Fats of Pig and Beef from Benin: Purification, Stability Study, Chemical and Nutritional Composition

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To cite this article:
Hermann Nounagnon, Bénédicta Kpadonou-Kpoviessi, Berenger Ladele, Pierre Dossou-Yovo, Joachim Gbénou, Salomé Kpoviessi. Fats of Pig and Beef from Benin: Purification, Stability Study, Chemical and Nutritional Composition. American Journal of Applied Chemistry. Vol. 6, No. 2, 2018, pp. 43-50. doi: 10.11648/j.ajac.20180602.12

Received: February 27, 2018; Accepted: March 13, 2018; Published: April 9, 2018

Abstract: Animal fats, are used in several western countries in industrial sectors as energy, oleochemistry, animal and human feed but stay under-utilized through Africa. The aim of the present study was to purify and stabilize fats of Bos taurus Linnaeus (BTL) and Sus scrofa domesticus Erxleben (SSDE) from Benin for various uses. Fats from BTL and SSDE were purified using local reagents, for the first time in Benin. The physicochemical properties, nutritional composition and the fatty acid profile were determined, with also, their preserving times. The GC/FID analyzes shown that 100 g of purified BTL fat contained 65.76% of saturated fatty acids (SFA), 31.83% of monounsaturated fatty acids (MUFA) and 2.41% of polyunsaturated fatty acids (PUFA). In the SSDE fat, 48.57% of SFA, 38.59% of MUFA and 12.84% of PUFA were obtained. The studied fats were rich in unsaturated fatty acids (UFA) such as oleic acid (34% and 26% and for SSDE and BTL respectively) and linoleic acid (11% and 1% for SSDE and BTL respectively). Their major saturated acids were palmitic and stearic acids, with respectively 25% and 35% for BTL fat and 25% and 21% for SSDE fat. The physicochemical parameters such as acid, peroxide, iodine and saponification indexes of the two fats were in agreement with the Codex Stan 211 (1999) recommendations, during all the conservation time. The preserving times of the two purified fats varied according to the temperature of conservation. These purified fats, with interesting physicochemical properties, could be used in agri-food, energy, oleochemistry, cosmeticology.

Keywords: Purified Fats, Sus scrofa domesticus Erxleben, Bos taurus Linnaeus, Stability, Fatty Acid Profile, Nutritional Properties

1. Introduction

Animal fats are substances belonging to the lipid class. They are basically extracted from adipose tissues or milk of animals. Pig (SSDE) and beef (BTL) BTL are animals raised throughout West Africa. In Benin, fish, poultry, pig (SSDE) and beef (BTL) are the most important sources of animal protein. The livestock capital of ruminants accounts for 89.6% of the total Benin livestock (ruminants, pigs, poultry, rabbits, and aulacodes). Ruminant farming, with a population of 2 to 3 million head, originally practiced mainly in the north of the country (more than 65 percent of the national population), tends to move towards the Center and South [1]. SSDE production is fairly developed and spread throughout the country's rural and urban areas. It is present in all departments of the national territory with a high concentration in those of the South and Central [2]. The local SSDE is the most breed by the producers and its meat, compared to that of the improved breed SSDE, is more
appreciated by consumers [3]. The improved breeds encountered are the Large White, the Landrace and their mixed breeds often raised in semi-modern to modern conditions. The use of SSDE meat during ceremonies such as weddings, baptisms, birthdays... the use of SSDE fat in traditional medicine and the frequent gathering of consumers around the many delicatessens located on both sides in the towns and villages of the country justify the high consumption of the meat of pigs [3]. Weighing up to 500 Kg for beef and 150 Kg for pig, the carcass of these animals after slaughter can contain up to 8.5% of lipid for beef and 12% for pig [4]. Unfortunately, these lipids, stoked in the adipose tissues, were not valued at their fair value in Benin. Throughout the literature, these fats could be refined from the adipose tissues [5] and their fatty acid profile revealed a high percentage of UFA (58.8% and 54.3% for pig and beef respectively) [6, 7] as major compound. They also contained palmitic and stearic acids (24% and 13% respectively for pig and beef) [6, 7] as major SFA [4] with oleic acid (43.5% and 45.5% respectively for pig and beef) [6, 7] as major compound.

2. Materials and Method

2.1. Chemicals and Reagents

Orthophosphoric acid (H$_3$PO$_4$, 85%) was obtained from Surechem Products LTD Needham Market Suffolk England. The caustic soda in pellets (NaOH, 97%) from Scharlab S. L. Spain. 40 methylester fatty acid components and all reagents (diethyl ether, ethanol 95%, phenolphthalein, potassium hydroxide, chlorhydric acid, cyclohexane, potassium bromate, potassium iodide, chloroforme, acetic acid, sodium thiosulfate, magnesium sulfate) were obtained from Sigma-Aldrich (Steinhein, Germany) and absolute ethanol obtained from Surechem Products LTD Needham Market Suffolk England. Aldrich (Steinhein, Germany) and absolute ethanol obtained from Surechem Products LTD Needham Market Suffolk England. The caustic soda in pellets (NaOH, 97%) from Scharlab S. L. Spain. 40 methylester fatty acid components and all reagents (diethyl ether, ethanol 95%, phenolphthalein, potassium hydroxide, chlorhydric acid, cyclohexane, potassium bromate, potassium iodide, chloroforme, acetic acid, sodium thiosulfate, magnesium sulfate) were obtained from Sigma-Aldrich (Steinhein, Germany) and absolute ethanol obtained from Surechem Products LTD Needham Market Suffolk England. All chemicals and reagents were of high quality and analytic grade.

2.2. Animal Material

The adipose tissues of BTL and SSDE were purchased in the Cotonou slaughterhouses and kept in a cooler whose temperature has been maintained between 0 and 4 ± 1°C. The samples were taken to the laboratory and store in the freezer at 4 ± 1°C.

2.3. Fats Purification

The adipose tissues of BTL and SSDE were refined using the chemical process. Small pieces of adipose tissues were heated at about 60 to 70°C. Then the melted fat was separated from meat residues by filtration and purified following the different steps:

- Degumming: removal of the mucilage by addition of phosphoric acid to the obtained oil heated at about 90 °C. Proteins and carbohydrates were separated from the oil by filtration.
- Neutralization: elimination of short carbon chains fatty acids and free acids that affect taste and accelerate denaturation of fat, by addition of 15% of sodium hydroxide solution (NaOH). The neutralized acids were separated from the oil by washing with water. - Discoloration: at about 90 °C, a mixture of kaolin and coal was added. Dyes, pigments and heavy metals as well as various impurities or undesirable compounds were complexed and separated. - Deodorization: then we heated the obtained fat at high temperature (about 240 °C). This last step allowed us to evacuate unpleasant odors and taste. Volatile compounds and pesticides were also eliminated. All these different steps allowed us to obtained pure fats.

2.4. Fat Analysis Methods

2.4.1. Physicochemical Properties Determination

The values of the smoke and melting points, acid (AI), saponification (SI), iodine (I$_2$) and peroxide (PVs) values were determined on the refined fat using standard methods (BS684-1.8, NFT60-204, NFT 60-206, NF ISO 3961 and NFT 60-220 respectively).

2.4.2. Unsaponifiable Matter Extraction

The content and isolation of the unsaponifiable matter were obtained by the method described by kpoviessi et al. [9] including saponification of oil in KOH/ethanol (2N) at reflux for 2 h, extraction of unsaponifiable matter with hexane, washing of the organic phase with NaOH/H$_2$O (3%), removing of traces of water, filtration, drying and determination of weight on a Mettler Toledo Balance.

2.4.3. GC-FID Determination of Fatty Acid Profile

Fatty acid profiles were obtained by gas-liquid chromatography of the fatty acid methyl ester derivatives. Fatty acids from the refined fats were methylated in a solution of KOH in methanol (0.1 mol/L) at 70°C for 60 min, then in a solution of HCl in methanol (1.2 mol/L) at 70°C for 20 min, and finally extracted with hexane. Fatty acid methyl esters (FAMEs) were separated and quantified with a gas-liquid chromatograph (GC Trace ThermoQuest, Milan, Italy) equipped with a flame ionization detector, an automatic injector and a fused silica capillary column (100m x 0.25 mm internal diameter) coated with a 0.2 mm film of biscyanopropyl-polysiloxane (Rtx-2560, Restek, Bellefonte, PA, USA). The system used H$_2$ as the carrier gas and operated at a constant pressure of 200 kPa. Splitless injection mode was used minimizing the risk of discrimination between FAs with very different volatilities. The initial oven temperature was 80°C; it increased at 25°C/min to 175°C (held for 25 min), then increased at 10°C/min to 205°C (held for 4 min), then increased at 10°C/ min to 225°C (held for 20min) and
finally decreased at 20°C/min to 80°C. The temperature of the flame ionization detector was maintained at 255°C. Hydrogen flow to the detector was 35 mL/min and air flow was 350 mL/min. A calibration mixture of fatty acid standards was processed in parallel. The data were analyzed by using the Chromquest 3.0 software. Each peak was identified and quantified by comparison of retention times with pure FAME standards. Fatty acids are expressed as the percent of total fatty acids quantified within an individual sample. A total of forty pure FAME standards were used.

3. Results and Discussion

3.1. Obtaining of Refined Fats

The refined fats were obtained from the adipose tissues after application in order, of different processes: degumming, neutralization, discoloration and deodorization. The kaolin and the coal used in the discoloration process were local products. These operations allowed to obtain, for BTL, a solid refined fat at room temperature (27 ± 1°C), whitish in color, neutral in taste and odorless. The fat of SSDE was pasty at room temperature; white-gray, neutral and odorless (Table 1).

| Parameters          | BTL fat | SSDE fat | Standard |
|---------------------|---------|----------|----------|
| Melting point (°C)  | 54      | 45 – 50° | 43       |
| Smoke point (°C)    | 207     | 210°     | 216      |
| Aspect              | Solid at| Solid or semi-solid at 20°C | Paste at Solid or semi-solid at 20°C |
| Color               | Whitish | White-gray to yellow | White-gray Gray to yellow |
| Taste               | Neutral | Neutral | Neutral |
| Odor                | No odor | Specific | No odor | Specific |
| Acid value (mg KOH / g) | 1.01±0.10 | 0.6² | 0.94±0.08 | 1.3³ |
| Peroxide value (meq O₂ / Kg) | 0.23±0.04 | ≤ 15.0 | 5.26±0.02 | ≤ 14.0³ |
| Iodine value (g I₂ / 100 g) | 35±0.01 | 40⁴ | 57±0.21 | 46.0 – 66.0⁴ |
| Saponification value (mg de KOH / g) | 201±5.80 | 197⁵ | 191.5±6.75 | 193.0 – 200.0⁵ |
| Unsaponifiable (%)  | 0.04±0.00 | ≤ 1.2⁶ | 0.29±0.03 | ≤ 1⁶ |

BTL: Bos taurus Linnaeus; SSDE: Sus scrofa domesticus Erxleben
² CODEX STAN 211, 1999 – Amended in 2009, 2013 and 2015 [16].
³ ALINORM 99/17 [10].
⁴ Nutrition Infos, Intimate toxins. 2013 [17].

3.2. Physicochemical Characteristics of Fats

The physicochemical characteristics including acid, saponification, iodine, peroxide values of the studied fats are listed in Table 1.

The acid value of BTL and SSDE fats, respectively 1.01 and 0.94 mg KOH / g of fat, were in accordance with the standard value (2.5 mg KOH / g) of ALINORM [10]. The refined fats could be used in food industry.

The saponification value of BTL fat (201 mg KOH / g) was in accordance with standard value (190 – 202 mg KOH / g) [10]. While that of SSDE (191.5 mg KOH / g) was closed to standard values (192.0 - 203.0 mg KOH / g). These two values were also closed to those of Palm oil (190 – 209 mg KOH / g) and could orient these fats in soap manufacturing.

The BTL fat iodine value, 35 g of I₂ / 100 g was lower than reported value 40 - 53 g of I₂ / 100 g of the standard [10]. However, that of 57 g of I₂ / 100 g of SSDE was within the range 55 - 65 g of I₂ / 100 g. With these values, BTL fat seemed to be less unsaturated than SSDE one.

For both fats, peroxide values (0.23 and 5.26 meq O₂ / Kg respectively for BTL and for SSDE) were much lower than the maximum value (10.0 meq O₂ / Kg) prescribed for food fat [10], showing the very good quality of the studied fats.

The unsaponifiable value of BTL and SSDE (0.04 ± 0.00 and 0.29 ± 0.05 respectively) were in accordance with standards values (≤ 1 for SSDE and ≤ 1.2 for BTL) [10].

3.3. Fatty Acids Profile of SSDE and BTL Fats

The fatty acid profile showed the presence of 19 and 23 fatty acids for SSDE and BTL respectively (Table 2).

| Fatty acids         | g TFA  | RDI   |
|---------------------|--------|-------|
| Caproic acid (C6:0) | 0.10 ± 0.01 | 0.10 ± 0.03 |
| Capric acid (C10:0) | –      | 0.10 ± 0.02 |
| Lauric acid (C12:0) | 0.21 ± 0.01 | 0.12 ± 0.01 |
| Myristic acid (C14:0) | 1.45 ± 0.00 | 3.03 ± 0.14 |
| Myristoleic acid (C14:1, c9) | – | 0.13 ± 0.01 |
| Pentadecylic acid (C15:0) | – | 0.50 ± 0.01 |
The unsaturated fatty acids (UFA) of BTL fat account for 34.24% of total fatty acids and this value was almost half that of SFA (65.76%). With the high percentage of SFA, BTL fat was solid at room temperature (27 ± 1°C), relatively stable, and stearic acid was higher (13.0 %) [6]. These differences could be explained by the variation in the animal feeds composition but the oleic acid percentage was less (43.5) and that of fat (< 1 %) [13]. The ω6 / ω3 ratio of 7.23 was higher than those generally obtained in vegetal oil and in the animal races [4]. The ratio SFA and UFA in SSDE fat (1.11) was higher than those generally obtained in vegetal oil (< 1 %) [13]. The ω6 / ω3 ratio of 4 ± 1°C respectively. The probability of an oxidative polymerization (of SSDE fat) was high when highly heated during frying [15]. Oleic (34.37%), stearic (21.36%) and palmitic (24.50%) fatty acids were the major components (Table 2). The ratio SFA and UFA of BTL fat (1.92) was higher than recommended value (1) of dieticians [12].

Among MUFA, palmitoleic acid percentage (1.06) was higher than those generally obtained in vegetal oil and fat (< 1 %) [13]. Palmitoleic and oleic acids content were inferior compared to reported values (2.5% and 45.5 % respectively), and stearic acid value was twice (17.1 %) [7]. The presence of vaccenic acid (0.61%), elaidic acid (0.38 %), trans vaccenic acid (2.97%) and the high percentage of trans fatty acids proved that the studied fat was from animal. The total value of trans fatty acids (3.54%) was close to that (5-10%) reported by Sonntag [13]. The ratio MUFA and PUFA (13.21) was very high and showed that this fat contained more MUFA and would be suitable for frying. The ratio ω6 / ω3 of 0.78 was lower than the recommended value of 4 [14]. The percentage of ω3 acid in BTL fat was higher than 1 and was an advantage for this fat.

Concerning SSDE fat, UFA account for 51.43% of total fatty acids and this value was slightly higher than that of SFA (48.57%). The high percentage of UFA can explain in part the pasty and solid appearance of this fat at room temperature (27 ± 1°C) and at 4 ± 1°C respectively. The probability of an oxidative polymerization (of SSDE fat) was high when highly heated during frying [15]. Oleic (34.37%), stearic (21.36%) and palmitic (24.50%) acids were the major components (Table 2). The ratio SFA and UFA of BTL fat (1.92) was higher than recommended value (1) of dieticians [12].

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Table 3. Comparative values of total fatty acids in BTL fat with those of the reference (mean ± sd, n = 3).

|                | Purified fats | CE Rules a | Standard b | Other studies |
|----------------|---------------|------------|------------|---------------|
| ∑ SFA          | 65.76 ± 0.94  | 54.00      | < 54.00    | > 44.5 c      | 44.1 d        |
| ∑ MUFA         | 31.83 ± 0.17  | 42.00      | < 43.00    | > 48.5 c      | 48.7 d        |
| ∑ PUFA         | 2.41 ± 0.21   | 3.00       | < 4.50     | > 3.43 c      | 7.4 d         |
| Linoleic acid  | 0.97 ± 0.02   | 2.00       | 3.00       | 3.23 c        | 7.2 d         |
| Linolenic acid | 0.38 ± 0.01   | 0.80       | < 1.50     | 0.10 c        | 0.2 d         |

SFA: saturated fatty acid; MUFA: mono unsaturated fatty acid; PUFA: poly unsaturated fatty acid

a Regulation (EC) No 608/2004 [19].
b CODEX STAN 211, 1999 – Amended in 2009, 2013 and 2015 [16].
c MRAD et al., 2009 [18].
d Sreenivasan et al., 1968 [7].

Table 4. Comparative value of total fatty acids in SSDE fat with those of the reference. (mean ± sd, n = 3).

|                | Purified fats | CE Rules a | Standard b | Other study c |
|----------------|---------------|------------|------------|---------------|
| ∑ SFA          | 48.57 ± 0.08  | 44.00      | < 44.26    | 39.26         |
| ∑ MUFA         | 38.59 ± 0.01  | 46.00      | < 49.50    | 46.71         |
| ∑ PUFA         | 12.84 ± 0.12  | 11.00      | < 11.50    | 13.21         |
| Linoleic acid  | 10.54 ± 0.02  | 10.50      | 8.00       | 11.2          |
| Linolenic acid | 0.50 ± 0.01   | 0.85       | < 1.50     | 1.3           |

SFA: saturated fatty acid; MUFA: mono unsaturated fatty acid; PUFA: poly unsaturated fatty acid

a Regulation (EC) No 608/2004 [19].
b CODEX STAN 211, 1999 – Amended in 2009, 2013 and 2015 [16].
c Iverson et al., 1965 [6].

3.4. Fat Stability at 4 ± 1°C and at Room Temperature 27 ± 1°C

3.4.1. BTL Fat Stability

To determine the BTL fat duration of conservation, the samples were exposed separately to room temperature (27 ± 1°C) and to freezer temperature (4 ± 1°C) for a period of 180 days.

The values of quality indices (acid and peroxide) determined during the storage period were shown in Figures 1 and 2.

![Figure 1. BTL, acid evolution at room temperature 27 ± 1°C and at 4 ± 1°C.](image-url)
When the refined fat of BTL was stored at 27 ± 1°C, the standard maximum value set at 2.5 mg KOH /g was reached after 59 days but when the fat was kept under 4 ± 1°C, the maximum value was reached after 94 days. At the same time, the peroxide values were determined. The maximum value of 14.0 meq O₂ / Kg was reached after 68 days of storage at 27 ± 1°C and after 110 days if the fat was kept at 4 ± 1°C. From the analysis of these data, it appears that the peroxide value increased at the same time as the acidity one, whatever the temperature at which the fat was conserved. These values were summarized in Table 5 and showed that this fat could be conserved at freezer temperature (4°C) during 94 days with preservation of quality properties and at room temperature during 59 days.

Table 5. Maximum time (day) of storage of BTL and SSDE fats at 4 ± 1°C and at 27 ± 1°C (mean ± sd, n = 3).

| Sample Parameter | BTL | SSDE |
|------------------|-----|------|
|                  | 4 ± 1°C | 27 ± 1°C | 4 ± 1°C | 27 ± 1°C |
| Acid value       | 94 day  | 59 day  | 54 day  | 43 day  |
| Peroxide value   | 110 day | 68 day  | 95 day  | 67 day  |

BTL: Bos taurus Linnaeus, SSDE: Sus scrofa domesticus Erxleben

3.4.2. SSDE Fat Stability

The quality values (acid and peroxide) of SSDE fat conserved at room temperature (27 ± 1°C) and at freezer temperature (4 ± 1°C) for 180 days were determined (Figures 3 and 4).
The following information were observed: at 27 ± 1°C, the maximum standard acid value (2.5 mg KOH / g fat) was reached after 43 days but at 4 ± 1°C, this value was reach after 54 days. The peroxide maximum recommended value (14.0 meq O₂ / Kg) was achieved after 67 days and 95 days respectively at room and freezer temperature. From the analysis of these data, it appears that the peroxide value increases at the same time as the acidity, whatever the temperature at which the fat was maintained. This fat could be conserved at freezer temperature (4 ± 1°C) during 54 days with preservation of quality properties and at room temperature during 43 days (Table 5). Literature report that SSDE refined fat could be conserved for 56 days at 10°C [11].

4. Conclusion

The report of our work is the first on the purification of fats from BTL and SSDE using local reagents in Benin. The physicochemical properties of studied fats satisfy in majority the international standards (Codex Alimentarius). The two fats were rich in oleic, stearic and palmitic acids with respectively 25.94%; 34.99%; 24.50% for BTL fat and 34.37%; 21.36%; 24.59% for SSDE fat. Their ω3 contents (1.25% and 1.56% respectively for BTL fat and SSDE fat) were interesting. The BTL fat could be stored for 94 days at 4 ± 1°C and for 59 days at 27 ± 1°C but SSDE fat for 54 days at 4 ± 1°C and 43 days at 27 ± 1°C, with preservation of their quality properties. These refined fats could be used in several sectors such as: energy, oleochemistry, cosmetology, animal feed, agri-food, etc.

Acknowledgements

The authors would like to thank Eric Mignolet and all the staff of faculty of Bioscience Engineering & Institute of Life Sciences of Université Catholique de Louvain, for the GC analysis.

Conflict of Interest

The authors declare no conflict of interest.

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