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Full Length Research Paper

Effect of fractionation on the physicochemical properties and the storage stability of Unniyappam

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'Unniyappam' or 'appam' is prepared out of rice and jaggery in ghee and is given to devotees at the Sabarimala Ayyappa temple in Kerala, India. It is one of the largest annual pilgrimage sites in the world with an estimate of over 40 to 50 million devotees visiting every year. Ghee is used in the Sabarimala temple as the frying medium to make Unniyappam. Ghee, the clarified milk fat prepared chiefly from cow or buffalo milk, is the most common and popular milk fat-based product in the Indian subcontinent since time immemorial. Modification of milk fat composition through the application of fractionation processes, which could result in fat fractions with favourable technical and nutritional properties, appears to be the most promising option for a better output. The Apparent merits of fractionated milk fat in the baking and confectionery sector have encouraged several scientists and efforts were made to incorporate fractions for preparations of sweets. The effect of fractionation was studied and it was shown that the products made with the inclusion of the S_{30} fraction yielded the best overall acceptability score. The Unniyappam made by incorporating of S_{30} fraction (18%) yielded the best results with greater textural and flavour scores attributing to higher overall acceptability. The Unniyappam prepared using the L30 fraction had a poor texture and surface was uneven and excessive burning was observed while frying. The potential of having a solid fraction for increased textural properties and overall acceptability was identified in many products and such initiatives will help to come up with the concept of tailoring milk fat to compete with other fats and oils in meeting the needs of the food industry.

Keywords: Sabarimala Unniyappam, Appam, Fractionation, Milk Fat, Ghee, Butter Oil, HMF, LMF, Solid fraction, Sweets, Traditional sweets

INTRODUCTION

India is the largest producer of milk in the world, producing 187.7 million tons (BAHS 2019) whereas the world's milk production amounts to 827.88MT (BAHS FAO 2019). India contributes to around 22.67%, of global milk production. Besides fluid milk consumption, the consumption of value-added dairy products is also growing due to higher incomes, urbanization, and demographic changes. Ghee is also gaining popularity in Australia, Arabian countries, the United States, the United Kingdom (UK), Belgium, New Zealand, the Netherlands, and many other African and Asian countries (Illingworth *et al*., 2009). The negative nutritional and physical image of the fat, especially certain saturated long-chain fatty acids, trans-fats, cholesterol, and poor spreadability and oxidative susceptibility, has driven the development of technologies to produce modified fat with different physicochemical or nutritional properties. Modified fats have a wide area of application. They can

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substitute the conventional costlier fats and be used in functional foods, such as nutraceuticals. Moreover, one can use different fractions derived from a single source of fat for different applications. Therefore, the modification of fat can be considered a tool that enhances natural fats' functionality and nutritional value and broadens their area of application (Gandhi *et al*., 2018).

MATERIALS AND METHODS

Preparation of Ghee (Sserunjogi *et al*., 1998).

Whole Milk
↓
Warming 40-50^{0}C/10min.
↓
Cream Separation
↓
Ghee Boiler
↓
Boiling at 110-120^{0}C
↓
Clarified fat
↓
Filtration
↓
Ghee (Sserunjogi *et al*., 1998).

Fractionation of Ghee

Fractionation processing (Deffense, 1993) was adopted for the fractionation of ghee. Melting will be carried out by heating ghee at 60^{0}C to remove the crystal memory. It will be then slowly cooled to 30^{0}C and held at this temperature for 24 h in an incubator for crystallization. The liquid will be separated from the crystals by decantation after centrifugation at 2000 rpm for 10 min in a temperature-controlled centrifuge (REMI) maintained at 30°C. The solid fraction obtained at 30^{0}C (S_{30}) will be regarded as a high melting fraction (HMF or S_{30}). The weight of the solid fraction thus obtained was determined and the percentage yield was calculated. Subsequently, the liquid portion obtained at 30^{0}C will be the L_{30} fraction (LMF or L_{30}).

Preparation of Sabarimala Unniyappam

The method followed by Travancore Devaswom Board, Kerala was used for preparing Unniyappam. In a wide bowl, add rice flour (1 cup), crushed cardamom, jaggery syrup (3/4-1cup), finely chopped and fried coconut pieces 2 tbsp, fired till seeds (1 tbsp), and mix well. Add water slowly and make a smooth batter, it should not be too thick or too thin. Set the batter for an hour.

After 1-3 hours, add ghee/fractionated to each mold of the pan and fry in ghee until the ghee becomes hot. Pour a spoonful of batter into each mold until 3/4 full and cook over a low to medium flame until the bottom side of the appam become crispy. Turn the appam to the other side and cook until the colour changes to a golden brown.

Sensory evaluation of products

A trained sensory panel assessed the coded ghee samples at random, according to the 9-point Hedonic scale ISI scorecard. Sensory evaluation of the ghee samples was carried out with a twelve-member panel (ages 22 to 50 years) who were Scientists, Students, and Technical staff of the Institute with previous knowledge of sensory evaluation of dairy products. Samples were analyzed and the respective panel's scores were recorded.

Moisture

The moisture content of the sample was estimated as per ISI: SP 18 (Part XI) 1981. About 5 g of the material was accurately weighed in a pre-weighed petri dish and placed in a hot air oven and dry for 2 hours at 100ºC. The dish with the sample was cooled in a desiccator and weight was noted. Heated again at 100ºC in an air oven for 30 minutes cooled and weighed. This process of heating for 30 minutes was repeated till the difference in weight between two successive observations was less than 1 mg. From the lost weight during the drying amount of moisture was calculated as follows:

$$\text{Moisture content (\% by weight)} = 100 \times \frac{W_1 - W_2}{W}$$

Where,

W_1=Weight in g of the dish with ghee before drying,

W_2= Weight in g of the dish with ghee after drying, and

W=Weight in g of the empty dish

Peroxide value:

This was determined through iodometric titration according to standard methods for the oils analysis AOCS (1998) [9], and the results were expressed in meq O2 /kg oil. Two grams of product samples were weighed into a 250ml stoppered conical flask. Thirty milliliters of acetic acid chloroform solvent mixture were added to each and swirled to dissolve. Then 0.5 ml saturated potassium iodide solution was added with a Mohr pipette and left to stand for 1 minute in the dark with occasional shaking added about 30 ml of distilled water was. This was titrated with 0.01 N sodium thiosulphate solution, with vigorous shaking until yellow u was almost gone. 0.5 ml starch solution was added as an indicator and titration was continued until the blue colour disappeared indicating the endpoint.

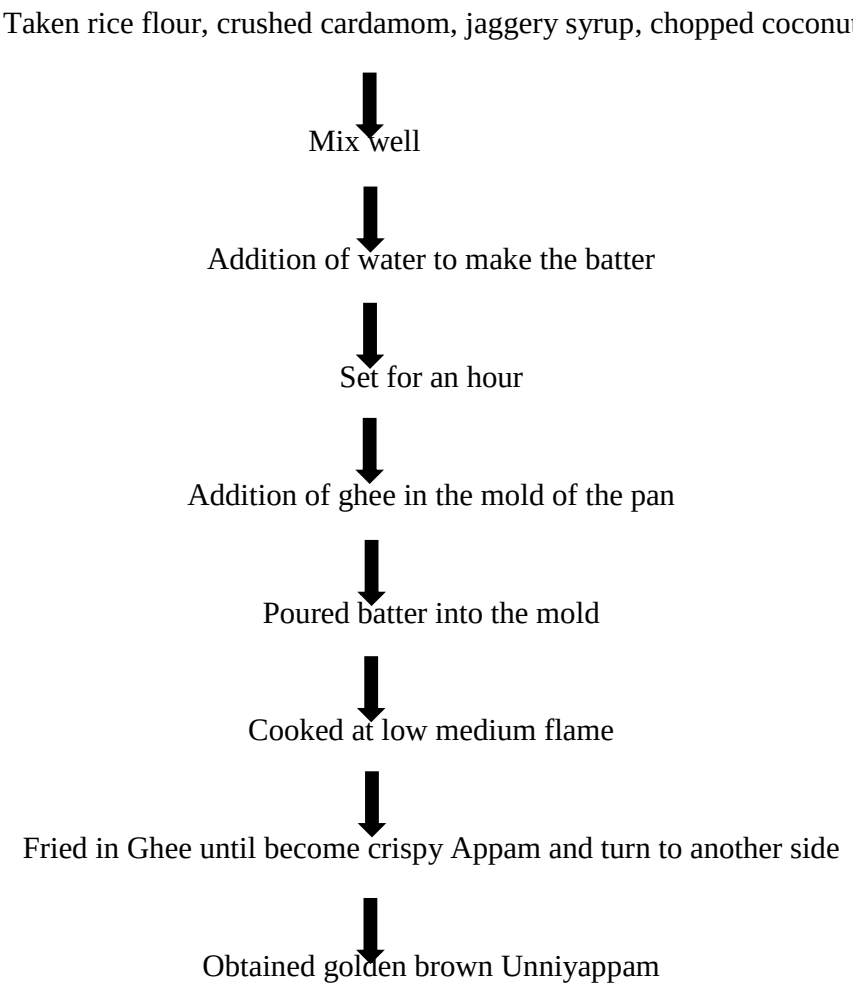

Figure 1. Flow chart for preparation of Unniyappam

Calculation of the peroxide value was done as below:

Peroxide value= drying amount of moisture was calculated as follows:

Peroxide value = Titre X N X100 /W

where W is the weight of the sample, Titre=ml of Sodium Thiosulphate used, N=Normality of sodium thiosulphate solution Figure 1.

Estimation of Free fatty Acid Content

Free fatty acid (FFA) levels, expressed as % oleic acid, of ghee samples, were determined by the method IS: 548 (Part 1) - 2010, which is described below:

Procedure: Ten grams of ghee sample was accurately weighed in a 250ml conical flask. 50ml of neutralized alcohol (at 70°C) was added to the flask containing ghee. The contents were brought to a boiling water bath. The solution, while hot, was titrated against 0.1N sodium hydroxide solution, shaking vigorously during titration. The endpoint of the titration was perceived when the addition of a single drop produced a slight but definite colour change (pink colour) for at least 15 seconds. The FFA levels were expressed as per oleic acid percentage.

Free fatty acid [% (as oleic acid)] = (V X N X 282 X 100]/ (W X 1000)
Substituting the value for normality as 0.1N

$$\text{FFA (\% oleic acid)} = \frac{2.82 \times V}{W}$$

Where V=Titrate value (ml)
N= Normality of NaOH
282= molecular weight of oleic acid
W=Weight of Sample(g) of fat

Microbiological analysis

The metalized polyester pouch containing the sample

Plate 1. Shelf-life Studies

was opened and 11g of the product was weighed and transferred to 99 ml of the sterile physiological saline aseptically. Further dilution to the desired level was carried out by serially transferring 1ml of diluted sample to 9 ml sterile diluent blanks (FSSAI, 2016) Plate 1.

Total bacterial count

The total bacterial count was determined by using standard plate agar (Hi-Media). Pipetted1 ml of the food homogenate (Mysore Pak /Unniyappam to sterile labeled Petri plates. The molten agar (10-12 ml) to 42-45^{0}C is poured into each plate. Mixed the media and dilutions by swirling gently, clockwise, anti-clockwise, to and fro thrice, and care was taken that the contents do not touch the lid. It was allowed to set. The count was taken after 24-48 hours of incubation at 37^{0}C (FSSAI, 2012). All colonies that appear on SPCA were counted and expressed as log 10 cfu/g.

Yeast and mold count

Yeast and mold count was determined by plating using potato dextrose agar (Hi-Media). Pipetted1 ml of the food homogenate (Mysore Pak/Unniyappam) to sterile labeled Petri plates. The molten agar (10-12 ml) to 42-45^{0}C is poured into each plate. Mixed the media and dilutions by swirling gently, clockwise, anti-clockwise, to and fro thrice, and care was taken that the contents do not touch the lid. It was allowed to set. The pH of the medium was adjusted to around 3.5 by adding 1.6 ml of sterile lactic acid solution (10 %) to 100 ml of potato dextrose agar. The count was taken after 2–5 days of incubation at 25 ºC (FSSAI, 2012). All colonies were counted and expressed as log 10cfu/g.

Coliform count

The coliform count was determined by plating using violet red bile salts agar (Hi-Media). Pipetted1 ml of the food homogenate (Mysore Pak/Unniyappam) to sterile labeled Petri plates. The molten agar (10-12 ml) to 42-45^{0}C is poured into each plate. Mixed the media and dilutions by swirling gently, clockwise, anti-clockwise, to and fro thrice, and care was taken that the contents do not touch the lid. It was allowed to set. The count was taken after 18-24 hours of incubation at 37 ^{0}C (FSSAI, 2012). All colonies were counted and expressed as log 10cfu/g.

RESULTS AND DISCUSSION

Effect of solid and liquid fractionated Gir Ghee on the sensory quality of Unniyappam

Control (T0) Unniyappam was prepared using whole Gir Ghee. Samples T_1, T_2, and T_3 were prepared by replacing whole ghee with solid fractions (S_{30}) at 6,12,18 percent levels whereas the samples T_4, T_5, and T_6 were prepared by replacing whole ghee with a liquid portion(L_{30}) of ghee at 06, 12, 18 percent levels.

Color and Appearance

The color and appearance score was the highest for S_{30}

Table 1. Effect of solid and liquid fractionated Gir Ghee on the sensory quality of Unniyappam

Treatments	Sensory characteristics			
	Color and appearance	Flavor	Body and texture	Overall acceptability
T_0	8.18^a	8.35^a	8.45^a	8.39^a
T_1	8.21^a	8.15^a	8.03^a	8.18^a
T_2	8.37^a	8.34^a	8.29^a	8.37^a
T_3	8.49^a	8.45^a	8.64^a	8.52^a
T_4	7.94^a	6.90^b	7.96^b	7.48^b
T_5	7.95^a	6.75^b	7.58^b	7.30^b
T_6	7.14^b	6.59^b	7.12^b	6.90^b
CD (P≤0.05)	0.59	0.49	0.62	0.67

Note:
Scores were given as per a 9-point hedonic scale
*All values are an average of three trails
Similar superscripts indicate non-significant (NS) at corresponding critical difference (CD)
T_0 = Control Product was prepared using whole ghee
T_1 =Product was prepared by replacing 06% of whole ghee with an S_{30} fraction
T_2 = Product was prepared by replacing 12% of whole ghee with an S_{30} fraction
T_3 = Product was prepared by replacing 18% of whole ghee with an S_{30} fraction
T_4= Product was prepared by replacing 06% of whole ghee with an L_{30} fraction
T_5= Product was prepared by replacing 12% of whole ghee with an L_{30} fraction
T_6= Product was prepared by replacing 18% of whole ghee with an L_{30} fraction

incorporated Unniyappam at 18% level T_3 (8.49) followed by T_2 (8.37) and T_1 (8.21). The score for color and appearance for L_{30} incorporated fractions was lower. There was no significant difference ($P < 0.05$) in the color and appearance among the samples.

Flavor

The flavor score was observed high for the S_{30} incorporated Unniyappam T_3 (8.45) followed and then by the control sample T_0 (8.35). The score for L_{30} incorporated fractions was lower. There was no significant difference ($P < 0.05$) observed between the control and S_{30} incorporated Unniyappam, but a significant difference was found between S_{30} incorporated Unniyappam and L_{30} incorporated Unniyappam.

Body and Texture

The textural values were recorded and the highest value observed was for S_{30} fraction T_3 (8.64) incorporated Unniyappam followed by T_0, T_2, and T_1 respectively. The scores were lower for L_{30} incorporated fractions. The values were 8.45, 8.03, 8.29, 8.64, 7.96, 7.58, and 7.12 for T_0, T_1, T_2, T_3, T_4, T_5, and T_6, respectively. There was no significant difference ($P<0.05$) among the scores of S_{30} fraction (T_1, T_2 and T_3) incorporated Unniyappam products Table 1.

Overall Acceptability

The overall acceptability of Unniyappam made by incorporating S30 fraction at 18% was the highest and was significantly higher ($P < 0.05$) to L30 incorporated preparations. The optimized Unniyappam with the inclusion of the S30 fraction at the 18 % level recorded the best score and was selected for further studies.

Storage Stability of Unniyappam

Effect of ghee fractionation on the Moisture content of Unniyappam during storage

The moisture content of the samples was recorded from the 0^{th} day to the 9^{th} day. It was observed that the moisture content decreased during storage. The moisture content has decreased for the S_{30} incorporated Unniyappam also. The values of the S_{30} incorporated Unniyappam was low in comparison with the Unniyappam using whole Gir Ghee and Market Ghee ($P<0.05$).

The values were in the range of 21.03 to 19.55% for the Gir Ghee used sample., 19.78 to 19.30 for the S_{30} incorporated Unniyappam, and 22.15 to 20.77% for Unniyappam prepared using market Ghee during storage. There was a significant difference in the moisture content of Unniyappam made out of Market Ghee whereas there was no significant difference in Unniyappam made out of Gir Ghee and S_{30} incorporated Ghee Table 2.

Effect of ghee fractionation on the Peroxide value content of Unniyappam during storage

The peroxide values of the Unniyappam samples were observed for the control and S_{30} incorporated fraction and are delineated in Table 3. It was found that the peroxide value increased during storage for all three variants of Unniyappam. There was no significant difference ($P<0.05$) in peroxide value amongst the three types of Unniyappam. The peroxide value for the Unniyappam using whole Gir ghee, and Market Ghee and S_{30} incorporated Unniyappam ranged from 5.14 to 6.80, 5.17 to 6.81, and 4.80 to 6.20 from 0^{th} day to 9^{th} day.

Table 2. Effect of ghee fractionation on the Moisture content of Unniyappam during storage

Treatments	Storage days			
	Moisture (percent)			
	0th	3rd	6th	9th
T_0	21.03[a]	20.17[a]	19.90[a]	19.55[a]
T_1	19.78[a]	19.52[a]	19.45[a]	19.30[a]
T_2	22.15[b]	21.98[b]	21.56[b]	20.77[b]
CD (P≤0.05)	1.82	1.32	1.53	1.22

Note:
*All values are an average of three trails
Similar superscripts indicate non-significant (NS) at corresponding critical difference (CD)
T_0 = Control (Whole ghee)
T_1 = Whole ghee replaced by 18% of S_{30} fraction of ghee
T_2= Product was prepared using market ghee

Table 3. Effect of ghee fractionation on the Peroxide value of Unniyappam during storage

Treatments	Storage days			
	Peroxide value (percent)			
	0th	3rd	6th	9th
T_o	5.14[a]	5.81[a]	6.21[a]	6.80[a]
T_1	4.80[a]	5.43[a]	5.83[a]	6.20[a]
T_2	5.17[a]	5.83[a]	6.43[a]	6.81[a]
CD (P≤0.05)	0.75	0.63	0.61	0.83

Note:
*All values are an average of three trails
Similar superscripts indicate non-significant (NS) at corresponding critical difference (CD)
T_0 = Control (Whole ghee)
T_1 = Whole ghee replaced by 18% of S_{30} fraction of ghee
T_2= Product was prepared using market ghee

Table 4. Effect of ghee fractionation on the Free fatty acid content of Unniyappam during storage

Treatments	Storage days			
	Free fatty acid content (percent oleic acid)			
	0th	3rd	6th	9th
T_o	1.85[a]	1.93[a]	2.05[a]	2.11[a]
T_1	1.79[a]	1.87[a]	1.99[a]	2.01[ac]
T_2	1.79[a]	2.12[a]	2.27[b]	2.35[b]
CD (P≤0.05)	1.79[a]	0.19	0.17	0.28

Note:
*All values are an average of three trails
Similar superscripts indicate non-significant (NS) at corresponding critical difference (CD)
T_0 = Control (Whole ghee)
T_1 = Whole ghee replaced by 18% of S_{30} fraction of ghee
T_2= Product was prepared using market ghee

Effect of ghee fractionation on the Free fatty acid value of Unniyappam during storage

The S_{30} fraction incorporated Unniyappam had 1.79 percent oleic acid, whereas the whole Gir ghee Unniyappam had 1.85 and Unniyappam prepared from market Ghee had 1.91 percent oleic acid on the 0th day (Table 4). The FFA values increased from the 0th day to the 9th day for all the varieties however there was no significant difference observed up to the 6thday. The FFA of unniyappam made of market ghee showed a significant difference on the 6th and 9th day whereas there was no significant difference in the S_{30} incorporated product.

Effect of storage on Microbiological quality of Unniyappam

Table 5 showed the microbiological quality of the Unniyappam samples. The Total plate count ($\log_{10}$ cfu/g) for S_{30} incorporated unniyappam samples was low compared to the whole gir and market ghee unniyappam

Table 5. Microbiological quality of Unniyappam after incorporating S30 Fractions

Treatments	Storage days				
	Total bacterial count (log_{10} cfu/g)				
	0th	3rd	6th	9th	12th
T_0	2.66 [a]	2.82[a]	3.01[a]	3.21 [a]	
	2.32 [a]	2.57 [a]	2.89 [a]	3.18 [a]	S
	2.75 [a]	2.84 [a]	3.15 [a]	3.38 [a]	
CD (P≤0.05)	0.82	0.85	1.14	1.53	
	Coliform (log_{10} cfu/g)				
T_0	Nil	Nil	Nil	Nil	
T1	Nil	Nil	Nil	Nil	S
T2	Nil	Nil	Nil	Nil	
	Yeast and mold (log_{10} cfu/g)				
T_0	Nil	Nil	Nil	1.09[a]	
T1	Nil	Nil	Nil	1.01 [a]	S
T2	Nil	Nil	Nil	1.12 [a]	
CD (P≤0.05)				0.10	

Note:
*All values are an average of three trails
Similar superscripts indicate non-significant (NS) at corresponding critical difference (CD)
T_0 = Control (Whole ghee)
T_1 = Whole ghee replaced by 18% of S_{30} fraction of ghee
T_2= Product was prepared using market ghee
S = Spoiled

(2.32, 2.66, 2.75cfu/g). The results were in good agreement with those reported by Sharma *et al.*, 2010. The coliform count was found nil throughout the study. The yeast and mould count was also within the limits and the unniyappam was rated very good up to nine days of storage at ambient temperature conditions. The good results might have been related to the initial food quality and the good control throughout the storage period. Similar results were also obtained by Yadav *et al*., 2013.

CONCLUSION

Economic fractionation of milk fat into liquid and solid fat fractions, which differ markedly from one another in chemical composition and physical characteristics, but within the statutory limits of food safety by FSSAI could increase the utilization of milk fat in many food applications. The superiority of utilizing Gir Ghee in Sweet preparation such as and Unniyappam was significant from the sensory evaluation of the products. The Apparent merits of fractionated milk fat in the baking and confectionery sector have encouraged several scientists and efforts were made to incorporate fractions for preparations of Unniyappam. The potential of having a solid fraction for increased textural properties and overall acceptability was identified in both the products. Such initiatives will help to come up with the concept of tailoring milk fat to compete with other fats and oils in meeting the needs of the food industry.

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