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Characterization of lipid oxidation process of beef during repeated freeze-thaw by electron spin resonance technology and Raman spectroscopy

Qingmin Chen[a,b], Yunfei Xie[a,b], Jinzhong Xi[a,b], Yahui Guo[a,b], He Qian[a,b], Yuliang Cheng[a,b], Yi Chen[c], Weirong Yao[a,b,*]

[a] State Key Laboratory of Food Science and Technology, Jiangnan University, PR China
[b] School of food science & Technology, Jiangnan University, PR China
[c] Scion, Private Bag 3020, Rotorua 3046, New Zealand

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1, 1, 3, 3-tetraethoxypropane (PubChem CID: 67147)
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ABSTRACT

In this study, electron spin resonance (ESR) and Raman spectroscopy were applied to characterize lipid oxidation of beef during repeated freeze–thaw (RFT). Besides the conventional indexes including peroxide values (PV), thiobarbituric acid-reactive substances (TBARS) and acid values (AV) were evaluated, the radical and molecular structure changes were also measured by ESR and Raman spectroscopy. The results showed that PV, TBARS and AV were increased ($P < 0.05$) after RFT. This suggested that lipid oxidation was occurred during RFT. With the increase of radical signal intensity, lower oxidation stability was presented by ESR. Raman intensity of ν(C=C) stretching region (1655 cm^{-1}) was decreased during RFT. Furthermore, lower Raman intensity ratio of I_{1655}/I_{1442}, I_{1655}/I_{1745} that determine total unsaturation was also observed. Significant correlations ($p < 0.01$) were obtained among conventional methods, ESR and Raman spectroscopy. Our result has proved that ESR and Raman spectroscopy showed great potential in characterizing lipid oxidation process of beef during RFT.

1. Introduction

The high protein and fat which are mainly constituent of meat are highly more prone to deterioration by chemical and enzymatic processes. Consequently, the use of frozen storage is still the preferred way for preservation, especially for long distance transport. Unfortunately, repeated freeze-thaw (RFT) cycles commonly occur in China during long-distance transport, mainly caused by multiple transfers and very poor cold-chain conditions (Qi et al., 2012). Many studies have confirmed that RFT accelerates the oxidation of lipids (Ali et al., 2015; Rahman, Hossain, Rahman, Amin, & Oh, 2015; Wang et al., 2017). The majority of researchers believe that the ice crystal formation during freezing contributes to the membrane disintegration (Martino & Zaritzky, 1988; Molina-Garcia et al., 2004), which results in the release of the primary catalysts of lipid oxidation in cell, and accelerated lipid oxidation. These oxidations contribute to the formation of hydrocarbon, aldehydes and ketones, which are responsible for the off-odour, off-flavour, rancidity, colour deterioration and the proteins oxidation (Andersen, Bertelsen, & Skibsted, 1990; Iglesias & Medina, 2008; Papuc, Goran, Predescu, & Nicorescu, 2017), resulting in the deterioration of meat quality. At present, most common methods used for the determination of lipid oxidation include the following: peroxide values (PV), thiobarbituric acid-reactive substances (TBARS) analysis (Gimenez, Gomez-Guillen, Perez-Mateos, Montero, & Marquez-Ruiz, 2011) and finally chromatography. Some other methodologies such as chemiluminescence (Yang et al., 2010), fluorescence emission (Nguyen, Thorarinsdottir, Thorkelsson, Gudmundsdottir, & Arason, 2012), hyperspectral imaging (Cheng, Sun, Pu, Wang, & Chen, 2015), infrared spectroscopy or magnetic resonance (Guillen & Goicoechea, 2007) are gaining wide attention now.

* Corresponding author at: State Key Laboratory of Food Science and Technology, Jiangnan University, PR China.
E-mail address: yaoweirongcn@jiangnan.edu.cn (W. Yao).

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Furthermore, electron spin resonance (ESR) and Raman spectroscopy are also popular as well for their advantages of rapidness and online monitoring.

The lipid oxidation process is generally composed of several radical mechanisms which finally resulting in the formation of compounds such as alkyl aldehydes and ketones. ESR is considered as a new and sensitive technique to determine radical changes for the study of lipid oxidation. As a technology of studying the early stages of lipid oxidation, which has proven to be a successful analytical approach to evaluate lipid oxidation in several foods (Quiles, Ramirez-Tortosa, Gomez, Huertas, & Mataix, 2002; Westermann, Bruggemann, Olsen, & Skibsted, 2009; Yi, Andersen, & Skibsted, 2011).

Raman spectroscopy, an emerging non-invasive technique has great potential for studying biochemical analysis of tissue on both macroscopic and microscopic scales. One of its main advantages is that it made it possible to provide information about concentration, structure, and interaction of biochemical molecules within intact cells and tissues (Marquardt & Wold, 2004). More researchers have realized that it can be applied to evaluate lipid oxidation. Carmona et al. had used Raman spectroscopy in their work to assess the stability against thermal oxidation of different types of commercial olive oils (Carmona, Lafont, Jimenez-Sanchidrian, & Ruiz, 2014). FT-Raman spectroscopy was utilized to monitor lipid changes in hake fillets during frozen storage (Sanchez-Alonso, Carmona, & Careche, 2012). Xu et al. overviewed a large number of non-destructive spectroscopic techniques to assess oxidative status lipid and lipid oxidation in fish and fish products (Xu, Riccioli, & Sun, 2015).

The objectives of this work are to try to characterise lipid oxidation process of beef during RFT by ESR and Raman spectroscopy. Conventional indexes (PV, TBARS and acid values) were measured to investigate lipid oxidation during RFT. Both ESR and Raman spectroscopy were also applied to monitor radical and molecular structure changes of lipid oxidation during RFT. Correlation analyses of Conventional methods, ESR and Raman spectra were performed to demonstrate the potential of ESR and Raman spectroscopy to characterise the lipid oxidation process during RFT.

2. Materials and methods

2.1. Materials

Trichloroacetic acid (TCA purity above 99%) and 2-thiobarbituric acid (TBA, 99%) were purchased from Sinopharm Chemical Reagent Inc. (Shanghai, China). 1, 1, 3, 3-tetraethoxypropane (TEP, 97%) were purchased from Energy Chemical Technology Inc. (Shanghai, China). *N*-*t*-butyl-α-phenylnitrone (PBN, 97%) were obtained from Sigma-Aldrich (Steinheim, Germany). All reagents were of analytical grade, and toluene was used to dissolve PBN solution.

2.2. Sample preparation

To assurance the homogeneity of thawing and decrease the difference between the parallel groups. Raw beef (chuck) purchased from local market was cut into $4 \times 4 \times 4\,cm^3$ pieces, then packed individually in ziplock bags. All these RFT samples were frozen at −20 °C for 3 days and thawing at 4 °C for 20 h for one freeze–thaw cycle. However, the control was kept frozen at −20 °C and thawed under the same condition before testing. Assays were made at least in triplicate.

2.3. Lipid extraction

Minced beef samples which is Forty grams each were mixed with 150 mL petroleum ether (30–60 °C) in conical flasks and placed on shaking bed in operation over night at room temperature. The solvent was evaporated using a vacuum rotatory evaporator. Lipid samples were obtained for measurements.

2.4. Lipid oxidation measurements

Peroxide values determination, a method to determine the number of moles of peroxide contained in 1 kg of lipid was performed according to the method proposed by (AOAC., 1990) with a slight modification. Lipid samples were dissolved in a blended solution of 30 mL chloroform-glacial acetic acid (3:2, v/v). A saturated solution of KI (1 mL) was added. The mixture was shaken by hand for 30 s and kept in the dark for another 5 min. After the addition of 100 mL distilled water, the mixture was titrated against sodium thiosulphate (0.1 mol/L) until the yellow colour almost disappeared. Then, about 1 mL of starch indicator (0.05%) solution was added. Titration was sustained until the blue colour just disappeared. Lipid oxidation accompanied with the formation of peroxide, which would oxidize Potassium iodide into free iodine. Then, free iodine can be quantified by sodium thiosulfate. A blank was also determined under similar conditions. PV (mmol/kg) was calculated as follows:

$$PV(mmol/kg) = \frac{C\times(V-V_0) \times 12.69 \times 39.4}{m}$$

where C is the sodium thiosulphate concentration (mol/L); V and V_0 represent the volumes of sodium thiosulphate exhausted by the samples and the blank, respectively (mL); and m is the mass of lipid sample (g).

TBARS test was according to slightly modified method by published work (Zhang et al., 2010), TBARS of the lipid were measured during freeze–thaw cycles. Samples (5 g) were homogenised in 10 mL 7.5% TCA-EDTA (0.1%) solution. This sample was shaken continuously for 0.5 h with a mechanical shaker and then filtered. Exactly 5 mL filtrate was added to 5 ml TBA (2.88 g/L) solution and transferred into a 25 mL colour metrical tube. The mixture was heated in a boiling water bath for 40 min for pink colour development. The tube was cooled for 1 h in room temperature and centrifuged for 5 min. The supernatant was added to 5 ml chloroform in another tube and then shaken. The mixed solution was left to stand for at least 1 h. The absorbance was measured at 532 nm using a spectrophotometer (UV-1800, SHIMADZU). TBARS content was calculated from a standard curve of malondialdehyde (MDA), freshly prepared by TEP acidification (1, 1, 3, 3-tetraethoxypropane) in the range 0.02–2 μg/mL ($y = 0.1979x + 0.0032$, $R^2 = 0.9997$) and expressed as MDA mg/per kg sample. The TBARS concentration was calculated as

$$TBARS(mg/kg) = \frac{S}{m} \times 10$$

where S is the corresponding mass concentration of MDA obtained from the standard curve, and m is the mass of the lipid (g) sample.

Analyses of acid value (AV) were carried out according to standard titration method described by GB/T500.44-2003. Briefly, for the determination of the content of AV, lipid sample dissolved in 50 mL of ethanol was incubated in a water bath (70 °C) until the solution was slightly boiling. And then titrated the mixture with 0.01 mol/L potassium hydroxide-ethanol solution until the phenolphthalein end point was obtained (the pink color of the phenolphtalein persisted for at least 30 s). AV was expressed as milligrams of potassium hydroxide required to neutralize the free fatty acids present in 1 g of the lipid sample (mg/g). All determinations were performed in duplicate.

The acid value was calculated as:

$$AV = \frac{V\times c\times 56.11}{m}$$

where V is the volume in milliliters of standard volumetric potassium hydroxide solution used; c is the concentration in moles per liter of the standard volumetric potassium hydroxide solution used; m is the mass in grams of the test sample of lipid samples.