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Quality and Oxidative Stability of Tallow Extracted by Dry- and Wet-Rendering

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***Abstract*— The oxidative stability of oil or fats is an essential parameter to determine the quality of products. Commonly, tallow is extracted by dry- and wet-rendering, both methods depend upon the water requirement. The study aims to examine the quality and oxidative stability of tallow from both methods, along with different temperatures (25°C and 4°C) and storage times. Tallow was analyzed for quality changes such as acid value (AV), peroxide value (PV), thiobarbituric acid (TBA), fatty acid profile, and differential scanning calorimetry (DSC) to indicate oxidative stability. According to research, acid value (AV) and thiobarbituric acid (TBA) were considered acceptable during storage for 180 days, as per the Codex Standard and FAO/WHO for Named Animal Fats CODEX STAN 211–1999 for edible fats and oils. However, peroxide oxide value (PV) did not meet the acceptable limit for dry- and wet-rendering and storage time of 180 days at 25°C, with values of 17.3 and 16.9 meq O_2/kg, respectively. This indicates that fat oxidation increases during storage ($p<0.05$), while temperature and rendering methods do not significantly impact fat hydrolysis inhibition for 180 days ($p>0.05$). Even at 4°C, both temperatures failed to preserve tallow quality, as confirmed by fatty acid profiles and differential scanning calorimetry. The result indicates that neither method nor temperature can prevent the inhibition of fat hydrolysis from natural oxidation for a longer storage time of 180 days.**

***Keywords*— Oxidative stability; tallow; dry- and wet-rendering; unsaturated fatty acid; fat oxidation**

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I. INTRODUCTION

Fat and oils are essential organic materials that are needed in the human body for nutritional and good sensory attributes for food products [1]. In addition, fats are important ingredients or sources to various manufacturers of food, feed, pharmaceuticals, and renewable energy [2-5]. The technology of applying fats in those products depends on their physical and chemical properties and nutritional properties, which is the composition of fatty acids [1]. Beef fats are a blending of saturated fatty acids (SFA) such as myristic, palmitic, and stearic acid and unsaturated fatty acids (USFA), including monounsaturated (palmitoleic acid and oleic acid) and polyunsaturated fatty acids (linoleic acid) [6]. However, unsaturated fatty acids play an important role in the oxidation reaction for lipid hydrolysis, which is associated with the product's final quality (tallow). Moreover, lipid oxidation generates unpleasant flavor, undesirable quality change, and low nutritional value aspects of the product due to the loss of essential fatty acids and vitamins [7]. The oxidations cannot be minimized by cold storage, good transportation, and proper packaging. However, oxidative rancidity also cannot be stopped by lowering storage temperature, as a result, determining the oxidation phase is essential to evaluate the quality of food changes [8-10]. Tallow is extracted animal fat generated from boiling or rendering processes and fat wastes. Commonly, tallow is obtained from ruminant tissues (beef, mutton, and lamb),

which mainly contain triglycerides (adipose tissue, skeletal muscles, or extracted from other animal wastes, including bones, or fat surrounding specific organs) [1,2]. The composition of tallow consists of fatty acids, including SFAs (saturated fatty acids) and USFA (unsaturated fatty acids). According to the number of double bonds, USFAs are categorized as monounsaturated and polyunsaturated fatty acids. Both types of these fatty acids, mainly oleic acid, and linolenic acids, are employed as major ingredients in various cosmeceutical and pharmaceutical products [11,12]. Thus, the quality of beef tallow is affected by fatty acid composition. Dry-and wet rendering is the oldest existing approach for the extraction of fats, mainly used for edible and inedible industries. These methods apply low and high temperatures in either batch or continuous processes. The waste material can be transformed into useful products such as fuels, soaps, rubber, and plastic using these methods. Both techniques are much more resistant to spoiling, in which the raw materials used are mainly susceptible to spoilage [13,14]. Several studies on the quality properties of tallow prepared by dry- and wet rendering have been explored for different sample fats such as ostrich [15], crocodile [16], and beef [17]. Since dry- and wet rendering employing animal fats has been frequently used, the quantitative information on the quality and oxidative stability of the extracted tallow method is still very limited and inconsistent. Therefore, the purpose of this study was to examine how the extracted tallow method affects the quality and oxidative stability of tallow. Additionally, the study aimed to identify the key quality indicators for both samples during periods of different temperatures (25°C and 4°C) and storage times (0, 7, 14, 30, 60, and 180 days).

II. MATERIAL AND METHODS

A. Sample preparation, storage conditions, and reagents

Tallow was processed using previous dry- and wet-rendering methods [16]. Briefly, fats of adipose tissue were obtained from public slaughterhouses. Fats were cut into small tubes (3x3x3 cm), thoroughly mingled, weighed 500 gr each, and rendered using dry and wet rendering. Subsequently, tallow was transferred into a 250 mL bottle (6.4 x 7.4 cm) and stored at 25°C and 4°C (Panasonic refrigerator) for 0, 7, 14, 30, 60, and 180 days. All reagents and organic solvents were purchased from Sigma ((St. Louis, MO, USA), while the gas chromatography (GC) column was purchased from Agilent, Santa Clara, USA). The deterioration progress was evaluated by acid value, peroxide value, and thiobarbituric acid (TBA) value, while fatty acid profiles and Differential Scanning Calorimetry (DSC) were determined on samples of 0 and 60 days for both methods.

B. Measurement of quality and oxidative stability

Acid Value (AV)

The acid value was determined in line with AOCS Official Method Cd 3d-63 [18]. Five grams of fat rendering was mixed thoroughly with 50 mL of 95% EtOH, followed by two drops of 1% phenolphthalein. The solution of 0.1 N NaOH was used to neutralize until the sample appeared pink in color. The acid values were calculated using the formula of AV (mg KOH/g oil) = (sample with oil – sample without oil) × 56.1 × N NaOH/weight of sample used (g).

Peroxide Value (PV)

The AOCS Official Method Cd 3-25 [18] was performed to determine the peroxide value. Five grams of fat rendering was mixed with 30 mL (CH_3COOH-$CHCl_3$) and followed by 0.5 mL saturated potassium iodide. The mixed solution was shaken gently for 1 min and subsequently added 0.5 mL amylum. The mixed solution was titrated using 0.01 N $Na_2S_2O_3$ until the sample was colorless. The peroxide value was calculated using the formula PV (meq O_2/kg oil) = S × M × 1000/g sample, where S = mL $Na_2S_2O_3$ (blank corrected) and M = molarity $Na_2S_2O_3$ solution.

Thiobarbituric acid (TBA)

According to Tarladgis et al. [19], the thiobarbituric acid was determined. Approximately 100 mg of fats rendering were weighed, dissolved, and made up to 25 mL with 1-butanol. Five mL of sample solution was transferred and mixed with an equal amount of TBA reagent. The mixed solution was subsequently heated at 95°C for 2 h, and cooled under tap water for about 15 min until it reached room temperature. The absorbance was determined at 530 nm against a blank sample. The values were calculated using the formula of TBA value (µmol/g oil) = (absorbance sample - absorbance blank) × 7.8 × weight of sample used (g).

Fatty Acid Profile

GC analysis was performed on 7890B Gas Chromatography System (Agilent Technologies, California, USA) equipped with the flame ionization detector. The utilized column was a silica column HP-88 (length: 30 m; diameter, 0.25 mm; with film thickness: 0.2 µm; Agilent, USA). The injection was made in split mode, using a split flow rate of 81 mL/min (split ratio of 1:50), and the volume injected was 1 µL. Injector and detector temperatures were set at 250°C and 280°C, respectively. The GC oven program was as follows: an initial oven temperature of 50°C (hold time of 1 min), to 200°C at 25°C/min, and the final temperature was 230°C at 3°C/min (hold time of 18 min), respectively and total run time was 35 min with an ultrahigh purity grade of helium and nitrogen were used as carrier gases at a flow rate of 1 mL/min. Specified CRM47885 FAME mix 37 standard was used for the identification and quantification of fatty acids produced. Fatty acids were identified by comparing retention times with authentic standards, and the results were reported as weight

percentages following integration and calculation using ChemStation (Agilent Technologies) [20].

Differential scanning calorimetry (DSC)

Sample of tallow (3-5 mg) before and after storage time were placed in aluminum pans, covers were sealed, under an oxygen atmosphere and analyzed being pressurized in an isobaric module (1,400 kPa) with DSC-60 Plus (Shimadzu, Japan). The isothermal temperature (25-100 °C) was used for the data collection. The obtained diagrams were analyzed using OriginPro 2022 software. For each sample, the measurement was triplicate [21].

Statistical analysis

All experiments were carried out in triplicate and expressed as mean±standard deviation (SD). A three-way analysis of variance (ANOVA; GraphPad 8 for Windows) was performed to determine the group means, with 2 x 2 x 6 (A= Rendering methods, B= Storage Temperature, C= Storage time). The fatty acids profile was analyzed using the Turkey test for multiple comparisons. The significance level was defined at $p<0.05$. Two-dimensional PCA was assigned to discriminate the fatty acid profile of the oil rendered using dry and wet methods before and after 180 days of storage at 4°C and 25°C. R-version 4.2.2 was used for PCA with the "ggplot2" and "ggfortify" packages [22].

III. Result and Discussion

A. Chemical properties

The deterioration of oxidative oil has been reported that the oxidation process occurs during the storage condition and time [22,23]. In this study, the oil quality was monitored based on the chemical properties, including acid value, peroxide value, thiobarbituric acid (TBA) value, and fatty acid composition, under different rendering methods and storage temperatures. Storage condition was selected at 25°C and 4°C due to Indonesian customers keeping tallow frequently at that temperature.

TABLE 1
Changes in acid value, peroxide value, and thiobarbituric acid value of fat rendered from beef tallow using dry and wet method during 180 days of storage at 25°C and 4°C

Lipid properties	Storage period (day)	Dry rendering		Wet rendering		SEM
		25°C	4°C	25°C	4°C	
Acid value (mg KOH/g)	0	0.49^{a}	0.49^{a}	0.49^{a}	0.49^{a}	0.02
	7	0.71^{b}	0.71^{b}	0.64^{b}	0.52^{b}	0.03
	14	1.05^{c}	1.01^{c}	1.05^{c}	0.94^{c}	0.02
	30	1.23^{d}	1.23^{d}	1.16^{d}	1.05^{d}	0.04
	60	1.23^{d}	1.20^{d}	1.27^{d}	1.20^{d}	0.03
	180	1.16^{d}	0.97^{d}	1.35^{d}	1.23^{d}	0.05
Peroxide value (meq O_2/kg)	0	1.80^{a}	1.80^{a}	1.33^{a}	1.40^{a}	0.08
	7	1.93^{a}	1.80^{a}	2.43^{b}	1.40^{a}	0.12
	14	2.13^{b}	1.80^{a}	3.80^{c}	2.47^{b}	0.23
	30	5.11^{c}	3.60^{b}	4.73^{d}	4.00^{c}	0.19
	60	7.13^{d}	4.33^{c}	7.20^{e}	4.40^{c}	0.43
	180	17.3^{e}	4.80^{d}	16.9^{f}	4.80^{d}	1.87
Thiobarbituric acid (μmol/g)	0	0.49^{a}	0.49^{a}	0.49^{a}	0.49^{a}	0.01
	7	0.49^{a}	0.49^{a}	0.55^{a}	0.49^{a}	0.02
	14	0.91^{b}	0.88^{b}	0.96^{b}	0.94^{b}	0.01
	30	1.20^{c}	1.20^{c}	1.22^{c}	1.22^{c}	0.01
	60	1.43^{d}	1.46^{d}	1.48^{d}	1.48^{d}	0.02
	180	1.59^{e}	1.59^{e}	1.64^{e}	1.53^{e}	0.02

SEM, standard error of mean.
In the same column, values with different letter superscripts mean significant difference ($p<0.05$).

AV, PV, and TBA values of all tallow samples showed similar patterns, comparable to one another, as shown in **Table 1.** AV is commonly used as an indicator of fat hydrolysis related to lipase activity originating from microorganisms. A previous study showed that the AV of tallows stored at 2°C increased from 0.19 to 1.27 mg KOH/g oil, while at -18°C lifted from 0.19 to 0.98 mg KOH/g oil during storage for 210 days [24]. In another study, AV of bovine skin fats that were extracted using pressurized hot water resulted in a range from 0.64 to 6.21 mg KOH/g oil at 60°C for 90 days [25]. The study shows that the AV of all sample tallow in storage for 180 days ($p>0.05$) were within the acceptable limits defined by the Codex Standard and FAO/WHO for Named Animal Fats CODEX STAN 211–1999 for edible fats and oils, where the standard value is 2.5 mg KOH/g of oil. The increasing AV was probably due to raised hydrolysis and oxidation of triacylglycerol [26]. PV is also one of the general approaches to measuring lipid oxidation in processed meat products. Lipid oxidation is an essential determination of shelf-life which occurs during food production and storage. Primary oxidation products oxidation generates minor substances, including molecular weight alcohols and short-chain hydrocarbons, as well as a small quantity of secondary oxidation products, aldehydes, and ketones [27-29]. PVs followed the same trend as AV in the case of all our samples. Interestingly, the lowest increase in PV was observed in tallow sample storage at 4°C compared to 25°C. This suggests that low temperature (4°C) slightly reduced the rate of hydroperoxides (ROOH) decomposition process ($p>0.05$). However, tallow extracted by both techniques has a PV minimum value and increases significantly during the whole 180 days of storage ($p<0.05$). The legal limit of PV tallow is a maximum of 10 meq O_2/kg refer to Codex Alimentarius.

TBA represents lipid peroxidation particularly the formation of secondary oxidation products, such as malondialdehyde, alkenals, and alkadienals [30]. Like PV, TBA is also a common method to measure lipid oxidation with reactive substances such as malonaldehyde, which will be spectrophotometrically quantifiable via the formation of colored compounds with TBA. In the result, the TBA value was increased significantly between each sampling time point with a similar uptrend as AV and PV. All tallow samples, either stored at 25°C or 4°C, did not exceed the acceptability limit within 180 days. According to **Table 1**, like AV and PV, for all tallow samples, TBA values were increased in the case of both extraction procedures.

Overall, the increase in values starts on the 14th day of storage. The storage time is highly significant during the entire length of storage, while the rendering method slightly affects AV, PV, and TBA values. However, the difference in temperatures did not give a significant difference until the end of the storage period. It suggests that ranges between 25°C and 4°C are not big differences in terms of temperature. Several studies reported that lowering temperature cannot inhibit the hydrolytic reaction of fats [31-33]. Thus, storage of both temperatures has no significant differences among AV, PV, and TBA values.

B. Fatty acid profiles

To further determine the quality and oxidative stability of tallow, fatty acid profiles were analyzed in selected samples 0, 60, and 180 days. Theoretically, fatty acid profile correlated to oxidative stability, particularly high amounts of PUFAs roles to the rapid deterioration of fats [34]. The change in fatty acid composition under different methods and temperatures during storage time is shown in **Table 2**. The main fatty acids obtained in tallow were saturated fatty acids (myristic acid C14:0, pentadecanoic acid C15:0, palmitic acid C16:0, and stearic acid C18:0) and unsaturated fatty acids (oleic acid C18:1, linoleic acid C18:2, and linolenic acid C18:3). Palmitic, oleic, and linoleic acids were the most abundant in tallow, as in previous studies [35-37].

TABLE 2
Fatty acid composition (%) of oil rendered from beef tallow using dry and wet method on day 0 and after 180 days of storage at 25°C and 4°C

Fatty acid	Dry rendering			Wet rendering			SEM
	day 0	day 180 at 25°C	day 180 at 4°C	day 0	day 180 at 25°C	day 180 at 4°C	
C14:0	2.46^{c}	3.72^{c}	3.60^{d}	3.86^{b}	3.88^{a}	3.54^{e}	0.03
C15:0	1.18^{b}	0.74^{d}	0.73^{cd}	1.24^{a}	0.76^{c}	0.72^{d}	0.05
C16:0	26.01^{d}	26.50^{c}	25.95^{e}	26.90^{b}	27.02^{a}	25.91^{f}	0.11
C17:0	1.68^{c}	0.71^{e}	1.34^{d}	1.75^{b}	1.77^{a}	1.77^{a}	0.09
C18:0	27.29^{e}	25.92^{d}	26.36^{c}	23.66^{f}	26.90^{b}	27.66^{a}	0.35
C18:1n9	30.54^{a}	23.74^{d}	24.11^{c}	29.83^{b}	23.64^{e}	23.60^{f}	0.74
C18:2n6	0.78^{b}	0.48^{d}	0.49^{cd}	0.83^{a}	0.48^{d}	0.50^{c}	0.04
C18:3n3	0.18^{a}	0.13^{c}	0.18^{a}	0.17^{b}	0.18^{ab}	0.19^{a}	0.01
SFA	56.64^{f}	57.59^{d}	57.98^{c}	57.41^{e}	60.33^{a}	59.59^{b}	0.31
USFA	31.50^{a}	24.34^{d}	24.74^{c}	30.79^{b}	24.30^{d}	24.28^{d}	0.77
MUFA	30.54^{a}	23.74^{d}	24.07^{c}	29.83^{b}	23.64^{e}	23.60^{e}	0.74
PUFA	0.96^{a}	0.60^{d}	0.67^{b}	0.96^{a}	0.66^{c}	0.68^{b}	0.04

SFA, saturated fatty acids; USFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SEM, standard error of mean.

According to the proportion of fatty acid in rendered oil, SFA was the most abundant fatty acid group, with C16:0 and C18:0 being the most predominant fatty acids. In contrast, another study found that beef tallow contained C17:0, which is the most predominant fatty acid [24]. The fatty acid composition of beef tallow depends on cattle breed, feed nutrition, and body part where fat is deposited. Among the USFA group, C18:1n9 was more abundant than C18:2n6 and C18:3n3. These fatty acid profiles resulted in the characteristics of the rendered oil. The dominancy of SFA led the oil to harden easily at ambient temperature as the melting point of these fatty acids is higher than at ambient temperature. The melting point of C16:0 and C18:0 is 62.9°C and 69.4°C, respectively [38]. However, the smooth texture of the hardened oil is caused by the presence of C18:1n9, a MUFA of more than 20%, with a melting point of 13.4°C [39]. The results show that the amount of SFA increased, while USFAs decreased during storage at 25°C. However, storage at 4°C, the content of USF and SFA slightly increased. The type of rendering technique had no significant effect on the change in total fatty acids at the storage time **(Table 2)**. Longer storage affected a reduction in C14:0, C15:0, C16:0, C17:0, and C18:0, along with increasing in C18:1, C18:2, and C18:3. High PUFA levels have a detrimental impact on tallow quality because double bonds in fatty acids are rapidly hydrolyzed, resulting in the generation of undesirable odor molecules [40].

C. Determination of deterioration using DSC analysis

DSC is a primary tool for assessing the oxidative deterioration of oils and fats with an approach to the degree of thermos-oxidation after conventional heating [41]. DSC is widely used as an analytic, diagnostic, and research tool and is also one of the most frequently used non-chemical methods for oxidative stability expressed as the oxidation induction time [42-44]. To confirm the oxidative decomposition of tallow, selected sample storage at 4°C on 0, 60, 120, and 180 days was examined. The DSC curve during storage is shown in **Fig 1.** At time 0, all samples show the classical profile of tallow with single endothermic, a major peak at low temperature. Change of profile pattern is evident for tallow samples at different storage times. A small reduction in the height of the peak was observed on storage up to 60, 120, and 180 days for dry rendering methods (**Fig 1a**). In wet rendering methods, every peak height shifted broadly (**Fig 1b**). Thus, changes in the transition profiles are proof when lipid oxidation noticeably increases, in reference to the PV pattern shown in **Tabel 1**. These substantial DSC curve profiles have never been reported before and might be attributed to the high amounts of oxidized molecules (both primary and secondary lipid oxidation products).

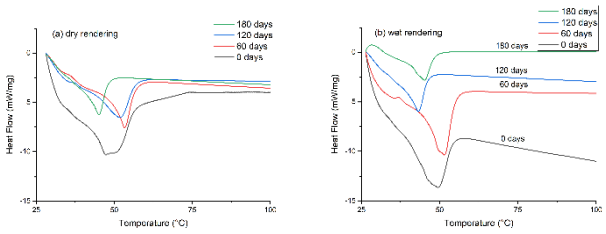

Fig 1. DSC curves of tallow samples produced by different methods of dry rendering (a) and wet rendering (b) at different storage times (days) for storage temperature at 4°C.

D. PCA (principal component analysis)

The discrimination among treatment groups according to their fatty acid profile is clearly shown in **Fig 2**. The first principal component (PC1) explains 63.8% of the variation, while PC2 explains 18.1% of the variability. Statistically, the first two principal components (PC1 and PC2, 81.9%) indicate that the data obtained are normally distributed and the model is acceptable, corresponding with previous studies [45,46]. The cluster pattern shows that different rendering methods had a significant effect on the fatty acid profile of the rendered oil. On the other hand, storage duration and storage temperature also had a significant impact on the fatty acid profile of the rendered oil. The loading plots of all fatty acids were affected by storage temperature and rendering methods. The fatty acid profile of freshly rendered oil using both methods is close to each other. Although the close distance was identified, the proportion of all identified fatty acids among those groups was statistically different (**Table 2**), indicating that different rendering methods resulted in different fatty acid profiles of freshly rendered oil from beef tallow. Higher SFA content was found in wet-rendered oil, while dry-rendered oil contained more USFA. Samples from dry rendering that were stored at 4°C were also quite identical to samples from wet rendering that were stored at 25°C in terms of fatty acid profile (**Fig 2**). Cold storage at 4°C for 180 days for oil rendered using the wet method tends closer to the higher proportion of C18:0 and SFA. These are also confirmed in **Table 2**, where the highest proportion of C18:0 and C18:3n3 were found in oil rendered using the wet method and stored at 4°C for 180 days. Among fatty acid groups, the proportion of USFA, MUFA, and PUFA declined significantly after storage, while the proportion of SFA increased to replace the proportion of unsaturated fatty acids. Interestingly, the proportion of C18:3n3 increased in oil rendered using the wet method after 180 days of storage at different storage temperatures, as the proportion of C18:2n6 decreased after storage in both oils rendered using the wet and dry method. This indicates that the oxidation of C18:2n6 occurred highly during storage. Flavia et al. [24] also found that the concentration of C18:2n6 in beef tallow decreased by 26% after being stored at 2°C for 180 days. Overall, dry rendering resulted in higher oxidation of unsaturated fatty acids during storage than wet rendering, and a storage

temperature of 4°C prevents rapid oxidation of unsaturated fatty acids in oil rendered from beef tallow.

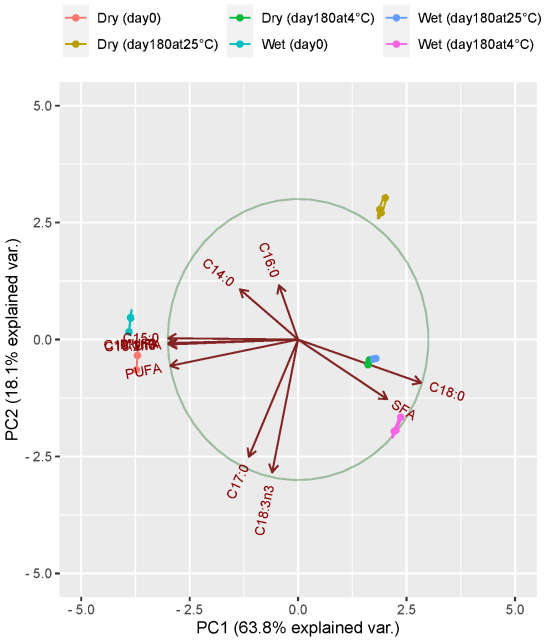

Fig 2. PCA plot showing the fatty acid loadings and differences in oil rendered from beef tallow using dry and wet methods at day 0 and after 180 days of storage at 4°C and 25°C.

IV. CONCLUSION

The quality and characteristics of tallow in different rendering methods and storage conditions (temperature and time) were evaluated. Quality and oxidation indicators such as AV, PV, TBA, fatty acid profile, and DSC show that temperature cannot prevent oxidative change in tallow. However, the storage time for 60 days at both temperatures was acceptable for edible fat/oil. In addition, both rendering methods do not affect the oxidative and quality of tallow.

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