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Enzyme Modified Ghee Flavour: Application and Shelf Life Studies

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ABSTRACT

Ghee is a fat rich dairy product. Ghee is prepared using direct cream method. The huge consumer acceptability of ghee is due to its flavour. Ghee has a pleasant, slightly cooked and caramalised flavour. Lipase enzyme has a great influence on the flavour characteristics of milk fat. Enzymes were used in combination for the flavour enhancement of ghee. Shelf life studies of enzyme modified ghee (EMG) flavour at 65°C for 24 days showed higher shelf life as compared to control (no enzyme) ghee. Due to higher intensity of EMG, a comparatively lower quantity of ghee was required to impart ghee flavour in food products like gulabjamun and jalebi.

Key words: *Enzymes, Ghee, Gulabjamun, Jalebi.*

INTRODUCTION

In 2015-16, the milk production of India was 155.5 million tonnes which rose to 163.7 million tonnes in 2016-17[9]. In 2018 combined butter and ghee (clarified butter) production in India is estimated to 5.6 MMT according to USDA GAIN reports, (2017)[11]. Almost 35 % of the milk processed in India is converted into ghee[3].

Ghee is the pure clarified milk fat. From Vedic times ghee has its presence in the Indian diet. It is a rich source of Vitamin A, D, E, K and essential fatty acids. It is used for the preparation of many medicines in Ayurveda. It acts as a coolant, digestive aid, capable of increasing mental power, curative of ulcers, eye diseases and improve vision. It is also used for religious rituals and customs. Conjugated Linoleic acid (CLA), an anti-carcinogenic compound is also present in ghee[5].

Ghee is made by different methods like desi, creamery butter, direct cream, pre-stratification and continuous (industrial approach). The demand for ghee in India is increasing rapidly especially due to its flavour. It is used as a cooking and frying medium. A wide range of Indian sweets prepared with the admixture of milk, cereals and nuts are cooked in ghee. Ghee is used for garnishing rice or spreading lightly on chapattis. No other fat can duplicate the flavour of ghee. Special status is given to those sweets and meals which are cooked in ghee and are considered for their distinct flavour attributes derived from ghee. Thus, it is the flavour of ghee which is cherished by all.

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Flavour of ghee is contributed by a wide range of compounds like free fatty acid (FFA), carbonyls, lactones, esters, ketones, aldehydes and other volatile compounds like alcohols and diols. Lipolysis of fatty acid glycerides during fermentation of milk or cream and processing treatments while preparing ghee are responsible for the formation of FFA. Flavour quality of ghee is closely related to FFA content. The fermentation products of lactose and citrate are responsible for the formation of carbonyls[6]. Flavour improvement in cream is noticed when cream is ripened with starter culture of *Streptoccoccus lactis subsp.diacetylactis*[7]. This increase in ghee flavour is because of the metabolites of the starter culture and increase in FFA and carbonyl contents. The oxidation of lipid also causes the formation of carbonyl compounds. Thermal decompostion of carbohydrates is responsible for the formation of polar (dicarbonyls) compounds i.e. diacetyl, furfural and hydroxymethylfurfural. Hydrolysis of lactogenic glycerides to hydroxy acids during processing favours the formation of lactones. A coconut like aroma is produced by lactones which is related with the characteristic aroma of ghee. The concentration of lactones increases with the rise of ghee clarification temperature but there is no change qualitatively[8].

Temperature for clarification of ghee greatly affect the flavour of ghee. Improvement in ghee flavour is noticed when cream is heated at 120 °C or above. The presence of more solids not fat (SNF) in direct cream ghee lead to the formation of slightly cooked or caramalised flavour. Pronounced cooked flavour is found in direct cream ghee heated at 130 °C. However, mild flavour is obtained from ghee when it is heated at 110° C. Caramalised flavour is absent in ghee prepared from creamery butter method due to low SNF content.

Though ripening of cream improves the ghee flavour, but it is time consuming, requires controlled storage and more manpower. Thus, an attempt is made to increase the intensity of ghee flavour by addition of enzymes under controlled conditions. The target is with the use of very less quantity of enzyme and less time, more flavour could be produced which could be used as a frying or cooking medium for other products. Enzyme modified ghee (EMG) flavour is the technology which involves the use of specific combination of enzymes acting either on ghee/butter/cream to produce intense natural ghee flavour.

MATERIAL AND METHODS

Chemicals and Materials

Enzymes were procured from M/s Amano Enzyme Inc., Japan (through Anthem Cellutions, Bengaluru) and were used for the preparation of EMG.

Analyti cal grade chemicals obtained from various reputed companies were used for chemical analysis and HPLC grade chemicals were procured from M/s Merck Life Science Pvt. Ltd., Mumbai and M/s Sigma Aldrich Bengaluru.

Preparation of samples

Control ghee

Fresh cream was standardised to 35- 40 percent (%) fat. It was then clarified at 120°C. After clarification, ghee was filtered to separate ghee residue and cooled to 30°C.

Enzyme modified ghee (EMG) flavour

Fresh cream was standardized to 35-40% fat. Enzymes were added in cream along with lactose, glucose and citrate. It was incubated for 3 hours at 45 °C with continuous stirring on magnetic stirrer. Clarification was done at 120 °C followed by filtration and cooling.

Physico-chemical analysis

Free fatty acid content and Peroxide value were determined as per the method described in SP: 18 (part XI) - 1981[11].

Sensory evaluation

The flavour profile of the ghee was evaluated by an expert panel of minimum 5 judges as per 9 point hedonic scale score card[2]. The 9 point hedonic scale indicates 9- like extremely, 8- like very much, 7- like moderately, 6- like slightly, 5- neither like nor dislike, 4- dislike slightly, 3- dislike moderately, 2- dislike very

much, 1- dislike extremely. About 40 ml of ghee was given to each sensory panellists. The judged parameters were flavour and its intensity.

Flavour intensity was determined by blending 20% EMG with refined sunflower oil. The amount of 20% was decided by blending 10, 20 and 30% of EMG with refined sunflower oil. Ghee flavour was detected at 20% level whereas at 10% blend it was not possible to perceive ghee flavour.

Statistical Analysis

Data obtained from sensory evaluation and FFA content were statistically analyzed by one way ANOVA using SPSS (Version 16.0) software. In the case of peroxide value, two way ANOVA was used. The differences among the treatment were measured at 5% level of significance.

RESULTS AND DISCUSSION

Shelf life studies of EMG flavour

Anti-oxidant BHA (Butylated Hydroxy Anisole) of 0.02% (as permitted by FSSAI) was added in both EMG flavour and control ghee. Thus four samples were prepared i.e. two samples with antioxidant and two samples without anti-oxidant. The samples were then packed in 100 ml poly ethylene terephthalate (PET) jars and sealed with aluminium foil before putting the screw cap on the top of bottle. All the four samples were stored at 65°C for accelerated storage study. Samples were drawn at an interval of four days for analysis. Samples were first taken for peroxide value (PV) evaluation followed by FFA and sensory analysis. The ghee samples were coded as: Control-control ghee with no enzyme, Control + BHA- Control ghee with antioxidant BHA, EMG - Enzyme modified ghee flavour, EMG + BHA- Enzyme modified ghee flavour with anti-oxidant BHA.

Changes in flavour during storage

The flavour score drastically reduced during storage period. There was no significant ($p>0.05$) difference in flavour score up to four days of storage. The initial score of 7.93 for control and 8.07 for EMG flavour became 3.03 and 4.10 respectively after 24 days of storage at 65 ℃ (Fig. I). After eight days of storage, flavour score of control sharply decreased to 4.13 and the ghee became unacceptable as rancid flavour was found in it. In case of EMG flavour, slight rancid flavour was found after sixteen days of storage. However, in case of anti-oxidant added samples flavour score greatly reduced after 20 days of storage. The flavour score of EMG flavour and control sample with anti-oxidant was quite comparable up to sixteen days of storage.

Changes in peroxide value during storage

The peroxide value at initial stage increased gradually and thereafter it increased suddenly especially in case of control sample as shown in Fig. II. The peroxide value is expressed in volume in ml of sodium thiosulfate used per gm. of ghee for titration of the ghee sample. Initially, the peroxide value of control sample and EMG flavour is 1.12 ml and 0.89 ml per gm. of fat respectively whereas after 24 days of storage it was 6.19 ml and 3.80 ml per gm. of fat for control and EMG respectively. After eight days of storage, control ghee became of poor quality and was completely unacceptable on sixteenth day of storage. However, EMG flavour became of poor quality on sixteenth day and was completely unacceptable on 20 days of storage. The BHA added samples showed a slightly lower peroxide value than the other two samples.

Changes in FFA content during storage

Free fatty acid of all the samples increased throughout the storage period. The initial values of FFA for control and EMG flavour were 0.29 and 5.56 which became 0.82 and 6.06 % oleic acid respectively after 24 days of storage at 65 ℃ as shown in Figure III. The initial high amount of FFA in EMG was because of addition of enzymes as well additives in cream before enzymatic hydrolysis.

Thus, from the flavour score, peroxide value and FFA contents of EMG flavour and control sample, it is evident that EMG sample had a higher shelf life than control sample. This might be due to higher production of maillard reaction products (MRPs) in EMG than control. This is also because of MRPs

have an oxygen radical absorbing capacity[1,2]. Also, citrate is used as an additive in EMG, which also perhaps acts as an anti-oxidant.

Application of EMG flavour in manufacture of different products

Developed EMG flavour was used as a flavouring agents for frying of different food products. The EMG flavour was blended 20 % with refined sunflower oil and used as frying medium to fry *Gulabjamun* and *Jalebi*. Sensory evaluation and ghee flavour intensity of these two fried products were evaluated and reported as follows:

Gulabjamun

Gulabjamun was prepared using "Gits" gulabjamun mix available in the market. A smooth dough was prepared by using required quantity of water as per instruction mentioned on the packet. Each ball of 10 gm. was prepared and care was taken to avoid cracks on the ball surface. It was then fried under low heat. Four frying mediums were used i.e. refined sunflower oil, 20% control ghee blended with refined sunflower oil, pure ghee and 20% EMG flavour blended with refined sunflower oil. After frying it was then soaked in hot 50% sugar syrup for 2 hrs.

Soaked *Gulabjamuns* were subjected for sensory evaluation to detect the presence of characteristic ghee flavour in the fried product. There was no significant ($p>0.05$) difference in the flavour of the *gulabjamun* samples (Table I). The highest flavour score was obtained when the *jamuns* were fried with ghee (100%) followed by 20% blended EMG flavour with oil. Ghee flavour was most perceived in the gulabjamuns fried in 100% control ghee. Ghee flavour was perceived in gulabjamun fried in 20% blended EMG with sunflower oil. However, no ghee flavour was perceived in gulabjamun fried in 20% blended control ghee sample. Thus, it can be concluded that due to the higher intensity of EMG flavour, ghee flavour was retained in gulabjamun.

Jalebi

Jalebi was also made using "Gits" jalebi mix. The jalebi was prepared from the mix as per the instructions mentioned on the packet of the mix. It was fried under medium to high heat flame. Four frying mediums were used i.e., refined sunflower oil, 20% control ghee blended with refined sunflower oil, ghee, 20% EMG flavour blended with refined sunflower oil. After frying, jalebi was then soaked in 50% hot sugar syrup for 1-2 minutes.

Sensory evaluation of jalebi revealed that there was no significant ($p>0.05$) difference in the flavour among of the samples (Table II). The highest flavour score was obtained in jalebi fried in ghee (100%) sample followed by frying in EMG blended with oil. Ghee flavour was most perceived in the jalebi fried in 100% control ghee. Ghee flavour was perceived when frying was done in 20% blended EMG flavour sample. Whereas no ghee flavour was perceived in the product fried in 20% blended control ghee sample. Therefore, it can be concluded that due to the higher intensity of EMG, ghee flavour was retained in jalebi.

Table I. Flavour scores of gulabjamun

Sample	Flavour
Oil (100%)	7.34±0.94 [a]
20% blended control ghee with oil	7.46±0.64 [a]
Ghee (100%)	7.89±0.53 [a]
20% blended EMG flavour with oil	7.88±0.76 [a]

Results are expressed as Mean±SD; means with different small letter superscript (a, b,) differ significantly ($p<0.05$) across the variants (rows)

Table II Flavour scores of jalebi

Parameter	Flavour
Oil (100%)	7.58±1.18 [a]
20% blended control ghee with oil	7.25±1.09 [a]
Ghee (100%)	8.03±0.46 [a]
20% blended EMG flavour with oil	7.62±1.05 [a]

Results are expressed as Mean±SD; means with different small letter superscript (a, b,) differ significantly ($p<0.05$) across the variants (rows)

CONCLUSIONS

The use of combination of enzymes produced a ghee which can be used to blend the ghee in the other products to impart the ghee flavour. It is now clearly evident that EMG has higher shelf life than control ghee. Thus, EMG can serve the purpose of most ideal blending agent for flavour enhancement in many food products.

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Conflict Of Interest

The authors declare no conflict of interest.

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