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Effect of thermal degradation on oxidative stability of ghee under conventional and sub-baric frying conditions

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Abstract
The present study is proposed with the specific objectives to study the physico-chemical, rheological, and sensory changes in G*hee* during conventional and sub-baric frying and proposed the superiority of sub-baric by repeated use of *Ghee*. For conventional methods the *Gulabjamun* balls were fried at 145 °C for two different time periods of 5 min and 10 min, for sub-baric frying conditions three levels of frying temperature *viz*. 120, 135 and 150 ºC had been selected, and the product was fried at each of the selected temperature for 5 min under a vacuum of 400 mm Hg. Thus, 15 samples of Ghee were collected after each set of frying and used for Chemico-physical and sensory evaluation. Two-way ANOVA was employed to determine the significance differences in data between the 3 temperatures and frying cycles for sub-baric frying experiments. As the frying cycles progressed, the Peroxide value, TBA value and viscosity of the Ghee samples increased over the frying cycles, while Iodine value decreased over the frying cycles. Generation of Free fatty acids in the Ghee samples were marginal, its appearance was significantly delayed in the sub-baric processed samples at lower temperatures. The colour values, primarily the L*, a* and b* values were indicative of a yellowish hue, the trend of Chroma and Yellowness Index derived from the colour data were indicative of a loss of yellow colour was lesser with progression of frying cycles under sub-baric frying condition. The study revealed that *Ghee* as a frying medium was more stable during repeat frying cycles under sub-baric frying process when compared to conventional frying, especially at the lower temperatures of sub-baric frying.

Keywords: Ghee, conventional, oxidative stability, sub-baric, thermal degradation

Introduction
Ghee is a popular traditional dairy product in India and many countries in Middle East, prepared by clarification of milk fat (cream or butter). Ghee is characterized with a typical savoury, nutty and buttery flavour and is considered to be a superior cooking medium as compared to oils, primarily because of the unique flavour it imparts to the product. Various studies have been done on the thermal stability of Ghee under conventional heating process [1-2].

Frying, a complex unit operation, is essentially a cooking process that has been widely used in the food industry. Deep-fat frying (DFF) of food is a common cooking technique, conventionally carried out by immersion of foods in frying oil, mostly at temperatures above the boiling point of water, bringing about a counter-flow of water bubbles and oil on the surface of the product [3]. DFF operations are usually performed at high temperature (> 140 °C) under atmospheric pressure. The frying process triggers a chain of heat induced reactions in the product such as gelatinization of 14 starch, denaturation of proteins, hydrolysis, development of flavours, Maillard browning, caramelization and inactivation of enzymes resulting in the development of typical physico-chemical, textural and sensory attributes, that enhances the overall acceptability of the fried food [4].

The interactive effect of high temperatures employed during the frying process with the atmospheric oxygen and moisture released during the frying process is reported to cause thermal breakdown to the constituents of the fat/oil, especially for medium rich in polyunsaturated fatty acids (PUFA). This breakdown sets a chain of reactions that ultimately result in the formation of several oxidative and hydrolytic products, polymerization, isomerization and/or cyclization of the constituents leading to irreversible changes to the physico-chemical and sensory profile of the frying medium [5].

Since the frying process is a simultaneous heat and mass transfer process, the alteration in the physical properties of the frying medium, especially, viscosity and conductivity have a direct impact on the heat and mass transfer efficiency of the system. Also, during frying, a two-way mass transfer is observed, moisture loss from the product to the frying medium along with a simultaneous fat uptake by the fried product [6]. However, as the frying cycles progress, the degradation products accumulate and is transferred to the product through the fat uptake, hampering the flavour and colour of the product. Further, the frying process has also been widely implicated in producing adverse health effects due to the chemicals formed in the product during frying (e.g., acrylamides) or due to degradation products generated in the frying medium, especially, on repeated cycles of frying [7]. Several attempts have been reported in literature to improve the thermal stability of frying medium, primarily through blending the PUFA rich oils with monounsaturated counterparts in suitable proportions [8] or by the addition of antioxidants, both natural and synthetic [9]. It has also evolved that sub-baric frying or vacuum frying that is carried out at pressures well below atmospheric level, could improve the oxidative stability of the frying medium [10]. The enhanced stability is attributed to the system being closed in nature, lower boiling point due to reduced pressures and lower exposure to oxygen. Concurrently, during sub-baric frying, it is reported that the boiling points of frying oil and moisture in food decreases, increasing the rate of heat transfer to the interior of food and shortening the frying time. The effect of sub-baric frying on the thermal stability of different vegetable oils is widely reported [11-12].

Reports indicate Ghee is susceptible to thermal oxidation while conventional frying methods and products of degradation include FFAs and polymers [13]. But however, there is no reported literature on the stability of Ghee under vacuum frying conditions. So, to fill that research gap the present study was conducted to analyse the effect of thermal degradation on oxidative stability of Ghee under sub-baric frying conditions and comparing with conventional frying conditions.

Materials and Methods

Cow Ghee was procured locally from the Nandini Milk Products, a subsidiary unit of Karnataka Milk Federation (KMF), Bengaluru, India. For preparation of Gulabjamun, cow milk sourced from the Experimental Dairy Plant of ICAR-National Dairy Research Institute, Bengaluru, India was standardized and converted to Khoa, which was used as base material for the preparation of the product. Maida (refined wheat flour) and baking soda were sourced from the local supermarket.

Gulabjamun balls preparation

The procedure for preparation of Gulabjamun standardized by [14] was adopted for the study. For that Khoa was prepared by the continuous evaporation with constant stirring of the standardized milk in an open steam-jacketed kettle (steam pressure: 196.13 kPa) to a semi solid dough - like mass. Figure 1 demonstrates the preparation of fried Gulabjamun balls. At first, the prepared Khoa (100g) along with Maida (30g), and 0.6% baking powder (only for conventional frying samples) and water (as per the afore-mentioned computation) was kneaded to a homogenous mass in an orbital mixer (Make: M/s Lalith Industries, Bangalore, India) for 5 min to obtain the product dough.

Frying of Gulabjamun under conventional frying conditions

The dough was manually divided into weighed portions of 10 g each and carefully rolled to smooth spherical balls. Ghee was heated in a thermostatically controlled atmospheric mini-fryer and the prepared Gulabjamun balls were carefully immersed into the hot Ghee bath. The Gulabjamun balls were fried at 145 °C for two different time periods of 5 min and 10 min, until a golden-\brown crust was obtained.

Frying of Gulabjamun under sub-baric frying conditions

The product dough was prepared without the addition of baking powder. Portioned and rolled Gulabjamun balls were prepared and fried in an in-house fabricated sub-baric thermal processor (SBTP) unit.

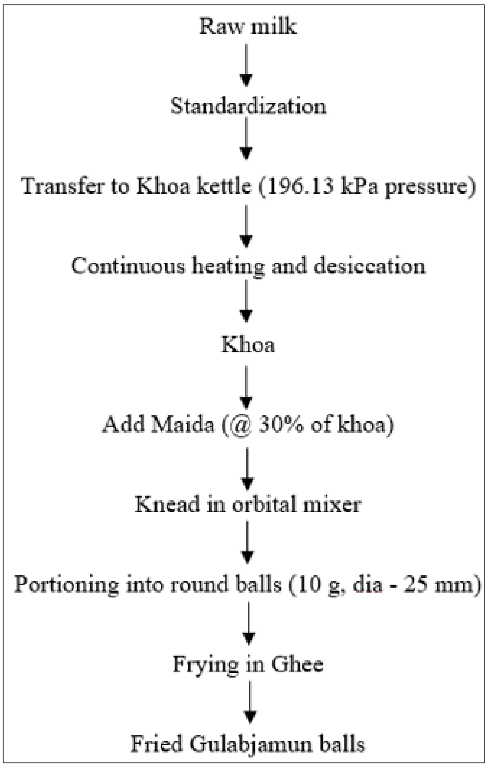

Fig 1: General flowchart for preparation of fried Gulabjamun

Three levels of frying temperature were selected for the study, *viz*. 120, 135 and 150 ºC, and the product was fried at each of the selected temperature for 5 min under a vacuum of 400 mm Hg. Frying experiments for each of the frying experiments, both under conventional and sub-baric conditions, the product was fried for three consecutive shifts for the prescribed duration (5 min / 10 min) for 5 cycles at hourly intervals, i.e., on the first day, for the first cycle, Gulabjamun balls were fried for 5 / 10 min, fried balls were withdrawn and a sample of 40 ml of the frying medium (Ghee) was collected for analysis after cooling of the Ghee bath. After a lapse of one hour from the end of the first frying cycle, fresh Gulabjamun balls were loaded into the fryer and the process repeated for the second cycle of frying and so on and so forth for five cycles for either method of frying. Adequate care was taken to ensure that for each frying cycle, the ratio of mass of product to Ghee was maintained constant. The five cycles of frying were repeated over three shifts spaced 8h apart to complete one set of frying experiments. Thus, 15 samples of Ghee were collected after each set of frying, which were immediately stored at -20 °C before analysis. Analysis of each sample was carried out within 3-5 days from collection.

Peroxide value: For determination of peroxide value of Ghee samples, the method as described in IS: 3508 (1966) was applied.

Thiobarbituric acid (TBA) value: For determination of thiobarbituric acid (TBA) value of Ghee samples, colorimetric method proposed 84 by [16] was applied. For that molten Ghee sample (0.1 g) was accurately weighed into a 15 ml stoppered centrifuge tube, to which 1 ml of trichloroacetic acid and 2 ml of TBA reagent were added. The contents were incubated in a boiling water bath for 15 min and then cooled under running tap water. Solvent (1 ml of glacial acetic acid and 2 ml of chloroform) was added to the tube and the contents were mixed well using a vortex mixer. It was then centrifuged for 4-5 min at 3000 rpm to obtain two clearly separated layers. The supernatant layer was decanted carefully into a cuvette and its OD was measured at 532 nm using a spectrophotometer. Readings were also recorded for a Blank sample prepared using only the reagents used for this analysis.

Iodine value: Iodine value was determined using the Wij's method as described [17]. A sample of clear Ghee (0.4 to 0.45 g) filtered of suspended residues was transferred into an Erlenmeyer flask. Carbon tetrachloride (20 ml) was added to the flask and the sample was dissolved in it by swirling the flask; this was followed by the addition of 25 ml of Wij's reagent. The flask was stoppered, its contents properly mixed and left standing in the dark for 1 hour. At the end of the hold, 20 ml of potassium iodide solution was added followed by addition of 150 ml of distilled water. The solution was properly mixed and then titrated against 0.1 N sodium thiosulfate solution until yellow-brown colour almost disappeared. About 1-2 ml of 1% starch indicator was added and titration was continued until the disappearance of the blue colour. An estimation of the blank sample using all the reagents listed above (without Ghee) was also carried out simultaneously.

Free fatty acid (FFA) value: The FFA content of the Ghee samples were determined by the titration method described in [15]. About 5 g of molten Ghee sample was taken in 250 ml conical flask along with 50 ml of freshly neutralized 95% ethanol and 1 ml of phenolphthalein indicator. The mixture was heated for about 5 min in a boiling water bath and titrated while hot against standard 0.1 N sodium hydroxide solution with vigorously shaking. The FFA content of the sample, expressed as % oleic acid.

Colour parameters: The colour parameters of the Ghee samples were derived using image analysis. The Ghee samples (5 g) were accurately weighed in to steriplan petridish (50 mm×15 mm), placed on the flat bed of the scanner (Canoscan 9000F mark II) and the scanned images of the Ghee samples were acquired following the protocol standardized by [18]. The images of the samples were acquired in photo mode using the supporting software of the scanner, imported to Adobe Photoshop and the *Lightness* (L) and chromatic parameters (a and b) of the image were obtained through the histogram method in Lab mode as described in [19]. The value obtained was converted in terms of CIELAB, lightness (L*) ranging from zero (black) to 100 (white), a* ranging from +60 (red) to -60 (green) and b* ranging from +60 (yellow) to -60 (blue). These values were used to derive colour descriptors like chroma, yellowness index by computation using appropriate equations [20].

Viscosity of Ghee: The viscosity of the Ghee samples was determined using a clean Cannon-Fenske viscometer. The viscosity of the Ghee samples was determined in terms of its kinematic viscosity following the standard procedure for recording efflux time for free-flowing sample equilibrated to 40 °C through a fixed distance in the viscometer stem [30]. The kinematic viscosity was calculated by multiplying the efflux time by the viscometer constant, determined by using viscosity of known liquid.

Butyro-refractometer (BR) reading: BR reading of the samples were determined by the method described in ISI, S.18 (Part XI)-1981. The temperature of refractometer was set to 40±0.1 °C using a thermostatically controlled hot water bath. The prism of the instrument was cleaned with a cotton plug moistened with ethyl alcohol and allowed to dry, and refractometer was calibrated with the standard provided by the company. A drop of molten Ghee sample was placed on lower prism of the refractometer and closed by placing upper prism over it and held for 2 min. The most distinct reading (after adjusting the mirror) at 40 °C was recorded as the BR reading of the sample.

Sensory evaluation of Ghee: The organoleptic quality of Ghee samples drawn in the study was also evaluated subjectively. A small volume of each sample of Ghee was presented to a panel of judges who were asked to rate the samples for its sensory attributes in terms of colour and appearance, flavour, body and texture, and overall acceptability on a 9-point hedonic scale, wherein a score of 1 represented 'dislike extremely' and score of 9 represented 'like extremely'.

Statistical analysis: Two-way ANOVA was employed to determine the significance differences in data between the 3 temperatures and frying cycles for sub-baric frying experiments. Two-way ANOVA was also applied to distinguish the data between sub-baric frying at 150 ºC and conventional frying at 145 ºC across the frying cycles. A value of $p \leq 0.05$ was considered to be significant.

Results and Discussion

During deep-fat frying, various deteriorative chemical processes, such as hydrolysis, oxidation, and polymerization take place and *Ghee* decompose to form volatile products and non-volatile monomeric and polymeric compounds. Chemical changes during frying increase FFA, carbonyl compounds, and polymeric compounds and decrease fatty acid unsaturation. *Ghee* samples drawn from the lot following the frying protocols across 5 frying cycles in 3 shifts for conventional and sub-baric frying were individually analyzed for its stability based on the selected chemical and physical parameters and sensory evaluation. In this study, to analyze the degree of thermal degradation of Ghee peroxide value, TBA value, iodine value, FFA value, viscosity, BR reading, colour index was employed.

Peroxide value: Peroxides (R-OOH) are known to be the primary reaction products of the fat oxidation and are formed during the initial stages of oxidation [21]. Hence, peroxide value (PV) is widely considered as an indicator of the progress of lipid oxidation. The mean value of peroxide value of Ghee samples subjected to conventional frying and sub-baric process across the selected 15 frying cycles is presented in Figure 2. It was observed that the average initial values of PV observed in Ghee (before frying was 0.79 meq O2 / kg fat. As the frying progressed, during the first 5 cycles of frying (i.e., shift 1) there was a marginal increase in the PV values to 1.4 to 2.6 meq O2 / kg fat for the Ghee subjected to sub-baric frying. Comparatively, the values for the samples subjected to conventional frying under atmospheric pressure, the PV values were obtained as 10.30 and 15.10 meq O2 / kg fat after frying for 5 and 10 min. The effect of temperature on the lipid oxidation process was observed in the sub-baric process in the 2nd shift and after the 10th cycle of frying the value for the sub-baric process was 3.20, 4.20 and 8.50 meq O2 / kg fat at 120, 135 and 150 °C, respectively.

Comparatively, the values for conventional frying for 5 and 10 min were significantly higher at 28.30 and 48.50 meq O2 / kg fat. After the 3rd shift, i.e., at the end of 15 cycles of frying, the peroxide values were 4.30, 8.00 and 17.50 meq O2 / kg fat for the sub-baric frying samples at 120, 135 and 150 ºC, respectively, while for the conventional samples, the values were 46.30 and 72.80 meq O2 / kg fat after frying for 5 and 10 min. Thus, the trend obtained clearly indicated that sub-baric frying reduced the rate of lipid oxidation in Ghee during frying even at high temperatures and is attributed to the reduced O2 present within the fryer environment. These results are in accordance with the studies on changes in characteristics of palm oil during vacuum and atmospheric frying conditions reported during frying of sweet potato [22]. The lower peroxide value for sub-baric frying has been attributed to the less amount of oxygen was in contact with frying medium while frying.

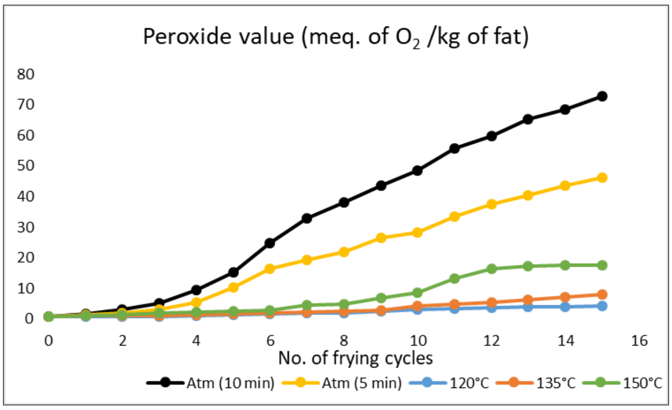

Fig 2: Peroxide value distribution of Ghee subjected to conventional and sub- baric frying

TBA value: Estimation of TBA value, as an indicator of lipid oxidation, is considered a very sensitive and useful method for quantifying lipid oxidation [17]. The TBA value of Ghee subjected to conventional and sub-baric frying is presented in Figure 3.

The fresh Ghee samples reported a mean TBA value of 0.014 and at the end of the 5th cycle of frying, the TBA value of the sample subjected to conventional frying for 5 and 10 min was 0.20 and 0.47, respectively.

In contrast, the values obtained for the samples drawn during sub - baric frying at 120, 135 and 150 °C was 0.017, 0.032 and 0.044, respectively, clearly pointing to a 90% reduction in the increase in TBA value due to sub-baric frying. For the samples processed under sub-baric condition at 120 and 135 °C, showed a progressive increase in its values, reporting a TBA values of 0.033 and 0.148, respectively at the end of the 15th cycle of frying. In comparison, the values for the Ghee drawn from the conventional frying process for 5 and 10 min and sub-baric frying process at 150 °C was similar at 0.46, 0.49 and 0.48, respectively. The interesting trend to note from Fig. 2 is that the conventional frying samples achieved a peak TBA values of 0.58 and 0.65 at the end of the 9th cycle of frying, thereafter, recording diminishing values with progressive frying cycles. This reduction in TBA value after reaching a critical threshold value could be attributed the effect of various factors such as high temperature interaction of malondialdehyde with multiple compounds rather than the development of secondary lipid oxidation products alone [23-25]. Sub-baric frying at 150 °C resulted in continued increase in TBA value up to the 15th cycle, implying that the sample was yet to reach the critical value.

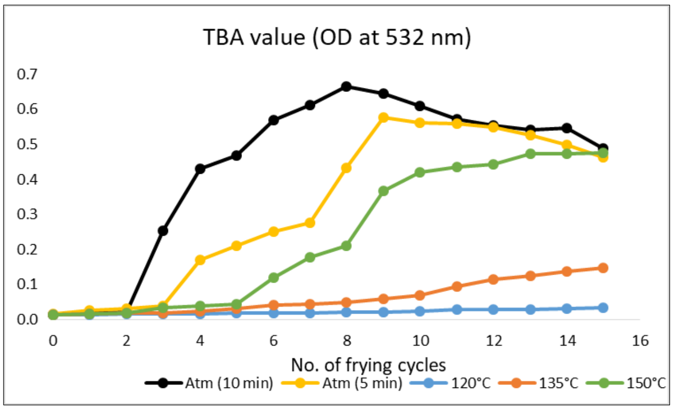

Fig 3: TBA value distribution of Ghee subjected to conventional and sub-baric frying

Free fatty acids (FFA): Presence of free fatty acids (FFA) in *Ghee* is considered as an indication of hydrolytic rancidity, though it is also postulated that some free fatty acids may appear in the oils because of oxidative breakdown pathways. During frying, the moisture released by the fried product combined with the high temperature trigger the hydrolytic breakdown of fat constituents [21]. Once generated, the amount of FFAs in the frying medium increase with each cycle and its oxidative product led to development of off-flavour.

The free fatty acid of *Ghee* subjected to conventional and sub-baric frying process in the study is presented in (Table 1). It can be seen from the data presented, that there was no significant change in the FFA content for all the frying treatments evaluated (conventional and sub-baric) after the 1st shift of frying. The first increase in FFA content was from 0.338 to 0.367% Oleic acid was reported after the 6th cycle of frying under conventional process for 10 min. The first increase in FFA content in the Ghee sample subjected to conventional frying process for 5 min was observed at the end of 8th cycle. In contrast, the Ghee subjected to sub-baric frying did not report any change in its FFA content till the end of the 13th, 11th and 9th cycle for frying at 120, 135 and 150 ºC, respectively.

Table 1: Mean free fatty acid value (% Oleic Acid) for Ghee subjected to conventional and sub-baric frying for 15 frying cycles

No. of frying cycles	Conventional frying (145 °C)		Sub-baric frying (400 mm Hg, 5 min)		
	Frying Time		Frying Temperature		
	5 min	10 min	120 °C	135 °C	150 °C
0	0.338 a	0.338 a	0.338 a	0.338 a	0.338 a
1	0.338 a	0.338 a	0.338 a	0.338 a	0.338 a
2	0.338 a	0.338 a	0.338 a	0.338 a	0.338 a
3	0.338 a	0.338 a	0.338 a	0.338 a	0.338 a
4	0.338 a	0.338 a	0.338 a	0.338 a	0.338 a
5	0.338 a	0.338 a	0.338 a	0.338 a	0.338 a
6	0.338 a	0.367 b	0.338 a	0.338 a	0.338 a
7	0.338 a	0.367 b	0.338 a	0.338 a	0.338 a
8	0.367 b	0.395 c	0.338 a	0.338 a	0.338 a
9	0.367 b	0.395 c	0.338 a	0.338 a	0.367 b
10	0.395 c	0.395 c	0.338 a	0.338 a	0.395 c
11	0.395 c	0.423 d	0.338 a	0.367 b	0.395 c
12	0.395 c	0.423 d	0.338 a	0.395 c	0.395 c
13	0.423 d	0.451 e	0.367 b	0.395 c	0.395 c
14	0.423 d	0.451 e	0.395 c	0.395 c	0.395 c
15	0.451 e	0.451 e	0.395 c	0.395 c	0.395 c

Data with identical superscripts in a column are not significantly different, $p<0.05$

Viscosity: The viscosity of *Ghee* samples subjected to conventional and sub-baric frying is presented in Figure 4. From the Fig. it is observed that the viscosity of *Ghee* progressively increased across all treatments evaluated with each frying cycle. However, the increase in viscosity was more rapid when the *Ghee* was heated under the conventional process for 10 min. The initial viscosity of *Ghee* samples was recorded as 34.58±0.61 cP; the viscosity of the *Ghee* samples at the end of 15th cycle of frying was 38.23±0.20, 38.23 ±0.37, 38.98 ±0.25 and 38.48±0.42 cP for conventional frying for 5 min and sub-baric frying at 120, 135 and 150 ºC, respectively. The corresponding reading obtained for the conventional frying for 10 min was 40.22±0.28 cP. It has been reported that the increase in viscosity of the frying medium is closely correlated to the polymerization of fat constituents [22, 26]. Had reported that the viscosity of frying oil under vacuum showed no significant change even after 8 days of frying cycles. However, the results recorded in the experiments contrasted with this report.

Butyro refractometer reading (BR)

Butyro refractometer reading is a measure of the index of refraction between air and liquid fat and varies with the nature of fat [17]. It was anticipated that the degradation of the fat constituents of *Ghee* during the frying cycles would alter its optical properties, influencing the BR value. However, it was observed that the frying protocols for both conventional and sub-baric process through the 15 cycles did not exert any significant influence on refractive index of *Ghee* and the value recorded remained steady at 41 at 40 °C through all the replications recorded in the study.

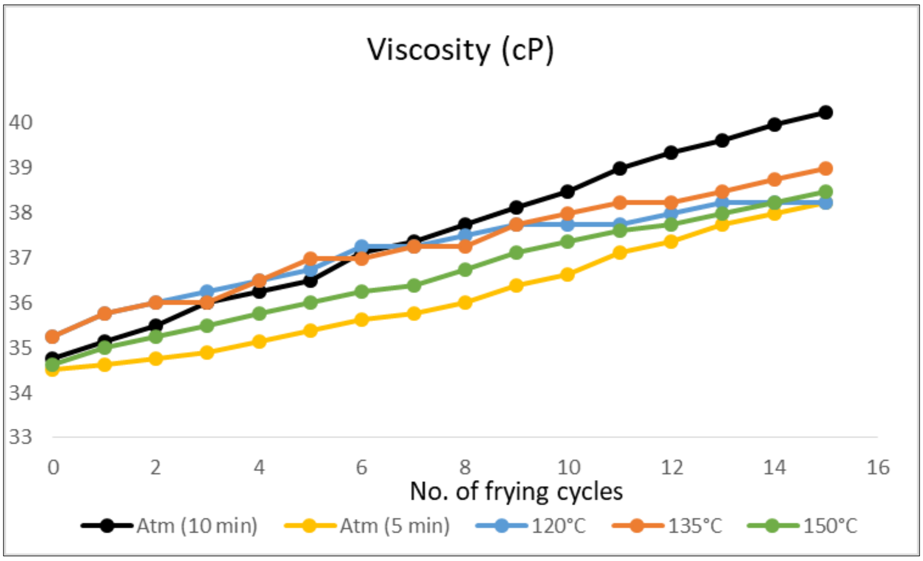

Fig 4: Viscosity distribution of *Ghee* subjected to conventional and sub-baric frying

Colour index: Colour is an important physical index for the quality and acceptance of frying medium, in fact it is generally accepted that visual observation (primarily colour) in addition to flavour is one of the criteria based on which frying oils are rejected during repeated cycles. The change in colour of the frying medium is attributed to two pathways, either due to the accumulation of polymers in the frying medium over the frying cycles or due to the migration or leaching of colour pigments from the fried product into the frying medium [21, 27].

The colour of the *Ghee* subjected to conventional and sub-baric frying at the end of each shift for all combination was quantified using image analysis, the primary L, a, b data obtained through the software was then converted to the standard colour numerical values of L^*, a^* and b^* values as indicated by Yam and Papadakis (2004). L^* values represent the lightness of the sample, ranging from dark (L^* = 0 being black) and light (L^* = 100 being white). The a^* value represents the green - red chromaticity, negative values moving towards the green spectrum on the colour scale and positive values channelling towards the red hue. Similarly, the b^* values represent the blue - yellow components on the colour scale, yellow being represented by positive values and the negative values indicating a blue colour scheme [28].

Among all the colour values expressed in terms of the L^*, a^* and b^* values only b^* was significantly affected which was extracted for the Ghee samples subjected to conventional and sub-baric frying process as per the frying protocols identified for this study, presented in Table 2.

It was noticed that as the frying cycles progressed, the b* values progressively reduced indicating a shift in sample colour from a brighter to a lighter shade of the same hue of yellow (since the a* values are constant). The decrease in the b* values was more prominent in the higher temperature frying samples (both conventional and sub-baric) from the 6th frying cycle onwards and there was no significant difference between the samples from conventional frying (for both 5 and 10-min durations) (7.53 and 9.65, respectively) and the sample subjected to sub-baric frying at 150 °C (8.00). The values indicate that the samples reached a near grey shade of colour. Conversely, the samples subjected to sub-baric frying at lower temperatures (120 and 135 °C), retained their colour better than the other samples, the b^* value at the end of the 15^{th} frying cycle was 38.59 and 21.88, respectively. This trend was further elucidated by deriving the yellowness index of the samples. (Figure 5)

The plot clearly indicates a rapid loss of yellowness in the samples subjected to frying at higher temperature when compared to the samples processed at 120 and 135 °C. Even though the sample processed under sub-baric frying at 150 °C equilibrated with the conventionally processed samples towards the 3rd shift, its progression towards that value of yellowness index was clearly slower than the conventionally processed samples. The interactive effect of temperature and oxygen was also found to be statistically significant for yellowness index.

The yellow hue of cow *Ghee* is attributed to its carotene content [18] and the loss of yellow colour and resultant bleached appearance of the *Ghee* on repeat frying cycles is indicative of a breakdown of this component due to thermal oxidation.

Chroma is a measure of the saturation of the colour and is representative of the vividity of the colour of the sample. The basic colour values obtained for the *Ghee* samples processed by the frying protocols were converted to chroma values and the results is presented in Figure 6. Since the a^* values of the samples were near negligible, the chroma values closely correlated with the b^* values obtained and followed the same trend.

Table 2: Mean values of yellow-blue colour index (b*) for Ghee subjected to conventional and sub-baric frying for 15 frying cycles

No. of frying cycles	Conventional frying (145°C)		Sub-baric frying (400 mm Hg, 5 min)		
	Frying Time		Frying Temperature		
	5 min	10 min	120 °C	135 °C	150 °C
0	48.94	49.65	53.65	53.88	47.53
1	46.59	47.76	49.41	47.53	44.71
2	44.47	42.82	47.06	45.41	40.00
3	40.71	32.94	46.59	43.53	39.53
4	34.82	24.47	43.76	40.94	34.35
5	24.00	16.71	47.06	40.71	32.94
6	23.06	11.53	46.12	37.88	31.06
7	20.71	9.41	42.82	36.00	27.76
8	18.35	8.71	41.88	36.00	22.12
9	13.18	9.18	38.59	34.35	18.35
10	10.82	8.71	40.00	32.94	13.65
11	8.47	8.94	44.24	32.00	9.88
12	8.00	9.18	40.47	29.41	8.94
13	7.76	9.41	41.41	26.82	8.94
14	7.53	8.94	39.53	25.41	8.00
15	7.53	9.65	38.59	21.88	8.00

Data with identical superscripts in a column are not significantly different, $p<0.05$

Sensory evaluation of the *Ghee* samples: The samples of *Ghee* subjected to the frying protocols (both conventional and sub-baric) after each frying cycle was presented to a panel of judges for sensory evaluation and the mean values obtained for the attributes of colour and appearance, flavor, body and texture. From that overall acceptability (OA) was calculated, which is presented in Table 3. From the data, it was clearly deduced that the conventional frying samples recorded poorer scores from the 1^{st} shift itself, primarily due to poor color and flavor. On the other hand, the sub-baric samples were more acceptable to the panel of judges. Fat uptake in the fried product is an important step in developing the quality attributes of the product [29], and poorer sensory profile of the frying medium may result in poorer quality attributes of the fried product also.

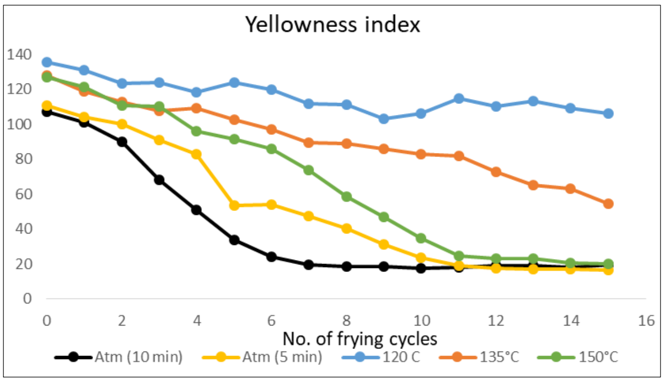

Fig 5: Yellowness index distribution of Ghee subjected to conventional and sub-baric frying

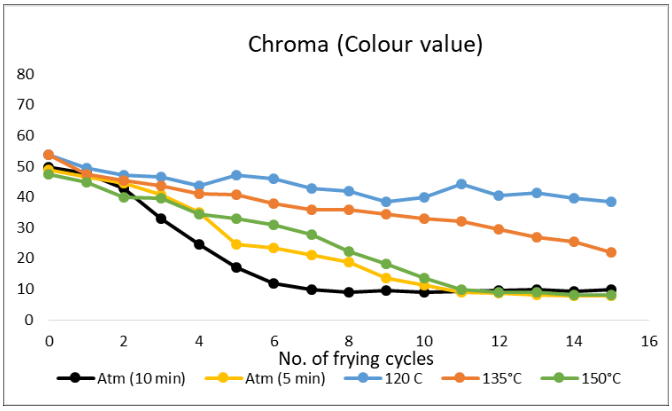

Fig 6: Chroma distribution Ghee subjected to conventional and sub-baric frying

Table 3: Sensory Scores obtained for overall acceptability of Ghee subjected to conventional and sub-baric frying

No. of frying cycles	Conventional frying (145°C)		Sub-baric frying (400 mm Hg, 5 min)		
	Frying Time		Frying Temperature		
	5 min	10 min	120 °C	135 °C	150 °C
0	8.9	8.9	8.9	8.9	.9
1	8.3	8.3	8.7	8.5	8.5
2	8.0	7.8	8.5	8.5	8.5
3	7.5	7.2	8.5	8.2	8.2
4	6.8	6.5	8.3	8.2	8.2
5	6.0	5.5	8.2	8.2	7.8
6	5.5	5.2	8.0	7.8	7.7
7	5.2	4.7	8.0	7.7	6.8
8	4.8	4.5	8.0	7.3	6.7
9	4.7	4.3	7.5	7.2	6.3
10	4.2	3.8	7.5	7.0	6.0
11	3.8	3.2	7.3	6.8	5.3
12	3.8	3.0	7.2	6.5	5.2
13	3.7	3.2	7.0	6.2	5.2
14	3.3	2.8	6.7	6.2	5.0
15	3.3	2.5	6.3	5.8	5.0

Data with identical superscripts in a column are not significantly different, $p<0.05$

Conclusions

As the frying cycles progressed, the peroxide value, TBA value and viscosity of the *Ghee* samples increased over the frying cycles, while iodine value decreased over the frying cycles. The BR values were unaffected by the frying protocols evaluated. Generation of Free fatty acids in the *Ghee* samples were marginal, its appearance was significantly delayed in the sub-baric processed samples at lower temperatures. The colour values, primarily the L^*, a^* and b^* values were indicative of a yellowish hue in the *Ghee* samples, the trend of chroma and yellowness index derived from the colour data were indicative of a loss of yellow colour with progression of frying cycles, possibly due to breakdown of the carotene content of the samples on repeat frying. An interactive effect of temperature and sub-baric environment on the physico-chemical indices was deduced from the trends of the experimental data and established by statistical significance. Sub-baric frying was observed to significantly slow down the degradation changes; at higher temperatures the rate of changes equilibrated for the 3rd shift. Sensory Analysis of the *Ghee* samples indicated that the samples subjected to conventional frying (at atmospheric pressure) were unacceptable after the 1st shift, while the sub-baric samples were found acceptable up to the 3rd shift. The overall acceptability scores were influenced by the colour and flavour of the *Ghee* samples after frying. The study revealed that *Ghee* as a frying medium was more stable during repeat frying cycles under sub-baric frying process when compared to conventional frying, especially at the lower temperatures recommended for sub-baric frying.

References

1. Patel S, Shende S, Arora S, Singh RRB, Rastogi S, Rawat AKS. Antioxidant potential of herbs and spices during deep frying of Ghee. International Journal of Dairy Technology. 2014;67:365-372.
2. Rahila MP, Surendra Nath B, Laxmana Naik N, Pushpadass HA, Manjunatha M, Franklin MEE; 2018.
3. Sanchez-Gimeno AC, Negueruela AI, Benito M, Vercet A, Oria R. Some physical changes in Bajo Aragon extra virgin olive oil during the frying process. Food Chemistry. 2008;110(3):654-658.
4. Sahin S, Sumnu SG. Advances in deep-fat frying of foods. 341 CRC Press, New York; c2009.
5. Serjouie A, Tan CP, Mirhosseini H, Man YBC. Effect of vegetable based oil blends on physicochemical properties of oils during deep-fat frying. American Journal of Food and Technology. 2010;5(5):310-323.
6. Haak L, Sioen I, Raes K, Van Camp J, De Smet S. Effect of pan-frying in different culinary fats on the fatty acid profile of pork. Journal of Food Chemistry. 2007;102(3):857-864.
7. Andrikopoulos NK, Kalogeropoulos N, Falirea A, Barbagianni MN. Performance of virgin olive oil and vegetable shortening during domestic deep-frying and pan-frying of potatoes. International Journal of Food Science and Technology. 2002;37(2):177-190.
8. Anwar F, Hussain AI, Iqbal S, Bhanger MI. Enhancement of the oxidative stability of some vegetable oils by blending with Moringa oleifera oil. Food Chemistry. 2007;103:1181-1191.
9. Aladedunye AF. Natural antioxidants as stabilizers of frying oils. European Journal of Lipid Science and Technology. 2014;116(6):688-706.
10. Shyu SL, Hau LB, Hwang LS. Effect of vacuum frying on the oxidative stability of oils. Journal of the American Oil Chemists Society. 1998;75(10):1393-1398.
11. Amany MB, Shaker MA, Azza AA. Vacuum frying: An alternative to obtain high quality potato chips and fried oil. Banat's Journal of Biotechnology. 2012;3:22-30.
12. Crosa MJ, Skerl V, Cadenazzi M, Olazábal L, Silva R, Suburú G, *et al*. Changes produced in oils during vacuum

and traditional frying of potato chips. Food Chemistry. 2014;146:603-607.
13. Bector BS, Narayanan KM. Chemical and nutritive changes in ghee during thermal oxidation. Indian journal of nutrition and dietetics. 1976;0022:296-302.
14. Kumar MG, Ravindra MR. Design of vacuum impregnation chamber for soaking of Gulabjamun in sugar syrup and optimization of wall thickness by Finite Element Analysis (FEA). IJEAB: Open Access Bi-Monthly International Journal: Infogain Publication, 2016, 2(1).
15. IS: 3508. Indian standards, Methods for sampling and test for Ghee (butter fat). Bureau of Indian Standards, New Delhi; c1966.
16. Patton S, Kurtz GW. 2-Thiobarbituric acid as a reagent for detecting milk fat oxidation. Journal of Dairy Science. 1951;34(7):669-674.
17. Sharma V, Arora S, Lal D, Wadhwa BK. A Laboratory Manual on Analysis of Milk Lipids (Ghee). Shelf-life assessment of Food undergoing oxidation-A Review, Critical Reviews in Food Science and Nutrition. 2007;56(11):1903-1912.
18. Wasnik PG, Menon RR, Nath SB, Balasubramanyam BV, Manjunatha M, Sivaram M. Application of particle analysis and colour parameters for detection of adulteration of cow Ghee with vanaspati derived from image analysis. Indian Journal Dairy Science, 2017, 70(2).
19. Yam KL, Papadakis S. A simple digital imaging method for measuring and analyzing colour of food surfaces. Journal of Food Engineering. 2004;61(1):137-142.
20. Pathare PB, Opara UL, Al-Said FA. Colour measurement and analysis in fresh and processed foods, A Review. Food Bioprocess Technology. 2013;6:36-60.
21. Choe E, Min DB. Chemistry of deep-fat frying oils. Journal of Food Science. 2009;72:77-86.
22. Kusucharid C, Jangchud A, Thamakorn P. Changes in characteristics of palm oil during vacuum and atmospheric frying conditions of sweet potato. Kasetsart Journal: Natural Science. 2009;43:298-304.
23. Dahle LK, Hill EG, Holman RT. The thiobarbituric acid reaction and the autoxidations of polyunsaturated fatty acid methyl esters. Archives of Biochemistry and Biophysics. 1962;98(2):253-261.
24. Janero D. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radical Biology and Medicine. 1969;9(6):515-540.
25. Gilbertson G. Oleoresin as flavor ingredients. The Flavour Industery. 1971;43:403-405.
26. Tseng YC, Moreira R, Sun X. Total frying-use time effects on soybean-oil deterioration and on tortilla chip quality. International Journal of Food Science and Technology. 1996;31(3):287-294.
27. Moreira RG. Vacuum frying versus conventional frying–An overview. European Journal of Lipid Science and Technology. 2014;116(6):723-734
28. Markovic I, Ilic J, Markovic D, Simonovic V, Kosanic N. Color measurement of food products using CIE L*a*b* and RGB color space. Journal of Hygienic Engineering and Design. 2013;4:50-53.
29. Bordin K, Tomihe Kunitake M, Kazue Aracava K, Silvia Favaro Trindade C. Changes in food caused by deep fat frying - A review. Archivos Latinoamericanos de Nutricion. 2013;63(1):5-13
30. BS EN ISO 3104: Methods of test for petroleum and its products: transparent and opaque liquids- determination of kinematic viscosity and calculation of dynamic viscosity. The Institute of Petroleum and BSI; c1996.