

**USE OF SUPERCRITICAL CO<sub>2</sub> EXTRACTED CAROTENOIDS  
FROM TOMATO POMACE IN SELECTED FOOD PRODUCTS**

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# Use of Supercritical CO<sub>2</sub> Extracted Carotenoids from Tomato Pomace in Selected Food Products

## ABSTRACT

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Tomato (*Lycopersicon esculentum*) is a fruit used mainly as a vegetable both in fresh as well as in processed forms. Lycopene, an important carotenoid in tomatoes is responsible for the red colour in tomatoes. The antioxidant capability of lycopene has led to promising results in decreasing the risk of some illnesses and diseases. Tomatoes are processed for the production of many useful food items such as juice, paste, puree, catsup, sauce, etc. Tomato pomace is the main by-product of such processing and does not have high economic utilization at present. The tomato processing waste (pomace) having peel, some pulp and seed has a high amount of lycopene and hence, is considered to be the potential raw material for production of natural lycopene. However, the pomace is a high moisture by-product and therefore, it is require to dry it appropriately for preservation and then used for lycopene extraction.

Supercritical fluid extraction (SFE) has emerged as a highly promising environmentally benign technology for selectively recovering thermally labile bioactive ingredients from natural sources. The nutraceutical produced by SFE using carbon dioxide (CO<sub>2</sub>) at near-ambient temperatures are preferred by consumers due to their superior quality and higher bioactivity without the problems of residual solvent and microbial contamination.

Lycopene is a natural antioxidant and having functional and nutraceutical properties as well as can be used as a natural colouring agent in food. It can be satisfactorily intended in the food systems as a functional ingredient.

The tomato carotenoid extract was characterized for its chemical properties and utilized in three different products i.e. ice cream, filling cream (biscuit) and jam. The optimization of level of incorporation of lycopene extract was carried by sensory analysis and colour measurement. The incorporation level of 0.1 % in ice cream, 0.6 % in filling cream (biscuit) and 0.08 % in jam was optimised. The stability of lycopene content was found to be greater in ice cream as compare to jam and filling cream (biscuit). The maximum lycopene content was observed initially in the ice

cream (0.151 mg/100g). The negligible decreasing trend of lycopene content was observed after 35 days to 49 days. The minimum lycopene content 0.148 mg/100g was observed at 49<sup>th</sup> days of storage. The lycopene content in filling cream (biscuit) and jam was 0.879 mg/100g and 0.124 mg/100g in the fresh sample respectively and during storage period, the lycopene content was decreased and observed 0.731 mg/100g and 0.091 mg/100g at 49<sup>th</sup> day of storage.

**KEY WORDS:** *Tomato pomace, drying, super critical fluid extraction, tomato carotenoid extract.*

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**CERTIFICATE**

This is to certify that the thesis entitled “**USE OF SUPERCRITICAL CO<sub>2</sub> EXTRACTED CAROTENOIDS FROM TOMATO POMACE IN SELECTED FOOD PRODUCTS**” submitted by Mr. **PIYUSHBHAI CHAMPAKSINH JADAV (Reg. No. 04-2030-2012)** in partial fulfillment of the requirement for the award of the degree of **Master of Technology in Food Processing Technology** to the Anand Agricultural University is a record of bonafide research work carried out by him under my guidance and supervision.

**Place : Anand**

**(S.H. Akbari)**

**Date : / /2014**

**Major Advisor**

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Date: - -2014

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(Piyush C. Jadav)

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## ABBREVIATIONS AND SYMBOLS

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| <b>Particular</b> | <b>Description</b>                     |
|-------------------|----------------------------------------|
| AI                | Active ingredient                      |
| ANOVA             | Analysis of Variance                   |
| CD                | critical difference                    |
| CO <sub>2</sub>   | carbon dioxide                         |
| CRD               | Completely Randomized Design           |
| CV                | Co-efficient of Variance               |
| DCM               | Dichloromethane                        |
| d.b.              | dry basis                              |
| D.F               | degree of Freedom                      |
| <i>et al.</i>     | et ally                                |
| e.g.              | example gratia                         |
| etc.              | et cetera                              |
| Fig.              | Figure                                 |
| g                 | Gram                                   |
| HPLC              | High Performance Liquid Chromatography |
| i.e.              | that is                                |
| kg                | Kilogram                               |
| L                 | Liter                                  |
| SE                | Solvent extraction                     |
| SEM               | Mean Square of Error                   |
| SFE               | Supercritical fluid extraction         |
| m.c.              | moisture content                       |
| MPa               | Mega Pascal                            |
| mg                | Milligram                              |

|        |                                |
|--------|--------------------------------|
| ml     | Milliliter                     |
| nm     | nano meter                     |
| P      | Pressure                       |
| p.     | Page                           |
| SE     | Solvent Extraction             |
| SFE    | Supercritical Fluid Extraction |
| S.S.   | Sum of Squares                 |
| T      | Temperature                    |
| UV-VIS | Ultra violet visible           |
| Vol.   | Volume                         |
| m/s    | meter per second               |
| mg/L   | milligram per litre            |
| v/v    | volume by volume               |
| w.b.   | wet basis                      |
| °C     | degree Celsius                 |
| %      | per cent                       |

# CHAPTER I

## INTRODUCTION

---

Tomato (*Lycopersicon esculentum*) is a fruit used mainly as a vegetable both in fresh as well as in processed form, in food preparations. More than 80% of processed tomatoes produced are consumed in the form of tomato juice, paste, puree, catch up and sauce (Gould, 1992). Tomatoes have been traditionally credited as rich source of carotenoids and vitamins, particularly beta-carotene, pro-vitamin A and vitamin C (Hanson *et al.*, 2004; Shi and Maguer, 2001).

In recent years, another important carotenoid in tomatoes, lycopene, has received considerable attention. Lycopene is responsible for the red colour in tomatoes (Rao and Agarwal, 2000). Tomatoes and tomato products are the major sources of lycopene compounds which can represent more than 85% of all the carotenoids present in the fruits. The antioxidant capability of lycopene has led to promising results in decreasing the risk of some illness and cancers (Delgado and paredes, 2003).

Lycopene extract from tomato is intended for use as a food colour. It provides the similar colour shades, ranging from yellow to red, as do the natural and synthetic lycopenes. Lycopene extract from tomato is also used as a food/dietary supplement in products where the presence of lycopene provides a specific value (e.g., antioxidant or other claimed health benefits).

Lycopene is presently of particular interest for the food and pharmaceutical industries, both as natural colourant and as a nutraceutical. People knowledge and curiosity of the health benefits of lycopene have skyrocketed. Consumer demand for healthful food products and demand for clean-label declarations provides an opportunity to develop a market for food-grade and pharmaceutical-grade lycopene products. Industrial production of lycopene from tomatoes is of great interest to pharmaceutical and food companies, as a nutraceutical and for functional food development.

Lycopene extract from tomato is intended for use in the food categories like, baked foods, breakfast cereals, dairy products including frozen dairy desserts, spreads, carbonated beverages, fruits and vegetable juices, candy, soups, salad dressings and other foods.

Lycopene is a natural antioxidant and having functional and nutraceutical properties as well as can be used as a natural colouring agent in food. It can be satisfactorily intended in the food systems as a functional ingredient.

Tomato skins can be a viable source of lycopene, as tomato peels contain about five times more lycopene than the whole tomato pulp per unit mass. Presently more than one third of the tomatoes delivered to processing plants ends as processing wastes, mainly constituted by seeds and skins. The recovery of this carotenoid could represent an alternative for the valorisation of the by-products of the tomato industry (Sharma and Maguer, 1996). By-products and waste materials from the food processing industry can be a money spinner if they are appropriately utilized using newer knowledge and technologies (Potty, 2009).

Commercial processing of tomato produces a large amount of waste at various stages. Tomato pomace constitutes the major part of the waste that comes from the pulper. The wet pomace contains 33% seed, 27% skin and 40% pulp (Sogi and Bawa, 1998). Tomato processing waste is rich in bioactive compounds, e.g., lycopene and other carotenoids, tomato fiber and tomato oil (tocopherols) from seeds. At present, pomace (peels and seeds) in tomato industry is mechanically removed and discarded.

Lycopene has received great attention in recent years because of its beneficial effect in the treatment of diseases such as skin cancer (Ribayo *et al.*, 1995) and prostate cancer (Steven and Clinton, 1998). Several recent studies indicated the effect of dietary lycopene in reducing the risk of various chronic cancers, such as prostate, lung, breast, colon, pancreas, stomach, rectum, esophagus, oral cavity and cervix (Agarwal and Rao, 2000; Omoni and Aluko, 2005). Lycopene has been shown to act as a potent antioxidant. There are also a number of other mechanisms for lycopene, such as gap-junction communication, hypocholesteromic, anti-cancerous and anti-

atherogenic effects, that may reduce the risk of various chronic diseases. Thus, lycopene acts by various mechanisms to protect body cells against oxidative damage.

Industrial production of lycopene from tomatoes appears to be in high demand by food companies for the development of functional foods. To be used as a nutraceutical and functional food product, lycopene must satisfy regulatory requirements and have optimum stability. Environmental friendly extraction and purification procedure for production of lycopene on an industrial scale with minimum loss of bioactivity is highly desirable for food and pharmaceutical applications. High-purity lycopene can be obtained from tomato fruits by various purification and separation processes including solvent extraction (SE), super critical fluid extraction (SCFE), distillation, membrane separation, chromatography and crystallization. Extraction with organic solvents is a well-established method in the food industry but the issue of safety during extraction, poor efficiency, high energy requirement, longer processing time and the residual content of chemical solvents are the limitations.

The present international trend of increasingly stringent regulation regarding residual organic solvents in processed food products clearly indicates that the conventional SE process may be phased out in the near future. In recent years, super critical fluid extraction (SCFE) has emerged as a highly promising environmentally benign technology for selectively recovering thermally labile bioactive ingredients from natural sources. SCFE has also been successfully developed for producing biologically active principles and nutraceuticals. The nutraceuticals produced by SCFE using carbon dioxide (CO<sub>2</sub>) at near-ambient temperatures are preferred by consumers due to their superior quality and higher bioactivity without the problems of residual solvent and microbial contamination. It yields tailor-made extracts of superior organoleptic profile and shelf life, with high potency of active ingredients. The role of SCFE for natural products is well recognized today.

Keeping the above view in mind, a systematic scientific study was undertaken with the following specific objectives.

## **Objectives**

1. Characterization of tomato pomace carotenoids extracted using SCFE system.
2. Utilization of tomato pomace carotenoids extract in selected food products according to its suitability/compatibility.
3. Evaluation of the carotenoid fortified food products for acceptability.
4. Evaluation of the storability of the carotenoid rich food items.

## CHAPTER II

### REVIEW OF LITERATURE

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This chapter illustrates the published literature related with this study. The review covers the characterization i.e., drying of tomato pomace, lycopene and its health benefits, supercritical fluid extraction, characterization of carotenoid extract, utilization of carotenoid extract in food products.

#### **2.1 Drying of Tomato Pomace**

Shi and Maguer (2001) reported that exposure to air at high temperatures during processing of tomato products cause the naturally occurring *trans*-lycopene to be isomerised and oxidized, resulting in a loss of red colour. The stability of lycopene during dehydration was also studied. The dehydration treatments of the ripe tomatoes were (a) by conventional air drying at 95 °C for 6-10 h; (b) by vacuum drying at 55 °C for 4-8 h and (c) by an osmotic treatment at 25 °C in 65 °Brix sucrose solution for 4 h, followed by vacuum drying at 55 °C for 4-8 h. Tomatoes also underwent air drying at 90, 110, 120, and 150 °C for 1-6 h, respectively. The content of lycopene in fresh and processed tomato samples was determined by HPLC, with an analytical 3 µm polymeric C30 column (C30 isocratic separation 4.6 mm i.d. x 250 mm, S-5). Four dehydration methods produced slight differences in the total lycopene content but resulted in quite different distribution of the isomer composition. Osmotic treatments retained more total lycopene and induced only slight changes in the distribution of *trans*- and *cis*-isomers. The osmotic-vacuum treatment had less effect on lycopene loss and isomerisation than vacuum drying and conventional air drying. Heat treatment under atmospheric conditions in the dehydration processes accounts for the lycopene degradation through isomerisation and oxidation. In the osmotic treatment, the predominating mechanism of change may be isomerisation of lycopene. Because the total lycopene content remained essentially constant, only the distribution of *trans*- and *cis*-isomers was changed. In the air drying, isomerisation and oxidation (autoxidation) were two factors that simultaneously affected the decrease of total

lycopene content, distribution of *trans* and *cis*-isomers, and biological potency. Osmotic treatment could reduce lycopene losses in comparison with other dehydration methods.

Favati *et al.*, (2003) collected fresh processing waste of tomato seeds and skins from an industry producing concentrated tomato sauce and dried up to a final moisture content of about 10 %. Three different drying systems were tested: sun drying, oven drying at 105 °C and drying with an industrial Sandvik apparatus. When using the Sandvik equipment, the drying process was conducted on batches of 2.5 kg of fresh product, with air at 60 °C for 100 minutes. The sun drying and oven drying did not give good results, because of growth of molds and fermentations in the first case, and browning of the matrix in the second. Better results were obtained using the Sandvik apparatus; working at 60 °C allowed to dry the product up to the desired moisture level in a reasonable time, without any significant modification of the moieties of interest.

Kaur *et al.*, (2006) worked on drying characteristics of tomato peel isolated from tomato pomace. It was found that the fresh skin has a high moisture content that makes it susceptible to microbial proliferation and spoilage. Therefore, skin can be preserved by drying and then used for lycopene extraction. Tomato peel was separated from pomace by sedimentation and dried in cabinet and fluidized-bed dryers at 50-70 °C. The drying of tomato peel took place under the falling rate period. A fluidized-bed dryer was much more efficient than a cabinet dryer to dry tomato peel.

Mayeaux *et al.*, (2006) concluded that the percentage of retained lycopene in tomato slurry decreased with time and temperature of drying and suggested that lycopene is not stable when exposed to temperatures above 100 °C. While microwaving and baking are less severe treatments that can degrade lycopene, frying could cause serious loss of lycopene in tomato.

Akbari (2011) studied on standardization of drying of tomato processing waste (pomace). The waste was procured from tomato processing industry and stored at -35 °C. after that the pomace was thawed for 120 min before drying. Three different dryers viz. hot air tray dryer, fluidized bed dryer and vacuum dryer were used at

different drying temperatures (50, 60, 70, 80 and 90 °C) for production of pomace powder. It was concluded that tomato pomace dried at 50 °C temperature in fluidized bed dryer had the maximum lycopene content (24.34 mg/100 g).

## **2.2 Lycopene and its Health Benefits**

Lycopene gained the attention of the scientific community in human health because of its antioxidant activity. Studies have shown that lycopene intake was associated with a decreased risk of various chronic cancers, such as prostate, lung, breast, colon, pancreas, stomach, rectum, esophagus, oral cavity, and cervix (Giovannucci, 1999; Rao and Agarwal, 2000; Weisburger, 2002; Omoni and Aluko, 2005). Weisburger (2002) had stated that there was a lower incidence of chronic diseases in populations with regular intakes of tomato products that are rich in lycopene. Lycopene has been shown to protect important bio-molecules, such as lipids, low-density lipoproteins, proteins, and DNA against oxidative damage, which is how lycopene contributes to the prevention of cancers, atherogenesis, and cell proliferation (Rao and Agarwal, 2000; Stahl *et al.*, 2001; Weisburger 2002).

Stahl *et al.*, (2001) conducted an experimental study on 19 human subjects. Skin was exposed to a solar simulator to induce erythema. It was observed and reported that a tomato-based diet was efficient in scavenging reactive oxygen species generated under photo oxidative stress and thus, lycopene has been shown to protect skin against UV-induced erythema. Lycopene has been shown to act as a potent antioxidant. There are also a number of other potential mechanisms for lycopene, such as gap-junction communication, hypocholesteromic, anti-cancerous, and anti-atherogenic effects, that may reduce the risk for various chronic diseases (Young and Lowe, 2001). Thus, lycopene acts by various mechanisms to protect body cells against oxidative damage, and thus, it may play a preventive role in different kinds of chronic cancers and cardiovascular diseases.

Giovannucci *et al.*, (2002) conducted a large scale study of tomato products, lycopene and prostate cancer risk. The study consisted of an ongoing prospective cohort of 47,365 U.S. males. The researchers reported that frequent intake of tomato products or lycopene was associated with a reduced risk of prostate cancer. In

addition, the researchers reported that the consumption of a primary source of bio-available lycopene, tomato sauce, was associated with an even greater reduction in prostate cancer risk.

### **2.3 Supercritical Fluid Extraction of Lycopene**

Rozzi *et al.*, (2002) studied supercritical fluid extraction of lycopene from tomato processing by-products. The results indicated that the percentage of lycopene extracted increased with elevated temperature and pressure until a maximum recovery of 38.8 % was reached at 86 °C and 34.47 MPa, after which the amount of lycopene extracted, decreased. Conditions for optimum extraction of lycopene from 3 g of raw material were determined to be 86 °C, 34.47 MPa, and 500 mL of CO<sub>2</sub> at a flow rate of 2.5 mL/min. These conditions resulted in the extraction of 61.0 % of the Lycopene (7.19 micro g lycopene/g).

Favati *et al.*, (2003) studied extraction of natural antioxidants (carotenoids and tocopherols) from by-products of the tomato processing industry. The combination of temperatures (60 and 80 °C) and pressures (30 and 50 MPa) as well as with the utilization of sunflower oil as co-solvent were studied. The most suitable SFE conditions were found to be the pressure of 50 MPa and the temperature of 60 °C with the use of sunflower oil as a co-solvent for better recovery of carotenoids and tocopherols.

Sabio *et al.*, (2003) worked on extraction of lycopene and  $\beta$  carotene from tomato processing waste using supercritical CO<sub>2</sub>. In this study, tomato skins and their mixtures with seeds were submitted to supercritical CO<sub>2</sub> extraction using flow apparatus at pressures of 250 and 300 bar and temperatures of 60 and 80 °C. Two different mean particle sizes (80 and 345  $\mu$ m) were used. Supercritical fluid extraction from skins at 300 bar and 80 °C allowed the recovery of 80 % of the lycopene and 88 % of the  $\beta$  carotene, using about 130 g of CO<sub>2</sub> per gram of matrix at the lower flow rate of CO<sub>2</sub>.

Suoqi *et al.*, (2003) studied extracting lycopene from tomato powder by supercritical propane and carbon dioxide. Tomato powder size of 5, 20 and 60 mesh,

extracting temperature of 30, 40, and 50 °C and pressure of 15, 25 and 35 MPa were chosen and cross combined for higher oleoresin yields. The results show 60 mesh sizes, pressure at 35 MPa and temperature of 50 °C the best combination. Also, it was revealed that oleoresin yield increases as pressure increase until 25 MPa when extraction was carried out at 40 °C and run time 1.5 h. Further rising pressure will not get more oleoresin. It was also concluded that higher temperature, pressure and longer run time favour high lycopene recovery.

Tabera *et al.*, (2004) investigated the counter-current supercritical fluid extraction process using the following variables and ranges: extraction pressure, 75-200 bar; extraction temperature, 35-50 °C; and ethanol as modifier, 0-10 %. Olive leaves were crushed in a laboratory blade cutter and extracted by maceration with hexane (1:5 w/v), for 48h at 25 °C. Higher extraction yields were attained at 200 bars.

Kaur *et al.*, (2008) evaluated the effect of extraction conditions on lycopene extractions from tomato processing waste skin using response surface methodology. Five independent variables, namely solvent/meal ratio (20:1, 30:1, 40:1, 50:1, and 60:1 v/w); number of extractions (1, 2, 3, 4 and 5); temperature (20, 30, 40, 50 and 60 °C); particle size (0.05, 0.15, 0.25, 0.35 and 0.43 mm); extraction time (4, 8, 12, 16 and 20 min) were used to study their effects on lycopene extraction. The experimental values of lycopene ranged between 0.639 and 1.98 mg/100 g. Maximum lycopene (1.98 mg/100 g) was extracted when the solvent/meal ratio, number of extractions, temperature, particle size and extraction time were 30:1 (v/w), 4, 50 °C, 0.15 mm and 8 min, respectively.

Supercritical fluid extraction of nutraceuticals enriched with carotenoids from tomatoes as the feed, tomato skin, tomato waste or the entire tomato fruit is possible without having to removal of the seeds. Super critical CO<sub>2</sub> at 300 bar and 60 °C extracts containing 14-21 % lycopene with a minimum of 81 % recovery from the enriched feed of tomato (Mukhopadhyay and Karamta, 2008).

Wen *et al.*, (2008) optimized operating parameters for supercritical carbon dioxide extraction of lycopene by response surface methodology and the optimal condition for lycopene extraction within the experimental range of the variables

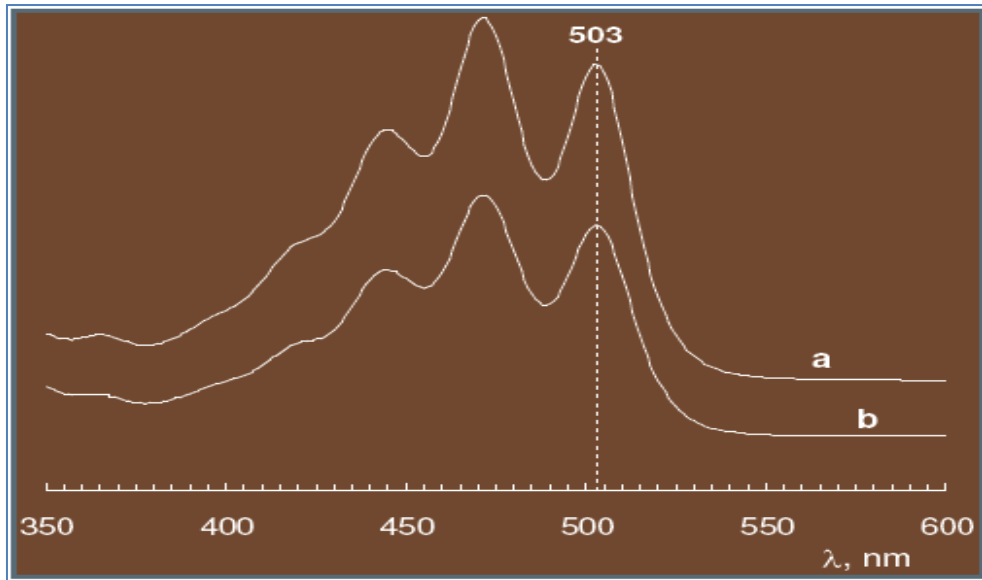
studied was at 57 °C, 40 MPa, and 1.8 h of extraction. At this condition, the predicted amount of lycopene extracted was 28.64 mg in 100 g dried tomato pomace (corresponding to 93 % lycopene recovery).

## **2.4 Characterization of Lycopene Extract**

### **2.4.1 Spectrometric measurement of lycopene**

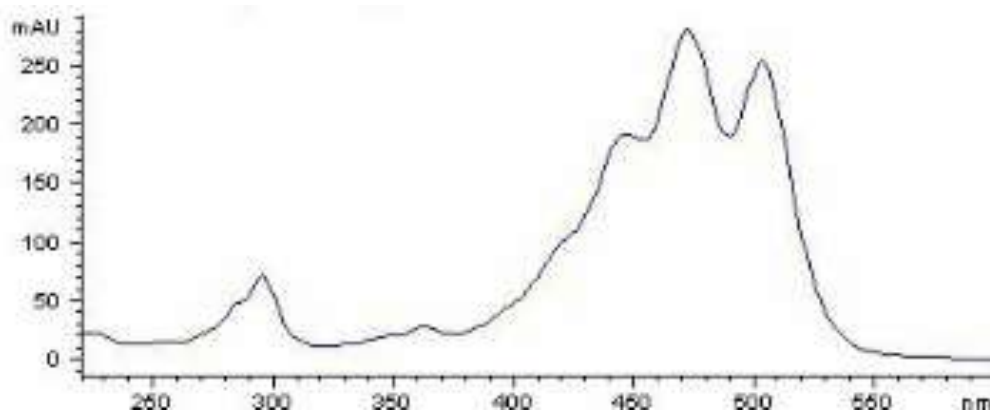
Lycopene concentration in the extracting solvents was determined by spectrophotometric measurement at room temperature in the wavelength range 350-600. A double-beam UV-Vis spectrophotometer (Perkin-Elmer Lambda 25) and quartz cells of 1-cm path length were used. The absorption spectra of the extract, independently of the solvent used and displayed the three characteristic peaks of lycopene at around 445, 472 and 503 nm. To minimise interference from other carotenoids measurements were made at 503 nm. Hexane, ethyl acetate or the ternary mixture hexane/acetone/ethanol (50:25:25 v/v) were used as solvent. The later was chosen because of its proven efficacy for the extraction of carotenoids from plant material (Olives *et al.*, 2006).

Lavecchia and Zuorro (2008) worked on enhancement of lycopene extraction from tomato peels by enzymatic treatment and concluded that the influence of solvent type, using hexane or ethyl acetate did not produce significant differences in lycopene extraction efficiency. In contrast, the mixture of hexane/acetone/ethanol (50:25:25) appeared to be much more effective, both in the presence and absence of enzymatic treatment. Since hexane is the only component of the mixture with a high affinity for lycopene, it follows that acetone and ethanol must play some auxiliary role in the overall extraction process. The absorption spectra of the extract, independently of the solvent used and/or application of enzymes, displayed the three characteristic peaks of lycopene at around 445, 472 and 503 nm (Fig. 2.1). To minimise interference from other carotenoid measurements were made at 503 nm.



**Fig. 2.1: UV-VIS absorption spectra of hexane extracts from tomato peels**

Naviglio *et al.*, (2008) studied extraction of pure lycopene from industrial tomato waste in water using the extractor Naviglio. In the study, extracts were filtered through 0.20 micron membrane (Millipore, Bedford, MA, USA) and anhydriified on sodium sulphate anhydrous in order to eliminate water traces and rough impurities and analysed by spectrophotometer in the 350-550 nm wavelength range (Fig. 2.2).



**Fig. 2.2: UV-Vis absorption spectra of lycopene extract**

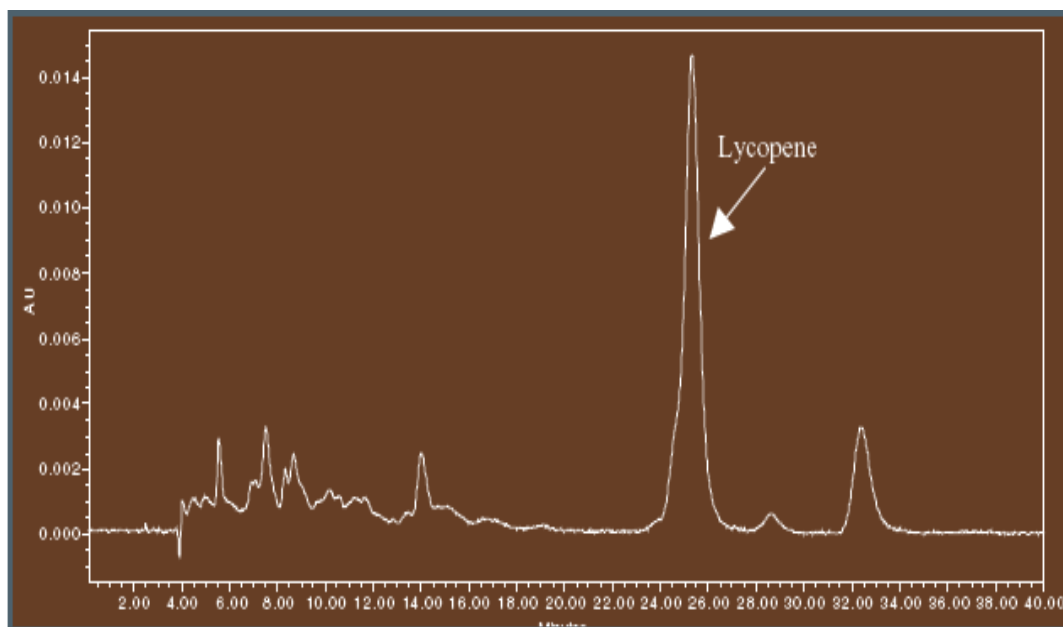
Mukhopadhyay and Karamta (2008) reported that the conventional method for the recovery of lycopene involves solvent extraction (SE) using chloroform, acetone, hexane, dichloromethane (DCM) etc. The conventional SE process suffers from

several drawbacks, e.g. low extraction efficiency, multi-step process for removal of traces of solvent and long time needed for pre-processing and post processing steps. In one such process, lycopene concentrate (obtained from the dried source with 50 ppm lycopene) contains only 5 % lycopene. The required pre-treatment for this process involves washing it with water at 70 °C prior to extraction, which needs to be repeated four times. This causes loss of lycopene while treating with hot water resulting in poor extraction efficiency. In another process, a mixed solvent such as hexane and DCM is used to extract a concentrate with around 5 % lycopene and again it involves separation of residual solvent from the extract. However, in this process, the pre-treatment with steam at 95 °C causes degradation of lycopene and makes the process inefficient. In another process, the concentration of lycopene in oleoresin is reported to be 3-6 % by using a multi-step SE process. Thus, it has been found that the conventional SE method for obtaining nutraceutical concentrate suffers from several drawbacks, such as

- (a) Poor extraction efficiency of AI
- (b) Poor selectivity of AI separation
- (c) Traces of residual solvents, which may be harmful to our body systems
- (d) Artifacts that may be formed due to the high temperature processing  
needed for removal of residual solvent
- (e) High energy requirements
- (f) Very high (16-24 h) batch time
- (g) Costly and high purity solvents are required.
- (h) Requirement of multiple solvents and multiple solvent removal steps

## 2.4.2 Purity Profile of Lycopene Using HPLC Technique

Mayeaux *et al.*, (2006) worked on the effects of cooking conditions on the lycopene content in tomatoes. In the thermal stability study using a pure lycopene standard, 50 % of lycopene was degraded at 100 °C after 60 min, 125 °C after 20 min, and 150 °C after less than 10 min. The samples were analyzed using HPLC system with a photo diode array detector and a 25 cm x 4.6 mm dia. 5- $\mu$ m C18 Discovery column. The mobile phase was methanol:acetone (90:10) and the flow rate was 0.8 mL/min. As shown in Fig. 2.3, the lycopene peak was monitored at 470 nm. It was found that only 64.1 % and 51.5 % lycopene was retained when the tomato slurry was baked at 177 °C and 218 °C for 15 min, respectively. At these temperatures, only 37.3 % and 25.1 % of lycopene was retained after baking for 45 min. In 1 min of the high power of microwave heating, 64.4 % of lycopene still remained. However, more degradation of lycopene in the slurry was found in the frying study. Only 36.6 % and 35.5 % of lycopene was retained after frying at 145 and 165 °C for 1 min, respectively.



**Fig. 2.3: A representative chromatogram of lycopene in raw tomato sample**  
(HPLC conditions: 5c-colomun 25cm x 4.6 mm5 $\mu$ m C18, mobile phase methanol:acetone 90:10; flow rate, 0.8 mL/min; detector wavelength, 470 nm.)

The lycopene concentration of the tomato sauce was measured using a HPLC system (Ajmera, 2006). A Hitachi series liquid chromatogram instrument was used,

consisting of a solvent delivery pump, controller, and UV/visible detector for the determination of lycopene. Separation was achieved by using an analytical polymeric C30 column (4.6 mm x 25 cm). A guard column packed with C30 stationary phase was used in-line for all separations. The mobile phase consisted of acetone: butyric acid: ethanol (50:25:50). A 60 µl sample was injected into the detector via a micro syringe. The flow rate was adjusted to 1 ml/min, and peak areas were measured at 450 nm. The lycopene calibration curve was generated from peak heights of standards run on HPLC. The peaks of lycopene were identified by comparing their retention times and peak area with that of a pure standard.

During HPLC experiments done by Chokshi and Joshi (2007), Methanol and Methyl tert-butyl ether were filtered and degassed with Helium. The 250 mm x 4.6 mm Develosil UG C30 column operated for the HPLC analysis. The mobile phase for solvent extraction is Methanol: THF: Water (67:27:6), flow rate 2ml/min. The routine detection of sample injection volume 20 µl is done at 475 nm-the adsorption maximum for lycopene in the mobile phase. The percentages of carotenoid compounds were calculated from peak areas measured.

## **2.5 Utilization of Carotenoid Extract in Food**

Hanan *et al.*, (2011) studied degradation kinetics and utilization of extracted tomato skin carotenoids in filling cream and glazing jelly preparation. Tomato skin carotenoid extract can be use as a colouring agent in some confectionary products such as filling cream. Filling cream was prepared using sucrose (62.55 %), shortening (34.78 %), lecithin (0.05 %), salt (0.04 %) and potassium sorbate (0.10 %) (Jeffery, 1993). The tomato skin carotenoid extract (200, 400, 600 and 800 µl/100 g of mixture) were added.

Machewad *et al.*, (2012) studied the extraction of pigments from safflower petals and its utilization in ice cream as a natural colour. The pigments which yield from yellow safflower (carthamidin) were 29.59% and safflower red (carthamin) was 0.77%. The pigment was utilised as a natural colour in ice cream to replace the synthetic colour. Ice cream was prepared by incorporation of carthamidin extract at the level of 0.03, 0.06 and 0.09 ml. The addition of carthamidin (0.06 ml) in the ice

cream was found to score higher overall acceptability in sensory evaluation. Further addition of the safflower yellow 0.09 mL scored less for colour of the ice cream.

## 2.6 Sensory Evaluation

Hanan *et al.*, (2011) studied the effect of tomato skin carotenoids on sensory quality of filling cream and glazing jelly. Filling cream and glazing jelly samples were prepared using sunset yellow (0.02 %) as a synthetic colour and tomato skin extract (0.18, 0.36, 0.54 and 0.72 %) as a normal colour were evaluated for their sensory attributes by 10 sensory panellists. The higher overall acceptability and colour scores observed in filling cream prepared using carotenoid extract at 0.36 and 0.54 % with significant difference compare to filling cream, which prepared using synthetic colour at concentration of 0.02 %. On the other hand, glazing jelly prepared using 0.54 % carotenoid extract had overall acceptability score (8.9) higher than that of the glazing jelly prepared using synthetic colour (8.2) with significant difference ( $p < 0.05$ ).

Satyanarayana *et al.*, (2012) studied organoleptic qualities of *jilebi* & *jangri* prepared with the application of water soluble annatto dye in a various concentration (2.5 – 40 mg norbixin/ kg product). The samples were evaluated for their colour, taste, aroma and overall acceptability using 9- point hedonic scale among 10 selected sensory panellists. The overall sensory quality of 5 mg/kg and 30 mg/kg *jilebi* & *jangri* samples were score higher among all samples ( $> 7.0$ ).

## 2.7 Shelf Life

Hanan *et al.*, (2011) studied degradation kinetics of total carotenoids pigments in tomato skin extract by storing the carotenoid extract at 50, 70, 100 and 121 °C. The highest level of carotenoids destruction was found to be 46.6 % and 29.3 % at 121 °C and 100 °C, respectively. Shi *et al.* (2007) reported that at low temperatures such as at 60 and 80 °C, the cis isomers was formed and tends to accumulate. At high temperature treatments such as at 100 °C and 121 °C the all stable -trans isomers converts to unstable -cis isomers.

Tola and Ramaswami (2013) studied stability of lycopene from watermelon pulp powder extracted at pH 3.85 and different storage temperature in combination to ambient air and 9 % CO<sub>2</sub> concentration. Lycopene degradation rate was observed for 15 days at storage temperature 25 °C. Watermelon pulp powder stored in the presence of ambient air oxygen showed least stability in the lycopene concentration. However, sample store at lower temperature (2 °C) under 9 % CO<sub>2</sub> concentration showed better stability.

## CHAPTER III

### MATERIALS AND METHODS

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This chapter encompasses the explanation of experimental plan of various steps of experiments performed throughout the study and the description of materials used and methods adapted for the experiments and analysis for achieving objectives of the study. The experimental design and the statistical analysis are explained. The entire study was conducted at College of Food Processing Technology and Bio Energy, Anand Agricultural University, Anand.

#### 3.1 Tomato Pomace

Fresh tomato pomace was procured in a bulk from tomato processing industry Green Fiber Pvt. Ltd., Navsari. Collected pomace was bagged and stored at -20 °C in deep freezer until used for analysis and experiments.

#### 3.2 Characterization of Tomato Pomace

##### 3.2.1 Proximate analysis of pomace

The moisture content, protein, crude fat, ash and crude fiber content of tomato pomace were estimated using standard analytical methods. The carbohydrate content was calculated by difference.

##### **Moisture content**

Moisture content of tomato pomace was determined using oven drying method (AOAC, 1996). The sample was dried in hot air oven at 105 °C for 5 h or till the constant weight achieved. After drying, the sample was taken out and then cooled in desiccator and weighed after the sample attained room temperature. The data were recorded and the moisture content was calculated using the following formula:

$$\text{Moisture Content (m. c.), \%} = \frac{W_m}{(W_m + W_d)} \times 100 \quad \text{----(3.1)}$$

Where,

m.c. = moisture content, per cent wet basis

$W_m$  = mass of water evaporated, g

$W_d$  = mass of dry matter, g.

### **Total ash**

The total ash content in tomato pomace sample was determined using method given by Ranganna (1986). About five gram pomace sample was taken into a crucible which was already weighed. After determination of moisture content, the same sample crucible was placed into muffle furnace (make: Patel Scientific Instruments, Ahmedabad; Fig. 3.1) at  $525 \text{ }^\circ\text{C} \pm 5 \text{ }^\circ\text{C}$  for 6 hours.



**Fig. 3.1: Muffle furnace for ash estimation**

Crucible was then quickly transferred to desiccators with porcelain plate and desiccant. Crucible was cooled prior to weighing. The ash content was calculated as follows:

$$\% \text{ Ash} = \frac{(W_a - W_c)}{W_f} \times 100 \quad \text{----(3.2)}$$

Where,

$W_a$  = mass after ashing with crucible, g

$W_c$  = mass of empty crucible, g

$W_f$  = mass of sample taken, g.

### Crude fat

For determination of crude fat in pomace, method given by Ranganna (1986) was used. The pomace sample after moisture content determination was transferred to a thimble. The thimble was placed into the fat extraction tube of Soxhlet apparatus, Socsplus (make – Pelican equipment, Chennai) (Fig. 3.2).



**Fig. 3.2: Soxhlet apparatus for fat estimation**

The sample was put in the thimble which is fitted in thimble guide and fixed it in to the weighed flask. Then, 80 ml hexane was poured through the sample into the Socsplus assembly. Sample was extracted for three hours. At the end of extraction period, hexane was distilled off by allowing it to collect in the soxhlet tube by means

of closing the knob. Trace of hexane was evaporated at low temperature under a current of hot air. Then extract was cooled and weighed. The percentage of extract in sample was calculated as follows:

$$\% \text{ Crude fat} = \frac{\text{wt. of extract}}{\text{wt. of sample}} \times 100 \quad \text{----(3.3)}$$

### Crude fibre

Fibra plus instrument (make: Pelican Equipments, Chennai; Fig. 3.3) was used for estimation of crude fibre content. Tomato pomace sample was treated with 1.25 % H<sub>2</sub>SO<sub>4</sub> followed by 1.25 % NaOH and washed thoroughly with distilled water after each treatment. Neutral residue left over was dried and weighed and then ignited in to muffle furnace. From the loss in weight of the residue, the percentage of crude fibre was calculated using following formula given by Ranganna (1986).



**Fig. 3.3: Assembly for fibre content estimation**

$$\% \text{ Crude Fiber} = \frac{W_r - W_a}{W_s} \times 100 \quad \text{----(3.4)}$$

*Where,*

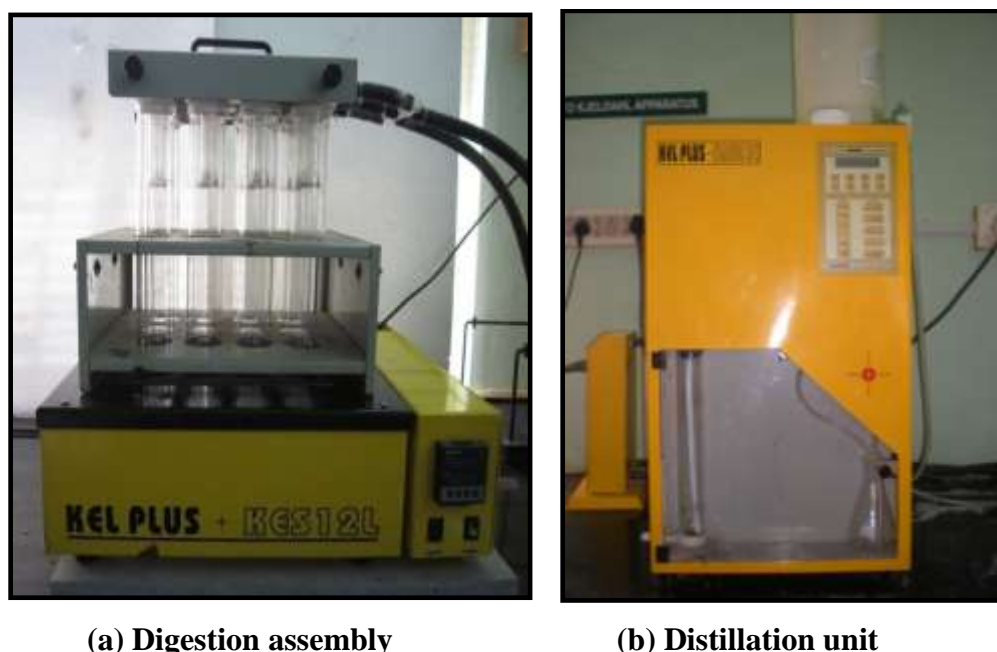
$W_r$  = mass of residue, g

$W_a$  = mass of ash, g

$W_s$  = mass of sample, g

## Protein content

Protein content of pomace was determined using Kjeldahl method as per AOAC (1996). The per cent nitrogen was estimated from Auto Kjeldahl method and the protein content was quantified by multiplying with factor 6.25. Kel-plus automatic Kjeldahl set up (make: Pelican Equipments, Chennai) was used which consisted of two units namely digester and distillation unit as shown in Fig. 3.4.



**Fig. 3.4: Assembly for protein estimation**

### 3.3 Drying of Pomace

The frozen pomace sample was drowned from deep freezer and then allowed to thaw for 120 min before drying. Pomace sample of about 5 mm bed thickness was dried using fluidized bed dryer (Fig. 3.5) at 0.75 m/s velocity and 50 °C temperature at Food Engineering Laboratory of College of FPT & BE up to 8 % m. c. (w. b.) achieved (Akbari, 2011). The dried pomace was then used for extraction using supercritical fluid extraction system. The dried sample was kept in the desiccators at cool and dark place. Then, the dried pomace was pulverized with the help of Willey mill. Dried powder was sieved to get particle size of 0.4 mm as per ASTM standard to get higher yield of carotenoid extract.

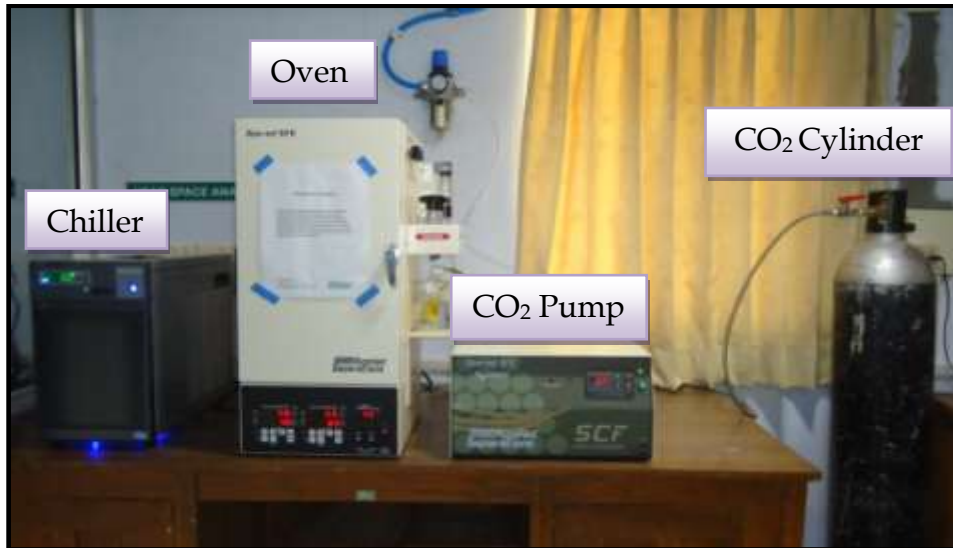


**Fig. 3.5 Fluidized bed dryer**

### **3.4 Supercritical Fluid Extraction of Lycopene**

Supercritical Fluid Extraction assembly (make: Applied Separations, USA) has five components: reciprocating compressor, CO<sub>2</sub> cylinder, CO<sub>2</sub> pump, oven module and chiller (Fig. 3.6).

Compressed air is necessary to drive the CO<sub>2</sub> pump for which a reciprocating compressor (make: Anest iwata, Noida) (Fig. 3.7) was used. The capacity of reciprocating compressor is 180 liters. CO<sub>2</sub> cylinder (46.7 liter capacity) was connected to CO<sub>2</sub> pump. This pump was used to pressurize CO<sub>2</sub> at required pressure with a maximum limit of 690 bar. From pump, pressurized CO<sub>2</sub> enters in oven module.



**Fig. 3.6: Super critical fluid extraction system**



**Fig. 3.7: Air compressor and CO<sub>2</sub> cylinder**

The SFE oven module (Fig. 3.8) consists of a rectangular chamber in which sample holding vessel was fixed at required temperature. In the oven, two sample holding vessels can be fixed at a time. Oven temperature, micro metering valve temperature, vessel temperature and set temperature are displayed at bottom panel of oven module. CO<sub>2</sub> inlet valve and vent valve are located at left side wall of oven and CO<sub>2</sub> outlet valve and micro metering valve are located at right side wall of the oven. The inlet valves supply supercritical CO<sub>2</sub> from the pump module to the extractor vessels whereas outlet valves supply supercritical CO<sub>2</sub> from the extraction vessel to

the metering valves. The vent valves are used to vent the supercritical CO<sub>2</sub> from the extractor vessels to the atmosphere. The micro-metering valves are flow control valves used to release supercritical CO<sub>2</sub> to the atmosphere. Thermocouple is provided inside oven module to measure the temperature of vessel. A flow meter is attached to the right side of the oven module which is used to monitor the flow of discharged CO<sub>2</sub> gas. The extract is collected in a glass vial which is fixed below micro metering valve.



**Fig. 3.8: Oven module**

Chiller is used for recirculating coolant and is connected to pump module. Recirculating coolant is required by the pump module for CO<sub>2</sub> pressurization. The recirculating coolant should be maintained at a controlled temperature of 0 °C or less. Mixture of ethylene glycol and water are used as a cooling medium.

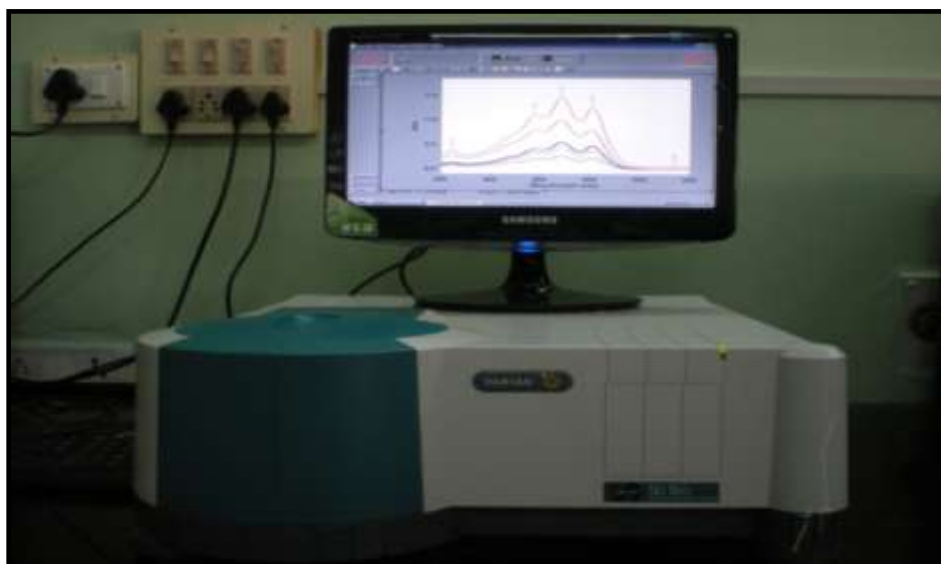
### **3.4.1 Experimental procedure for supercritical fluid extraction of lycopene from dried tomato pomace**

Extraction of lycopene from dry tomato pomace powder was experimentally done using supercritical fluid extraction system using CO<sub>2</sub>. Carbon dioxide was pressurized with a high-pressure pump and then charged into the extraction vessel to desired pressure. The extraction vessel was packed with dried and sieved pomace powder. Polypropylene wool and frits were used for proper packing and empty space was filled with glass beads. The extraction vessel was heated in the oven and its temperature was controlled by a thermocouple. The supercritical CO<sub>2</sub> with dissolved compounds passed through a heated micrometer valve and was subsequently expanded at ambient pressure and temperature leaving the extract in a glass vial.

## **3.5 Characterization of Lycopene Extract**

### **3.5.1 UV visible spectrophotometric analysis of lycopene**

Lycopene concentration in the extracting solvents was determined by spectrophotometric measurement at room temperature in the wavelength range 350-600 nm. A double-beam UV-Vis spectrophotometer (make: Varian-carry-50, Fig. 3.9 ) and quartz cells of 1-cm path length were used.



**Fig. 3.9: UV visible spectrophotometer**

The absorption spectra of the extract, independently of the solvent (hexane) used, displayed the three characteristic peaks of lycopene at around 445, 472 and 503 nm. To minimise interference from other carotenoids, measurements were made at 503 nm ( Olives *et al.*, 2006; Lavecchia and Zuorro, 2008; Naviglio *et al.*, 2008). The absorbance at 503 nm gives lycopene concentration of the sample. The data were recorded and the per cent lycopene was calculated using the following formula:

$$\text{Lycopene (\%)} = \frac{A_{503}}{a_{503}} \times \frac{v}{1000} \times \frac{100}{w} \quad \text{----(3.5)}$$

where,

$A_{503}$  = absorbance noted at 503 nm

$a_{503}$  = specific extinction coefficient of lycopene in n-hexane  
(e.g. 310.5)

v = total volume of the solvent and extract, ml

w = weight of the sample, g

### 3.5.2 HPLC Analysis of Lycopene

The purity of lycopene obtained by Super Critical Fluid Extraction System from pomace samples was determined using High-Performance Liquid Chromatography - HPLC (Make: Varian, USA) (Fig. 3.10), as a major quality evaluation parameter in the study. HPLC consisted of a solvent delivery pump, controller and having UV/visible, PDA and RI detector.

For this study, The stock solution of 180 ppm was prepared by dissolving 5 mg lycopene standard in 25 ml HPLC grade hexane, then sonicated for two minutes in sonicator (Make: Life care instruments, Mumbai) for complete dissolution (Fig. 3.11) and stored at -20 °C. Then, the stock solution was further diluted to different concentrations with HPLC grade acetonitril solvent. Again, the sample was sonicated for two minutes to ensure proper and homogeneous mixing. The standard solution was injected by 25 µL syringe. Several trials were carried out using acetonitril and methanol to get good resolution and peak height with a flow rate of 1 mL/min (Ajmera, 2006; Shi and Maguer, 2001). From these experiments, mobile phase system

was selected on the basis of good separation and peak height for the experiments. Separation was achieved by using thermo BDS Hypersil C18 column (4.6 mm x 250 mm x 5  $\mu$ m) using UV detector at 472 nm for all experiments.



**Fig. 3.10: High-Performance Liquid Chromatography**

**Fig. 3.11: Sonicator**

Calibration curve was prepared using different known concentrations (1.0, 1.5, 2.0, 3.0, 5.0 and 10 ppm) of standard lycopene solution. The lycopene calibration curve was generated from peak heights of standards run on HPLC.

Per cent purity of extracted lycopene of the dried tomato pomace powder derived from the SCFE System using CO<sub>2</sub> was measured using a HPLC system. For this, all the samples were run on the standardized conditions.

### **3.5.3 Colour measurement**

The colour value was measured by Lovibond Tintometer (Model F) (Fig.3.12). The lovibond glass cell was cleaned with carbon tetrachloride and allowed to dry. The sample was poured in to a lovibond glass cell (*W600/OG/1''*). Having placed the sample in position, focus the viewing tube until a sharp image of the aperture is obtained. Slide the tabs controlling the coloured filters to the right. The proportions of Red, Yellow and Blue must be adjusted until a colour match obtained (Anon., 1984).



**Fig.3.12: Lovibond Tintometer**

Colour of sample in terms of Lovibond unit as follow:

$$\text{Colour reading} = (aY + bR)$$

Where,

a = sum total of the various yellow slides used

b= sum total of the various red slides used

Y+R = Mode of expressing the colour of the light coloured sample

### **3.5.4 Refractive index**

Few drops of the sample of carotenoid extract was placed on the prism. Closed the prism and allowed to stand for 1-2 min. Adjusting the instrument and lighting to obtain the most distinct reading possible, the refractive index number was determined (AOAC, 2000).



**Fig.3.13: Digital Refractometer**

### **3.6 Utilization of Carotenoid Extract into Food Product**

Carotenoid extract was obtained using optimized conditions and incorporated into various food products for lycopene enrichment. The products were selected on the basis of (a) feasibility of the product (b) solubility of carotenoid extract in byproduct and (c) sensory attribute of food product.

Based on above three criteria, following three products were selected;

1. Ice cream
2. Filling cream (Biscuit)
3. Jam

#### **3.6.1 Ice cream**

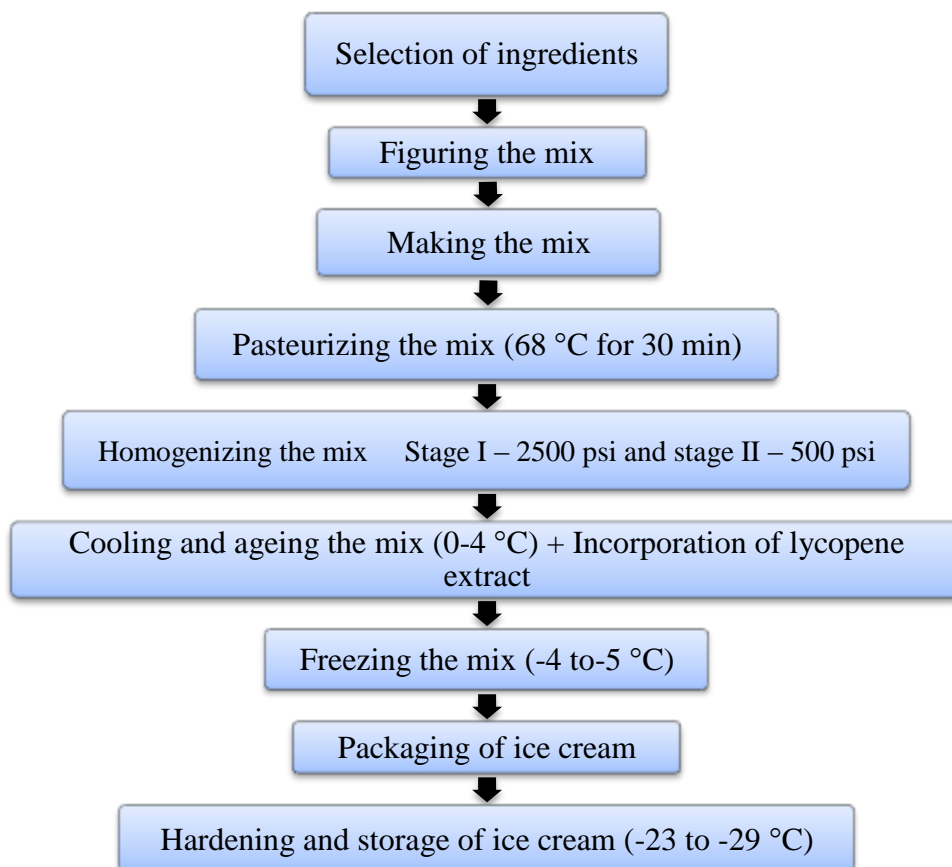
##### **a) Ice cream formulation and process**

Plain ice cream was prepared using the composition given in the following Table 3.1 and standard process described by De (2007), Arbuckle (1977).

**Table 3.1: Composition of ice cream**

| Standard                                  | per cent                                  |
|-------------------------------------------|-------------------------------------------|
| Fat                                       | 10                                        |
| MSNF                                      | 11                                        |
| Sugar                                     | 15                                        |
| Commercial stabilizer                     | 0.3                                       |
| Total Solids                              | 36                                        |
| Level of incorporation (Lycopene extract) | 0.02, 0.04, 0.06, 0.08, 0.1,0.2,0.3 & 0.4 |

Commercially available mango flavoured ice cream having sunset yellow colour was treated as control. The lycopene extract was added @ 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3 and 0.4 per cent (w/v) to the plain ice cream mix and processed further for organoleptic test.



**Fig.3.14: Process flow chart of ice cream preparation**

## b) Organoleptic evaluation

The different samples of ice cream prepared by above mentioned process were subjected to sensory evaluation of colour, aroma, taste and overall acceptability by trained panelists consisting of 10 persons using 9 point hedonic scale (Ranganna, 1986).

### 3.6.2 Filling cream (Biscuit)

#### a) Filling cream (Biscuit) formulation and process

Filling cream (Biscuit) was prepared using the composition given in the Table.3.2. Filling cream (Biscuit) was prepared using the formula according to Jeffery (1993). The used ingredients were sucrose (62.55 %), Shortening (34.78 %), Lecithin (0.05 %) and salt (0.04 %). The sucrose added to creamed shortening and beaten for 5 min. other ingredients then added and further beaten for 3 min. Finally different concentrations of carotenoid extract were added. Commercially available biscuit having Sunset yellow coloured filling cream was treated as control sample. Thereafter the prepared filling creams were sensory evaluated.

**Table.3.2: Composition of Filling cream (Biscuit)**

| <b>Ingredients</b>                           | <b>per cent</b>                           |
|----------------------------------------------|-------------------------------------------|
| Sucrose                                      | 62.55                                     |
| Shortening                                   | 34.78                                     |
| Lecithin                                     | 0.05                                      |
| Salt                                         | 0.04                                      |
| Level of incorporation<br>(Lycopene extract) | 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0 and 4.0 |



**Fig. 3.15: Biscuits with filled cream**

**b) Organoleptic evaluation**

The different samples of filling cream (biscuit) prepared by above mentioned process were evaluated organoleptically for their colour, aroma, taste and overall acceptability by trained panelists consisting of 10 persons using 9 point hedonic scale (Ranganna, 1986).

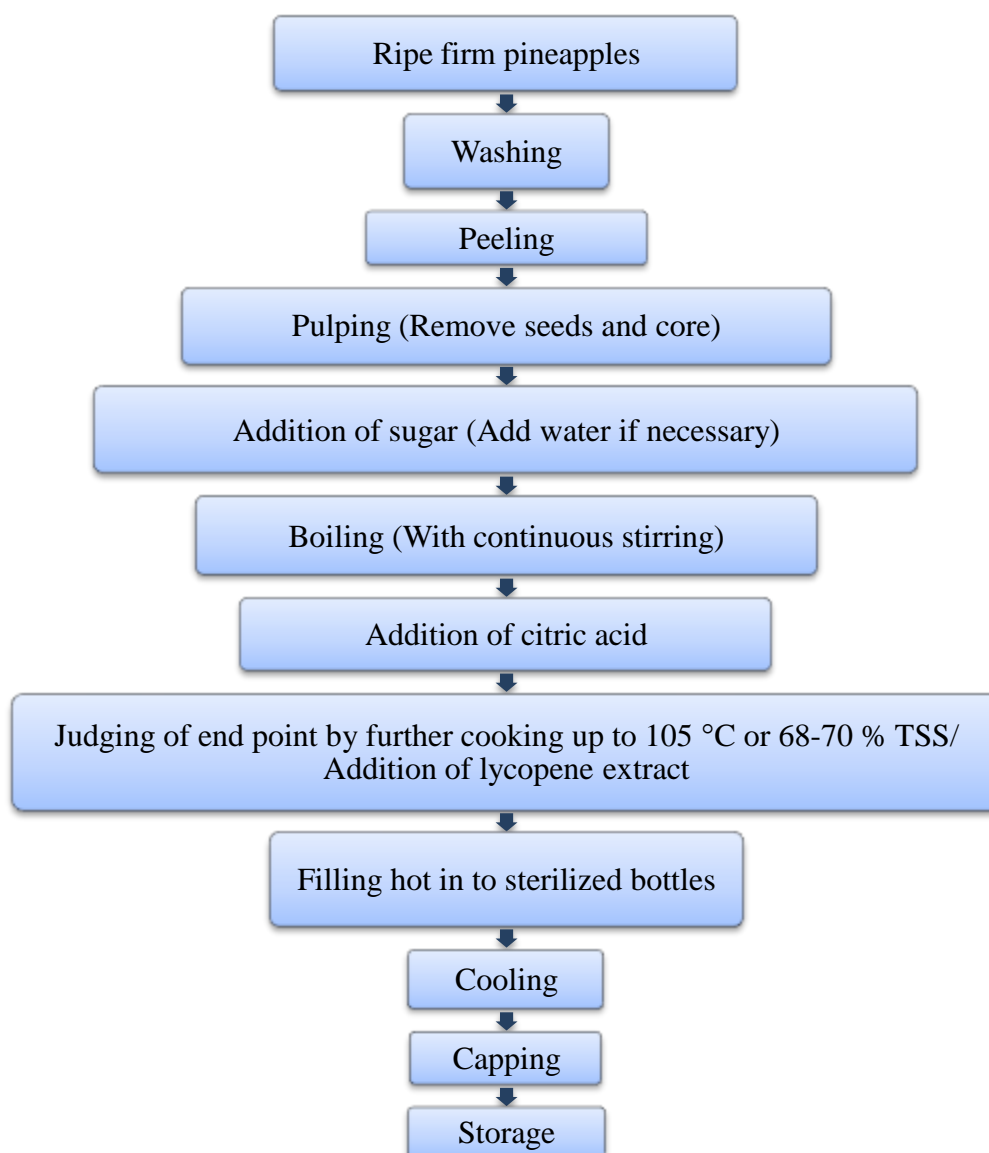
**3.6.3 Jam**

**a) Jam formulation and process**

Jam (Pineapple) was prepared using the composition given in the Table.3.3. The standard process described by Girdhari lal and Siddappa (1960) and as mentioned below (Fig. 3.16) was used to prepare jam. Commercially available pale yellow coloured pineapple jam was selected as control. For the test samples, the carotenoid extract was added @ 0.06, 0.08, 0.1, 0.2, 0.3 and 0.4 per cent (w/w) to the plain jam and processed further.

**Table.3.3: Composition of jam**

| <b>Ingredients</b>                        | <b>per cent</b>                   |
|-------------------------------------------|-----------------------------------|
| Pineapple pulp                            | 50                                |
| Sugar                                     | 50                                |
| Pectin                                    | 0.5                               |
| Citric acid                               | 0.5                               |
| Level of incorporation (Lycopene extract) | 0.06, 0.08, 0.1, 0.2, 0.3 and 0.4 |



**Fig.3.16: Process flow chart of Jam**

#### **a) Organoleptic evaluation**

The different samples of jam prepared by above mentioned process were subjected to sensory evaluation of colour, aroma, taste and overall acceptability by trained panelists consisting of 10 persons using 9 point hedonic scale (Ranganna, 1986).

#### **3.6.4 Carotenoid extraction from prepared food products**

The carotenoid extraction was carried out by Mojonnier fat extraction method for ice cream and filling cream (biscuit). 5 g of food sample was taken in Mojonnier flask and then into it 10 ml 95 % alcohol was added, flask was then sealed with H<sub>2</sub>O- soaked cork, and was shaken for 15 s. For the extraction of carotenoid from the food product, 25 ml of ethyl ether was added and then the flask was shaken vigorously for 1 min, then 25 ml of petroleum ether was added and again the flask was shaken vigorously for 1 min. The flask was then centrifuged to 600 rpm for > 30 s to obtain aqueous and ether phase. Extraction was carried out 2 to 3 times. Ether was then evaporated at < 100 °C to get the fat containing carotenoid extract (AOAC, 1992).

The carotenoid extraction for the jam was carried out by the method followed by Ranganna (1986). 5 to 10 g of sample was extracted repeatedly with acetone using pestle and mortar until the residue becomes colourless. Acetone extract was then transferred to a separating funnel containing 20 ml of petroleum ether and mixed gently. 20 ml of 5 % sodium sulphate solution was then added to the same separating funnel. The petroleum ether extract containing carotenoids was then poured in to a amber coloured bottle containing about 10 g anhydrous sodium sulphate. Petroleum ether was then decanted in to a 100 ml volumetric flask. The extract was made up to desired volume and was taken for absorbance measurement by UV Vis spectrophotometer at 503 nm.

#### **3.6.5 Quantification of Carotenoid Concentration**

Carotenoid concentration was determined by scanning 2 mL of ubiquitous extract on a UV Visible Spectrophotometer between 350 nm and 550 nm and recording absorbance values at 425 nm, 447 nm, and 470 nm. These wavelengths correspond to predominant carotenoids present in tomato carotenoid extract, for

example Lycopene (503). Concentration was calculated using methods outlined by Gross (1991) using 3105 as an average extinction coefficient for all carotenoids and reported in parts per million.

$$\text{Carotenoid concentration } \left( \frac{\text{mg}}{\text{l}} \right) = \frac{\text{Absorbance} * \text{Extraction volume}}{\text{Sample weight} * 100 * \text{extinction coefficient}}$$

### **3.6.5 Sensory analysis**

The experimental samples were served to the panel. The panelists were instructed to rate each sample on the 9 - point hedonic scale as shown in APPENDIX-1, which included score for colour, aroma, taste, and overall acceptability. The final score for each attribute was obtained by averaging the score of all the panelists.

### **3.6.6 Statistical analysis**

The mean values generated from analysis of each of quality attribute obtained from two replications during the experimentation were subjected to statistical analysis using Completely Randomized Design (CRD) as per the procedure given by Steel and Torrie (1980). By statistical analysis, the influence of each parameter on the specific characteristic was tested.

### **3.6.8 Shelf life study**

The food samples were prepared again at optimized conditions as mentioned above. The jam was packed in PET jar bottles and filling cream (biscuit) was packed in metalized pouch. Jam and Filling cream (Biscuit) was stored at a temperature of 30 °C in an incubator (Make: Nova Instruments Pvt. Ltd., Ahmedabad) and Ice cream was stored in deep freezer at -18 °C. The storage study was carried out for 45 days and samples were analyzed for its lycopene content at 7 days interval.

**Statistical Design:** Completely Randomised Design

Treatment: 7

Storage period i.e. 7, 14, 21, 28, 35, 42 and 49 days

Repetition: 4

Observation: Lycopene content

## CHAPTER IV

### RESULTS AND DISCUSSION

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The results obtained from the investigations are presented in this chapter under various sections dealing with characterization of tomato pomace powder, tomato carotenoid extract, utilization of tomato carotenoid extract into various food products, characterization of carotenoid rich food items, evaluation of the shelf life of the carotenoid rich food item.

#### 4.1 Characterization of Tomato Pomace

Tomato pomace sample was drawn from stored pomace and thawed at room temperature for the proximate analysis.

##### Proximate analysis

The data obtained regarding the proximate analysis i.e., moisture content, total ash, protein, crude fat, crude fiber and carbohydrate are presented in Table 4.1. All determinations were replicated thrice and average values are reported. The moisture content of tomato pomace was found 84.71 %, protein 7.13 %, crude fat 0.31 %, crude fiber 2.65 %, total ash 0.7 % and carbohydrates by difference was found to be 4.51 %.

**Table 4.1: Proximate composition of tomato pomace**

| Constituent      | Average value (%) |
|------------------|-------------------|
| Moisture content | 84.71             |
| Protein          | 07.13             |
| Crude Fat        | 00.31             |
| Crude Fiber      | 02.65             |
| Total Ash        | 00.70             |
| Carbohydrate     | 04.51             |

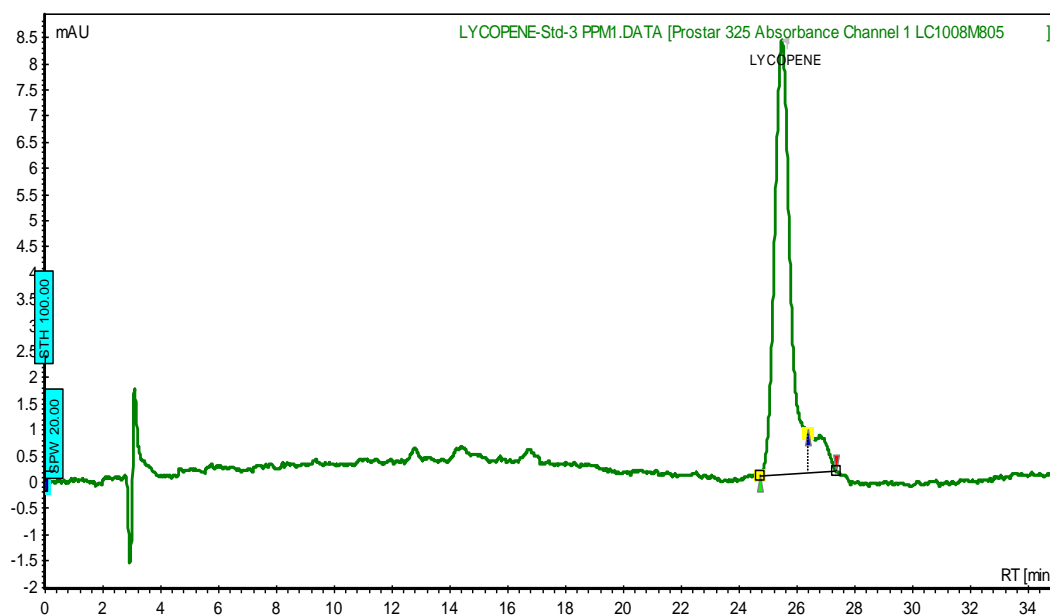
## 4.2 Characterization of Tomato Carotenoid Extract

The obtained tomato carotenoid extract by super critical fluid extraction system was characterized for their Lycopene, Refractive index and Colour value. The data obtained regarding are presented in Table 4.2. All determinations were replicated thrice and average values are reported. The lycopene content of carotenoid extract was found 170.661 mg/100g, Refractive index at 20 °C was 1.48042 and Colour value was found to be 111.8.

**Table 4.2: Characterization of Tomato Carotenoid Extract**

| Carotenoid extract | Absorbance at 503nm | Lycopene content(mg/100g) | Refractive index at 20 °C | Colour value (aY+bR) |
|--------------------|---------------------|---------------------------|---------------------------|----------------------|
|                    | 0.335               | 170.661                   | 1.48042                   | 111.8                |

The following Fig.4.1 shows the HPLC chromatogram of the carotenoid extract from the tomato pomace.



**Fig. 4.1: HPLC chromatogram of the carotenoid extract from the tomato pomace**

### 4.3 Utilization of Carotenoid in Food

Three different categories of food products viz. ice cream, filling cream (biscuit) and jam were chosen for utilization of carotenoid extract according to their feasibility study.

#### 4.3.1 Ice cream

Ice cream samples were prepared by incorporating the tomato carotenoid extract at different level of 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3 and 0.4 % in the standard recipe mentioned and described under section 3.7.1 and the market sample (sunset yellow color) was selected for sensory evaluation and colour measurement.

##### 4.3.1.1. *Effect of incorporation of lycopene extract on sensory quality of ice cream*

Sensory evaluation of ice cream was conducted using method of 9-point hedonic scale. The mean score of different sensory parameters (colour and appearance, taste, aroma and overall acceptability) of ice cream prepared by incorporating lycopene extract at different levels are presented in the Table 4.3 and the effect on sensory quality is presented in Fig.4.2.

**Table 4.3: Mean sensory score for ice cream prepared by incorporation of tomato carotenoid extract**

| Incorporation level % | Sensory score         |       |       |                       |
|-----------------------|-----------------------|-------|-------|-----------------------|
|                       | Colour and appearance | Taste | Aroma | Overall acceptability |
| Control               | 7.2                   | 7.25  | 7.0   | 7.25                  |
| 0.02                  | 5.1                   | 5.55  | 5.1   | 5.35                  |
| 0.04                  | 5.45                  | 5.50  | 5.4   | 5.7                   |
| 0.06                  | 6.1                   | 6.0   | 5.85  | 6.305                 |
| 0.08                  | 6.0                   | 6.7   | 6.4   | 6.3                   |
| 0.1                   | 7.15                  | 7.3   | 7.1   | 7.3                   |
| 0.2                   | 5.65                  | 5.8   | 5.5   | 5.85                  |
| 0.3                   | 5.6                   | 5.7   | 5.4   | 5.8                   |

|                          |              |              |              |              |
|--------------------------|--------------|--------------|--------------|--------------|
| 0.4                      | 4.2          | 4.2          | 3.95         | 4.35         |
| <b>S.Em</b>              | <b>0.199</b> | <b>0.187</b> | <b>0.220</b> | <b>0.188</b> |
| <b>CD<sub>0.05</sub></b> | <b>0.56</b>  | <b>0.52</b>  | <b>0.6</b>   | <b>0.52</b>  |
| <b>CV %</b>              | <b>10.81</b> | <b>9.89</b>  | <b>12.12</b> | <b>9.93</b>  |

#### **a. Colour and appearance**

The mean score for colour and appearance of the ice cream varied from 4.2 to 7.15 with different levels of incorporation of carotenoid extract. It was observed that the ice cream prepared by incorporation level of 0.1 % (w/v) with carotenoid extract scored the maximum (7.15) and also high over the control. As the level of incorporation was increased from 0.1 % to above, the colour and appearance of the ice cream became inferior. Similarly, the colour and appearance of the ice cream prepared by incorporation level of 0.06 and 0.08 % (w/v) were found to be acceptable (>6.0) among the panelists (Table 4.3).

#### **b. Taste**

The mean score for taste varied from 4.2 to 7.3 with different levels of incorporation of carotenoid extract. It was observed that the ice cream prepared by incorporation level of 0.1 % (w/v) with carotenoid extract scored the maximum (7.3) and high over the control sample. Ice cream prepared by incorporation level of 0.06 and 0.08 % (w/v) were found to be acceptable (>6.0) among the panelists for taste point of view. It is evident from the score that increase in incorporation level beyond 0.1 % was not liked from taste point of view. As the level of incorporation was increased from 0.1 % to above, the gradual increase in off taste was observed.

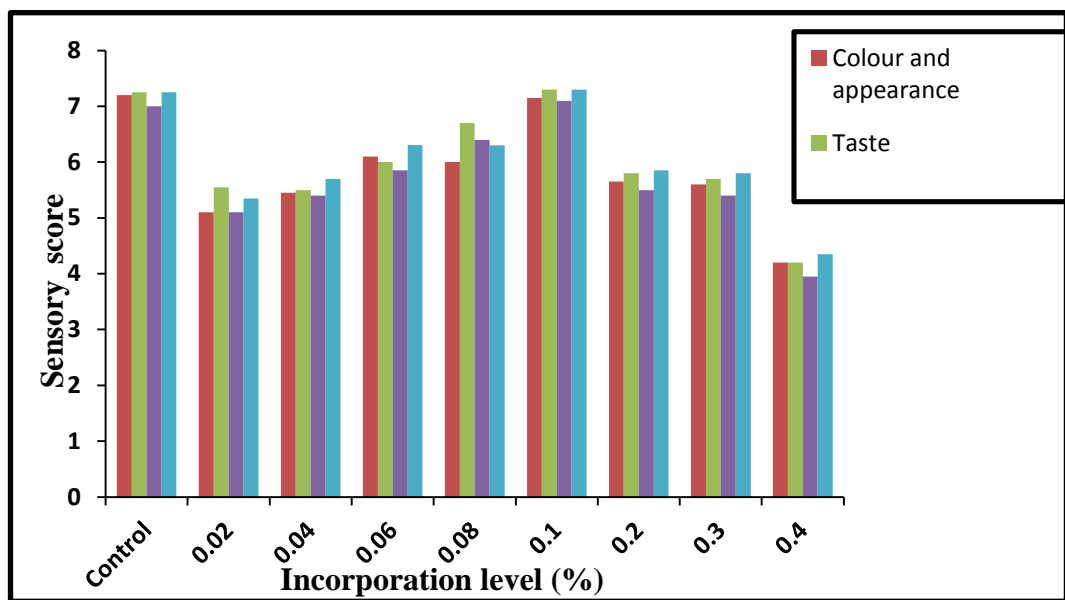
#### **c. Aroma**

The mean score for aroma varied from 3.95 to 7.1 with different levels of incorporation of carotenoid extract. It was observed that the ice cream prepared by incorporation level of 0.1 % (w/v) with carotenoid extract scored the maximum (7.1) and high over the control. Ice cream prepared by incorporation level of 0.08 % (w/v) was found to be accepted (>6.0) among the panelists for aroma point of view. It is evident from the score that increase in incorporation level beyond 0.1 % was not

liked from aroma point of view. As the level of incorporation was increased from 0.1 % to above, the gradual increase in off smell was observed.

**d. Overall acceptability**

The mean score for overall acceptability varied from 4.35 to 7.3. The maximum value corresponded to the sample prepared with incorporating level of 0.1 % with carotenoid extract scored the maximum, while the sample prepared with a incorporation level of 0.4 % with carotenoid extract scored the minimum. Ice cream prepared by incorporation level of 0.06 and 0.08 % (w/v) were found to be acceptable among the panelists as the scores are more than 6.0. The liking for ice cream prepared with incorporation level beyond 0.1 % was gradually decreased with increase in incorporation level as they were found inferior in colour and appearance, taste and aroma.



**Fig.4.2: Effect of incorporation of lycopene extract on sensory quality of ice cream**

Statistical analysis was carried out for the whole sensory data and is presented in Table 4.3. The results showed that increasing incorporation level from 0.02 to 0.4 % (w/v) with carotenoid extract had a significant influence (at 5 % level of significance) on colour and appearance, taste, aroma and overall acceptability of the ice cream samples.

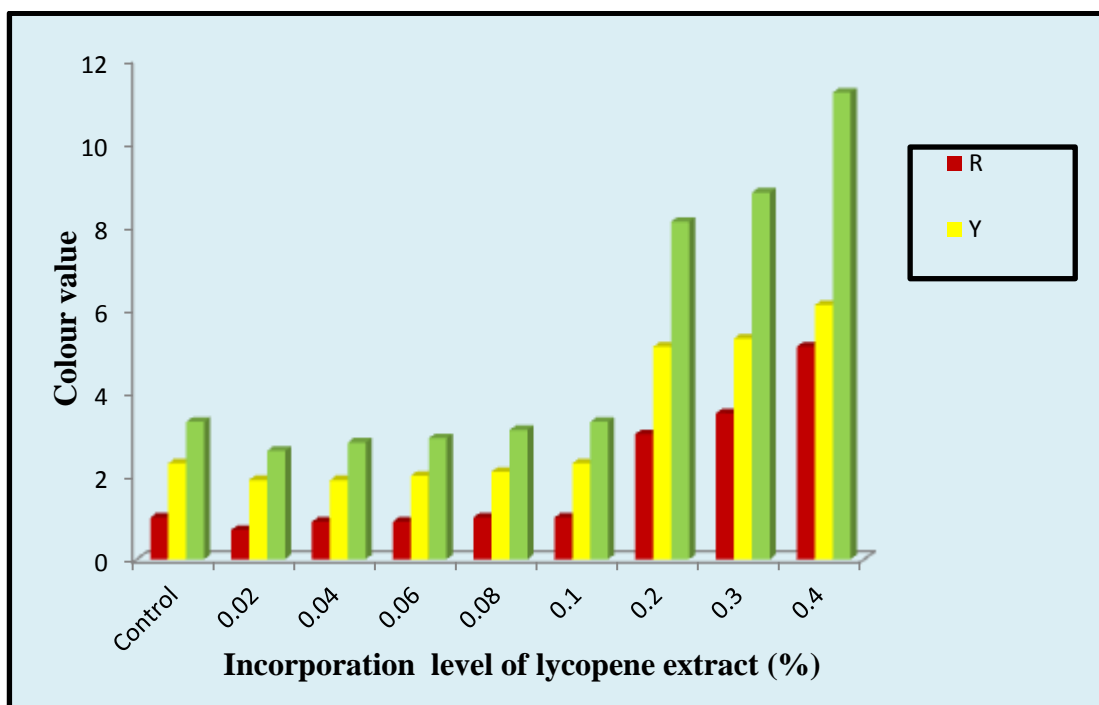
#### 4.3.1.2 Colour value measured by Lovibond Tintometer

The data of colour value obtained are presented in Table 4.4. All determinations were replicated thrice and average values are reported. The colour values were measured in the form of RYB. The obtained colour value of samples prepared by incorporation level of 0.1 % was found to be similar as control sample.

**Table 4.4: Colour values of different samples of ice cream measured by Lovibond Tintometer**

| Incorporation level (%) | Observations |     |   |   |              |
|-------------------------|--------------|-----|---|---|--------------|
|                         | R            | Y   | B | W | Colour value |
| Control                 | 1.0          | 2.3 | 0 | 0 | 3.3          |
| 0.02                    | 0.7          | 1.9 | 0 | 0 | 2.6          |
| 0.04                    | 0.9          | 1.9 | 0 | 0 | 2.8          |
| 0.06                    | 0.9          | 2.0 | 0 | 0 | 2.9          |
| 0.08                    | 1.0          | 2.1 | 0 | 0 | 3.1          |
| 0.1                     | 1.0          | 2.3 | 0 | 0 | 3.3          |
| 0.2                     | 3.0          | 5.1 | 0 | 0 | 8.1          |
| 0.3                     | 3.5          | 5.3 | 0 | 0 | 8.8          |
| 0.4                     | 5.1          | 6.1 | 0 | 0 | 11.2         |

R- Red, Y- Yellow , B- Blue, W- White



**Fig.4.3: Effect of incorporation of lycopene extract on colour value of ice cream**

#### ***4.3.1.3 Optimization of incorporation level of lycopene extract in ice cream***

By reviewing the results obtained for sensory quality and colour values, the ice cream prepared by incorporating the carotenoid extract in various levels i.e. 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3 and 0.4 % (w/v), it can be inferred from the sensory scores that the incorporation level of 0.1 % found to be similar score as the control sample for colour and other sensory attributes.

Also, it can be inferred from the sensory scores that the incorporation level of 0.1 % found to be similar score as the control sample for colour values measured by lovibond tintometer.

It was observed that the ice cream samples prepared with incorporation level of 0.06 and 0.08 % found to be accepted by the sensory scores but the colour values measured by the lovibold tintometer found to be lower compare to control sample and ice cream prepared by incorporation level of 0.1 %.

It was also observed that with increase in the incorporation level of carotenoid extract in ice cream, had strongly affect to the sensory quality of the ice cream. Incorporation level beyond 0.1 % (w/v) of carotenoid extract in ice cream, the sensory score has decreased for taste and colour, which was responsible to reduce the liking of

ice cream. Hence, incorporation level beyond 0.1 % (w/v) of carotenoid extract in ice cream was not found desirable.

The ice cream fortified with carthamidin (0.06 ml) was found to score higher overall acceptability (Machewad, *et al.* 2012). Thus, for ice cream incorporation level of 0.1 % (w/v) of carotenoid extract in formulation was found to higher sensory score and found optimum.

#### 4.3.2 Filling cream (Biscuit)

Filling cream (Biscuit) samples were prepared by incorporating the tomato carotenoid extract at different level of 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0 and 4.0 % in the standard recipe mentioned and described under section 3.7.2 and the market sample was selected for sensory evaluation and colour measurement.

##### 4.3.2.1 Effect of incorporation of lycopene extract on sensory quality of filling cream (Biscuit)

Sensory evaluation of filling cream (biscuit) was conducted using method of 9-point hedonic scale. The mean score of different sensory parameters of filling cream (biscuit) prepared by incorporating carotenoid extract at different levels are presented in Table 4.5 and the effect on sensory quality is presented in Fig.4.4.

**Table 4.5: Mean sensory score for filling cream (biscuit) prepared by incorporation of tomato carotenoid extract**

| Incorporation level % | Sensory score         |       |       |                       |
|-----------------------|-----------------------|-------|-------|-----------------------|
|                       | Colour and appearance | Taste | Aroma | Overall acceptability |
| Control               | 7.0                   | 6.9   | 7.2   | 7.22                  |
| 0.2                   | 5.9                   | 5.75  | 6.375 | 6.47                  |
| 0.4                   | 6.05                  | 5.95  | 6.35  | 6.45                  |
| 0.6                   | 7.05                  | 6.95  | 7.25  | 7.25                  |
| 0.8                   | 6.3                   | 6.1   | 6.3   | 6.4                   |
| 1.0                   | 5.6                   | 5.5   | 6.35  | 6.45                  |

|                          |              |              |              |              |
|--------------------------|--------------|--------------|--------------|--------------|
| 2.0                      | 4.65         | 4.55         | 6.0          | 6.1          |
| 3.0                      | 4.25         | 4.15         | 5.0          | 5.1          |
| 4.0                      | 3.65         | 3.55         | 4.5          | 4.6          |
| <b>S.Em</b>              | <b>0.184</b> | <b>0.193</b> | <b>0.169</b> | <b>0.163</b> |
| <b>CD<sub>0.05</sub></b> | <b>0.51</b>  | <b>0.54</b>  | <b>0.47</b>  | <b>0.46</b>  |
| <b>CV%</b>               | <b>10.4</b>  | <b>11.16</b> | <b>8.72</b>  | <b>8.33</b>  |

#### **a. Colour and appearance**

The mean score for colour and appearance of the filling cream (biscuit) varied from 3.65 to 7.05 with different levels of incorporation of carotenoid extract. It was observed that the filling cream (biscuit) prepared by incorporation level of 0.4 %, 0.6 % and 0.8 % were found to be accepted among the sensory panelists. It was observed that the filling cream (biscuit) prepared by incorporation level of 0.6 % (w/w) with carotenoid extract scored the maximum (7.05) and high over the control. Incorporation level beyond 0.6 %, reduced the liking of the product gradually from colour and appearance point of view. As the level of incorporation was increased from 0.6% to above, the colour and appearance of the filling cream (biscuit) became inferior ( Table 4.5 ).

#### **b. Taste**

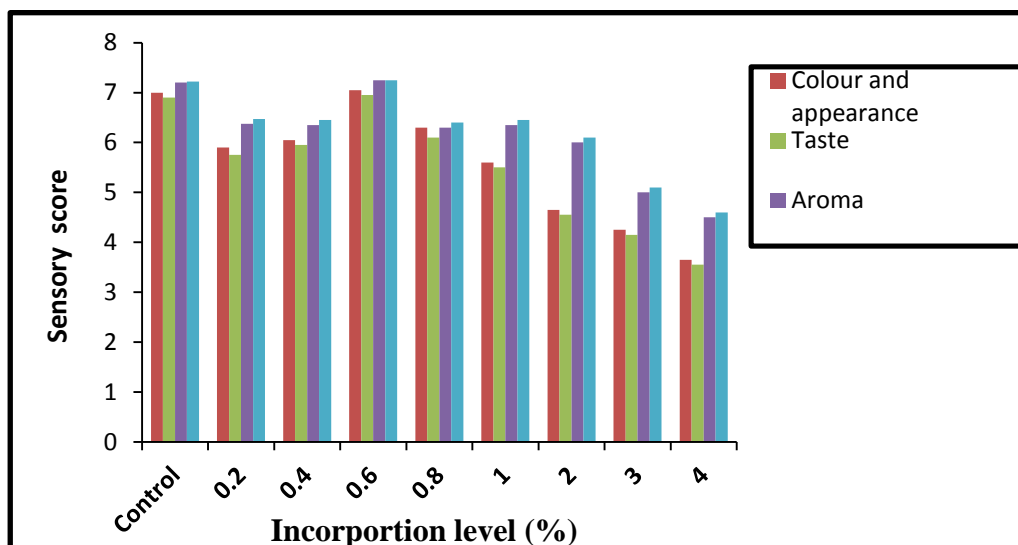
The mean score for taste varied from 3.55 to 6.95 with different levels of incorporation of carotenoid extract. It was observed that the filling cream (biscuit) prepared by incorporation level of 0.6 % and 0.8 % (w/w) were found to be accepted among the sensory panelists. It was observed that the filling cream (biscuit) prepared by incorporation level of 0.6 % (w/w) with carotenoid extract scored the maximum (6.95) and high over the control (market sample). It is evident from the score that increase in incorporation level beyond 0.6 % was not liked from taste point of view. As the level of incorporation was increased from 0.6 % to above, the gradual increase in off taste was observed.

### c. Aroma

The mean score for taste varied from 4.5 to 7.25 with different levels of incorporation of carotenoid extract. It was observed that the filling cream (biscuit) prepared by incorporation level of from 0.2 % to 2.0 % (w/w) with carotenoid extract were accepted by sensory panelists. It was observed that the filling cream (biscuit) prepared by incorporation level of 0.6 % (w/w) with carotenoid extract scored the maximum (7.25) and high over the control. It is evident from the score that increase in incorporation level beyond 2.0 % was not liked. As the level of incorporation was increased from 0.6 % to above, the gradual increase in off smell was observed.

### d. Overall acceptability

The mean score for overall acceptability varied from 4.6 to 7.25. It was observed that the filling cream (biscuit) prepared by incorporation level of from 0.2 % to 2.0 % (w/w) with carotenoid extract were accepted by sensory panelists. The maximum value corresponded to the sample prepared with incorporating level of 0.6 % with carotenoid extract scored the maximum, while the sample prepared with a incorporation level of 4.0 % with carotenoid extract scored the minimum (4.6). The liking for filling cream (biscuit) prepared with incorporation level beyond 0.6 % was gradually decrease with increase in incorporation level as they were found inferior in colour and appearance, taste and aroma.



**Fig.4.4: Effect of incorporation of lycopene extract on sensory quality of the biscuit filling cream**

Statistical analysis was carried out for the whole sensory data and is presented in Table 4.5. The results showed that increasing incorporation level from 0.2 to 4.0 % (w/w) with carotenoid extract had a significant influence (at 5 % level of significance) on colour and appearance, taste, Aroma and overall acceptability of the filling cream (biscuit) samples.

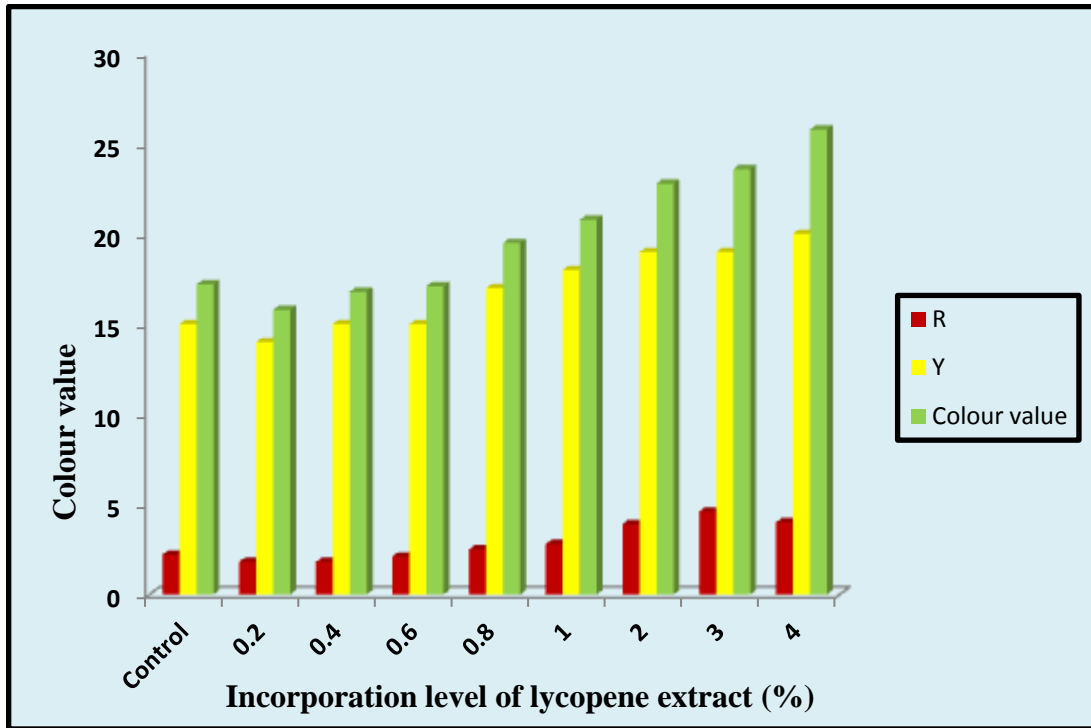
#### **4.3.2.2 Colour value measured by Lovibond Tintometer**

The data obtained regarding colour value are presented in Table 4.6. All determinations were replicated thrice and average values are reported. The colour values were measured in the form of RYB. The obtained colour value of samples prepared by incorporation level of 0.6 % was found to be similar as control sample.

**Table 4.6: Colour values of different samples of filling cream (biscuit) measured by Lovibond Tintometer**

| <b>Incorporation level (%)</b> | <b>Observations</b> |          |          |          |                     |
|--------------------------------|---------------------|----------|----------|----------|---------------------|
|                                | <b>R</b>            | <b>Y</b> | <b>W</b> | <b>B</b> | <b>Colour value</b> |
| Control                        | 2.2                 | 15.0     | 0        | 0        | 17.2                |
| 0.2                            | 1.8                 | 14.0     | 0        | 0        | 15.8                |
| 0.4                            | 1.8                 | 15.0     | 0        | 0        | 16.8                |
| 0.6                            | 2.1                 | 15.0     | 0        | 0        | 17.1                |
| 0.8                            | 2.5                 | 17.0     | 0        | 0        | 19.5                |
| 1.0                            | 2.8                 | 18.0     | 0        | 0        | 20.8                |
| 2.0                            | 3.9                 | 19.0     | 0        | 0        | 22.8                |
| 3.0                            | 4.6                 | 19.0     | 0        | 0        | 23.6                |
| 4.0                            | 4.0                 | 20.0     | 0        | 0        | 25.8                |

Red, Y- Yellow, B- Blue, W- White



**Fig.4.5: Effect of incorporation of lycopene extract on colour value of filling cream (Biscuit)**

#### ***4.3.2.3 Optimization technique of incorporation level of lycopene extract in filling cream (biscuit)***

By reviewing the results obtained for sensory quality and colour values, the filling cream (biscuit) prepared by incorporating the carotenoid extract in various levels i.e. 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0 and 4.0 % (w/w), it can be inferred from the sensory scores that the incorporation level of 0.6 % found to similar score as the control sample for colour and other sensory attributes.

Also, it can be inferred from the sensory scores that the incorporation level of 0.6 % found to be similar score as the control sample for colour values measured by lovibond tintometer were also found to be similar to control sample. It was observed that the filling cream (biscuit) samples prepared with incorporation level of 0.2 %, 0.4%, 0.6 %, 0.8 % and 2.0 % found to be accepted by the sensory scores but the colour values measured by the lovibond tintometer found to be lower compare to control sample and filling cream (biscuit) prepared by incorporation level of 0.6 %.

It was also observed that with increase in the incorporation level of carotenoid extract in filling cream (biscuit), had strongly affect to the sensory quality of the

filling cream (biscuit). Incorporation level beyond 0.6 % (w/w) of carotenoid extract in filling cream (biscuit), the sensory score was decreased for taste and colour attribute, which was responsible to reduce the liking of filling cream (biscuit). Hence incorporation level beyond 0.6 % (w/w) of carotenoid extract in filling cream (biscuit) was not found desirable.

Thus, for filling cream (biscuit), incorporation level of 0.6 % (w/w) of carotenoid extract in formulation was found optimum.

### 4.3.3 Jam

Jam samples were prepared by incorporating the tomato carotenoid extract at different level (0.06, 0.08, 0.1, 0.2, 0.3 and 0.4 %) in the standard recipe mentioned and described under section 3.7.3 and the commercially available sample (Kissan Brand) was treated as control for sensory analysis and colour measurement.

#### 4.3.3.1. Effect of incorporation of lycopene extract on sensory quality of Jam

Sensory evaluation of jam was conducted using method of 9-point hedonic scale. The mean score of different sensory parameters of jam prepared by incorporating carotenoid extract at different levels are presented in Table 4.7 and the effect on sensory quality is presented in Fig.4.6.

**Table 4.7: Mean sensory score for jam prepared by incorporation of tomato carotenoid extract**

| Incorporation level % | Sensory score         |       |       |                       |
|-----------------------|-----------------------|-------|-------|-----------------------|
|                       | Colour and appearance | Taste | Aroma | Overall acceptability |
| Control               | 7.25                  | 7.2   | 7.3   | 7.15                  |
| 0.06                  | 6.86                  | 7.0   | 6.96  | 6.94                  |
| 0.08                  | 7.3                   | 7.5   | 7.3   | 7.4                   |
| 0.1                   | 6.2                   | 6.45  | 6.3   | 6.2                   |
| 0.2                   | 6.1                   | 6.15  | 5.7   | 5.95                  |
| 0.3                   | 5.35                  | 5.25  | 5.1   | 5.15                  |

|                          |              |              |              |              |
|--------------------------|--------------|--------------|--------------|--------------|
| 0.4                      | 4.35         | 4.2          | 4.4          | 4.0          |
| <b>S.Em</b>              | <b>0.201</b> | <b>0.185</b> | <b>0.157</b> | <b>0.193</b> |
| <b>CD<sub>0.05</sub></b> | <b>0.57</b>  | <b>0.52</b>  | <b>0.44</b>  | <b>0.54</b>  |
| <b>CV%</b>               | <b>10.13</b> | <b>9.94</b>  | <b>7.98</b>  | <b>9.85</b>  |

#### **a. Colour and appearance**

The mean score for colour and appearance of the jam varied from 4.35 to 7.3 with different levels of incorporation of carotenoid extract. It was observed that the jam prepared by incorporation level of 0.08 % (w/w) with carotenoid extract scored the maximum (7.3) and high over the control. Incorporation level of 0.06 %, 1.0 % and 0.2 % were found to be acceptable among the sensory panelists. Incorporation level of beyond 0.08 %, reduced the liking of the product gradually from colour and appearance point of view. As the level of incorporation was increased from 0.08 % to above, the colour and appearance of the jam became inferior.

#### **b. Taste**

The mean score for taste varied from 4.2 to 7.5 with different levels of incorporation of carotenoid extract. Incorporation level of 0.06, 0.08, 1.0 and 0.2 % were found to be acceptable among the sensory panelists. It was observed that the jam prepared by incorporation level of 0.08 % (w/w) with carotenoid extract scored the maximum (7.5) and high over the control sample. It is evident from the score that increase in incorporation level beyond 0.08 % was not liked from taste point of view. As the level of incorporation was increased from 0.08 % to above, the gradual increase in off taste was observed and hence was not acceptable by the panelists.

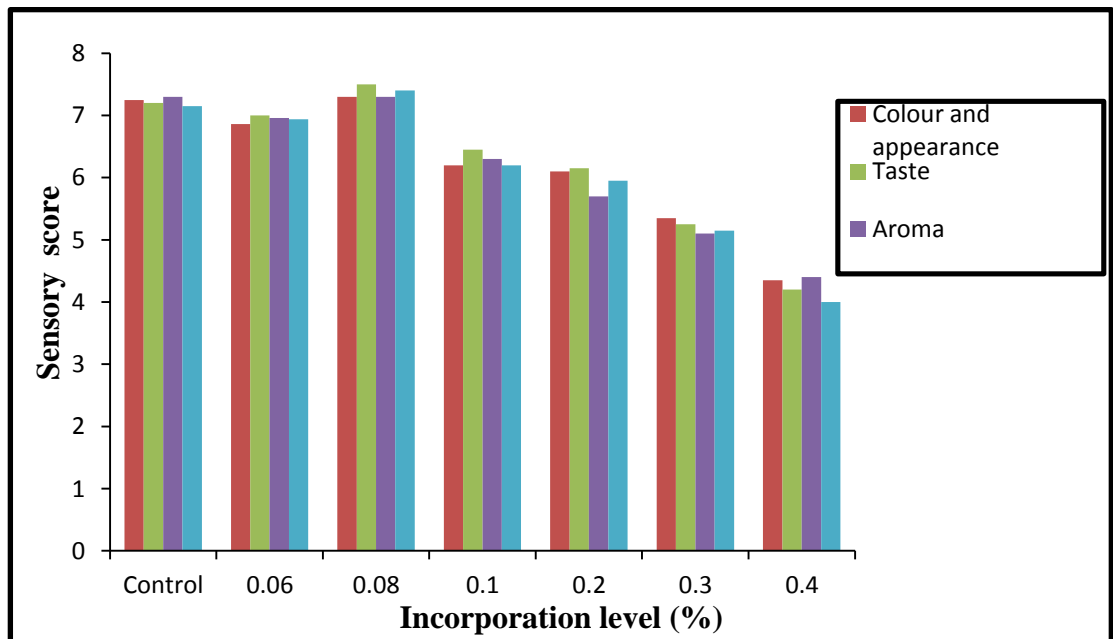
#### **c. Aroma**

The mean score for taste varied from 4.4 to 7.3 with different levels of incorporation of carotenoid extract. Incorporation level of 0.06 %, 0.08 % and 1.0 % were found to be acceptable among the sensory panelists. It was observed that the jam prepared by incorporation level of 0.08 % (w/w) with carotenoid extract scored the maximum (7.3) and same as the control. From the table it is clear that increase in

incorporation level beyond 0.08 % was not liked. As the level of incorporation was increased from 0.08 % to above, the gradual increase in off smell was observed.

#### d. Overall acceptability

The mean score for overall acceptability varied from 4.0 to 7.4. Incorporation level of 0.06 %, 0.08 % and 1.0 % were found to be acceptable among the sensory panelists. The maximum value corresponded to the sample prepared with incorporating level of 0.08 % with carotenoid extract scored the maximum, while the sample prepared with a incorporation level of 0.4 % with carotenoid extract scored the minimum. The liking for jam prepared with incorporation level beyond 0.08 % was gradually decrease with increase in incorporation level as they were found inferior in colour and appearance, taste and aroma.



**Fig.4.6: Effect of incorporation of lycopene extract on sensory quality of the jam**

Statistical analysis was carried out for the whole sensory data and is presented in Table 4.7. The results showed that increasing incorporation level from 0.08 to 0.4 % (w/w) with carotenoid extract had a significant influence (at 5 % level of significance) on colour and appearance, taste, Aroma and overall acceptability of the jam samples.

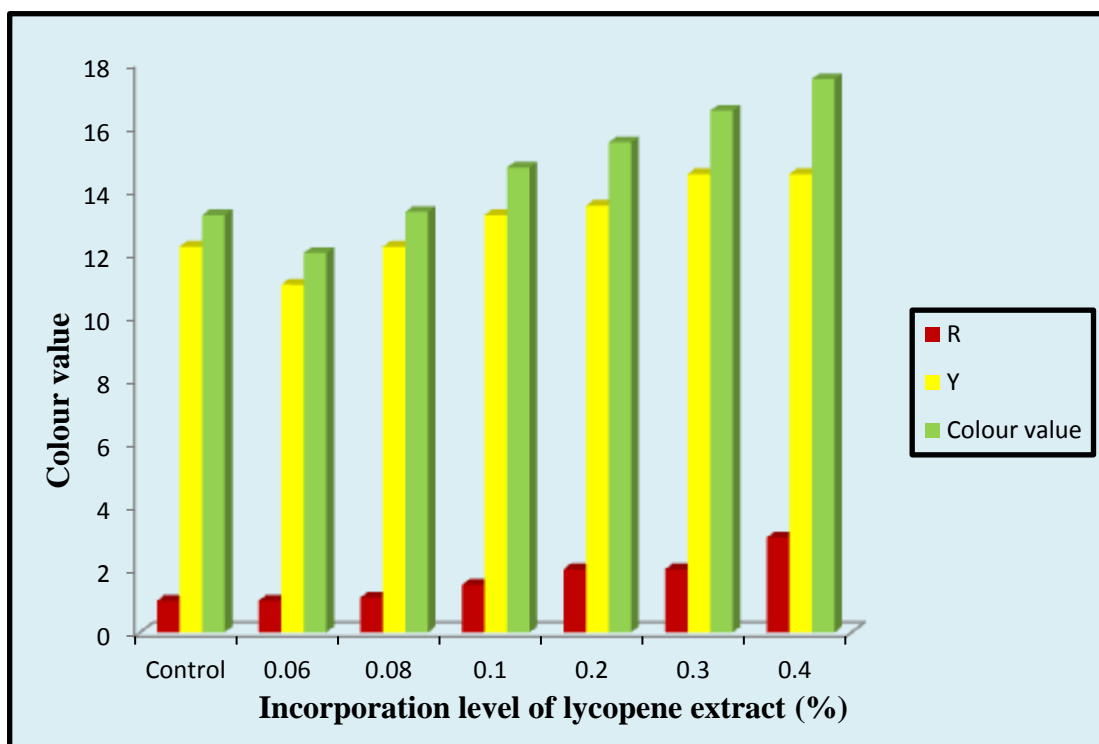
#### 4.3.3.2 Colour values measured by Lovibond Tintometer

The data obtained regarding colour value are presented in Table 4.8. All determinations were replicated thrice and average values are reported. The colour values were measured in the form of RYB. The obtained colour value of samples prepared by incorporation level of 0.08 % was found to be similar as control sample.

**Table 4.8: Colour values measured by Lovibond Tintometer of different samples of jam**

| Incorporation Level (%) | Observations |      |   |   |              |
|-------------------------|--------------|------|---|---|--------------|
|                         | R            | Y    | W | B | Colour value |
| Control                 | 1.0          | 12.2 | 0 | 0 | 13.2         |
| 0.06                    | 1.0          | 11.0 | 0 | 0 | 12.0         |
| 0.08                    | 1.1          | 12.2 | 0 | 0 | 13.3         |
| 0.1                     | 1.5          | 13.2 | 0 | 0 | 14.7         |
| 0.2                     | 2.0          | 13.5 | 0 | 0 | 15.5         |
| 0.3                     | 2.0          | 14.5 | 0 | 0 | 16.5         |
| 0.4                     | 3.0          | 14.5 | 0 | 0 | 17.5         |

Red, Y- Yellow, B- Blue, W- White



**Fig. 4.7: Effect of incorporation of lycopene extract on colour value of jam**

#### ***4.3.3.3 Optimization of incorporation level of lycopene extract in jam***

By reviewing the results obtained for sensory quality and colour values, the jam prepared by incorporating the carotenoid extract in various levels i.e. 0.06, 0.08, 0.1, 0.2, 0.3 and 0.4 % (w/w), it can be inferred from the sensory scores that the incorporation level of 0.08 % found to be more or less similar score as the control sample for colour and other sensory attributes.

Also, it can be inferred from the sensory scores that the incorporation level of 0.08 % found to be similar score as the control sample for colour values measured by lovibond tintometer. It was observed that the jam samples prepared with incorporation level of 0.06 %, 0.08% and 0.1 %, found to be accepted by the sensory scores but the colour values measured by the lovibold tintometer found to be lower compare to control sample and jam prepared by incorporation level of 0.08 %.

It was also observed that with increase in the incorporation level of carotenoid extract in jam, had strongly affect to the sensory quality of the jam. Incorporation level beyond 0.08 % (w/w) of carotenoid extract in jam, the sensory score has decreased for taste and colour, which was responsible to reduce the liking of jam.

Hence, incorporation level beyond 0.08 % (w/w) of carotenoid extract in jam was not found desirable.

Thus, for jam, incorporation level of 0.08 % (w/w) of carotenoid extract in formulation was found optimum.

#### 4.4 Storage Study of Lycopene in different Food Products

Optimized food samples viz. ice cream, filling cream (biscuit) and jam were stored for shelf life study of lycopene. Ice cream was stored at -18 °C temperature, filling cream (biscuit) was stored in metalized pouches at 30 °C temperature and jam was stored in a PET jar at 30 °C temperature. Samples were analyzed for lycopene content at 7 days interval for a period of 49 days.

**Table 4.9: Variation in lycopene content as influenced by storage in different food products**

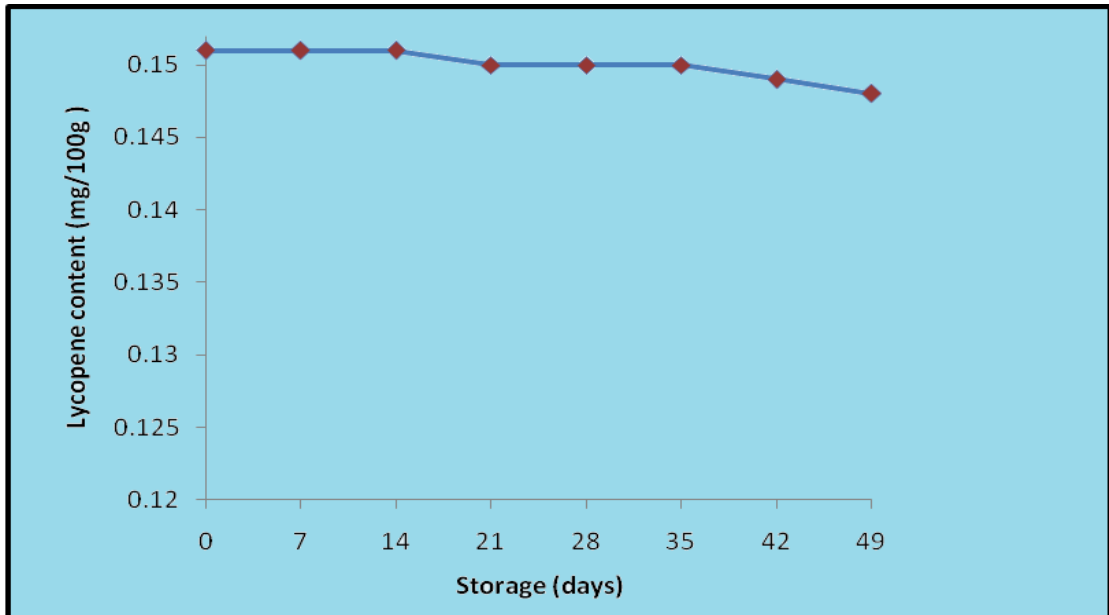
| Storage period (days)    | Lycopene content (mg/100g)  |                             |                             |
|--------------------------|-----------------------------|-----------------------------|-----------------------------|
|                          | Ice cream                   | Filling cream (Biscuit)     | Jam                         |
| 0                        | 0.151                       | 0.879                       | 0.124                       |
| 7                        | 0.151                       | 0.856                       | 0.117                       |
| 14                       | 0.151                       | 0.843                       | 0.116                       |
| 21                       | 0.150                       | 0.830                       | 0.114                       |
| 28                       | 0.150                       | 0.818                       | 0.112                       |
| 35                       | 0.150                       | 0.792                       | 0.108                       |
| 42                       | 0.149                       | 0.766                       | 0.095                       |
| 49                       | 0.148                       | 0.731                       | 0.091                       |
| <b>S.Em</b>              | <b>1.11×10<sup>-4</sup></b> | <b>3.42×10<sup>-4</sup></b> | <b>4.0×10<sup>-4</sup></b>  |
| <b>CD<sub>0.05</sub></b> | <b>2.64×10<sup>-4</sup></b> | <b>8.28×10<sup>-4</sup></b> | <b>9.67×10<sup>-4</sup></b> |
| <b>CV%</b>               | <b>0.145</b>                | <b>0.077</b>                | <b>0.738</b>                |

The absorbance spectra of different food products were measured (503 nm) at interval of 7 days up to 49 days of storage period (Appendix II). The data were

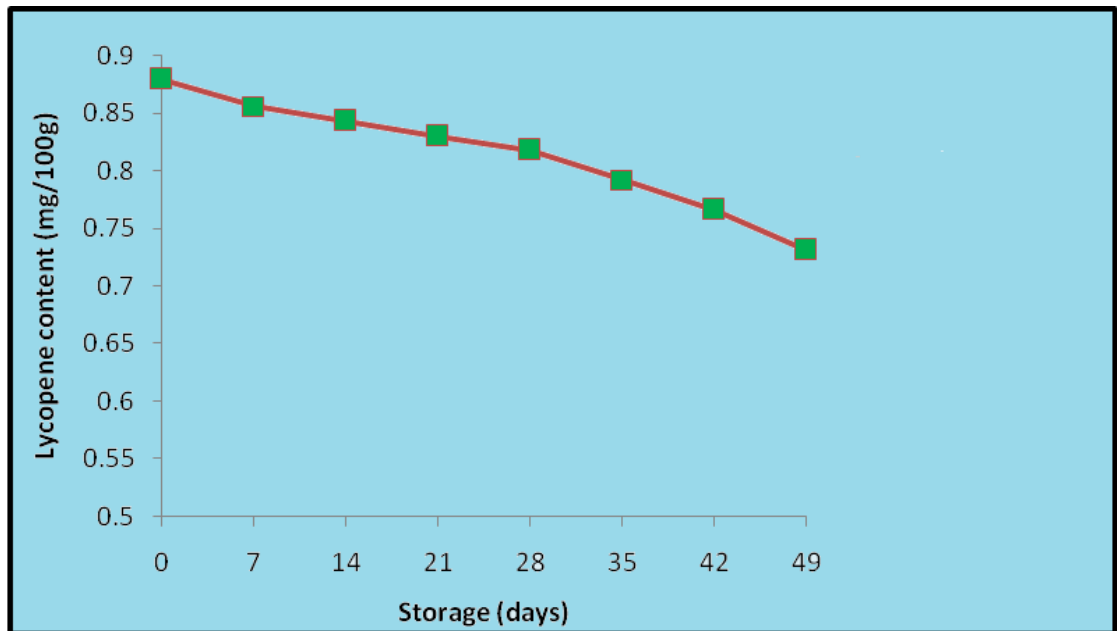
statistically analyzed. From the above Table 4.9, the results indicated that the lycopene content in ice cream was remain same up to 35 days of storage period. There was no significant difference was observed. Then, lycopene content was gradually decreased. The maximum lycopene content was observed initially in the ice cream (0.151 mg/100g) and minimum lycopene content (0.148 mg/100g) was observed at 49<sup>th</sup> day. The lycopene content in filling cream (biscuit) was significantly highest (0.879 mg/100g) at 0 day as compare to rest of the storage period and the lowest lycopene content (0.731 mg/100g) was observed at 49<sup>th</sup> day. There is significantly declining trend was observed over the period of storage. The maximum lycopene content in jam (0.124mg/100g) was recorded at 0 day as compare to rest of the storage period and the lowest lycopene content (0.091 mg/100g) was observed at 49<sup>th</sup> day. Significantly declining trend was observed over the period of storage. The data indicate that as the storage period was increased, the lycopene content was decreased for food products viz. jam and filling cream (biscuit) stored at 30 °C. The lycopene content decreased quite faster in case of jam and filling cream (biscuit) as compare to ice cream. The lycopene content remain almost same for entire period of storage for ice cream. It was found that the decrease in lycopene content in ice cream, filling cream (biscuit) and jam was 1.98%, 16.83 % and 26.61 % respectively after 49 days. Tola and Ramaswamy (2013) also found that the lycopene degradation in watermelon pulp powder for 15 days at storage temperature 25 °C. However, sample stored at lower temperature (2 °C) showed better stability of lycopene content in their study.

The degradation of lycopene may be due to the degradation of *trans*-lycopene in to *cis*-lycopene (isomerization). Light and heat affect to tomato carotenoid extract can result in destruction of lycopene. The same results were observed by Hanan *et al.* (2011) and Machewad *et al.* (2012) during their study.

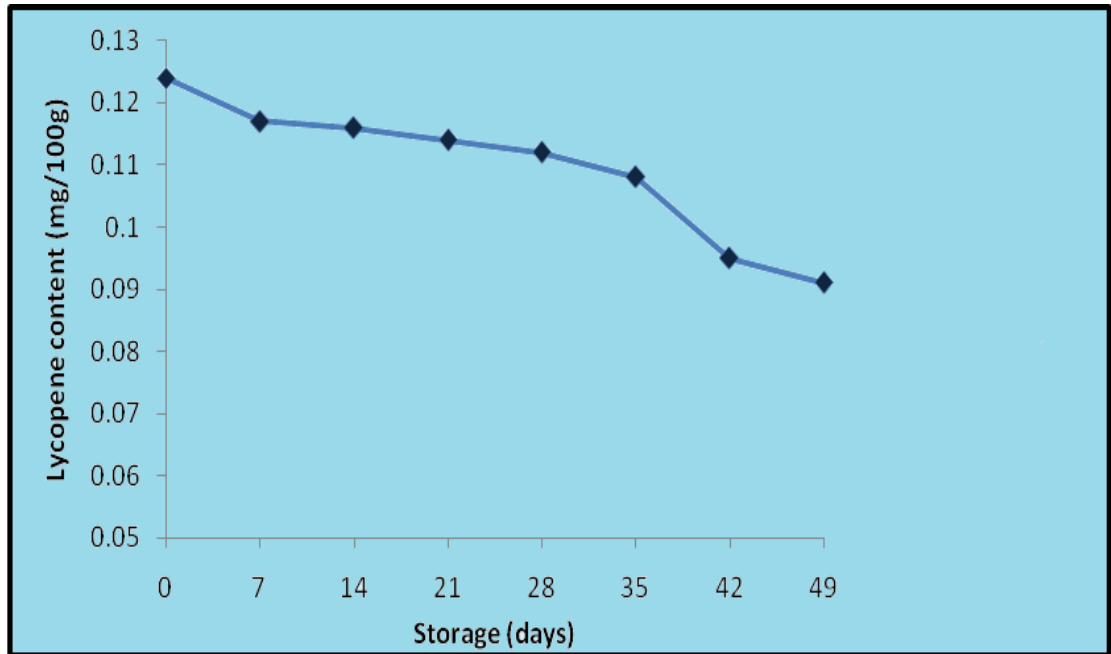
The graphical representation of effect on lycopene content as influenced by storage in the ice cream, filling cream (biscuit) and jam are illustrated in Fig.4.8, Fig.4.9 and Fig.4.10 respectively.



**Fig. 4.8: Changes in lycopene content in the ice cream during storage**



**Fig. 4.9: Changes in lycopene content in the filling cream (biscuit) during storage**



**Fig.4.10: Changes in lycopene content in the jam during storage**

## CHAPTER V

### SUMMARY AND CONCLUSIONS

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Tomato (*Lycopersicon esculentum*) is a fruit used mainly as a vegetable both in fresh as well as in processed forms, in food preparations. More than 80 % of the processed tomatoes produced are consumed in the form of tomato juice, paste, puree, catsup and sauce. Tomatoes and tomato-based foods are considered healthy for the reason that they are low in calories, but possess a remarkable combination of antioxidant micronutrients. Tomatoes have been traditionally credited as rich source of carotenoids and vitamins, particularly beta-carotene, pro-vitamin A, ascorbic acid, and vitamin C. In recent years, another important carotenoid in tomatoes, lycopene, has received considerable attention.

Lycopene is a naturally occurring pigment in fruits and vegetables known as a carotenoid. It gives tomatoes their rich red colour. Tomatoes and tomato products are the major sources of lycopene compounds which are considered to be important contributors of carotenoids to the human diet. The antioxidant capability of lycopene has led to promising results in decreasing the risk of some illnesses and cancers. Animals and humans do not synthesize lycopene, and thus depend on dietary sources.

Commercial processing of tomato produces a large amount of pomace which is rich in bioactive compounds, e.g., lycopene and other carotenoids, tomato fiber and tomato oil (tocopherols) from seeds. Presently, pomace (peels and seeds) in tomato industry is mechanically removed and discarded. Unutilized wastes not only add to the disposal problem but also aggravate environmental pollution. This by-product is used for animal feed with no economical benefit to the industry. It has high moisture content and incurs a drying expense if required to be preserved.

Environmental friendly extraction and purification procedure for production of lycopene on an industrial scale with minimal loss of bioactivity is highly desirable for food and pharmaceutical applications. Extraction with organic solvents is a well-established method in the food industry but the issue of safety during extraction, poor

efficiency, high energy requirement, longer processing time and the residual content of chemical solvents are the limitations. Therefore, a critical and demanding step is the selection of proper and safe extraction system.

Lycopene is presently of particular interest for the food and pharmaceutical industries, both as natural colourant and as a nutraceutical. People knowledge and curiosity of the health benefits of lycopene have skyrocketed. Consumer demand for healthful food products and demand for clean-label declarations provides an opportunity to develop a market for food-grade and pharmaceutical-grade lycopene products. Industrial production of lycopene from tomatoes is of great interest to pharmaceutical and food companies, as a nutraceutical and for functional food development.

Lycopene extract from tomato is intended for use in the food categories like, baked foods, breakfast cereals, dairy products including frozen dairy desserts, spreads, carbonated beverages, fruits and vegetable juices, candy, soups, salad dressings and other foods. Lycopene is a natural antioxidant and having functional and nutraceutical properties as well as can be used as a natural colouring agent in food. It can be satisfactorily intended in the food systems as a functional ingredient.

Keeping the above view in mind, a systematic scientific study was undertaken with the following specific objectives.

5. Characterization of tomato pomace carotenoids extracted using SCFE system.
6. Utilization of tomato pomace carotenoids extract in selected food products according to its suitability/compatibility.
7. Evaluation of the carotenoid fortified food products for acceptability.
8. Evaluation of the storability of the carotenoid rich food items.

The tomato pomace was procured and stored in the deep freezer. This was then characterized for its chemical constituents. The tomato pomace was dried using optimized fluidized bed drying method at 50 °C for production of pomace powder with about 8 % moisture content (w.b.).

Lycopene was extracted from dried tomato pomace powder using supercritical extraction system having CO<sub>2</sub> as solvent at optimized conditions i.e., temperature 70 °C, pressure 300 bar and time 90 min, keeping particle size of 0.4 mm.

The obtained carotenoid extract was characterized for its lycopene content, refractive index and colour value. The lycopene extract was utilized in different levels of incorporation in selected food product for their acceptability, Viz. Ice cream, Filling cream (Biscuit) and Jam. The optimization of the same was carried out on sensory analysis and colour measurement.

The food products which were optimized by sensory score and colour measurement were evaluated for their shelf life for the period of 49 days.

The following conclusions could be drawn from the present investigation:

1. The tomato pomace as obtained from tomato processing industry was found to be moisture content of 84.71 %, protein 7.13 %, crude fat 0.31 %, crude fiber 2.65 %, total ash 0.7 % and carbohydrates by difference was found to be 4.51 %.
2. The carotenoid extract obtained using supercritical fluid extraction system was found lycopene content of 170.661 mg/100g, refractive index at 20 °C of 1.48 and colour value of 111.8.
3. The ice cream prepared by incorporating the carotenoid extract at the rate of 0.1 % was found nearer score as the commercially available sample for colour and other sensory attributes.
4. The filling cream (biscuit) prepared by incorporating the carotenoid extract at the rate of 0.6 % was found similar score as the commercially available sample for colour and other sensory attributes.
5. The jam prepared by incorporating the carotenoid extract at the rate of 0.08 % was found similar score as the commercially available sample for colour and other sensory attributes.

6. It was found that lycopene content in ice cream remained unchanged up to 35 days. After which there was a gradual and insignificant decrease. This could be because of the low temperature of the product.

In case of jam the change in lycopene is gradual as the product is not exposed to air but is affected by light. Hence, the degradation is quite slow.

In case of filling cream (biscuit) the lycopene loss is very rapid as the product is in contact with air and light both.

7. It was found that the decrease in lycopene content in ice cream, filling cream (biscuit) and jam was 1.98%, 16.83 % and 26.61 % respectively after 7 weeks.

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## APPENDIX I

### Sensory evaluation sheet

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Name of the product:

Date:

Name of the evaluator:

Name of the test: Hedonic

test

1. Please test this sample according to your preference.
2. Use the appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample.
3. Remember, you are the only one who can tell what you like.
4. An honest expression of your feeling will help us.

#### Scale:

Like extremely-9

Like very much-8

Like moderately-7

Like slightly-6

Neither like nor dislike-5

Dislike slightly-4

Dislike moderately-3

Dislike very much-2

Dislike extremely-1

| Sample code | Colour and Appearance | Aroma | Taste | Overall Acceptability |
|-------------|-----------------------|-------|-------|-----------------------|
| Control     |                       |       |       |                       |
| 1           |                       |       |       |                       |
| 2           |                       |       |       |                       |
| 3           |                       |       |       |                       |
| 4           |                       |       |       |                       |
| 5           |                       |       |       |                       |

Remark:

Signature:

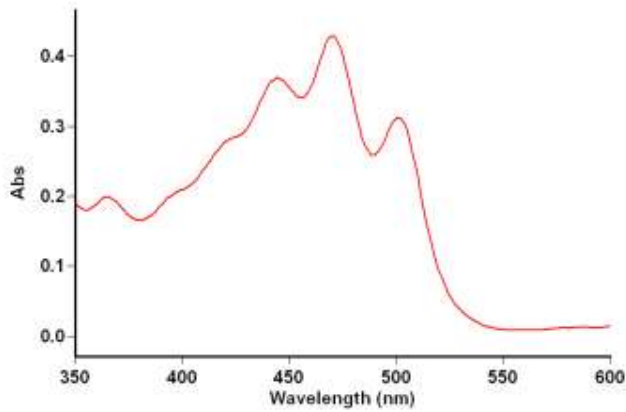
## APPENDIX II

### UV visible spectra of different Food products

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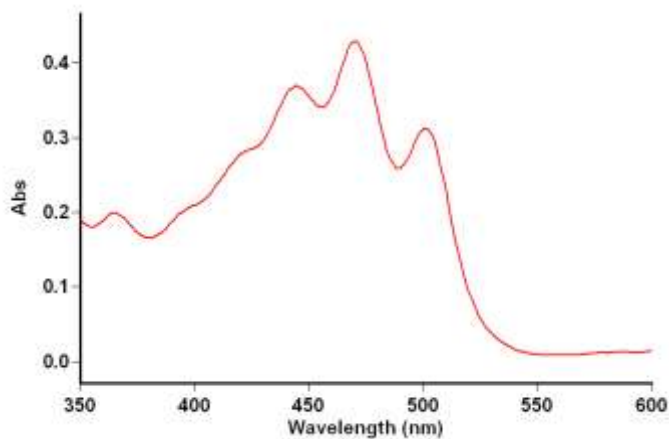
#### Ice cream

0 day



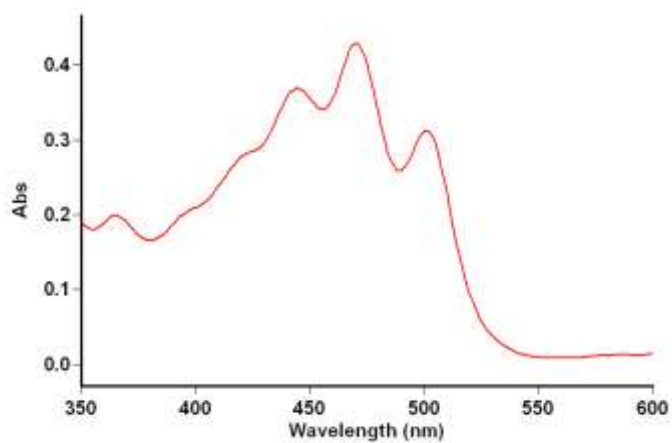
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.1           | 0.313 |
| 470.0           | 0.429 |
| 445.0           | 0.369 |
| 365.1           | 0.199 |

7 day



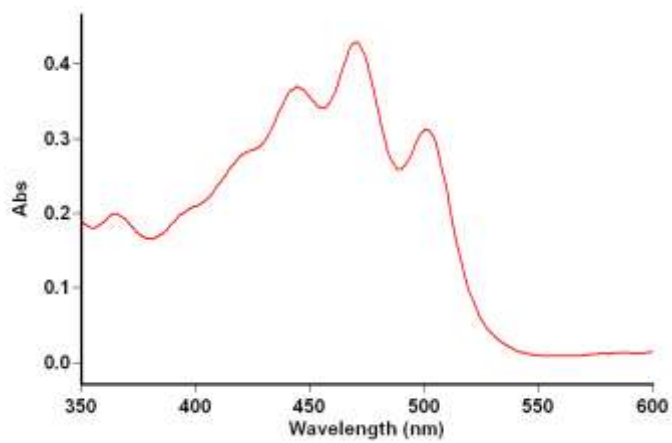
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.1           | 0.313 |
| 470.0           | 0.429 |
| 445.0           | 0.369 |
| 365.1           | 0.199 |

14 day



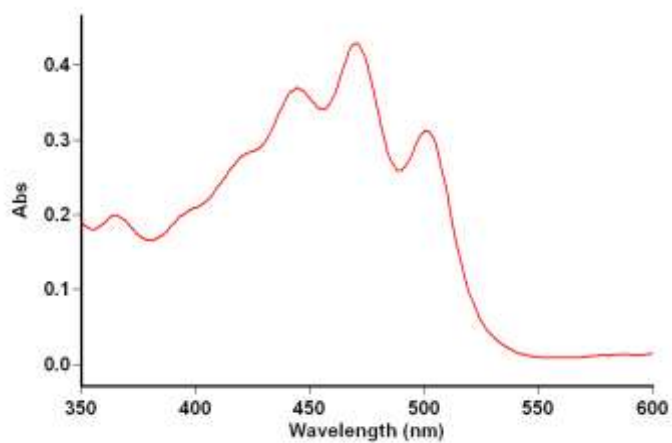
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.1           | 0.313 |
| 470.0           | 0.429 |
| 445.0           | 0.369 |
| 365.1           | 0.199 |

Day 21



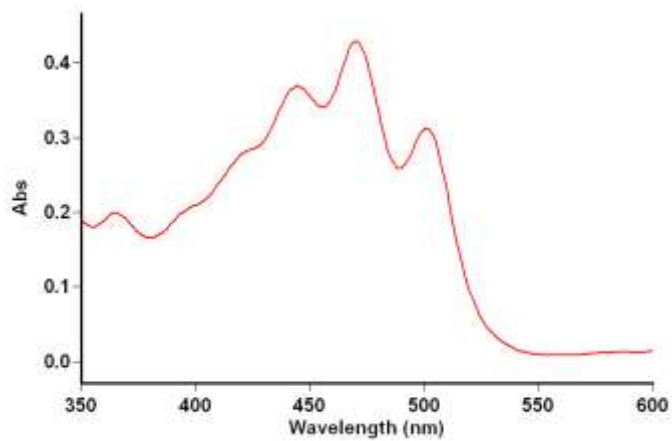
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.1           | 0.312 |
| 470.0           | 0.429 |
| 445.0           | 0.369 |
| 365.1           | 0.199 |

Day 28



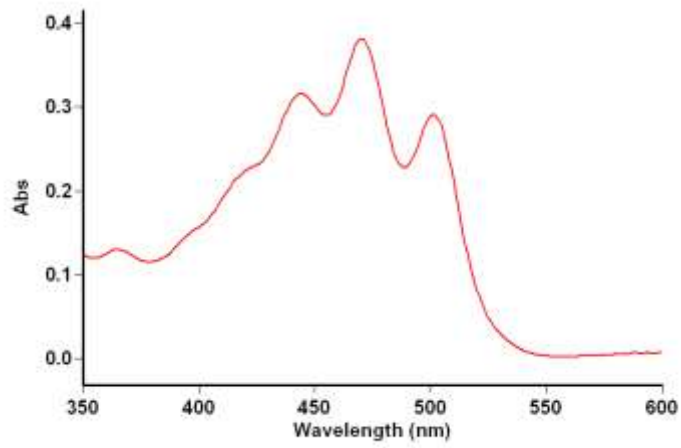
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.1           | 0.312 |
| 470.0           | 0.429 |
| 445.0           | 0.369 |
| 365.1           | 0.199 |

Day 35



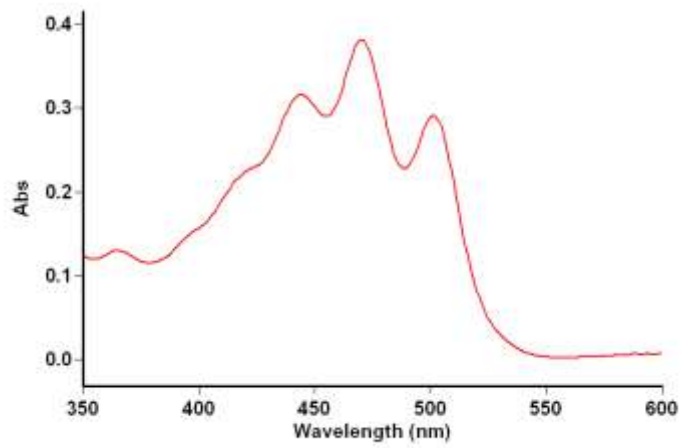
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.1           | 0.312 |
| 470.0           | 0.429 |
| 445.0           | 0.369 |
| 365.1           | 0.199 |

Day 42



| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.0           | 0.310 |
| 470.0           | 0.381 |
| 443.9           | 0.316 |
| 365.1           | 0.130 |

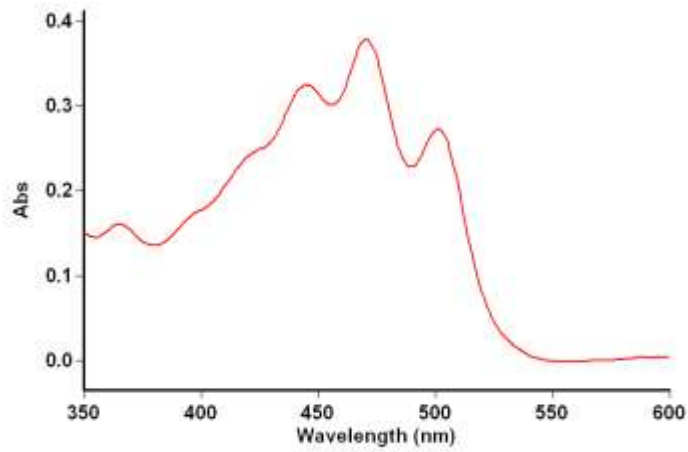
Day 49



| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.0           | 0.307 |
| 470.0           | 0.345 |
| 443.9           | 0.315 |
| 365.1           | 0.121 |

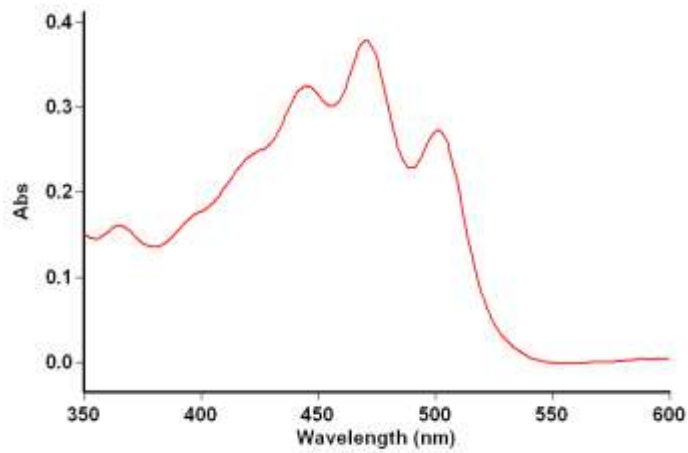
## Filling cream (Biscuit)

Day 0



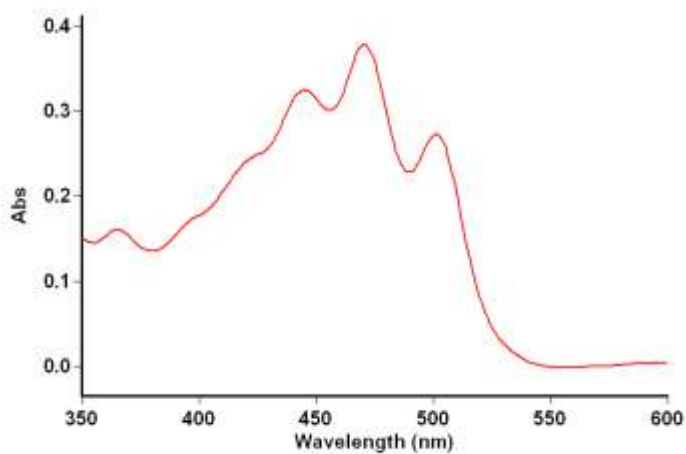
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.0           | 0.273 |
| 471.1           | 0.378 |
| 445.0           | 0.325 |
| 365.1           | 0.161 |

Day 7



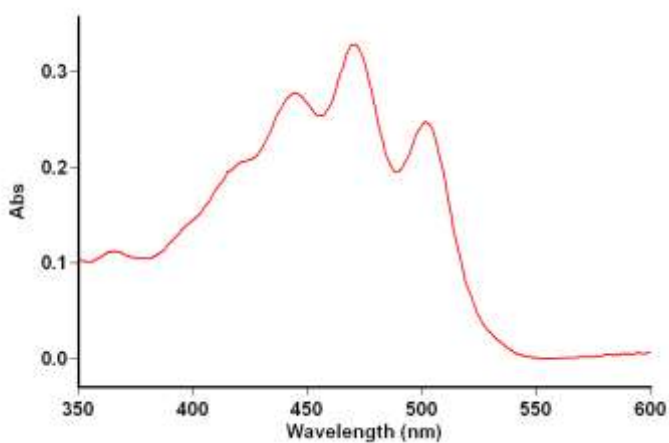
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.0           | 0.266 |
| 471.1           | 0.374 |
| 445.0           | 0.318 |
| 365.1           | 0.132 |

Day 14



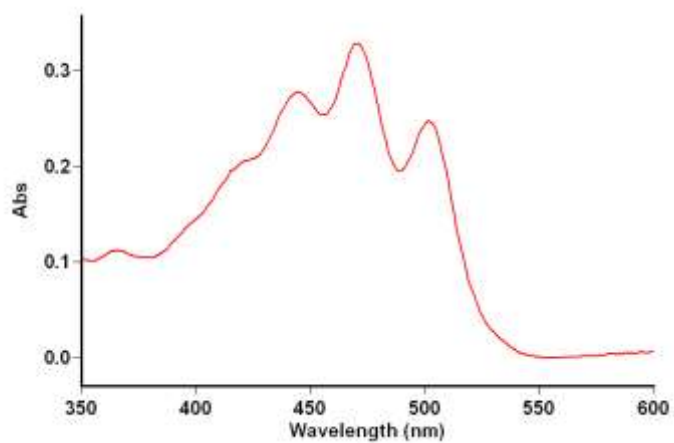
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.0           | 0.262 |
| 471.1           | 0.372 |
| 445.0           | 0.315 |
| 365.1           | 0.141 |

Day 21



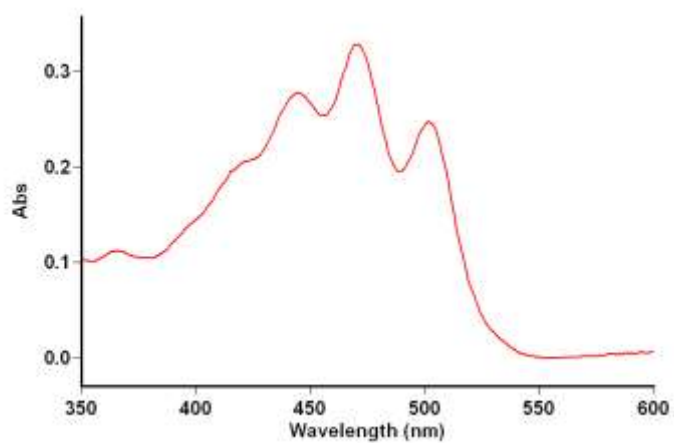
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.0           | 0.258 |
| 471.1           | 0.329 |
| 445.0           | 0.278 |

Day 28



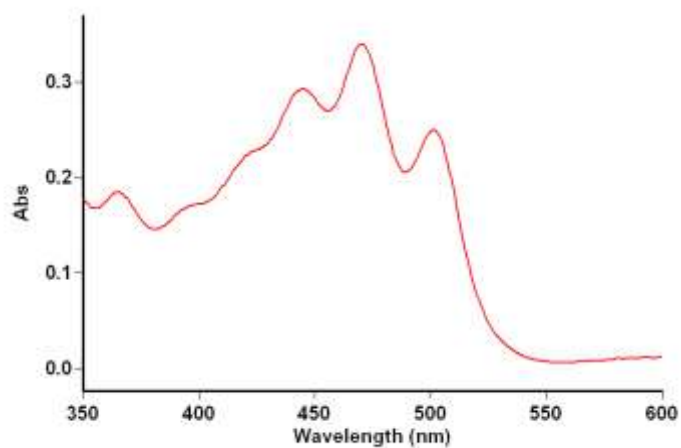
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.0           | 0.254 |
| 471.1           | 0.321 |
| 445.0           | 0.278 |

Day 35



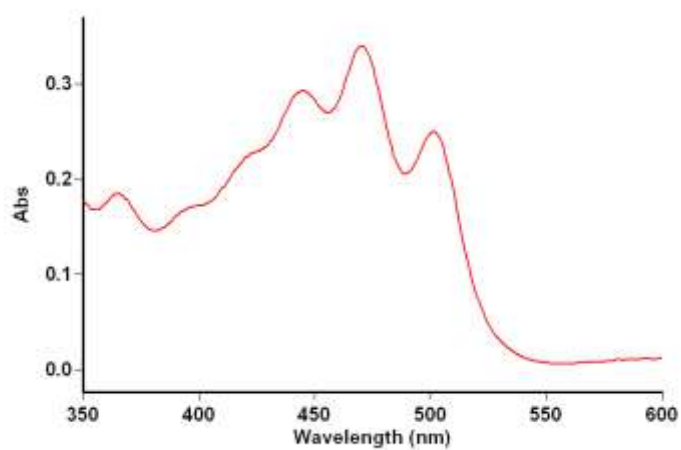
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.0           | 0.246 |
| 471.1           | 0.319 |
| 445.0           | 0.271 |

Day 42



| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.1           | 0.238 |
| 470.0           | 0.339 |
| 445.0           | 0.292 |
| 365.1           | 0.185 |

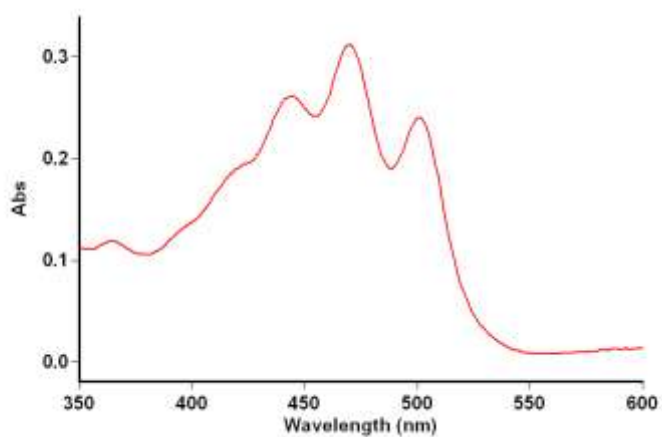
Day 49



| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.1           | 0.227 |
| 470.0           | 0.339 |
| 445.0           | 0.292 |
| 365.1           | 0.185 |

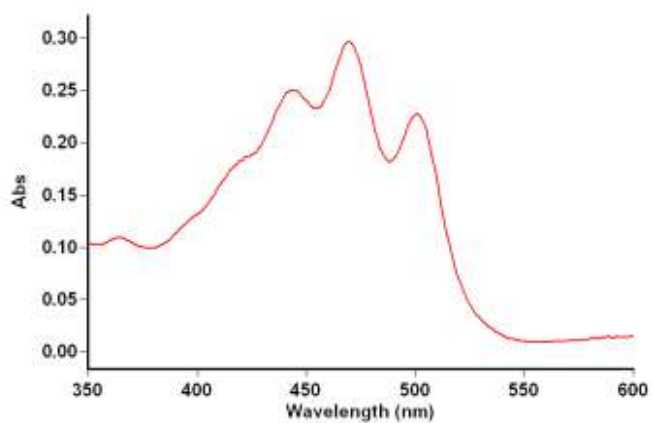
# Jam

Day 0



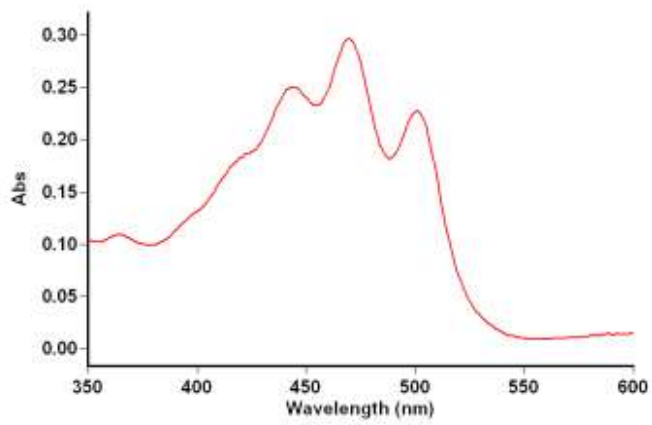
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.1           | 0.241 |
| 470.0           | 0.312 |
| 443.9           | 0.261 |

Day 7



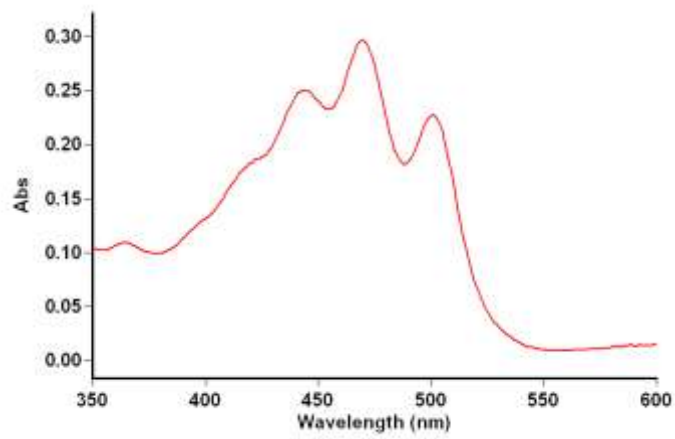
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.1           | 0.229 |
| 470.0           | 0.296 |
| 443.0           | 0.250 |

Day 14



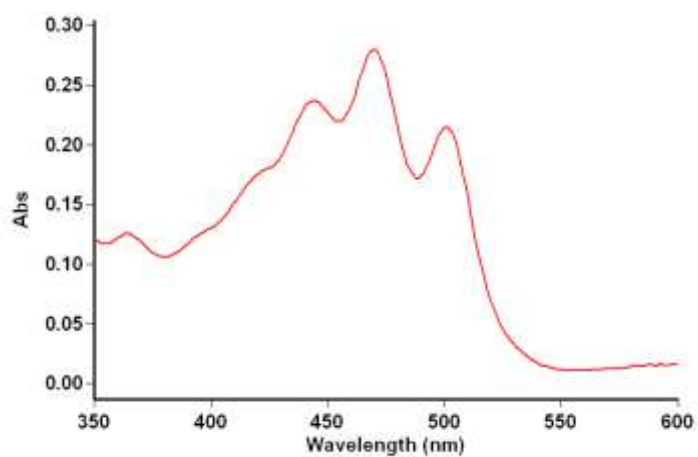
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.1           | 0.226 |
| 470.0           | 0.291 |
| 443.0           | 0.242 |

Day 21



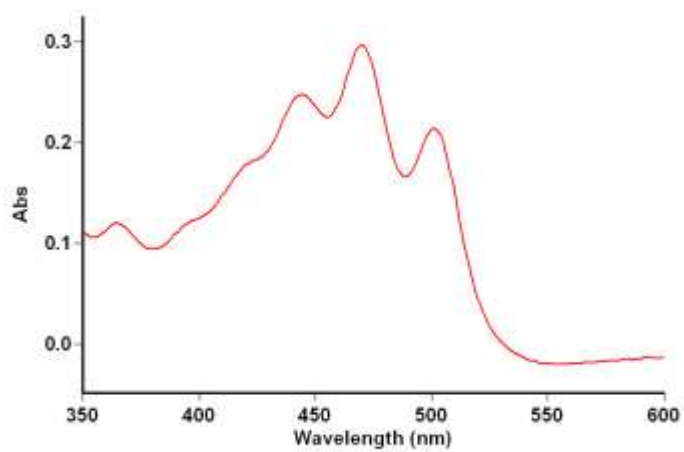
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.1           | 0.223 |
| 470.0           | 0.289 |
| 443.0           | 0.242 |

Day 28



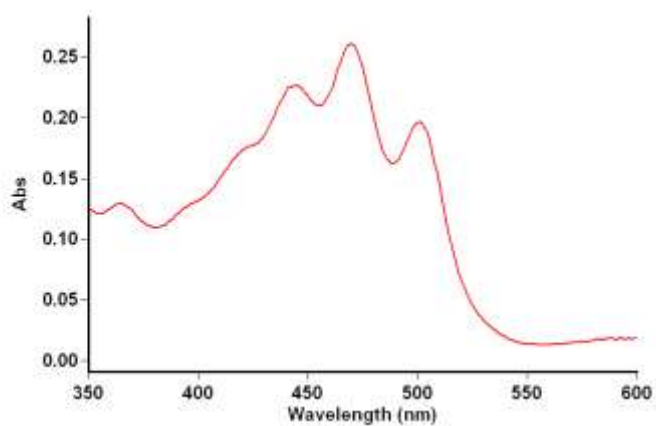
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.1           | 0.218 |
| 470.0           | 0.280 |
| 443.9           | 0.237 |

Day 35



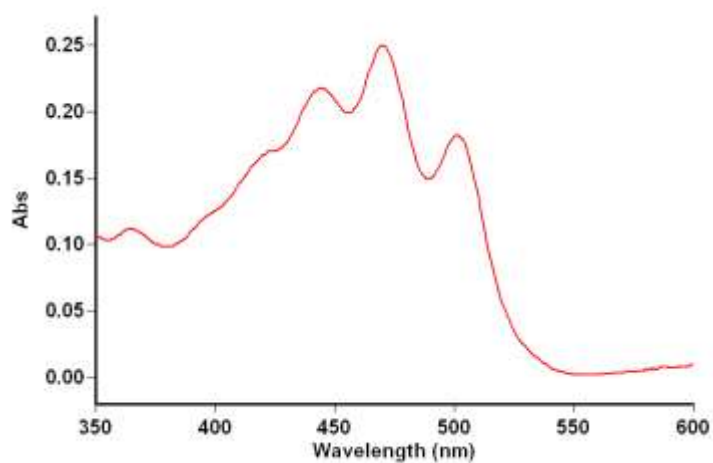
| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.1           | 0.210 |
| 470.0           | 0.296 |
| 445.0           | 0.247 |
| 365.1           | 0.120 |

Day 42



| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.1           | 0.185 |
| 470.0           | 0.261 |
| 443.9           | 0.227 |

Day 49



| Wavelength (nm) | Abs   |
|-----------------|-------|
| 503.1           | 0.177 |
| 470.0           | 0.250 |
| 443.9           | 0.218 |

## APPENDIX III

### (a) ANOVA for effect of incorporation level of carotenoid extract on sensory attributes of ice cream

| <b>Colour and appearance</b> |           |           |           |                      |                        |            |           |             |
|------------------------------|-----------|-----------|-----------|----------------------|------------------------|------------|-----------|-------------|
| <b>Factor</b>                | <b>DF</b> | <b>SS</b> | <b>MS</b> | <b>F<sub>c</sub></b> | <b>F<sub>tab</sub></b> | <b>SEM</b> | <b>CD</b> | <b>Test</b> |
| <b>Treatment</b>             | 8         | 71.40556  | 8.925694  | 22.47028             | 2.05488                | 0.199      | 0.56      | *           |
| <b>Error</b>                 | 81        | 32.175    | 0.397222  |                      |                        |            |           |             |
| <b>Total</b>                 | 89        | 103.5806  |           |                      |                        |            |           |             |
| <b>CV %</b>                  | 10.81     |           |           |                      |                        |            |           |             |
| <b>Taste</b>                 |           |           |           |                      |                        |            |           |             |
| <b>Factor</b>                | <b>DF</b> | <b>SS</b> | <b>MS</b> | <b>F<sub>c</sub></b> | <b>F<sub>tab</sub></b> | <b>SEM</b> | <b>CD</b> | <b>Test</b> |
| <b>Treatment</b>             | 8         | 73.13889  | 9.14236   | 26.121               | 2.05488                | 0.187      | 0.52      | *           |
| <b>Error</b>                 | 81        | 28.35     | 0.35      |                      |                        |            |           |             |
| <b>Total</b>                 | 89        | 101.488   |           |                      |                        |            |           |             |
| <b>CV %</b>                  | 9.89      |           |           |                      |                        |            |           |             |
| <b>Aroma</b>                 |           |           |           |                      |                        |            |           |             |
| <b>Factor</b>                | <b>DF</b> | <b>SS</b> | <b>MS</b> | <b>F<sub>c</sub></b> | <b>F<sub>tab</sub></b> | <b>SEM</b> | <b>CD</b> | <b>Test</b> |
| <b>Treatment</b>             | 8         | 77.8722   | 9.7340    | 20.088               | 2.054882               | 0.220      | 0.6       | *           |
| <b>Error</b>                 | 81        | 39.25     | 0.4845    |                      |                        |            |           |             |
| <b>Total</b>                 | 89        | 117.1222  |           |                      |                        |            |           |             |
| <b>CV %</b>                  | 12.12     |           |           |                      |                        |            |           |             |
| <b>Overall acceptability</b> |           |           |           |                      |                        |            |           |             |
| <b>Factor</b>                | <b>DF</b> | <b>SS</b> | <b>MS</b> | <b>F<sub>c</sub></b> | <b>F<sub>tab</sub></b> | <b>SEM</b> | <b>CD</b> | <b>Test</b> |
| <b>Treatment</b>             | 8         | 67.2835   | 8.4104    | 20.513               | 2.054882               | 0.188      | 0.52      | *           |
| <b>Error</b>                 | 81        | 28.97225  | 0.3576    |                      |                        |            |           |             |
| <b>Total</b>                 | 89        | 96.25581  |           |                      |                        |            |           |             |
| <b>CV %</b>                  | 9.93      |           |           |                      |                        |            |           |             |

**(b) ANOVA for effect of incorporation level of carotenoid extract on sensory attributes of filling cream (biscuit)**

| <b>Colour and appearance</b> |           |           |           |                      |                        |            |           |             |
|------------------------------|-----------|-----------|-----------|----------------------|------------------------|------------|-----------|-------------|
| <b>Factor</b>                | <b>DF</b> | <b>SS</b> | <b>MS</b> | <b>F<sub>c</sub></b> | <b>F<sub>tab</sub></b> | <b>SEM</b> | <b>CD</b> | <b>Test</b> |
| <b>Treatment</b>             | 8         | 113.722   | 14.21528  | 41.832               | 2.0548                 | 0.184      | 0.51      | *           |
| <b>Error</b>                 | 81        | 27.525    | 0.3398    |                      |                        |            |           |             |
| <b>Total</b>                 | 89        | 141.247   |           |                      |                        |            |           |             |
| <b>CV %</b>                  | 10.4      |           |           |                      |                        |            |           |             |
| <b>Taste</b>                 |           |           |           |                      |                        |            |           |             |
| <b>Factor</b>                | <b>DF</b> | <b>SS</b> | <b>MS</b> | <b>F<sub>c</sub></b> | <b>F<sub>tab</sub></b> | <b>SEM</b> | <b>CD</b> | <b>Test</b> |
| <b>Treatment</b>             | 8         | 112.139   | 14.01736  | 37.410               | 2.0548                 | 0.193      | 0.54      | *           |
| <b>Error</b>                 | 81        | 30.35     | 0.374691  |                      |                        |            |           |             |
| <b>Total</b>                 | 89        | 142.488   |           |                      |                        |            |           |             |
| <b>CV %</b>                  | 11.16     |           |           |                      |                        |            |           |             |
| <b>Aroma</b>                 |           |           |           |                      |                        |            |           |             |
| <b>Factor</b>                | <b>DF</b> | <b>SS</b> | <b>MS</b> | <b>F<sub>c</sub></b> | <b>F<sub>tab</sub></b> | <b>SEM</b> | <b>CD</b> | <b>Test</b> |
| <b>Treatment</b>             | 8         | 65.330    | 8.1663    | 28.412               | 2.0548                 | 0.169      | 0.47      | *           |
| <b>Error</b>                 | 81        | 23.281    | 0.2874    |                      |                        |            |           |             |
| <b>Total</b>                 | 89        | 88.611    |           |                      |                        |            |           |             |
| <b>CV %</b>                  | 8.72      |           |           |                      |                        |            |           |             |
| <b>Overall acceptability</b> |           |           |           |                      |                        |            |           |             |
| <b>Factor</b>                | <b>DF</b> | <b>SS</b> | <b>MS</b> | <b>F<sub>c</sub></b> | <b>F<sub>tab</sub></b> | <b>SEM</b> | <b>CD</b> | <b>Test</b> |
| <b>Treatment</b>             | 8         | 61.668    | 7.7085    | 28.658               | 2.0548                 | 0.163      | 0.46      | *           |
| <b>Error</b>                 | 81        | 21.7875   | 0.26898   |                      |                        |            |           |             |
| <b>Total</b>                 | 89        | 83.455    |           |                      |                        |            |           |             |
| <b>CV %</b>                  | 8.33      |           |           |                      |                        |            |           |             |

(c) ANOVA for effect of incorporation level of carotenoid extract on sensory attributes of jam

| <b>Colour and appearance</b> |           |           |           |                      |                        |            |           |             |
|------------------------------|-----------|-----------|-----------|----------------------|------------------------|------------|-----------|-------------|
| <b>Factor</b>                | <b>DF</b> | <b>SS</b> | <b>MS</b> | <b>F<sub>c</sub></b> | <b>F<sub>tab</sub></b> | <b>SEM</b> | <b>CD</b> | <b>Test</b> |
| <b>Treatment</b>             | 6         | 84.254    | 14.042    | 34.428               | 2.246                  | 0.201      | 0.57      | *           |
| <b>Error</b>                 | 63        | 25.696    | 0.407     |                      |                        |            |           |             |
| <b>CV %</b>                  | 10.13     |           |           |                      |                        |            |           |             |
|                              |           |           |           |                      |                        |            |           |             |
| <b>Taste</b>                 |           |           |           |                      |                        |            |           |             |
| <b>Factor</b>                | <b>DF</b> | <b>SS</b> | <b>MS</b> | <b>F<sub>c</sub></b> | <b>F<sub>tab</sub></b> | <b>SEM</b> | <b>CD</b> | <b>Test</b> |
| <b>Treatment</b>             | 6         | 97.5      | 16.25     | 47.23                | 2.246                  | 0.185      | 0.52      | *           |
| <b>Error</b>                 | 63        | 21.675    | 0.344     |                      |                        |            |           |             |
| <b>Total</b>                 | 69        | 119.175   |           |                      |                        |            |           |             |
| <b>CV %</b>                  | 9.94      |           |           |                      |                        |            |           |             |
|                              |           |           |           |                      |                        |            |           |             |
| <b>Aroma</b>                 |           |           |           |                      |                        |            |           |             |
| <b>Factor</b>                | <b>DF</b> | <b>SS</b> | <b>MS</b> | <b>F<sub>c</sub></b> | <b>F<sub>tab</sub></b> | <b>SEM</b> | <b>CD</b> | <b>Test</b> |
| <b>Treatment</b>             | 6         | 92.15     | 15.358    | 61.53                | 2.246                  | 0.157      | 0.44      | *           |
| <b>Error</b>                 | 63        | 15.725    | 0.249     |                      |                        |            |           |             |
| <b>Total</b>                 | 69        | 107.875   |           |                      |                        |            |           |             |
| <b>CV %</b>                  | 7.98      |           |           |                      |                        |            |           |             |
|                              |           |           |           |                      |                        |            |           |             |
| <b>Overall acceptability</b> |           |           |           |                      |                        |            |           |             |
| <b>Factor</b>                | <b>DF</b> | <b>SS</b> | <b>MS</b> | <b>F<sub>c</sub></b> | <b>F<sub>tab</sub></b> | <b>SEM</b> | <b>CD</b> | <b>Test</b> |
| <b>Treatment</b>             | 6         | 101.7     | 16.95     | 45.44                | 2.246                  | 0.193      | 0.54      | *           |
| <b>Error</b>                 | 63        | 23.5      | 0.373     |                      |                        |            |           |             |
| <b>Total</b>                 | 69        | 125.2     |           |                      |                        |            |           |             |
| <b>CV %</b>                  | 9.85      |           |           |                      |                        |            |           |             |