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Evaluation of Liquorice (*Glycyrrhiza glabra*) for Enhancing Shelf-Life of Ghee against Oxidative Deterioration

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A B S T R A C T

Liquorice has been widely appreciated as an important medicinal plant. Its rhizomes and roots have been used for centuries in traditional medicine. Liquorice was used as an additive in ghee and its antioxidant potential was evaluated to enhance the shelf-life of ghee. Liquorice was analysed for its total phenolic content and radical scavenging activity. Liquorice contained 1030.00±69.76 mg GAE/100g total phenolic content and gave 83.20±1.29 % DPPH radical scavenging activity. Addition of liquorice at initial stage of heat clarification was found more effective than at final stage of heat clarification. Optimum rate for use of liquorice in treatment of ghee was found 0.3 per cent. Liquorice was found to be capable of retarding oxidative degradation in ghee and even more effective than BHA.

Introduction

Ghee under ambient conditions of storage undergoes oxidative deterioration. Addition of synthetic antioxidants is common approach to extend shelf-life of ghee, but use of synthetic antioxidants which posse potential health risk necessitated attention towards natural antioxidants. Spices are considered very good sources of natural antioxidants, but very limited work has been reported for utilization of spices as a possible antioxidant in ghee. Plants produce a variety of phenolic antioxidants. Among the various phenolic compounds, the flavonoids are perhaps the most important group (El-sherif *et al.*, 2011; Rice-Evans *et al.*, 1995). Flavonoids are components of a wide variety of edible plants, fruits, vegetables and grains, and are an integral part of the human diet (Berge and Daniel, 1988). Spices and herbs are added to food, not only for flavor, but also for preservation (Patel *et al.*, 2013). *Glycyrrhiza glabra*, also known as licorice and sweet wood, is native to the Mediterranean and certain areas of Asia. Historically, the dried rhizome and root of this plant were employed medicinally by the Egyptian, Chinese, Greek, Indian and Roman civilizations as an expectorant and carminative. *Glycyrrhizaglabra Linn* isahar dyperennial shrub, attaining a height upto 2.5m. The

taproot is approximately 1.5 cm long and subdivides into 3-5 subsidiary roots, about 1.25 cm long, from which the horizontal woody stolons arise. These may reach 8 m and when dried and cut, together with the root, constitute commercial liquorice. It may be found peeled or unpeeled. The pieces of root break with a fibrous fracture, revealing the yellowish interior with a characteristic odour and sweet taste (Roshan *et al.*, 2012).

The roots of *Glycyrrhiza glabra* Linn contain glycyrrhizin, which is a saponin glycoside that is 60 times sweeter than cane sugar. More than 300 flavonoids have been isolated from Glycyrrhizin species. These flavonoids belong to various types, including flavanones or flavanols, chalcones, isoflavans, isoflavenes, flavones or flavonols, isoflavones and isoflavanones (Karahan *et al.*, 2016). Flavonoid rich fractions include eliquirtin, isoliquertin liquiritigenin and rhamnoliquirilin and five new flavonoids-glucoliquiritinapio-side, prenyllicoflavone A, shinflavanone, shinpterocarpinand 1-methoxyphaseolin[3,4] isolated from dried roots (Visavadiya *et al.*, 2009). The presence of many volatile components such as pentanol, hexanol, linalooloxide A and B, tetramethylpyrazine, terpinen-4-ol,α-terpineol, geraniol and other sintherootsis reported. Presence of propionic acid, benzoicacid, ethyl linoleate, methyle-thylketine, 2, 3-butanediol, furfuraldehyde, furfurylformate,1-methyl-2 formylpyrrole, trimethylpyrazie, maltol and anyother compounds is also isolated from the essential oil (Roshan *et al.*, 2012).

Several researches concluded that volatile components in essential oils and phenolic constituents from roots of liquorice inhibited the growth of a range of micro-organisms and inhibition of lipid peroxidation. Liquorice is useful in conventional and naturopathic medicine for both mouth ulcers and peptic ulcers. It is also useful to treat cough, bronchitis, ulceration of urinary tract and anaemia (Rajurkar and Hande, 2011; Karahan *et al.*, 2016). Some limited work is reported for utilization of their extracts prepared in organic solvents in ghee (Patel *et al.*, 2013). Minor components are critical to the activity due to their synergistic effect might be lost during preparation of extracts using organic solvent. Even addition of small amount of extracts gave high pungency to the product, but no work is reported for utilization of liquorice as such in ghee.

This research aims to evaluate the potential of liquorice (*Glycyrrhiza glabra*) for enhancing shelf-life of ghee against oxidative deterioration. Keeping this idea as a central goal, the study was divided into four phases (a) Assess the compatibility of liquorice as an additive in ghee, (b) select the stage of addition of liquorice in preparation of ghee, (c) optimize the rate of liquorice in preparation of ghee, (d) comparison of liquorice with synthetic antioxidant (BHA).

Materials and Methods

Chemicals and glassware

All the chemicals and glassware used in the present study were of analytical (AR) grade and standard quality supplied by authorized dealers.

Collection and preparation of plant material

Liquorice (*Glycyrrhiza glabra*) was brought from the local market of Anand, Gujarat, India. They were converted into coarse size particles and transferred in to zip lock plastic cover, then put them in air tight plastic bottle and stored at refrigeration temperature. Fresh and dried roots of liquorice are shown in Plate 1.

Evaluation of liquorice for its antioxidant potential

Preparation of extract

0.5 g powder of liquorice was treated with 10 ml of methanol-water (8:2, v/v) in a shaking water bath at 35°C for 24 h as described by Song *et al.*, (2010). The mixture was then centrifuged at 4,000 rpm for 10 min. The supernatant (liquorice extract) was recovered for the determination of the total phenolic content and radical scavenging activity.

Analysis of total phenolic content

Total phenolic content of liquorice extract was analysed by Folin-Ciocalteu (FC) reagent according to the procedure described by Singleton and Rossi (1965). 0.05 ml sample of liquorice extract was taken in a test tube and volume was made up to 1 ml with distilled water. To this 0.5 ml each of diluted FC reagent (1:1) and 10 ml 7.5 % sodium carbonate solution were added. The contents were mixed using vortex mixer. This was incubated under dark at room temperature for 30 min. For blank preparation 1 ml of distilled water was taken instead of sample. The absorbance was measured against blank at 750 nm using spectrophotometer. The result was expressed in terms of mg of GAE per 100 gm of dried liquorice.

Radical-scavenging activity by DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) assay

The radical-scavenging activity of liquorice extract was determined as the ability to scavenge DPPH radicals according to the procedure of Brand-Williams *et al.*, (1995). 0.05 ml of sample of liquorice extract was taken in a test tube and the volume was made up to 1 ml with methanol. 3 ml of 0.1 mM methanolic solution of DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand at 37^{o}C for 15 min. in a water bath. The control was prepared by taking 1 ml methanol and 3 ml of 0.1 mM methanolic DPPH solution. Methanol was used (as blank) for the baseline correction. Absorbance of the control and sample were measured at 517 nm using a spectrophotometer against blank (methanol). DPPH activity was expressed as the inhibition percentage and was calculated using the following formula.

Radical-scavenging activity (% inhibition) = [(Ac – A)/Ac] x 100

Where, Ac = Absorbance of control and A = Absorbance of sample

Preparation of ghee and addition of liquorice

White butter was procured from a commercial dairy plant of Amul Dairy, Anand. Ghee was prepared by creamery butter method described by De (2004). Ghee was clarified at 120^{o}C temperature. Prepared ghee was divided into two portions of 100g each. To portion 1 nothing was added which served as control. To the second portion, liquorice was added at the rate of 0.5% (w/w) respectively. The mixtures of ghee and liquorice were thoroughly mixed using glass rod and allowed to stand at 80^{o}C for 30 min. in a hot air oven. The treated ghee samples were filtered through 6 layers of muslin cloth. The ghee samples were assessed for its compatibility in ghee stored. The colour characteristic of ghee samples was also observed.

Selection of stage to add liquorice in preparation of ghee

For selection of stage for addition of liquorice in preparation of ghee, liquorice was added at two different stages during manufacture of ghee.

(1) Addition at the initial stage of heat clarification of butter (in melted butter)

(2) Addition at the final stage -of heat clarification of butter (105^{o}C temperature)

For evaluating two different stages in preparation of ghee (initial stage of clarification and final stage of clarification) for treatment with the liquorice, butter sample (120 g) was taken in to each of three of 500 ml glass beakers.

The beakers containing butter were arranged in round shaped sand bath at equal distance from the centre of the sand bath. Sand bath was heated by gas fired burner.

When butter was melted completely liquorice was added in 1 beaker. In another beaker liquorice was added when their temperature reached to 105°C (nearer to heat clarification). One sample was not treated with any liquorice to serve as a control.

The heating was continued till temperature reached to 120°C.In the entire process of ghee preparation each samples were mixed with stainless steel spatula and then content of each beaker was filtered through 6 folded muslin cloth, ghee was collected in 150 ml glass beakers and stored in incubator at 80°±2°C. Sand bath was used to prepare ghee samples simultaneously under similar and uniform heating conditions. Total four replications were conducted.

The ghee samples were analysed for peroxide value when fresh and at an interval of 2 days for 12 days. Simultaneously, the samples were also monitored for changes in flavour score by sensory evaluation using 9-point hedonic scale. The stage for addition of liquorice giving better stability against oxidative deterioration of ghee was selected for further study.

Optimization of rate for addition of liquorice in preparation of ghee

Liquorice was added according to results obtained from the selected stage at the rate of 0.1, 0.2, 0.3 and 0.4% (expected yield of ghee from butter). Liquorice treated ghee was prepared as per above method. The sample of ghee without addition of liquorice was also prepared to serve as control. All the 5 ghee samples were stored in an incubator at 80º±2ºC. The ghee samples were analysed for peroxide value when fresh and at an interval of 2 days for 12 days then after at an interval of 1 day for 22 days. Simultaneously, the samples were also monitored for changes in flavour score by sensory evaluation using 9-point hedonic scale. Total three replications were conducted. The rate at which liquorice giving better stability against oxidative deterioration of ghee was selected for further study.

Comparison of liquorice with synthetic antioxidant (BHA)

In this phase, ghee samples were prepared with liquorice at the selected stage and optimized rate on the basis of expected yield of ghee from butter as per procedure described in phase b (selection of stage). Simultaneously, butylated hydroxyl anisole (BHA) was added directly into the freshly prepared ghee at the rate of 0.02% (w/w). The sample of ghee without addition of liquorice served as a control. All the 3 ghee samples were stored in an incubator at 80º±2ºC.

Oxidative changes taking place in ghee were monitored by analysing the ghee samples for peroxide value when fresh and at an interval of 2 days for 22 days. Simultaneously, the samples were also monitored for changes in flavour score by sensory evaluation using 9-point hedonic scale. Total three replications were conducted.

Determination of oxidative changes in ghee

Peroxide value of ghee

The peroxide value of ghee was determined by the method (Iodometric method) as described in IS: SP: 18 (part XI) (1981).

Sensory evaluation of ghee

All samples of ghee made in the laboratory were evaluated for their sensory characteristics on a 9-point hedonic scale by a panel of 9 experienced judges. Sensory evaluation was developed considering the rancidity of ghee. The nine experienced judges, who were familiar with rancidity (off-flavour) of ghee, were academic staff aged 30 to 56 years. Each judge evaluated the ghee for flavour score (i.e. rancidity) using the 9-point hedonic scale.

Statistical analysis

The collected data were subjected to statistical analysis. Data were analysed by completely randomized design and critical difference test at 5% level of significance ($p<0.05$) as per the procedure mentioned by Steel and Torrie (1980).

Results and Discussion

Assessing the compatibility of liquorice as an additive in ghee

The first and foremost point for consideration was the compatibility of liquorice to be used for study. Liquorice was added at the rate of 0.5% w/w of ghee and then the compatibility of liquorice added ghee sample was assessed based on their flavour. Colour characteristic of liquorice added ghee sample was also examined by visual observation. The results indicated that both the samples of ghee treated with liquorice were found acceptable for their flavour in sensory evaluation by members of the panel of the 9judges.

It was evident from the examination of the colour characteristic of ghee samples that both the ghee samples (liquorice added ghee sample + control ghee sample) acquired golden yellow colour. Colour of liquorice did not impart any objectionable colour to the ghee. The colour characteristic of the fresh ghee samples (control ghee and liquorice added ghee) is presented in Plate 2.

Evaluation of liquorice for its antioxidant potential

Since use of liquorice was found compatible in ghee, it was evaluated for its antioxidant potential. To evaluate the antioxidant potential, liquorice was analysed for its total phenolic content and radical scavenging activity. Total three replications were conducted. Liquorice contained 1030.00 ± 69.76mg GAE/100 g total phenolic content and gave 83.20 ± 1.29% DPPH radical scavenging activity.

Tupe *et al.*, (2013) found that methanolic extract of liquorice contained 71.29±4.67 mg GAE per g total phenolic and 87.64±0.7% DPPH activity. Extraction and identification of natural antioxidants from liquorice (*Glycyrrhiza glabra*) was done by El-sherif *et al.,* (2011) and found that liquorice extract (ethanolic) contained 353.93 mg per 100g (gallic acid). The DPPH-radical-scavenging activity of the methanolic extracts of licorice from different habitats determined by Karahan *et al.,* (2016). The IC_{50} values of the extracts were found to be between 588±0.86 µg/ml and 2190±1.73 µg/ml.

The total phenolics content and radical scavenging activity of liquorice evaluated in the study was more or less within the range reported in the literature. However, some

deviations in total phenolics content might be attributed to variations variety of the plants (Benabdallah *et al.,* 2016; Abdulkadir *et al.,* 2015), prevailing agroclimatic conditions of the area in which plant is grown (Jaiswal *et al.,* 2014), agronomic practises followed in the plant farming (Azhar *et al.,* 2011; Pakade *et al.,* 2013), maturity of the plant at the stage of harvesting (Jinesh *et al.,* 2010), method followed for post-harvest processing of the plant (Pakade *et al.,* 2013; Al-juhaimi and Ghafoor, 2011), the type and concentration of solvent (Hasim *et al.,* 2015; Wangcharoen and Gomolmanee, 2011)as well as polarity of solvent (Rahman *et al.,* 2013; Basak *et al.,* 2014)used for analysis, the method followed for the estimation of the total phenolics content (Jinesh *et al.,* 2010; Wangcharoen and Gomolmanee, 2011; Rahman *et al.,* 2013), etc. However, some deviations in radical scavenging activity might be attributed to variations in chemical composition of the different plants with respect to phenolics and other antioxidants as well as pro-oxidants content (Tupe *et al.,* 2013; Kaur and Mondal, 2014; Fukumoto and Mazza, 2000; Bouayed and Bohn, 2010).

Selection of stage to add liquorice in preparation of ghee

Ghee is almost anhydrous milk fat and obtained by clarification of milk fat at high temperature usually at 110 to 120°C temperature (Ganguli and Jain, 1973). Exposure of the spice to such a high temperature may adversely affect the stability of major and/ or minor antioxidant components present in the spices. Even possibility also exists about interaction of antioxidants present in spices with ghee residue, leading to decrease in effectiveness of the antioxidants. At the same there is possibility of improvement in extraction of these antioxidants due to their better leaching from the spices to ghee at higher temperature. Therefore, it is necessary to find out appropriate stage for addition of liquorice in process method adopted for preparation of ghee. From examination of manufacturing process for ghee it can be envisaged that there are three possible stages to add liquorice into ghee, as listed below.

(1) Initial stage of heat clarification of butter in to ghee (in melted butter)

(2) Final stage of heat clarification of butter in to ghee (near 105°C temperature)

(3) After the heat clarification and separation from ghee residue (in hot ghee at 80°C)

Adopting the first and second possibility is the most appropriate in the study particularly when small number of samples to be treated alike, to avoid sample to sample variation in intensity of heat clarification. Sand bath was used to prepare ghee samples simultaneously under similar and uniform heating conditions.

Performance of liquorice added at two different stages in ghee preparation

Effect of two different stages of addition on performance of liquorice for retarding oxidative deterioration of ghee was measured in terms of changes in peroxide value and flavour score of ghee during storage at 80°±2°C.

Peroxide value of liquorice treated ghee during storage

The changes in peroxide value of different ghee samples (control and treated with liquorice) during storage at 80°±2°C are presented in Table 1and graphically presented in Figure 1.

The peroxide value of different fresh ghee was in the order of ghee treated with liquorice

at initial stage of clarification <control ghee < ghee treated with liquorice at final stage of clarification. However, the order of peroxide value of different ghee samples changed at the end of the 12 days storage at 80°±2°C. The order of peroxide value of different ghee samples was just reversed at the end of the storage was ghee treated with liquorice at initial stage of clarification < ghee treated with liquorice at final stage of clarification < control ghee.

It was revealed from statistical analysis that different stage in preparation of ghee used for treatment of ghee with the liquorice differed significantly ($P<0.05$) in their effect on changes in peroxide value of ghee. Similarly, period of storage also differed significantly ($P<0.05$) in their effect on changes in peroxide value of ghee. The interaction effect indicated that the stage in preparation of ghee used for treatment with liquorice and period of the storage differed significantly from each other in their effect on peroxide value of ghee over a period of storage. Thus, it became evident that the effect of stage in preparation of ghee used for treatment with liquorice and period of storage were dependent on each other.

The peroxide value of all the three types of ghee samples increased at almost steady rate up 6^{th} day of the storage. The rate of rise in peroxide value became steep from 6^{th} day onwards in case of control ghee sample. However, in ghee treated with liquorice at final stage of clarification slightly steep rise was noticed from 10^{th} day of the storage. On the other hand in ghee treated with liquorice at initial stage of clarification, no noticeable steep rate of rise in peroxide value was noticed at any stage during the entire storage period.

Among the fresh ghee samples (0 day) peroxide value of control ghee sample was statistically at par with sample of ghee treated with liquorice at initial stage of clarification whereas it was significantly ($P<0.05$) lower than sample of ghee treated with liquorice at final stage of clarification. Only on 2^{nd} day of the storage peroxide value of all three types of ghee samples became statistically at par. Moreover, after 2^{nd} day of the storage period peroxide value of control ghee sample remained higher than samples of ghee treated with liquorice, irrespective of their stage of addition in preparation of ghee. From 4^{th} day of storage, control ghee sample was significantly ($P<0.05$) higher than both ghee samples treated with liquorice at initial and final stage of clarification.

Among the sample of ghee treated with liquorice at two different stages of clarification (initial and final) in preparation of ghee the peroxide values of ghee sample treated with liquorice at initial stage of clarification remained lower all throughout the storage period than that of sample of ghee treated with liquorice at final stage of clarification. However, peroxide values of both these ghee samples were statistically at par up to 6^{th} day of the storage except on 0 day. On subsequent storage period peroxide value of ghee sample treated with liquorice at initial stage of clarification was significantly ($P<0.05$) lower than that of sample of ghee treated with liquorice at final stage of clarification.

From the forgoing resume it became evident that treating the ghee with liquorice at initial stage of clarification reduced the peroxide formation more effectively compared to the ghee with liquorice at final stage of clarification. No report is available in the literature for evaluating effect of treatment of ghee with liquorice at different stages in preparation of ghee on changes in peroxide value of ghee during storage. Therefore, results obtained in present study could not be

compared as such with the reports in the literature.

Flavour score of liquorice treated ghee during storage at 80°±2°C

The changes in flavour score of different ghee samples (control and treated with liquorice) during storage at 80°±2°C are presented in Table 2 and graphically presented in Figure 2.

The flavour score of different fresh ghee was in the order of control ghee > ghee treated with liquorice at initial stage of clarification > ghee treated with liquorice at final stage of clarification. However, the order of flavour score of different ghee samples changed at the end of the 12 days storage at 80°±2°C. The order of flavour score of different ghee samples was in the order of ghee treated with liquorice at initial stage of clarification > ghee treated with liquorice at final stage of clarification > control ghee.

The flavour score of all the three types of ghee samples decreased at almost equal rate on 2nd day of the storage. The rate of decline in flavour score of control ghee sample became steep from 4th day onwards. However, in case of ghee treated with liquorice at initial stage of clarification as well as at final stage of clarification no steep rate of decline in flavour score was noticed at any stage during the entire storage period. The flavour score of control ghee sample went below the acceptable level (<6) on 8th day of the storage. However, flavour score of ghee samples treated with liquorice at initial stage and final stage of clarification remained acceptable even on 12th day of the storage.

It was revealed from statistical analysis that different stage in ghee preparation used for treatment of ghee with the liquorice differed significantly ($P<0.05$) in their effect on changes in flavour score of ghee. Similarly, period of storage also differed significantly ($P<0.05$) in their effect on changes in flavour score of ghee. The interaction effect indicated that the stage in preparation of ghee used for treatment with liquorice and period of the storage differed significantly from each other in their effect on flavour score of ghee over a period of storage. Thus, it became evident that the effect of stage in preparation of ghee used for treatment with liquorice and period of storage were dependent on each other.

Among the fresh ghee samples (0 day) control sample of ghee had significantly ($P<0.05$) higher flavour score compared to samples of ghee treated with liquorice, irrespective of their stage of addition in preparation of ghee. Moreover, differences between flavour scores all three types of ghee were statistically at par on 2nd day of the storage. However, from 4th day of the storage flavour score of control ghee sample became significantly ($P<0.05$) lower than both ghee samples treated with liquorice at initial and final stage of clarification.

Among the sample of ghee treated with liquorice at two different stages of clarification (initial and final) in preparation of ghee the flavour score of ghee sample treated with liquorice at initial stage of clarification remained higher all throughout the storage period than that of sample of ghee treated with liquorice at final stage of clarification. Moreover, flavour score of both ghee samples were statistically at par up to 10th day of the storage. However on 6th and 12th day of storage flavour score of ghee sample treated with liquorice at initial stage of clarification was significantly ($P<0.05$) higher than that of sample of ghee treated with liquorice at final stage of clarification. From the forgoing resume it became evident that treating the ghee with liquorice at initial stage of clarification reduced the peroxide formation more effectively compared to the

ghee with liquorice at final stage of clarification. No report is available in the literature for evaluating effect of treatment of ghee with liquorice at different stages in preparation of ghee on changes in flavour score of ghee during storage. Therefore, results obtained in present study could not be compared as such with the reports in the literature.

The quality of natural extracts and their antioxidative performances depends not only on the quality of the original plant, the geographic origin, climatic condition, harvesting date and storage but also environmental and technological factors affect the activities of antioxidants from residual sources (Moure *et al.*, 2001).

Kim *et al.*, (2006) studied the effect of heat on the antioxidant capacity of grape seed extracts and came to the conclusion that the antioxidant capacity of these extracts increased through the liberation of phenolic compounds by heat. Hence the additive effects are shown well by partitioning easily and spreading evenly well in favourable medium provided by heating. Nwaichi and Anyanwu (2013) studied phytoscreening and the effect of heat treatment on the antioxidant activity in three medicinal plant parts *Tetrapleura tetraptera*, *Piper guineense* and *Xylopia ethiopica*. The recorded results clearly indicate total phenolic content and antioxidant activities decreased due to thermal treatment. Horvathova *et al.*, (2007) reported that the when powders and oils were analysed in case of ginger this antioxidant activity was reduced on heating (120°C for 1 hour).

As per the views expressed by Nicoliy *et al.*, (1999) polyphenols with an intermediate oxidation state can exhibit higher radical scavenging efficiency than the non-oxidized ones. The higher antioxidant properties of the partially oxidized polyphenols could be attributed to their increased ability to donate a hydrogen atom from the aromatic hydroxyl group to a free radical and/or to the capacity of their aromatic structures to support the unpaired electron through delocalization around the π-electron system. Processing and/or prolonged storage times can promote or enhance the progressive enzymatic or chemical oxidation of phenolic compounds; these reactions proceed at different rates depending on some intrinsic food variables as well as on processing conditions (a_w, pH, time, temperature, oxygen availability, etc.). Thus, the increase or the decrease in the overall antioxidant properties of polyphenol-containing products are consequences of the same oxidation reactions.

Therefore, in present study better performance of liquorice used in treatment of ghee at initial stage of clarification during preparation of ghee compared to final stage of the clarification might be attributed to increased liberation of phenolic compounds, easy partitioning, spreading evenly and formation of polyphenols with an intermediate oxidation state of antioxidants by heat. Thus, findings of present study were in corroboration with findings reported and views expressed by various authors as presented above.

Optimization of rate for addition of liquorice in preparation of ghee

The typical aroma and taste associated spices in turn adversely affect the organoleptic property of the products like ghee, because people are not accustomed with it. Moreover, it has been reported that the effect of antioxidant depends on its concentration, both at low and high concentration they may become pro-oxidant (Gordon, 1990). Therefore, it was essential to optimize rate of addition of the liquorice for use in treatment of ghee.

Table.1 Changes in peroxide value of liquorice treated ghee during storage

Storage period (days)	Peroxide value of ghee during storage at 80°±2°C (meq of O_2 per kg fat)		
	Control	Liquorice added at initial stage of clarification	Liquorice added at final stage of clarification
0	0.19	0.15	0.44
2	0.53	0.64	0.67
4	1.44	0.95	1.11
6	1.93	1.50	1.59
8	4.04	1.88	2.13
10	6.19	2.65	2.98
12	9.33	4.04	4.93

Source of variation	Storage period (P) (days)	Treatment (T) (Stage of addition)	Interaction (P×T)
SEm	0.12	0.08	0.21
CD (0.05)	0.34	0.22	0.58
CV%	17.62		

Table.2 Changes in flavour score of liquorice treated ghee during storage

Storage period (days)	Flavour score of ghee during storage at 80°±2°C (Out of 9)		
	Control	Liquorice added at initial stage of clarification	Liquorice added at final stage of clarification
0	9.00	8.88	8.85
2	8.58	8.60	8.55
4	7.75	8.20	8.23
6	6.60	7.93	7.83
8	5.60	7.55	7.48
10	4.10	7.18	7.08
12	2.40	6.90	6.65

Source ofvariation	Storage period (P) (days)	Treatment (T) (Stage of addition)	Interaction (P×T)
SEm	0.05	0.03	0.08
CD (0.05)	0.13	0.09	0.23
CV%	2.25		

Table.3 Changes in peroxide value of ghee liquorice during storage after treating with at different rates

Storage period (days)	Peroxide value of ghee during storageat 80°±2°C (meq of O_2 per kg fat)				
	Rate of liquorice used in treatment of ghee				
	0.0%	0.1%	0.2%	0.3%	0.4%
0	0.19	0.26	0.31	0.38	0.57
2	0.77	0.65	0.72	0.79	0.90
4	1.67	0.88	0.96	1.04	1.16
6	3.68	1.23	1.26	1.33	1.56
8	4.73	1.83	2.28	1.72	1.80
10	6.71	2.36	2.38	2.16	2.20
12	8.71	3.27	2.78	2.48	2.54
13	10.88	3.65	3.54	2.66	2.76
14	12.79	4.53	3.84	2.87	3.24
15	14.72	4.89	4.28	3.18	3.67
16	17.41	6.50	4.55	3.64	3.80
17	20.35	6.95	7.01	4.18	4.09
18	24.71	7.53	8.81	4.55	4.59
19	27.76	8.30	10.54	4.76	4.90
20	31.02	10.95	11.37	5.16	6.36
21	32.97	13.23	12.71	5.57	8.17
22	35.80	15.81	15.08	5.95	11.10

Source of variation	Storage period (P) (days)	Treatment (T) (Rate of addition)	Interaction (P×T)
SEm	0.23	0.13	0.52
CD (0.05)	0.65	0.35	1.45
CV%	13.75		

Table.4 Changes in flavour score of ghee during storage after treating with liquorice at different rates

Storage period (days)	Flavour score of ghee during storageat 80°±2°C (Out of 9)				
	Rate of liquorice used in treatment of ghee				
	0.0%	0.1%	0.2%	0.3%	0.4%
0	9.00	9.00	9.00	9.00	8.53
2	8.70	8.60	8.67	8.73	8.37
4	7.97	8.03	8.03	8.40	8.27
6	6.77	7.30	7.50	8.13	7.87
8	5.57	6.20	7.13	7.70	7.40
10	4.53	5.10	6.47	7.23	6.77
12	3.27	4.23	6.00	6.87	6.30
13	2.67	4.00	6.00	6.70	6.17
14	2.00	3.70	5.67	6.40	6.03
15	1.53	3.27	5.17	6.13	5.80
16	1.37	3.03	4.90	6.00	4.70
17	1.23	2.67	4.30	6.00	4.07
18	1.00	1.93	3.67	5.40	3.53
19	1.00	1.20	3.27	5.07	3.07
20	1.00	1.00	2.20	4.20	2.40
21	1.00	1.00	1.00	3.57	1.33
22	1.00	1.00	1.00	2.87	1.00

Source of Variation	Storage period (P)(days)	Treatment (T) (Rate of addition)	Interaction (P×T)
SEm	0.09	0.05	0.21
CD (0.05)	0.26	0.14	0.58
CV%	7.26		

Table.5 Changes in peroxide value of ghee during storage at 80°C after treating with different antioxidants

Storage period (days)	Peroxide value of ghee (meq of O_2 per kg fat)		
	Control	Antioxidants used in ghee	
		Liquorice (0.3%)	BHA (0.02%)
0	0.26	0.39	0.07
2	1.15	0.96	0.26
4	1.90	1.37	0.43
6	2.19	1.77	0.81
8	3.85	2.12	1.08
10	5.66	2.35	1.56
12	6.68	2.65	2.70
14	9.81	3.15	4.70
16	12.87	3.82	6.01
18	22.74	4.51	9.25
20	38.03	5.43	10.53
22	33.68	6.24	12.39

Source of variation	Storage period (P) (days)	Treatment (T) (antioxidant)	Interaction (P×T)
SEm	0.11	0.05	0.18
CD (0.05)	0.30	0.15	0.52
CV%	5.11		

Table.6 Changes in flavour score of ghee during storage at 80°C after treating with different antioxidants

Storage period (days)	Flavour score of ghee during storage at 80°±2°C (Out of 9)		
	Control	Antioxidants used in ghee	
		Liquorice (0.3%)	BHA (0.02%)
0	9.00	7.83	9.00
2	8.77	8.20	9.00
4	8.27	7.70	9.00
6	7.30	7.60	8.60
8	6.60	7.50	8.20
10	5.77	7.33	7.67
12	4.90	7.27	6.90
14	2.70	6.87	5.27
16	1.33	6.60	4.07
18	1.00	6.50	3.00
20	1.00	4.57	2.57
22	1.00	4.17	2.00

Source of variation	Storage period (P) (days)	Treatment (T) (antioxidant)	Interaction (P×T)
SEm	0.17	0.08	0.29
CD (0.05)	0.48	0.24	0.82
CV%	8.48		

Fig.1 Changes in peroxide value of liquorice treated ghee during storage

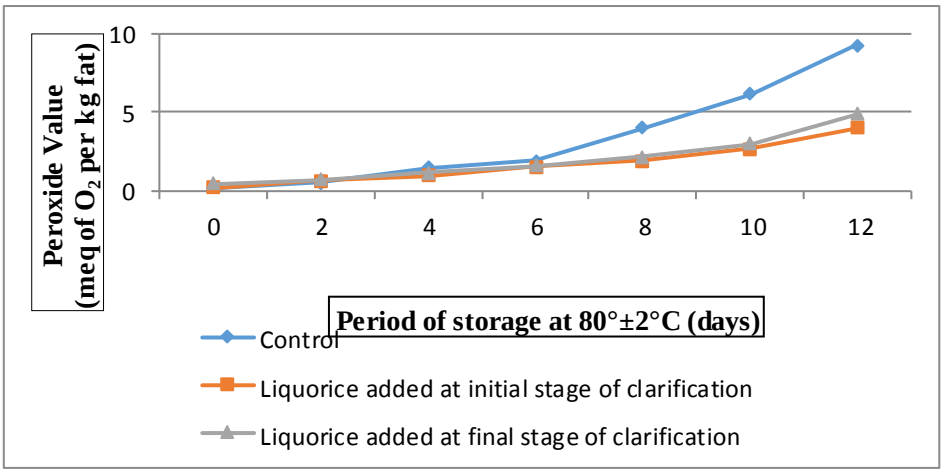


Fig.2 Changes in flavour score of liquorice treated ghee during storage

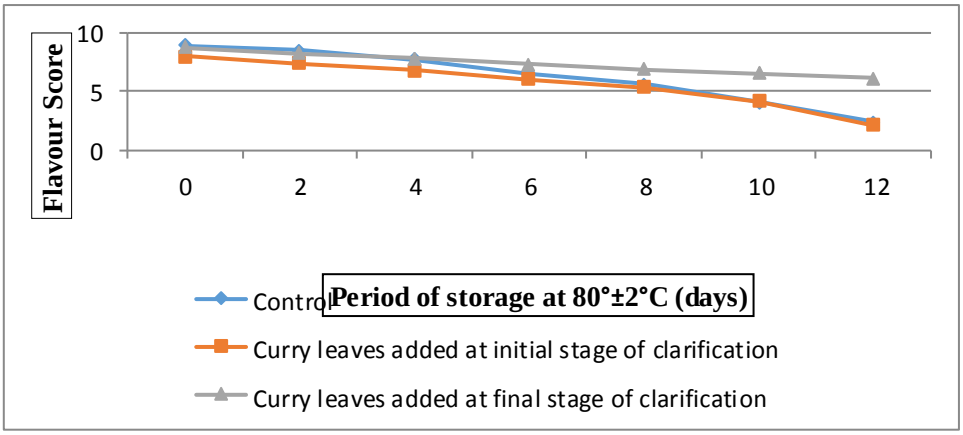


Fig.3 Changes in peroxide value of ghee during storage after treating with liquorice at different rates

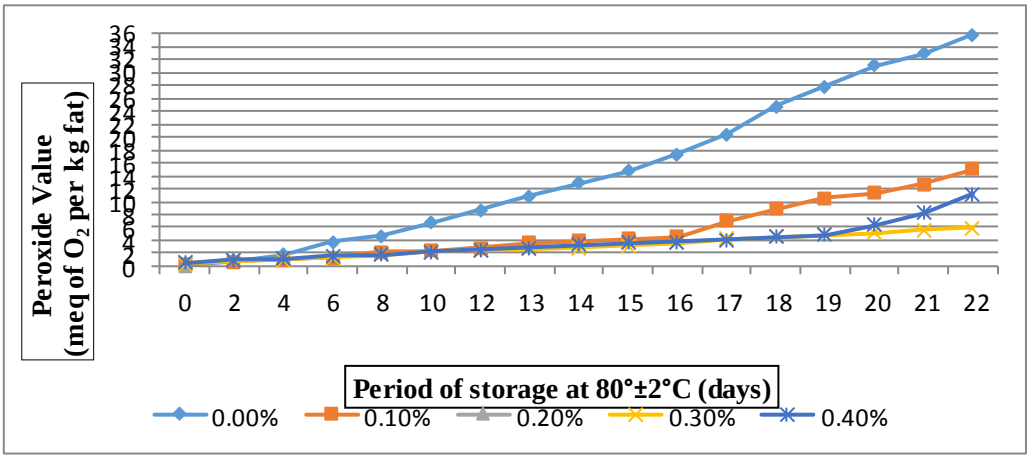


Fig.4 Changes in flavour score of ghee during storage after treating with liquorice at different rates

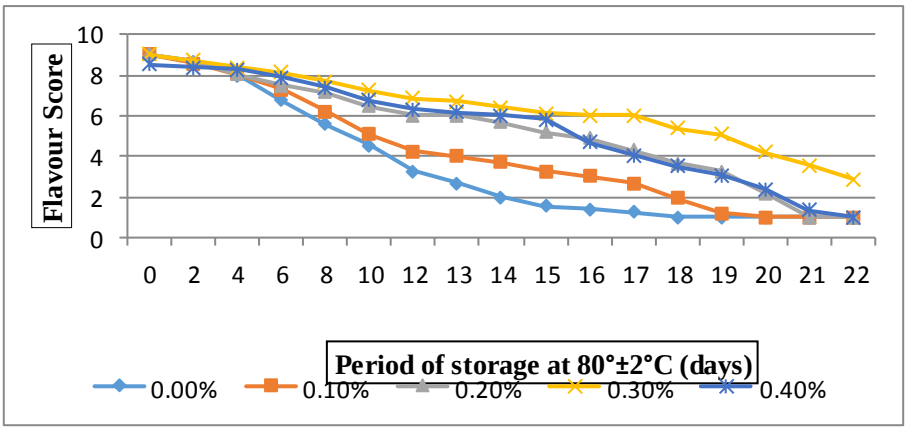


Fig.5 Changes in peroxide value of ghee during storage at 80°C after treating with different antioxidants

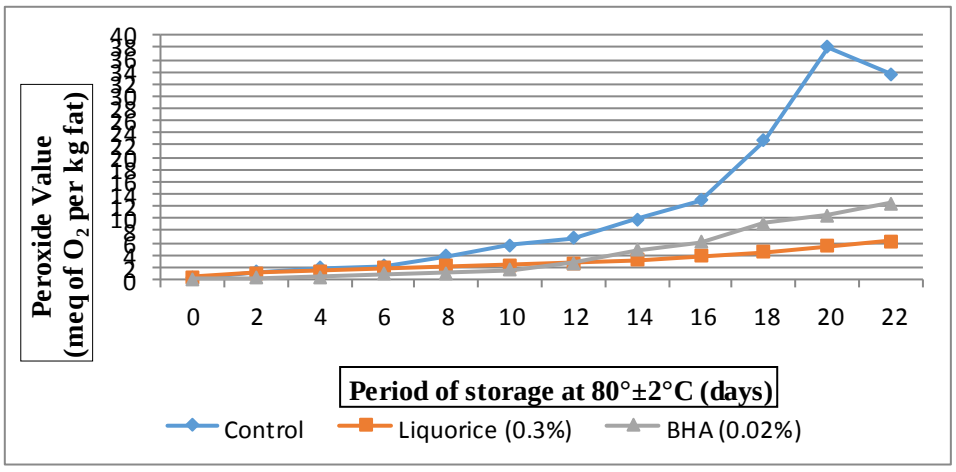


Fig.6 Changes in flavour score of ghee during storage at 80°C after treating with different antioxidants

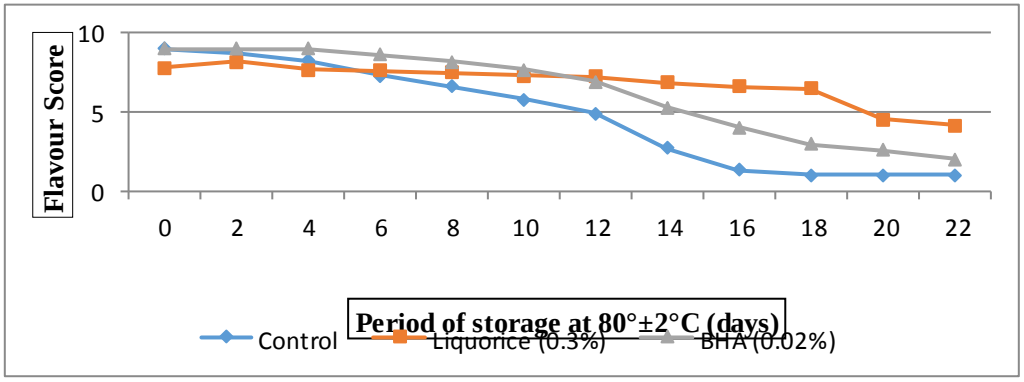
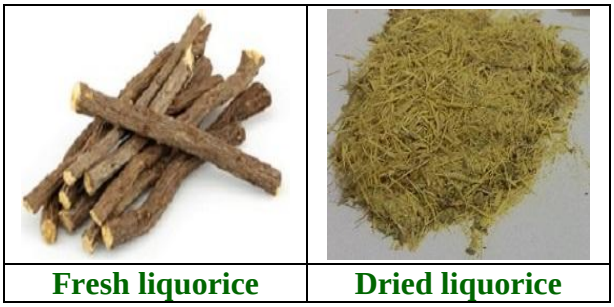
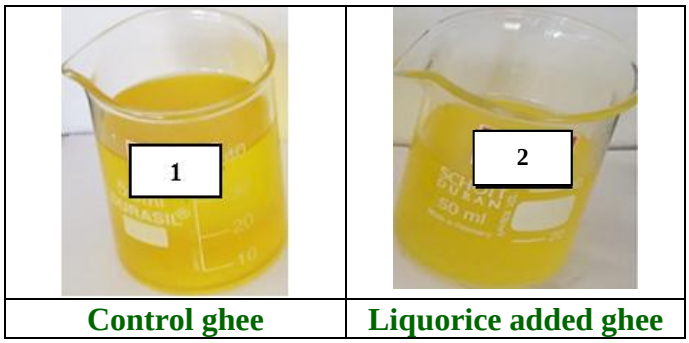


Plate.1 Fresh and dried roots of liquorice



Fresh liquorice	Dried liquorice

Plate.2 The colour characteristic of the fresh ghee samples



Control ghee	Liquorice added ghee

Effect of rate of liquorice used in treatment of ghee

Effect of different rates of addition of liquorice on its performance in retarding oxidative deterioration of ghee was measured in terms of changes in peroxide value and flavour score of ghee during storage at 80°±2°C.

Changes in peroxide value of liquorice treated ghee during storage at 80°±2°C

The results obtained for rate of liquorice addition on changes in peroxide value of ghee during storage are presented in Table 3 and the trend is presented in Figure 3.

The peroxide value of different fresh ghee sample was in the order of control ghee < ghee treated with 0.1% liquorice < ghee treated with 0.2% liquorice < ghee treated with 0.3% liquorice < ghee treated with 0.4% liquorice. However, the order of peroxide value of different ghee samples changed at the end of the 22 days storage at 80°±2°C. At the end of the storage the order of peroxide value of different ghee samples was ghee treated with 0.3% liquorice < ghee treated with 0.4% liquorice < ghee treated with 0.2% liquorice < ghee treated with 0.1% liquorice < control ghee.

The control ghee sample showed steeped rise in peroxide value on 16th day of the storage. Whereas, samples of ghee treated with liquorice at 0.1, 0.2, and 0.4 per cent rate showed steeped rise in peroxide value on 17th, 18th and 20th day of the storage. Interestingly, sample of ghee treated with 0.3 per cent liquorice peroxide value of ghee increased at a steady rate, no sharp rise in peroxide value was observed in entire period of storage.

Among the fresh ghee samples (0 day), control sample of ghee had the lowest

peroxide value compared to samples of ghee treated with liquorice at different rates of addition. It was noticed that among fresh ghee samples peroxide value increased with increase in rate of liquorice addition. Even up to 6th day of the storage same trend continued among liquorice treated ghee samples. However, from 4th of the storage peroxide value of control ghee sample became significantly higher ($P<0.9$) compared to all the ghee samples treated with liquorice and remained significantly higher during subsequent storage up to end.

It was revealed from statistical analysis that different rate of liquorice used in treatment of ghee differed significantly ($P<0.05$) in their effect on changes in peroxide value of ghee during storage. Similarly, storage period also differed significantly ($P<0.05$) in their effect on changes in peroxide value of ghee. The interaction effect indicated that the rate of liquorice used in treatment of ghee and period of storage differed significantly from each other in their effect on peroxide value of ghee over a storage period. Thus, it became evident that the effect of rate of liquorice used in treatment of ghee and storage period were dependent on each other.

Among the sample of ghee treated with liquorice at different rate of addition (0.1, 0.2, 0.3 and 0.4%), on 8th of the storage the peroxide value of ghee treated with 0.3 per cent liquorice was lowest and also remained lowest during remaining period of the storage. Though, peroxide values of liquorice treated ghee samples was statistically at par up to 6th day of the storage, thereafter from 14th day of storage period peroxide values of ghee sample treated with 0.3 per cent liquorice was significantly ($P<0.05$) lower than all the liquorice treated ghee samples. Thus, it can be sum-up that treatment of ghee with liquorice at four different rate used in the treatment of ghee, all the four rates were able to reduce the peroxide formation in ghee during storage 80^{o}±2°C, compared to formation of peroxide in control ghee. However, best control in formation of peroxide in ghee during storage was given by 0.3 per cent liquorice. Any increase or decrease in the rate of liquorice from 0.3 per cent resulted in to reduction in effectiveness of the treatment in controlling the peroxide formation in ghee during storage 80^{o}±2°C.

From the forgoing resume it became very clearly evident that treatment of ghee with 0.3 per cent liquorice was most effective for reducing the peroxide formation in ghee during storage 80^{o}±2°C, compared to the ghee with liquorice at 0.1, 0.2 and 0.4 per cent rate. Any deviation from 0.3 per cent resulted in to decrease in performance of the treatment. Therefore, in the present study 0.3 per cent liquorice was considered as optimum in treatment of ghee for better control of peroxide formation during storage. No report is available in the literature for evaluating effect of treatment of ghee with liquorice at different rates in preparation of ghee on changes in peroxide value of ghee during storage. Therefore, results obtained in present study could not be compared as such with the reports in the literature.

Duga (1976) pointed out that some antioxidants provide increased protection with increasing concentration, while others have optimal levels after which higher levels exert pro-oxidant effects. According to Fukumoto and Mazza (2000) most phenolic compounds had pro-oxidant activity at low concentrations. Bouayed and Bohn (2010) opined that high concentrations of antioxidants including BHT and BHA in food items, can also increase spoilage of food items due to pro-oxidant activities. Similar views were expressed by Ling *et al.*, (2010). These authors stated that high concentrations of antioxidants may have pro-oxidant activity.

Moure *et al.*, (2001) suggested that potent antioxidants can autoxidize and generate reactive substances and thus also act as pro-oxidants, depending on the systems, According to Gordon (1990) as well as Cao and Cutler (1993) at high concentrations of antioxidant, their pro-oxidant effects could arise due to the involvement of the phenolic compounds in initiation reactions (i.e., formation of radicals). As per Cillard *et al.*, (1980) hydroxyl radical absorbance capacity of antioxidants decreases at high concentrations due to their involvement in initiation reactions such as at high concentrations.

Changes in flavour score of liquorice treated ghee during storage at 80°±2°C

The results obtained for rate of liquorice addition on changes in flavour score of ghee during storage are presented in Table 4and the trend is presented in Figure 4.

The flavour score of different fresh ghee sample was in the order of control ghee = ghee treated with 0.1% liquorice = ghee treated with 0.2% liquorice = ghee treated with 0.3% liquorice > ghee treated with 0.4% liquorice. On 13th day of the storage the order of flavour score of different ghee samples was ghee treated with 0.3% liquorice > ghee treated with 0.4% liquorice > ghee treated with 0.2% liquorice > ghee treated with 0.1% liquorice > control ghee.

It was revealed from statistical analysis that different rate of liquorice used in treatment of ghee differed significantly ($P<0.05$) in their effect on changes in flavour score of ghee during storage. Similarly, storage period also differed significantly ($P<0.05$) in their effect on changes in flavour score of ghee. The interaction effect indicated that the rate of liquorice used in treatment of ghee and storage period significantly differed from each other in their effect on flavour score of ghee over a storage period. Thus, it became evident that the effect of rate of liquorice used for treatment of ghee and storage period were dependent on each other.

Among the fresh ghee samples (0 day) control ghee sample as well as ghee samples treated with liquorice at the rate of 0.1, 0.2 and 0.3 acquired full flavour score (9 out of 9). Only the ghee samples treated with liquorice at 0.4 per cent got slightly lower flavour score compared other samples in the group. On 2nd day of the storage flavour score of all the ghee samples decreased, but relative trend between the samples was almost similar as noticed in case of the fresh ghee samples. On 4th of the storage flavour score of control ghee was lowest among all the ghee samples. The flavour score of control ghee became significantly lower than all the liquorice treated ghee samples.

The flavour score of control ghee decreased at a rapid rate from beginning of the storage and showed steeped descale on 17th day of the storage. Flavour score of the control sample reached to a significantly lower level compared to liquorice treated ghee on 4th day of the storage.

Among the samples of ghee treated with liquorice at different rate of addition (0.1, 0.2, 0.3 and 0.4 per cent), the sample of ghee treated with 0.1 per cent liquorice also showed almost similar trend in changes in flavour score of ghee on storage as that of the control ghee sample, except slight variation between 13th to 19th days of the storage. Interestingly, sample of ghee treated with 0.3 per cent liquorice flavour score of ghee increased at a steady rate, no sharp rise in flavour score was observed up to 17th day of the storage. The samples of ghee treated with 0.2 and 0.4 per cent liquorice also followed almost similar trend in changes in flavour

score of ghee as that of the 0.3 per cent treated ghee sample up to 13th day of the storage. However, their trend change towards the control and 0.1 per cent treated sample of ghee after on storage up to 15th day of the storage, however, their trend changed thereafter and tended toward fatter rate of decline. The flavour score of ghee treated with 0.3 per cent liquorice remained highest all throughout the storage.

Among all the samples of ghee, the flavour score of control ghee went below the acceptable level on 8th day of the storage. On the other hand flavour score of ghee treated with 0.1, 0.2, 0.3 and 0.4 per cent liquorice went below the acceptable level on 10th, 14th, 18th and 15th day of the storage. Thus, among different levels of liquorice used in treatment of ghee, best control for retention of flavour score of ghee during storage was achieved by 0.3 per cent liquorice. Any increase or decrease in the rate of liquorice from 0.3 per cent resulted in to reduction in effectiveness of the treatment in controlling the deterioration ghee flavour during storage 80^{o}±2°C.

From the forgoing resume it became very clearly evident that treatment of ghee with 0.3 per cent liquorice was most effective for restricting flavour deterioration of ghee during storage 80^{o}±2°C, compared to the ghee with liquorice at 0.1, 0.2 and 0.4 per cent rate. Any deviation from 0.3 per cent resulted in to decrease in performance of the treatment. Therefore, in the present study 0.3 per cent liquorice was considered as optimum in treatment of ghee for better retention of flavour during storage.

The variation in rate of the liquorice on any side (towards lower or higher) resulted in to reduction in the effectiveness of the treatment. The reduction in the effectiveness of the treatment on lowering the amount of liquorice in treatment of ghee might be attributed insufficient availability of antioxidants for the action and/or possible pro-oxidant effect of the antioxidant due to their presence in low concentration. On the other hand decrease in the effectiveness of the treatment on increasing the amount of liquorice in treatment of ghee might be attributed possible pro-oxidant effect of the antioxidant due to their presence in higher concentration.

Liquorice contains phytochemicals such as alkaloids, flavonoids, phenols and tannins. The flavour of liquorice is due the presence of volatile components.

The volatile components of liquorice includes pentanol, hexanol, linalooloxide A and B, tetramethylpyrazine, terpinen-4-ol, α-terpineol, geraniol and others (Roshan *et al.*, 2012). Therefore, decrease in flavour score of ghee upon addition of liquorice observed in the present study, up to 0.4 per cent may be attributed to above mentioned constituents of liquorice.

No report is available in the literature for evaluating effect of treatment of ghee with liquorice at different rates in preparation of ghee on changes in flavour score of ghee during storage. Therefore, results obtained in present study could not be compared as such with the reports in the literature.

Comparison of liquorice with synthetic antioxidant (BHA)

After selecting the appropriate stage and optimum rate of liquorice addition in preparation of ghee, last phase of the study work was carried out to compare the performance of the liquorice with butylated hydroxyl anisole (BHA), a synthetic antioxidant permitted in ghee under FSSAI rules for reducing the oxidative deterioration of ghee during storage.

Effect of antioxidants on oxidative changes in ghee on storage at 80°C

For comparing the effect of liquorice addition in ghee with BHA added ghee, oxidative changes in ghee samples during storage at 80°±2°C were analysed for peroxide value and flavour score at regular interval of 2 days for 22 days.

Effect of antioxidants on peroxide value of ghee during storage at 80°C

The results obtained for changes in peroxide value of ghee during storage at 80°±2°C are presented in Table 5 and the trend is presented in Figure 5.

The peroxide value of different fresh ghee samples was in the order of BHA > control >liquorice. However, after the storage at 80°±2°C for 22 days the order of peroxide value of ghee was control >BHA >liquorice.

It was revealed from statistical analysis that different antioxidants (BHA and liquorice) used in treatment of ghee differed significantly ($P<0.05$) in their effect on changes in peroxide value of ghee during storage. Similarly, period of storage also differed significantly ($P<0.05$) in their effect on changes in peroxide value of ghee. The interaction effect indicated that different antioxidants used in treatment of ghee as well as period of the storage differed significantly from each other in their effect on peroxide value of ghee over a period of storage. Thus, it became evident that the effect of different antioxidants used in treatment of ghee and period of storage were dependent on each other.

Among the fresh ghee samples (0 day) peroxide value of control ghee sample was statistically at par with sample of ghee treated with liquorice and then after became significantly ($P<0.05$) higher on subsequent storage up to end of the storage. Whereas control ghee sample was significantly ($P<0.05$) higher than the sample of ghee treated with BHA during the entire storage period. However, liquorice added ghee sample was significantly ($P<0.05$) higher than BHA treated ghee sample up to 10th day of storage then both the samples became statistically at par with each other. Moreover, liquorice added ghee sample was significantly ($P<0.05$) lower than BHA treated ghee sample after 10thday of storage.

In control sample of peroxide value rise at very rapid from beginning, showed steeped rise in peroxide value on 14th day of the storage, reached to the maximum level (38.03 meq of O_2 per kg fat) on 20th day of the storage and then falling down (33.68 meq of O_2 per kg fat) on 22nd day of the storage. In ghee sample treated with liquorice peroxide value rised slowly during the entire storage period and reached to 6.24 meq of O2 per kg fat on 22nd day of the storage. In sample of ghee treated with BHA peroxide value rise very gradually up to 10th day, on further storage its rate of rise in peroxide value was accelerated, much steeped rise in peroxide value was noticed from 14th day of the storage and peroxide value reached to 12.39 meq of O_2 per kg fat on 22nd day of the storage.

On the basis of above findings, it can be inferred that addition of liquorice exerts resistance towards the oxidative deterioration of ghee. From survey of literature it appears that no work is reported so far dealing with evaluation for relative effectiveness of BHA and liquorice in controlling formation of peroxides in ghee during storage at 80°±2°C. Therefore, results obtained in the present study could not be compared as such with the reports in the literature. The peroxide value of ghee containing coriander extracts was significantly higher than BHA throughout 21

days of storage at 80 ± 1 °C (Patel *et al.*, 2013).

Effect of antioxidants on flavour score of ghee during storage at 80°C

The results obtained for changes in flavour score of ghee during storage 80°±2°C are presented in Table 6 and the trend is presented in Figure 6.

The flavour score of different fresh ghee samples was in the order of control = BHA >liquorice. However, after the storage at 80°±2°C for 22 days the order of flavour score of ghee was liquorice >BHA > control.

Among all the fresh ghee samples control ghee and the ghee added with BHA received full flavour score (9 out of 9). In control ghee sample flavour score decreased at slower rate from beginning, decline in flavour score was steeped from 4th day of the storage, it went below the acceptable level on 10th day and then went down on further storage. In ghee treated with liquorice alone, flavour score decreased very gradually up to 19th day of the storage and the flavour score reached below the acceptable level on the 20th day of the storage. However, on further storage flavour score of the BHA added ghee sample started declining at rapid rate and sharp decline in flavour score was noticed from 10th day of the storage. The flavour score of BHA treated ghee sample went below the acceptable level on 14th day of the storage and continued to decline on further storage.

It was revealed from statistical analysis of data that the treatments (BHA and liquorice used in ghee as antioxidants) and storage period both were significant (P<0.05) on changes in flavour score of ghee during storage. The interaction between storage period as well as antioxidants used in ghee was statistically non-significant. Thus, results revealed that treatments and storage period have significant effect on changes in flavour score of ghee during storage. However, the interaction effect of storage period and treatments was non-significant.

Flavour score of control ghee sample remained significantly (P<0.05) higher than that of the liquorice added ghee sample up to 4th day of storage then after liquorice added ghee sample remained significantly (P<0.05) higher than that of the control ghee sample on subsequent storage period up to the end of the storage. However, flavour score of control ghee was statistically at par with BHA added ghee sample up to 2 days but then after the flavour score of BHA added ghee sample was remained significantly (P<0.05) higher than that of the control ghee. Moreover, flavour score of BHA added ghee sample remained significantly (P<0.05) higher than that of the liquorice added ghee sample up to 10th day of storage then after liquorice added ghee sample remained significantly (P<0.05) higher than that of the BHA added ghee sample on subsequent storage period up to the end of the storage.

From survey of literature it appears that no work is reported with evaluation for relative effectiveness of BHA and liquorice in controlling flavour deterioration in ghee during storage at 80°±2°C. Therefore, results obtained in the present study could not be compared as such with the reports in the literature.

The present study was conducted to evaluate potential of liquorice (*Glycyrrhiza glabra*) as an antioxidant in ghee to extend the shelf-life by retarding oxidative reactions during its storage. The total phenolic content and radical scavenging activity of liquorice evaluated in the study was more or less within the range reported in the literature. For addition of liquorice in treatment of ghee initial stage of

heat clarification found more effective than the final stage of heat clarification. Treatment of ghee with different rate of liquorice (0.1%, 0.2%, 0.3% and 0.4%), the rate 0.3 per cent was found most effective in reducing peroxide formation and flavour deterioration in ghee during storage. Liquorice was found to be capable of retarding oxidative degradation in ghee and even more effective than BHA. Hence, liquorice could be used as a natural antioxidant to preserve the food system apart from providing other beneficial benefits and would be preferred over BHA to minimize adverse effects on mankind.

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