vi. The minimum losses of total soluble solids (11.31 to 8.43 °Brix) and less degradation of ascorbic acid (55.96 to 49.43 $mg.ml^{-1}$) was observed in (T7) followed by other treatments

vii. The less browning index was observed in (T7) during the storage period of initial to 40 days and more retention of total carotenoids during 40 days of storage was also noticed in sweet orange juice samples preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate, filled in glass bottles at refrigerated condition (4±1 °C)

viii. The total flavonoids in sweet orange juice was found to be retained in 0.5 $mg.ml^{-1}$ of sodium benzoate and upon storage, less decrease in total flavonoids content were observed in these samples at refrigerated temperature (4±1 °C).

ix. Sensory score was also found to be more in T7 and least microbial population, followed by yeast and mould counts were observed as storage period increases from initial to 40 days of storage

x. Sweet orange juice preserved with 0.5 $mg.ml^{-1}$ of sodium benzoate filled in glass bottles and stored at a refrigerated condition (T7) recorded more shelf-life of 130 days with a benefit cost ratio of ₹1.50 upon investment

FUTURE LINE OF WORK

- Shelf-life studies of sweet orange juice using advanced non-thermal preservation techniques like pulsed electric field, ultra-filtration, cross-filtration, micro-filtration, high hydro-static pressure, UV-light, ultrasound and thermosonication
- Shelf-life studies using natural antimicrobials like lactoperoxidase, herb leaves, oils, spices, chitozan, pimaricin and organic acids
- Shelf-life studies using tetra packs, multilayer PET bottles with oxygen scavenger and liquid nitrogen drop on head space, tinplate cans of low carbon mild steel of 99.75% purity

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APPENDICES

Appendix-A

General specifications of various instruments/equipment used in the present investigation

1. Technical specifications power operated screw press type juice extractor

Sl. No.	Particulars	Specifications
1	Type	LZ-0.5
2	Capacity(t/h)	0.1-0.5 t/h
3	Power (kW)	1.5
4	Voltage (V)	380 V
5	Nt. Wt (kg)	85
6	Size (mm)	1020×300× 900
7	Filter aperture (mm)	0.6
8	Spiral diameter (mm)	Ø 88
9	Screw rotation speed	600 rpm
10	Cost	36,000

2. Technical specifications round cap top and bottom sealing machine

Sl. No.	Modal	No	Rs 8*10	Rs 10*14	Rs 14*16
1	Max. diameter of tin can to be seamed	mm	200	250	350
		inch	8	10	14
2	Max. height of tin to be seamed	mm	250	350	400
		inch	10	10	10
3	hp required	rpm 1440	0.5	1.2	0

3. Technical specifications glass bottle corking machine

Sl.No	Type	Available	Capacity
1	Hand lever operated for glass bottle corking	Pneumatic, foot, hand	150-200 bottles per day

4. Technical specifications of Homogenizer

Sl. No	Parameter	Specifications
1	Make	GOMA
2	Model No.	H-102
3	Capacity	70 lph/lpm
4	Pressure range	0-420 kg/ cm^2 (or) 0-6000 psi
5	Input power	320
6	No of stages	2

5. Technical specifications PC based double beam spectrophotometer 2302

Sl. No.	Parameters	Specifications
1	Measurement modes	% REL and % TRN
2	Measurement principles	DUAL BEAM
3	Sphere dimensions	152 mm diameter
4	Light source	Pulsed xenon
5	Wavelength range	360 nm to 740 nm
6	Wavelength interval	10nm
7	Photometric range	0-200%
8	Optical configuration	D/8 or D/0 viewing geometry
9	Wavelength accuracy	0.1 nm (Average)
10	Repeatability	Less than 0.1 DE CIELAB (white tile)
11	Inter instrument agreement (LAV)	0.15DE (Maximum)
12	Measurement time	2 sec
13	Large area view (LAV)	25.4 mm diameter

14	Medium area view (MAV)	5.0 mm×10.0 mm
15	Small area view (SAV)	12.0 mm diameter
16	Very small area view	3 mm diameter
17	UV filter	IN/OUT
18	Visible cut-off filter	420 nm/465 nm/560 nm
19	Specular component	Included/excluded
20	Operating temperature	150-320 °C
21	Relative humidity	<90% non-condensing
22	Electrical requirements	110-120V AC/50-60 Hz 230-240V AC/50-60 Hz
23	Weight	21 kg approx.
24	Size	54 cm×29 cm×23 cm

6. Technical specifications of systronics pH meter

Sl. No.	Particulars	Specifications
1	pH range	0 to 14.00
2	Resolution	0.01 pH
3	Repeatability	±0.01 pH±1 digit
4	mV range	0 to ±1999 mV
5	Resolution	1mV
6	Make	Systronics
7	Temperature range	0 to 99.9 °C
8	Resolution	0.1 °C
9	Repeatability	± 0.2 °C±1 digit
10	Power requirement	230 V AC±10%, 50 Hz, 10 VA

7. Technical specifications of rotronic water activity meter

Sl. No.	Particulars	Specifications
1	Temperature measurement	5 to 50 °C
2	Humidity measurement	0 to100% RH
	Reproducibility	
3	Humidity measurement	<0.5% RH
4	Temperature measurement	<0.2 °C
5	Long term stability	<1% RH/ year
6	Supply voltage	6 to 15 DC
7	Current consumption	12 mA
	Output signals	
8	Digital	DIO
9	Analogue	ROV: 0….1V=0..100% RH, 5…50 °C
10	Load per analogue output	>1000 Ohm
	Adjustment points	
11	Humidity	35%, 80%, 10%, 5%, or 0% RH
12	Temperature	T_{min}, T_{max}

8. Technical specifications of Zetasizer Nano Series

Sl.No	Size measurement	
1	Maximum particle size range (diameter):	0.3nm - 10µm** 0.3nm - 5µm††
2	Minimum sample volume:	12 µL 20µL
3	Minimum sample concentration:	0.1mg/mL Lysozyme 0.1ppm polystyrene latex (60nm)
4	Maximum sample concentration: Size measurement in continuous flow for use as	40%w/v*
5	SEC chromatography detector	Flow rate maximum 1mL/min

Zeta potential measurement		
1	Particle size range (diameter):	3.8nm - 100µm†
2	Zeta potential range:	No practical limits exist
3	Electrophoretic mobility range	Minimum Zero No practical upper limit exists
4	Conductivity range:	0 to 200mS/cm
5	Sample concentration range:	Up to 40%w/v† 750µL (Folded capillary cell)
6	Minimum sample volume:	750µL (Dip cell) 150µL (High concentration cell)

	Molecular weight measurement	
1	Absolute molecular weight range using Debye plot:	980Da - 2x107 Da† 9,800Da - 2x107 Da†
2	Molecular weight range estimated from Hydrodynamic diameter:	342Da - 2x107 Da†
3	Minimum sample volume:	12 µL 20 µL
4	Minimum volume for automated measurement:	4mL

* Peak mode range (diameter), 0.6nm - 8.9 microns, sample dependent

** Temperature accuracy, 0.1°C at 25°C, 0.2°C at 0°C and 0.5°C at 90°C, 1°C at 120°C

† Sample dependent

9. Technical specifications of Colour Flex Hunter's lab Colorimeter

Sl. No.	Particulars	Specifications
1	Port size	31.8 mm (45°/0°) 14.3 mm (diffuse/8°)
2	Reproducibility	0.15(Avg. DE^* on BCRA II tile set)
3	Spectral range	400–700 nm
4	Spectral resolution	10 nm
5	Spectral band pass	< 12 nm
6	Photometric	0–150%
7	Lamp life	> 500,000 flashes
8	Display (cm)	6.4×6.4 LCD
9	Colour spaces	CIE XYZ CIE L^* a^* b^*, CIE L^*, c^*, h^* Hunter *L, a, b* ΔE^*, ΔE, ΔE cmc
10	Indices	Whiteness, Yellowness, Meta merism, Tint, Brightness, ISO Greyscale, Gray Stain
11	Illuminates	A, C, D65, TL84, F2 and 10 Degree observer
12	Approximate size	36 cm (depth)×13 cm (width)×16 cm (height)
13	Weight	4.5 Kg

10. Technical specifications of digital hand-held refractometer

Sl. No.	Particulars	Specifications
1	Model	3840 (PAL-Maple)
2	Brix scale (range minimum)	0
3	Brix scale (range maximum)	85
4	Resolution	0/1 % Brix
5	Temperature compensation	50 to 212 °F (10-100 °C)
6	Temperature range	10-100 °C
7	Temperature accuracy	±1 °C
8	Temperature resolution	0.1 °C
9	Sample volume	0.3 ml
10	Response time	3 seconds
11	Product type	Refractometer (Digital)
12	Display	3 digit LCD
13	Dimensions	2” W×4-1/4”H×1-1/4” D
14	Power	2 AA batteries

APPENDIX B

Sensory analysis of freshly processed sweet orange juice Nine point hedonic scale method for organoleptic evaluation of sweet orange juice with different preservation methods and packaging materials

Description: 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.

Sl. No.	Treatments	Attributes					
		Colour and appearance	Sweetness	sourness	flavour	Aroma	Overall acceptability
1	T1						
2	T2						
3	T3						
4	T4						
5	T5						
6	T6						
7	T7						
8	T8						
9	T9						
10	T10						
11	T11						
12	T12						

Nine point hedonic scale method for organoleptic evaluation of sweet orange juice with different preservation methods and packaging materials

Description: 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.

Sl. No.	**Treatments**	**Attributes**					
		Colour and appearance	**Sweetness**	**sourness**	**flavour**	**Aroma**	**Overall acceptability**
1	T1						
2	T2						
3	T3						
4	T4						
5	T5						
6	T6						
7	T7						
8	T8						
9	T9						
10	T10						
11	T11						
12	T12						

APPENDIX- C

1. T1-Pasteurized juice filled in glass bottles and stored at ambient condition (30±2 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	:	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	:	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges	:	15,000
F	Selling price of orange juice in market per lit (Rs)		₹64
G	Total value of juice	:	**₹9,60,000**
II	**Labour cost:**	:	
A	Number of labours required Per day	:	2
B	Total no of labours required for 300days		600
C	Total labour charges (Per day/Per person @ 175)	:	**1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated screw press type juice extractor	:	**₹6,000**
B	Repair and maintenance charge of equipments	:	**₹1,000**
C	Total energy consumption for 300 days for power operated screw press type juice extractor is 3.8 kWh		**1,140**
D	Total cost of energy consumed by equipments		**₹4,560**
IV	**Packaging material cost**	:	
A	Cost of single bottle (Rs)		**₹5**
B	No of bottles required for filling 15,000 litres of juice (Nos)	:	**15,000**
C	Cost of 15,000 glass bottles (Rs) **(A*B)**	:	**₹75,000**
V	Gross returns obtained by selling 15,000 litres sweet orange juice in glass bottle	:	**₹9,60,000**
VI	Total cost incurred in obtaining 15,000 litres sweet orange juice		**₹9,41,560**
VII	**B:C ratio**	:	**₹1.01:1**

2. T2- Pasteurized juice filled in PET bottles and stored at ambient condition (30±2 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	**:**	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	**:**	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges	**:**	₹15,000
F	Selling price of orange juice in market per litre (Rs)	**:**	₹56
G	Total value of juice	**:**	**₹8,40,000**
II	**Labour cost:**		
A	Number of labours required Per day	**:**	2
B	Total no of labours required for 300 days		₹600
C	Total labour charges (Per day/Per person @ 175)	**:**	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated screw press type juice extractor	**:**	**₹6,000**
B	Repair and maintenance charge of equipments	**:**	**₹1,000**
C	Total energy consumption for 300 days (@ 3.8 kWh for power operated screw press type juice extractor)	**:**	**₹1,140**
D	Total cost of energy consumed by equipments		**₹4,560**
IV	**Packaging material cost**		
A	Cost of single PET bottle (Rs)	**:**	**₹2.5**
B	No of bottles required for filling 15,000 litres of juice (Nos)	**:**	**15,000**
C	Cost of 15,000 PET bottles (Rs)	**:**	**₹37,500**
V	Gross returns obtained by selling 15,000 litres sweet orange juice in PET bottle	**:**	**₹8,40,000**
VI	Total cost incurred in obtaining 15,000 litres sweet orange juice		**₹9,04,060**
VII	**B:C ratio**	**:**	**₹0.92:1**

3. T3-Pasteurized juice filled in tin cans and stored at ambient condition (30±2 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	**:**	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	**:**	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges	**:**	15,000
F	Selling price of orange juice in market per litre (Rs)		₹62
G	Total value of juice	**:**	**₹9,30,000**
II	**Labour cost:**	**:**	
A	Number of labours required per day	**:**	2
B	Total no of labours required for 300 days		₹600
C	Total labour charges (Per day/Per person @ 175)	**:**	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated screw press type juice extractor	**:**	**₹6,000**
B	Repair and maintenance charge of equipments	**:**	**₹1,000**
C	Total energy consumption for 300 days (@ 3.8 kWh for power operated screw press juice extractor)		**1,140**
D	Total cost of energy consumed by equipments		**₹4,560**
IV	**Packaging material cost**	**:**	
A	Cost of single tin can (Rs)		**₹4**
B	No of tin cans required for filling 15,000 litres of juice (Nos)	**:**	**15,000**
C	Cost of 15,000 tin cans (Rs)	**:**	**₹60,000**
V	Gross returns obtained by selling 15,000 litres sweet orange juice in tin cans	**:**	**₹9,30,000**
VI	Total cost incurred in obtaining 15,000 litres sweet orange juice		**₹9,26,560**
VII	**B:C ratio**	**:**	**₹1.0:1**

4. T4- Homogenized juice filled in glass bottles and stored at ambient condition (30±2 °C).

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	**:**	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	**:**	₹25
D	Total cost of sweet oranges **(B*C)**	**:**	**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges (Litres)	**:**	₹15,000
F	Selling price of orange juice in market per litre (Rs)		₹74
G	Total value of juice **(E*F)**	**:**	**₹11,10,000**
II	**Labour cost:**	**:**	
A	Number of labours required Per day	**:**	2
B	Total no of labours required for 300 days		600
C	Total labour charges (Per day/Per person @ 175)	**:**	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated screw press type juice extractor	**:**	**₹6,000**
B	Depreciated cost of homogenizer		**₹22,000**
C	Repair and maintenance charge of equipments	**:**	**₹2,000**
D	Total energy consumption for 300 days @ 3.8 and 0.17 kWh for power operated screw press type juice extractor and homogenizer per day, respectively		**1,191**
E	Total cost of energy consumed by equipments		**₹4,764**
IV	**Packaging material cost**	**:**	
A	Cost of single glass bottle (Rs)		**₹5**
B	No of bottles required for filling 15,000 litres of juice (Nos)	**:**	**15,000**
C	Cost of 15000 glass bottles (Rs) **(A*B)**	**:**	**₹75,000**
V	Gross returns obtained by selling 15,000 litre sweet orange juice in glass bottle	**:**	**₹11,10,000**
VI	Total cost incurred in obtaining 15,000 litre sweet orange juice		**₹9,64,764**
VII	**B:C ratio**	**:**	**₹1.15:1**

5. T5 - Homogenized juice filled in PET bottles and stored at ambient condition (30±2 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	:	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	:	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30000 kg of sweet oranges (Litres)	:	15,000
F	Selling price of orange juice in market per litre (Rs)		₹67
G	Total Value of Juice **(E*F)**	:	**₹10,05,000**
II	**Labour cost:**	:	
A	Number of labours required per day	:	₹2
B	Total no of labours required for 300 days		600
C	Total labour charges (Per day/Per person @ 175)	:	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of screw press juice extractor	:	**₹6,000**
B	Depreciated cost of homogenizer		**₹22,000**
D	Repair and maintenance charge of equipments	:	**₹2,000**
E	Total energy consumption for 300 days @ 3.8 and 0.17 kWh for screw press and homogenizer respectively, per day)		**1,191**
F	Total cost of energy consumed by equipments		**₹4,764**
IV	**Packaging material cost**	:	
A	Cost of single PET bottle (Rs)		**₹2.5**
B	No of bottles required for filling 15000 litres of juice (Nos)	:	**15,000**
C	Cost of 15,000 PET bottles (Rs) **(A*B)**	:	**₹37,500**
V	Gross returns obtained by selling 15,000 litre sweet orange juice in PET bottle	:	**₹10,05,000**
VI	Total cost incurred in obtaining 15,000 litre sweet orange juice		**₹9,27,264**
VII	**B:C ratio**	:	**₹1.08:1**

6. T6-Homogenized juice filled in tin cans and stored at ambient condition (30±2°C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	**:**	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	**:**	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30000 kg of sweet oranges (Litres)	**:**	15,000
F	Selling price of orange juice in market per litre (Rs)		₹70
G	Total value of Juice **(E*F)**	**:**	**₹10,50,000**
II	**Labour cost:**	**:**	
A	Number of labours required Per day	**:**	2
B	Total no of labours required for 300 days		600
C	Total labour charges (Per day/Per person @ 175)	**:**	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated power operated screw press juice extractor	**:**	**₹6,000**
B	Depreciated cost of homogenizer		**₹22,000**
C	Repair and maintenance charge of equipments	**:**	**₹2,000**
D	Total energy consumption for 300 days (@, 3.8 and 0.17 for power operated screw press and homogenizer respectively, per day)		**1,191**
E	Total cost of energy consumed by equipments		**₹4,764**
IV	**Packaging material cost**	**:**	
A	Cost of single tin can (Rs)		**₹4**
B	No of tins required for filling 15,000 litres of juice (Nos)	**:**	**15,000**
C	Cost of 15,000 tin cans (Rs) **(A*B)**	**:**	**₹60,000**
V	Gross returns obtained by selling 15,000 litres sweet orange juice in tin cans	**:**	**₹10,50,000**
VI	Total cost incurred in obtaining 15,000 litres sweet orange juice		**₹9,49,764**
VII	**B:C ratio**	**:**	**₹1.10:1**

7. T7- Juice preserved with sodium benzoate filled in glass bottles and stored at ambient condition (30±2 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	**:**	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	**:**	₹ 25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges (litres)	**:**	15,000
F	Selling price of orange juice in market per litre (Rs)		₹90
G	Total value of Juice	**:**	**₹13,50,000**
II	**Labour cost:**	**:**	
A	Number of labours required per day	**:**	2
B	Total no of labours required for 300days		600
C	Total labour charges (Per day/Per person @ 175)	**:**	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated screw press juice extractor	**:**	**₹6,000**
B	Repair and maintenance charge of equipments	**:**	**₹1,000**
C	Total energy consumption for 300 days (@ 3.8 kWh for power operated screw press type juice extractor)		**1,140**
D	Total cost of energy consumed by equipments		**₹4,560**
IV	**Preservatives cost**		
A	Cost of 1 kg of sodium benzoate (Rs)		**₹500**
B	Total quantity of sodium benzoate used for preserving 15,000 litres of juice @ 0.5 mg.ml		**7.5 kg**
C	Total cost of 7.5 kg for preserving 15,000 litres of sweet orange juice (Rs)**(A*B)**		**₹6250**
V	**Packaging material cost**	**:**	
A	Cost of single bottle (Rs)		₹5
B	No of bottles required for filling 15,000 liters of juice (Nos)	**:**	**15,000**
C	Cost of 15,000 glass bottles (Rs) **(A*B)**	**:**	**₹75000**
VI	Gross returns obtained by selling 15,000 litres sweet orange juice in glass bottle	**:**	**₹13,50,000**
VII	Total cost incurred in obtaining 15,000 litres sweet orange juice		**₹9,47,810**
VIII	**B:C ratio**	**:**	**₹1.42:1**

8. T8 - Juice preserved with sodium benzoate filled in PET bottles and stored at ambient condition (30±2 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	**:**	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	**:**	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges(litres)	**:**	15,000
F	Selling price of orange juice in market per litre (Rs)		₹84
G	Total value of juice	**:**	**₹12,60,000**
II	**Labour cost:**	**:**	
A	Number of labours required Per day	**:**	2
B	Total no of labours required for 300days		600
C	Total labour charges (Per day/Per person @ 175)	**:**	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated screw press juice extractor	**:**	**₹6,000**
B	Repair and maintenance charge of equipments	**:**	**₹1,000**
C	Total energy consumption for 300 days (@ 3.8 kWh for power operated screw press type juice extractor per day)		**1,140**
D	Total cost of energy consumed by equipments		**₹4,560**
IV	**Preservatives cost**		
A	Cost of 1 kg of sodium benzoate (Rs)		**₹500**
B	Total quantity of sodium benzoate used for preserving 15,000 litres of juice @ 0.5 mg.ml		**7.5 kg**
C	Total cost of 7.5 kg for preserving 15000 litres of sweet orange juice (Rs)**(A*B)**		**₹6,250**
V	**Packaging material cost**	**:**	
A	Cost of single PET bottle (Rs)		**₹2.5**
B	No of bottles required for filling 15,000 litres of juice (Nos)	**:**	**15,000**
C	Cost of 15,000 PET bottles (Rs)	**:**	**₹37,500**
VI	Gross returns obtained by selling 15,000 litres sweet orange juice in PET bottles	**:**	**₹12,60,000**
VII	Total cost incurred in obtaining 15,000 litres sweet orange juice	**:**	**₹9,10,310**
VIII	**B:C ratio**	**:**	**₹1.38:1**

9. T9- Juice samples preserved with sodium benzoate filled in tin cans and stored at ambient condition (30±2 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	**:**	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	**:**	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges (litres)	**:**	15,000
F	Selling price of orange juice in market per litre (Rs)		₹88
G	Total value of juice	**:**	**₹13,20,000**
II	**Labour cost:**	**:**	
A	Number of labours required per day	**:**	2
B	Total no of labours required for 300days		600
C	Total labour charges (Per day/Per person @ 175)	**:**	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated screw press juice extractor	**:**	**₹6000**
B	Repair and maintenance charge of equipments	**:**	**₹1,000**
C	Total energy consumption for 300 days (@ 10 kWh for refrigerator per day and 3.8 for power operated screw press juice extractor)		**1,140**
D	Total cost of energy consumed by equipments		**₹4,560**
IV	**Preservatives cost**		
A	Cost of 1 kg of sodium benzoate (Rs)		**₹500**
B	Total quantity of sodium benzoate used for preserving 15000 litres of juice @ 0.5 mg.ml		**7.5 kg**
C	Total cost of 7.5 kg for preserving 15,000 litres of sweet orange juice (Rs)**(A*B)**		**₹6250**
V	**Packaging material cost**	**:**	
A	Cost of single tin can (Rs)		**₹4**
B	No of tin cans required for filling 15,000 litres of juice (Nos)	**:**	**15,000**
C	Cost of 15,000 tin cans (Rs)	**:**	**₹60,000**
VI	Gross returns obtained by selling 15,000 litres sweet orange juice in tin cans	**:**	**₹13,20,000**
VII	Total cost incurred in obtaining 15,000 litres sweet orange juice		**₹9,32,810**
VIII	**B:C ratio**	**:**	**₹1.41:1**

10. T10- Juice samples preserved with nisin filled in glass bottle and stored at ambient condition (30±2 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	**:**	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	**:**	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges	**:**	15,000
F	Selling price of orange juice in market per litre (Rs)		₹82
G	Total value of juice	**:**	**₹12,30,000**
II	**Labour cost:**	**:**	
A	Number of labours required Per day	**:**	2
B	Total no of labours required for 300days		600
C	Total labour charges (Per day/Per person @ 175)	**:**	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated screw press juice extractor	**:**	**₹6,000**
B	Repair and maintenance charge of equipments	**:**	**₹1,000**
C	Total energy consumption for 300 days (@ 3.8 kWh for power operated screw press type juice extractor)		**1,140**
D	Total cost of energy consumed by equipments		**₹4,560**
IV	**Preservatives cost**		
A	Cost of bio preservative nisin for 5g (Rs)		**₹9,000**
B	Quantity of nisin required for processing 15000 litres of sweet orange juice @ 0.001 $mg.ml^{-1}$		**15g**
C	Total cost of 15 g of nisin for preserving 15000 litres of sweet orange juice (Rs)		**₹27,000**
V	**Packaging material cost**	**:**	
A	Cost of single bottle (Rs)		₹5
B	No of bottles required for filling 15,000 litres of juice (Nos)	**:**	**15,000**
C	Cost of 15,000 glass bottles (Rs) **(A*B)**	**:**	**₹75,000**
VI	Gross returns obtained by selling 15,000 litres sweet orange juice in glass bottle	**:**	**₹12,30,000**
VII	Total cost incurred in obtaining 15,000 litres sweet orange juice		**₹9,77,560**
VIII	**B:C ratio**	**:**	**1.28:1**

11. T11- Juice samples preserved with nisin filled in PET bottle and stored at ambient condition (30±2 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	:	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	:	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges (litres)	:	₹15,000
F	Selling price of orange juice in market per litre (Rs)		₹75
G	Total value of juice	:	**₹11,25,000**
II	**Labour cost:**	:	
A	Number of labours required per day	:	2
B	Total no of labours required for 300 days		600
C	Total labour charges (Per day/Per person @ 175)	:	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated Cost of power operated screw press juice extractor	:	**₹6,000**
B	Repair and maintenance charge of equipments	:	**₹1,000**
C	Total energy consumption for 300 days (@ 10 kWh for refrigerator Per day and 3.8 for power operated screw press juice extractor)	:	**1,140**
D	Total cost of energy consumed by equipments	:	**₹4,560**
IV	**Preservatives cost**		
A	Cost of bio preservative nisin for 5g (Rs)	:	**₹9,000**
B	Quantity of nisin required for processing 15000 litres of sweet orange juice @ 0.001 mg.ml	:	**15g**
C	Total cost of 15 g of nisin for preserving 15000 litres of sweet orange juice (Rs)	:	**₹27,000**
V	**Packaging material cost**	:	
A	Cost of single PET bottle (Rs)		**₹2.5**
B	No of bottles required for filling 15,000 litres of juice (Nos)	:	**15,000**
C	Cost of 15,000 PET bottles (Rs)	:	**₹37,500**
VI	Gross returns obtained by selling 15,000 litres sweet orange juice in PET bottle	:	**₹11,25,000**
VII	Total cost incurred in obtaining 15,000 litres sweet orange juice	:	**₹9,31,060**
VIII	**B:C ratio**	:	**₹1.20:1**

12. T12- Juice samples preserved with nisin filled in tin cans and stored at ambient condition (30±2 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	:	100
B	**Total quantity handled (kg)**		₹30,000
C	Cost of sweet oranges per kg (Rs)	:	25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30000 kg of sweet oranges(litres)	:	15000
F	Selling price of orange juice in market per litre (Rs)		₹78
G	Total value of juice	:	**₹11,70,000**
II	**Labour cost:**	:	
A	Number of labours required Per day	:	2
B	Total no of labours required for 300days		600
C	Total labour charges (Per day/Per person @ 175)	:	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated screw press type juice extractor	:	**₹6,000**
B	Repair and maintenance charge of equipments	:	**₹1,000**
C	Total energy consumption for 300 days (@ 3.8 kWh for power operated screw press type juice extractor)		**1,140**
D	Total cost of energy consumed by equipments		**₹4,560**
IV	**Preservatives cost**		
A	Cost of bio preservative nisin for 5g (Rs)		**₹9,000**
B	Quantity of nisin required for processing 15,000 liters of sweet orange juice @ 0.001 mg.ml		**15g**
C	Total cost of 15 g of nisin for preserving 15,000 liters of sweet orange juice (Rs)		**27,000**
V	**Packaging material cost**	:	
A	Cost of single tin can (Rs)		**₹4**
B	No of tins required for filling 15,000 litres of juice (Nos)	:	**₹15,000**
C	Cost of 15,000 tin cans (Rs) **(A*B)**	:	**₹60,000**
VI	Gross returns obtained by selling 15,000 litres sweet orange juice in tin cans	:	**₹11,70,000**
VII	Total cost incurred in obtaining 15,000 litres sweet orange juice		**₹9,53,560**
VIII	**B:C ratio**	:	**₹1.22:1**

Estimate for Annual (300 Working Days)

1. T1- Pasteurized juice filled in glass bottles and stored at refrigerated condition (4±1 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	:	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	:	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges	:	15,000
F	Selling price of orange juice in market per litre (Rs)		₹78
G	Total value of juice	:	**₹11,70,000**
II	**Labour cost:**	:	
A	Number of labours required per day	:	2
B	Total no of labours required for 300 days		600
C	Total labour charges (Per day/Per person @ 175)	:	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated type screw press juice extractor	:	**₹6,000**
B	Depreciated cost of refrigerator	:	**₹41,666**
C	Repair and maintenance charge of equipments	:	**₹1,360**
D	Total energy consumption for 300 days (@ 10 kWh for refrigerator Per day and 3.8 for power operated screw press type juice extractor)		**4,140**
E	Total cost of energy consumed by equipments		**₹16,560**
IV	**Packaging material cost**	:	
A	Cost of single bottle (Rs)		₹5
B	No of bottles required for filling 15,000 litres of juice (Nos)	:	**15,000**
C	Cost of 15000 glass bottles (Rs) **(A*B)**	:	**₹75,000**
V	Gross returns obtained by selling 15,000 litres sweet orange juice in glass bottle	:	**₹11,70,000**
VI	Total cost incurred in obtaining 15,000 litres sweet orange juice		**₹9,95,586**
VII	**B:C ratio**	:	**₹1.17:1**

2. T2 – Pasteurized juice filled in PET bottles and stored at refrigerated condition (4±1 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	**:**	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	**:**	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges	**:**	₹15,000
F	Selling price of orange juice in market per litre (Rs)	**:**	₹72
G	Total value of Juice	**:**	**₹10,80,000**
II	**Labour cost:**		
A	Number of labours required per day	**:**	2
B	Total no of labours required for 300 days		₹600
C	Total labour charges (Per day/Per person @ 175)	**:**	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of screw press juice extractor	**:**	**₹6,000**
B	Depreciated cost of Refrigerator	**:**	**₹41,666**
C	Repair and maintenance charge of Equipments	**:**	**₹1,360**
D	Total energy consumption for 300 days (@ 10 kWh for refrigerator Per day and 3.8 for power operated screw press type juice extractor)		**4,140**
E	Total cost of energy consumed by equipments		**₹16,560**
IV	**Packaging material cost**	**:**	
A	Cost of single PET bottle (Rs)		**₹2.5**
B	No of bottles required for filling 15,000 litres of juice (Nos)	**:**	**15,000**
C	Cost of 15,000 PET bottles (Rs)	**:**	**₹37,500**
V	Gross returns obtained by selling 15,000 litres sweet orange juice in PET bottles	**:**	**₹10,80,000**
VI	Total cost incurred in obtaining 15,000 litres sweet orange juice		**₹9,58,086**
VII	**B:C ratio**	**:**	**₹1.12:1**

3. T3- Pasteurized juice filled in tin cans and stored at refrigerated condition (4±1 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	:	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	:	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges	:	15,000
F	Selling price of orange juice in market per litre (Rs)		₹75
G	Total value of juice	:	**₹11,25,000**
II	**Labour cost:**	:	
A	Number of labours required per day	:	2
B	Total no of labours required for 300 days		₹600
C	Total labour charges (Per day/Per person @ 175)	:	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated screw press juice extractor	:	**₹6,000**
B	Depreciated cost of refrigerator	:	**₹41,666**
C	Repair and maintenance charge of Equipments	:	**₹1,360**
D	Total energy consumption for 300 days (@ 10 kWh for refrigerator Per day and 3.8 for power operated screw press type juice extractor)		**4,140**
E	Total cost of energy consumed by equipments		**₹16,560**
IV	**Packaging material cost**	:	
A	Cost of single tin can (Rs)		**₹4**
B	No of tin cans required for filling 15,000 litres of juice (Nos)	:	**15,000**
C	Cost of 15,000 tin cans (Rs)	:	**₹60,000**
V	Gross returns obtained by selling 15,000 litres sweet orange juice in tin cans	:	**₹11,25,000**
VI	Total cost incurred in obtaining 15,000 litres sweet orange juice		**₹9,80,586**
VII	**B:C ratio**	:	**₹1.14:1**

4. T4- Homogenized juice sample filled in glass bottles and stored at refrigerated condition (4±1 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	:	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	:	₹25
D	Total cost of sweet oranges **(B*C)**	:	**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges (Litres)	:	₹15,000
F	Selling price of orange juice in market per litre (Rs)		₹85
G	Total value of juice **(E*F)**	:	**₹12,75,000**
II	**Labour cost:**	:	
A	Number of labours required per day	:	2
B	Total no of labours required for 300days		600
C	Total labour charges (Per day/Per person @ 175)	:	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated screw press type juice extractor	:	**₹6,000**
B	Depreciated cost of homogenizer		**₹22,000**
C	Depreciated cost of refrigerator		**₹41,666**
D	Repair and maintenance charge of equipments	:	**₹2,360**
E	Total energy consumption for 300 days @ 10 kWh for refrigerator Per day, 3.8 and 0.17 for power operated screw press juice type extractor and homogenizer respectively)		**4.191**
F	Total cost of energy consumed by equipments		**₹16,764**
IV	**Packaging material cost**	:	
A	Cost of single glass bottle (Rs)		**₹5**
B	No of bottles required for filling 15,000 litres of juice (Nos)	:	**15,000**
C	Cost of 15,000 glass bottles (Rs) **(A*B)**	:	**₹75,000**
V	Gross returns obtained by selling 15,000 litre sweet orange juice in glass bottle	:	**₹12,75,000**
VI	Total cost incurred in obtaining 15,000 litre sweet orange juice		**₹10,18,790**
VII	**B:C ratio**	:	**₹1.25:1**

5. T5 - Homogenized juice sample filled in PET bottles and stored at refrigerated condition (4±1 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	:	100
B	**Total Quantity handled (kg)**		30,000
C	Cost of sweet oranges per Kg (Rs)	:	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges (Litres)	:	15000
F	Selling price of orange juice in market per litre (Rs)		₹82
G	Total value of juice **(E*F)**	:	**₹12,30,000**
II	**Labour cost:**	:	
A	Number of labours required per day	:	₹2
B	Total no of labours required for 300days		600
C	Total Labour charges (Per day/Per person @ 175)	:	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated screw press type juice extractor	:	**₹6,000**
B	Depreciated cost of homogenizer		**₹22,000**
C	Depreciated cost of refrigerator		**₹41,666**
D	Repair and maintenance charge of equipments	:	**₹2,360**
E	Total energy consumption for 300 days (@ 10 kWh for refrigerator Per day, 3.8 and 0.17 for power operated screw press type juice extractor and homogenizer respectively)		**4191**
F	Total cost of energy consumed by equipments		**₹16,764**
IV	**Packaging material cost**	:	
A	Cost of single PET bottle (Rs)		**₹2.5**
B	No of bottles required for filling 15000 litres of juice (Nos)	:	**15,000**
C	Cost of 15,000 PET bottles (Rs) **(A*B)**	:	**₹37,500**
V	Gross returns obtained by selling 15,000 litres sweet orange juice in PET bottle	:	**₹12,30,000**
VI	Total cost incurred in obtaining 15,000 litre sweet orange juice		**₹9,81,290**
VII	**B:C ratio**	:	**₹1.20:1**

6: T6- Homogenized juice sample filled in tin cans and stored at refrigerated condition (4±1 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	**:**	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	**:**	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30000 kg of sweet oranges (Litres)	**:**	15,000
F	Selling price of orange juice in market per litre (Rs)		₹80
G	Total value of juice **(E*F)**	**:**	**₹12,00,000**
II	**Labour cost:**	**:**	
A	Number of labours required per day	**:**	2
B	Total no of labours required for 300days		600
C	Total labour charges (Per day/Per person @ 175)	**:**	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated power operated screw press type juice extractor	**:**	**₹6,000**
B	Depreciated cost of homogenizer		**₹22,000**
C	Depreciated cost of refrigerator		**₹41,666**
D	Repair and maintenance charge of equipments	**:**	**₹2,360**
E	Total energy consumption for 300 days (@ 10 kWh for refrigerator Per day, 3.8 and 0.17 for power operated screw press type juice extractor and homogenizer respectively)		**4,191**
F	Total cost of energy consumed by equipments		**₹16,764**
IV	**Packaging material cost**	**:**	
A	Cost of single tin can (Rs)		**₹4**
B	No of tins required for filling 15,000 litres of juice (Nos)	**:**	**15,000**
C	Cost of 15,000 tin cans (Rs) **(A*B)**	**:**	**₹60,000**
V	Gross returns obtained by selling 15000 litres sweet orange juice in tin cans	**:**	**₹12,00,000**
VI	Total cost incurred in obtaining 15,000 litres sweet orange juice		**₹10,03,790**
VII	**B:C ratio**	**:**	**₹1.22:1**

7. T7- Juice samples preserved with sodium benzoate filled in glass bottles and stored at refrigerated condition (4±1 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	:	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	:	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges (litres)	:	15,000
F	Selling price of orange juice in market per litre (Rs)		₹100
G	Total value of juice	:	**₹15,00,000**
II	**Labour cost:**	:	
A	Number of labours required per day	:	2
B	Total no of labours required for 300 days		600
C	Total labour charges (Per day/Per person @ 175)	:	**₹10,5000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated screw press type juice extractor	:	**₹6,000**
B	Depreciated cost of refrigerator	:	**₹41,666**
C	Repair and maintenance charge of equipments	:	**₹1,360**
D	Total Energy consumption for 300 days (@ 10 kWh for refrigerator Per day and 3.8 for power operated power operated screw press type juice extractor)		**4,140**
E	Total cost of energy consumed by equipments		**₹16,560**
IV	**Preservatives cost**		
A	Cost of 1 kg of sodium benzoate (Rs)		**₹500**
B	Total quantity of sodium benzoate used for preserving 15000 litres of juice @ 0.5 mg.ml		**7.5 kg**
C	Total cost of 7.5 kg for preserving 15,000 litres of sweet orange juice (Rs)**(A*B)**		**₹6250**
V	**Packaging material cost**	:	
A	Cost of single bottle (Rs)		₹5
B	No of bottles required for filling 15,000 litres of juice (Nos)	:	**15,000**
C	Cost of 15000 glass bottles (Rs) **(A*B)**	:	**₹75,000**
VI	Gross returns obtained by selling 15,000 litres sweet orange juice in glass bottle	:	**₹15,00,000**
VII	Total cost incurred in obtaining 15,000 litres sweet orange juice		**₹10,01,836**
VIII	**B:C ratio**	:	**₹1.50:1**

8. T8- Juice samples preserved with sodium benzoate filled in PET bottles and stored at refrigerated condition (4±1 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	**:**	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	**:**	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges(litres)	**:**	15,000
F	Selling price of orange juice in market per litre (Rs)		₹94
G	Total value of juice	**:**	**₹14,10,000**
II	**Labour cost:**	**:**	
A	Number of labours required Per day	**:**	2
B	Total no of labours required for 300days		600
C	Total labour charges (Per day/Per person @ 175)	**:**	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated screw press type juice extractor	**:**	**₹6,000**
B	Depreciated cost of Refrigerator	**:**	**₹41,666**
C	Repair and maintenance charge of equipments	**:**	**₹1,360**
D	Total energy consumption for 300 days (@ 10 kWh for refrigerator per day and 3.8 for power operated screw press type juice extractor)		**4,140**
E	Total cost of energy consumed by equipments		**₹16,560**
IV	**Preservatives cost**		
A	Cost of 1 kg of sodium benzoate (Rs)		**₹500**
B	Total quantity of sodium benzoate used for preserving 15,000 litres of juice @ 0.5 mg.ml		**7.5 kg**
C	Total cost of 7.5 kg for preserving 15,000 litres of sweet orange juice (Rs)**(A*B)**		**₹6,250**
V	**Packaging material cost**	**:**	
A	Cost of single PET bottle (Rs)		**₹2.5**
B	No of bottles required for filling 15,000 litres of juice (Nos)	**:**	**15,000**
C	Cost of 15,000 PET bottles (Rs)	**:**	**₹37,500**
VI	Gross returns obtained by selling 15,000 litres sweet orange juice in PET bottle	**:**	**₹14,10,000**
VII	Total cost incurred in obtaining 15,000 litres sweet orange juice	**:**	**₹9,64,336**
VIII	**B:C ratio**	**:**	**₹1.46:1**

9. T9- Juice samples preserved with sodium benzoate filled in tin cans and stored at refrigerated condition (4±1 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	**:**	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	**:**	₹25
D	Total cost of sweet oranges **(B*C)**		**₹75,0000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges (litres)	**:**	15,000
F	Selling price of orange juice in market per litre (Rs)		₹97
G	Total value of juice	**:**	**₹14,55,000**
II	**Labour cost:**	**:**	
A	Number of labours required per day	**:**	2
B	Total no of labours required for 300days		600
C	Total labour charges (Per day/Per person @ 175)	**:**	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated screw press type juice extractor	**:**	**₹6,000**
B	Depreciated cost of refrigerator	**:**	**₹41,666**
C	Repair and maintenance charge of equipments	**:**	**₹1,360**
D	Total energy consumption for 300 days (@ 10 kWh for refrigerator per day and 3.8 for power operated screw press type juice extractor)		**4,140**
E	Total cost of energy consumed by equipments		**₹16,560**
IV	**Preservatives cost**		
A	Cost of 1 kg of sodium benzoate (Rs)		**₹500**
B	Total quantity of sodium benzoate used for preserving 15,000 litres of juice @ 0.5 mg.ml		**7.5 kg**
C	Total cost of 7.5 kg for preserving 15,000 litres of sweet orange juice (Rs)**(A*B)**		**₹6,250**
V	**Packaging material cost**	**:**	
A	Cost of single tin can (Rs)		**₹4**
B	No of tin cans required for filling 15,000 litres of juice (Nos)	**:**	**15,000**
C	Cost of 15,000 tin cans (Rs)	**:**	**₹60,000**
VI	Gross returns obtained by selling 15,000 litres sweet orange juice in tin cans	**:**	**₹14,55,000**
VII	Total cost incurred in obtaining 15,000 litres sweet orange juice		**₹9,86,836**
VIII	**B:C ratio**	**:**	**₹1.47:1**

10. T10- Juice samples preserved with nisin filled in glass bottle and stored at refrigerated condition (4±1 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	**:**	100
B	**Total quantity handled (kg)**		30,000
C	Cost of sweet oranges per kg (Rs)	**:**	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges	**:**	15,000
F	Selling price of orange juice in market per litre (Rs)		₹92
G	Total value of juice	**:**	**₹13,80,000**
II	**Labour cost:**	**:**	
A	Number of labours required per day	**:**	2
B	Total no of labours required for 300days		600
C	Total labour charges (Per day/Per person @ 175)	**:**	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated screw press type juice extractor	**:**	**₹6,000**
B	Depreciated cost of refrigerator	**:**	**₹41,666**
C	Repair and maintenance charge of equipments	**:**	**₹1,360**
D	Total energy consumption for 300 days (@ 10 kWh for refrigerator per day and 3.8 for power operated screw press type juice extractor)	**:**	**4,140**
E	Total cost of energy consumed by equipments		**₹16,560**
IV	**Preservatives cost**		
A	Cost of Bio preservative nisin for 5g (Rs)		**₹9,000**
B	Quantity of nisin required for processing 15,000 litres of sweet orange juice @ 0.001 mg.ml		**15g**
C	Total cost of 15 g of nisin for preserving 15,000 liters of sweet orange juice (Rs)		**₹27,000**
V	**Packaging material cost**	**:**	
A	Cost of single bottle (Rs)		₹5
B	No of bottles required for filling 15,000 litres of juice (Nos)	**:**	**15,000**
C	Cost of 15,000 glass bottles (Rs) **(A*B)**	**:**	**₹75,000**
VI	Gross returns obtained by selling 15,000 litres sweet orange juice in glass bottle	**:**	**₹13,80,000**
VII	Total cost incurred in obtaining 15,000 litres sweet orange juice		**₹10,22,586**
VIII	**B:C ratio**	**:**	**₹1.35:1**

11. T11- Juice samples preserved with nisin filled in PET bottle and stored at refrigerated condition (4±1 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	**:**	100
B	**Total Quantity handled (kg)**		30,000
C	Cost of sweet oranges per Kg (Rs)	**:**	₹25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges (litres)	**:**	₹15,000
F	Selling price of orange juice in market per litre (Rs)		₹ 88
G	Total value of juice	**:**	**₹1,32,0000**
II	**Labour cost:**	**:**	
A	Number of labours required Per day	**:**	2
B	Total no of labours required for 300days		600
C	Total labour charges (Per day/Per person @ 175)	**:**	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated Cost of power operated screw press type juice extractor	**:**	**₹6,000**
B	Depreciated cost of refrigerator	**:**	**₹41,666**
C	Repair and maintenance charge of Equipments	**:**	**₹1,360**
D	Total energy consumption for 300 days (@ 10 kWh for refrigerator per day and 3.8 for power operated screw press type juice extractor)	**:**	**4,140**
E	Total cost of energy consumed by equipments	**:**	**₹16,560**
IV	**Preservatives cost**		
A	Cost of bio preservative nisin for 5g (Rs)	**:**	**₹9,000**
B	Quantity of nisin required for processing 15000 litres of sweet orange juice @ 0.001 mg.ml	**:**	**15g**
C	Total cost of 15 g of nisin for preserving 15000 litres of sweet orange juice (Rs)	**:**	**₹27,000**
V	**Packaging material cost**	**:**	
A	Cost of single PET bottle (Rs)		**₹2.5**
B	No of bottles required for filling 15,000 litres of juice (Nos)	**:**	**15,000**
C	Cost of 15,000 PET bottles (Rs)	**:**	**₹37,500**
VI	Gross returns obtained by selling 15,000 litres sweet orange juice in PET bottle	**:**	**₹13,20,000**
VII	Total cost incurred in obtaining 15,000 litres sweet orange juice	**:**	**₹9,85,086**
VIII	**B:C ratio**	**:**	**₹1.33:1**

12. T12- Juice samples preserved with nisin filled in tin cans and stored at refrigerated condition (4±1 °C)

I	**No of working days**		300
A	Quantity of sweet oranges handled per day	**:**	100
B	**Total quantity handled (kg)**		₹30,000
C	Cost of sweet oranges per kg (Rs)	**:**	25
D	Total cost of sweet oranges **(B*C)**		**₹7,50,000**
E	Total quantity of juice obtained by processing 30,000 kg of sweet oranges (litres)	**:**	15000
F	Selling price of orange juice in market per litre (Rs)		₹90
G	Total value of juice	**:**	**₹13,50,000**
II	**Labour cost:**	**:**	
A	Number of labours required Per day	**:**	2
B	Total no of labours required for 300days		600
C	Total labour charges (Per day/Per person @ 175)	**:**	**₹1,05,000**
III	**Cost of equipments used for processing sweet orange juice**		
A	Depreciated cost of power operated screw press type juice extractor	**:**	**₹6,000**
B	Depreciated cost of refrigerator	**:**	**₹41,666**
C	Repair and maintenance charge of equipments	**:**	**₹1,360**
D	Total energy consumption for 300 days (@ 10 kWh for refrigerator Per day and 3.8 for power operated screw press type juice extractor)		**4140**
E	Total cost of energy consumed by equipments		**₹16,560**
IV	**Preservatives cost**		
A	Cost of bio preservative nisin for 5g (Rs)		**₹9,000**
B	Quantity of nisin required for processing 15,000 liters of sweet orange juice @ 0.001 mg.ml		**15g**
C	Total cost of 15 g of nisin for preserving 15,000 liters of sweet orange juice (Rs)		**27,000**
V	**Packaging material cost**	**:**	
A	Cost of single tin Can (Rs)		**₹4**
B	No of tins required for filling 15,000 litres of juice (Nos)	**:**	**₹15,000**
C	Cost of 15,000 tin cans (Rs) **(A*B)**	**:**	**₹60,000**
VI	Gross returns obtained by selling 15,000 litres sweet orange juice in tin cans	**:**	**₹13,50,000**
VII	Total cost incurred in obtaining 15,000 litres sweet orange juice		**₹10,07,586**
VIII	**B:C ratio**	**:**	**₹1.34:1**