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Effects of high pressure treatment and temperature on lipid oxidation and fatty acid composition of yak (*Poephagus grunniens*) body fat

Qiang Wang [a,b,*,1], Xin Zhao [a,1], Yanrong Ren [a], Enguo Fan [e], Haijun Chang [c], Hongbin Wu [d]

[a] Department of Biological and Chemical Engineering, Chongqing University of Education, Chongqing 400067, PR China
[b] Institute of food Safety and Nutrition, Chongqing University of Education, Chongqing 400067, PR China
[c] College of Environmental and Biological Engineering, Chongqing Technology and Business University, Chongqing 400067, PR China
[d] Institute of Agro-products Processing Science and Technology, Xinjiang Academy of Agricultural And Reclamation Science, Shihezi 832000, PR China
[e] Institut für Biochemie und Molekularbiologie, Universität Freiburg, Freiburg 79104, Germany

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ABSTRACT

Effects of high-pressure treatment (100 MPa to 600 MPa) on lipid oxidation and composition of fatty acids in yak body fat at 4 °C and 15 °C were investigated for up to 20 days storage. 400 and 600 MPa treatments increase the level of thiobarbituric acid-reactive substances (TBARS) 335% and 400% ($p < 0.05$), respectively. Composition analysis shows that 600 MPa treatment induces a lower ($p < 0.05$) percentage of polyunsaturated fatty acids, and C22:6 decreased significantly. A significant decrease in PUFA/SFA and n-6/n-3 PUFA values was observed at the end of storage. Samples treated at the lower pressures gave good sensory acceptability. It is concluded that a higher-pressure treatment is important in catalyzing lipid oxidation and the evolution of fatty acids in pressure-treated yak body fat.
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1. Introduction

Composition and variation of fatty acids, including saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), functional unsaturated fatty acids (FUFAs) and nutraceutical fatty acids (n-3, n-6 PUFAs), during storage and lipid processing are one of the major nutritional and scientific concerns (Chen, Nguyen, Semmens, Beamer, & Jaczynski, 2007; Wood et al., 2008). While autoxidation during storage is one of the major causes of food spoilage that decreases the nutritional quality of food and generates potential toxic products (Jill, Warner, & Martin, 2007; Manuel, Francisco, & Rosario, 1998). Investigations to analyze lipid autoxidation products and factors influencing their production (Lucy, Horsfall, & Mayo, 2006; Manat, Soottawat, Wonnop, & Cameron, 2006; Saldanha & Bragagnolo, 2008) suggest that temperature and time for food processing, conditions for transport and storage, other factors including pH, light, oxygen, water activity, or a combination of these factors, and particularly heat processing all contribute to fat autoxidation and fatty acid degradation.

Recently, effects of high pressure on lipid oxidation in meat or lipids (Bolumar, Andersen, & Orlien, 2011; Ma, Ledward, Zamri, Frazier, & Zhou, 2007) have been investigated because high pressure is increasingly used to extend the shelf life and improve the quality and functional properties of lipids and meat products (Hugas, Garriga, & Monfort, 2002; Marcos, Kerry, & Mullen, 2010). It was found that meat becomes more susceptible to lipid oxidation at high pressures, and as a consequence deterioration and alteration of fatty acid composition (Angsupanich & Ledward, 1998; He et al., 2012; Ma et al., 2007; Orlien, Hansen, & Skibsted, 2000). Cheah and Ledward (1996) found that pressure treated pork slightly affects lipid oxidation below 300 MPa, but increases proportionally at higher pressures. Angsupanich and Ledward (1998) found an increased oxidation rate at 400 MPa or higher at ambient temperature in cod muscle. In contrast, Beltran, Pla, Yuste, and Mor-Mur (2003) found that treatments of minced chicken breast muscle at pressure up to 500 MPa had no effects on oxidation rates at chill temperatures, and concluded that the pressure-induced oxidative stability of chicken muscle was more stable compared with turkey muscle.

Yaks (*Poephagus grunniens* or *Bos grunniens*) live in extremely harsh conditions at altitudes from 2000 m to 5000 m above sea level and provide the main sources of livelihood for local people. Yak kidney fat contains several functional fatty acids and has a valuable composition (Wang et al., 2009; Zhang, Wang, & Fan, 2009), which suggests the potential development of yak fat into commercial products for the food industry. A higher UFA content is found in yak body fat than SFA (Wang et al., 2009) and considerable attention focuses on the factors influencing the composition and the UFA/SFA ratio of yak fat during processing. A number of researchers (Ana, Estrella, & Manuel, 2009; Jofré, Aymerich, & Garriga, 2008) found that high-pressure

* Corresponding author at: Department of Biological and Chemical Engineering, Chongqing University of Education, Xuefu Road 9, Chongqing 400067, PR China. Tel./fax: +86 23 88607574.
E-mail address: gogo1443@sina.com (Q. Wang).
[1] These two authors contribute equally.

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treatment increases susceptibility to pressure-induced lipid autoxidation and a high-pressure treatment has a positive role on the production of ham, where yak body fat is an important high-quality component. However, the changes in fatty acid composition and sensory acceptability of pressurized yak body fat have not been reported. Therefore, the present study was designed to investigate the pressure-induced autoxidation of yak body fat using thiobarbituric acid-reactive substances (TBARS) as a relative susceptibility index. And the stability of the UFA profiles of yak fat under different high-pressure treatments was also investigated.

2. Materials and methods

2.1. Samples

The fat samples of yak, which grazed on grassland at an average elevation of 3700 m above sea level (Gannan State, China), were selected randomly from 32 healthy yaks (3 to 5 years old). The subcutaneous fat and kidney fat were mixed at 4 °C, and the fat sample was sectioned into 100 equal aliquots (200 g aliquot^{-1}) of the same thickness and area and stored at −4 °C before processing.

2.2. Thiobarbituric acid-reactive substances (TBARS)

15 μL trichloroacetic acid (TCA) solution (7.5% TCA, 0.1% EDTA, and 0.1% propyl gallate) was mixed with 5 g samples for 45 s, then centrifuged at 16,300 ×*g* (Model 3740, KUBOTA Co., Japan), and filtered. A 5 μL filtrated sample was mixed with 5 mL of 0.02 M TBA (2-thiobarbituric acid) solution, and the mixture was placed in a water bath at 90 °C for 40 min (Aline, Bente, & Leif, 2008). The absorbance was measured at 532 nm and 600 nm using an UV–vis spectrophotometer (Model UV-2450, Shimadzu Co., Japan) and the difference (A532 nm to A600 nm) was used to correct the absorbance for turbidity.

2.3. Preparation of fatty acid methyl esters (FAME)

The FAME preparation procedure was performed according to Savage, Dutta, and McNeil (1999) after modification. Briefly, 20 g of fat sample was extracted for 3 h with 90 mL chloroform–methanol solution (2:1, v/v) in a circumfluence instrument at 70 °C with shaking. After the mixture became clear, approximately 80 ml of the extract was collected and evaporated under N_2 at room temperature for 10 min. The extract was then mixed with 2 mL of 0.01 M NaOH (in anhydrous methanol) at 60 °C for 30 min under continuous shaking. After the solution became colorless and clear, the samples were cooled to 4 °C under running water. Then, a mixture of 1 mL $NaHSO_4$ (10%), 1 mL NaCl (25%), 3 mL distilled water, and 1 mL hexane were added. After a vigorously shaking, the mixture was left to stand for 10 min until different layers were observed. The upper FAMEs-containing hexane layer was collected and the lower aqueous layer was passed through the extraction procedure once more. Finally, the hexane layers were pooled and anhydrous Na_2SO_4 (1 g) added. The FAME sample was stored at −20 °C and before capillary gas chromatography analysis, the sample was first exposed to a stream of N_2 then ultrafiltered with a 0.5 μm organic phase ultrafiltration filter membrane.

2.4. FAME analysis by GC

Identification of individual fatty acids was based on comparison of their retention times with those of standard FAMEs (Sigma Chemical Co. Ltd., USA). Quantification was performed by comparing their peak areas with those of an internal standard (C14:0). The data were calculated using the normalized peak area percentages of total fatty acid content. The results were expressed as a percentage of the amount of the total methyl esters. 1 μL FAMEs sample was injected into a 7890A GC instrument (Agilent Co., USA) equipped with an HP-88 column (60 m × 0.25 mm i.d.; film thickness 0.2 μm). The inlet temperature was set at 250 °C and that for the flame ionization detector (FID) at 270 °C. The oven temperature program was set at 40 °C for the first 2 min, 190 °C for 3 min, then increased to 220 °C for 6 min. The carrier gas was nitrogen with a flow rate of 1.2 mL/min. The split ratio was 20:1.

2.5. High pressure processing and storage

High-pressure processing and storage were carried out as described by Ma et al. (2007) after modifications. The samples were cut into 60 mm × 20 mm × 20 mm portions and treated for 30 min at 100, 200, 400, or 600 MPa in a high pressure unit (Stansted Fluid Power Ltd., Stansted, UK), respectively. The samples were stored in the dark at 4 °C or 15 °C in a sealed low oxygen permeable environment for up to 20 days. The analysis started at 5th day of storage. Each treatment was carried out with three replicates.

2.6. Sensory evaluation

Fifty panelists (25 women and 25 men) recruited from the staff and graduate students of the University tested stored yak fat without any knowledge of the product formulation. The samples were presented to the panelists as oblique 2 cm-thick slices. The test was done in individual booths under fluorescent light. A hedonic scale (anchored at both ends) rating test was used to evaluate each sample according to the following criteria: appearance (1 = not characteristic, 10 = characteristic), flavor (1 = very mild, 10 = very strong), stickiness (1 = very mild, 10 = very strong), and juiciness (1 = very dry, 10 = very juicy). The evaluation was carried out on a non-structured scale with fixed extremes. Each point was converted later into a numerical scale, and the panelists were also invited to provide their own comments on the samples. The conversion relationship of the sensory score and the evaluated grade are shown in Table 1.

2.7. Statistical analysis

Lipid autoxidation was evaluated periodically. The results reported are the means of three replicates (expressed as the mean ± standard error). The TBARS values and fatty acid composition data from various treatments were analyzed by one-way analysis of variance (ANOVA), using a SPSS computer software package (version 13.0, SPSS Inc., Chicago, IL, U.S.A.). Levels for significant differences were set at $p < 0.05$.

3. Results and discussion

3.1. TBARS values during initial oxidation

Table 2 shows the effect of high-pressure treatment on the TBARS values of yak fat during storage at 4 °C and 15 °C, respectively. The TBARS values increased with increased pressure treatment (from 100 to 600 MPa) and storage time (0–20 days) at both 4 °C and 15 °C. After 20 days at 4 °C, the TBARS values of samples treated at 400 MPa and 600 MPa increased from 0.52 and 0.46 to 1.95 and 2.31, respectively. The increases of TBARS values were 335% and 400% ($p < 0.05$), which were much higher than the increase of 199% at 0.1 MPa. Except for the 0.1 MPa treatment, TBARS after pressure treatment increased more than two folds from day 0 to day 20 ($p < 0.05$). Table 2 also shows the

Table 1
Corresponding relationship between the evaluated grade and the overall sensory score.

Grade	1	2	3	4	5	6	7	8
Score	0–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40

Evaluated grade (1 = dislike extremely, 8 = like extremely).

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