ANTIOXIDANT ACTIVITY OF TURMERIC IN GHEE

A
THESIS
SUBMITTED TO THE
ANAND AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF DEGREE

OF

Master of Science

IN

DAIRY CHEMISTRY

BY

SONI MEHULKUMAR DINESHCHANDRA
B.Sc. (Chemistry)

DAIRY CHEMISTRY DEPARTMENT
SHETH M. C. COLLEGE OF DAIRY SCIENCE
ANAND AGRICULTURAL UNIVERSITY
ANAND – 388 110 (GUJARAT)
INDIA

2011

Registration No. 04-0670-2007
DEDICATED
TO
MY
BROTHERS
ANTIOXIDANT ACTIVITY OF TURMERIC IN GHEE

Soni Mehul D.

Name of Student

Dr. K. D. Aparnathi

Name of Major Advisor

DAIRY CHEMISTRY DEPARTMENT
SHETH M. C. COLLEGE OF DAIRY SCIENCE
ANAND AGRICULTURAL UNIVERSITY
ANAND – 388 110, GUJARAT

ABSTRACT

Ghee undergoes oxidative deterioration and one of the most common approaches to inhibit it is by addition of antioxidants. Turmeric is reported to inhibit oxidative rancidity in several food products. However, the potential of turmeric has not been trapped as a natural antioxidant for preventing oxidative rancidity in ghee. Therefore, present study was planned to investigate antioxidant properties of turmeric to extend the shelf life of ghee. The study was divided into four phases. In the first phase two different varieties of turmeric were evaluated for their antioxidant activity in ghee. In the second phase, selection for stage of addition of turmeric was studied. In third phase rate of addition of the turmeric was
tested. In fourth phase comparison of turmeric with the permitted synthetic antioxidant (Butylated Hydroxy Anisole) was made.

In first phase samples of turmeric powder obtained from its two varieties viz. yellow (Madras) and orange (Alleppey) was evaluated for their ability to prevent oxidative deterioration of ghee. In the selection of variety the turmeric powder of each variety was added to the samples of ghee at the rate of 0.5 per cent. Sample of ghee without addition of turmeric was kept as such to serve as control. All the samples of ghee were stored at elevated temperature (80 ± 2°C) to accelerate the oxidation. The samples of ghee were subjected to sensory evaluation for flavour using 9 point hedonic scale. The samples of ghee were also analyzed for changes in peroxide value during the storage. Among the two varieties of turmeric the orange variety gave better result which extended the flavour score to acceptable level by two days.

In second phase two possible stages were envisaged for addition of turmeric in ghee viz before heat clarification of butter in to ghee and after heat clarification of butter in to ghee. In each set of experiments turmeric was added at the rate of 0.5 per cent. In one set of experiment, turmeric added before heat clarification of butter fat in to ghee. The prepared samples of ghee were subjected to sensory evaluation by panel of judges
using 9 point hedonic scale when fresh and after the interval of every three days of storage. The samples were also analyzed for peroxide value when fresh and after the interval of two days of storage. The results indicated two different stages of addition for turmeric were found to have similar effect on oxidative stability of ghee. The addition of turmeric after clarification was adopted to avoid any variation in flavour score due to difference which may lightly occur during preparation of ghee, due to variation in control of clarification in different samples of ghee.

In the third phase of the study work was carried out to select the rate of turmeric addition in ghee for extending its oxidative stability during storage. For selection of the rate for turmeric addition in ghee, the fresh ghee samples were prepared in the laboratory from the white butter. The turmeric was added to the ghee at the rate of 0.2, 0.4, 0.6, 0.8 and 1.0 per cent. The sample of ghee without addition of ghee was kept as control sample. The prepared samples of ghee were stored at 80 ± 2°C and monitored for changes in flavour score by subjecting the samples to sensory evaluation by panel of judges using 9 point hedonic scale at an interval of 7 days. The addition of turmeric in ghee at the rate of 0.6 per cent was most effective in retaining flavour score of ghee during storage.
In final phase of the study work was carried out to compare effectiveness of the 0.6 per cent turmeric from orange variety with 0.02 per cent BHA a synthetic antioxidant legally permitted for addition in ghee to delay oxidative deterioration of ghee. The results for changes in flavour score of ghee indicated that the difference between BHA added and turmeric added fresh ghee samples were non significant. However, the difference between BHA added and turmeric added ghee samples become significant during further storage period. The initial flavour score of the all the samples of ghee were almost similar up to third day of storage period. Control sample decline at a faster rate and reached below unacceptable level (< 6) on between 5th and 7th day of the storage. The flavour score of turmeric added ghee sample decreased below acceptable level on 9th day of the storage and that of the BHA added ghee sample went below the acceptable level between 11th and 13th day of storage. The results obtained for changes in peroxide value very well corroborated with those obtained for the changes in flavour of the corresponding samples.

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. The results indicated that all the prepared samples of ghee viz. control, BHA added and turmeric added fulfilled the
requirements for all the quality standards prescribed under PFA, as well as under Agmark.

From findings of the study entailed to conclude that turmeric has antioxidant properties and delays oxidative deterioration of ghee. Addition of turmeric from orange variety at the rate of 0.6 per cent does not have any adverse effect on quality standards for ghee as prescribed under PFA, as well as Agmark. The fact that turmeric acts as an antioxidant by prevention and intervention processes makes it very unique as a natural antioxidant for fat rich products like ghee. Further work using curcuminoids isolated from turmeric is required.
Dr. K. D. Aparnathi  
Professor & Head,  
Dairy Chemistry Department,  
Sheth M. C. College of Dairy Science,  
Anand Agricultural University,  
Anand – 388 110  
Gujarat, India.

CERTIFICATE

This is to certify that the thesis entitled “Antioxidant Activity of Turmeric in Ghee” submitted by Soni Mehulkumar Dineshchandra in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in DAIRY CHEMISTRY of the Anand Agricultural University 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

Date: March 20, 2011

(K. D. Aparnathi)

Major Advisor
ACKNOWLEDGEMENT

I express my sincere thanks to Anand Agricultural University for giving me the opportunity of M. Sc. study.

At this auspicious moment, I am at a loss of words to express my deep sense of gratitude and indebtedness to my Major Advisor Dr. K. D. Aparnathi., Professor & Head, Dairy Chemistry Department, S. M. C. College of Dairy Science, Anand Agricultural University, Anand for his keen interest, most valuable and inspiring guidance and constant encouragement throughout the course of investigation. The years I have spent in his counsel and company will have an ever lasting positive impact on my professional career.

It gives great pleasure to express heartful thanks to my minor advisor Dr. J. B. Prajapati Professor & Head, Dairy Microbiology Department for his keen interest, invaluable guidance and profound support at all time during my work tenure.

I pay my due respect and thanks to Dr. B. P. Shah, Principal, S. M. C. College of Dairy Science, Anand for the help and facilities provided.

I am very much thankful to the members of my advisory committee Dr. J. B. Prajapati, Mrs. Sunita V. Pinto, Dr. V. B. Darji and Mr. B. M. Mehta for their learned advice, helpful criticism and kind cooperation.

With great appreciation, I would like to thank Mr. Bhavbhuti Mehta, Mr. Amit Kumar Jain, Mr. S. C. Parmar, Mr. A. I. Shaikh, Mr. D. H. Patel, Mr. A. V. Jadhav, Ms Sudhaben and all staff members.
I would like to thank to Amit, Umang, Ripan, Kinjal, and all P.G. students of SMC college of Dairy Science for their co-operation during the study and successful completion of this research work.

The help of my brother Mr. Snehal Soni, for providing every facility to me is thankfully acknowledged.

I would like to extend my deepest sense of appreciation and love to my parents and elder brother Snehal and younger brother Jigar. Without their love, moral support, sacrifice and blessings, my dream would not have been a reality.

Place: Anand

Date: (Soni M. D.)
<table>
<thead>
<tr>
<th>CHAPTERS</th>
<th>TOPICS</th>
<th>PAGE NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. INTRODUCTION</td>
<td></td>
<td>1-4</td>
</tr>
<tr>
<td>2. REVIEW OF LITERATURE</td>
<td></td>
<td>5-36</td>
</tr>
<tr>
<td>2.0 Lipid oxidation</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>2.1 Inhibiting oxidation</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>2.1.1 Phospholipids</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>2.1.2 Ghee residue</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>2.1.3 Browning compounds</td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>2.1.4 Tocopherol</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>2.1.5 Vitamin A and Carotene</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>2.2 Antioxidant</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>2.2.1 Types of antioxidants</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>2.2.1.1 Synthetic antioxidants</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>2.2.1.2 Natural antioxidants</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>2.2.2 Addition of natural antioxidants:</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>a. Amla (Indian gooseberry) juice</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>b. Aromatic herbs</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>c. Betel, curry and drumstick leaves</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>d. Mango seed kernel</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>e. Seed phospholipids</td>
<td></td>
<td>26</td>
</tr>
</tbody>
</table>
f. Tomato seed powder 28
g. Onion skin extract 28
h. Tulsi leaves 28
i. Sorghum grain powder 29
j. Spices and condiments 29

2.2.3 Herbs and spices 30
2.2.4 Turmeric 32
   2.2.4.1 Varieties of turmeric 33
   2.2.4.2 Turmeric as an antioxidant 34

3. MATERIALS AND METHODS 37-43

3.1 Collection of market ghee samples 37
3.2 Preparation of ghee 37
3.3 Collection and preparation of turmeric sample 37
3.4 Sensory evaluation 38
3.5 Chemicals and Glasswares 39
3.6 Chemical methods for analysis of ghee 39
   3.6.1 Determination of moisture 39
   3.6.2 Determination of refractive index of ghee 40
   3.6.3 Determination of peroxide value of ghee 41
   3.6.4 Determination of free fatty acids of ghee 41
   3.6.5 Determination of Reichert Meissl value of ghee 42
3.7 Statistical analysis 43
4. RESULT AND DISCUSSION 44-76

4.1 Selection of turmeric variety 46
4.2 Selection of stage for turmeric addition in ghee 53
4.3 Selection of rate for turmeric Addition in ghee 60
4.4 Analysis of fresh ghee samples for quality standards 65
4.5 Comparison of turmeric with BHA as an antioxidant in ghee 67

5. SUMMARY AND CONCLUSION 77-89

6. REFERENCES i-xxii
## LIST OF TABLE

<table>
<thead>
<tr>
<th>TABLE NO.</th>
<th>TITLE</th>
<th>PAGE NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 4.1</td>
<td>Effect of turmeric variety on changes in flavour score of ghee during storage at 80 ± 2 °C</td>
<td>48</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>Effect of turmeric variety on peroxide values of ghee during storage at 80 ± 2 °C</td>
<td>50</td>
</tr>
<tr>
<td>Table 4.3</td>
<td>Effect of stage of turmeric addition on flavour score of ghee during storage at 80 ± 2 °C</td>
<td>55</td>
</tr>
<tr>
<td>Table 4.4</td>
<td>Effect of stage of turmeric addition on peroxide value of ghee during storage at 80 ± 2 °C</td>
<td>57</td>
</tr>
<tr>
<td>Table 4.5</td>
<td>Effect of rate of turmeric addition of turmeric on flavour score of ghee during storage at 80 ± 2 °C</td>
<td>61</td>
</tr>
<tr>
<td>Table 4.6</td>
<td>Analysis of fresh ghee samples for quality standards</td>
<td>65</td>
</tr>
<tr>
<td>Table 4.7</td>
<td>Effect of comparison between turmeric and BHA on flavour score for enhancing oxidative stability of ghee</td>
<td>69</td>
</tr>
<tr>
<td>Table 4.8</td>
<td>Effect of turmeric and BHA on peroxide value of ghee during storage at 80 ± 2 °C</td>
<td>71</td>
</tr>
<tr>
<td>FIG. NO.</td>
<td>TITLE</td>
<td>PAGE NO.</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------------------------------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Figure 4.1</td>
<td>Effect of turmeric variety on changes in flavor score of ghee during storage at 80 ± 2 °C</td>
<td>49</td>
</tr>
<tr>
<td>Figure 4.2</td>
<td>Effect of turmeric variety on peroxide values of ghee during storage at 80 ± 2 °C</td>
<td>51</td>
</tr>
<tr>
<td>Figure 4.3.</td>
<td>Effect of stage of turmeric addition on sensory score of ghee during storage at 80 ± 2 °C</td>
<td>56</td>
</tr>
<tr>
<td>Figure 4.4.</td>
<td>Effect of stage of turmeric addition on peroxide value of ghee during storage at 80 ± 2 °C</td>
<td>58</td>
</tr>
<tr>
<td>Figure 4.5.</td>
<td>Effect of rate of turmeric addition of turmeric on flavour score of ghee during storage at 80 ± 2 °C</td>
<td>62</td>
</tr>
<tr>
<td>Figure 4.6</td>
<td>Effect on flavour score of ghee for comparison between turmeric and BHA</td>
<td>70</td>
</tr>
<tr>
<td>Figure 4.7</td>
<td>Effect on turmeric and BHA on peroxide value of ghee during storage at 80 ± 2 °C</td>
<td>72</td>
</tr>
<tr>
<td>PLATE NO.</td>
<td>TITLE</td>
<td>AFTER PAGE NO.</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------------------------------------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>4.1</td>
<td>Turmeric powder from two different varieties of turmeric</td>
<td>44</td>
</tr>
<tr>
<td>4.2</td>
<td>Ghee samples with added turmeric from two different varieties</td>
<td>46</td>
</tr>
<tr>
<td>4.3</td>
<td>Ghee samples with added turmeric at two different stages of clarification</td>
<td>53</td>
</tr>
<tr>
<td>4.4</td>
<td>Ghee samples with added turmeric at different rates</td>
<td>60</td>
</tr>
<tr>
<td>4.5</td>
<td>Ghee samples with added turmeric, added BHA and control</td>
<td>68</td>
</tr>
</tbody>
</table>
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 flavour. 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.

Many chemical and biochemical reactions can lead to deterioration of product quality or impairment of product safety. The physico-chemical reactions
Introduction

that occur on processing and storage of ghee bring some undesirable changes in texture, flavour 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-flavours.

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.

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 esch, 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 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 (Honglian et al., 2001).
Introduction

In ancient Indian literature, turmeric is referred to as “Haridra” which is being used for coloring, flavouring, and digestive properties (Patel and Srinivasan, 2004). It is principally known for its yellow-orange coloring power, having a musky flavour and aroma, which necessitates classifying it as a spice. Turmeric is often used as an inexpensive alternative to saffron. The primary product of *C. longa*, true turmeric, is the cured, dried rhizome. Cured and dried turmeric of commerce are available in the form of bulbs and fingers. Turmeric is reported to inhibit oxidative rancidity in several food products. In addition turmeric has plethora of health effect benefits (Purseglove et al., 1981). However, the potential of turmeric 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 turmeric as a natural antioxidant for preventing oxidative rancidity in ghee. Keeping this idea as a central goal, the study is planned with the following objectives.

OBJECTIVES

(a) To select variety of turmeric for addition in ghee.
(b) To select stage for addition of turmeric in ghee during its preparation.
(c) To select concentration of turmeric for addition in ghee.
(d) To compare the antioxidant effectiveness of turmeric with permitted synthetic antioxidant (BHA).
CHAPTER 2
REVIEW OF LITERATURE

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-flavour 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.

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 triacylglycerols, 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; Noguchi and Niki, 1999) as well as by exogenous agents (UV, ionisation radiation, heat).

Lipid oxidation is a chemical reaction that usually takes place at ambient temperatures between atmospheric oxygen and organic compound and is generally referred to as autoxidation. An important feature of autoxidation is that it is autocatalytic. The rate of spontaneous oxidation is slow at the beginning and increases as the reaction progresses (Chan, 1987). Autoxidation is a free radical chain reaction consisting of initiation, propagation and termination steps (reactions 1-4).

Initiation $X^* + RH \rightarrow R^* + XH$ (1)
In the initiation step, polyunsaturated lipids (RH) may form alkyl radicals (R*) which react very rapidly with oxygen to form peroxyl radicals (ROO*). In the propagation step, a chain reaction with more lipids produces hydroperoxides (ROOH), i.e. primary oxidation products. These hydroperoxides decompose in the presence of metals to produce alkoxyl radicals (RO*), which cleave into a complex mixture of aldehydes and other products, i.e. secondary oxidation products (Frankel, 1995).

2.1 Inhibiting 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
Review of Literature

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.

\[
\text{LOO} + \text{AH} \rightarrow \text{LOOH} + \text{A} \\
\text{LO} + \text{AH} \rightarrow \text{LOH} + \text{A} \\
\]

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, nordihydroguaiaretic acid and flavonoids arise from this process. Aromatic amines inhibit the autoxidation via the same electron-transfer mechanism.

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.

2.1.1 Phospholipids

Several studies have indicated that phospholipids of milk and from other sources increased the shelf-life of ghee. Amongst all the milk components, the effects of phospholipids have been studied extensively.

The phospholipids content of milk fat have been found to vary between 0.2 to 1.0 per cent (Jenness and Patton, 1959) whereas in ghee prepared from cows' and buffaloes' milk, the average phospholipids content was 38.0 and 42.5 mg per cent respectively (Rama Murthy and Narayanan, 1966). The level of unsaturation is higher in phospholipids than that of natural lipids (Smith and Lowry, 1962). Consequently they are inherently more susceptible to autoxidation than neutral fats (Smith and Lowry, 1962). However, phospholipids are also shown to possess antioxidant properties. They serve as synergistic antioxidants (Smith et al., 1958).

The ionized phosphate group enters the initial stages of oxidative process, involving the unsaturated bonds in fatty acids molecule. After a time when these charged phosphate groups can no longer stabilize the hydroperoxides being formed, the system deteriorated rapidly and rampant oxidative deterioration became apparent (Brunner, 1974).

Rama Murthy et al., (1968) studied the keeping quality of cow and buffalo ghee in relation to their phospholipids content. Ghee samples prepared by heating
buffaloes’ and cows’ milk butter at 120 ºC for 0, 10, 20 min contained 8.2, 75.0 and 105.0 and 8.5, 50.8 and 125.1 mg phospholipids per 100g fat respectively. The storage studies for 6 months at 37 ºC revealed that the ghee samples containing lower levels of phospholipids developed peroxides at faster rate than those containing higher levels of phospholipids. It was noted that cow ghee showed lower peroxide development than corresponding buffalo ghee especially when the samples had low phospholipids. It was concluded that the heat treatment given during the manufacture of ghee governed the level of phospholipids in ghee which on storage presumably increased the shelf-life of ghee.

This latter contention evoked the interest of Pruthi et al., (1970) to investigate the effect of addition of various concentrations of purified milk phospholipids on the keeping quality of buffalo butter fat prepared practically free of phospholipids. For such studies, purified phospholipids were added at the rate of 0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8 and 1.0 g / 100 g of phospholipids free ghee and when stored at 80 ºC attained the peroxide value of 5 (milliequivalents of peroxide oxygen per kg of fat) at 42.0, 95.7, 127.2, 139.0, 135.2, 182.0, 228.0 and 237.8 h, respectively. These findings thus revealed that phospholipids have got antioxidant properties. Further it was also observed that phospholipids containing ghee samples acquired brown color long before the end of induction period.

However, these studied was not clear whether one or more or all of the phospholipids were involved in the antioxidant effect. Therefore, Pruthi et al.,
Pruthi et al., (1971) resolved the milk phospholipids into cephalin, lecithin and sphingomyelin by preparative thin layer chromatography on silica gel-G. Each fraction was added to phospholipids free butterfat at 1 mg/g fat. After the samples had been heated to 160 °C for 16 h, color development was measured.
Review of Literature

spectrophotometrically at 550 nm. It was found that cephalin had maximum potential for browning, the relative browning potential of cephalin, lecithin and sphingomyelin fractions were 71, 7 and 3 respectively.

Santha and Narayanan, (1979) isolated the phospholipids from the lipid constituents of ghee residue by heating creamery butter at 120 ºC. They observed that addition of ghee residue was responsible for the antioxidant property due to its phospholipids having different fractions tested namely, lecithin, cephalin, sphingomyelin and cerebrosides (all at 0.5 per cent concentrations), the cephalin fraction was the only fraction with marked antioxidant property.

To understand whether the milk phospholipids possess antioxidant properties due to their synergistic action with tocopherol which is present as a natural antioxidant in butterfat in small qualities (Bector and Narayanan, 1975) or due to chelating action with metals like copper and iron which may otherwise catalyse the oxidative rancidity. Bector and Narayanan, (1972) undertook a study to know the mechanism of antioxidative action of phospholipids in combination with different proportions of α-tocopherol and copper. Peroxide development was monitored at 80 ± 0.5 ºC after adding 0.05 to 0.4 per cent milk phospholipids, 0.006 to 0.030 per cent of α-tocopherol and 0.1 to 0.8 ppm of copper, individually or in combination. They found that with increasing addition of phospholipids, the development of peroxide was reduced, whereas with α-tocopherol there was an antioxidant effect at low concentration and an oxidizing
effect at higher concentration. The addition of phospholipids and α-tocopherol to the fat increased the antioxidant properties of α-tocopherol. The maximum synergistic effect was obtained with 0.018 per cent α-tocopherol. In the presence of copper, there was an increase in peroxide formation but simultaneous addition of phospholipids increased the oxidative stability of butterfat but only in presence of low level of copper (upto 0.4 ppm). This indicated that antioxidant property of phospholipids was shown only when copper was present at lower concentrations. Addition of phospholipids upto copper concentration of 0.4 ppm in butterfat had beneficial effect beyond which phospholipids addition had no effect. This suggests that the inactivation of metal by the phospholipids probably takes place when copper is present in minute quantities. Thus milk phospholipids have antioxidant properties. They act synergistically with α-tocopherol in oxidation of butterfat and they also possess metal chelating properties.

All the studies reported above definitely prove that milk phospholipids (cephalin) exhibit antioxidant properties. These phospholipids in milk occur in a complex with proteins in fat globule membrane and the temperature of clarification of cream or butter during ghee making release the phospholipids from the complex. Narayanan et al., (1966) reported that the phospholipids content of ghee depends on the temperature of clarification. Cows’ and buffaloes’ ghee prepared from cream or butter by heating to 120 ºC had a bland flavor and
Review of Literature

contained only traces of phospholipids, the rest being left in ghee residue. Holding of cream of butter at 120 °C for 10 min or 20 min removed more water from ghee residue and transferred more phospholipids to ghee.

Ghee prepared by heating butter to 120 °C without any holding time had much less content of phospholipids than the ghee prepared at the same temperature but held for some time. Ghee filtered at 110 °C had more phospholipids than ghee filtered at 60 °C. Kuchroo and Narayanan, (1977), however, observed that increase in phospholipids of ghee occurred only upto 40 min holding time at 120 °C, beyond which a progressive decline in phospholipids of ghee was observed. With increase in heating period, there was progressive browning in ghee. At later stages, it became dark brown with pungent fishy odor. There was a decrease in the per cent distribution of phosphatidylcholine, phosphatidylethanolamine, phosphotidylserine, sphingomyelin and phosphatidylinositol whereas an increase in lysophospholipids was seen when the period of heating was increased from 40 to 90 min.

Pruthi, (1980) studied the phospholipids content of ghee prepared at higher temperatures of clarification. While preparing ghee from butter that had been momentarily heated to 120, 130, 140 and 150 °C, he found that phospholipids content of ghee increased as the temperature of clarification increased. The highest phospholipids content was in ghee samples prepared at 150 °C (average 263.3. mg/100g) followed by 140 °C (average 177.7 mg/100g) and 130 °C
Review of Literature

(average 76.5 mg/100g) and the lowest at 120 ºC (average 22.0 mg/100g). He also observed that the moisture content of ghee residue decreased progressively with an increase in the temperature of ghee making. Thus, he concluded that the phospholipids content of ghee is reciprocally related to the moisture content of ghee varied with the method of preparation, clarification temperature and heating time. He observed higher phospholipids content in ghee prepared by creamery butter method than that of direct cream method. Ghee clarified at higher temperature (120 ºC) also contained higher phospholipids than the ghee clarified at lower temperature (105 ºC) and similarly 10 min holding time transferred more phospholipids in ghee samples than 5 min holding time.

2.1.2 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 ºC 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 ºC 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.

2.1.3 Browning compounds

Browning compounds formed due to interaction of various milk constituents in the ghee making process have been shown to have profound effect on oxidative stability of ghee. Nath and Murthy, (1988) initiated the study of effect of browning compounds on oxidative stability of ghee. They observed that ghee made from cream washed three times with water to remove free amino acids and carbonyls had a lower oxidative stability when compared to ghee made from normal cream. Addition of browning compounds like dihydroxy acetone, leucine, methyl glyoxal + leucine and glyoxal + leucine to milk fat heated to 120 °C for 5 minutes had significant antioxidative effect and a protection factor (ratio of time to reach peroxide value of 1.0 in samples containing antioxidant to that in control) were 2.25, 2.00 and 1.75, respectively. Moreover protection factor (PF) offered by dihydroxy acetone and leucine was found to be high in case of ghee
prepared from washed cream (PF = 1.63) than that in ghee prepared from unwashed cream (PF = 1.15). Addition of casamino acids, methyl glyoxal + casamino acids and diacetyl + amino acids to ghee had a protection factor of 1.25, 2.00 and 1.75, respectively, indicating that casamino acid + methyl glyoxal had the best antioxidative activity. Thus, it was concluded that browning compounds produced due to interaction of dicarboxyls and amino acids have considerable antioxidative activity.

2.1.4 Tocopherol

Ghee contains natural antioxidant such as tocopherols. Tocopherols are present in minor quantities in ghee. The values being 26.4 and 30.5 µg/g of buffalo and cow ghee respectively (Bector and Narayanan, 1975). Bector and Narayanan, (1972) reported that the tocopherols possess antioxidative property due to their synergistic effect with milk phospholipids. They also showed that in presence of added copper, tocopherol enhances the antioxidant properties of added phospholipids. The role of oxidation products of tocopherol act as an antioxidation process of milk fat. The resistance of milk fat against oxidation reduced upon addition of oxidation products of tocopherol. The reason attributed was the pro-oxidant activity of oxidation products of tocopherol. Addition of phospholipids and butylated hydroxyl toluene reduced this pro-oxidant activity due to synergistic effects.
2.1.5 Vitamin A and Carotene

Schuller, (1957) believed that carotene itself was an antioxidant but that its breakdown products, formed during the induction period, functioned thereafter as pro-oxidants. Smith et al., (1958) also established the antioxidative role of carotene.

Santha and Narayanan, (1979) reported that in lipid constituents of ghee residue, vitamin A was one of the constituents responsible for antioxidative properties.

2.2 Antioxidant

The term antioxidant is used and defined differently by different authors in the free radical literature. In more precise chemical terms, 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 (Halliwell and Gutteridge, 1995). 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 prevents 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•, or peroxyl-ROO• radicals), formed in human tissue cells by many endogenous and
Review of Literature

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, 1997; Krinsky, 1992).

To prevent oxidation of lipids, antioxidants are often used in the food industry. Synthetic antioxidants (e.g., butylated hydroxytoluene, butylated hydroxyanisole) have been progressively replaced by natural antioxidants (Qi and Sim, 1998). Tocopherols are a reliable choice and have been tested successfully in several food products. Supplementation of animal diets with tocopherols increases the content of this natural antioxidant in animal food products and prevents lipid oxidation in broiler meat and egg products (Ajuyah et al., 1993; Wahle et al., 1993; Cherian et al., 1996). Researchers are looking for other natural antioxidants, as alternatives to tocopherols. Carotenoids are another group of natural antioxidants (Burton, 1989; Jørgensen and Skibsted, 1993). The antioxidant activity of canthaxanthin (CX) is controversial. Some authors have reported the antioxidant activity of CX in model systems (Palozza and Krinsky, 1992; Jørgensen and Skibsted, 1993), but its antioxidant activity in animal food products after dietary supplementation has been tested only in broilers, without clear results (Barroeta and King, 1991; Woodall et al., 1996).
Phenolic compounds are also able to quench $^1\text{O}_2$ (Mukai et al., 1991), although in a slower rate. On the other hand, phenolic compounds possess the best chemical structures for free radical scavenging activities due to their reducing properties (Rice-Evans et al., 1997).

In the evaluation of antioxidants, varied results can be obtained by methods which measure products at different stages of lipid oxidation. Thus, it is important that more than one method is used (Frankel, 1995; Lampi et al., 1997).

In brief, the most common methods to measure lipid hydroperoxides are peroxide value (PV) and conjugated dienes (CD). The aldehydes can be measured by anisidine value (AV) analysis and thiobarbituric acid (TBA) test. The volatile aldehydes can be determined by head-space gas chromatography (GC) or as derivatives by high-performance liquid chromatography (HPLC). The above mentioned methods for measurement of lipid oxidation products have been widely applied to follow autooxidation of lipids as well as free radical initiated lipid oxidation in the presence of carotenoids.

2.2.1 Types of antioxidants

To prevent or retard the oxidative deterioration of foods, natural or synthetic antioxidants have been used as additives in fats and oils.
2.2.1.1 Synthetic antioxidants

Some of the more popular synthetic antioxidants are phenolic compounds such as butylated hydroxyanisole (BHA), butylated hydroxy toluene (BHT), tertiary butyl hydroquinone (TBHQ) and esters of gallic acid e.g. propyl gallate (PG). These are most suitable antioxidant for vegetable oils and fairly stable to heat (Rawls et al., 1970; Frankel et al., 1977; Frankel et al., 1985). The addition of the antioxidants to food is usually restricted to 0.02 per cent on fat basis (AOAC, 1997). For addition in ghee BHA is permitted at the rate of 0.02 per cent under PFA (2010).

The synthetic antioxidants have been tested for their toxicological behaviors, but some of them are coming, after a long period of use, under heavy pressure as new toxicological data impose some caution in their use. It is generally accepted, however, that natural antioxidants are more potent, efficient and safer than synthetic antioxidants. Since about 1980 natural antioxidants have appeared as an alternative to synthetic antioxidants (Honglian et al., 2001).

2.2.1.2 Natural antioxidants

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. Simple phenols, polyphenolics and phenolic acid derivatives are the antioxidants that are common to all plant sources (Pokorny, 1999).

2.2.2 Addition of natural antioxidants:

A favourable trend towards natural products has developed due to reports from medicinal centers 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 materials used for protecting ghee against oxidative deterioration is given below:

2.2.2.1. 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).

2.2.2.2. Aromatic herbs

Amr (1990) 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.

2.2.2.3. 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).

2.2.2.4. 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
Review of Literature

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.
2.2.2.5. 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 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.

2.2.2.6. 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).

2.2.2.7. 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.

2.2.2.8. Tulsi leaves

Sharma, (1997) isolated the antioxidant principles of Tulsi (Ocimum sanctum Linn.) leaves via a pre-extract. The antioxygenic 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.

2.2.2.9. 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.

2.2.2.10. 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
Review of Literature

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.

2.2.3 Herbs and spices

The mere mention of natural antioxidants brings about an association with spices and herbs, because they are the most important targets in the search for natural antioxidants from the point of view of safety. Man has used them not only for flavouring foods but also for antiseptic and medical properties since the prehistoric era. The antioxidative activity of allspice, aniseed, black pepper, caraway, cinnamon, clove, ginger, mace, marjoram, nutmeg, oregano, paprika, peppermint, red paprika, rosemary, sage, savory, thyme, turmeric and white pepper have been reported (Clifford, 1999).

The essential oils from a number of herbs and spices were also studied for antioxidative activity, e.g. oregano, rosemary, sage, clove, coriander, cumin, fennel, thyme, marjoram, laurel, caraway, peppermint, basil, cinnamon, nutmeg, dill and black pepper. Although the compounds in the essential oils such as carvone from caraway, eugenol from clove, thymol from thyme and thujone from sage possess antioxidant activity, the aromatic character of these
Review of Literature

compounds limits the use of the essential oils as antioxidants in foods (Madsen and Bertelsen, 1995). It was also found that there was a reduced antioxidant activity in extracts prepared from an equivalent amount of spice as opposed to that prepared from the whole spice, confirming that a wide range of compounds acting together are important as antioxidants in the plant material, which further may act synergistically (Chipault et al., 1952; Madsen et al., 1998).

From time immemorial, herb extracts were used for preserving the quality of soybean oil, beef, meat, poultry, fish, and lard. Simple phenols, polyphenolics and phenolic acid derivatives are the antioxidants that are common to all plant sources. However, fortification by herbal extract in dairy products is a newly emerged area (Bandopathyay et al., 2008).

Chipault et al., (1952) investigated antioxidant activity of spices in various fats. In general, alcoholic and ether extracts of spices are less active than native spices. Allspice, clove, sage, oregano, rosemary, and thyme possess antioxidant activity in all types of fats examined. Clove appears to be the most active antioxidant in vegetable oils; however, extracts of rosemary and sage are the most effective.

Chang et al., (1977) were able to prepare an odourless and flavourless natural antioxidant from rosemary (Rosmarinus officinalis L.) and sage (Salvia officinalis L.). These antioxidants can be successfully extracted into different organic solvents such as benzene, chloroform, diethyl ether and methanol.
The extract of rosemary leaves contain a phenolic diterpene, namely, carnosol (Haulihan et al., 1984). Rosmaridiphenol (at 0.02%) has shown antioxidant activity similar to BHT at the same level in prime steam lard. Carnosic acid and rosmaric acid are the most active antioxidant constituents of rosemary and rosmaric acid possesses an activity comparable to that of caffeic acid. In animal fats, Carnosic acid has been described as the most active antioxidative constituent of rosemary (Schuler, 1990). Commercial antioxidant extracts (molecular or vacuum distilled) from rosemary are available as a fine powder. Depending on their content of active antioxidants, they are recommended for use at concentrations ranging between 200 and 1000 mg/kg of the processed product (Chang et al., 1977).

The diethyl ether extract of rosemary was purified and evaluated for its antioxidant activity (peroxide value) at 0.02% (w/w) in potato chips, sunflower oil and corn oil. It showed a very low peroxide value and provided excellent flavour stability to the products tested (Fereidoon and Marian, 2004).

2.2.4 Turmeric

*Curcuma*, a very important genus in the family Zingiberaceae, consists of about 110 species. Many species of *Curcuma* are economically valuable, the most important being *Curcuma longa*, known as turmeric commercially. Turmeric is a rhizomatous perennial plant. The rhizomes of turmeric contain coloured
substances known as curcuminoids and collectively termed as curcumin (Sarker and Nahar, 2007).

![Curcuminoid Structure]

**Curcuminoids**

<table>
<thead>
<tr>
<th>Curcumin (Curcumin I)</th>
<th>$R_1 = R_2 = OCH_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demethoxycurcumin (Curcumin II)</td>
<td>$R_1 = OCH_3$ and $R_2 = H$</td>
</tr>
<tr>
<td><em>bis</em>-Demethoxycurcumin (Curcumin III)</td>
<td>$R_1 = R_2 = H$</td>
</tr>
</tbody>
</table>

### 2.2.4.1 Varieties of turmeric

Around 30 turmeric varieties are grown in India, but only two designations are commercially significant in the world market: “Alleppey” and “Madras,” both named after the places of export from India. The deep yellow to orange-yellow Alleppey turmeric, is grown in Kerala (Shobha et al., 1998; ASTA, 2002). Alleppey turmeric contains about 3.5 to 5.5% volatile oil, and 4.0 to 7.0% curcumin (Buescher et al., 2000; Weiss 2002; ASTA, 2002). It has a peppery, earthy odour and a slightly aromatic, bitter taste. The Madras type contains only 2% of volatile oil and 2% of curcumin. Madras turmeric is comprised of as many as nine cultivars including Guntur, Salem, Rajahmundry, Nizamabad, and
Cuddappah. India keeps most of its Madras turmeric for domestic use and exports most of the Alleppey turmeric (Govindarajan, 1980; ASTA, 2002).

2.2.4.2 Turmeric as an antioxidant

Curcuminoids are natural phenolic compounds with potent antioxidant properties, which were reported as early as 1975. Curcumin is a non-toxic, highly promising natural antioxidant due to excellent antioxidant properties. It is reported to be more potent in preventing lipid peroxidation than α-tocopherol or the commonly used synthetic antioxidant BHT (Majeed, 1995). Both turmeric and curcumin inhibit generation of superoxide and hydroxyl free radicals. The antioxidant properties of curcumin in the prevention of lipid peroxidation are also well recognized (Sreejayan and Rao, 1994).

A complex of the three curcuminoids was found to be more effective as an antioxidant than each of the components (curcumin, demethoxycurcumin, or bisdemethoxycurcumin) used alone. The antioxidant property of the dye is attributed to its phenolic structure (Ravindran and Srinivasan, 2007). In addition, tetrahydrocurcumin, a colourless, heat resistant, antioxidative compound, has been found in turmeric (Osawa et al., 1989).

The antioxidant property of the dye is attributed to its phenolic structure. It is suggested that the antioxidant mechanism of curcuminoids may include one or more of the following interactions:

(1) Scavenging or neutralizing of free radicals.
Review of Literature

(2) Interacting with oxidative cascade and preventing its outcome.

(3) Oxygen quenching and making it less available for oxidative reactions.

(4) Inhibition of oxidative enzymes like cytochrome P-450.

(5) Chelating or disarming oxidative properties of metal ions like iron (Fe).

(Agarwal, et. al., 2007)

In a study (Ramaswamy and Banerjee, 1948), turmeric dye exhibited excellent antioxidant properties on coconut oil, groundnut oil, cottonseed oil, and sesame oil. Five Antioxidative components of turmeric (Curcuma longa) oleoresin were detected and identified by comparing with authentic compounds on TLC and HPLC and studied by TLC fluorescent method develop to separate and evaluate the activity of the individual antioxidative components (Choiu et.al., 1984). In a study of the diethyl ether extracts of 23 spices, turmeric extract proved to be the second most active (Yoshika and Miyagi, 1990).

Turmeric is reported to inhibit oxidative rancidity in salted cooked fish and is said to be more potent than garlic and onion (Ramanathan and Das, 1993). Turmeric powder, extracts and curcumin exhibit antioxidant property as observed by the induction period and oxygen absorption of coconut, groundnut, safflower, sesame, mustard, cotton seed oil and ghee at 95°C to 22°C for period up to 144h. In foods, the antioxidant property of turmeric was effective in preventing peroxide developments (Khanna, 1999).
The use of 0.5 to 1.0% levels reduces the formation of peroxides; thus, this product has been used to increase the shelf life of oils and fats. Water and alcohol extracts are as effective as butylated hydroxy anisole in methyl linoleate systems. In butter cakes containing 13.1% moisture and 38% crude fat, turmeric has shown an important antimycotic activity. In this product, turmeric prevents the development of oxidation and cakes do not show rancid characteristics (Shalini and Srinivas 1987).

It appears from the above review that despite of proven antioxidant properties of turmeric no attempts have been made to trap its potential as a natural antioxidant for preventing oxidative rancidity in ghee.
CHAPTER 3
MATERIALS AND METHODS

3.1 Collection of Market Ghee Samples

Market samples of ghee from organized and unorganized 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 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 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. The samples were filled in clean and dry beakers, cooled to room temperature and thereafter stored in incubator at 80 °C.

3.3 Collection and Preparation of Turmeric Sample

Two different varieties of turmeric samples Madras (yellow) and Alleppey (orange) were procured from Anand local market. Alleppey (orange) variety was taken and boiled for 40-45 minutes and after boiling it was let for drying for two or three days at 50 °C. After drying it was crushed and ground.
3.4 Sensory Evaluation

All the market samples of ghee were evaluated for their sensory characteristics on a 9 point hedonic scale by a panel of nine judges. The samples were evaluated for their flavour. The 9 point hedonic scale score card for sensory analysis is given below.

Score card

**Sensory Scorecard for Ghee on 9 point hedonic scale**

<table>
<thead>
<tr>
<th>Sensory attribute</th>
<th>Samples of ghee</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Flavour</td>
<td></td>
</tr>
</tbody>
</table>

Comment if any:

<table>
<thead>
<tr>
<th>Hedonic rating</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Like extremely</td>
<td>9</td>
</tr>
<tr>
<td>Like very much</td>
<td>8</td>
</tr>
<tr>
<td>Like moderately</td>
<td>7</td>
</tr>
<tr>
<td>Like slightly</td>
<td>6</td>
</tr>
<tr>
<td>Neither like nor dislike</td>
<td>5</td>
</tr>
<tr>
<td>Dislike slightly</td>
<td>4</td>
</tr>
<tr>
<td>Dislike moderately</td>
<td>3</td>
</tr>
<tr>
<td>Dislike very much</td>
<td>2</td>
</tr>
<tr>
<td>Dislike extremely</td>
<td>1</td>
</tr>
</tbody>
</table>

Date:________________     Name of the Panelist:____________________
3.5 Chemicals and Glasswares

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

3.6 Chemical Methods for Analysis of Ghee

For chemical methods different methods are used. 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 (IS: 3508-1966).

Ten grams 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 ± 1 °C for approximately 1 h. The dish was removed from the oven and cooled to room temperature in a desiccator. 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 weighings did not exceed 1 mg.

The moisture content of ghee was calculated as follows:

\[
\text{Moisture content, per cent by weight} = 100 \frac{(W_1-W_2)}{W_1-W}
\]

Where,

\( W_1 \) = Weight in g of the dish with ghee before drying
Materials and Methods

$W_2$ = 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

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 care 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 are 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 (IS: 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 milliequivalents of peroxide oxygen per kg of ghee.

Peroxide value (milliequivalents of peroxide oxygen/kg of ghee) = \( \frac{2T}{W} \)

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).
Materials and Methods

In a clean and dry 50 ml conical flask, 10 g 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 \times \frac{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

A Reichert-Meissl value was determined by the method specified in IS: 3508-1966. Ghee (5 g) 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. The blank tests were conducted in the same way without using ghee sample.
Reichert- Meissl value = 1.10 (T1-T2)

Where

T1= volume in ml of 0.1N NaOH used for sample for titration (water soluble)
T2= volume in ml of 0.1N NaOH used for blank (water soluble)

3.7 Statistical Analysis

All the parameters under study was analyzed by statistical methods. Storage study data obtained for Peroxide value and flavour score were analyzed using Two- Factor CRD. The level of significance was P< 0.05 levels and CD per cent was also calculated whenever required.
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. Ghee is a rich source of energy, fat soluble vitamins, essential fatty acids and pleasing flavour. 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. 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. 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 esch, 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 (Honglian et al., 2001).

In ancient Indian literature, turmeric is referred to as “Haridra” which is being used for coloring, flavoring, and digestive properties (Patel and Srinivasan, 2004). Turmeric is reported to inhibit oxidative rancidity in several food products. In addition turmeric has plethora of health effect benefits (Purseglove et al., 1981). However, the potential of turmeric has not been trapped as a natural antioxidant for preventing oxidative rancidity in ghee. The antioxidant/pro-oxidant action of turmeric on lipid oxidation has been of interest in food lipids. In literature conflicting opinions are prevailing about antioxidant potential of turmeric, but not proved much in food systems (Majeed, 1995; Sreejayan and Rao, 1994).

No attempts have been made to trap potential of turmeric as a natural antioxidant for preventing oxidative rancidity in ghee. Therefore, present study was planned to study the antioxidant properties of turmeric to extend the shelf life of ghee. The study was divided into four phases. In the first phase two different varieties of turmeric was evaluated for their antioxidant activity in ghee. In the second phase, selection for stage of addition of turmeric was studied. In third phase rate of addition of the turmeric was tested. In fourth phase
Result & Discussion

comparison of turmeric with the permitted synthetic antioxidant (Butylated Hydroxy Anisole) was made.

4.1 Selection of turmeric variety

Curcuma, a very important genus in the family Zingiberaceae, consists of about 110 species. Many species of Curcuma are economically valuable, the most important being Curcuma longa, known as turmeric commercially. Around 30 turmeric varieties are grown in India, but only two designations are commercially significant in the world market: “Alleppey” and “Madras,” both named after the places of export from India. The deep yellow to orange-yellow Alleppey turmeric, is grown in Kerala (Shobha et al. 1998; ASTA, 2002). Alleppey turmeric contains about 3.5 to 5.5% volatile oil, and 4.0 to 7.0% curcumin (ASTA, 2002; Buescher and Yang, 2000; Weiss, 2002). It has a peppery, earthy odour and a slightly aromatic, bitter taste. The Madras type contains only 2% of volatile oil and 2% of curcumin. Madras turmeric is comprised of as many as nine cultivars including Guntur, Salem, Rajamundry, Nizamabad, and Cuddappah. India keeps most of its Madras turmeric for domestic use and exports most of the Alleppey turmeric (ASTA, 2002; Govindarajan, 1980).

Addition of turmeric for enhancing the shelf life of ghee, the important point for consideration was the selection of variety of turmeric in the ghee. Therefore, in first phase samples of turmeric powder obtained from its two
Result & Discussion

varieties *viz.* Madras and Alleppey was evaluated for their antioxidant activity in ghee.

In the selection of variety the turmeric powder of each variety was added to the samples of ghee at the rate of 0.5 per cent. For this study four fresh samples were obtained from four different sources *viz.* (1) *Anubhav* Dairy of the SMC College of Dairy Science, (2) Private commercial dairy plant in Anand city (3) commercial co-operative dairy plant in Anand city and (4) Vidya dairy in Anand city. Each sample of ghee was warmed up to 65 °C and divided in to three parts. In first part turmeric from yellow variety was added at the rate of 0.5 %, in second part turmeric from orange variety was added at the rate of 0.5 % and the third part was kept as such to serve as control. All the three samples of ghee were mixed thoroughly with the help of glass road and filtered through four folded muslin cloth. All the samples of ghee were stored at elevated temperature (80 ± 2 °C) to accelerate the oxidation.

The samples of ghee were subjected to sensory evaluation for flavour using 9 point hedonic scale. The average results obtained from four different samples for the changes in flavour score of the ghee samples are given in Table 4.1 and presented in Figure 4.1. The samples of ghee were also analyzed for peroxide value at an interval of two days for period of 131 days. The average results obtained from four different samples for changes in peroxide value of the ghee samples are given in Table 4.2 and graphically represented in Figure 4.2.
Table 4.1 Effect of turmeric variety on changes in flavor score of ghee during storage at 80 ± 2 °C

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>Control</th>
<th>Yellow turmeric</th>
<th>Orange turmeric</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.8</td>
<td>8.1</td>
<td>8.1</td>
</tr>
<tr>
<td>3</td>
<td>7.5</td>
<td>7.5</td>
<td>8.2</td>
</tr>
<tr>
<td>5</td>
<td>7.3</td>
<td>7.8</td>
<td>8.1</td>
</tr>
<tr>
<td>7</td>
<td>6.1</td>
<td>7.1</td>
<td>7.7</td>
</tr>
<tr>
<td>9</td>
<td>5.3</td>
<td>6.8</td>
<td>7.7</td>
</tr>
<tr>
<td>11</td>
<td>5.1</td>
<td>6.3</td>
<td>7.1</td>
</tr>
<tr>
<td>13</td>
<td>4.6</td>
<td>5.2</td>
<td>6.2</td>
</tr>
<tr>
<td>15</td>
<td>3.7</td>
<td>4.5</td>
<td>5.5</td>
</tr>
<tr>
<td>17</td>
<td>3.3</td>
<td>4.0</td>
<td>4.7</td>
</tr>
</tbody>
</table>

ANOVA TABLE

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Storage period</th>
<th>Treatment (Turmeric variety)</th>
<th>Storage period x Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.150</td>
<td>0.086</td>
<td>0.259</td>
</tr>
<tr>
<td>C. D</td>
<td>0.421</td>
<td>0.243</td>
<td>0.729</td>
</tr>
<tr>
<td>Test</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>CV %</td>
<td></td>
<td>8.11</td>
<td></td>
</tr>
</tbody>
</table>

The statistical analysis of the data indicated that the period of storage, treatments of samples and interaction between period of storage and treatments of samples were significant. The difference between yellow and orange varieties of turmeric was non significant when fresh samples ghee were analyzed for flavour score on day one. However, the difference between two varieties became significant during storage from day two and remained significant all through out the storage period.
Figure 4.1. Effect of turmeric variety on changes in flavor score of ghee during storage at 80°C ± 2

The results also indicated that the initial flavour score of the control sample of ghee was slightly lower than that of the sample added with turmeric. During the storage the flavour score of control sample decline at a greater rate and reached below unacceptable level (< 6) on 9th day of the storage. Flavour scores of the turmeric added samples of ghee remained higher compared to that of the control sample. The flavour score of ghee sample added with yellow turmeric went below the acceptable level on 13th days of storage, where as, flavour score of ghee sample added with orange turmeric went below the acceptable level on 15th days of storage. Thus, among the two varieties of turmeric the orange variety gave better result which extended the flavour score to acceptable level by two days.
Table 4.2 Effect of turmeric variety on peroxide unit values of ghee during storage at 80 ± 2 °C

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>Sample of ghee</th>
<th>Storage period (days)</th>
<th>Sample of ghee</th>
<th>Storage period (days)</th>
<th>Sample of ghee</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Yellow turmeric</td>
<td>Orange turmeric</td>
<td>Control</td>
<td>Yellow turmeric</td>
</tr>
<tr>
<td>1</td>
<td>1.75</td>
<td>1.75</td>
<td>1.65</td>
<td>45</td>
<td>74.32</td>
</tr>
<tr>
<td>3</td>
<td>4.00</td>
<td>4.20</td>
<td>3.25</td>
<td>47</td>
<td>77.15</td>
</tr>
<tr>
<td>5</td>
<td>6.00</td>
<td>5.30</td>
<td>4.65</td>
<td>49</td>
<td>79.87</td>
</tr>
<tr>
<td>7</td>
<td>8.00</td>
<td>7.95</td>
<td>6.60</td>
<td>51</td>
<td>81.65</td>
</tr>
<tr>
<td>9</td>
<td>11.20</td>
<td>9.30</td>
<td>8.30</td>
<td>53</td>
<td>85.32</td>
</tr>
<tr>
<td>11</td>
<td>13.80</td>
<td>13.5</td>
<td>11.10</td>
<td>55</td>
<td>86.42</td>
</tr>
<tr>
<td>13</td>
<td>14.90</td>
<td>15.0</td>
<td>12.20</td>
<td>57</td>
<td>82.90</td>
</tr>
<tr>
<td>15</td>
<td>30.55</td>
<td>18.75</td>
<td>16.90</td>
<td>59</td>
<td>88.92</td>
</tr>
<tr>
<td>17</td>
<td>33.10</td>
<td>22.15</td>
<td>18.90</td>
<td>61</td>
<td>91.00</td>
</tr>
<tr>
<td>19</td>
<td>30.70</td>
<td>24.05</td>
<td>18.30</td>
<td>63</td>
<td>95.30</td>
</tr>
<tr>
<td>21</td>
<td>32.35</td>
<td>26.85</td>
<td>19.70</td>
<td>65</td>
<td>98.30</td>
</tr>
<tr>
<td>23</td>
<td>39.50</td>
<td>31.35</td>
<td>25.30</td>
<td>67</td>
<td>102.80</td>
</tr>
<tr>
<td>25</td>
<td>51.65</td>
<td>36.50</td>
<td>32.80</td>
<td>69</td>
<td>107.77</td>
</tr>
<tr>
<td>27</td>
<td>59.20</td>
<td>49.70</td>
<td>41.30</td>
<td>71</td>
<td>126.42</td>
</tr>
<tr>
<td>29</td>
<td>62.95</td>
<td>56.95</td>
<td>48.45</td>
<td>73</td>
<td>140.42</td>
</tr>
<tr>
<td>31</td>
<td>68.10</td>
<td>63.20</td>
<td>53.65</td>
<td>75</td>
<td>147.22</td>
</tr>
<tr>
<td>33</td>
<td>79.75</td>
<td>71.70</td>
<td>60.70</td>
<td>79</td>
<td>148.92</td>
</tr>
<tr>
<td>35</td>
<td>67.15</td>
<td>62.95</td>
<td>56.40</td>
<td>81</td>
<td>152.70</td>
</tr>
<tr>
<td>37</td>
<td>79.60</td>
<td>63.70</td>
<td>55.05</td>
<td>83</td>
<td>155.72</td>
</tr>
<tr>
<td>39</td>
<td>77.30</td>
<td>78.72</td>
<td>63.90</td>
<td>85</td>
<td>152.95</td>
</tr>
<tr>
<td>41</td>
<td>79.40</td>
<td>82.32</td>
<td>66.52</td>
<td>87</td>
<td>154.97</td>
</tr>
<tr>
<td>43</td>
<td>75.17</td>
<td>80.80</td>
<td>60.97</td>
<td>89</td>
<td>150.40</td>
</tr>
</tbody>
</table>
The statistical analysis of the data indicated that the period of storage, treatments of samples and interaction between period of storage and treatments of samples were significant. The difference between yellow and orange varieties of turmeric was non significant when fresh samples of ghee were analyzed for flavour score up to ninth day. However, the difference between two varieties became significant during storage from eleventh day and remained significant during further storage period.

![ANOVA TABLE](image)

**Figure 4.2** Effect of turmeric variety on peroxide values of ghee during storage at 80 ± 2 °C
**Result & Discussion**

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 turmeric. The peroxide value of ghee samples containing orange turmeric remained the lowest almost throughout the period of storage, except slightly higher during the last few days of extended storage. The peroxide value of ghee samples containing yellow turmeric remained almost similar to that of the samples of ghee containing orange turmeric up to 11 days of the storage. However, on further storage the rate of increase in peroxide value of ghee samples containing yellow turmeric remained higher than that of the samples of ghee containing orange turmeric. Thus the performance of turmeric of orange variety in controlling the oxidative deterioration of ghee was better than the turmeric of yellow variety.

*Curcuma*, a very important genus in the family Zingiberaceae, consists of about 110 species. Many species of *Curcuma* are economically valuable, the most important being *Curcuma longa*, known as turmeric commercially. Around 30 turmeric varieties are grown in India, but only two designations are commercially significant in the world market: “Alleppey” and “Madras,” both named after the places of export from India. The deep yellow to orange-yellow Alleppey turmeric, is grown in Kerala (Shobha *et al*., 1998; ASTA, 2002). Alleppey turmeric contains about 3.5 to 5.5% volatile oil, and 4.0 to 7.0% curcumin (ASTA, 2002; Buescher and Yang, 2000; and Weiss, 2002). It has a peppy, earthy odour.
and a slightly aromatic, bitter taste. The Madras type contains only 2% of volatile oil and 2% of curcumin. Madras turmeric is comprised of as many as nine cultivars including Guntur, Salem, Rajamundry, Nizamabad, and Cuddappah. India keeps most of its Madras turmeric for domestic use and exports most of the Alleppey turmeric (ASTA, 2002; Govindarajan, 1980).

The difference in effect of turmeric the relative performance of turmeric obtain from their yellow and orange varieties towards oxidative stability of ghee and other fats, oil and others foods has not been reported. The better performance of turmeric from orange variety found in present study may be attributed to its higher curcumin content compared to the turmeric from yellow variety as discussed above. Therefore, in the present study orange variety was selected for further study.

4.2 Selection of stage for turmeric addition in ghee

From examination of manufacturing process for ghee it can be envisaged that there are two possible ways to add turmeric 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 turmeric at any of the above mentioned stage some advantages and limitations appeared to be associated. It was investigated that on addition of turmeric before heat clarification of butter fat in to ghee may give better extraction of active components in to fat, start their antioxidative effect
right from the stage of heating the fat at elevated temperature (115 °C for no hold) used for the clarification. On the other hand it may happen that the elevated temperature use the clarification of butter fat in too ghee may adversely affect stability of the antioxidant components present in the turmeric. Moreover, the addition of turmeric before heat clarification of butter fat may also lead to loss of the active component in the ghee residues and lower transfer in to fat phase (ghee). Similarly, in addition of turmeric after heat clarification of butter in to ghee may also have possibilities opposite effects. This addition of turmeric after the clarification may not give appropriate dissolution of active components of turmeric in to fat phase. However, this stage of addition may give better stability of the active components.

Therefore, in the present investigation work was carried out to select stage for addition of turmeric to enhance the shelf life of ghee as second phase of the study. To find out the effect of stage for turmeric addition in ghee on oxidative stability of the ghee, turmeric was added at two different stages of the ghee preparation. In each set of experiments turmeric was added at the rate of 0.5 per cent. In one set of experiment, turmeric added before heat clarification of butter fat in to ghee. The samples of butter was taken in to clarification pan and the turmeric was added when butter get melted. After clarification of butter fat in to ghee, the prepared sample of ghee was filtered through muslin cloth. In second set of the experiment, turmeric was added after filtration of heat clarified butter fat (ghee) at 65 °C, the sample was thoroughly mixed with the help of dry glass
rod for uniform mixing of the turmeric, followed by filtration through four folded muslin cloth. The samples of ghee without addition of turmeric were also prepared to serve as control samples. Total four replications were conducted.

The prepared samples of ghee were subjected to sensory evaluation by panel of judges using 9 point hedonic scale when fresh and after the interval of every three days of storage. The samples were also analyzed for peroxide value when fresh and after the interval of two days of storage. The results obtained for sensory evaluation of fresh and stored samples of ghee are presented Table 4.3 and graphically presented in Figure 4.3. The results obtained for peroxide value of fresh and stored samples of ghee are presented Table 4.4 and graphically presented in Figure 4.4.

**Table 4.3. Effect of stage of turmeric addition on flavour score of ghee during storage at 80 ± 2 °C**

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>Samples of ghee</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>1</td>
<td>8.2</td>
<td>8.1</td>
<td>8.0</td>
</tr>
<tr>
<td>3</td>
<td>6.5</td>
<td>7.5</td>
<td>7.4</td>
</tr>
<tr>
<td>5</td>
<td>5.9</td>
<td>7.1</td>
<td>6.9</td>
</tr>
<tr>
<td>7</td>
<td>5.0</td>
<td>6.7</td>
<td>6.8</td>
</tr>
<tr>
<td>9</td>
<td>4.5</td>
<td>6.4</td>
<td>6.1</td>
</tr>
<tr>
<td>11</td>
<td>4.0</td>
<td>5.8</td>
<td>5.7</td>
</tr>
</tbody>
</table>

**ANOVA Table**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Storage period</th>
<th>Treatment (Stage of addition)</th>
<th>Storage period × Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.183</td>
<td>0.130</td>
<td>0.318</td>
</tr>
<tr>
<td>C. D</td>
<td>0.521</td>
<td>0.369</td>
<td>0.903</td>
</tr>
<tr>
<td>Test</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>CV %</td>
<td></td>
<td>9.81</td>
<td></td>
</tr>
</tbody>
</table>
Result & Discussion

The statistical analysis of the data indicated that the period of storage, treatments of samples and interaction between period of storage and treatments of samples were significant. The difference between stages for addition of turmeric was non significant when fresh samples ghee was analyzed for flavour score on day one. The difference between two stages for addition of turmeric also remained non significant during entire storage period.

![Graph showing effect of stage of turmeric addition on sensory score of ghee during storage at 80 ± 2 °C](image)

**Figure 4.3. Effect of stage of turmeric addition on sensory score of ghee during storage at 80 ± 2 °C**

The results indicated that the initial flavour score of the control sample of ghee was slightly higher than that of the sample added with turmeric. During the storage the flavour score of control sample declined at a faster rate and reached below unacceptable level (< 6) on 5th day of the storage. Flavour scores of the turmeric added samples of ghee remained higher compared to that of the control sample. The flavour score of ghee samples decreased at the almost similar rate
when turmeric was added before or after clarification. In case of ghee samples added with turmeric the flavour score went below the acceptable level on 11th day of the storage, irrespective of the stage of addition (before or after clarification).

Table 4.4. Effect of stage of turmeric addition on peroxide unit value of ghee during storage at 80 ± 2°C

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>Samples of ghee</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>After Clarification</td>
<td>Before Clarification</td>
</tr>
<tr>
<td>1</td>
<td>1.60</td>
<td>1.57</td>
<td>1.65</td>
</tr>
<tr>
<td>3</td>
<td>1.87</td>
<td>2.10</td>
<td>2.12</td>
</tr>
<tr>
<td>5</td>
<td>7.35</td>
<td>4.35</td>
<td>6.15</td>
</tr>
<tr>
<td>7</td>
<td>8.55</td>
<td>6.14</td>
<td>7.47</td>
</tr>
<tr>
<td>9</td>
<td>17.55</td>
<td>14.20</td>
<td>11.72</td>
</tr>
<tr>
<td>11</td>
<td>25.77</td>
<td>18.87</td>
<td>17.15</td>
</tr>
</tbody>
</table>

ANOVA TABLE

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Storage period</th>
<th>Treatment (Stage of addition)</th>
<th>Storage period × Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.590</td>
<td>0.417</td>
<td>1.022</td>
</tr>
<tr>
<td>C. D.</td>
<td>1.677</td>
<td>1.186</td>
<td>2.904</td>
</tr>
<tr>
<td>Test</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>CV %</td>
<td></td>
<td></td>
<td>23.53</td>
</tr>
</tbody>
</table>

The statistical analysis of the data indicated that the period of storage, treatments of samples and interaction between period of storage and treatments of samples were significant. The difference between stages for addition of turmeric was non significant up to third day of the storage period when samples
of ghee was analyzed for peroxide value. However, the difference between two stages for addition of turmeric became significant during remaining storage period.

![Graph showing peroxide value over storage period](image)

**Figure 4.4. Effect of stage of turmeric addition on peroxide value of ghee during storage at 80 ± 2 °C**

The peroxide values (milli equivalents per kilogram) of the control ghee sample and the samples with added turmeric irrespective of stage of addition of turmeric 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 turmeric added ghee samples remained well below the control sample throughout the storage period. The peroxide values of the ghee samples with added turmeric before or after clarification remained almost at par throughout entire storage period. Thus, the results obtained for effect of addition of turmeric before or after the clarification of butterfat in to ghee are very well
collaborated with those obtained for the flavour of the corresponding sample as discussed above.

Thus two different stages of addition for turmeric were found to have similar effect on oxidative stability of ghee. The difference in effect of stage for turmeric addition on the relative performance of turmeric towards oxidative stability of ghee has not been reported.

Turmeric contains lipid soluble curcumin and water soluble turmeric (Sreejayan and Rao, 1994; Srinivas et al., 1992). Hence the additive effects are shown well by partitioning easily and spreading evenly well in favourable medium provided by cooking. Cooking helps the fish fat to get into the medium and solubilise the curcumin part of turmeric. Cooking also alters the physicochemical nature of the membranes thereby, the solubility and site of action is favoured by having more access to the radical and thus better activity.

In practical application practice for addition of turmeric before clarification of butter fat in to ghee may be adopted to avoid additional step of filtration, which otherwise required in case of turmeric addition after clarification. However, in present study if turmeric was added before clarification of butter fat in to ghee, some variation between the samples may arise due to chances of variation in clarification process. Therefore, in further study of present investigation the addition of turmeric after clarification was adopted to avoid any variation in flavour score due to difference which may
lightly occur during preparation of ghee, due to variation in control of clarification in different samples of ghee.

4.3 Selection of rate for turmeric addition in ghee

In the third phase of the study work was carried out to select the rate of turmeric addition in ghee for extending its oxidative stability during storage. As discussed under section 4.4 above it was decided to add turmeric after clarification of butter fat in to ghee in selection of its rate for the addition in ghee. For selection of the rate for turmeric addition in ghee, the fresh ghee samples were prepared in the laboratory from the white butter following the process as described under section 3.2. Each sample of ghee was divided in to six parts. The turmeric was added to the ghee at the rate of 0.2, 0.4, 0.6, 0.8 and 1.0 per cent. The sample of ghee without addition was kept as control sample. Total four replications were conducted.

The prepared samples of ghee were stored at 80°C ± 2 and monitored for changes in flavour score by subjecting the samples to sensory evaluation by panel of judges using 9 point hedonic scale at an interval of 7 days. The results obtained for effect of concentration of turmeric on changes in flavour score of ghee during storage are presented in Table 4.5 and graphically presented in Figure 4.5.
Figure 4.5. Effect of rate of turmeric addition of turmeric on flavour score of ghee during storage at 80 °C
Result & Discussion

Table 4.5. Effect of rate of turmeric addition of turmeric on flavour score of ghee during storage at 80 ± 2 °C

<table>
<thead>
<tr>
<th>Rate of turmeric addition (%)</th>
<th>Storage period (days)</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td></td>
<td>8.2</td>
<td>7.1</td>
<td>5.4</td>
<td>4.8</td>
</tr>
<tr>
<td>0.2</td>
<td></td>
<td>8.1</td>
<td>7.0</td>
<td>5.9</td>
<td>4.9</td>
</tr>
<tr>
<td>0.4</td>
<td></td>
<td>8.1</td>
<td>7.3</td>
<td>6.4</td>
<td>5.3</td>
</tr>
<tr>
<td>0.6</td>
<td></td>
<td>8.0</td>
<td>7.5</td>
<td>7.0</td>
<td>5.8</td>
</tr>
<tr>
<td>0.8</td>
<td></td>
<td>7.8</td>
<td>7.1</td>
<td>6.8</td>
<td>5.6</td>
</tr>
<tr>
<td>1.0</td>
<td></td>
<td>7.6</td>
<td>6.9</td>
<td>6.7</td>
<td>5.4</td>
</tr>
</tbody>
</table>

ANOVA TABLE

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Storage period (Concentration)</th>
<th>Storage period × Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.127</td>
<td>0.156</td>
</tr>
<tr>
<td>C. D.</td>
<td>0.360</td>
<td>-</td>
</tr>
<tr>
<td>Test</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>CV %</td>
<td>9.36</td>
<td></td>
</tr>
</tbody>
</table>

The statistical analysis of the data indicated that the period of storage has significant effect. However, concentration of turmeric addition to ghee samples and interaction between period of storage and concentration of turmeric addition to ghee samples were non significant.

The results indicated that the flavour score of control sample of fresh ghee was higher than that of the turmeric added ghee samples. The flavour score of ghee decreased gradually up to 0.6 per cent addition of turmeric in the ghee. However, score remained to highly acceptable level up to 0.6 per cent addition of
The results showed that the addition of turmeric in ghee at the rate of 0.6 per cent was most effective in retaining flavour score of ghee during storage. It is interesting to note that effectiveness of turmeric decreased in stabilizing ghee against oxidative stability when added at the rate > 0.6 per cent. The possible reason could be attributed to prooxidant effect of antioxidant...
components of turmeric when added at higher concentration. Similar effect for use of tocopherol at high concentration was reported by Frankel (1985). The author reported that natural tocol mixtures are usually used at levels up to 500 ppm. Above around 1000 ppm α-tocopherol acts as a proxidant. Another speculation for the low effect of turmeric at high concentration could be proxidant effect of lipid of turmeric when it is incorporated at higher concentration in ghee. The fresh dried rhizomes of turmeric contain 5-10 per cent fat (Spices board, 2002).

No data are reported in the literature for effect of rate of turmeric addition of turmeric on flavour score of ghee during storage at 80 ± 2 °C. The flavour score of the ghee remained highly acceptable on addition of turmeric up to 0.6 per cent. Moreover, addition of turmeric in ghee at the rate of 0.6 per cent found best in retaining the flavour score of ghee during storage. Therefore, this rate of addition turmeric was selected for further study in this investigation.

4.4 Analysis of fresh ghee samples for quality standards

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 4.6. The samples were also analyzed for peroxide value when fresh and also during the storage at interval of two days. The samples of ghee were also subjected to sensory evaluation for flavour score when fresh and also during the storage at interval of two days.
**Table 4.6.** Analysis of fresh ghee samples for quality standards

<table>
<thead>
<tr>
<th>Quality standards</th>
<th>Control</th>
<th>BHA added</th>
<th>Turmeric added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content (%)</td>
<td>0.17</td>
<td>0.19</td>
<td>0.18</td>
</tr>
<tr>
<td>B.R. reading at 40 °C</td>
<td>41.7</td>
<td>40.4</td>
<td>40.2</td>
</tr>
<tr>
<td>RM value</td>
<td>33.2</td>
<td>34.6</td>
<td>36.0</td>
</tr>
<tr>
<td>FFA content (%)</td>
<td>0.16</td>
<td>0.20</td>
<td>0.17</td>
</tr>
<tr>
<td>Baudouin test</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

The moisture content of ghee samples varied on an average from 0.17 to 0.19 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 41.7 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 to 36 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 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 FFA content of ghee samples varied on an average from 0.16 to 0.20 (unripened cream) per cent among different treatments. 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 of control, BHA added and turmeric added was found negative. Thus, all the samples fulfilled the legal standards for this test.
The above results indicated that all the prepared samples of ghee in this study viz. control, BHA added and turmeric 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 and turmeric added fulfilled the legal requirements for all the quality standards prescribed under Agmark. The addition of turmeric from orange variety at the rate of 0.6 per cent did not affect quality standard as prescribed under PFA and Agmark standard. Replication vise analysis of fresh ghee samples for quality standards are given below.

4.5 Comparison of turmeric with BHA as an antioxidant in ghee

After selecting variety of turmeric, stage for addition of the turmeric during preparation of ghee and concentration of turmeric for addition in the ghee; in final phase of this study work was carried out to compare effectiveness of the turmeric in enhancing oxidative stability of ghee during storage with synthetic antioxidant legally permitted for addition in ghee. In India BHA is permitted under PFA for addition in ghee as an antioxidant at the rate of 0.02 per cent. Therefore, in present study effect of 0.6 per cent addition of orange variety of turmeric was made with addition of 0.02 per cent BHA in ghee.

For comparison of turmeric with BHA as an antioxidant, ghee was prepared from butter following the method as described under section 3.2. The samples of ghee were divided in to three parts. In one part of the ghee the orange
turmeric was added at the rate of 0.6 per cent immediately after filtration, sample was thoroughly mixed and filtered with four folded muslin cloth when temperature reached to 65 °C. In second part of the ghee BHA was added at the rate of 0.02 per cent and thoroughly mixed to dissolve the BHA. The sample of ghee without any addition kept as a control. The samples of ghee were stored at 80 ± 2 °C. Four replications were taken for this investigation.
Table 4.7 Effect of comparison between turmeric and BHA on flavour score for enhancing oxidative stability of ghee

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>Samples of ghee</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>BHA</td>
<td>Turmeric</td>
</tr>
<tr>
<td>1</td>
<td>8.0</td>
<td>8.12</td>
<td>8.2</td>
</tr>
<tr>
<td>3</td>
<td>8.12</td>
<td>8.12</td>
<td>8.12</td>
</tr>
<tr>
<td>5</td>
<td>6.62</td>
<td>7.62</td>
<td>7.37</td>
</tr>
<tr>
<td>7</td>
<td>5.75</td>
<td>7.25</td>
<td>7.0</td>
</tr>
<tr>
<td>9</td>
<td>4.75</td>
<td>6.37</td>
<td>5.87</td>
</tr>
<tr>
<td>11</td>
<td>4.50</td>
<td>6.12</td>
<td>5.75</td>
</tr>
<tr>
<td>13</td>
<td>3.75</td>
<td>5.37</td>
<td>5.12</td>
</tr>
</tbody>
</table>

ANOVA TABLE

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Storage period</th>
<th>Treatment (BHA vs Turmeric)</th>
<th>Storage period × Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.177</td>
<td>0.116</td>
<td>0.307</td>
</tr>
<tr>
<td>C. D</td>
<td>0.501</td>
<td>0.328</td>
<td>-</td>
</tr>
<tr>
<td>Test</td>
<td>*</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>CV %</td>
<td></td>
<td></td>
<td>9.34</td>
</tr>
</tbody>
</table>

The statistical analysis of the data indicated that the period of storage and treatments of samples were significant. However, interaction between period of storage and treatments of samples was non significant. The difference between BHA added and turmeric added ghee samples was non significant when samples of ghee was analyzed for flavour score up to seventh day of storage. However, the difference between BHA added and turmeric added ghee samples become significant during further storage period.
The results indicated that the initial flavour score of the all three samples of ghee viz. control, BHA added and turmeric added were almost similar up to third day of storage period. Flavor score of control sample decline at a faster rate and reached below unacceptable level (< 6) on between 5th and 7th day of the storage. Flavour scores of the turmeric added samples of ghee remained higher compare to that of the control sample. The flavour score of turmeric added ghee sample decreased below acceptable level on 9th day of the storage and that of thee BHA added ghee sample went below the acceptable level between 11th and 13th day of storage.
Result & Discussion

Table 4.8 Effect of turmeric and BHA on peroxide value of ghee during storage at 80 ± 2 °C

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>Samples of ghee</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>BHA</td>
<td>Turmeric</td>
</tr>
<tr>
<td>1</td>
<td>0.32</td>
<td>0.30</td>
<td>0.32</td>
</tr>
<tr>
<td>3</td>
<td>1.75</td>
<td>1.65</td>
<td>1.55</td>
</tr>
<tr>
<td>5</td>
<td>4.00</td>
<td>2.32</td>
<td>3.20</td>
</tr>
<tr>
<td>7</td>
<td>11.05</td>
<td>3.10</td>
<td>5.67</td>
</tr>
<tr>
<td>9</td>
<td>11.92</td>
<td>4.10</td>
<td>6.55</td>
</tr>
<tr>
<td>11</td>
<td>12.95</td>
<td>4.47</td>
<td>7.37</td>
</tr>
<tr>
<td>13</td>
<td>14.10</td>
<td>4.97</td>
<td>7.62</td>
</tr>
</tbody>
</table>

ANOVA TABLE

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Storage period</th>
<th>Treatment (BHA vs Turmeric)</th>
<th>Storage period x Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.241</td>
<td>0.158</td>
<td>0.418</td>
</tr>
<tr>
<td>C. D</td>
<td>0.682</td>
<td>0.447</td>
<td>1.182</td>
</tr>
<tr>
<td>Test</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>CV %</td>
<td></td>
<td></td>
<td>16.05</td>
</tr>
</tbody>
</table>

The statistical analysis of the data indicated that the period of storage, treatments of samples and interaction between period of storage and treatments of samples were significant. The difference between BHA added and turmeric added ghee samples was non significant when samples of ghee was analyzed for peroxide value (milli equivalents per kilogram) up to third day of storage. However, the difference between BHA added and turmeric added ghee samples become significant during further storage period.
The peroxide values of the control ghee sample and the samples with added turmeric irrespective of stage of addition of turmeric 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 turmeric added ghee samples remained well below the control sample throughout the storage period. The peroxide values of the ghee samples with added turmeric before or after clarification remain almost at par throughout entire storage period. Thus, the results obtained for effect of addition of turmeric before or after the clarification of butterfat in to ghee are very well collaborated with those obtained for the flavour of the corresponding sample as discussed above.
No data are reported in the literature for effect of addition of turmeric on peroxide value of ghee during storage.

D’Souza and Ramachandra Prabhu (2006) studied the effects of turmeric on fish lipid peroxidation during standard cooking practices and on time-dependent changes in the peroxidation of fish homogenate. The antioxidant effect of α-tocopherol was also studied to confirm the relevance of the study. The results suggest that turmeric may be considered as a safe, cheap and readily usable antioxidant for food preparations. Effects of turmeric on lipid peroxidation on raw fish homogenate and cooked fish homogenate in saline phosphate buffer were evaluated by the authors. Homogenate when treated with turmeric and was cooked in phosphate buffer saline at 100 °C for 15 minutes showed highly significant decrease (P<0.01) in peroxide level as compared to control. It is seen that fish homogenate treated with turmeric showed lipid peroxide level of 16.7±0.1 m mole MDA/gm tissue to 17.9±0.1 nmole MDA/gm tissue, while in control the level of peroxides have increased from 17.16 ± 0.1nmole MDA/gm tissue to 25.06 ± 0.1nmoleMDA/gm tissue (p<0.01) over a four hour incubation period. α-tocopherol exhibited a similar pattern with 16.50±0.02nmole MDA/gm tissue to 18.67±0.3 nmole MDA/gm tissue as compared to control (p <0.01) over the four hour intervals. Turmeric treated group exhibited profound reduction in lipid peroxides formed which was time-dependent becoming evident from the second hour onward. Turmeric reduced the lipid peroxides hourly by 2.2%, 8%, 13.6%, 20.5%, and 28.5% respectively as
compared to controls. Vitamin E treated homogenate group showed reduction of lipid peroxides hourly as 3.8%, 8%, 15%, 18.9%, and 25.4% respectively as compared to controls. The author concluded that turmeric with the active curcuminoids and water soluble turmeric has antioxidant properties and hence effectively inhibits the free radical damage to biomolecules. The fact that turmeric acts as an antioxidant by prevention and intervention processes makes it very unique as a natural antioxidant.

Antiperoxidant effects of curcumin on several natural lipids of microsomes, brain lipids and synthetic lipids on liposomes are well established (Tonnesen et al., 1993). Studies have shown that ingestion of turmeric reduced the levels of lipid peroxides and resulted in higher activities of superoxide dismutase, catalase and glutathione peroxidase in liver homogenate (Reddy and Lokesh, 1992 and 1994). Studies have shown that curcumin is a potent inhibitor of lipid peroxidation catalysed by iron and its chelates in rat brain homogenate and rat liver microsomes (Sreejayan Rao, 1993).

Curcuminoids are potent inhibitors of experimentally induced lipid peroxidation as that of α-tocopherol (Sreejayan, and Rao, 1994). Curcumin has been found to be more potent than α-tocopherol as an antioxidant in the inhibition of lipid peroxidation of rat liver microsomes (Sharma, 1976). Antioxidant activity of turmeric and α-tocopherol is mainly attributed to the phenolic group. The phenolic group provides a labile hydrogen atom for
abstraction by free radical like peroxyl or alkoxy radicals and gets converted to phenoxy radical. The antioxidant potency depends on the stability and reactivity of this phenoxy radical (Roberfroid, et al., 1987). In α-tocopherol the phenoxy radical is stabilized by steric and electronic factors (Bareclay et al., 1993). In curcumin, the resonance is stabilized due to several possible tautomeric forms, along the extended conjugated system of double bonds (Priyadarshini, 1997).

Gordon (2001) reported that the Peroxide value at which oxidation of oils can be detected as an off-flavour 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-flavours 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 10. 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 favor scores. For soybean oil, a PV of 1.0 or less indicates freshness; 1 to 5 PV, low oxidation; 5 to 10 PV, moderate oxidation; >10 PV, high oxidation; and >20 PV, poor flavour. 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 flavour because of the transitory nature of peroxides and their breakdown to non peroxide materials. Although a linear relationship has been observed between peroxide values and favor scores during the initial stages of lipid oxidation.

It can be concluded from this study that turmeric with the active curcuminoids have antioxidant properties and hence effectively inhibits the free radical damage to food components such as lipids. The fact that turmeric acts as an antioxidant by prevention and intervention processes makes it very unique as a natural antioxidant.
Ghee is by far the most ubiquitous indigenous milk product. It is a rich source of energy, fat soluble vitamins, essential fatty acids and pleasing flavour. However, it undergoes oxidative deterioration. Therefore, constant research endeavors are made to extend the shelf-life by various approaches. One the most common approaches is addition of antioxidants. Turmeric is reported to inhibit oxidative rancidity in several food products. In addition turmeric has plethora of health effect benefits. However, the potential of turmeric has not been trapped as a natural antioxidant for preventing oxidative rancidity in ghee. The antioxidant/pro-oxidant action of turmeric on lipid oxidation has been of interest in food lipids. In literature conflicting opinions are prevailing about antioxidant potential of turmeric, but not proved much in food systems.

No attempts have been made to trap potential of turmeric as a natural antioxidant for preventing oxidative rancidity in ghee. Therefore, present study was planed to study the antioxidant properties of turmeric to extend the shelf life of ghee. The study was divided into four phases. In the first phase two different varieties of turmeric was evaluated for their antioxidant activity in ghee. In the second phase, selection for stage of addition of turmeric was studied. In third phase rate of addition of the turmeric was tested. In fourth phase comparison of
Summary and Conclusion

turmeric with the permitted synthetic antioxidant (Butylated Hydroxy Anisole) was made.

5.1 Selection of turmeric variety

The Alleppey turmeric is orange in colour, whereas, the Madras turmeric is yellow in colour. Addition of turmeric in ghee to enhance shelf life of the ghee, an important point for consideration was the selection of variety of turmeric. Therefore, in first phase samples of turmeric powder obtained from its two varieties viz. Madras and Alleppey was evaluated for their ability to prevent oxidative deterioration of ghee.

In the selection of variety the turmeric powder of each variety was added to the samples of ghee at the rate of 0.5 per cent. For this study four fresh samples of ghee were wormed up to 65 °C and divided in to three parts. In first part turmeric from yellow variety was added at the rate of 0.5 per cent, in second part turmeric from orange variety was added at the rate of 0.5 per cent and the third part was kept as such to serve as control. All the samples of ghee were stored at elevated temperature (80 °C ± 2) to accelerate the oxidation. The samples of ghee were subjected to sensory evaluation for flavour using 9 point hedonic scale. The samples of ghee were also analyzed for changes in peroxide value during the storage.

The results for sensory evaluation indicated that the period of storage, treatments of samples and interaction between period of storage and treatments
Summary and Conclusion

of samples were significant. The difference between yellow and orange varieties of turmeric was non significant when fresh samples ghee was analyzed for flavour score on day one. However, the difference between two varieties became significant during storage from day two and remains significant all throughout the storage period.

The results for sensory evaluation also indicated that the initial flavour score of the control sample of ghee was slightly lower than that of the sample added with turmeric. During the storage the flavour score of control sample declined at a greater rate and reached below unacceptable level (< 6) on 9th day of the storage. Flavour scores of the turmeric added samples of ghee remained higher compared to that of the control sample. The flavour score of ghee sample added with yellow turmeric went below the acceptable level on 13th days of storage, where as, flavour score of ghee sample added with orange turmeric went below the acceptable level on 15th days of storage. Thus, among the two varieties of turmeric the orange variety gave better result which extended the flavour score to acceptable level by two days.

The results for changes in peroxide value indicated that the period of storage, treatments of samples and interaction between period of storage and treatments of samples were significant. The difference between yellow and orange varieties of turmeric was non significant when fresh samples of ghee was analyzed for flavour score up to ninth day. However, the difference between two
Summary and Conclusion

varieties became significant during storage from eleventh day and remained significant during further storage period.

During accelerated storage, the peroxide value (milliequivalents/kg) of control sample increased at a rapid rate compared to the samples of ghee added with turmeric. The peroxide value of ghee samples containing orange turmeric remained the lowest almost throughout the period of storage, except slightly higher during the last a few days of extended storage. The peroxide value of ghee samples containing yellow turmeric remained almost similar to that of the samples of ghee containing orange turmeric up to 11 days of the storage. However, on further storage the rate of increase in peroxide value of ghee samples containing yellow turmeric remained higher than that of the samples of ghee containing orange turmeric. Thus the performance of turmeric of orange variety in controlling the oxidative deterioration of ghee was better than the turmeric of yellow variety.

On the basis of relative performance to delay oxidative deterioration of ghee, in the present study orange variety was selected for further study.

4.2 Selection of stage for turmeric addition in ghee

From examination of manufacturing process for ghee two possible ways were envisaged viz before heat clarification of butter in to ghee and after heat clarification of butter in to ghee. In addition of turmeric at any of these stage some advantages and limitations appeared to be associated.
Therefore, in the present investigation work was carried out to select stage for addition of turmeric to enhance the shelf life of ghee as second phase of the study. To find out the effect of stage for turmeric addition in ghee on oxidative stability of the ghee, turmeric was added at two different stages of the ghee preparation. In each set of experiments turmeric was added at the rate of 0.5 per cent. In one set of experiment, turmeric added before heat clarification of butter fat in to ghee. The sample of butter was taken in to clarification pan and the turmeric was added when butter get melted. After clarification of butter fat in to ghee, the prepared sample of ghee was filtered through muslin cloth. In second set of the experiment, turmeric was added after filtration of heat clarified butter fat (ghee) at 65 °C, the samples was thoroughly mixed with the help of dry glass rod for uniform mixing of the turmeric, followed by filtration through four folded muslin cloth. The samples of ghee without addition of turmeric were also prepared to serve as control samples. Total four replications were conducted. The prepared samples of ghee were subjected to sensory evaluation by panel of judges using 9 point hedonic scale when fresh and after the interval of every three days of storage. The samples were also analyzed for peroxide value when fresh and after the interval of two days of storage.

The results obtained for changes in flavour score indicated that the period of storage, treatments of samples and interaction between period of storage and treatments of samples were significant. The difference between stages for addition of turmeric was non significant when fresh samples of ghee were
Summary and Conclusion

analyzed for flavour score on day one. The difference between two stages for addition of turmeric also remained non significant during entire storage period. The changes in flavour score also indicated that the initial flavour score of the control sample of ghee was slightly higher than that of the sample added with turmeric. During the storage the flavour score of control sample declined at a faster rate and reached below unacceptable level (< 6) on 5th day of the storage. Flavour scores of the turmeric added samples of ghee remain higher compare to that of the control sample. The flavour score of ghee samples decreased at the almost similar rate when turmeric was added before or after clarification. In case of ghee samples added with turmeric the flavour score went below the acceptable level on 11th day of the storage, irrespective of the stage of addition (before or after clarification).

The results obtained for changes peroxide value ghee indicated that the period of storage, treatments of samples and interaction between period of storage and treatments of samples were significant. The difference between stages for addition of turmeric was non significant up to third day of the storage period when ghee samples were analyzed for peroxide value. However, the difference between two stages for addition of turmeric became significant during remaining storage period. The peroxide values (milli equivalents per kilogram) of the control ghee sample and the samples with added turmeric irrespective of stage of addition of turmeric were more or less similar during first three days of storage. On further storage peroxide values of control ghee sample increased
Summary and Conclusion

with very faster rate. The peroxide value of turmeric added ghee samples remained well below the control sample throughout the storage period. The peroxide values of the ghee samples with added turmeric before or after clarification remain almost at par throughout entire storage period. Thus, the results obtained for effect of addition of turmeric before or after the clarification of butterfat in to ghee are very well collaborated with those obtained for the flavour of the corresponding sample as discussed above.

Thus two different stages of addition for turmeric were found to have similar effect on oxidative stability of ghee. In practical application practice for addition of turmeric before clarification of butter fat in to ghee may be adopted to avoid additional step of filtration, which otherwise required in case of turmeric addition after clarification. However, in present study if turmeric was added before clarification of butter fat in to ghee, some variation between the samples may arise due to chances of variation in clarification process. Therefore, in further study of present investigation the addition of turmeric after clarification was adopted to avoid any variation in flavour score due to difference which may lightly occur during preparation of ghee, due to variation in control of clarification in different samples of ghee.

4.3 Selection of rate for turmeric addition in ghee to increase its oxidative stability

In the third phase of the study work was carried out to select the rate of turmeric addition in ghee for extending its oxidative stability during storage. For
selection of the rate for turmeric addition in ghee, the fresh ghee samples were prepared in the laboratory from the white butter following the process as described under section 3.2. Each sample of ghee was divided into six parts. The turmeric was added to the ghee at the rate of 0.2, 0.4, 0.6, 0.8 and 1.0 per cent. The sample of ghee without addition of ghee was kept as control sample. Total four replications were conducted. The prepared samples of ghee were stored at 80°C ± 2 and monitored for changes in flavour score by subjecting the samples to sensory evaluation by panel of judges using 9 point hedonic scale at an interval of 7 days.

The statistical analysis of the data indicated that the period of storage has significant effect. However, concentration of turmeric addition to ghee samples and interaction between period of storage and concentration of turmeric addition to ghee samples were non-significant.

The results indicated that the flavour score of control sample of fresh ghee was higher than that of the turmeric added ghee samples. The flavour score of ghee decreased gradually up to 0.6 per cent addition of turmeric in the ghee. However, score remained to highly acceptable level up to 0.6 per cent addition of turmeric. On further increase in rate of turmeric addition in ghee resulted in sharp declined in flavour score of fresh ghee samples.

On storage of ghee samples at 80 ± 2°C the flavour score of control ghee sample decreased sharply and went below acceptable on 11th day of storage. On
the other hand flavour score of ghee samples added with 0.2, 0.4, 0.6, 0.8, 1.0 per cent turmeric went below acceptable level on 13th, 15th, 17th, and 18th day of storage respectively. Thus, the results indicated that the addition of turmeric in ghee at the rate of 0.6 per cent was most effective in retaining flavour score of ghee during storage.

It was noted that effectiveness of turmeric decreased in stabilizing ghee against oxidative stability when added at the rate > 0.6 per cent. The flavour score of the ghee remained highly acceptable on addition of turmeric up to 0.6 per cent. Moreover, addition of turmeric in ghee at the rate of 0.6 per cent found best in retaining the flavour score of ghee during storage. Therefore, this rate of addition turmeric was selected for further study in this investigation.

4.4 Analysis of fresh ghee samples for quality standards

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. The samples were also analyzed for peroxide value when fresh and also during the storage at interval of two days. The samples of ghee were also subjected to sensory evaluation for flavour score when fresh and also during the storage at interval of two days.

The moisture content of ghee samples varied on an average from 0.17 to 0.19 per cent among different treatments. The B.R. reading of ghee samples at 40 °C varied on an average from 40.2 to 41.7 among different treatments. The RM
value of ghee samples varied on an average from 33 to 36 among different treatments. The FFA content of ghee samples varied from 0.16 to 0.20 per cent. The Baudouin test of control, BHA added and turmeric added was found negative.

The above results indicated that all the prepared samples of ghee in this study viz. control, BHA added and turmeric 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 and turmeric added fulfilled the legal requirements for all the quality standards prescribed under Agmark. The addition of turmeric from orange variety at the rate of 0.6 per cent did not affect quality standard as prescribed under PFA and Agmark standard.

4.5 Comparison of turmeric with BHA as an antioxidant in ghee

After selecting variety of turmeric, stage for addition of the turmeric during preparation of ghee and concentration of turmeric for addition in the ghee; in final phase of this study work was carried out to compare effectiveness of the turmeric in enhancing oxidative stability of ghee during storage with synthetic antioxidant legally permitted for addition in ghee. In India BHA is permitted under PFA for addition in ghee as an antioxidant at the rate of 0.02 per cent. Therefore, in present study effect of 0.6 per cent addition of orange variety of turmeric was made with addition of 0.02 per cent BHA in ghee.
Summary and Conclusion

For comparison of turmeric with BHA as an antioxidant, ghee was prepared from butter following the method as described under section 3.2. The samples of ghee were divided into three parts. In one part of the ghee the orange turmeric was added at the rate of 0.6 per cent immediately after filtration, sample was thoroughly mixed and filtered with four folded muslin cloth when temperature reached to 65 °C. In second part of the ghee BHA was added at the rate of 0.02 per cent and thoroughly mixed to dissolve the BHA. The sample of ghee without any addition was kept as a control. The samples of ghee were stored at 80±2°C. Four replications were taken for this investigation.

The results for changes in flavour score of ghee indicated that the period of storage and treatments of samples were significant. However, interaction between period of storage and treatments of samples was non significant. The difference between BHA added and turmeric added ghee samples was non significant when samples of ghee was analyzed for flavour score up to seventh day of storage. However, the difference between BHA added and turmeric added ghee samples become significant during further storage period. The initial flavour score of the all thee samples of ghee viz. control, BHA added and turmeric added were almost similar up to third day of storage period. Control sample declined at a faster rate and reached below unacceptable level (< 6) on between 5th and 7th day of the storage. Flavour scores of the turmeric added samples of ghee remained higher compared to that of the control sample. The
flavour score of turmeric added ghee sample decreased below acceptable level on 9\textsuperscript{th} day of the storage and that of thee BHA added ghee sample went below the acceptable level between 11\textsuperscript{th} and 13\textsuperscript{th} day of storage.

The results for changes in peroxide value of ghee indicated that the period of storage, treatments of samples and interaction between period of storage and treatments of samples were significant. The difference between BHA added and turmeric added ghee samples was non significant when samples of ghee was analyzed for peroxide value (milli equivalents per kilogram) up to third day of storage. However, the difference between BHA added and turmeric added ghee samples become significant during further storage period. The peroxide values of the control ghee sample and the samples with added turmeric irrespective of stage of addition of turmeric 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 turmeric added ghee samples remained well below the control sample throughout the storage period. The peroxide values of the ghee samples with added turmeric before or after clarification remained almost at par throughout entire storage period. Thus, the results obtained for effect of addition of turmeric before or after the clarification of butterfat in to ghee are very well collaborated with those obtained for the

From findings of the study it can be concluded that turmeric with the active curcuminoinds have antioxidant properties and hence effectively inhibits
Summary and Conclusion

the free radical damage to food components such as lipids. The fact that turmeric acts as an antioxidant by prevention and intervention processes makes it very unique as a natural antioxidant.
References


References


References


References


References


Spices Board (2002). Quality requirements of spices for export.


References


www.agmarknet.in/gheegmr.pdf