Lipolytic and oxidative changes in acoustically assisted frozen fattened duck liver

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Abstract. The delicate structure of the fattened duck liver requires rapid freezing as quickly as possible. Acoustically assisted freezing meets both the requirement for quick freezing and energy efficiency. For the purpose of the experiment, a fattened duck liver "foie gras" was used. The degree of lipolysis (acid value) and primary (peroxide value) and secondary (2-thiobarbituric acid value) products of lipid oxidization were examined at 2 h post mortem. The oxidative changes were evaluated after conventional (CF) and acoustically assisted freezing (AAF) and at the 6-th and 12-th month of storage at -18°C. Both time storage and type of freezing affect the degree of lipolysis. The duck liver after AAF had higher degree of lipolysis at the 12-th month of storage at -18°C. The peroxide value increased almost 2 times in both fattened duck livers (CF and AAF) during the 6 months of storage at -18°C and stabilizes until the 12-th month. A conclusion was made that the acoustically assisted freezing of the fattened duck liver did not promote higher lipid oxidation compared to the conventional type of freezing.

1 Introduction

Fattened duck liver "*foie gras*" has a unique sensory profile, which makes it highly valued by consumers. In order to preserve it to the maximum extent, various techniques are applied, the most common being freezing [1].

Frozen foods are characterized by an extended storage period due to the death of microorganisms and the slowing down of oxidation processes [2]. Besides the positive aspects of freezing, if it is not done properly, it could be detrimental to the product. The efficiency of freezing is determined by the thermodynamic properties, chemical composition, dimensions and shape of the product [3].

In the process of freezing, water crystallizes, the faster the freezing, the smaller the size of the ice crystals. Large ice crystals tear the structure of the product and worsen its sensory characteristics [4]. The disturbed cell's structure initiates oxidation processes, as a result of the released cellular components, such as: enzymes, free fatty acids, amino acid residues, heme iron, etc. [5]. Found in cell walls, phospholipids are liable to oxidation [6].

Lipid peroxidation is the second most important process after microbial spoilage that deteriorates the sensory characteristics and safety of meat products. Once initiated, chain-radical processes do not stop until all substrates are oxidized to end products. Hexanal, propanal and 4-hydroxy-nonenal (malondialdehyde/MDA) are the most common and studied end products of lipid oxidation [7].

The malondialdehyde change the sensory characteristics of meat and fish products when presented in amounts above 1-2 mg MDA/kg [8]. At further concentrations the consumers indicate a rancid and warm-over flavor. In addition to influencing the taste and aroma, MDA exhibits strong genotoxin properties and initiate the formation of carcinomas in the colon [9].

Since the amount of fat in fattened duck liver can exceed 50%, it is important to establish a freezing method that maximally inhibit oxidative processes [10]. In this line of thought, intensifying the freezing process is a step to increase the quality and safety of frozen foods. Rapid and ultra-rapid freezing are the directions in which technique and technology are developing. In addition to preserving the quality of the frozen product, they are classified as "green" technologies based on the lower consumption of electricity [11]. The innovative thing about acoustically assisted freezing is that under the influence of ultrasound, a much larger number of crystallization centers are formed, which results in many, but small ice crystals. In this way, the structure of the tissue and cells can be preserved and the oxidative processes can be indirectly inhibited [12].

A previous study found that acoustically assisted freezing of fattened duck liver preserved cell structure and color characteristics even after 12 months of storage at -18°C [13]. The lack of studies regarding oxidative changes in acoustically assisted frozen *foie gras* is the basis of the present study. For this purpose, the characteristics of the chilled fattened duck liver 2 h *post mortem* were studied, as well as the lipolytic and oxidative

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changes occurring after acoustically assisted freezing and storage for 6 and 12 months at -18°C.

2 Materials and methods

The fattened duck liver was provided by a local slaughterhouse (Brezovo village, Bulgaria). The livers were placed in flaky ice immediately after evisceration and transported to the laboratory in chilled conditions (0 - 4°C). The fattened duck livers were separated into three groups. First group was examined instantly (0 - 4°C, 2 h *post mortem*). From the second and third group were prepared 10 samples (150-200 g each) each (CF, AAF) for a total of 20 samples.

After packing under vacuum, the fattened duck livers with temperature in the center 1 - 3°C were headed for freezing. Vacuum packages marked with "AAF" were placed in the freezer (shock freezer cabinet equipped with acoustic module AEF APACH SH05/SH20). The packages were pre-massaged with acoustic waves for 20 min at 3°C and > 500 Hz, followed by acoustically assisted freezing at -25°C until -18°C in the center (> 500 Hz) in agreement with patent WO 2021/148721 A1.

The third group marked as a control sample "CF" was freeze intensively at -35°C until -18°C in the center.

Both groups CF and AAF frozen fattened duck liver were stored for 6 and12 months.

After 6 months of storage at -18°C, 5 pieces of each group (CF and AAF) were thawed to temperature in the center 1 - 3°C, homogenized and immediately tested. The same procedure was completed at the 12^{-th} month of storage at -18°C.

2.1 Lipid extraction

Total lipids were extracted according the method of Bligh and Dyer [14] with modifications. Equal parts of homogenized sample and chloroform: methanol (2: 1, v/v) were mixed and incubated at 4°C overnight.

2.2 Lipolytic changes

The degree of lipolysis was evaluated by a titration of 1 gram of fat with 0.1N KOH and expressed as acid value (AV) in accordance to ISO 660: 2020 [15].

2.3 Oxidative changes

The concentration of primary products of lipid peroxidation or the peroxide value (POV) was determined following the recommendations of Shantha & Decker [16]. Briefly, 0,1 g fat was mixed with 50 µl fresh $FeCl_2$, 50 µl NH_4SCN and 9.8 ml $CHCl_3$: CH_3OH (3: 5, v/v). Tubes were shaken until fat is dissolved and incubated for 10 min in dark. The absorbance is measured at 507 nm against black prepared without the solution of $FeCl_2$.

For determination of the end products of lipid peroxidation, the content of malondialdehyde/MDA was measured according to the method of Botsoglou et al. [17]. Briefly, 5 g homogenized sample was mixed with 50 ml 0.9% NaCl. After 3 min at room temperature, 50 ml 10% Trichloroacetic acid was added to precipitate the extract and the solution was filltered. Four milliliters of the supernatants were mixed with 1 ml fresh 1% 2-thiobarbituric acid in 0.9% NaCl and incubated at 70°C for 30 min. The absorbance is measured at 532 nm against black prepared with distilled water instead of supernatant.

Both methods were performed using a double beam UV-VIS spectrophotometer Camspec model M550 (Camspec Ltd, Cambridge, UK).

2.4 Statistical analysis

All statistical analysis were executed using Microsoft® Excel® 2016 MSO software. Analysis of variables (ANOVA: Two-Factor with Replication) with probability $p \leq 0.05$ was performed [17]. Mean samples were prepared with 5 number of replicates (n = 5).

3 Results and discussion

3.1 Lipolytic changes in acoustically assisted and conventionally frozen fattened duck liver during the storage at -18°C

At 2 h *post mortem* the acid value (AV) is normal for fresh chilled fattened duck liver (Fig. 1) and it's in agreement with results of other authors [10].

After 6 months of storage at -18°C increase of 1.3 and 1.4 times ($p \leq 0.05$) in AV was evaluated for samples CF and AAF, respectively. By the time of the 12^{-th} month of storage at -18°C the AV decrease in both frozen fattened duck liver samples (CF and AAF).

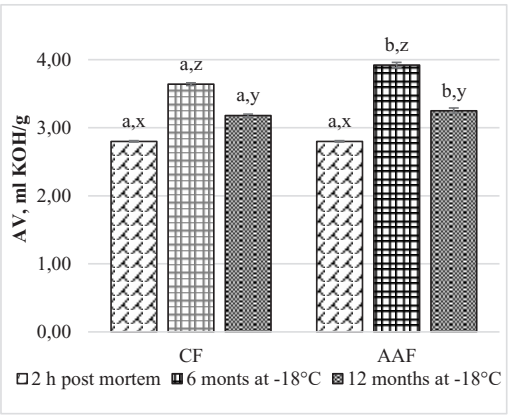

Fig. 1. Acid value (AV) of frozen fattened duck livers during the storage at -18°C

The high fat content of *gras*, the degree of unsaturation of the fatty acids and the freezing process are contributing to the destruction of the native structure which influences the processes of hydrolysis and oxidation [6, 12]. Freezing slows down, but cannot stop, ongoing lipolytic processes in meat and offal.

Previous study reported that the cell structure of acoustically assisted frozen fattened duck liver was less damaged by the formed ice crystals [13]. This suggests

that the free fatty acids content should be lower due to the preserved cell membranes consisted manly phospholipids [6]. Our results opposing this hypothesis and suggests that the found preserved cell structure by Vlahova-Vangelova et al. [13] was mostly proteins [5]. The obtained results show that during the 6-month storage of the fattened duck liver, lipolytic changes occur, associated with an increase in the content of free fatty acids. At the end of the experiment (12 months, -18°C), the AV decreased, probably due to oxidative degradation of fatty acids to primary and secondary products of lipid oxidation [9].

3.2 Oxidative changes in acoustically assisted and conventionally frozen fattened duck liver during the storage at -18°C

Assessed lipid hydroperoxide content (POV) in the chilled fattened duck liver at 2 h *post mortem* is low corresponding to the early period of the storage (Fig. 2). After 6 months of storage at -18°C, POV increased ($p \leq 0.05$) in both frozen fattened duck liver (CF and AAF). The increase of the primary products of lipid peroxidation compared to the initial values was 1.8 times ($p \leq 0.05$) in the conventionally frozen liver (CF) and 1.85 times ($p \leq 0.05$) in the acoustically assisted frozen sample (AAF). By the time of the 12-th month of frozen storage (-18°C), the content of lipid hydroperoxides slightly ($p \leq 0.05$), but statistically significantly decreased (Fig. 2). The evaluated trend at the 12-th month of storage at -18°C between the two examined samples – CF and AAF showed no statistically significant differences in POV ($p \geq 0.05$).

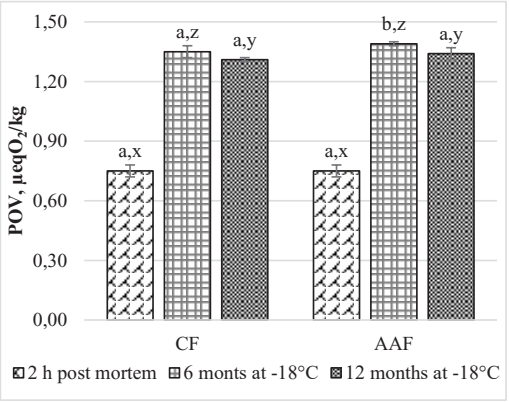

Fig. 2. Peroxide value (POV) of frozen fattened duck livers during the storage at -18°C

Possible explanation for the increase of POV at 6-th month of frozen storage can be the ongoing oxidative changes associated with the formation and accumulation of lipid hydroperoxides. The registered decrease at 12-th month can be explained by the formation of lower molecular-weight end products from the lipid hydroperoxides [7, 19].

All of the above increase followed by a decrease in AV and POV have a strong connection with the active enzymatic system in the frozen fattened duck liver. Enzyme activity is slowed down at -18°C but it's not completely stopped. In the conditions where most of the water is frozen the concentration of electrolytes increase in the remaining liquid water [4]. The activity of phospholipases is regulated by the calcium ions, whose concentration increases in the process of freezing. As a result, the free fatty acid content increases, but they are labile and easily step into oxidative reactions forming lipid hydroperoxides [6].

Typical and close to zero for the chilled fattened duck liver (2 h *post mortem*) was the TBA value (Fig. 3). Evaluated by us results were confirmed by another author [20]. After 6 months of storage at -18°C, the content of end products of lipid peroxidation (TBA value) significantly increased ($p \leq 0.05$) in both frozen fattened duck liver samples (CF and AAF). For the same studied period, also are the highest values of POV, related to the formation of primary products of lipid peroxidation (Fig. 2). The established changes related to the significant accumulation of lipid hydroperoxides and MDA are proof of the ongoing oxidative changes in the lipid fraction of both frozen fattened duck liver (CF and AAF).

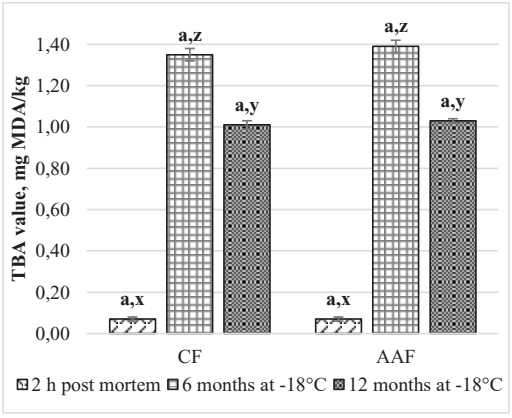

Fig. 3. 2-thiobarbituric acid value (TBA value) of frozen fattened duck livers during the storage at -18°C

According to the data obtained, in the case of frozen fattened duck liver, the end products of lipid peroxidation accumulate up to the 6-th month of frozen storage. The high content of fats in the liver, their degree of unsaturation, as well as the significant content of heme pigments make it an unstable product from the point of view of lipid peroxidation [7, 9, 19].

With prolonging the storage time up to 12-th month the content of MDA decreased (Fig 3.). Possible reason for our results can be the inclusion of malondialdehyde in processes of protein oxidation or the formation of dimers [9, 20]. MDA is major oxidant of heme pigments, which contributes to fading of the pink-red color [7]. Vlahova-Vangelova et al. [13] reported a higher value of the red component of the color a* in acoustically assisted frozen fattened duck liver compared to conventionally frozen.

Those results in some sense confirm that acoustically assisted freezing preserves the heme pigments more than the conventional. This allows us to hypothesize that preservation of the native structure of heme pigments could possibly inhibit of lipid

peroxidation, as the released heme iron upon destruction of pigments is potent initiator [7].

4 Conclusions

Both investigated factors (type of freezing and storage time) and their interaction influence the acid value of the frozen fattened duck livers. The peroxide value increased trough the time of frozen storage but it is not affected by the type of freezing. The evaluated trend in TBA values showed that the acoustically assisted freezing of the fattened duck liver did not inhibit nor promote higher lipid oxidation compared to the conventional type of freezing.

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