E-ISSN: 2663-1067
P-ISSN: 2663-1075
www.hortijournal.com
IJHFS 2025; 7(1): 93-96
Received: 13-10-2024
Accepted: 18-11-2024

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Oxidative stability of ghee clarified at various time: Temperature combinations

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DOI: https://doi.org/10.33545/26631067.2025.v7.i1b.248

Abstract
Ghee is a clarified dairy fat esteemed for its unique flavor and durability. This research investigated the oxidative stability and sensory attributes of ghee clarified at 115 °C and 120 °C for durations of 5, 10, and 15 minutes, thereafter held at room temperature and 60 °C for one month. The results from Thiobarbituric acid (TBA) tests indicated that lipid oxidation increased with extended storage duration and elevated temperatures. Samples clarified for a shorter time exhibited increased oxidation compared to those clarified for 15 minutes (G3 and G6), with G6 showing the minimal oxidation. The clarification and storage conditions did not influence the flavor, texture, color, appearance, or general acceptance of the samples. The results indicate that clarifying ghee at 120 °C for 15 minutes reduces oxidation during storage while preserving sensory quality. This enhances the shelf life and oxidative stability of the ghee.

Keywords: Ghee, oxidative stability, TBA, sensory

1. Introduction
Ghee is a well-known traditional dairy product in India and many Middle Eastern countries, produced by the clarification of milk fat (Butter or cream). Ghee is characterized by its characteristic buttery, pleasant, and nutty flavor and is considered an excellent frying medium compared to other oils, particularly for the peculiar taste it imparts to meals. A multitude of studies has been undertaken regarding the thermal stability of ghee under various settings. (Patel *et al.*, 2014; Rahila *et al.*,2018; Prateek *et al.*, 2023) [1, 2, 3].

Milk fat is a complex lipid found in nature. It is sometimes referred to as clarified butter fat, anhydrous milk fat, or processed milk fat. Triglycerides, predominantly in a mixed form, constitute the majority, accompanied by free fatty acids, phospholipids, sterols, sterol esters, fat-soluble vitamins, carbonyls, hydrocarbons, and carotenoids. Ghee may also include a substantial quantity of conjugated linoleic acids, a substance with anti-cancer properties (clement *et al.*, 1994) [4].

Lipid oxidation is a principal contributor to the degradation of food quality and has traditionally been a matter of concern for food scientists and manufacturers. The presence of catalytic systems such as metals, light, microbes, heat, enzymes and metalloproteins, leads to the generation of off-flavors and the destruction of fat-soluble vitamins, essential fatty acids and other bioactive compounds. Lipids can undergo thermal oxidation, autoxidation, photooxidation, and enzymatic oxidation, all of which typically involve free radicals or reactive oxygen species. (Vercellotti *et al.*, 1992; Shahidi & Nahrung, 2000) [5, 6].

Numerous chemical techniques have been established to assess oxidative degradation in fats and oils (Gray 1978; Shahidi & Zhong 2005) [7, 8]. The techniques described for assessing oxidative degradation of diverse fats and oils are predicated on the chemical alterations occurring in the primary and secondary phases of oxidative degradation. The initial molecules produced in the primary phase of the oxidation process are peroxides, particularly hydroperoxides, which can subsequently yield secondary oxidation products such as aldehydes, ketones, hydroxyl compounds, epoxides, and polymers. These compounds exhibit a diverse range of physicochemical properties, mostly varying in volatility, polarity, and molecular weight. Carbonyl compounds are proposed to be the primary contributors to off-flavours linked to the rancidity of numerous food products (Shahidi & Zhong (2005) [8]. To assess lipid degradation in the secondary stage, methodologies such as thiobarbituric acid

(TBA) value, q-anisidine value (q-AnV), and carbonyl value (CV) measurement have been documented in numerous research (Holm & Ekbom-Olsson 1972; Ronald 2001; Shahidi & Wanasundara, 2002) [9, 10, 11]. Malonaldehyde (MA) is produced during lipid oxidation as a consequence of polyunsaturated fatty acid breakdown. It is typically employed as a marker for the lipid oxidation process. In this assay, the MA reacts with TBA to produce a pink MA-TBA complex, which is quantified spectrophotometrically at its absorption maximum of 530–535 nm (Shahidi and Zhong 2005) [8].

Hence the purpose of the current study was to assess the oxidative stability of ghee clarified under various time-temperature combinations and stored at both ambient and higher temperature.

2. Materials and Methods

2.1. Preparation of ghee

Freshly prepared cow milk cream with 40-42% fat was obtained from University Dairy Plant of Kerala Veterinary and Animal Sciences University, Mannuthy. Ghee samples was prepared by using creamery butter method by clarifying butter at different temperature and time combinations. For this, cream with 40-42% fat was taken, cooled to 8º to 10ºC and aged overnight at this temperature. The aged cream was churned into butter at 10°C in a domestic mixer grinder and the butter was clarified at different temperature and time combinations (Table 1), followed by filtering through cheese cloth.

The samples were stored at room temperature and elevated temperature(60ºC) for a period of one month for further analysis of oxidative stability.

Table 1: Different temperature and time combinations used in clarification of ghee

Sample	Temperature	Time (minutes)
G1	115 ºC	5
G2	115 ºC	10
G3	115 ºC	15
G4	120 ºC	5
G5	120 ºC	10
G6	120 ºC	15

2.2. Thiobarbituric acid value

The TBA value of all ghee samples was assessed using the method of Patton& Kurtz (1951) [12] with slight modifications. The method in brief is discussed below:

Reagents

1. Trichloro acetic acid (TCA) –35% TCA was prepared in distilled water
2. TBA reagent -0.36 g of TBA (2-Thiobarbituric acid) and 0.1 g of anhydrous sodium sulphate were weighed accurately and dissolved in distilled water. The volume was made up to 100 mL using volumetric flask.

2.2.1 Procedure

Approximately 0.1 g of melted ghee samples was precisely measured into a centrifuge tube, to which 1 mL of trichloroacetic acid and 2 mL of TBA reagent were added. The materials were agitated vigorously. The tubes were subjected to a boiling water bath for 15 minutes. The mixture was subsequently cooled, combined with 1 mL of glacial acetic acid and 2 mL of chloroform, and centrifuged at 500 g for 5 minutes to achieve two distinctly separated layers. The optical density (OD) of the supernatant was quantified at 532 nm utilizing a UV/Visible spectrophotometer. The results were quantified as TBA reactive chemicals (TBARS) per 0.1 g of ghee. Blank was concurrently produced without ghee.

2.3 Sensory evaluation

All samples of ghee made in the laboratory were evaluated for their sensory characteristics after a storage period of one month on a 9-point hedonic scale (ranging from 9=like extremely to 1= dislike extremely) by a panel of 5 experienced judges consisted of faculty, technical staff and Master students. Each judge evaluated the ghee samples for flavor, body and texture, color and
appearance and overall acceptability using the 9-point hedonic scale.

3. Results and Discussion

Estimation of TBA value, as an indicator of lipid oxidation, is considered a very sensitive and useful method for quantifying lipid oxidation (Sharma *et al.,* 2007) [13]. The results obtained from three different replications of the samples are given in Table 2 and Figure 1. There was no significant difference in TBA values of different fresh ghee samples. Ashok & Bector (1985) [14] assessed the TBA values of ghee with the technique described by Patton and Kurtz (1951) [12]. They documented TBA values for fresh ghee samples in the Range: 0.020–0.045, with a mean of 0.031. Hence, the TBA values of fresh ghee samples acquired in this study fell within the range documented by Ashok & Bector (1985) [14].

As the time-temperature combination of ghee clarification began to increase, there was a corresponding fall in the TBA values. The TBA values were found to be greater in all of the samples that were stored at a higher temperature (60°C) in comparison to the typical room temperature. The rate of formation of secondary products is faster during accelerated storage than during normal storage (Mehta *et al.*,2015).[15]

The samples that were post-clarified to 115°C for five minutes (G1) and 120°C for five minutes (G4) exhibited high TBA values in comparison to the samples that were maintained at the same temperatures for fifteen minutes (G3 &G6).

Accordingly, one of the essential aspects that must be taken into consideration for the preservation of ghee is the temperature at which it is clarified. At room temperature, the order of oxidative stability was G6> G5> G4> G3> G2> G1. At 60°C, the order was G6> G3> G5> G4> G2> G1. Therefore, clarifying ghee to a higher temperature up to 120°C to a time period up to 15 minutes can decrease the oxidation process of ghee during storage.

Ghee samples prepared with different clarification temperatures and time combinations had no significant effect on the sensory scores in terms of flavor, color and appearance, body and texture and overall acceptability (Table 3).

Table 2: Thiobarbituric acid value of ghee samples

Sample	TBARS/0.1g fat					
	G1	G2	G3	G4	G5	G6
Fresh ghee (0^{th} day)	0.05^{a}± 0.006	0.03 ^{a}±0.006	0.03^{a}± 0.010	0.04^{a} ± 0.004	0.03^{a} ±0.061	0.02^{a}± 0.011
Ghee Stored for I month at Room temperature	0.773^{a}± 0.078	0.546^{ab}±0.087	0.279^{bc}±0.121	0.445^{b} ±0.011	0.269^{bc}±0.070	0.106^{c}±0.090
Ghee Stored for 1 month at Elevated temperature (60^{0})	0.821^{a}±0.097	0.767^{a}±0.075	0.321^{bc}±0.084	0.499^{b}±0.054	0.354^{bc}±0.054	0.221^{c}±0.012

Figures are mean ± standard error of three replications. $^{a-c}$ Means with different superscript vary significantly (p<0.05) within a row.

Table 3: Sensory attributes of ghee samples

Ghee samples stored at room temperature						
Sensory Parameters	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**
Flavor	8.12 ±0.04^{a}	8.33±0.06^{a}	8.63 ±0.12^{a}	8.32 ±0.04^{a}	8.55± 0.13^{a}	8.43±0.08^{a}
Color & Appearance	8.32 ±0.03^{a}	8.14 ±0.06^{a}	8.31 ±0.11^{a}	7.962± 0.06^{a}	8.03±0.11a	8.05± 0.07^{a}
Body &Texture	8.38 ±0.0^{a}	8.21 ±0.08^{a}	8.51± 0.05^{a}	7.9 ±-.09^{a}	8.16±0.09a	8.23±0.04^{a}
Overall acceptability	8.05 ±0.04^{a}	8.32 ±0.08^{a}	8.1 ±0.05^{a}	8.01 ±0.10^{a}	8.23±0.03^{a}	8.49±0.04^{a}
Ghee samples stored at 60° C						
Flavor	7.91 ± 0.07^{a}	8.12±0.06^{a}	8.15 ±0.12^{a}	8 ±0.04^{a}	8.09±0.02^{a}	8.14±0.05^{a}
Colour &Appearence	8.12 ±0.04^{a}	7.97 ±0.06^{a}	8.01 ±0.11^{a}	8.15 ± 0.06^{a}	8.21±0.04^{a}	8.36±0.07^{a}
Body &Texture	8.12 ±0.03^{a}	8.07 ±0.08^{a}	8.03 ± 0.05^{a}	8.11±0.09^{a}	8.18±0.05^{a}	8.21±0.01^{a}
Overall acceptability	8.03 ±0.0^{a}	8.09 ±0.08^{a}	6=8.19 ±0.05^{a}	8.17 ±0.10^{a}	8.24±0.01^{a}	8.30±0.06^{a}

Figures are mean ± standard error of three replications

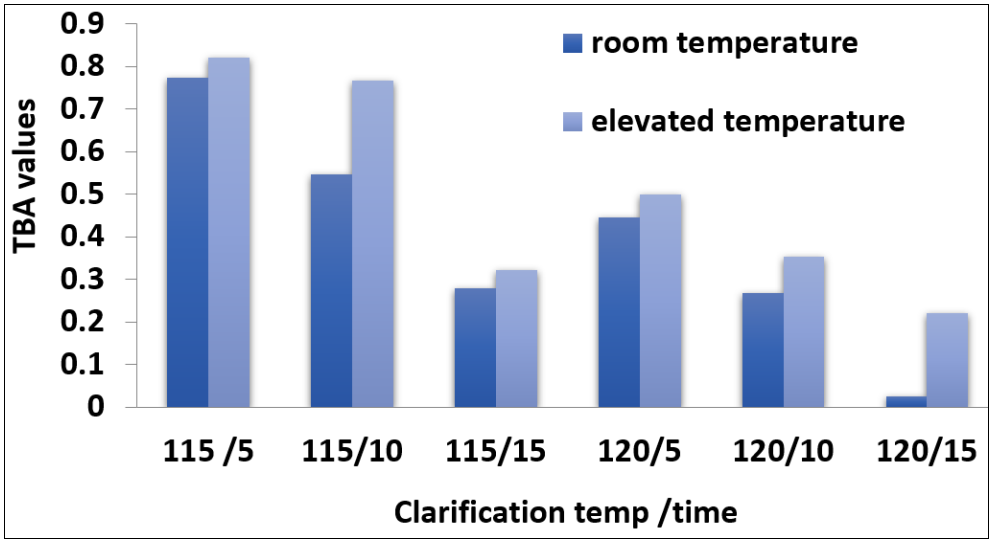

Fig 1: Graphical representation of TBA values

4. Conclusion

Ghee, a traditional dairy product common in India and the Middle East, is created by refining milk fat and is esteemed for its distinctive flavor and culinary attributes. This study examines the oxidative stability of ghee produced under diverse time-temperature settings and stored at varying temperatures. Diverse chemical techniques, such as the Thiobarbituric Acid (TBA) assay, were utilized to evaluate oxidative deterioration. The results demonstrated that elevated clarification temperatures and extended durations enhanced the oxidative stability of ghee, as TBA readings reflected reduced oxidation in samples clarified at 120°C for 15 minutes. Sensory tests revealed no significant variations in flavor, appearance, or general acceptability among the ghee samples. The results indicate that ideal clarifying conditions might improve the shelf life and quality of ghee.

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