"ANTIOXIDANT ACTIVITY OF LYCOPENE FROM TOMATO FOR ENHANCING SHELF LIFE OF GHEE"

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"ANTIOXIDANT ACTIVITY OF LYCOPENE FROM TOMATO FOR ENHANCING SHELF LIFE OF GHEE"

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ABSTRACT

Ghee, a fat rich dairy product with pleasing flavor, is prone to oxidative deterioration during storage. In recent years use of natural antioxidant to retard such spoilage is gaining popularity from safety aspects. Tomato is a source of lycopene which has high antioxidant potential with plethora of health benefits. Therefore, the present study was undertaken to examine potential of tomato as an antioxidant to enhance shelf life of ghee.

Various aspects dealt in the study include: examination of tomatoes cultivars (globe, round or oblong) to extend shelf life of ghee, evaluation different parts of tomatoes (skin, pulp and paste) for their antioxidant activity in ghee, selection of stage for addition of selected part of tomato in ghee (before or after heat clarification of butter into ghee), optimization for rate of addition of selected part of tomato in ghee, comparison of selected part of tomato, BHA and synergistic effect of tomato part along with BHA for enhancing shelf life of ghee and analysis of ghee samples for the quality standards prescribed by FSSAI, PFA and AGMARK.

For present investigation sample of ghee were prepared from white butter by heat clarification at a temperature of 115^{o}C with no hold. Samples of ghee received different treatments in accordance to aspect to be studied. The ghee sample without any treatment was kept as control sample for comparison. All the prepared samples of ghee were stored at 80°C ± 2^{o}C to accelerate the oxidation. The samples of ghee were monitored regularly for changes in flavor score by sensory evaluation, as well as for extent of oxidation by determination of peroxide value of ghee during storage of ghee at an interval of every two days.

Tomato from these three cultivars *viz*. Globe (*Ruby*), Oblong (*Shaktiman*), Round (*Heemshikhar*) were examined for their antioxidant activity in ghee. The three cultivars of tomato differed significantly for their effect on changes in flavor score and peroxide value of ghee during storage of ghee. The changing in flavor score of ghee and changes in peroxide value of ghee during storage suggested that globe cultivar of tomato was best to extend the shelf life of ghee.

Three different parts obtained from glob cultivar *viz*. skin, pulp and paste were evaluated for their antioxidant activity in ghee. The changing in flavor score of ghee and changes in peroxide value of ghee during storage suggested that skin obtained from globe tomato was best to extend the shelf life of ghee.

Two different stages in preparation of ghee *viz*. before and after heat clarification of butter fat were tested for suitability to add skin from glob cultivar of tomato for its antioxidant activity in ghee. The changing in flavor score of ghee and changes in peroxide value of ghee during storage suggested that addition of tomato skin after heat clarification of butter into ghee was better to extend the shelf life of ghee, compared to the addition before heat clarification.

For optimization of rate of addition of tomato skin in ghee, the skin was added separately at the rate of 0.2, 0.4, 0.6, 0.8 and 1.0 per cent. The changing in flavor score of ghee and changes in peroxide value of ghee during storage suggested that addition rate of tomato skin at rate of 0.6 per cent was optimum to extend the shelf life of ghee.

Finally potency of globe cultivar tomato skin (@ 0.6%), BHA (@ 0.02%) and combination of tomato skin (@ 0.6%) along with BHA (@ 0.02%) was compared for extending the shelf life of ghee. The changing in flavor score of ghee and changes in peroxide value of ghee during storage suggested that the addition of tomato skin in ghee extend shelf life of ghee against oxidative deterioration during storage, but its potency was lower than that of the BHA. However, the addition of tomato skin along with BHA in ghee effectively extended the shelf life of ghee against oxidative deterioration during storage and its potency was much higher than that of the BHA alone.

After getting confirmation antioxidant potential of tomato skin from globe cultivar of tomato and its potential synergistic action with BHA the samples of ghee were tested for fulfillment of requirements for quality as prescribed under FSSAI and/or AGMARK. The fresh samples of ghee were analyzed for various quality standards *viz*. moisture content, B.R. reading at 40°C, RM value, Polenske value, FFA content and Baudouin test. All the samples of ghee fulfilled requirements for quality standards prescribed under FSSAI and AGMARK. The addition of tomato skin from globe variety at the rate of 0.6 per cent did not affect quality standard as prescribed under FSSAI and/or AGMARK for ghee.

It can be summarized from this study that tomato among three different cultivars *viz.* globe, round and oblong; the globe cultivar of tomato was best to extend shelf life of ghee. Among different parts of tomato *viz.* paste, pulp and skin, the skin was best to extend shelf life of ghee. For addition of the tomato skin during preparation of ghee, the addition of skin after heat clarification of butter fat better than the addition before the heat clarification. Among the different rates for addition of tomato skin in ghee (0.2 to 1.0%), the addition of at the rate of 0.6 per cent was found optimum to extend the shelf life of ghee. Analysis of ghee samples revealed that the addition of tomato skin at the rate of 0.6 per cent did not affect parameters of quality prescribed for ghee under FSSAI and AGMARK. The comparison for antioxidant potential of tomato skin, BHA and tomato skin along with BHA in extending the shelf life of ghee revealed that the use of tomato skin along with BHA was most effective. Therefore, the present study entailed to

conclude that tomato skin which is generally, thrown as waste during tomato processing can be used as an effective antioxidant for ghee.

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CERTIFICATE

This is to certify that the thesis entitled "**ANTIOXIDANT ACTIVITY OF LYCOPENE FROM TOMATO FOR ENHANCING SHELF LIFE OF GHEE**" submitted by TANMAY HAZRA in partial fulfillment of the requirements for the degree of MASTER IN TECHNOLOGY in DAIRY CHEMISTRY of Anand Agricultural University, Anand is a record of bonafide research work carried out by him under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

Place: Anand **(K. D. Aparnathi)**

Date: **Major Advisor**

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Place: Anand (TANMAY HAZRA)

Date:

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CHAPTER 1

INTRODUCTION

Ghee is clarified milk fat and also termed as anhydrous milk fat. It is obtained by clarification of the fat at higher temperature. Ghee is by far the most ubiquitous indigenous milk product and prominent in the hierarchy of Indian dietary, being a rich source of energy, fat soluble vitamins, essential fatty acids and pleasing flavor. Therefore, it enjoys a supreme status. In India, ghee is considered as an excellent cooking or frying medium. In addition, ghee is used for numerous religious rites by Hindus and it has also many medicinal uses (Rajorhia, 2003). Ghee contains several components (conjugated linoleic acid, sphingomyelins, butyric acid and β-carotene), which have therapeutic potential against carcinogenesis (Parodi, 1996).

Ghee chemically may be defined as complex lipids of triacylglycerol, together with small quantity of free fatty acids, phospholipids, sterols, hydrocarbons, carbonyl compounds, fat soluble vitamins (A, D, E and K), carotenoid pigments, moisture and traces of elements like copper and iron. On an average cow or buffalo ghee contains 99.0-99.5% fat and less than 0.5% moisture (Parodi, 1996).

Many chemical and biochemical reactions can lead to deterioration of product quality or impairment of product safety. The physico-chemical reactions that occur on processing and storage of ghee bring some undesirable changes in texture, flavor and colour attributes. It has a shelf-life of 6 to 8 months even at ambient tropical temperatures (Gunstone and Padley, 1997). The deterioration in the quality of ghee generally arises from two pathways and known as hydrolytic rancidity and oxidative rancidity. The major type of rancidity which affects an isolated fat is caused by the action of oxygen. Milk fat easily undergoes autoxidation. In the presence of oxygen and under the usual processing and storage conditions the unsaturated fatty acids in milk fat undergo autoxidation. The oxidation of milk lipids results in a number of adverse effects on its quality. The major effect of autoxidation of milk fat is the development of various off-flavors (Parodi, 1996).

Ghee is the costliest edible fat from natural source. However, it undergoes oxidative deterioration which adversely affects its economical and nutritional value. These in turn determine the storage stability and are of paramount importance from economic

view points. Generally ghee is produced during the flush season, when excess milk is converted in to skimmed milk powder. The fat obtained in this process is converted in to ghee for use in the lean season. Now demand for ghee in other countries is growing, probably reflecting the migration of people from the subcontinent of India this needs long term storage of ghee. Therefore, constant research endeavors are made to extend the shelf-life by various approaches. One of the most common approaches is addition of antioxidants (Hathway, 1966).

Antioxidants can be classified according to their source as natural or synthetic. Continuous use of synthetic antioxidants may cause health hazards such as teratogenic, carcinogenic and mutagenic effects in experimental animals and primates. It is because of these reasons that constant endeavors have been made for the use of natural antioxidants (Hathway, 1966; Maeura *et al*., 1984; Heijden *et al*., 1986; Van, 1986).

The use of chemical antioxidants to extend shelf life of food products has been widely practiced. However, use of these additives is regulated and limited by law. It is generally accepted that natural antioxidants are more potent, efficient and safer than synthetic antioxidants. Therefore, now consumers demand less use of synthetic additives (Membre *et al.*, 2001). Consequently, in recent years many attempts have been made for search of natural antioxidant compounds that can properly serve the demand of consumers and needs of the food manufacturers. Various fruits and vegetables have been recognized for their antioxidant activity and can serve as a source of natural antioxidants. The antioxidant activity of fruits and vegetables is attributed to their phytochemicals content such as carotenoids, poly-phenols, tocopherol, ascorbate etc. Therefore, it is evident that the use of fruits and vegetables as source of natural antioxidants is a promising alternative to the use of synthetic antioxidants (Van, 1986).

Carotenoids are tetraterpenoid organic pigments that are naturally occurring in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms. Carotenoids are effective physical quenchers of singlet oxygen and act as an antioxidant. Lycopene is a member of the carotenoid family. Among various carotenoids, lycopene is the most efficient singlet oxygen quencher. The physical quenching rate of lycopene was two times higher than β-carotene and 10 times higher

than α-tocopherol. The most common and rich source of lycopene is "Tomatoes." Therefore, the term lycopene is derived from the scientific name of tomato, *Lycopersicon esculentum* (Choksi and Joshi, 2007).

Lycopene has plethora of health benefits. However, the potential of tomato as a source of lycopene has not been trapped as a natural antioxidant for preventing oxidative rancidity in ghee. Therefore, the present study is contemplated with a view to evaluate the potential of tomato (as a source of lycopene) as a natural antioxidant for preventing oxidative rancidity in ghee. Keeping this idea as a central goal, the study is planed with the following objectives.

Objectives

(a) To evaluate cultivar of tomato for incorporation in ghee.

(b) To evaluate the different parts of tomato for incorporation in ghee.

(c) To select the stage of addition of tomato in ghee.

(d) To decide the rate for addition of selected part of tomato in ghee.

(e) To compare of tomato, BHA and synergistic effect of tomato with BHA for enhancing shelf life of ghee.

CHAPTER 2
REVIEW OF LITREATURE

Fats, oils and lipid-based foods deteriorate through several degradation reactions both on heating and on long term storage. The main deterioration processes are oxidation reactions. Oxidation is generally treated as the most frequently occurring form of lipid deterioration, which leads to the development of off-flavor compounds (rancidity), polymerization, reversion and other reactions causing reduction of shelf life and nutritive value of the food product. The retardation of these oxidation processes is important for all the persons involved in entire food chain from the factory to the consumer. Oxidation may be inhibited by various methods. One of the most common methods of protection against oxidative deterioration of lipid-based foods is use of specific additives which inhibit oxidation. These additives are known as antioxidants. These inhibitors represent a class of substances that vary widely in chemical structure and have diverse mechanisms of action(Pokorny, 1999) .

Increasing appreciation of the nutritional effects of highly unsaturated fatty acids including eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6) and there very high susceptibility to oxidation encourage the search for more effective antioxidants (Pokorny, 1999).

2.0 LIPID OXIDATION

Oxidation is generally treated as the most frequently occurring form of lipid deterioration, which leads to the development of rancidity, off-flavour compounds, polymerisation, reversion, and other reactions causing reduction of shelf life and nutritive value of the food product. Lipids occur in almost all food stuffs, and most of them (more than 90%) are in the form of tri-acylglycerols, which are esters of fatty acids and glycerol. Two major components involved in lipid oxidation are unsaturated fatty acids and oxygen. Oxidative degradation of lipids may be initiated by active oxygen and related species, which are more active than triplet oxygen molecules present in air, (Simic *et al.,* 1992) as well as by exogenous agents (UV, ionisation radiation and heat).

There are two different mechanisms by which the fatty oxidation reaction can proceed:

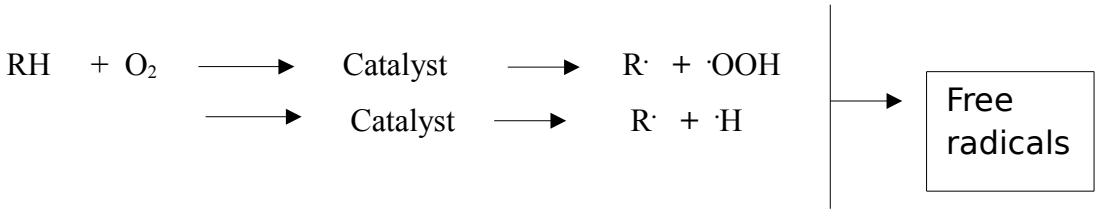
A. fatty acid free radical autoxidation by molecular oxygen, and

B. fatty acid oxidation by singlet oxygen or "ene" reaction

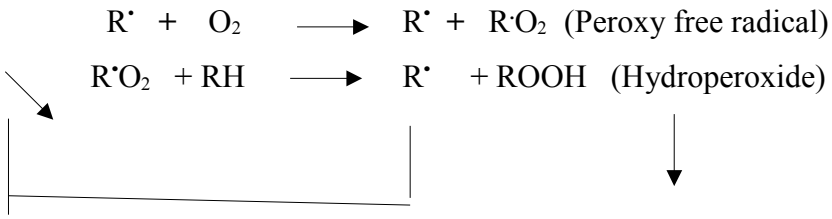
The intermediates in both the cases, however, are lipid hydroperoxides only. These are odorless and tasteless compounds which readily decompose to small molecules yielding off-flavors (Frankel, 1980).

The free radical autoxidation is best described in terms of initiation, propagation and termination reactions. The initiation can occur by the action of external energy sources such as heat, light or high energy radiation or by chemical initiation involving metal ions or metallo proteins such as haem. The classical route depends on the production of free radicals ($R^{\cdot}$) from lipid molecules (RH) by their interaction with oxygen in the presence of a catalyst. The free radical ($R^{\cdot}$) produced in the initiation step can then react to form a lipid peroxy radical ($ROO^{\cdot}$) which can react further to give the hydroperoxide (ROOH). The reaction of propagation steps also provides further a free radical $R^{\cdot}$, making it a self propagating chain process. In this way a small amount of catalyst e.g. copper ions, can initiate the reaction, which then produce many hydroperoxide molecules, which ultimately breakdown to a variety of saturated and unsaturated compounds to giving rise to oxidized flavours. The self-propagating chain can be stopped by termination reactions, where two similar or dissimilar radicals combine to give products which do not feed the propagating reaction (Hamilton, 1989).

Initiation:



Propagation:



Decomposition

Termination:

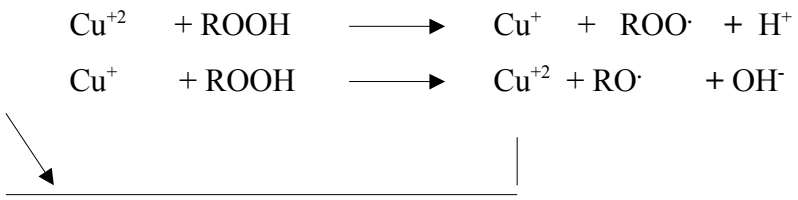
The hydroperoxides formed in the autoxidation of unsaturated fatty acids are unstable and readily decompose. The main products of hydro peroxides decomposition are saturated and unsaturated aldehydes. The mechanism suggested for the formation of aldehydes involves cleavage of the isomeric hydroperoxide to the alkoxy radicals, which undergo carbon to carbon fission to form aldehydes (Frankel *et al*., 1961). In addition to aldehydes, other secondary products of lipid oxidation such as unsaturated ketones (Stark and Forss, 1962),saturated and unsaturated alcohols (Hoffmann, 1962; Stark and Forss, 1964 ; Stark and Forss, 1966), saturated and unsaturated hydrocarbons (Horvat *et al*., 1965 ; Forss *et al*., 1967) and semi aldehydes (Frankel *et al*., 1961) have been observed in the decomposition of hydroperoxides of oxide lipid system.

Khan, (1965) has proposed a new concept of initiation of autoxidation which overcomes some of the energy problems faced by the above theory. According to him, the electronic structure of oxygen is favorable for the formation of transition cyclic *pi* complexes with a group of carbon atoms in the vicinity of double bond. Such complex formation allows for free movement by reducing electron density and accordingly, it explains the mechanism better than the free radical chain reaction.

The explanation for actual reaction of oxygen with lipid substrates has remained obscure with the free radical mechanism. It is unlikely that unexcited ground state triplet oxygen would react with the unexcited singlet unsaturated lipid. The activation energy for the reaction is relatively high. The univalent stepwise reduction of triplet state oxygen is highly endothermic. The singlet state oxygen will react readily with the singlet state lipid to yield singlet state peroxide. Thus activation of the ground state triplet oxygen by some means is necessary to initiate the formation of peroxides

which then decompose to propagate the chain reaction. Activation of oxygen can be achieved by electronic excitation from the triplet ground state to excited singlet state. The singlet oxygen then reacts readily with unsaturated fatty acids. The oxidation by singlet oxidation or photo-oxidation proceeds by a different mechanism than free radical autoxidation and has been recognized as an alternative to the free radical chain reaction (Khan, 1976; Aurand *et al*., 1977 ; Korycka-Dhal and Richardson, 1980). Singlet oxygen reacts directly with double bonds by concerted addition, the so called "ene" reaction. Oxygen is thus inserted at either end carbon of a double bond, which is shifted to yield an allylic hydroperoxide in the trans configuration. According to this mechanism, oleate produces a mixture of 9- and 10- hydroperoxides, linoleate produces a mixture of 9-, 10-, 12-, and 13-hydroperoxides and linolenate produces a mixture of 9-, 10-, 11-, 12-, 13-, 15- and 16- hydroperoxides. Once the initial hydroperoxides are formed, the free radical chain reaction prevails as the main mechanism.

Metals in traces are believed by some workers to be essential to initiate the autoxidation reaction (Uri, 1961). These react with molecular oxygen and give it a negative charge, making its further reaction with unsaturated fats (Heaton and Uri, 1961)



They appear to function by increasing the rate of formation of free radicals. Metal ions may react with molecular oxygen to form radical ions which can initiate chain reactions. Direct reaction of metal with fatty substrate to yield free radicals may also occur. Metals which show high catalytic activity, such as copper and cobalt abstract hydrogen from the unsaturated substrate directly, while metals like iron and nickel do so from the hydroperoxides formed. All these metals have an inner incomplete group of electron, hence they can pass into a higher valence state but still retain the power to react further with oxygen which they transfer to the substrate, thus oxidizing it, while themselves reversing to the lower valence oxidation state (Skellon, 1950).

2.1 NUTRITIONAL ASPECTS OF OXIDIZED FAT

Oxidised fat contains several classes of compounds which have toxic effects. These toxic compounds may be categorized as lipid peroxides, hydroxyl fatty acids, carbonyl compounds like malonaldehyde, cyclic monomers, dimmers, polymers, polycyclic aromatic compounds and oxidized sterols (Logani and Davies, 1980; Nawar, 1985; Addis, 1986 ; Alexander, 1986). Acute effect of consuming oxidized fat is diarrhea. The other groups of compounds responsible for acute adverse biological effects are secondary lipid oxidation products. Tissue congestion, fatty degeneration and neurosis were more severe in mice dosed with autoxidised methyl linoleate containing secondary oxidation products than with methyl linoleate hydroperoxides (Alexander, 1986). Chronic effects of consuming oxidized fat have been summarized by Sanders (1989) which include diarrhea, poor growth rate, myopathy, hepatomegaly, steatites, yellow fat disease, hemolytic anemia and secondary deficiency of vitamin A and E. Long term effects associated with the consumption of oxidized fats are initiation and promotion of tumor growth. These are mainly because of fatty acid hydroperoxides which are mutagenic (Mac Gregor *et al*., 1985) and malonaldehydes which is both mutagenic and carcinogenic (Shamberger *et al*., 1974).

The new point of concern is the involvement of lipid peroxides in atherogenesis leading to atherosclerosis. Further, the oxidation products of cholesterol have also shown to be atherogenic in experimental animals (Imai *et al*., 1981). Oxidized cholesterol may be carcinogenic or may promote tumor growth (Alexander, 1986). It has been suggested that high incidence of coronary heart disease amongst Asian Indian men of Indian descent in Britain may be related to their intake of oxidized cholesterol in ghee (Jacobson, 1987).

2.2 INHIBITING/DELAYING THE LIPID OXIDATION

Optimum oxidative stability can be achieved by minimizing exposure of lipids and lipid-containing food products to air, light and higher temperatures during processing and storage. Theoretically, the most elegant way of preserving fatty foods from oxidative spoilage is to remove all oxygen from the food during manufacture and from the packaging container. Modern packaging material and equipment ,allows inert-gas vacuum packaging, but residual oxygen levels of less than 1 % are extremely difficult to obtain in a production environment(Lolinger, 1991).The free radical chain

process of autoxidation can be retarded by two categories of inhibitors: chain-breaking inhibitors (or antioxidants) and preventive inhibitors .

The chain-breaking antioxidants AH scavenge the free radicals (LOO·, LO·) interrupting the propagation step [reactions (a) and (b)] and forming an antioxidant radical A· of such a low reactivity that no further reaction with lipids can occur (Frankel ,1980).

$$LOO^{\cdot} + AH \rightarrow LOOH + A^{\cdot} \quad \text{.....(a)}$$

$$LO^{\cdot} + AH \rightarrow LOH + A^{\cdot} \quad \text{.....(b)}$$

Radical scavengers usually donate one electron to the unpaired electron of the free radical and thus reduce it. Polyphenols are very active in this respect and the radical-scavenging activities of gallates, nordihydroguairetic acid and flavonoids arise from this process. Aromatic amines inhibit the autoxidation via the same electron-transfer mechanism (Frankel ,1980).

Quinones (vitamin K, ubiquinone, α-tocopheryl, quinone) are also chain-breaking inhibitors (antioxidants) of autoxidation (Scott, 1985) acting as electron-acceptor antioxidants by competing with oxygen for alkyl radicals. Alkyl radicals react extremely rapidly with oxygen under atmospheric conditions. Peroxide decomposers such as thioethers, methionine and thiodipropionic acid and its esters prevent the formation of free radicals for initiation of new chain reactions (Frankel, 1980).

2.3 ANTIOXIDANTS

Britton, (1995) defined that to be an effective antioxidant, a molecule such have to remove radicals from the system either by reacting with them to yield harmless products or by disrupting free radical chain reactions.

Antioxidants may inhibit oxidation by scavenging free radicals at various steps of oxidation. Lipid oxidation can be inhibited first, by reacting with ROO stops chain propagation and inhibit the formation of ROOH. Secondly, by reacting with alkoxyl radicals RO decreases the decomposition of hydroperoxides and the formation of aldehydes.

Tsuchihashi *et al*., (1995) proposed that the antioxidant potency is determined by several factors such as intrinsic chemical reactivity of the antioxidant toward the

radical, site of generation and reactivity of the radicals, site of antioxidant, concentration and mobility of the antioxidant at the microenvironment, stability and fate of antioxidant-derived radical, and interaction with other antioxidants. Classically lipid antioxidants have been divided into two groups: primary or chain-breaking antioxidants, and secondary or preventive antioxidants. In broader terms, Halliwell and Gutteridge (1995) defined an antioxidant as "any substance that when present at low concentrations compared to those of an oxidizable substrate significantly delays or inhibit oxidation". This emphasizes the source of oxidative damage in the characterization of an antioxidant (Halliwell *et al*., 1995). Krinsky, (1992) defined biological antioxidants broadly as compounds that protect biological systems against the potentially harmful effects of processes or reactions that can cause excessive oxidations". In general, oxidizable substrates include lipids, proteins, carbohydrates and DNA. In addition, some antioxidants, such as vitamins E and C, are known to have synergistic interactions through their recycling mechanisms, whereby the combination of compounds has a better antioxidant activity than the sum of separate activities (Niki, 1987).

Lipids are generally susceptible to oxidation, which produces undesirable volatile compounds and causes detrimental flavour effects in foods (Gordon, 1990). Moreover, reactive oxygen species (such as hydroxyl-$OH^{\bullet}$, or peroxyl-$ROO^{\bullet}$ radicals), formed in human tissue cells by many endogenous and exogenous causes, produce extensive oxidative damage, which in turn, may contribute to aging, cancer, and other human diseases (Aruoma, 1999; Reaven and Witzum, 1996). To control and reduce oxidative damage, nature makes use of several types of compounds, known as antioxidants, which react rapidly with free radicals at one of the stages of an oxidative sequence, to retard or decrease the extent of oxidative deterioration (Akoh and Min, 1998; Krinsky, 1992). To prevent or retard the oxidative deterioration of foods, natural or synthetic antioxidants have been used as additives in fats and oils.

2.3.1 Synthetic Antioxidants

Synthetic antioxidants are intentionally added to foods to inhibit lipid oxidation. Synthetic antioxidants approved for use in food include BHA, BHT, PG (also octyl and dodecyl gallate), ethoxyquin, ascorbyl palmitate, and TBHQ .

BHA and BHT are fairly heat stable and are used in heat processed foods. Heat-stable TBHQ is useful in frying applications. BHA and BHT are strongly lipophilic and are used extensively in oil-in-water emulsions. BHA and BHT are also typically used together in mixtures, acting synergistically (Haumann,1990).

2.3.2 Natural Antioxidants

Consumers are concerned about the safety of their food and about potential effects of synthetic additives on their health. Despite the superior efficacy, low cost, and high stability of synthetic antioxidants in foods, the suspicion that these compounds may act to promote carcinogenicity has led to a decrease in their use (Namiki, 1990).

The empirical use of natural compounds as antioxidants is very old. Smoking and spicing in the home for preservation of meat, fish, cheese and other fat-rich foods is an age old practice. Natural antioxidants are found in almost all plants, microorganisms, fungi and even in animal tissues. The majority of natural antioxidants are phenolic compounds, except tocopherols. The most important groups of natural antioxidants are the tocopherols, flavonoids and phenolic acids and carotenoids (Pokorny, 1989). These are excellent antioxidant at low concentrations, but at high concentrations its ability to reduce metal initiators can actually lead to a prooxidant effect (Frankel, 1995). Extensive research has been dedicated to identification of antioxidants from various natural sources. Ascorbic acid and tocopherols are the most important commercial natural antioxidants. Other sources of natural antioxidants include carotenoids, flavonoids, amino acids, proteins, protein hydrolysates, Maillard reaction products, phospholipids, and sterols. Numerous naturally occurring phenolic antioxidants have been identified in plant sources and vegetable extracts. Enzymes also play important roles as antioxidants.

2.3.2.1 Phospholipids

The antioxidative action of phospholipids is not well understood. It is likely that antioxidant activity differs among the various phospholipids as a result of the wide variance in functional groups and structures. Possible actions include regeneration of primary antioxidants, metal chelating, and decomposition of hydro peroxides. Phospholipids have been shown to be synergists. Moreover, phosphatidylcholine(PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS), display antioxidant activity that is possibly linked to chelating ability (King,1996). Lecithin, once an important commercial antioxidant, now sees limited use because of inefficiency as an antioxidant and poor heat stability. Burkow*et al,*(1992) found lecithin to have antioxidant activity in cod liver oil.

2.3.2.2 Sterols

Sterols have been documented to have antioxidant activity. It is thought that sterols interact with oil surfaces and inhibit oxidation. Sterols may be oxidized at oil surfaces and inhibit propagation by acting as hydrogen donors(King*,* 1996).

2.3.2.3 Proteins and related substances

Numerous amines, amino acids, peptides, and protein hydrolyzates have antioxidant activity. Amines have been shown to possess antioxidant activity. Recently, spermine and spermidine isolated from fish sources were used to inhibit fish oil oxidation (Sasaki *et al.*,1996). Numerous amines such as hypoxanthine and xanthine can be readily isolated from marine sources. Amino acids have chelating abilities, but also exhibit antioxidant activity when used alone. Glycine, methionine, histidine, tryptophan, proline, and lysine are effective antioxidants in oil (Rajalakshmi and Narasimhan ,1996).Iron-binding proteins such as ferritin and transferritin have antioxidant function (Sasaki *et al*., 1996).

2.3.2.4 Enzymatic antioxidants

Glucose oxidase, superoxide dismutase, catalase, and glutathione peroxidase act as antioxidants by removing from the lipid environment either oxygen or highly oxidative species. The enzymes just named act biologically to eliminate cellular free radicals, to keep reactive oxygen species at low concentrations, and to catalyze the destruction of hydrogen peroxide. Thus, they constitute an important biological defense mechanism against free radical damage. Glucose oxidase is an enzyme that

removes oxygen by using it to produce gluconic acid and hydrogen peroxide from glucose (Belitz and Grosch 1987).

2.3.2.5 Ascorbic acid

Ascorbic acid is attractive as an antioxidant because it has GRAS status with no usage limits, is a natural or nature-identical product, and is highly recognized as an antioxidant nutrient by the consumer (Foyer, 1993).

Ascorbic acid acts as a primary or a secondary antioxidant(Foyer ,1993). In vivo, ascorbic acid donates hydrogen atoms as a primary antioxidant. Ascorbic acid is also capable of scavenging radicals directly by converting hydroperoxides into stable products. Ascorbic acid can scavenge oxygen, shift the redox potential of food systems to the reducing range, act synergistically with chelators, and regenerate primary antioxidants (Madhavi *et al.,* 1996). The vitamin is commonly used as a synergist to donate hydrogen to primary antioxidants such as tocopherol. Tocopheroxyl radicals are reduced back to tocopherol by ascorbic acid .Ascorbic acid and its salts (sodium ascorbate and calcium ascorbate) are water soluble and are not applicable as antioxidants for oils and fats(Johnson, 1995). They are used extensively to stabilize beverages.

2.3.2.6 Maillard reaction products (MRPs)

Maillard reaction products (MRPs) are an excellent example of natural, process induced oxidation inhibitors that arise as a result of cooking (Madhavi *a*nd Kulkarni, 1996). MRPs are formed during the cooking of low-moisture foods at temperatures above 80^{o}C. They are produced from the reaction of amines and reducing sugars. Lipids, vitamins, and other food constituents also participate in Maillard reactions. Identification of the compounds responsible for antioxidant activity has proved difficult because of the complexity of the Maillard reaction, the vast number and variety of MRPs, and the diversity of the model systems that can be studied. MRPs have been shown to have antioxidant activity in model systems as well as in some fat-containing foods (Madhavi *a*nd Kulkarni, 1996). Lingnert *et al* .,(1983) used processing parameters and the Maillard reaction to prevent oxidation in cookies. Theories on the mechanism of antioxidant activity of MRPs conflict as well. Kawakishi *et al*.,(1987) hypothesized that the protective effects of melanoidins

against autoxidation were likely to depend on their ability to chelate metals. Amadori compounds may behave like reductones, which inhibit autoxidation.

2.3.2.7 Tocopherols and tocotrienols

Tocopherols and tocotrienols comprise the group of chromanol homologs that possess vitamin E activity in the diet. They are natural monophenolic compounds with varying antioxidant activities. The antioxidative mechanism of tocopherol is well understood.Tocopherol .donates a hydrogen to a peroxy radical resulting in a tocopheryl semiquinone radical. This radical may further donate another hydrogen to produce methyl -tocopherylquinone , or react with another tocopheryl semiquinone radical to produce an tocopherol dimer. The methyl tocopherylquinone is unstable and will yield tocopherylquinone. Numerous other decomposition products with various degrees of antioxidant activity can arise from oxidation of tocopherols (Schuler, 1990).

2.3.2.8 Carotenoids

Carotenoids are isoprenoid compounds, biosynthesized by tail-to-tail linkage of two C_{20} geranylgeranyl diphosphate molecules. This produces the parent C_{40} carbon skeleton from which all the individual variations are derived. This skeleton can be modified 1) by cyclization at one end or both ends of the molecule to give different end groups, 2) by changes in hydrogenation level and 3) by addition of oxygen-containing functional groups. Carotenoids that contain one or more oxygen atoms are known as xanthophylls, the parent hydrocarbons as carotenes. For clarity, and to avoid confusion in nomenclature, the use of both end-group prefixes for a carotene is now recommended. For example, β-carotene is referred to as β,β-carotene (Britton., 1995).

The most characteristic feature of the carotenoid structure is the long system of alternating double and single bonds that forms the central part of the molecule . This constitutes a conjugated system in which the π-electrons are effectively delocalized over the entire length of the polyene chain. This feature is responsible for the molecular shape, chemical reactivity and light-absorbing properties, and hence color, of carotenoids (Britton.,1995) and he also suggested that the following structural properties could contribute to antioxidant functions of carotenoids: 1) A multiplicity of closely spaced energy levels between the excited state and ground state of the

carotenoid, such that the carotenoid can dissipate excited state energy via small collision exchanges with the solvent, 2) minimal tendency for the excited-state carotenoid to sensitize other molecules, 3) resonance states in the excited state carotenoid, allowing delocalization and stabilization of the excited state and 4) multiple potential sites on the carotenoid for attack by active oxygen.

Each double bond in the polyene chain of a carotenoid can exist in two configurations, *trans* or *cis* geometrical isomers. The presence of a cis double bond creates greater steric hindrance between nearby hydrogen atoms and/or methyl groups, so that *cis* isomers are generally less stable thermodynamically than the trans form. Most carotenoids occur in nature predominantly or entirely in the all-trans form (Britton, 1995).Different carotenoids are found these carotenoids have a great ant oxidative capacity and health benefits, so scientists are concentrating their research on different antioxidant.

a- β-carotene

Beta-carotene is the only carotene that can be converted into vitamin A by the body (Rock., 2004). Vitamin A is crucial to health, playing an important role in vision, bone growth, reproduction, and cell division (Landrum and Bone ,2004). Vitamin A also helps maintain skin and mucous membranes that defend against bacteria and viruses. It also helps regulate the immune system. Carrots are a great source of beta-carotene (beta-carotene is the pigment that gives fruits and vegetables their orange, red, and yellow colors), a medium sized carrot contains about 10,000 IU (international units) of beta-carotene, this important antioxidant can be found in other whole foods, including squash, cantaloupe, pumpkin, sweet potatoes, and many other yellow, orange, and red vegetables. Beta-carotene is an important antioxidant can prevent heart disease and cancer (Landrum and Bone, 2004).

b- Lutein and Zeaxanthin

Lutein and zeaxanthin belong to the xanthophyll family of carotenoids and are the two major componentsof the macular pigment of the retina. Lutein and zeaxanthin are the only carotenoids found in both the macula and lens of the human eye, and have dual functions in both tissues – to act as powerful antioxidants and to filter high-energy blue light. Lutein is found in high amounts in human serum.2 In the diet it is found in highest concentrations in dark green, leafy vegetables (spinach, kale, collard greens,

and others), corn, and egg yolks((Sesso and Gaziano 2004) .Zeaxanthin is the major carotenoid found in corn, orange peppers, oranges, and tangerines. Lutein and zeaxanthin differ from other carotenoids in that they each have two hydroxyl groups, one on each side of the molecule. Zeaxanthin is a stereoisomer of lutein, differing only in the location of a double bond in one of the hydroxyl groups. Lutein and zeaxanthin are powerful antioxidants(Rock, 2004), and lutein is widely known as the primary nutrient for protecting ocular function.

c- Canthaxanthin

Canthaxanthin and astaxanthin are carotenoids, can be extracted from natural sources such as crustaceans and algae (Baker 2001). These carotenoids are used in poultry feed and has effective ant oxidative capacity.

d-Lycopene

Effective antioxidant found in tomato, gac fruits and others red fruits.

2.4. LYCOPENE

Lycopene is a photochemical, belongs to carotenoid family. It is responsible for deep red color of several fruits and vegetables (Burri, 2002).

2.4.1 Sources of Lycopene

Lycopene is a natural pigment synthesized by plants, some photosynthetic organisms like algae, some types of fungus and some bacteria. Humans cannot produce lycopene and they are dependent on dietary sources to meet their requirements of lycopene. Fruits and vegetables rich in lycopene include gac, tomatoes, watermelon, pink grapefruit, pink guava and papaya . The gac (*Momordica cochinchinensis*) has the highest content of lycopene of any known fruit or vegetable. However, due to rarity of gac outside its native region of Southeast Asia tomatoes and tomato-based products account for more than 85% of the dietary intake of lycopene for most people (Burri, 2002). Lycopene for food use is also manufactured by fermentation of *Blakeslea trispora*. Lycopene can also be obtained by chemical synthesis (Ernst., 2002).

2.4.2 Biosynthesis of Lycopene

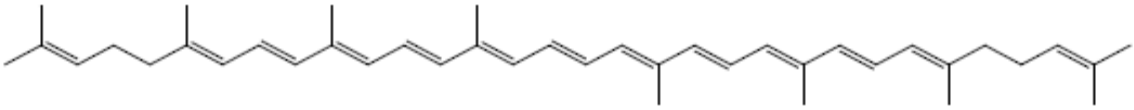
The biosynthesis of lycopene in eukaryotic plants and in prokaryotic Cyanobacteria is similar. Synthesis begins with mevalonic acid, which is converted into dimethylallyl pyrophosphate. This is then condensed with three molecules of isopentenyl pyrophosphate, to give the twenty carbon isopentenyl pyrophosphate. Two molecules of this product are then condensed in a tail-to-tail configuration to give the forty carbon phytoene, the first committed step in carotenoid biosynthesis. Through several desaturation steps, phytoene is converted into lycopene. The two terminal isoprene groups of lycopene can be cyclized to produce β-carotene, which can then be transformed into a wide variety of xanthophylls (Cunningham & Gantt., 2007).

2.4.3 Properties of Lycopene

Lycopene is a lipophilic compound with hydrophobic characteristics due to its acyclic structure and 11 linear conjugated double bonds that make it more soluble in organic solvents (Shi, 2000). It is freely soluble in ethyl acetate, chloroform, hexane, benzene, methylene chloride, acetone; partially soluble in ethanol and petroleum ether and insoluble in water (Kong *et al*., 2010). Lycopene extract from tomato is a dark-red viscous liquid. A solution in *n*-hexane shows an absorption maximum at approximately 472 nm (Wenli *et al*., 2001). With its acyclic structure, large array of conjugated double bonds and hydrophobicity, lycopene exhibits a range of unique and distinct biological properties. Of these properties, its antioxidant properties continue to arouse substantial interest (Rao and Ali, 2007). The system of conjugated double bonds allows lycopene molecules to efficiently quench the energy of singlet oxygen and to scavenge a large spectrum of free radicals. Of all naturally occurring carotenoids, lycopene is the most potent antioxidants. Lycopene has a singlet-oxygen-quenching ability twice as high as that of β-carotene and ten times higher than that of α-tocopherol (Shi *et al*., 2008).

2.4.4 Chemistry of Lycopene

Lycopene is a symmetrical tetraterpenoid assembled from 8 isoprene units . Lycopene is a highly unsaturated open straight chain hydrocarbon, with chemical formula $C_{40}H_{56}$. It contains 13 double bonds of which 11 are conjugated and 2 are non-conjugated. The conjugated bonds are arranged in a linear array. The molecular weight of lycopene is 536.9 (Rao and Ali ,2007).



Lycopene

Unlike some other carotenoids, lycopene lacks the terminal β-ionone ring in its structure. Therefore it lacks pro-vitamin A activity (Rao and Ali, 2007).The color of lycopene is due to its many conjugated double carbon bonds which allow the molecule to absorb visible light of longer wavelengths. Lycopene absorbs most of the visible spectrum, so it appears red. The all-*trans*-isomer of lycopene is the most predominant geometrical isomer in fruits and vegetables (about 94–96% of total lycopene in red tomato fruit) and is the most thermodynamically stable form. In nature, lycopene exists in all-*trans* form and seven of these bonds isomerize to the mono- or poly-*cis* form under the influence of heat, light, oxygen, or certain chemical reactions (Shi *et al*., 2008). Lycopene is a lipophilic compound with hydrophobic characteristics due to its acyclic structure and 11 linear conjugated double bonds that make it more soluble in organic solvents (Shi , 2000). It is freely soluble in ethyl acetate, chloroform, hexane, benzene, methylene chloride, acetone; partially soluble in ethanol and petroleum ether and insoluble in water (Kong *et al.,* 2010). Lycopene extract from tomato is a dark-red viscous liquid. A solution in *n*-hexane shows an absorption maximum at approximately 472 nm (Wenli *et al*., 2001).

2.4.5 Lycopene as Antioxidant and its Mechanism of Function

With its acyclic structure, large array of conjugated double bonds and hydrophobicity, lycopene exhibits a range of unique and distinct biological properties. Of these properties, its antioxidant properties continue to arouse substantial interest (Rao and Ali, 2007) . The system of conjugated double bonds allows lycopene molecules to efficiently quench the energy of singlet oxygen and to scavenge a large spectrum of free radicals. Of all naturally occurring carotenoids, lycopene is the most potent antioxidants,it has a singlet-oxygen-quenching ability twice as high as that of β-carotene and ten times higher than that of α-tocopherol.

The mechanism of action for lycopene towards the reactive species can be predicted through three possible mechanisms (Shi *et.al*., 2002).

(1) adduct formation,

(2) electron transfer to the radical and

(3) allylic hydrogen abstraction, is also shown below

Lycopene + R$^{\bullet}$ = R-Lycopene

Lycopene + R$^{\bullet}$ = Lycopene$^{\bullet+}$ + R^{-}

Lycopene + R$^{\bullet}$ = Lycopene$^{\bullet}$+ RH

Adduct formation is the formation of resonance-stabilized carbon centered-peroxyl radicals where the free radical will attach to the polyene chain, the highly conjugated double bonds of lycopene, to form a lycopene-peroxyl radical adduct (ROO-lycopene•). This reaction is described in (1) where the lipid peroxyl radical (ROO$^{\bullet}$) reacts with lycopene

(1) Lycopene + ROO$^{\bullet}$ → ROO-lycopene•

Antioxidant based on electron transfer reactions. Electron transfer, is the reaction with formation of carotenoid radicals such as lycopene cation radical (lycopene+•), anion radical (lycopene-•) or alkyl radical (lycopene•). Nitrogen dioxide radical ($NO_2^{\bullet}$) from smoking, an environmental pollutant and the powerful oxidant trichloromethylperoxyl ($CCl_3O_2^{\bullet}$) may convert lycopene into radical cations (reaction 2 and 3).

(2) $NO_2^{\bullet}$ + Lycopene ⟶ NO_2^{-} + Lycopene

(3) $CCl_3O_2^{\bullet}$ + Lycopene ⟶ [CCl_3O_2- Lycopene]$^{\bullet}$ ⟶ CCl3O- +Lycopene+•

In addition, the reaction between lycopene and superoxide radical (O2-•) through electron transfer can form the lycopene anion radical (reaction 4)

(4) Lycopene + $O_2^{-\bullet}$ ⟷ Lycopene$^{-\bullet}$ + O_2

Table 2.1: Comparison of the antioxidant activities of different carotenoids

Carotenoids	Rate constant for quenching of singlet oxygen (kq*10^{9})$mol^{-1}s^{-1}$)
Lycopene	**31**
β-carotene	**25**
α-carotene	**19**
Lutein	**08**
Bixin	**14**
Zeaxanthin	**10**
Canthaxanthin	**21**

(Kessy *et al.* , 2011)

2.4.7 Interaction of Lycopene with Other Antioxidant

In lipid bilayer of cellular membrane, lycopene is expected to be a poor antioxidant due to its lesser interaction with aqueous phase radicals. However, the role of lycopene as a lipid phase antioxidant should not be neglected. The combinations of lycopene and other antioxidants such as vitamin C, vitamin E and β-carotene has exhibited higher scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical than their individual antioxidant activity (Matos *et al*.,2001) . Besides, lycopene combined with other antioxidants also gave a better inhibiting effect towards diene hydroperoxides produced from linoleic methyl ester with 2,2-azobis (2,4-dimethylvaleronitrile) (AMVN) induced oxidation (Bhuvaneswari *et al.*,2002).

Lycopene is the strongest reducing agent and able to reduce the radical cations of lutein and zeaxanthin, but not β-carotene. Lycopene in combination with other antioxidants such as vitamins E and C, polyphenols and other carotenoids have wide potential for human health (Giovannucci ,1995 and Hulten *et.* al ,2001).

2.5 TOMATO THE MIRACLE FRUIT

Tomato (*Lycopersicon esculentum*), among antioxidant-rich commodities, has achieved a spectacular status because of its rich composition and widespread consumption. It is one of the major vegetable crops, grown in almost every country of the world. Studies found that regular intake of cooked tomato as a part of the vegetable regimen appears to be the major nutritional factor accounting for lower risk of prostate cancer, digestive tract cancer and coronary heart diseases in the Mediterranean region, particularly in southern Italy (Giovannucci *et al.*,1995). Supported by overwhelming and convincing epidemiological studies, tomato has been called a functional food and an effective preventive strategy against major lifestyle diseases such as cardiovascular diseases and cancer, and it is said to protect cells from DNA damage (Sesso *et al*., 2003).Tomato contain 93.9% moisture, 0.62% ash, total fiber 0.82%,protein 1.82%,glucose 0.82%,fructose 1.01%,others material minor amounts(Grolier, 2000).The composition of tomato changes from agriculture practice, maturity of fruits and species of fruits (Cano *et al*.,2003). Tomatoes have been ranked first as a source of lycopene (71.6%), second as a source of vitamin C (12.0%), pro-vitamin A carotenoids (14.6%) and other carotenoids (17.2%), and third as a source of vitamin E (6.0%) (Kargl *et al*., 1960).

2.5.1 Anti Oxidative Components in Whole Tomato

Antioxidative components of tomato are mjorly two types a) fat soluble carotenoids (99-99.8%),b water soluble flavonoids ,ascorbic acids(0.5-0.2%) (Cano *et al*.,2003).

2.5.1.1 Fat soluble anti oxidative components in tomato

The visible red color of the tomato fruit is due to its major carotenoid, lycopene, making 90% to 98% of the total carotenoids (Shi *et al.,*1999). It also contains colorless carotenoid precursors such as phytoene and phytofluene (15-30%), xanthophylls (free and esterified, 6%) and minor tomato hydrocarbon carotenes such as β-carotene, γ-carotene, z-carotene, (Gross,1991). The carotenoid composition varies in some tomato strains. In ripened tomato lycopene (major), β-carotene, γ-carotene are the major carotenoids(99.6%).The distribution of carotenoids in tomato fruit is not uniform. The outer pericarp constitutes the largest amount of total carotenoids and lycopene, while the locule contains a high proportion of carotene (Shi *et al*.,1999). About 12 mg of lycopene per 100 g fresh weight was found in tomato skin, while the whole tomato fruit contained only 3.4 mg/100 g fresh weight (Shi , 2000). Thus, tomato skin is a rich source of lycopene, indicating that lycopene is attached to the insoluble fiber portion of tomatoes, whereas β-carotene is equally distributed in tomatoes skin and pulp(Shi *et al*.,1999). Lycopene is responsible for deep red color of several fruits and vegetables, the most notably tomatoes. The tomato originated from South America and its most widely known scientific name is Lycopersicon esculentum, but it can also be identified as *Solanumly copersicon*, as originally classified by Linnaeus in 1753, because of the similarity between tomatoes and potatoes. The most common and rich source of lycopene is tomatoes. Therefore, the term lycopene is derived from the scientific name of tomato, *Lycopersicon esculentum* (Choksi and Joshi , 2007).

2.5.1.2 Water soluble antioxidative components in tomatoes

Tomatoes also contain moderate amounts of water-soluble phenolics, flavonoids (quercetin, kaempferol and naringenin) and the hydrocinnamic acids (caffeic, chlorogenic, ferulic and *p*-coumaric acids), mainly concentrated in skin (Martinez-Valverde *et al*., 2002; Minoggio *et al*.,2003). Rutin, caffeoyl, quinic and chalconaringenin have also been identified in tomato fruit (Proteggente *et al*., 2002). Vitamin C content in tomato is moderate (84 to 590 mg/kg), but its contribution to diet is significant because of its high consumption.

2.5.2 Tomatoes Parts to Prevent Oxidative Spoilage in Different Fat Rich Food Products

Though in literature the antioxidative capacity of tomato and its different parts has been proved in different way but still prevention of oxidative deteoration of different oil or ghee by whole tomato or its parts are very few in literature.

2.5.2.1 Tomato seed powder

Tomato seed powder added at 5.0 per cent level in ghee inhibited oxidation and ensured its stability practically to the same extent as 0.01 per cent of BHT or BHA (Guleria *et al*., 1983).

2.5.2.2 Lycopene powder from tomato

In a study, changes in an extra virgin olive oil treated with lycopene during storage were analyzed. Pure lycopene (0.5 and 1.0 mg) obtained from tomato was added to two separate bottles, each containing 100 ml of extra virgin olive oil, while another bottle containing the same oil was stored without any treatment. Samples enriched with pure lycopene showed PV (peroxide value) remarkably lower than the sample without lycopene, and the total phenol contents were higher in treated oil samples than in the reference sample. The concentration of added lycopene decreased very slowly; after about 8 months its residual value was over 60% with respect to the initial concentration (Montesano *et al*., 2006).But still no research has been performed to check the effect of whole tomato on the oxidative deteoration of ghee.

2.6 ANTIOXIDANTS REPORTED FOR GHEE

Ghee is an anhydrous milk fat, occupies a prominent place in the Indian diet. Chemically ghee is a complex lipid of mixed glycerides together with a small amount of free fatty acids, phospholipids, sterols and their esters, fat soluble vitamins (A,D, Eand K), carotenoids, carbonyl compounds, hydrocarbons, charred casein, moisture and traces of trace elements like copper and iron.

Table 2.2: Agmark Standards of Ghee

PARAMETERS	SAMPLE GRADE

	SPECIAL	GENERAL
Baudouin test	Negative	Negative
B.R reading at 40^{o}c	41.5-45	41.5-45
Reichert Meissl value	Not less than 23	Not less than 23
Polenske value	0.5-1.2	0.5-1.2
Moisture content	Not more than 0.3%	Not more than 0.3%
Percentage of Free Fatty Acids (as oleic acid)	Not more than 1.4	Not more than 1.4
Phytosterol Acetate test	Negative	Negative

(www.agmark.net)

Table 2.3: FSSAI Standards of Ghee

PARAMETERS	SAMPLE	
	Milk fat/Butter Oil/Ghee	Anhydrous milk fat
Baudouin test	Negative	Negative
B.R reading at 40^{o}c	40-44	40-44
Reichert Meissl value	Not less than 24	Not less than 24
Milk fat m/m	Not less than 99.6 %	Not less than 99.6%
Moisture content (m/m)	Not more than 0.4%	Not more than 0.1%
Percentage of free fatty acids (as oleic acid)	Not more than 0.4 %	Not more than 0.3%

(FSSAI , 2006)

Oxidative rancidity is the major pathway by which ghee undergoes deterioration. This is referred to as autoxidation because the rate of oxidation increases as the reaction proceeds under usual processing and storage conditions. Several workers have done exhaustive work to improve the stability of ghee against autoxidation through feeding specific feed to milch animals (Rama Murthy and Narayanan, 1972; Tondon, 1977; Hagrass *et al*., 1983), altering processing parameters (Rama Murthy *et al*.,1968; Singh *et al*., 1979), using proper packaging materials and storage conditions (Chauhan and Wadhwa, 1987 ; Amr, 1990a), adding milk components (Gupta Sudha *et al*., 1979; Bhatia *et al*.,1978 ; Santha and Narayanan, 1979; Megha, 1981), adding synthetic antioxidants (Kuchroo and Narayanan, 1972; Chatterjee, 1977), incorporating natural antioxidants from edible plant materials, spices and condiments, aromatic herbs, etc (Sethi and Aggarwal, 1952 ; Ahmad *et al*.,1960 ; Parmar and Sharma, 1986; Amr, 1990b). Continuous use of synthetic antioxidants may cause health hazards such as teratogenic, carcinogenic and mutagenic effects in experimental animals and primates (Hathway, 1966; Maeura *et al*., 1984). It is because of these reasons that constant endeavors have been made for the use of natural antioxidants.

2.6.1 Synthetic Antioxidants Reported for Ghee

Synthetic antioxidants are monohydric or polyhydric phenols with various ring substitutions. These phenolic antioxidants perform the function of capturing free radicals (Love, 1992). Use of these compounds is very well recognized. Some of the examples are butylated hydroxyl anisole, butylated hydroxyl toluene, amino phenols (n-butyl-p-aminophenol, n-cyclohexyl-p-aminophenol) and other aromatic compounds like hydroquinone, gossypol, sesamolin etc. Sampath and Anantkrishnan (1957) reported that the addition of butylated hydroxyl anisol (BHA) and ethyl gallate (EG) in small quantities greatly reduced the peroxide development and loss of vitamin A in ghee.

Kuchroo and Narayanan, (1972) studied the effect of some synthetic antioxidants, namely Propyl gallate (PG), Octyl gallate (OG), Oodecyl gallate (DOG), BHA and Butylated Hydroxyl Toluene (BHT), singly (0.005-0.02 per cent) or in combination of two (mixture not exceeding 0.02 per cent) with or without phospholipids, on oxidation of ghee. The results revealed that the efficiency of antioxidants decreased in the sequence PG>OG>DOG>BHT>BHA and on addition of mixtures of two antioxidants (0.01 per cent each), the protective factor decreased in the sequence: BHA + PG>BHT +PG >BHA + BHT > BHT+OG >BHA + OG > BHT+DOG>BHA+DOG. Among the mixtures of antioxidants studied, only BHA + PG and BHA+ BHT gave a synergistic effect. The addition of phospholipids either to an individual or mixture of antioxidants increased protective factor. Among the antioxidants studied phospholipids gave synergistic effect only with BHA+ PG and BHT+PG mixture. Further, Kuchroo and Narayanan, (1973) found BHT to be more effective than BHA over 12 months of storage period.

Chatterjee, (1977) observed that under commercial conditions, BHA (0.01 per cent) could improve the aroma, flavour and shelf life of ghee irrespective of season of production. Gupta Sudha *et al.,* (1979) found that antioxidant potentialities of certain compounds tested were in order: hydroquinone> catechol> resorcinol and again: palmitoyl ascorbate> PG>OG>BHA. The strong effect of hydroquinone could be due to formation is a stable quinone which can terminate the chain reaction. The effectiveness of palmitoyl ascorbate was explained to be due to the formation of diketone which is a stable structure.

Temperature of clarification during conversion of butter into ghee significantly affected the loss of antioxidants (Lal and Narayanan, 1980). They also concluded that the direct addition of antioxidants to ghee was slightly less effective in suppressing the development of peroxides in comparison to that when added to butter before clarification into ghee. The stability of antioxidants decreased with increase in storage period of ghee (Lal and Narayanan, 1980; Sree and Lal, 1990). The losses of antioxidants observed during storage might be ascribed to the scavenging of antioxidants by the free radicals formed during autoxidation of ghee (Sree and Lal, 1990). Rao *et al* .,(1984) observed that addition of BHA or BHT (0.02 per cent) or BHA + BHT (0.01 per cent each) had a retarding effect on the development of both free fatty acids and peroxide values in buffaloes' ghee during storage at room temperature (18-36 ºC). All the three antioxidant treatments had a similar effect upon free fatty acid development but peroxide development was retarded to a greater extent by BHT or BHA plus BHT than by BHA. Pande and Verma ,(1989) studied the efficacy of BHA, BHT and Tertiarybutyl hydroquinone (TBHQ) as antioxidants in ghee for different storage periods. They found that when ghee added with these antioxidants was stored in amber colored plastic bottles, BHA exhibited better antioxidant property in the first month of storage while BHT and TBHQ had similar antioxidant effect after the end of 60 days and only TBHQ exhibited maximum effect after 90 days of storage. Thus, they indicated that TBHQ could be used for long term storage of ghee.

2.6.2 Natural Antioxidants Reported for Ghee

A favorable trend towards natural products has developed due to reports from medicinal centres regarding the potential teratogenic, carcinogenic and mutagenic effects of synthetic antioxidants in experimental animals including primates. Hence, due to increased reservations such as government regulations and toxicity of using synthetic antioxidants, the use of naturally occurring antioxidants hold good promise (Hathway, 1966). The complied literature on edible plant and milk materials used for protecting ghee against oxidative deterioration is given below:

2.6.2.1 *Amla* (Indian gooseberry) juice

The juice of *amla* fruit (*Emblica officinalis*) has marked antioxidant property when added at the rate of 1.25 per cent in ghee. It retards the onset of rancidity to the same

extent as did 0.1 per cent propyl gallate and 0.01 per cent citric acid. The antioxidant property of *amla* juice was attributed to its high ascorbic acid and gallates content (Ahmad *et al*., 1960).

2.6.2.2 Aromatic herbs

Amr ,(1990b) studied the effect of addition of four aromatic herbs on oxidative stability of ghee made from Ewe's milk. Aromatic herbs namely, rosemary (*Rosmarinus officinalis*), sage (*Artemisia herballa*), fennel (*Foeniculum vulgare*) and rue (*Ruta graveatons*) were added at 7.5 per cent level to the ghee, only rosemary showed an antioxidative effect equivalent to that of BHA+ BHT (1:1, 250 ppm). All these herbs had an antioxidant effect at least for first 24 h of storage.

2.6.2.3 Betel, curry and drumstick leaves

Betel and curry leaves when added at 1.0 per cent level to ghee showed higher resistance to oxidative deterioration than BHA and BHT mixture. The antioxidative properties of betel and curry leaves were attributed to phenolic compounds, predominantly hydroxyl chavicol (Patel and Rajorhia, 1979). These leaves also contained some ascorbic acid which might work as synergist (Sethi and Aggarwal, 1956). When betel, curry and drumstick leaves were added at 1.0 and 3.0 per cent levels to ghee, which was subsequently stored for 12 m at ambient temperature, only curry leaves could protect ghee from hydrolytic rancidity and none could prevent oxidative deterioration (Thakar *et al*., 1984).

2.6.2.4 Mango seed kernel

A study was initiated by Parmar, (1984) to elucidate the effect of addition of mango (*Mangifera indica*) seed kernels or its pre-extract on oxidative stability of ghee. Dried mango seed kernel powder (MSKP) added at 1.0, 1.5, 2.0 and 2.5 per cent (w/v) levels and butylated hydroxyl anisole added at 0.02 per cent level to buffaloes' milk ghee had antioxidant potentialities in the orders :2.5 per cent MSKP > 2.0 per cent MSKP >1.5 per cent MSKP > 0.02 per cent BHA > 1.0 per cent MSKP. When a pre-extract (PE) prepared by heating MSKP and ghee at 4.0, 6.0, 8.0 and 10.0 per cent (v/v), the antioxidant potentialities of PE at all these levels were found to be greater than that of 0.02 per cent BHA. The main antioxidant principles were indicated to be various types of phospholipids and the phenolic compounds of mango seed kernels. In

addition to these compounds, the other possible agents were stated to be sterols, vitamin C, carotene and the interaction products of carbohydrates and protein generated during the heating process (Parmar, 1984; Parmar and Sharma, 1986, 1990). Dinesh *et al.,* (2000) isolated the antioxidant principles namely phenolics and phospholipids from MSKP using organic solvents. These compounds were dissolved in ghee to prepare phenolic and phospholipids extracts separately and in combination. Addition of extract in combination was more effective than individual extract. Moreover the phenolics were more effective than phospholipids in prolonging the induction period of ghee. Addition of extracts either individually or in combination at a level of 5 per cent or above were more effective in increasing the stability of ghee than addition of BHA at a 0.02 % level. It was concluded that the phenolic compounds in MSKP seemed to be the main antioxidative compounds which along with phospholipids gave the maximum stabilizing effect to ghee against oxidative deterioration.

2.6.2.5 Seed phospholipids

El-Sokkary and Ghoneim ,(1951) suggested that addition of soybean and sunflower seed at 0.5 per cent level were very effective in delaying the oxidative rancidity. Bhatia *et al*., (1978) isolated phospholipids from sunflower seed, groundnut seed and cotton seed and added to ghee. The antioxidant potentiality of whole phospholipids from these sources was in order: sunflower> groundnut >soybean > cotton seed. This was in order of decreasing phosphatidyl ethanolamine content. Individual classes of phospholipids such as phosphatidyl choline, phosphatidyl ethanolamine and phosphatidic acid were added to ghee, which was stored at 37 ºC. Phosphatidyl ethanolamine effectively prevented the increase in peroxide formation within 96 h followed by phosphatidic acid and then phosphatidyl choline. Antioxidant properties of phosphatidly ethanolamine and phosphatidyl choline were independent of the seed source. Gupta Sudha *et al* .,(1979) isolated lecithin and phenolic compounds from gram seeds (*Cicer arietinum*). They observed that phospholipids from this source could be good antioxidant for ghee. Lecithin was found to be less effective as compared to cephalin.

Kaur *et al* .,(1982) compared the seed phosphatides and synthetic compounds as antioxidants for cow and buffalo ghee. They found that antioxidant efficiency of sunflower seed oil phosphatides and synthetic compounds was in order: phosphatidyl ethanolamine> propyl gallate> palmitoyl ascorbate> BHA >phosphatidyl choline. The authors concluded that seed phospholipids were more effective than many synthetic antioxidants in controlling oxidative and lipolytic deterioration of ghee during storage.

2.6.2.6 Spices and condiments

Sesame seed powder exerts a protective action on ghee (Dhar and Aggarwal, 1949). A number of spices and condiments namely red chilli, cinnamon leaf, turmeric, clove, black pepper, nutmeg fruit, betel leaf and dry ginger have been found to possess good antioxidant properties when heated with ghee (Sethi and Aggarwal, 1952).

Semwal *et al.,* (1997) studied anti- or pro-oxygenic activity of turmeric (*Curcuma longa*) by adding its fractions (volatile oil and curcumin) in ghee at 37 ºC. The ground spice and water-soluble fraction of the spice showed antioxygenic activity. On the other hand curcumin, water-insoluble fraction, acetone soluble, ethanol soluble and insoluble fractions of turmeric showed moderate pro-oxidant activity. Volatile oil of turmeric also exhibited slight antioxygenic activity. Combination of alpha-tocopherol and curcumin showed moderate pro-oxygenic activity.

2.6.2.7 Tomato seed powder

Tomato seed powder added at 5.0 per cent level in ghee inhibited oxidation and ensured its stability practically to the same extent as 0.01 per cent of BHT or BHA (Guleria *et al*., 1983).

2.6.2.8 Onion skin extract

Jain ,(1996) elucidated the effect of addition of antioxidant principles of onion (*Allium cepa*) skin via pre-extract on the oxidative stability of ghee. The antioxygenic compounds of onion skin were extracted into methanol and dried. The dried material was mixed with ghee at a rate of 0.5 per cent (w/v). Addition of such extracts at different levels was found to be almost at par with addition of BHA at 0.02 per cent in protecting ghee. Quercetin and anthocyanin, the phenolic compounds appeared to be the main contributory factors in enhancing the oxidative stability of ghee.

2.6.2.9 Tulsi leaves

Sharma, (1997) isolated the antioxidant principles of *Tulsi* (*Ocimum sanctum* Linn.) leaves via a pre-extract. The ant oxygenic compounds of *Tulsi* leaves were extracted into methanol and then vacuum dried. The dried materials were further fractionated into water insoluble fraction which was then treated with mixture of silica gel and charcoal and designated at SCF. Addition of SCF pre-extract at the level of 0.6 per cent (w/v) was found to be more effective than the addition of BHA at the level of 0.02 per cent. The phenolic compounds appeared to be the main contributory factors in enhancing the oxidative stability of ghee.

2.6.2.10 Sorghum grain powder

Kaur *et al.,* (2001) studied the use of Sorghum (*Sorghum bicolor*, L.) grain powder in enhancing the oxidative stability of ghee. Direct addition of Sorghum grain powder (SGP) at different levels in ghee was elevated the phospholipids as well as water extractable phenolic compounds of ghee. The results also revealed that addition of SGP at a level of 1 % (w/v) and above have higher effect than the addition of permitted level of BHA. The proactive action of SGP in ghee could be attributed to the transfer of phospholipids and phenolic compounds present in SGP.

2.6.2.11 Ghee residue

Ghee residue is obtained as a by-product during making. It is a dark brown or brown material left after filtration of ghee .Narayanan *et al.,* (1966) found that cows' and buffaloes' ghee prepared from cream or butter by heating to 120 ^{o}C had a bland flavor and contained only traces of phospholipids and the rest being left in the ghee residue. Therefore, a systematic study was undertaken by Rama Murthy *et al.,* (1969) to see the effect of addition of different concentrations of ghee residue on phospholipids free ghee. In such studies, ghee residue having 6.012 per cent phospholipids was added at different levels (0 to 50 per cent) to phospholipids free ghee and stored at 37^{o}C for varying periods. It was observed that in control samples (phospholipids free ghee without ghee residue), the peroxides appeared at an early stage of storage (2 months) than those samples with added ghee residue. For instance, the peroxide values (millimoles of peroxide per kg fat) in buffalo ghee samples at 6 months of storage were, control (8.4), ghee with 1.0 per cent ghee residue (1.6), ghee with 2.0 per cent

ghee residue (1.1) and ghee with 5.0 per cent ghee residue (0.2). It was also observed that ghee samples with 5.0 per cent ghee residue showed better oxidative stability than those having lower levels of ghee residue. It was further noted that in all the control samples, the rate of increase of peroxide value was considerably higher in buffalo ghee (0.3, 2.8 and 8.4 at 2, 3 and 6 months of storage respectively). The higher peroxide values in control samples of buffalo ghee as compared to cow ghee were interpreted to be due to the differences in the natural antioxidants content like tocopherol in cows' and buffaloes' ghee. Santha and Narayanan, (1978) reported that the antioxidant property of ghee residue was dependent on the temperature of clarification, and method of preparation of ghee. With increase in temperature of clarification the antioxidant property decreased. The antioxidant efficiency of ghee residue obtained by different methods of preparation decreased in the following sequence: creamery butter ghee residue> desi butter ghee residue> direct cream ghee residue. The lipid fraction especially the acetone-insoluble fraction of ghee residue had the greatest antioxidant effect (Pagote and Bhandari, 1988). Santha and Narayanan, (1979) reported that phospholipids, the lipid constituents of ghee residue had maximum antioxidant property followed by α-tocopherol and vitamin A. Among the non-lipid constituents, the amino acids- proline, lysine, cysteine hydrochloride and tryptophan showed antioxidant property. The addition of lactose, glucose, galactose and their interaction products with protein and phospholipids to ghee increased the oxidative stability of ghee (Pagote and Bhandari, 1988). Hence, it was concluded that the antioxidant property of ghee residue is due to its above mentioned various constituents.

2.7 EPILOGUE

Although it was reported that consumption of tomato has a great antioxidative activity on health and Montesano *et al.*, 2006 reported that using lycopene from tomato could decrease oxidative rancidity in extra virgin olive oil. Guleria *et al*., 1983 reported that using tomato seed powder could decrease oxidative rancidity in ghee. But using whole tomato or its parts for checking antioxidative activity in ghee was not reported so far in any literature.

CHAPTER 3

MATERIALS AND METHODS

3.1 COLLECTION OF MARKET GHEE SAMPLES

Market samples of ghee from organized sectors were collected from the various parts of the Anand city. The samples of ghee were repacked in beakers and used for analysis.

3.2 COLLECTION AND PREPARATION OF TOMATO SAMPLE

Different cultivars of tomato depending on shape (ROUND, GLOBE, OBLONG) were collected from different market of Anand. Locally name of Round tomato- Heemshikhar, Ruby (Globe), Shaktiman (Oblong) . All tomatoes were cut and all seeds and free water were removed manually. Resulting tomatoes were crushed for preparing tomato paste.

3.3 PREPARATION OF GHEE

White butter was collected from the Dairy Technology department, Vidya dairy, Anand and local market of Anand. Butter so obtained was washed gently with cold water and then heated in a stainless steel vessel (pan) to remove moisture. Heating was continued till the curd become golden brown and the final temperature was not allowed to exceed 115 °C for no hold. The prepared ghee was allowed to settle and filtered through a double folded muslin cloth at 105^{o}C. The samples were filled in clean and dry beakers, cooled to room temperature and thereafter stored in incubator at 80 °C ± 2^{o} C.

3.3.1 Selection Cultivar of Tomato for Addition in Ghee

In the selection of variety of tomato three different cultivars (according to shape) were collected from different market of Anand viz- Round tomato- (Heemshikhar), Ruby – (Globe) and Shaktiman-(Oblong) (Appendix 2 plate 1) and made tomato paste described on 3.2.After that three varities of tomato paste were added to three parts of butter @ 0.4% (expected yield of ghee from butter) when butter started to melt and

one part of butter remain left without addition of tomato paste and made four samples of ghee as described in section (3.3).

Then all four samples were stored in incubators at 80 °C ± 2° C and observed flavor score and peroxide value when fresh and at an interval of every two days, the study was observed 15 days and four replications were taken. In this phase we had selected globe type tomato from the results.

3.3.2 Evaluate the Different Parts of Tomato for Addition in Ghee

In the selection of different parts of the tomato for addition in ghee - paste, skin and pulp (Appendix 3 plate 2) of globe variety/cultivars were added to the three samples of butter at the rate of 0.4 per cent (expected yield of ghee from butter) and one part of butter remain left without addition of tomato used as control and made ghee of four samples as described in previously section (3.3).

Then all four samples were stored in incubators at 80 °C± 2° C and observe flavor score and peroxide value in interval of two days, the study was observed 15 days and four replications were taken. In this phase we had selected skin from results as it was given best oxidative stability and flavor attribute compare of other two parts of tomatoes.

3.3.3 Selection of Stage for Addition of Selected Part of Tomato in Ghee

In the selection tomato skin was added at two different stages (after clarification or before clarification) of the ghee preparation. In each set of experiments tomato skin was added at the rate of 0.4 per cent. In one set of experiment, tomato skin added before heat clarification of butter fat in to ghee. The sample of butter was taken in to clarification pan and the tomato skin was added when butter got melted and made ghee described in 3.3. After clarification of butter fat in to ghee, the prepared sample of ghee was filtered through muslin cloth. In second set of the experiment, tomato skin was added after filtration (105^{o}C) of heat clarified (115^{o}C no hold) butter fat in to ghee, the sample was thoroughly mixed with the help of dry glass rod for uniform mixing of the tomato skin, followed by refiltration (at 65^{o}C) through four folded muslin cloth. The samples of ghee without addition of tomato skin were also prepared to serve as control samples described at section 3.3. All the samples were stored at 80^{o}C±2^{o}C. Total six replications were conducted. All the samples were subjected for sensory evolution for flavor score and analyzed for peroxide value when fresh and

interval of every two days. On basis of flavor score and peroxide value score we had selected addition of tomato skin after heat clarification of butter into ghee.

3.3.4 Optimization for Rate of Addition of Selected Part of Tomato in Ghee

The tomato skin were added to the butter after heat clarification at the rate of 0.2, 0.4, 0.6, 0.8 and 1.0 per cent (expected yield of ghee from butter) and one part is remain left without addition of anything as control sample (Appendix 4 plate 3) and then all six samples were stored in incubators at 80 °C±2^{o}C and observe flavor score and peroxide value when fresh and interval of every two days, the study was observed 15 days and 3 replication were taken. In this phase we had selected 0.6% skin added ghee sample on the basis of peroxide value and flavor score.

3.3.5 Comparison of Tomato Skin, BHA and Synergistic Effect of Tomato Skin with BHA for Enhancing Shelf life of Ghee.

In this phase 0.6% skin of tomato skin was added one sample of ghee , BHA @ 0.02% added in one part of ghee , 0.6% tomato skin and 0.02% BHA added in one part of ghee and compare with control sample and stored in 80^{o} C±2^{o}C. Sample were subjected to sensory evaluation and analyzed for peroxide value when they were fresh and interval of every two days. These studies remained for 15 days and found BHA+ tomato skin gave best result even from BHA and tomato added sample on basis of flavor score and peroxide value.

3.4 SENSORY EVALUATION

All the the samples of ghee made in laboratory were evaluated for their sensory characteristics on a 9 point hedonic scale by a panel of nine judges. The samples were evaluated for their flavor. The 9 point hedonic scale score card for sensory analysis is given below in apendice 1.

3.5 CHEMICALS AND GLASSWERES

During the entire study, Borosil brand of Glassware's and analytical grade chemicals were used. Glassware's and other materials were sterilized by the usual procedures whenever required.

3.6 METHODS FOR ANALYSIS OF GHEE

For chemical analysis different parameters were analyzed. *Viz.* Moisture, Refractive index, Peroxide value, Reichert Meissl value, and free fatty acid.

3.6.1 Determination of Moisture

Moisture content of ghee samples was determined by the method as described by Indian Standards Institution (ISI: 3508- 1966).

10 gm of ghee sample was weighted in a clean dry aluminum dish. The dish with ghee sample was placed in hot air oven maintained at 105°C ±1 ºC for approximately 1 h. The dish was removed from the oven and cooled to room temperature in desiccators. The dish was then weighed. The steps of heating, cooling and weighing were repeated after half an hour each time until the difference between the two successive weighing did not exceed 1 mg.

The moisture content of ghee was calculated as follows:
Moisture content, per cent by weight = 100 (W1-W2)/W1-W
Where,
W1= Weight in g of the dish with ghee before drying

W2= Weight in g of the dish with ghee after drying, and

W= Weight in g of empty dish

3.6.2 Determination of Refractive Index of Ghee (B.R reading)

Refractive Index of ghee samples was determined by the method as described by Indian Standards Institution (ISI: 3508- 1966).

The sample shall be rendered optically clear, and free from water and other suspended impurities. The correctness of the instrument shall be tested before taking reading by carrying out tests with fluid of known refractive index. At temperature of 40°C or over, the prisms of most instruments never reach the temperature indicated by the registering thermometer, and at temperatures greatly removed from the standard temperature for the instrument, there is a small error due to the change of the refractive index of the glass. At these high temperatures check the instruments experimentally with a liquid of known temperature coefficient, and apply the correction thus found to instrument readings given by the sample.

It shall be borne in mind that the presence of free fatty acids considerably lowers the refractive index. Ghee shall completely fill the space between the two prisms, and shall show no air bubbles. The reading shall be taken after ghee has been kept in the prism for 2 to 5 minutes and after it has been ensured that it has attained constant temperature by taking two or more readings. Take sure that the ghee has reached the temperature of the instrument before. The reading is taken. Before commencing to take readings circulate through prisms a stream of water at constant temperature and measure accurately the constant temperature at which the readings were taken.

3.6.3 Determination of Peroxide Value

The peroxide value of ghee was determined by the method (iodometric method) as described by the Indian Standards Institution (ISI: 3508-1966).

One gram of ghee sample was taken in a 150 x 25 mm test tube and 1 g of potassium iodide and 20 ml of the solvent mixture (prepared by mixing two volumes of glacial acetic acid, AR and one volume of chloroform, AR) was added. The contents were heated to boil within 30 sec in a boiling water bath and allowed to boil for not more than 30 sec. The test tubes were transferred to a 250 ml conical flask containing 20 ml of freshly prepared 5 per cent potassium iodide solution. The test tube was rinsed well with about 25 ml of distilled water and all washings were transferred to the above flask. The contents were titrated against 0.002 N Sodium thiosulphate solutions using 2 ml of starch indicator, near to end point. A blank was also performed without using ghee sample.

The peroxide value of ghee was calculated as mill equivalents of peroxide oxygen per kg of ghee.

Peroxide value (mill equivalents of peroxide oxygen/kg of ghee) = 2T/W

Where,
T= Volume in milliliters of 0.002 N Sodium thiosulphate, and

W= Weight in g of sample

3.6.4 Determination of Free Fatty Acids

The free fatty acids content of ghee was determined by the method as described by Indian Standards Institution (IS: 3508- 1966).

In a clean and dry 50 ml conical flask, 10 gm of ghee sample was taken. In a second flask about 50 ml of ethyl alcohol (95 per cent v/v) was taken and brought to boil, while hot it was neutralized to the end point of phenolphthalein (1 per cent solution in ethyl alcohol) with 0.1 N sodium hydroxide. The neutralized alcohol was poured on ghee in a flask, the contents were mixed thoroughly and brought to boil and still hot, the contents were titrated with 0.1 N sodium hydroxide. The end point was noted when the addition of a single drop produced a slight but definite pink color persisting for at least 15 seconds.

The free fatty acids content was calculated as follows:
Free fatty acids (as per cent, oleic acid) = 2.82 x T/ W
Where,
T= Volume in ml of 0.1 N Sodium hydroxide required for titration, and
W= Weight in g of ghee sample taken.

3.6.5 Reichert Meissl Value and Polenske Value

A Reichert-Meissl value was determined by the method specified in ISI: 3508-1966. Ghee (5 gm) was saponified using glycerol and 50 % NaOH, diluted with water and acidified, and thereafter steam- distilled in a glass apparatus at a controlled rate. The condensed and cooled distillate was filtered and the water-soluble acids that pass through were estimated by titration with 0.1N NaOH to give the Reichert Meissl value. For Polenske value wash the condenser , cylinder and head through the 15ml neutral alchol and collected the alcoholic solution. Same performances were done three times. Then titrate with 0.1N NaOH for water insoluble fatty acids. The blank tests were conducted in the same way without using ghee sample.

Reichert- Meissl value = $1.10 (T_1-T_2)$
Where
T_1= volume in ml of 0.1N NaOH used for sample for titration (water soluble)
T_2= volume in ml of 0.1N NaOH used for blank (water soluble).

For Polenske value = $T_3 – T_4$

Where,

T_3 = volume in ml of 0.1N NaOH used for sample for titration (water insoluble)

T_4 = volume in ml of 0.1N NaOH used for blank (water insoluble).

3.6.6 Baudouin Test

Baudouin test was done by the method specified in ISI: 3508-1966. 5 gm of melted ghee was taken in test tube add 5 ml concentrated HCl and add 0.4ml furfural solution. The test tube is shaken and allow the mixture to separate for 2 minute, Pink red color shown adulterated with vanaspati.

3.7 STATISTICAL ANALYSIS

Storage study data obtained for Peroxide value and flavor score on different stages of study were analyzed using CRD. The level of significance was $P< 0.05$ levels and CD per cent was also calculated

$$\mathbf{Y_{ij} = \mu + T_i + R_j + C_{ij}}$$

Where,

Y_{ij} = response due to the i^{th} treatment in j^{th} replication

μ = general mean

T_i = effect due to i^{th} treatment

R_i = effect due to j^{th} replication

C_{ij} = uncontrolled variation due to i^{th} treatment in j^{th} replication.

The analysis of variance was carried out as per Snedecor and Cochran (1967).

CHAPTER 4

RESULTS AND DISCUSSIONS

Ghee is clarified milk fat and also termed as anhydrous milk fat, constitutes an important part of Indian life. Ghee is chiefly used in India as a part of the diet and as a cooking medium. It is valued for its pleasant cooked, caramelized flavor and granular texture. Ghee is made up mainly of fat, which gives energy to the body and forms an integral part of the body's cells. It helps to maintain the body's temperature. Recent studies have indicated that milk fat contains some anti carcinogenic substances such as conjugated linoleic acid, butyric acid, and vitamins etc. Ghee contains the fat-soluble vitamins A, D, E and K. Indian Ayurvedic medical literature mentions various types of medicated ghee that can be used for the treatment of many diseases such as asthma, anti-aging, cough, dermatitis, digestive problems, heart, hysteria, leprosy, leucoderma, and piles.

However, ghee undergoes oxidative deterioration which adversely affects its economical and nutritional value. These in turn determine the storage stability and are of paramount importance from economic view points. Therefore, constant research endeavors are made to extend the shelf-life by various approaches. One the most common approaches is addition of antioxidants. Antioxidants can be classified according to their source as natural or synthetic antioxidants. Continuous use of synthetic antioxidants may cause health hazards such as teratogenic, carcinogenic and mutagenic effects in experimental animals and primates. It is because of these reasons that constant endeavors have been made for the use of natural antioxidants (Hathway, 1966; Maeura *et al*., 1984; Van , 1986). The use of these additives is regulated and limited by law. It is generally accepted that natural antioxidants are more potent, efficient and safer than synthetic antioxidants. Therefore, now consumers demand less use of synthetic additives (Membre *et al.*, 2001). Consequently, in recent years many attempts have been made for search of natural antioxidant compounds that can properly serve the demand of consumers and needs of the food manufacturers. Various herbs and spices have been recognized for their antioxidant activity and used throughout the past as an alternative approach to preserve foods. Several studies have revealed the results on the antioxidant action of spices, fruits and vegetables (Honglian *et al.*, 2001).

Tomatoes are now eaten freely throughout the world, and their consumption is believed to benefit for the heart, among other organs. They contain the carotene lycopene, one of the most powerful natural antioxidants. In some studies, lycopene, in tomatoes, has been found to help prevent prostate cancer (Rao and Agarwal ,1999). Lycopene and other carotenoids which found in tomato have also been shown to improve the skin's ability to protect against harmful UV rays (Mohanty *et al.,* 2001). Research shows lycopene from tomato has a potential effect to prevent heart disease and it can also drop LDL cholesterol (Rao and Balachandran ,2003). Lycopene can control high blood pressure (Paran and Engelhard, 2001). Health benefit action of tomato think to be for lycopene and all these health benefit action of lycopene come due to its high antioxidative action (Rao and Agarwal, 1999).

However, the potential of fresh tomato has not been trapped as a natural antioxidant for preventing oxidative rancidity in ghee. The antioxidant/pro-oxidant action of tomato (source of lycopene and other natural carotenoids as antioxidant) on lipid oxidation has been of interest in food lipids. No literature so far found about the potential antioxidative activity of tomato to inhibit oxidative rancidity in food especially in ghee.

Therefore, present study was planned to study the antioxidant potential of tomato to extend the shelf life of ghee. The study was divided into five phases. In the first phase three different varieties/cultivars of tomatoes were evaluated for their antioxidant activity in ghee. In the second phase, different parts of tomatoes (skin, pulp and paste) were studied for their antioxidant activity in ghee. In the third phase, selection for stage of addition of tomato was studied. In fourth phase rate of addition of the tomatoes were tested for best antioxidative action in ghee. In fifth phase comparison of tomato skin, BHA and synergistic effect of tomato skin with BHA for enhancing shelf life of ghee was studied.

4.1 SELECTION CULTIVAR OF TOMATO FOR ADDITION IN GHEE

Tomato originated from South America and its most widely known scientific name is *Lycopersicon esculentum*. This fruit contains a variety of carotenoids, among all these carotenoids lycopene is the major carotenoids found in tomato (Hart and Scott, 1995), and the scientific name of tomato is derived from this lycopene (Tonucci *et al*., 1995). The amount of lycopene in fresh tomato fruits is influenced by variety, agricultural practices, maturity and the

environmental conditions under which the fruits matured. Tomatoes of the common variety *Lycopersicon esculentum* typically contain about 3 to 10 mg (max 25 mg) of lycopene per 100 g of ripe fresh fruit (Hart and Scott, 1995).

Tomato varieties are roughly divided into several categories, based on shape and size- "Slicing" or "Globe" tomatoes are the usual tomatoes of commerce; along with a thinner skin and higher shelf life; “Globe” tomatoes are of the category of canners used for a wide variety of processing and fresh eating. Usually “Oblong”, and are shaped like large strawberries, are bred with a lower solid content and higher moisture content use in tomato sauce and paste. Cherry tomatoes are small and “Round” type, often sweet tomatoes generally eaten whole in salads. Usually name of these tomatoes are determined locally by local farmers for example in Anand area name of these tomatoes are Ruby (Globe), Shaktiman (Oblong) and Heem sikhar (Round). Among these three types/cultivars/varities of tomato globe cultivar of tomato harvested in summer and round cultivar of tomato harvested in winter, oblong cultivar of tomato generally harvested in autumn.

Addition of tomato for enhancing the shelf life of ghee, the important point for consideration was the selection of cultivar of tomato .Therefore, in first phase samples of tomato from these three cultivars *viz*, Globe (Ruby), Oblong (Shaktiman), Round (Heemshikhar) were evaluated for their antioxidant activity in ghee and to check that whether these three cultivar would have same result for oxidative stability in ghee or not.

In the selection of the cultivar tomato, paste of each variety was prepared by cutting tomatoes and all seeds as well as free water were removed manually. Resulting tomatoes were crushed for preparing tomato paste. The paste of tomato so obtained from each cultivar of tomato was added separately to the samples of butter at the rate of 0.4 per cent (expected yield of ghee from butter). For the study fresh samples of butter was obtained from commercial dairy plant. The sample of butter was divided in to four parts. The samples of butter was taken in to clarification pan and when butter get melted in first part tomato paste from Globe variety was added at the rate of 0.4 percentage (expected yield of ghee from butter), in second part tomato paste from Round variety was added at the rate of 0.4 percentage (expected yield of ghee from butter), in third part tomato paste from Oblong variety was added at the rate of 0.4 percentage (expected yield of ghee from butter). The fourth part was kept as such (no addition of tomato

paste) to serve as control and ghee were made described in 3.3(section). After clarification of butter fat in to ghee, the prepared samples of ghee were filtered(105^{O}C) through muslin cloth. All the samples of ghee were stored at elevated temperature (80 ^{O}C± 2 °C) to accelerate the oxidation. Total four replications were conducted.

4.1.1 Effect of Cultivar of Tomato on Changes in Flavor Score of Ghee During Storage

The samples of ghee were subjected to sensory evaluation for flavor using 9 point Hedonic scale, when fresh and also at regular interval of every two days. The average results obtained from four different replications for the changes in flavor score of the ghee samples are given in Table 4.1 and presented in Figure 4.1.

The statistical analysis of the data indicated that the period of storage, cultivar of tomatoes and interaction between period of storage and cultivar of tomatoes were significant (P< 0.05). Thus, statistical analysis of results revealed that cultivar of tomatoes and storage period have significant effect on changes in flavor score of ghee during storage. The interaction effect of period of storage and cultivar of tomatoes was also significant.

Table 4.1: Effect of cultivar tomato on changes in flavor score of ghee during storage

Storage periods (days)	**Cultivar of tomato added in ghee**			
	None (control)	**Globe**	**Oblong**	**Round**
1	8.40	8.60	8.40	8.50
3	8.10	8.60	8.20	8.50
5	6.40	7.90	7.20	7.40
7	5.10	7.30	6.40	6.20
9	4.60	6.80	5.50	5.30
11	4.10	6.20	4.80	5.10
13	3.50	5.30	4.60	4.90
15	2.40	4.90	3.90	4.50

ANOVA TABLE

Source of Variation	**Storage period**	**Cultivar of tomato**	**Storage period × Treatment**
S Em	0.091	0.064	0.181
C D	0.254	0.179	0.507
Test	*	*	*
C V percentage	5.87		

(*=significant,* *=highly significant, ns=non significant)

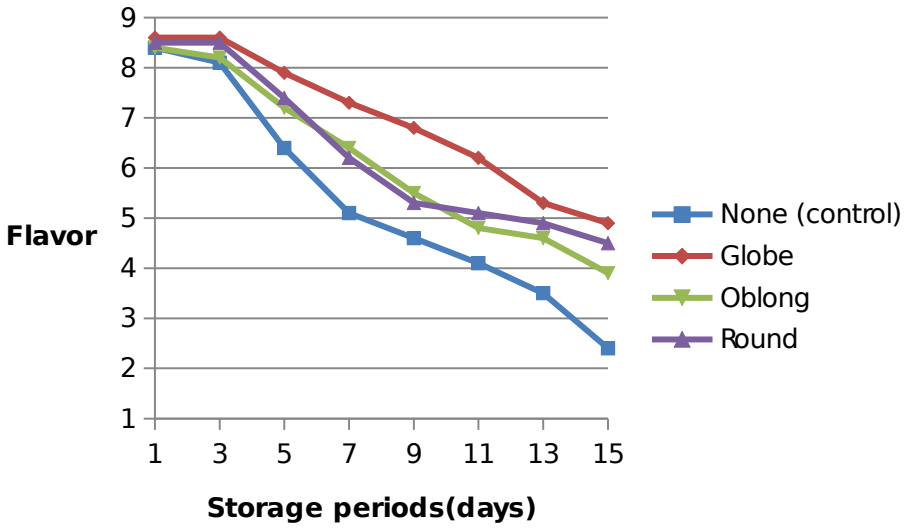


Figure 4.1: Effect of cultivar tomato on changes in flavor score of ghee during storage

Comparison of CD values of ghee sample added with tomato of different cultivar revealed that differences in flavor score of all the sample of fresh ghee (first day of storage) were statistically non significant to each other, except ghee sample added with Globe cultivar of tomato. The addition of tomato from Globe cultivar significantly improved the flavor score of the fresh ghee sample. Such improvement in the flavor score of the ghee sample might be attributed ability of constituent(s) from Globe cultivar tomato to prevent formation of compounds during clarification of milk fat in to ghee, which adversely affects the flavor of ghee. However, no report is available in the literature about effect of such addition of tomato flavor score of fresh ghee.

On third day of storage flavor score of ghee sample added with Globe and Round tomato significantly higher score than that of the control sample, but difference in flavor score of ghee added with Oblong tomato was statistically at par to that of the control sample. Similarly, on third day of storage difference in flavor score of ghee sample added with Round and Globe tomato was statistically at par with each other. However, flavor score difference of ghee sample added with Round and Globe tomato was significantly higher as compare to the flavor score of sample added with Oblong tomato. Throughout last twelve days of storage flavor score difference of all the sample added with different cultivars of tomato were remain

significantly higher as compare to control sample and all these tomato added ghee samples flavor score difference were remain significant to each other.

Comparing CD value it was found that during whole storage period flavor of control sample decreased significantly for every interval of storage right from beginning to the last day of storage. All three samples of ghee added with three different cultivar of tomato's flavor score decreased significantly for every interval of storage right after third day of storage.

The results given in graphical presentation also indicated that the initial flavor score of the control sample of ghee was slightly lower than that of the sample added with round type of tomato and globe type of tomato but as equal as oblong tomato. During the storage the flavor score of control sample declined at a greater rate and reached below 6 on seventh day of the storage. Flavor scores of the tomato added samples of ghee remained higher compare to that of the control sample thorough out whole study periods. The flavor score of ghee sample added with oblong and round went below 6 level on ninth day of storage, whereas, flavor score of ghee sample added with globe tomato went below 6 level on thirteen day of storage. Thus, among the three cultivar of tomato the globe variety gave better result which extended the flavor score to acceptable level by six days compare to control and four days as compare to round and oblong tomato. Thus, effectiveness of the three cultivars of tomato in retaining the flavor score of ghee was in decreasing order of Globe > Round > Oblong.

4.1.2 Effect of Cultivar Tomato on Changes in Peroxide Value of Ghee During Storage

The samples of ghee were also analyzed for peroxide value when fresh and at an interval of every two days. The average results obtained from four different replications for changes in peroxide value of the ghee samples are given in Table 4.2 and graphically represented in Figure 4.2

The statistical analysis of the data indicated that the period of storage, cultivar of tomatoes and interaction between period of storage and cultivar of tomatoes were significant ($P< 0.05$). Thus, statistical analysis of results revealed that cultivar of tomatoes and storage period have significant effect on changes in flavor score of ghee during storage. The interaction effect of period of storage and cultivar of tomatoes was also significant.

Table 4.2 Effect of tomato variety/cultivar on peroxide value of ghee during storage

Storage period (days)	Cultivar of tomato added in ghee			
	None (control)	Globe	Oblong	Round
1	1.50	1.27	1.55	1.40
3	2.05	1.65	1.80	1.65
5	6.85	4.00	4.55	4.30
7	8.30	5.10	6.87	6.87
9	8.40	7.40	8.42	7.92
11	11.75	8.60	9.60	8.85
13	18.30	13.60	14.92	13.93
15	24.40	17.00	19.25	17.50

ANOVA TABLE

Source of Variation	Storage period	Treatment (Tomato variety)	Storage period × Treatment
S. Em	0.310	0.219	0.620
C. D	0.872	0.616	1.743
Test	*	*	*
C V percentage	14.77		

(*=significant,* *=highly significant, ns=non significant)

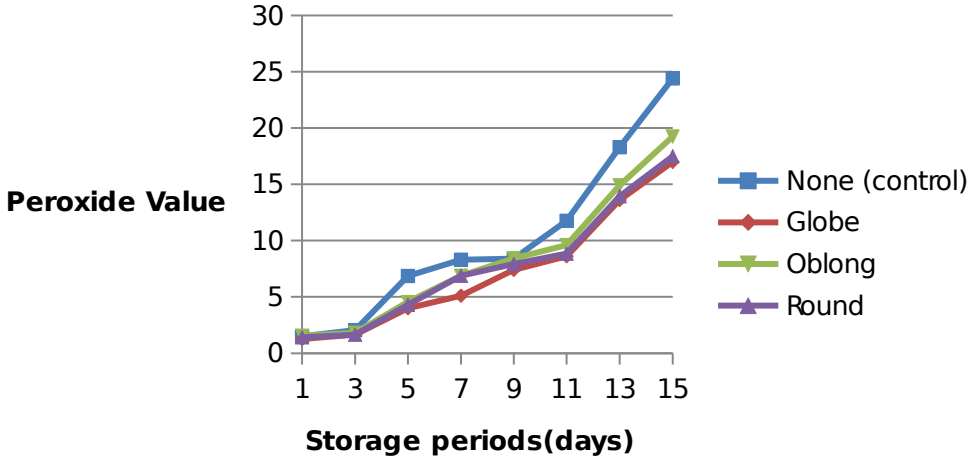


Fig 4.2: Effect of cultivar of tomato on changes in peroxide values of ghee during storage

During first day of storage when fresh ghee samples analyzed for peroxide value there were no significantly difference found among all the samples- control sample as well as ghee samples added with different cultivars of tomato. This trend followed up-to third day of storage. During fifth day of storage it was observed that peroxide value of control sample were significantly higher than that of samples added with different cultivars of tomato. But peroxide

value of three samples added with three different cultivars of tomato were remained non significant to each other in this period. During seventh day of storage peroxide value difference between the sample added with oblong tomato and round tomato was non-significant to each other, and peroxide value of globe tomato added sample was significantly lower than peroxide value of oblong as well as round tomato added ghee sample. But peroxide value of control sample had significantly higher than that of the samples added with different cultivar of tomato on seventh day of storage on seventh day of storage. On ninth day of storage peroxide value of control sample had significantly higher than that of samples added with both round as well as globe tomato but sample added with oblong tomato had not significantly differed to control sample. For last six days of storage it was found that control sample had significantly higher peroxide value than that of others three samples added with three different cultivars of tomato. For last six days of studies peroxide value difference between globe and round tomato added samples were statistically non significant, but peroxide value of oblong tomato added sample were remain comparatively higher than that of other two varieties of tomato added in ghee. Thus, effectiveness of the three cultivars of tomato of ghee on peroxide value during fifteen days of storage was shown in decreasing order of Globe > Round > Oblong.

Peroxide value of control sample increased significantly for first five days and from ninth to fifteenth days of studies, but there was no significantly difference found in peroxide value between seventh and ninth day of studies. Peroxide value for ghee samples added with tomato had no significantly difference on first three days of studies, there after peroxide value increased significantly throughout whole study periods. Interaction effect is significant as days increase and peroxide value also increased.

From graph we could draw conclusion that during accelerated storage, the peroxide value (milli equivalents/kg) of control sample increased at a rapid rate compared to the samples of ghee added with different cultivar of tomato. The peroxide value of ghee samples containing globe tomato was lowest almost the whole period of storage. Initially peroxide value of oblong type of tomato remains slightly higher compare to control sample. But at ninth day of storage peroxide value of control sample of ghee and samples of ghee added with different cultivar of tomato was minimum but from eleventh days of storage clear difference of PV

(peroxide value) were observed between control samples and samples added with different cultivars of tomato . From ninth day of studies peroxide value of control sample was increased very rapidly as compare to others three ghee samples added with different cultivar of tomato. Peroxide value of control sample was increased almost three times for last six days of storage compare to first nine days of storage. Peroxide value of ghee samples added with tomato increased rapidly from eleven days of storage. Peroxide values of three ghee samples added with globe, round and oblong tomato were very near at first five days of storage and after that peroxide value of ghee sample added with oblong and round were almost same up to thirteenth days of storage period. At fifteenth day peroxide value of globe sample was lowest as compare of all other three samples.

Changes in peroxide value of all the samples of ghee during storage were well collaborated with changes in flavor score of the corresponding samples of ghee. The control sample having highest peroxide value and corresponding lowest flavor score compare to samples of ghee added with different cultivars of tomato. The sample of ghee added with globe added tomato lowest peroxide value correspondingly highest flavor score almost entire storage periods.

The effect of different cultivars of tomato towards oxidative stability of ghee has not been reported so far in any literature. On the basis of result for per-oxide value and flavor score best performance of Globe tomato found in present study may be attributed to its higher lycopene content compared to the oblong and round variety. According to local farmer utilization of N_2 fertilizer is higher for harvesting of Ruby tomato as compare to others two types of tomatoes, using of Nitrogen fertilizers were reported to increase the lycopene and others carotenoids content of tomatoes (Grolier, 2000).

The literature says that size of tomato influence the quantity of lycopene (Hart and Scott, 1995), as different agriculture practice and time of harvesting of tomato influence the size of tomato and size does matter the secondary metabolism of lycopene in tomato (Hart and Scott, 1995) hence that's why three different cultivar of tomato had different effect on oxidative stability of ghee during storage.

Heinonen *et al.,* (1989) reported that the lycopene concentration in tomatoes was highest in the summer (from June to August) and lowest in the winter (from October to March).In India

globe cultivars of tomato is found during (harvested in) summer it might another cause for higher lycopene content as compare to other to cultivars of tomato.

Lycopene is bound in lipid matrix of tomato and structure of different lipid matrix is different according to size and shape of tomato, availability of lycopene depends on shape of matrix (Hussein and El-Tohamy, 1990), as globe tomato has thinner skin as compare to other two varieties so it might be that availability of lycopene is more in globe tomato than other two varieties of tomato.

Changing in flavor score of ghee and changes in peroxide value of ghee during storage had conclusively suggested that globe tomato variety was best to retain the flavor ghee by inhibiting oxidative deteoration .Therefore among three cultivar of tomato used in this study cultivar from globe variety was selected for further study.

4.2 EVALUATE DIFFERENT PARTS OF TOMATO FOR ADDITION IN GHEE

Fresh tomatoes may also be important in supplying dietary antioxidant, especially lycopene (carotenoids) (Vinson *et al* .,1998). In the human diet, tomatoes are the predominant sources of lycopene, which exhibits the highest anti oxidative properties among all dietary carotenoids (Miller *et al*., 1996). Lycopene as antioxidants can neutralize free radicals, which cause oxidative damage to biological molecules, especially to lipids, proteins, and nucleic acids (Vinson et al., 1998). Lycopene has been recognized as the most efficient singlet oxygen quencher among the carotenoids of biological importance (Di Mascio *et al*., 1989). Common tomato variety contains about 3 to 10 mg of lycopene per 100 g of ripe fresh fruit (Hart and Scott, 1995).

In ripening tomatoes, it is the last carotenoid to form. Its biosynthesis increases dramatically after the breaker stage (i.e., after the color of the berries starts changing from green to pink) as chloroplasts undergo transformation to chromoplasts (Kirk and Tilney-Bassett, 1978). Chromoplasts rich in lycopene predominate in the outer part of the pericarp. At the cellular level, lycopene is localized in the chloroplasts of tomato fruits and can be found among the thylakoid membranes in the photosynthetic pigment-protein complex (Sharma and Le Maguer, 1996).

According to Guleria *et al*., 1983 tomato seed powder added at 5.0 per cent level in ghee inhibited oxidation , but there were no report found that which part of fresh tomato give better oxidative stability in ghee, though literature shows that tomato skin has highest amount of lycopene(Chandra and Ramalingam ,2011 and Al-Wandawi *et al*.,1985) . Addition of tomato (as a source of lycopene) for enhancing the shelf life of ghee, the important point for consideration was the selection of part of tomato for addition during preparation of the ghee. Therefore, in second phase different parts of tomato viz. skin, paste (skin and pulp) and pulp were evaluated for their antioxidant activity in ghee.

For the study four fresh samples of butter were obtained from different sources (different commercial dairy plant of Anand city). Each sample of butter was divided in to four parts. The samples of butter were taken in to clarification pan and when butter got melted in first part tomato skin was added at the rate of 0.4 percentage(expected yield of ghee from butter) in second part tomato pulp was added at the rate of 0.4 percentage (expected yield of ghee from butter), in third part tomato paste was added at the rate of 0.4 percentage, and the fourth part was kept as such to serve as control and made the ghee described in section 3.3. After clarification(115^{o}C) of butter fat in to ghee, the prepared samples of ghee were filtered(105^{o}C) through muslin cloth. All the samples of ghee were stored at elevated temperature (80 ^{o}C± 2 °C) to accelerate the oxidation. Total four replications were taken.

4.2.1 Effect of Different Parts of Tomato on Changes in Flavor Score of Ghee During Storage

The samples of ghee were subjected to sensory evaluation on 9 HEDONIC scales on first day and at an interval of every two days for period of 15 days. The average results obtained from four different replication for changes in flavor score of the ghee samples are given in Table 4.3 and graphically represented in Figure 4.3.

Table 4.3: Effect of tomato parts on changes in flavor score of ghee during storage

Storage period (days)	Parts of tomato added in ghee			
	None (control)	Skin	Pulp	Paste
1	8.20	8.90	8.90	8.80
3	8.10	8.10	8.30	8.60
5	6.00	7.60	7.40	7.70
7	4.90	6.30	7.20	7.10
9	4.20	6.10	5.90	5.90

11	4.10	5.50	5.50	5.40
13	3.50	5.00	4.80	5.20
15	2.75	4.60	4.40	4.10

ANOVA TABLE

Source of Variation	Storage period	Treatment (Tomato variety)	Storage period × Treatment
S. Em	0.102	0.072	0.205
C. D	0.287	0.203	0.574
Test	*	*	*
C V percentage	6.59		

(*=significant,* *=highly significant, ns=non significant)

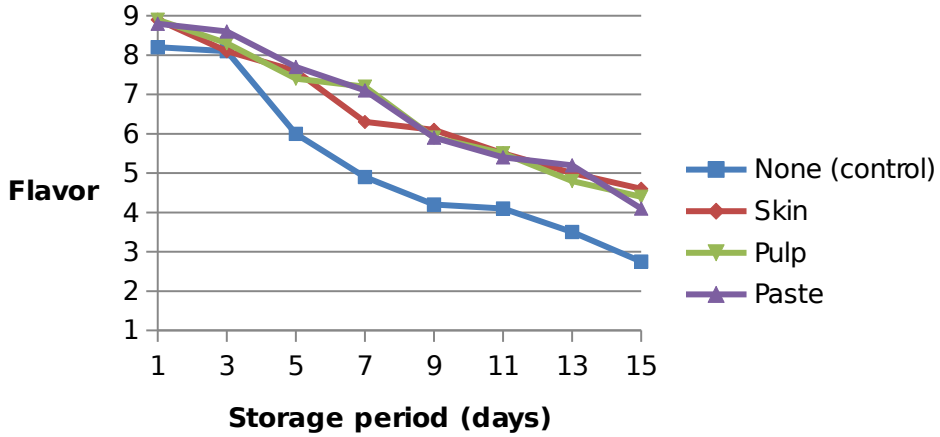


Fig 4.3: Effect of parts of tomato on changes in flavor score of ghee during storage

The statistical analysis of the data indicated that the period of storage, parts of tomatoes and interaction between period of storage and parts of tomato were significant (P< 0.05). Thus, statistical analysis of results revealed that parts of tomato and storage period have significant effect on changes in flavor score of ghee during storage. The interaction effect of period of storage and parts of tomato was also significant.

During first day of study when fresh ghee sample were gone for sensory evolution (flavor score) it was found that there was significantly lower flavor score of control sample than samples added with different parts of tomato but flavor score of three samples of ghee added with different parts of tomato was remain non significant to each other (basis of CD value). During third day of storage flavor score of control sample of ghee had significantly lower flavor score than flavor score of paste added sample but control sample had non-significant

flavor difference with samples added with both skin as well as pulp. Flavor score of paste added ghee sample had significantly higher flavor score than both pulp and paste added ghee samples but on third day of storage non significantly flavor score difference found between the pulp as well as skin added ghee samples with each others . From third day and onwards flavor score of control sample was significantly lower flavor score than that of flavor score of ghee added with different parts of tomato. On fifth day flavor score of paste added sample was significantly higher as compare to the pulp added sample but non-significant flavor score difference found with skin added sample of ghee. On seventh day flavor score of pulp added sample was significantly higher as compare to the skin added sample but non-significant flavor score difference with paste added sample of ghee. For ninth and eleventh days of observation it was found that flavor score difference of all three ghee samples added with different parts of tomato were remain non significant to each other though flavor scores of skin added sample were little bit higher than other two samples. On thirteen day of storage paste added sample were significantly higher than pulp added sample but non-significant flavor difference found to skin added sample. On fifteenth day of storage flavor score of ghee sample added with skin was significantly higher than paste added sample but non-significant difference (lower) found to the sample added with pulp.

For control sample flavor score decreased significantly except between first to third days of storage and ninth to eleventh days of storage where change in flavor score was non-significant from previous day's observation. For ghee samples added with both tomato skin as well as tomato pulp was significantly decreased throughout whole study except between seventh to ninth day for skin added and eleventh to thirteenth day for pulp added sample respectively where flavor score was decreased non- significantly from previous day's observation. For paste added sample flavor score decreased non- significantly for first three days of study and storage between eleventh to thirteenth day of storage except these days flavor score decreased significantly rest of the storage periods.

From graphical analysis we found initial flavor score of control sample was little bit lower than sample added with different parts of tomato and among these three sample of ghee added with tomato parts , samples with skin and pulp had same score(8.9) little bit higher compare to sample added with tomato paste(8.8). During the storage periods flavor of control sample

decreased very rapidly and came below 6 at seventh day of storage, during these seven days of storage flavor score of all three ghee samples added with different parts of tomato were very close to each others. Sample of ghee added with pulp and paste decreased to below 6 at ninth of the day of storage for both two cases flavor score were same. On ninth day of storage flavor score was just little higher for skin added sample (6.1). On eleventh day flavor score of ghee sample added with skin went below 6. At the end of fifteen days of studies all the flavor score of tomato samples were very close to each other but sample of ghee added with skin was little bit higher than other two samples. So from graphical analysis we found that skin added ghee sample gave best result that could retain the flavor of ghee 2 days more in accelerated storage as compare to pulp and paste. Thus, effectiveness of the three parts of tomato in retaining the flavor score of ghee was in decreasing order of Skin > Paste = Pulp.

4.2.2 Effect of Different Parts of Tomato on Changes in Peroxide Value of Ghee During Storage

The samples of ghee were also analyzed for peroxide value when fresh and at an interval of every two days. The average results obtained from four different replications for changes in peroxide value of the ghee samples are given in Table 4.4 and graphically represented in Figure 4.4.

The statistical analysis of the data indicated that the period of storage, parts of tomatoes and interaction between period of storage and parts of tomato were significant ($P< 0.05$). Thus, statistical analysis of results revealed that parts of tomato and storage period have significant effect on changes in flavor score of ghee during storage. The interaction effect of period of storage and parts of tomato was also significant.

Table 4.4: Effect of parts of tomato on changes in peroxide value of ghee during storage

Storage period (days)	Cultivar of tomato added in ghee			
	None (control)	Skin	Pulp	Paste
1	1.52	1.40	1.42	1.57
3	2.4	2.42	1.97	1.90
5	7.52	3.2	4.25	4.00
7	8.57	4.87	5.37	5.12
9	8.80	6.60	8.17	7.42
11	13.75	8.85	8.67	8.67
13	18.35	12.15	13.40	13.65
15	24.23	16.02	17.00	17.75

ANOVA TABLE			
Source of Variation	**Storage period**	**Treatment (Tomato variety)**	**Storage period × Treatment**
S. Em	0.434	0.307	0.868
C. D	1.221	0.864	2.443
Test	*	*	*
C V percentage	21.32		

(*=significant,* *=highly significant, ns=non significant)

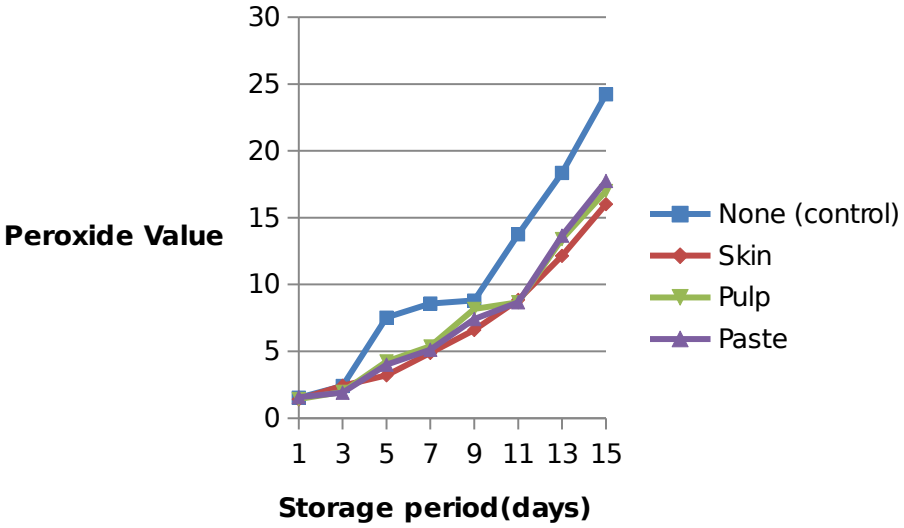


Figure 4.4: Effect of parts of tomato on changes in peroxide unit of ghee during storage

For first three days of storage differences in peroxide value among all the four samples were found non-significant. From third days onwards control sample peroxide value was significantly higher than that of different tomato part added samples. Skin added ghee sample's peroxide value was non-significantly higher from peroxide value of other two parts added sample throughout whole study (except first three days of study). Same trends followed to each other for other two samples of ghee added with other two different parts of tomato.

The peroxide value of control sample increased significantly for first five days and last six days of storage but peroxide value did not increase significantly on fifth to ninth days of storage. For skin added sample first three days peroxide value did not differed significantly but afterwards peroxide value significantly increased from previous day. For pulp added sample peroxide value non- significantly increased for first three days of study and between ninth to

eleventh days of studies, but peroxide value significantly increased on rest of the study periods. For paste added sample peroxide value non- significantly increased on first three days of studies and between fifth to seventh days of studies, but peroxide value significantly increased on rest of the study periods. We could concluded the effectiveness of different parts of tomato on the peroxide value of ghee on decreasing order Skin> Pulp> Paste.

From graphical representation we found during accelerated storage, the peroxide value (milli equivalents/kg) of control sample increased at a rapid rate compared to the samples of ghee added with tomato. Though peroxide values of four samples were same initially and these trends continue initially for three days of storage. At fifth day of storage clear difference shown between the samples added with different parts of tomato and control sample. Throughout whole storage period sample of ghee added with skin was lowest peroxide value than other three samples. At eleventh day peroxide value of three sample added with different tomato part was very close to each other. At fifteenth day peroxide value of three samples added with different tomato were almost same but peroxide value of sample added with skin was lowest (little bit) among all other two samples (pulp and paste).

Changes in peroxide value of all the samples of ghee during storage were well collaborated with changes in flavor score of the corresponding samples of ghee. The control sample having highest peroxide value and corresponding lowest flavor score compare to samples of ghee added with different parts of tomato. The sample of ghee added with skin had lowest peroxide value correspondingly highest flavor score after the fifteenth day of storage.

The skin and the pericarp of the tomato fruit are particularly rich in lycopene and other carotenoids (Al-Wandawi *et al*., 1985). Hart and Scott, (1995) reported that among skin, pulp and seed fraction of tomato, tomato skin contain highest percentage of lycopene and skin of contain one third of total lycopene in tomato. According to Al-Wandawi *et al*. ,(1985) tomato skin contains 12 mg of lycopene per 100 g (wet basis), whereas whole mature tomato contains only 3.4 mg lycopene per 100 g (wet basis). The concentration of lycopene in tomato skin is about three times higher than in whole mature tomatoes (Hart and Scott, 1995). D'Souza *et al*., (1992) also found that the skin and the pericarp of tomato fruits were rich in lycopene. Sharma and Le Maguer, (1996) found that skins were a rich source of lycopene, as they contained about five times more lycopene (53.9 mg/ 100 g) than the whole tomato pulp (11 mg/ 100 g). These tomato

skins might be used as other food additives as not only lycopene but also it are a rich source of other carotenoids that might provide antioxidative properties in our diet (Toor and Savage , 1995). So from above discussion and from results we could say that skin of tomato is a great source of lycopene and so it might that peroxide value of skin added sample were less as compared to other ghee samples added with different parts of tomato.

Also skin contains less moisture as compare paste and pulp (Hart and Scott, 1995). Among the different tomato fractions analyzed, skin showed the highest level of antioxidants(mainly lycopene) and less moisture therefore it might possible that free radical scavenging activity is highest in skin than other tomato fractions. On the other side tomato pulp contain more free moisture than tomato skin (Chandra and Ramalingam 2011), these free moisture could hinder the anti-oxidant activity of lycopene. So the result (flavor score and peroxide value) of pulp and paste (skin+ pulp) added ghee sample were worst than skin added sample.

Using of tomato skin as natural antioxidant is very economical in commercial point of view as during commercial processing of tomato, produces a large amount of waste (pomace) at various stages. The wet pomace contains 33percentage seed, 27percentage skin and 40percentage pulp while the dried pomace contain 44percentage seed and 56percentage skin (Sogi and Bawa, 1998).Tomato pomace consists of skin that could be utilized for extracting lycopene (Kaur *et al.,* 2008).So tomato skin which has been wasted during processing must be a effective antioxidant from health point of view and economical point of view.

So considering the results of peroxide value, flavor score the result supported on the line of discussion that higher lycopene content in skin, so peroxide value was lowest in skin added sample.

Changing in flavor score of ghee and changes in peroxide value of ghee during storage had conclusively suggested that globe tomato skin was best to retain the flavor ghee by inhibiting oxidative deteoration .Therefore among three parts of tomato used in this study tomato skin was selected for further study.

4.3 SELECTION OF STAGE FOR ADDITION OF TOMATO SKIN IN GHEE

From examination of manufacturing process for ghee it can be envisaged that there are two possible ways to add tomato skin into ghee, as listed below.

(1) Before heat clarification of butter in to ghee
(2) After heat clarification of butter in to ghee

During addition of tomato at any of the above mentioned stage some advantages and limitations appeared to be associated, from the stage of heating the fat at elevated temperature (115 ºC for no hold) used for the clarification. Temperature of clarification during conversion of butter into ghee significantly affected the loss of antioxidants (Lal and Narayanan, 1980). On the other hand it may happen that the elevated temperature uses the clarification of butter fat in to ghee may adversely affect stability of the antioxidant components (lycopene) present in the tomato. Moreover, the addition of tomato before heat clarification of butter fat may also lead to loss of the active component (lycopene) in the ghee residues. Similarly, in addition of tomato after heat clarification of butter in to ghee may also have possibilities opposite effects as this addition of tomato after the clarification may not give appropriate dissolution of active components (lycopene) of tomato in to fat phase. However, this stage of addition may give better stability of the active components especially lycopene which is the main carotenoid in tomato skin.

Therefore, in the present investigation work was carried out to select stage for addition of tomato skin to enhance the shelf life of ghee as third phase of the study. To find out the effect of stage for tomato skin addition in ghee on oxidative stability of the ghee, tomato skin was added at two different stages (after clarification or before clarification) of the ghee preparation. In each set of experiments tomato skin was added at the rate of 0.4 per cent (expected yield of ghee from butter). In one set of experiment, tomato skin added before heat clarification of butter fat in to ghee. The sample of butter was taken in to clarification pan and the tomato skin was added when butter got melted and made ghee described in 3.3. After clarification of butter fat in to ghee, the prepared sample of ghee was filtered through muslin cloth. In second set of the experiment, tomato skin @ 0.4% (expected yield of ghee from butter) was added after filtration(105^{o}C) of heat clarified butter fat in to ghee (115^{o}C), the sample was thoroughly mixed with the help of dry glass rod for uniform mixing of the tomato skin, followed by refiltration(65^{o}C) through four folded muslin cloth. The samples of ghee

without addition of tomato skin were also prepared to serve as control samples described at section 3.3. All the samples were stored at $80^{o}C \pm 2^{o}C$. Total six replications were conducted.

4.3.1 Effect of Stage of Tomato Skin Addition on Flavor Score during Storage

The samples of ghee were subjected to sensory evaluation in 9 HEDONIC scale when fresh and at an interval of every two days. The average results obtained from six different replications for changes in flavor score of the ghee samples are given in Table 4.5 and graphically represented in Figure 4.5.

Statistical data revealed that addition of tomato skin on irrespectively on two different stages and storage period have significant ($P< 0.05$) effect on changes in flavor score of ghee during storage. The interaction effect of period of storage and addition of tomato skin on two different stages was also significant.

Table 4.5: Effect of stage of tomato skin addition on flavor score during storage

Storage period (days)	**Different stage of tomato added in ghee**		
	Control (None)	**After**	**Before**
1	8.40	8.60	8.60
3	8.10	8.10	8.30
5	5.40	7.60	7.10
7	5.10	7.20	6.90
9	4.90	7.00	5.80
11	4.30	5.60	5.20
13	3.50	5.10	4.30
15	2.40	4.20	3.90
ANOVA TABLE			
Source of Variation	**Storage period**	**Treatment (Tomato variety)**	**Storage period × Treatment**
S. Em	0.089	0.055	0.155
C. D	0.251	0.153	0.434
Test	*	*	*
C V percentage	6.28		

(*=significant,* *=highly significant, ns=non significant)

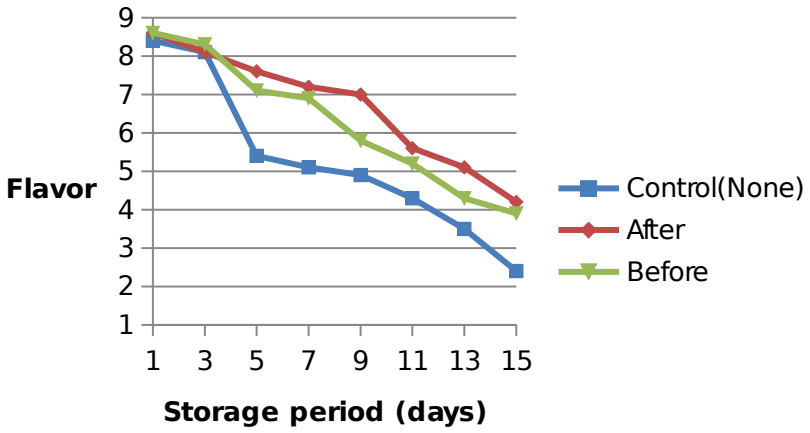


Fig 4.5: Effect of stage of tomato skin addition on flavor score of ghee during storage

During first three days of study flavor value of control sample was non-significantly differed (lower) from the samples of ghee added with tomato skin irrespectively two different stage of addition of tomato skin (before or after), but for rest of the study periods flavor score was significantly lower than sample of ghee added with tomato skin irrespectively two stage of addition. On first day when ghee samples were fresh flavor score of the ghee samples added with tomato skin irrespectively on two different stages of addition(after or before) were same. After that throughout whole storage flavor score of ghee sample added with tomato skin after clarification was significantly higher than that of the sample added with tomato skin before heat clarification (except third day of storage).

For control sample flavor score decreased significantly from previous day (comparing CD value) throughout whole study. For both tomato added samples of ghee before and after heat clarification flavor score decreased significantly as days passed except storage between fifth to seventh days for skin added before heat clarification sample and seventh to ninth days for after heat clarification sample ,in these time periods flavor score non significantly decreased from previous day's flavor score. From this study also found interaction effect was significant as day passed flavor score decreased. For addition of tomato skin in ghee at two different stages the effect of flavor score AFTER> BEFORE (on decreasing order).

The graphical results indicated that the initial flavor score of the control sample of ghee was slightly lower than that of the samples of ghee added with tomato skin at two different stages

(after or before clarification). During the storage the flavor score of control sample declined at a faster rate and reached below 6 on fifth day of the storage. Flavor scores of the tomato skin (irrespective two stages of addition) added samples of ghee remained higher compare to that of the control sample throughout whole storage periods. The flavor score of ghee samples decreased at almost similar rate for both of the cases whether tomato skin was added before or after clarification. The score of ghee added with tomato skin before heat clarification went below 6 at the ninth day of storage where as ghee sample added with tomato skin after clarification went below 6 at the eleventh day of storage. Flavor scores of tomato added ghee sample (before and after clarification) were very close to each other for last four days of study. However flavor score of ghee sample added with tomato after heat clarification was higher throughout whole study periods and this treatment could retain the flavor score two days more than the sample added with tomato skin before heat clarification.

4.3.2 Effect of Stage of Addition of Tomato Skin on peroxide value of Ghee during storage

The samples of ghee were analyzed for peroxide value at an interval of every two days for period of 15 days. The average results obtained from three different replications for changes in peroxide value of the ghee samples are given in Table 4.6 and graphically represented in Figure 4.6

The statistical analysis of the data indicated that the period of storage, addition of tomatoes skin irrespectively on two stages and interaction between period of storage and addition of tomato skin on two different stages were significant ($P< 0.05$). Thus, statistical analysis of results revealed that addition of tomato skin on irrespectively on two different stages and storage period have significant effect on changes in flavor score of ghee during storage. The interaction effect of period of storage and addition of tomato skin on two different stages was also significant.

Table 4.6: Effect of stage of tomato skin addition on peroxide unit value of ghee during storage

Storage period (days)	Different stage of tomato added in ghee		
	Control (None)	After	Before
1	1.86	1.65	1.70
3	2.86	2.60	2.65
5	10.20	3.71	4.48

7	12.12	4.71	6.40
9	12.45	6.73	7.36
11	17.52	8.85	9.58
13	18.60	12.66	16.41
15	24.37	16.18	19.60

ANOVA TABLE

Source of Variation	**Storage period**	**Treatment (Tomato variety)**	**Storage period × Treatment**
S. Em	0.276	0.169	0.478
C. D	0.773	0.473	1.339
Test	*	*	*
C V percentage	13.71		

(*=significant,* *=highly significant, ns=non significant)

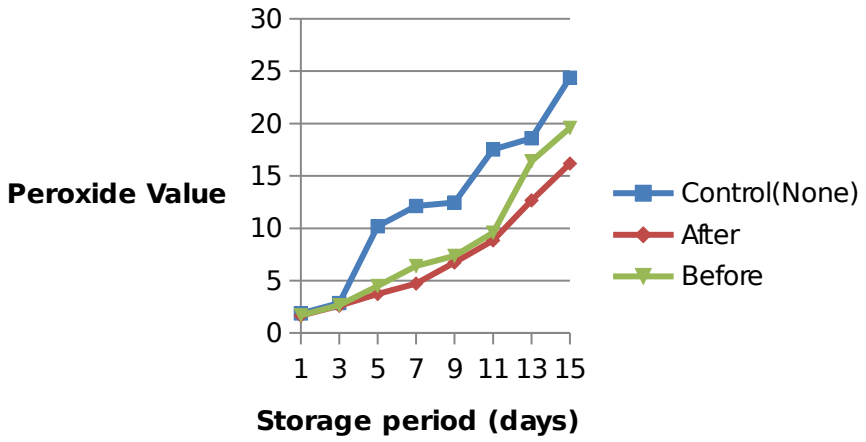


Fig 4.6: Effect of stage of tomato skin addition on peroxide value of ghee during storage

Except for first three days of storage through-out whole storage periods peroxide value of control sample was significantly higher than that of tomato skin added sample irrespectively two different stages of addition. For first three days of study peroxide value difference between two samples added with tomato skin irrespectively two different stage of addition was non-significant to each other and this trend followed up to seventh day of storage. On fifth day of storage peroxide value of two tomato skin added samples (on different stages) was at-per to each other.

On basis of study the effect of stage of addition of tomato skin on the peroxide value of ghee arranged in decreasing order AFTER CLARIFICATION >BEFORE CLARIFICATION .

Peroxide value of control sample significantly changed (increased) throughout whole study from previous day. For tomato added sample both before and after heat clarification, peroxide value increased significantly from previous day whenever day's passed. From these study (statistically) also found interaction effect is significant as day passed flavor score decrease.

Graphical data also represented that the peroxide values (milli equivalents per kilogram) of the control ghee sample and the samples added with tomato irrespective of stage of addition were more or less similar during first three days of storage. On further storage, peroxide values of control ghee sample increased with very faster rate. The peroxide value of tomato skin added ghee samples remained below the control sample throughout the whole storage period. The peroxide values of the ghee samples added with tomato skin before or after clarification remained almost very close throughout entire storage period. From thirteenth day peroxide value of ghee samples added with tomato before and after heat clarification became significantly differed from each other, from thirteenth day peroxide value of both the samples increased sharply. At fifteenth day sample of ghee added with tomato skin after clarification was lower as compare to sample of ghee added with tomato skin before clarification.

Changes in peroxide value of all the samples of ghee during storage were well collaborated with changes in flavor score of the corresponding samples of ghee. The control sample having highest peroxide value and corresponding lowest flavor score compare to samples of ghee added with tomato skin at two different stages. The sample of ghee added with tomato tomato skin after clarification had lowest peroxide value correspondingly highest flavor score almost entire storage periods.

Thus two different stages of addition for tomato were found to have very close effect on oxidative stability of ghee. Still peroxide value of the ghee sample added with tomato after clarification was little bit lower than the sample added with tomato skin before clarification. These results might be due to the thermodynamic behavior of "lycopene"- that is the main carotenoid present in tomato skin.

Physical factor like high temperature, exposure to light, oxygen incorporation during heat treatment are very much effective for the thermodynamic behavior (during heat treatment) of lycopene (Nguyen and Schwartz,1999).During heat treatment solubility of lycopene increase

in fat but research shows that level of trans lycopene decreased so oxygen scavenging activity decreased in lycopene(Shi *et al*.,2002). The level of all-trans-lycopene decreased by 78percentage after 120 minutes of heating at 100^{o}C (Lee and Chen, 2002). Shi *et al*.,(2002) found that dissolved extracted lycopene into canola oil and heated the sample at 25°C, 100°C, or 180°C, and observed that increasing the temperature from 100°C to 180°C decrease lycopene content 50percentage-76percentage. According to Sharma and Maguer,(1996) heating tomato pulp at 100°C for 120 minutes decreased lycopene content from 185.5 to 141.5 mg/100 g of total solids. Shi *et al*.,(2003) subjected tomato puree to intensive heat treatment—90°C, 110°C, 120°C, and 150°C for 1–6 hours and concluded the result that the higher the temperature and contact time , the faster the degradation of lycopene and he also reported that the temperature greater than 100°C and longer heating period (10-15 min) lead to a larger percentage of lycopene degradation. According to Kanasawud and Crouzet , (1990) heating of lycopene increase solubility in fat but decrease the oxygen scavenging ability of lycopene and more the contact time of lycopene with heat less the anti-oxidative capability as during heat treatment high temperature will break down lycopene molecules into small fractions like 2-methyl-2-hepten-6-on, neral, 6-methyl-3,5-heptadien-2-one and thus decreased oxygen scavenging ability of lycopene at greater extend . Cole and Kapur ,(1957) showed that more than 90percentage of lycopene was degraded upon heat treatment at 100°C for 3 h in the presence of oxygen, whereas only 5percentage was lost in the presence of CO2 after 3 h of treatment. Shi and Maguer, (2000) observed that longer contact time of lycopene with heat (more than 100^{o}C) in presence of oxygen more the oxidative degradation of lycopene. So addition of tomato before heat clarification of butter fat in to ghee may give better extraction of lycopene in to fat, mean while contact time of heat (more than 100^{o}C) increase so degradation of lycopene (both dynamic as well as oxidative) increase, on other hand high heat change the configuration of lycopene that decrease the oxygen scavenging ability of lycopene. So in this phase optimum anti-oxidative activity could not be available during addition of tomato before heat clarification from butter to ghee.

On the other hand the addition of tomato skin after the clarification may not give appropriate dissolution of lycopene in to fat phase and so availability of lycopene is less from tomato skin to ghee. However, this stage of addition may give better stability of lycopene as heat contact time is low so possibilities of degradation of lycopene and other active antioxidant component

of tomato skin would be lower as compare to the phase of addition of tomato skin before heat clarification. It might possible that optimum transformation of lycopene from skin to ghee was not possible during addition of tomato skin after heat clarification ,as literature shows that more the heat contact time more the transformation of lycopene, and research shows that availability of lycopene is more in heated(cooked tomato skin) tomato skin than fresh one(Shi *et al*.2002). So oxygen scavenging ability of lycopene might not highest level in this phase. But throughout whole study addition of tomato skin (as a source of lycopene) after heat clarification gave better result among all these three samples.

In practical application addition of tomato skin after heat clarification increase an additional step of clarification but heat contact time is less for tomato skin (lycopene) and activity of lycopene or other active components of tomato skin might be more

No report still found about the effect of different stages of adding tomato skin in on oxidative deteoration of ghee during accelerated storage. But Soni, (2011) reported that addition of turmeric in ghee after heat clarification gave better oxidative stability of ghee during storage than before addition of turmeric in ghee.

Changing in flavor score of ghee and changes in peroxide value of ghee during storage had conclusively suggested that addition of tomato skin after heat clarification of butter into ghee was best to retain the flavor ghee by inhibiting oxidative deteoration .Therefore among two different stages for addition of tomato skin into ghee used in this study after addition was selected for further study.

4.4 SELECTION OF RATE FOR ADDITION OF TOMATO SKIN IN GHEE

All carotenoids has both anti oxidant as well as pro-oxident activity at a particular rate , a carotenoids which is act as antioxidant at a particular rate and particular environment act as a pro-oxident at higher rate and other environment except Asthaxanthin has no pro-oxidant property . Antioxidant property of carotenoids depends on its structure and concentration as at higher concentration it acts as pro-oxidant. Though carotenoids are a great source of natural antioxidant but its anti-oxidative capacity depends on concentration and other environmental factor. For best anti-oxidant activity proper concentration of carotenoids are very much needed as lower concentration it's proper anti-oxidant activity cannot be shown and on higher

concentration it acts like a pro-oxidant (Martin *et .al.*,2011). Tomato skin contains highest lycopene. So considering all this point we have to select the right rate of tomato skin as a source of lycopene without any pro-oxidant activity.

Therefore in the fourth phase of the study work was carried out to select the rate of tomato skin addition in ghee for extending its oxidative stability during accelerated storage. As discussed above it was decided to add tomato skin after clarification of butter fat in to ghee then aim was to select of rate of tomato skin for better oxidative stability in ghee.

For selection of the rate for tomato skin addition in ghee, the fresh ghee samples were prepared in the laboratory from the white butter following the process as described under section 3.3. Each sample of ghee was divided in to six parts and tomato skin was added to the ghee at the rate of 0.2, 0.4, 0.6, 0.8 and 1.0 per cent (gm/100gm of ghee, expected rate for yield of ghee) after filtration(105^{o}C) of heat clarified butter fat in to ghee, the samples were thoroughly mixed with the help of dry glass rod for uniform mixing of the tomato skin, followed by refiltration(65^{o}C) through four folded muslin cloth. The sample of ghee without addition was kept as control sample. Total three replications were conducted. The prepared samples of ghee were stored at 80°C ± 2^{o}C.

4.4.1 Effect of Rate of Tomato Skin Addition on Flavor Score of Ghee During Storage

The samples of ghee were subjected to flavor score analysis on 9 HEDONIC scale when fresh and at an interval of every two days for period of fifteen days. The average results obtained from three different replications for changes in peroxide value of the ghee samples are given in Table 4.7 and graphically represented in Figure 4.7.

Table 4.7: Effect of rate of tomato skin addition on flavor score of ghee during storage

Storage Periods (day)	Amount of tomato skin added (g/100 g ghee)					
	0.0	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
1	8.40	8.63	8.63	8.60	8.60	8.50
3	8.10	8.40	8.60	8.60	7.56	8.50
5	5.90	7.23	7.73	7.90	7.13	6.60
7	4.80	6.00	6.26	7.20	6.73	5.70
9	4.70	5.50	6.10	6.40	6.10	5.53
11	4.30	4.80	5.73	5.90	5.60	5.00
13	3.50	4.70	5.50	5.80	5.20	4.26

15	2.40	3.40	4.30	5.03	4.80	3.40

ANOVA TABLE

Sources of Variation	Storage period	Treatment (Tomato skin rate of addition)	Storage period × Treatment
S. Em	0.084	0.073	0.206
C. D	0.237	0.205	0.580
Test	*	*	*
C V percentage	5.74		

(*=significant,* *=highly significant, ns=non significant)

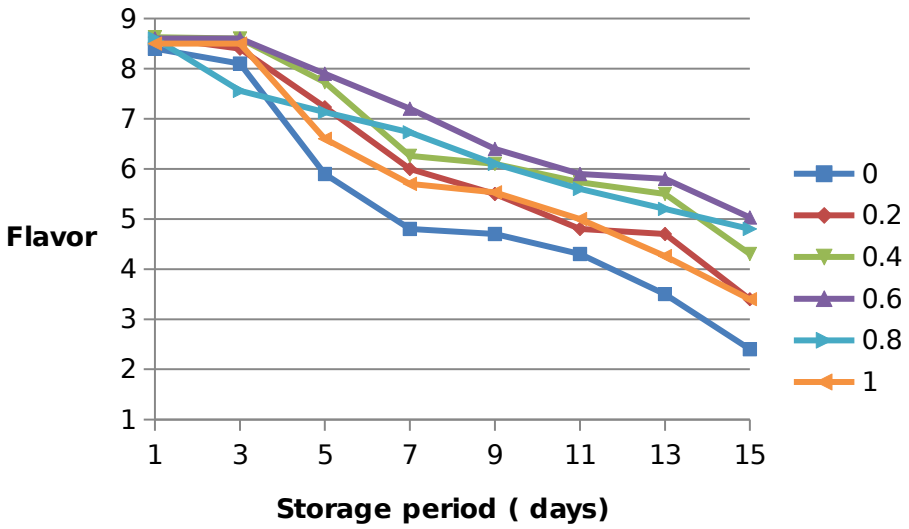


Fig 4.7: Effect of rate of tomato skin addition on flavor score of ghee during storage

The statistical analysis of the data indicated that the period of storage, different rate of tomato skin and interaction between period of storage and different rate of tomato skin were significant (P< 0.05). Thus, statistical analysis of results revealed that different rate of tomato skin and storage period have significant effect on changes in flavor score of ghee during storage.

On first day flavor score of control sample was at- per with flavor score of sample added with 0.2 percentage and 0.4percentage tomato skin added sample but no significant difference found with flavor score of ghee samples added with others different concentration of tomato skin. On third day of storage flavor score of ghee sample added with 0.8percentage tomato

skin had significantly lower flavor score than control sample but control sample had significantly lower flavor score than other tomato skin added ghee samples added with different rate of tomato skin (except 0.8percentage).Rest of the study period flavor score of control sample had significantly lower flavor score than that of the sample added with different concentration of tomato skin.

Throughout whole study flavor score of control sample decreased significantly from previous day except between seventh to ninth day of storage and this same trend followed for the ghee sample added with 0.8percentage tomato skin . Throughout whole study flavor score of 0.2percentage skin added ghee sample decreased significantly from previous day except between eleventh to thirteenth day of storage. Samples of ghee added with 0.4percentage , 0.6percentage and 1.0percentage tomato skin and not change flavor score for first three days of study. From fifth day flavor score of 0.4percentage tomato skin added sample decreased significantly rest of the study periods except storage between seventh to ninth days . From fifth day flavor score of 0.6percentage and 1percentage tomato skin added sample decreased significantly rest of the study periods except storage between eleventh to thirteenth days of storage for 0.6percentage added sample and between seventh to ninth days for 1.0percentage skin added samples.

Graphical data also indicated that initial flavor score of all six samples were almost at par and during first three days of storage flavor score of six samples were almost same. Flavor score of control sample decreased drastically and came unacceptable during fifth day of studies. Flavor score of ghee sample added with 1.0 percentage and 0.2percentage tomato skin came below 6 on seventh and ninth days of study respectively. Flavor score of samples added with tomato skin @ 0.4percentage, 0.6percentage and 0.8percentage (respectively) were almost at par for whole storage study and flavor score of these three samples came below 6 during eleventh of the days of storage. During last four days of study flavor score of all sample decreased sharply except sample of ghee added with 0.6percentage tomato skin. So according to flavor score we can choose 0.6percentage concentration of tomato skin was superior by observing whole fifteen days of storage.

4.4.1: Effect of Rate of Tomato Skin Addition on peroxide value of ghee during storage

The samples of ghee were also analyzed for peroxide value at an interval of every two days for period of fifteen days. The average results obtained from three different replications for changes in peroxide value of the ghee samples are given in Table 4.8 and graphically represented in Figure 4.8.

Table 4.8: Effect of rate of tomato skin addition on peroxide value of ghee during storage

Storage Periods (day)	**Amount of tomato skin added (g/100 g ghee)**					
	0.0	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
1	1.56	1.33	1.26	1.20	1.63	1.66
3	2.79	2.86	2.36	2.20	3.12	2.92
5	6.93	3.76	3.10	2.96	7.60	5.93
7	7.90	6.26	5.20	5.20	7.90	8.56
9	8.06	7.90	6.43	6.46	8.40	9.06
11	11.90	8.93	8.93	8.66	12.90	14.23
13	17.86	11.66	11.66	11.66	18.20	19.43
15	24.67	17.06	16.40	13.40	24.67	26.64

ANOVA TABLE

Sources of Variation	**Storage period**	**Treatment (Tomato skin rate of addition)**	**Storage period × Treatment**
S. Em	0.263	0.228	0.644
C. D	0.740	0.641	1.813
Test	*	*	*
C V percentage	12.70		

(*=significant,* *=highly significant, ns=non significant)

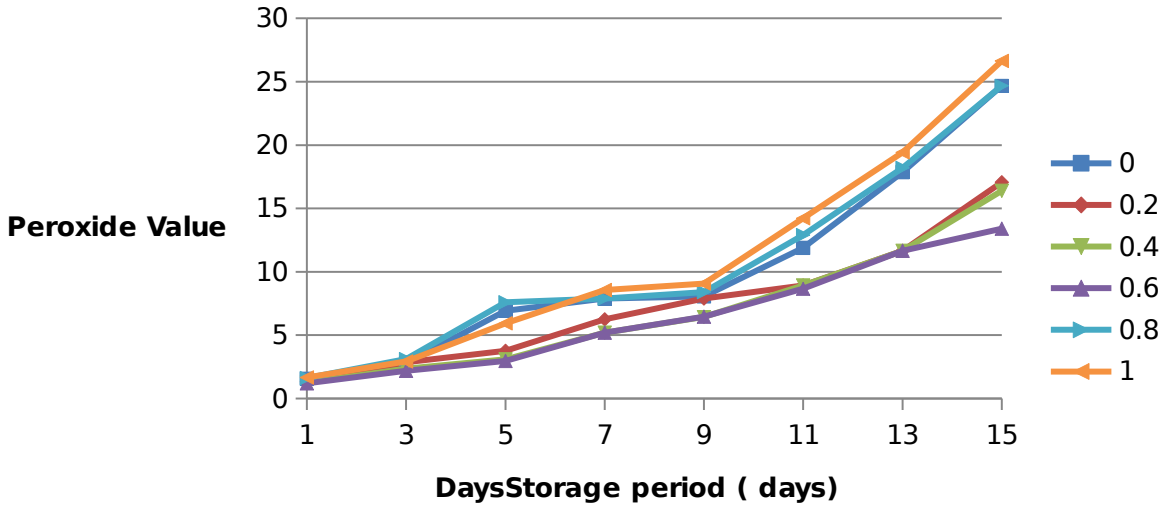


Fig 4.8: Effect of rate of tomato skin addition on peroxide value of ghee during storage

The statistical analysis of the data indicated that the period of storage, different rate of tomato skin and interaction between period of storage and different rate of tomato skin were significant ($P< 0.05$). Thus, statistical analysis of results revealed that different rate of tomato skin and storage period have significant effect on changes in flavor score of ghee during storage.

For control sample peroxide value significantly increased throughout whole storage periods except storage between seventh to ninth day. For ghee samples added with tomato skin @ 0.2percentage. @ 0.4percentage ,@ 0.6percentage and @ 1percentage respectively, peroxide value significantly increased throughout whole storage periods. For 0.8 percentage skin added samples peroxide value increased significantly except between seventh to ninth days of storage.

Graphical data showed that initial peroxide value of all six samples were same during first three days of studies. After third day peroxide value of control sample and sample added with 1percentage tomato skin increased drastically through whole storage period and after fifteenth day peroxide value of sample added with 1.0 percentage tomato skin was higher than that of control sample. Even from fifth day peroxide value of ghee sample added with 0.8percentage tomato skin increased sharply and peroxide value of this sample was same with control sample at the end of fifteenth days of storage. Peroxide value of samples of ghee added with tomato skin @ 0.2percentage, 0.4percentage and 0.6percentage was same up to the thirteenth day of whole study. At end of fifteenth day sample of ghee added with 0.6percentage tomato skin had lowest peroxide value. Careful examination of data for changes in peroxide value of ghee sample during storage clearly rebuild that addition of skin of tomato skin @ 0.2- 0.6 percentage exhibited antioxidant property in ghee , as peroxide value of theses samples remained below the peroxide value of control sample. On other hand addition of tomato skin skin @ rate of 0.8 and 1.0 percentage had shown pro-oxidant effect , as peroxide values of these ghee samples remained higher than that of the control ghee during later stage of storage(during (seventh and ninth day)

So from this study we selected 0.6percentage tomato skin for further phase of study.

The concentration of tomato skin added ghee samples can be arranged according to peroxide value after 15 days of studies-

1percentage> 0.8percentage=control>0.2percentage>0.4percentage>0.6percentage (concentration according to decreasing order effect peroxide value of ghee during ghee).

Changes in peroxide value of all the samples of ghee during storage were well collaborated with changes in flavor score of the corresponding samples of ghee. As day increased for storage peroxide value increase but flavor score decrease on other hand. For control sample highest flavor score lowest peroxide value but for tomato skin added sample @ 0.6% lowest peroxide value and highest flavor score.

No data are reported in literature about pro oxidant activity of tomato skin (as a source of lycopene) in fat rich food product when added at high concentration. The pro-oxidant potency of these compounds is determined by several factors, including oxygen tension, carotenoid concentration, and interactions with other antioxidants (Palazzo, 1998).According to Krinsky , (2000) that in vivo trial did not suggest clearly the pro-oxidant activity of lycopene. But some studies shows that pro oxidant activity of lycopene increase of 30microgram /gm of triglyceride (Jomova *et al., 2012).* According to Palozza, (1998) lycopene also allows for the formation of peroxyl radicals capable of acting as pro-oxidants and pro-oxidant activity of lycopene depends on its concentration as well as physical environment where it is acting.

Similar observation have been reported about pro-oxidant effect of tocopherol when used at high concentration; above around 1000 ppm α-tocopherol acts as a pro-oxidant (Frankel , 1985).Available data currently shows that the antioxidant activity of these compounds(carotenoids) may shift into pro-oxidant activity, depending on the redox potential of the carotenoid molecules as well as on the biologic environment in which they act(Martin *et al.*,2011). From the above results from present study it could be concluded higher concentration of tomato skin means higher amount of lycopene in ghee and at end of 15 days of storage we found that 1.0percentage concentration(tomato skin) of sample ghee had highest peroxide value among all the samples even from control sample and other side 0.8percentage(tomato skin) sample had same peroxide value with control sample , these data clearly supported that at higher amount carotenoids acts as pro-oxidant(Martin *et .al.,2011)*,

thus the exact amount of lycopene in ghee that act as pro-oxidant has not been found in literature. On other side ghee sample added with 0.4percentage, and 0.6percentage tomato skin could retain flavor score up-to 9 days but at 15 days of storage the sample added with 0.6 percentage had higher flavor score than 0.4percentage (tomato skin) added sample, but per oxide value was lower in case of 0.6percentage (tomato skin) sample at the end of 15 days of storage.

This phase of study clearly suggested though tomato skin (the best source of lycopene) that can delay oxidative rancidity in ghee at proper concentration but at higher concentration it acts as a pro-oxidant. Pro-oxidant activity of lycopene on specific concentration is still under study. This phase clearly suggested higher concentration of tomato skin as a source of lycopene(highest carotenoids in tomato skin) acts as a pro-oxidant and at lower concentration proper antioxidant activity is not shown, so it is needed proper concentration of tomato skin (source of lycopene)that give best result.

Changing in flavor score of ghee and changes in peroxide value of ghee during storage had conclusively suggested that globe tomato skin variety skin addition @ 0.6 percentage was best to retain the flavor ghee by inhibiting oxidative deteoration .Therefore among five different concentration of tomato skin used in this study addition of tomato skin @ 0.6 percentage was selected for further study.

4.5. COMPARISON OF TOMATO SKIN, BHA AND SYNERGISTIC EFFECT OF TOMATO SKIN WITH BHA FOR ENHANCING SHELF LIFE OF GHEE

The synthetic antioxidants are mono or dihydric phenols and react with a per-oxyradical to give a phenoxy radical (ArO), stabilized by extensive delocalization of the odd electron over the aromatic system. Butylated hydroxyanisole (BHA) shows good solubility in fat and reasonable stability in fried and baked products. It is very effective with animal fats but less so with vegetable oils. In India at present BHA is permitted synthetic anti oxidant for use in ghee for under legal rules. The maximum limit for its addition in ghee is 0.02 percentage (FSSA , 2006). Therefore in present study work was carried out to evaluate for efficiency of tomato skin compare to that of the BHA.

Various antioxidant show synergistic effect in preventing oxidative deteoration of fats and oil. Since BHA is a permitted antioxidant for ghee, it was felt worthwhile to test combination of tomato skin and BHA for their synergistic effect in preventing oxidative deteoration of ghee during storage.

Therefore in fifth phase of study work was carried out to compare efficacy of tomato skin, BHA and combination of tomato skin + BHA (synergistic effect) as an anti oxidant in ghee. Ghee was prepared from butter following the method as described under section 3.3. The samples of ghee were divided in to four parts. In one part of the ghee the Globe (Ruby) tomato skin was added at the rate of 0.6 per cent (expected yield of ghee from butter) immediately after filtration (105^{o}C), sample was thoroughly mixed and refiltered (65^{o}C) with four folded muslin cloth. In second part of the ghee BHA was added at the rate of 0.02 per cent and thoroughly mixed to dissolve the BHA and refiltered through muslin cloth .In another part of ghee 0.02percentage BHA+ tomato skin @ 0.6percentage was added and filtered with muslin cloth. The sample of ghee without any addition kept as a control. The samples of ghee were stored at 80°C ± 2 °C. Four replications were taken for this phase of study.

4.5.1 Effect of Addition of Tomato skin, BHA, and BHA+ tomato skin on Flavor Score of Ghee During Storage

The samples of ghee were subjected to sensory evolution on 9 HEDONIC scale by different judges when these samples were fresh and at an interval of every two days for period of 15 days. The average results obtained from four different samples for changes in flavor score of the ghee samples are given in Table 4.9 and graphically represented in Figure 4.9.

Table 4.9: Effect of addition of tomato skin, BHA and BHA+ tomato skin on flavor score of ghee during storage

Storage Periods (days)	Particulars of addition			
	None(control)	Tomato skin	BHA	BHA+ tomato skin
1	8.20	8.60	8.80	8.90
3	8.10	8.60	8.37	8.90
5	6.40	7.90	8.10	8.60
7	5.10	6.30	7.95	7.95
9	4.60	6.10	6.57	7.60
11	4.10	4.17	5.87	6.95
13	3.50	3.50	5.50	6.40

15	2.40	3.30	4.80	5.60
ANOVA TABLE				
Sources of Variation	**Storage period**	**Treatment (Tomato skin rate of addition)**	**Storage period × Treatment**	
S. Em	0.093	0.066	0.186	
C. D	0.261	0.185	0.522	
Test	*	*	*	
C V percentage	5.76			

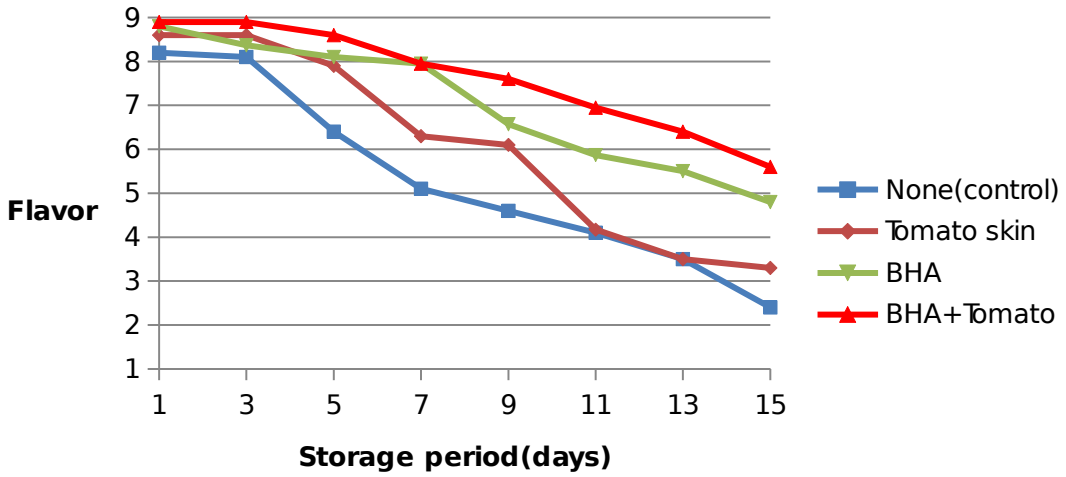


Fig 4.9: Effect of addition of tomato skin, BHA, and BHA+ tomato skin on flavor score of ghee during storage.

The statistical analysis of the data indicated that the period of storage, different particulars added in ghee and interaction between period of storage and particular of addition were significant ($P< 0.05$). Thus, statistical analysis of results revealed particular of addition and storage period have significant effect on changes in flavor score of ghee during storage. The interaction effect of period of storage and particular of addition was also significant.

Throughout whole storage periods flavor score of control sample significantly decreased from previous day except initially first three days of storage. For tomato skin added ghee sample change in flavor score from previous day was non-significant for first three days and storage between seventh to ninth day but rest of the study period flavor score decreased significantly

from previous day. For BHA added ghee sample flavor score significantly decreased throughout whole study except storage between fifth to seventh day where change in flavor score was at-per from previous day. For BHA+ tomato skin added ghee sample flavor score significantly decreased throughout whole study except first three days of storage where change in flavor score was non-significant from previous day.

For first nine days of study flavor score of control sample had significantly lower score from samples added with BHA, tomato skin and BHA+ tomato skin respectively. But during ninth to thirteenth days of study control sample had not any significant flavor score difference with tomato skin added ghee sample but it had significant flavor score difference(lower flavor score) with BHA added ghee sample and BHA+ tomato skin added ghee sample respectively. On fifteenth day of study flavor score of control sample was significantly lower than others three samples. Flavor score of tomato skin added ghee sample had significantly lower flavor score with sample of ghee added with BHA or BHA+ tomato skin throughout the whole study. Flavor score of BHA+ tomato skin added ghee sample had comparatively higher flavor score than that of sample added with BHA alone for almost whole study period except on seventh day of observation where flavor score for both the samples were same.

The graphical results indicated that the initial flavor score of the all four samples of ghee viz. control, BHA added ,tomato skin added and tomato skin + BHA added were almost similar up to third day of storage period. Flavor score of control sample declined at a faster rate and reached below 6 on seventh day of the storage. Flavor scores of the tomato skin added sample of ghee remained higher compare to that of the control sample throughout whole storage period. The samples added with tomato skin alone and BHA alone came below 6 respectively on eleventh day of storage. During whole storage study flavor score of ghee sample added with tomato skin and ghee sample added with BHA were almost similar (very near) ; but throughout whole study sample of ghee added with tomato skin +BHA was higher than all others ghee sample. Flavor score of ghee sample added with tomato skin +BHA came below 6 on fifteenth day of storage.

4.5.2: Effect of Addition of Tomato skin, BHA, and BHA+ tomato skin on Peroxide Value of ghee during storage

All four ghee samples were stored at 80^{o}C and analyzed for peroxide value when fresh and every two days of interval. Changes of peroxide value of ghee during storage is shown in Table-4.10 and Figure 4.10

Table 4.10: Effect of addition of tomato skin, BHA, and BHA+ tomato skin on peroxide value of ghee during storage

Storage periods (days)	**Particulars of Addition**			
	Control	**Tomato skin**	**BHA**	**BHA+ tomato skin**
1	1.57	1.65	1.27	1.05
3	1.94	1.70	1.32	1.15
5	6.85	4.25	1.57	1.62
7	8.57	5.20	2.25	2.10
9	9.00	6.60	2.42	2.57
11	11.90	8.80	7.22	2.97
13	18.30	11.66	7.40	5.20
15	25.10	16.95	9.50	5.55
ANOVA TABLE				
Sources of Variation	**Storage period**	**Treatment (Tomato skin rate of addition)**	**Storage period × Treatment**	
S. Em	0.184	0.130	0.367	
C. D	0.517	0.365	1.033	
Test	*	*	*	
C V percentage	12.04			

(*=significant,* *=highly significant, ns=non significant)

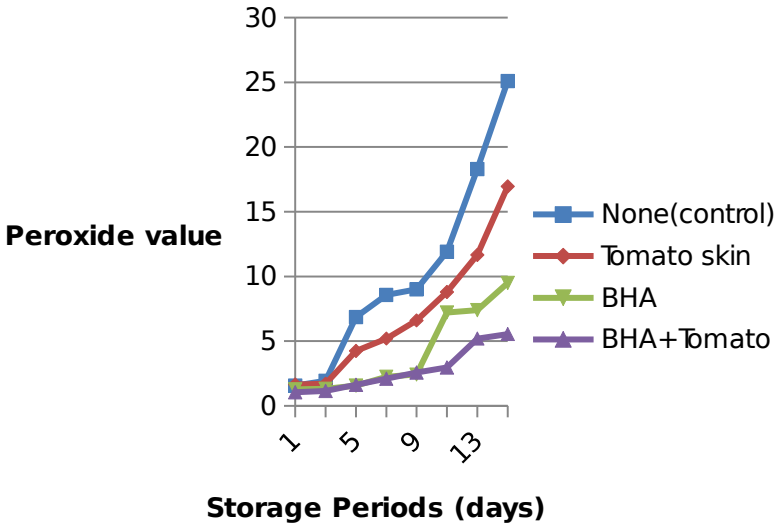


Fig 4.10: Effect of addition of tomato skin, BHA, and BHA+ tomato skin on peroxide value of ghee during storage

The statistical analysis of the data indicated that the period of storage, different particulars added in ghee and interaction between period of storage and particular of addition were significant ($P< 0.05$). Thus, statistical analysis of results revealed particular of addition and storage period have significant effect on changes in flavor score of ghee during storage. The interaction effect of period of storage and particular of addition was also significant.

For control sample peroxide increased significantly through-out whole of storage. For first three days of studies and storage between seventh to ninth days as well as storage between thirteenth to fifteenth days peroxide value of tomato skin added ghee sample increased non-significantly from previous day of observation but left of the study periods peroxide value increased significantly from previous day of observation. For BHA added ghee sample peroxide value increased non-significantly from previous day from third to seventh day of storage but peroxide value significantly increased from previous day. For BHA+ tomato skin added sample peroxide value increased non significantly from previous day for first eleven days of storage and between last two days of storage study but between eleventh to thirteenth day of storage peroxide value increased significantly from previous day.

When all four fresh ghee samples were analyzed for per oxide value it was found from data that control sample did not differ significantly from peroxide value of ghee sample added with

tomato skin but peroxide value of control sample was significantly higher than the peroxide value of both BHA added sample or BHA+ tomato skin added sample. These trends follow up-to 3days of studies. From third day of storage to throughout whole study periods a significantly higher peroxide value shown between control sample and sample of ghee added with tomato skin or sample of ghee added with BHA or sample of ghee added with BHA+ tomato skin. On other side peroxide value of ghee samples added with BHA or BHA+ tomato skin had no significant difference between each other through out first nine days of storage study. There after last six days of storage peroxide value of BHA added ghee samples had significantly higher peroxide value than BHA+ tomato skin added ghee sample. Except for first three days tomato skin added ghee sample had higher peroxide value than BHA added ghee sample, but from BHA + tomato skin added sample peroxide value was significantly higher in tomato skin added sample through-out entire study.

According to graphical representation during accelerated storage, the peroxide value (milli equivalents/kg) of control sample increased at a rapid rate compare to the samples of ghee added with tomato skin alone , BHA alone , and tomato skin +BHA combine. For first three days of storage peroxide value of four samples were almost very near. From fifth day of storage, peroxide value of control sample increased sharply and remained higher than that of the other three ghee samples throughout whole storage. For first nine days of storage peroxide value were very close for the sample of ghee added with BHA and BHA+ tomato skin respectively. Peroxide value of ghee sample added with tomato skin was lower than control sample but higher peroxide value than sample added with BHA +tomato skin or only BHA added sample. Addition of BHA+ tomato skin increase induction period of ghee almost 3 times more than control sample. Throughout all storage period peroxide value of ghee added with BHA+ tomato skin was lowest than other three sample. After ninth day peroxide value of BHA added sample increased sharply on other hand sample added with BHA+ tomato skin increased slowly from eleventh day of storage. At fifteenth day of storage peroxide value was lowest in ghee sample added with tomato skin +BHA combine. For performance wise arranging the results on oxidative stability of ghee found BHA + tomato skin > BHA> tomato skin (arranging on descending order).

Changes in peroxide value of all the samples of ghee during storage were well collaborated with changes in flavor score of the corresponding samples of ghee. The control sample having highest peroxide value and corresponding lowest flavor score compare to samples of ghee added with BHA or tomato skin or BHA+ tomato skin. The sample of ghee added with BHA+ tomato skin lowest peroxide value correspondingly highest flavor score for entire storage periods.

BHA is more effective antioxidant than tomato skin though literature shows that lycopene (which is the most vital antioxidant present in tomato skin) is more effective oxygen scavenger than BHT (Agarwal and Rao, 1998) but no record found on comparison between the antioxidative activity of BHA with lycopene (tomato skin as a source of lycopene).These variation of result might be due to the degradation of lycopene during storage. Lycopene content of tomato skin powder decreased in the presence of oxygen during storage (Kaufman *et al*., 1957). Analyses of lycopene content in stored tomato skin powder samples by Wong and Bohart, (1957) showed that air-packed samples retained the lowest levels of lycopene; all air-packed samples showed a progressive decrease in lycopene content during the storage period. Low storage temperature, low oxygen contents and avoidance of light exposure in storage will also limit the extent of the oxidation of lycopene, on other side high temperature of storage degradation of lycopene increase (Granado *et al*., 1992; Clinton *et al*., 1996; Porrini *et al.,* 1998).In literature there were no report found about the comparison of anti oxidative activity between BHA and tomato skin (as a source of lycopene)

On other side it is proved the synergetic effect of BHA+ tomato skin skin was better than BHA alone and as well as tomato skin for protecting ghee from oxidative rancidity. Literature suggested that the antioxidant activity of lycopene in multilamellar liposomes is superior to other lipophilic natural antioxidants e.g. α-tocopherol, α-carotene, β-cryptoxanthin, zeaxanthin, β-carotene and lutein.It is noteworthy that equimolar mixtures of carotenoids are more effective than any single compound alone. This strong synergistic effect is most pronounced with lycopene and lutein together (Stahl, 1998). So it is better to use combination of antioxidant than one type of antioxidant it might be natural or synthetic.

BHA shows marked synergism with Butylated hydroxytoluene (BHT) and Propyl gallate (PG) and can be used at a maximum level of 200 ppm. Among the mixtures of antioxidants studied,

only BHA + PG (Propyl gallet) and BHA+ BHT gave a synergistic effect. The addition of phospholipids either to an individual or mixture of antioxidants increased protective factor.

Among the antioxidants studied phospholipids gave synergistic effect only with BHA+ PG and BHT+PG mixture. Further, Kuchroo and Narayanan, (1973) found BHT to be more effective than BHA over 12 months of storage period of ghee but it is band in INDIA. American Meat Institute Foundation proposed an antioxidant mixture known as AMIF-72 that contains 20percentage BHA, six percentage Propyl-gallate, and four percentage of citric acid in propylene glycol shows highest antioxidant ability (synergetic effect). Lycopene is the strongest reducing agent and able to reduce the radical cations of lutein and zeaxanthin, but not β-carotene. Lycopene in combination with other antioxidants such as vitamins E and C, polyphenols and other carotenoids have wide potential for human health (Giovannucci.1995; Hulten *et al*, 2001). Among various carotenoids lutein has highest synergistic effect with lycopene (Hulten *et* al., 2001). The antioxidant activity of lycopene is highlighted by its singlet oxygen quenching property and its ability to trap peroxyl radicals (Kuhad *et al*., 2008). This singlet oxygen quenching ability of lycopene is twice as high as that of β-carotene and 10 times higher than that of α-tocopherol and Butylated hydroxyl toluene (BHT) (Agarwal and Rao,1998).But there no studies were conducted to compare between lycopene and BHA to give better oxidative stability in ghee. Also no literature found about the synergistic effect of lycopene with BHA. Chandra and Ramalingam, (2011) reported that tomato skin content highest amount of lycopene than others part of tomato skin.

Shahidi and Wanasundara, (2002) reported that a fat is considered to be rancid at a peroxide value of 15. But on fifteenth day of storage it was found peroxide value of tomato skin added ghee sample and control sample crossed 15 but peroxide values of BHA added as well as BHA+ tomato skin added ghee samples were lower than 15 . So we had to measure of peroxide value for BHA added sample and BHA+ tomato skin added sample till peroxide value of those two samples went above 15.

Table 4.11: Comparison of BHA and BHA+ tomato skin added sample on peroxide value during extended storage period

Storage Periods In Days	Sample Of Ghee	
	BHA	BHA+ tomato

		skin
15	11.20	5.55
17	13.60	6.90
19	13.90	8.30
21	14.20	8.60
23	14.80	9.70
25	16.60	8.40
27	17.20	8.90
29	18.60	11.60
30	19.30	14.80
31	21.40	14.80
33	23.90	16.60

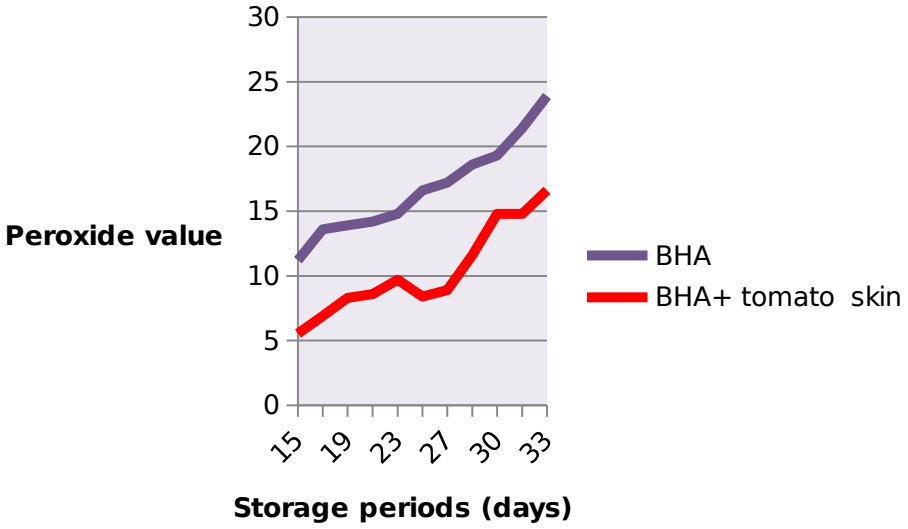


Figure 4.11: Effect of BHA and BHA+ tomato skin on peroxide value of ghee during further storage

Table 4.11 and Graph 4.11 clearly shows that tomato skin +BHA added sample was more effective on peroxide value than BHA added ghee sample during further storage. Peroxide value of BHA added sample went more than 15 on twenty fifth day of storage study on other hand peroxide value of ghee sample added with tomato skin +BHA went above 15 on thirty three days of study. Hence we could conclude by table 4.12 that peroxide value of all four samples went above 15 on following days of studies.

Table 4.12: Days of storage when peroxide value of each sample added with different particulars went above15

Sample of Ghee	Storage period to reach Peroxide Value > 15 (days)
Control sample	13
Tomato skin added sample	15
BHA added sample	25
BHA+ tomato skin added sample	33

So it is proved in present study that synergetic effect of tomato skin + BHA is more effective than BHA on oxidative rancidity of ghee. So it was clear that addition of BHA with tomato skin increased the efficacy of the BHA on oxidative stability of ghee during storage. Addition of BHA increased the protection factor of ghee Figure 4.13 and Table 4.13gave the comparison of different particulars addition on protection factor of ghee as induction period was 5 for this test-

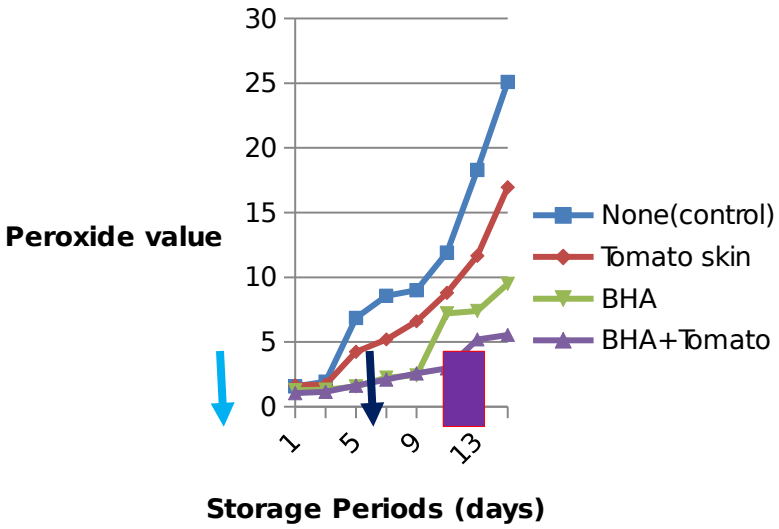


Figure 4.12: Addition of different particulars in ghee on change in induction period of ghee samples during storage.

Table 4.13: The comparison of addition of different particulars in ghee on of protection factor of ghee during storage

Ghee samples	Induction period(days)	Protection factor (PF)
Control	5	-
Tomato skin	7	1.4
BHA	10	2.0

Tomato skin + BHA	14	2.8

Time taken to attain a peroxide value of 5 was taken as an induction period. The protection factor of the tomato skin, BHA and BHA + tomato skin added samples of ghee was express as the ratio between induction periods of the ghee samples added with antioxidant agents to that of the control ghee sample.

Results revealed that addition of tomato skin delayed the peroxide formation, thereby increasing the induction period of ghee. The induction period of ghee containing tomato skin (7 days) was longer than the induction period of control ghee samples (5 days) . The combination of tomato skin with BHA prolong the induction period (14). Thus the combination of tomato skin with BHA gave better performance with maximum anti-oxidant effect (PF 2.8) compare to tomato skin (PF 1.4) or BHA (PF 2) better performance of tomato skin + BHA may be attributed to their synergistic effect as an antioxidant.

Data clearly showed that addition of tomato skin + BHA in ghee increased the protection factor of ghee two times more than tomato skin added sample and almost one and half time more than BHA added sample. Correspondingly addition of tomato skin + BHA increased the induction time of ghee almost three times more than the control sample.

Changing in flavor score of ghee and changes in peroxide value of ghee during storage had conclusively suggested that tomato skin + BHA was best to retain the flavor ghee by inhibiting oxidative deteoration .Therefore among three different particulars used in this study BHA + tomato skin study gave best result.

4.6: ANALYSIS OF FRESH GHEE SAMPLES FOR QUALITY STANDARDS AND SENSORY ATTRIBUTES

After getting confirmation that tomato skin would be a natural antioxidant that could inhibit oxidative rancidity in ghee and tomato skin + BHA would be more potent antioxidant than BHA alone. Now we had to check whether adding of tomato skin or adding of tomato skin+ BHA could meet various standards for ghee prescribed by PFA or AGMARK. So four samples of ghee were made from white butter described in 3.3 and divided into four parts. Then one by one part tomato skin @ 0.6percentage, tomato skin skin@0.6percentage + BHA @

0.02percentage and in third part BHA@ 0.02percentage was added respectively but one part remain left without addition of anything that was the control sample.

All the fresh samples of ghee were refiltered and went for various chemical and as well as sensory parameters evaluation.

The fresh samples of ghee were analyzed for various quality standards *viz*. moisture content, B.R. reading at 40 °C, RM value, FFA content and Baudouin test and their results presented in Table 4.13. The samples were also analyzed for peroxide value when fresh and also during the daily storage.

Table: 4.14. Analysis of fresh ghee samples for quality standards

Parameters	Particulars of addition			
	None (control)	BHA	Tomato skin	Tomato skin + BHA
Moisture	0.17	0.16	0.16	0.15
B.R reading at 40^{o}c	41.9	40.6	40.8	40.2
R.M. value	33.2	33.4	33.0	33.4
Polenske value	1.1	1.3	1.1	1.0
FFA content (percentage)	0.20	0.20	0.16	0.19
Baudouin test	Negative	Negative	Negative	Negative

The moisture content of ghee samples varied on an average from 0.15 to 0.17 per cent among different treatments. Moisture content of ghee is reported to vary from 0.3 per cent maximum (Serunjogi *et al.,* 1998). As per PFA standards moisture content of ghee should not be more than 0.5 per cent (PFA, 2008). As per Agmark standards moisture content of ghee should not be more than 0.3 per cent (www.agmarknet.in). Thus, the level of moisture content obtained in present study was in general agreement with reported values and legal standards for all the samples.

The B.R. reading of ghee samples at 40 °C varied on an average from 40.2 to 41.9 among different treatments. B.R. reading of ghee is reported to vary from 39.2-43.1 (Achaya, 1948).

As per PFA standards B.R. reading of ghee should be 40.0 to 43.5 for areas other than cotton tract areas in Gujarat (PFA, 2008). As per Agmark standards B.R. reading of ghee should be 40.0 to 43.0 for areas other than cotton tract areas in Gujarat. Thus, B.R. reading obtained in present study was in general agreement with reported values and legal standards for all the samples.

The RM value of ghee samples varied on an average from 33.2 to 33.4 among different treatments. RM value of ghee is reported to vary from 25.7-39.1 (Achaya, 1948). As per PFA standards RM value of ghee should be minimum 24.0 for areas other than cotton tract areas in Gujarat (PFA, 2008). As per Agmark standards RM value of ghee should be minimum 28 for areas other than cotton tract areas in Gujarat. Thus, RM value obtained in present study was in general agreement with reported values and legal standards for all the samples.

The Polenske value of ghee samples varied on an average from 1.0 to 1.3 among different treatments. In Agmark it is reported 1 to 2. . Thus, Polenske value obtained in present study was in general agreement with reported values and legal standards for all the samples.

The FFA content of ghee samples varied on an average from 0.16 to 0.20 per cent among different treatments and control sample in present study. FFA content of ghee is reported to vary from 0.23 to 0.28 (Sharma, 1981). As per PFA standards FFA content of ghee should not be more than 3.0 per cent (PFA, 2008). According to Agmark free fatty acid contains (as oleic acid) should not be more than 1.4 for special grade ghee, 2.50 for general grade ghee and 3.0 for standard grade ghee. Thus, the level of FFA content obtained in present study was in general agreement with reported values and legal standards for all the samples.

According to PFA and Agmark standard for ghee Baudouin test should be negative. The Baudouin test for control ghee sample, BHA added ghee sample, tomato skin + BHA added ghee sample and alone tomato skin added ghee sample were found negative. Thus, the standard of Baudouin test in present study was in general agreement with reported values and legal standards for all the samples.

The above results indicated that all the prepared samples of ghee in this study viz. control, BHA added, tomato skin and tomato skin +BHA added fulfilled the legal requirements for all the quality standards prescribed under PFA. Similarly, the results also indicated that all the

prepared samples of ghee in this study viz. control, BHA added, tomato skin added and tomato skin +BHA added sample fulfilled the legal requirements for all the quality standards prescribed under Agmark. The addition of tomato skin from Globe (Ruby) variety at the rate of 0.6 per cent did not affect quality standard as prescribed under PFA and Agmark standard. So all the chemical parameters meet the legal quality standards then we subjected to sensory score of ghee on 9 point HEDONIC scale.

Table 4.15: Sensory analysis of fresh ghee samples on various parameters

Sensory standards	Particulars of addition			
	None (Control)	BHA	Tomato skin	Tomato skin + BHA
Flavor	8.0	8.5	8.2	8.5
Texture	8.0	8.0	8.0	8.0
Color	8.5	8.5	8.5	8.5
Ghee residue	8.0	8.0	8.0	8.0
Overall acceptability	8.0	8.0	8.0	8.0

Color of ghee depends on method of production. By the direct cream method the color of cow ghee is deep yellow color (Dey, 1991).Color score of these four sample ranges from 8-8.5 and hence it was clear that addition of tomato skin would not affect any objectionable color to ghee (Appendix 4 Plate 3). Though lycopene (main carotenoid present in skin) is a color carotenoid (Shi *et al*.,2002) but it would not create any problem in product at appropriate concentration.

Normally a well prepared ghee has pleasant cooked and rich flavor (Dey, 1991). Flavor score of four samples were 8 respectively. So it was clear that addition of tomato skin as a source of natural antioxidant @0.6percentage would not mask the typical ghee flavor.

Texture of ghee should be firm and non greasy and grain size should be uniform and large. Thus texture of ghee depends on fatty acid of milk, feed, season and cooling-heating temperature. Texture score of the entire four ghee sample were 8 respectively. So it was clear that addition of tomato skin would not create any textural problem in ghee.

Tomato are now eaten freely throughout the world it has several health benefit it can prevent cancer, ulcer, heart diseases etc. Shi *et al*., (2002) reported tomato skin content many

carotenoids and lycopene is the main carotenoids (90percentage-95percentage of total carotenoids) in tomato skin. Apart from lycopene tomato skin content others carotenoids like phytene, leuteine etc (Trombly and Porter, 1953). Structurally, lycopene is a highly unsaturated aliphatic hydrocarbon. Its chain contains 13 carbon-carbon double bonds, 11 of which are conjugated and arranged in a linear array. The two central methyl groups of lycopene are in a 1,6-positionrelative to each other. All other methyl groups are in the 1,5-position. For this typical structure lycopene has high oxygen scavenging activity. Lycopene is the most powerful antioxidant than other carotenoids. The system of conjugated double bonds allows lycopene molecules to efficiently quench the energy of notably deleterious forms of oxygen (singlet oxygen) and to scavenge a large spectrum of free radicals. Of all naturally occurring carotenoids, lycopene is the most efficient quenchers of singlet oxygen (Di Mascio *et al*., 1989; Conn *et al.,* 1991). Interactions between lycopene and oxygen radicals can be considered second-order rate reactions. Lycopene is less efficient, and electron transfer is observed in both directions (Conn *et al*., 1992). Another mechanism of anti oxidative activity is that lycopene can also play a role in scavenging hypochlorous acid (HOCl) (Pennathur *et al*., 2010).In vitro study it was found that lycopene is a great natural antioxidant, that can prevent oxidative degradation to biological molecule like fat and DNA(Palozza, 1998). But antioxidative activity of lycopene not so much tried in food system. Lycopene in tomato skin increase by different agriculture practice, and lycopene in tomato skin present mainly outer pericrap of tomato skin cell specially higher amount of lycopene found in tomato skin skin(Chandra and Ramalingam, 2011), and these skin mainly thrown during tomato skin processing. Considering all information we had found that using of tomato skin in ghee as a source of natural antioxidant not only beneficial for health point of view but also beneficial for economical point of view.

Gordon, (2001) reported that the peroxide value at which oxidation of oils can be detected as an off-flavor varies widely depending on the nature of the oil. Samples of olive oil may not be perceived as rancid till the Peroxide value reaches 20meq per kg whereas fish oil may develop off-flavors at Peroxide value < 1meq per kg. For soybean oil, a Peroxide value of 1.0 or less indicates freshness; 1 to 5 Peroxide value, low oxidation; 5 to 10 Peroxide value, moderate oxidation; >10 Peroxide value, high oxidation; and >20 Peroxide value, poor flavor. These

quality estimates are specific for soybean oil and higher or lower Peroxide values may be acceptable for other oils.

Shahidi and Wanasundara ,(2002) reported that a fat is considered to be rancid at a peroxide value of 15. Peroxide value is one of the most widely used chemical tests for the determination of fats and oils quality. PV has shown good correlation with organoleptic flavor scores. For soybean oil, a PV of 1.0 or less indicates freshness; 1 to 5 PV (Peroxide vale), low oxidation; 5 to 10 PV, moderate oxidation; >10 PV, high oxidation; and >20 PV, poor flavor. These quality estimates are specific for soybean oil, and higher or lower PVs may be acceptable for other oils. Still, a peroxide determination does not provide a full and unqualified evaluation of fats and oils flavor because of the transitory nature of peroxides and their breakdown to non peroxide materials. There was a linear relationship has been observed between peroxide values and flavor scores during the initial stages of lipid oxidation.

It can be concluded from this study that tomato skin among three different cultivars *viz.* globe, round and oblong tomato skin; globe cultivar of tomato skin gave best results on oxidative stability of ghee during storage. Among different parts of tomato skin *viz.* paste, pulp and skin the skin gave best result on oxidative stability of ghee during storage. Addition of tomato skin after heat clarification of butter into ghee was more effective than addition of tomato skin before heat clarification of butter into ghee on oxidative stability of ghee during storage. Among the different concentration of tomato skin added to ghee (0.2 to 1.0 %), the addition of at the rate of 0.6% gave best result on oxidative stability of ghee during storage higher than this concentration tomato skin had adverse effect on ghee during storage and lower than this concentration were found less effective .Analysis of ghee samples for added of tomato skin @ 0.6 % revealed that the resultant ghee samples fulfilled the standards prescribed for ghee under PFA,FSSAI and AGMARK and had no adverse effect on At last stage in comparison for antioxidant potential of tomato skin, BHA and combination of tomato skin + BHA, combination of tomato skin + BHA found most effective followed by BHA and then tomato skin. It can be concluded from present study that tomato skin derived from globe variety of tomato skin has a potential as an anti oxidant for ghee and it has highly promising synergistic antioxidant potential when combine with BHA.

So tomato skin that generally thrown during tomato processing , is a rich source of carotenoids specially lycopene is very effective natural antioxidant, that would be used as an alternative of synthetic antioxidant as it has not only antioxidant activity but also plethora of health benefits. So more researches are needed in this topic.

CHAPTER 5

SUMMARY AND CONCLUSION

Ghee is an important fat rich dairy product, known for its pleasing flavour. However, oxidative rancidity deteriorates the flavour of ghee. The most common approach to prevent the oxidative the rancidity is to employee antioxidant. Use of natural antioxidant is gaining popularity due to safety aspects of human health. Tomato is rich source of lycopene which is reported as to have antioxidant potential with plethora of health benefits .Therefore, the present study was undertaken to evaluate potential of tomato has an antioxidant to enhance shelf life of ghee. The findings of the study are summarized below.

The entire study was divided into five phases. In the first phase three different varieties/cultivars (globe, round and oblong) of tomatoes were evaluated for their antioxidant activity in ghee. In the second phase, different parts of tomatoes (skin, pulp and paste) were studied for their antioxidant activity in ghee. In the third phase, selection for stage of addition (before heat clarification of butter into ghee and after heat clarification of butter into ghee) of tomato was studied. In fourth phase rate of addition of the tomato in ghee was optimized. In fifth phase comparison of tomato skin, BHA and synergistic effect of tomato skin with BHA for enhancing shelf life of ghee was studied. The samples of ghee were analyzed for the standards prescribed by PFA, FSSAI and AGMARK.

5.1 SELECTION CULTIVAR OF TOMATO FOR ADDITION IN GHEE

Tomato from these three cultivars *viz*. globe (*Ruby*), oblong (*Shaktiman*), round (*Heemshikhar*) were evaluated for their antioxidant activity in ghee. In selection for cultivar of tomato, tomato paste obtained from each cultivar of tomato was added separately to the samples of butter at the rate of 0.4 per cent of the expected yield of ghee and butter was converted into ghee at heat clarification at 115^{o}C for no hold. All the samples of ghee were filtered and stored at elevated temperature (80°C ± 2°C) to accelerate the oxidation and analyzed for flavor score by sensory evolution and for peroxide value when fresh and also when fresh and at an interval of every two days.

The cultivars of tomato differed significantly for their effect on changes in flavor score and peroxide value of ghee during storage of ghee.

The initial flavor score of the control sample of ghee was slightly lower than that of the sample added with round type of tomato and globe type of tomato but at par with that of the oblong tomato. During the storage the flavor score of control sample declined at a greater rate and reached below 6 on seventh day of the storage. Flavor scores of the tomato added samples of ghee remained higher compare to that of the control sample thorough out whole study periods. The flavor score of ghee sample added with oblong and round went below 6 level on ninth day of storage, whereas, flavor score of ghee sample added with globe tomato went below 6 level on thirteen day of storage. Thus, among the three cultivar of tomato the globe variety gave better result which extended the flavor score to acceptable level by six days compare to control and four days as compare to round and oblong cultivars of tomato.

For first three days of storage peroxide value of all the samples control as well as tomato added samples were very close to each other. On fifth day of storage peroxide value of control sample were significantly higher than that of samples added with different cultivars of tomato. From ninth day onwards peroxide value of oblong cultivar tomato added ghee sample was highest among three different cultivar tomato added ghee samples. Through-out whole study period peroxide value of globe cultivar tomato added ghee sample remained lowest.

The changing in flavor score of ghee and changes in peroxide value of ghee during storage suggested that globe cultivar of tomato was best to extend the shelf life of ghee. Therefore, among the three cultivars evaluated in the present investigation, globe cultivar of tomato was selected for further study.

5.2 EVALUATE DIFFERENT PARTS OF TOMATO FOR ADDITION IN GHEE

Three different parts obtained from glob cultivar *viz*. skin, pulp and paste were evaluated for their antioxidant activity in ghee. In selection of part of tomato for addition in ghee sample of butter was divided in to four parts tomato skin, pulp and paste were added separately in each part of the butter at the rate of 0.4 per cent of the expected yield of ghee and one of the parts of butter was kept as such to serve as control. After clarification of butter fat in to ghee, the prepared samples of ghee were filtered through muslin cloth. All the samples of ghee were stored at elevated temperature (80^{o} C± 2°C) to accelerate the oxidation and analyzed for flavor score by

sensory evolution and for peroxide value when fresh and also at an interval every of two days. The different parts tomatoes differed significantly for their effect on changes in flavor score and peroxide value of ghee during storage of ghee.

Initial flavor score of control sample was little bit lower than sample added with different parts of tomato. During storage flavor score of control sample decreased very rapidly and came below 6 at seventh day of storage. Sample of ghee added with pulp and paste decreased to below 6 at ninth of the day of storage for both two cases flavor score were same. On eleventh day flavor score of ghee sample added with skin went below 6. At the end of fifteen days of study flavor score of skin added sample was higher among all the four samples.

From third days onwards peroxide value of control ghee sample was significantly higher than that of the samples with parts of tomato. From fifth day of storage period, peroxide value of skin added sample was lowest than that of the ghee samples added with tomato pulp or paste. For entire study period peroxide value of ghee sample added with paste as well as pulp was remain non significant to each other. On fifteenth day of storage peroxide value of ghee sample added with skin had lowest peroxide value than others samples.

The changing in flavor score of ghee and changes in peroxide value of ghee during storage suggested that globe tomato skin was best to extend the shelf life of ghee. Therefore, among the three parts of tomato tested in the present investigation, skin of tomato was selected for further study.

5.3 SELECTION OF STAGE FOR ADDITION OF TOMATO SKIN IN GHEE

Two different stages in preparation of ghee *viz*. before and after heat clarification of butter fat were evaluated for addition skin from glob cultivar of tomato for its antioxidant activity in ghee. In selection of stage of addition of tomato skin, the tomato skin was added at the rate of 0.4 per cent of the expected yield of ghee. In one case skin was added in melted butter before its heat clarification in to ghee and in second case skin was added after heat clarification of butter fat in to ghee (when ghee reached to 105^{o}C from 115^{o}C after clarification). The samples of ghee without addition of tomato skin were also prepared to serve as control. All the samples were stored at 80^{o}C. All the samples were

analyzed for flavor score by sensory evolution and for peroxide value when fresh and also at an interval every of two days.

The different stage of addition of tomato skin, the period of storage and interaction between period of storage and different stages for addition of tomato skin differed significantly for their effect on changes in flavor score and peroxide value of ghee during storage of ghee.

The flavor score of ghee added with tomato skin before heat clarification went below 6 on ninth day of storage, whereas, the flavor score of ghee sample added with tomato skin after clarification went below 6 on eleventh day of storage. Flavor score of ghee sample added with tomato skin after heat clarification was higher throughout whole study periods.

Except for first three days of storage through-out whole storage periods peroxide value of control sample was significantly higher than that of tomato skin added sample irrespectively two different stages of addition. From ninth day onwards ghee sample added with tomato skin after heat clarification had lower peroxide value than samples of ghee added with tomato skin before the heat clarification of butter fat.

The changing in flavor score of ghee and changes in peroxide value of ghee during storage suggested that addition of tomato skin after heat clarification of butter into ghee was best to extend the shelf life of ghee. Therefore, among two different stages evaluated in the present investigation for addition of tomato skin into ghee, the addition after heat clarification was selected for further study.

5.4 SELECTION OF RATE FOR ADDITION OF TOMATO SKIN IN GHEE

Different rates for addition of skin from glob cultivar of tomato were evaluated for their antioxidant activity in ghee. To optimize rate of tomato skin addition in ghee, ghee samples were prepared in the laboratory from white butter. Sample of ghee was divided in to six parts and tomato skin was added separately to the ghee at the rate of 0.2, 0.4, 0.6, 0.8 and 1.0 per cent, when temperature of ghee reached to 105°C. The samples were mixed thoroughly and filtred through four folded muslin cloth. The sample of ghee without addition was kept as control sample. The prepared samples of ghee were stored at 80°C ± 2^{o}C.All the samples analyzed for flavor score by sensory evolution and for peroxide value when fresh and also at an interval every of two days

The different concentration of tomato skin in ghee differed significantly for their effect on changes in flavor score and peroxide value of ghee during storage of ghee.

Flavor score of control sample decreased drastically went below 6 on fifth day of storage. Flavor score of ghee sample added with 0.2 and 1.0 per cent tomato skin came below 6 on ninth and seventh day of storage respectively. Flavor score of samples added with tomato skin at the rate of 0.4, 0.6 and 0.8 per cent was almost at par for entire storage period and their flavour score went below 6 on eleventh day of storage. At end of fifteenth day of study flavor score of ghee sample added with 0.6 per cent tomato skin had highest flavor score.

From third day of storage peroxide value of control sample and sample added with 1.0 per cent tomato increased drastically and became highest at the end of storage. On fifteenth day peroxide value of sample added with 1.0 per cent tomato skin was higher than that of control sample. Similarly, on fifth day peroxide value of ghee sample added with 0.8 per cent tomato skin increased sharply and was at par with the peroxide value of control sample at the end of fifteenth days of storage. On fifteenth day of storage sample of ghee added with 0.6 per cent tomato skin had lowest peroxide value.

The changing in flavor score of ghee and changes in peroxide value of ghee during storage suggested that amongst addition rates of the tomato skin tested in present investigation (0.2 to 1.0%), the addition rate of 0.6 per cent was found optimum to extend the shelf life of ghee. Therefore, addition of tomato skin at the rate of 0.6 per cent was selected for further study.

5.5. COMPARISON OF TOMATO SKIN, BHA AND COMBINATION OF TOMATO SKIN WITH BHA FOR ENHANCING SHELF LIFE OF GHEE

After standardizing the entire protocol for use of tomato to extent shelf life of ghee, work was carried out to compare efficacy of glob cultivar tomato skin, BHA and combination of tomato skin with BHA for extending the shelf life of ghee, by retarding oxidative deterioration. For the comparison ghee samples were prepared in the laboratory from white butter. Sample of ghee was divided in to four parts, after heat clarification of the butter fat. In one part tomato skin from glob cultivar was added at the rate of 0.6 per cent immediately after when temperature of ghee reached to 105°C, mixed thoroughly and filtered. In the second part BHA was added at the rate of 0.02 per cent by following the same method as

described for the addition of tomato skin. Similarly in part tomato skin at the rate of 0.6 per cent and BHA at the rate of 0.02 per cent were added. The sample of ghee without any addition kept as a control. The samples of ghee were stored at 80 ± 2 °C. All the samples were analyzed for flavor score by sensory evolution and for peroxide value when fresh and also at an interval every of two days

The different particulars added in ghee, the period of storage and interaction between period of storage and different concentration of tomato skin had significant effect on changes in flavor score and peroxide value of ghee during storage.

The initial flavor score of the all four samples of ghee were almost similar up to third day of storage period. Flavor score of control sample declined at a faster rate and reached below 6 on seventh day of the storage. Flavor scores of the tomato skin added sample of ghee remained higher compare to that of the control sample during the entire storage period. The flavour scores of ghee added with tomato skin alone and BHA alone went below 6 on eleventh day of storage and that of tomato skin with BHA went below 6 on fifteenth day of storage

For first three days of storage peroxide value of all the four samples were almost very close to each other. On fifth day of storage, peroxide value of control sample increased sharply and remained higher than that of the other three ghee samples during remaining part of the storage period. Almost entire storage period peroxide value of ghee sample added with tomato skin was lower than that of the control sample but higher than that of ghee sample added with BHA alone or tomato skin with BHA. Similarly, peroxide value of ghee added with tomato skin along with BHA remained lowest during almost entire storage period.

The addition of tomato skin, BHA and tomato skin with BHA in ghee extended induction period of oxidation in ghee samples by 2, 5 and 9 days respectively; when compared with induction period of the control ghee sample. Thus, addition of tomato skin, BHA and tomato skin with BHA in ghee offered a protection factor of 1.4, 2.0 and 2.8 respectively against oxidative deterioration of the ghee.

The changing in flavor score of ghee and changes in peroxide value of ghee during storage suggested that the addition of tomato skin in ghee extend shelf life of ghee against oxidative deterioration during storage, but its potency was lower than that of the BHA. However, the addition of tomato skin along with BHA in ghee extended the

shelf life of ghee against oxidative deterioration during storage and its potency was much higher than that of the BHA alone. Thus, use of tomato skin along with BHA gave highly promising results for extending the shelf life of ghee against oxidative deterioration during storage.

5.6: ANALYSIS OF GHEE SAMPLES FOR QUALITY STANDARDS

After getting confirmation that tomato skin form glob cultivar would be a promising natural antioxidant that could inhibit oxidative rancidity in ghee and the tomato skin along with BHA would be very potent antioxidant compared to BHA alone, the samples of ghee were tested for fulfillment of requirements prescribed under FSSAI and AGMARK standards. The moisture content of the ghee samples varied on an average from 0.15 to 0.17 per cent. The B.R. reading of ghee samples at 40°C varied from 40.2 to 41.9. The RM value of ghee samples varied from 33.2 to 33.4. The Polenske value of ghee samples varied 1.0 to 1.3. The FFA content of ghee samples varied on an average from 0.16 to 0.20 per cent. Baudouin test for all the sample of ghee was negative. Thus, all the samples of ghee fulfilled requirements for quality standards prescribed under FSSAI and AGMARK. The addition of tomato skin from globe variety at the rate of 0.6 per cent did not affect quality standard as prescribed under FSSAI and AGMARK.

It can be summarized from this study that tomato among three different cultivars *viz.* globe, round and oblong; the globe cultivar of tomato was best to extend shelf life of ghee. Among different parts of tomato *viz.* paste, pulp and skin, the skin was best to extend shelf life of ghee. For addition of the tomato skin during preparation of ghee, the addition of skin after heat clarification of butter fat better than the addition before the heat clarification. Among the different rates for addition of tomato skin in ghee (0.2 to 1.0%), the addition of at the rate of 0.6 per cent was found optimum to extend the shelf life of ghee. Analysis of ghee samples revealed that the addition of tomato skin at the rate of 0.6 per cent did not affect parameters of quality prescribed for ghee under PFA, FSSAI and AGMARK. The comparison for antioxidant potential of tomato skin, BHA and tomato skin along with BHA in extending the shelf life of ghee revealed that the use of tomato skin along with BHA was most effective. Therefore, the present study entailed to conclude that tomato skin which is generally, thrown as waste during tomato processing can be used as an effective antioxidant for ghee.

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APPENDIX 1: 9 HEDONIC SCALE SCORE CARD

SCORE CARD

Hedonic rating	Score
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Sensory attribute	Samples of ghee					
	1	2	3	4	5	6
Flavor						

Comment if any

Date:_____ **Name :**_____

APPENDIX 2: PLATE 1 DIFFERENT CULTIVARS OF TOMATO

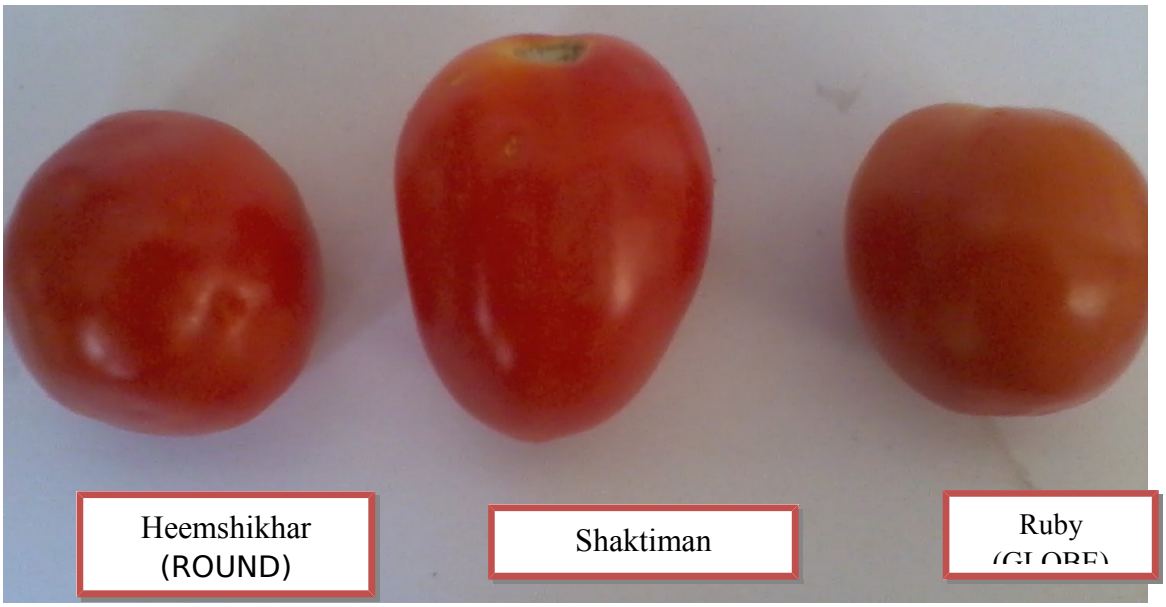
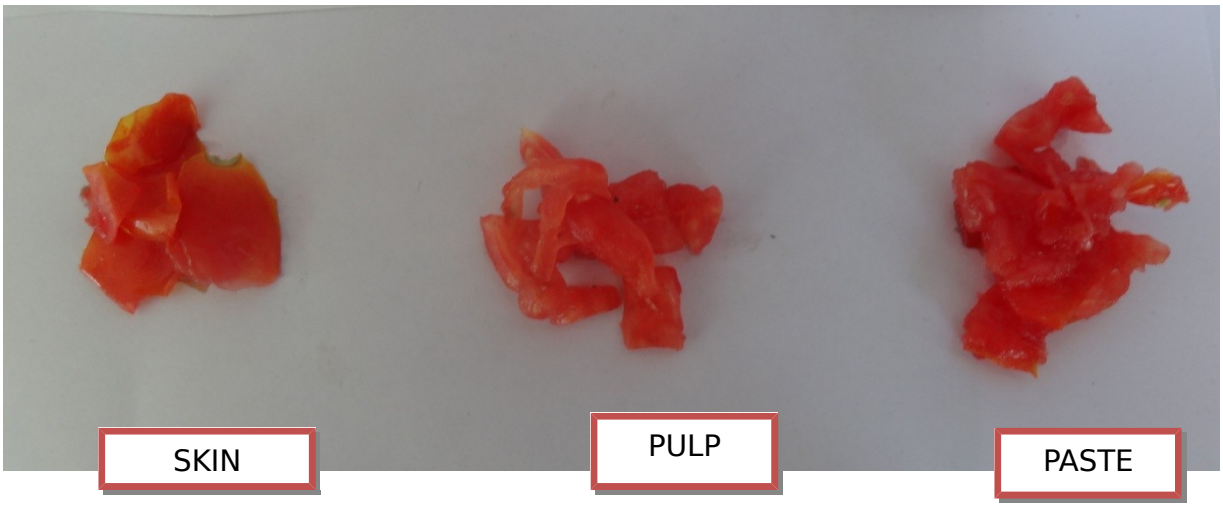


PLATE A.1 THREE DIFFERENT CULTIVARS OF

APPENDIX 3: PLATE 2 DIFFERENT PARTS OF TOMATO



APENDIX 4: PLATE A.2 THREE DIFFERENT PARTS OF TOMATO

ADDED GHEE SAMPLE

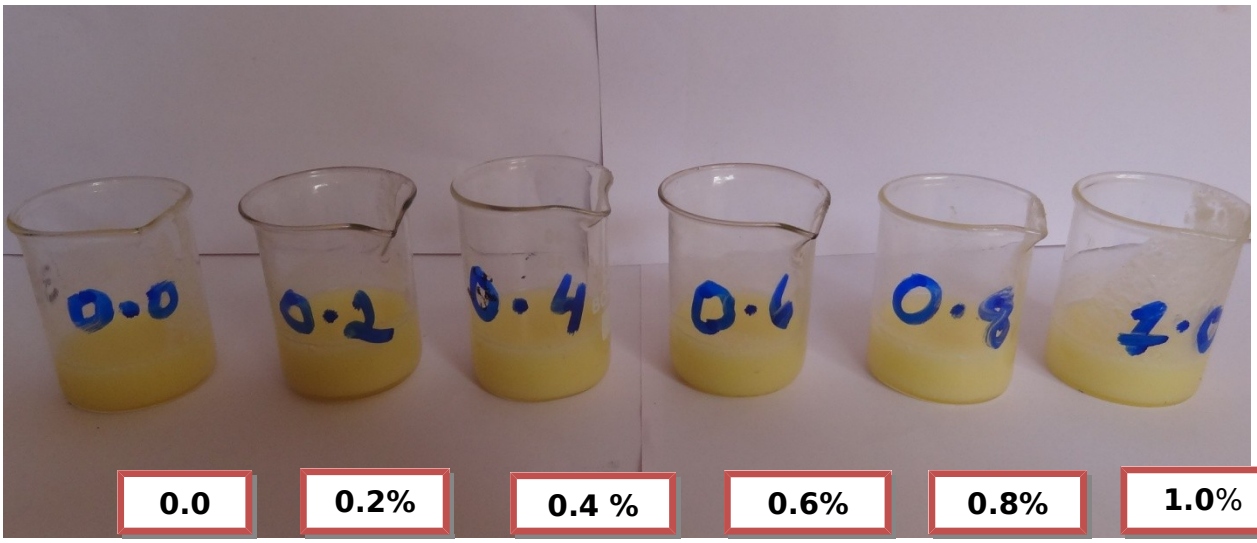


PLATE A.3 DIFFERENT CONCETRATION OF TOMATO SKIN ADDED GHEE SAMPLE (no color difference from control to other different concentration of