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# Influence of storage temperature and duration on lipid and protein oxidation and flavour changes in frozen pork dumpling filler

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ABSTRACT

This study was conducted to evaluate the effect of storage temperature and duration on oxidation and flavour changes in frozen pork dumpling filler. Freshly prepared dumplings were stored for 0, 30, 60, 90, and 180 d at −7 °C, −18 °C, and an oscillation between −7 °C and −18 °C. The samples stored at −7 °C for 180 d had significantly higher levels of TBARS and protein carbonyls than those stored at −18 °C and the fluctuating −7 °C/−18 °C ($P < 0.05$). The percentage of unsaturated fatty acids in total lipids decreased with extended storage times. The volatile compounds with pleasant odours decreased with time, while the compounds with pungent tastes and smells increased ($P < 0.05$). The sensory results showed that the dumplings stored at higher frozen temperatures for long periods of time had significantly lower acceptability scores ($P < 0.05$). The results suggest that oxidation is a primary cause of quality deterioration in pork dumpling filler during frozen storage.



## 1. Introduction

Dumplings (jiaozi), a traditional Chinese food consisting mainly of meat, vegetables, spices and wheat flour, are a very popular food in China and in many Asian cuisines in Western countries. Meat is one of the most important ingredients in dumpling filler. For distribution and quality preservation, dumplings are usually stored frozen. However, gradual losses in the quality attributes can still occur during frozen storage. Deterioration, especially in flavour and texture, is due to oxidation, osmotic removal of water and protein denaturation (Benjakul, Visessanguan, Thongkaew, & Tanaka, 2003). Furthermore, the presence of polyunsaturated lipids in the meat may also lead to the development of rancidity and flavour deterioration, which limits the shelf-life of frozen foods (Songsaeng, Sophanodora, Kaewsrithong, & Ohshima, 2010). The extent of quality losses in frozen meat is dependent upon many factors, including the rate of freezing, storage temperature, and temperature fluctuations (Muela, Sañudo, Campo, Medel, & Beltrán, 2010).

Lipid oxidation and physicochemical changes are the major causes of deterioration in meat; it involves changes in flavour, texture, colour, and nutritive value as well as the formation of potentially toxic compounds (Kanner, 1994). During frozen storage, the oxidation processes are slowed but not stopped. In fact, some lipid-soluble radicals may even be more stable at lower temperatures and thereby propagate oxidation (Kanner, 1994). Protein oxidation is also responsible for many modifications in foods (Liu, Xiong, & Butterfield, 2000), such as protein aggregation or fragmentation, decreased amino acid bioavailability, and decreased protein solubility (Santé-Lhoutellier, Astruc, Marinova, Grève, & Gatellier, 2008). Oxidative processes acting on proteins can decrease the number of sulfhydryl groups by forming disulphide bridges. Active oxygen species also attack the side chains of basic amino acids (histidine, arginine, and lysine) and can convert them into carbonyl derivatives. These carbonyl groups can react with free amino groups to form amide bonds (Santé-Lhoutellier et al., 2008). Xia, Kong, Liu, and Liu (2009) suggested that multiple freeze–thaw cycles could significantly increase sulfhydryl and carbonyl contents, especially in pork subjected to five freeze–thaw cycles.

Flavour is one of the most important quality attributes of meat and meat products, and hundreds of compounds in meat contribute to flavour and aroma. During storage, however, many of these compounds are altered and new ones are generated (Calkins & Hodgen, 2007; Rhee, Anderson, & Sams, 2005). Lipid oxidation has been considered the primary cause of flavour deterioration in meat and meat products (Shahidi, 1994), but the potential contribution of reactions involving protein degradation to such flavour changes cannot be ruled out (Rhee et al., 2005).

Much attention has been given to lipid oxidation regarding both its reaction mechanisms and the quality changes it induces during meat processing and storage (Fuentes, Ventanas, Morcuende, Estévez, & Ventanas, 2010; Shahidi & Zhong, 2010). However, little information is available on the oxidation of lipids and flavour changes in frozen dumplings as an action of different frozen storage temperatures and durations.

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The aim of the present study was to assess the contributions of lipid and protein oxidation to flavour deterioration in pork dumpling filler at various frozen storage temperatures and durations. Free fatty acids, flavour compounds and sensory quality changes were investigated.

## 2. Materials and methods

### 2.1. Chemicals

Bovine serum albumin, 2,4-dinitrophenylhydrazine (DNPH), 2,6-di-tert-butyl-methylphenol (BHT) and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were purchased from Wantai Biomedicals Inc. (Harbin, China) and were at least analytical grade.

### 2.2. Sample preparation

Pork shoulder and neck meat were obtained within 12 h of slaughter from Beidahuang Meat Corporation (Harbin, Heilongjiang, China). The samples were kept on ice and transported to the laboratory and used on the same day. Three batches of dumplings were manufactured in a pilot plant (10–12 °C), and each batch was used as a replicate. Fresh pork shoulder and neck meat were ground through a 4-mm plate and mixed with pork backfat and other ingredients according to the product formulations. Specifically, the following dumpling filler formulation was prepared: 800 g of minced lean meat, 200 g of back fat, 6 g of soybean oil, 30 g of salt, 40 g of soy sauce, 100 g of water, 20 g of green onion, 20 g of ginger and 9 g of spice powder. In the dumpling filler preparation, the fresh pork trimmings and ingredients were mixed by blending for 5 min with a Kitchen Aid mixer (SYM-10, Guangzhou Shanyou Machinery Equipment Co., Ltd, Guangdong, China) at a speed of 160 rpm. Dumplings, each with 10 g of filler, were shaped by hand with dough as dumpling wrappers, then were frozen at −25 °C for at least 20 h to ensure that the core temperature of the dumplings was below −18 °C. The dumplings were placed in styrene foam trays, wrapped with an oxygen permeable polyethylene film (8 μm of thickness and 18,500 $cm^3/m^2$/24 h/atm of oxygen permeability; Weiguang Plastic Co., Ltd., Jiangsu, China), and stored for 0, 30, 60, 90, and 180 d at −7 °C (retail temperature), −18 °C (typical frozen storage temperature), and at an oscillation between −7 °C and −18 °C (transportation temperature; temperature altered every three days). A strict sanitation procedure was followed to avoid microbial contamination. For each storage temperature, 160 dumplings were used for analysis, and for each storage time, 40 dumplings were used. Twenty dumplings from each storage time and temperature group were evaluated for lipid oxidation, protein oxidation, free fatty acid content and volatile flavour compounds, and 20 dumplings were cooked for a sensory panel evaluation.

### 2.3. Lipid oxidation and protein oxidation

#### 2.3.1. Peroxide value

The peroxide value (PV) of the dumpling filler was determined according to the method of Vareltzis, Hultin, and Autio (2008) with some modifications. The dumpling filler (2.0 g) was added to 20 mL of chloroform:methanol (2:1, v/v) containing 0.5 g/kg of BHT to prevent any further peroxidation during the preparation of the samples. The mixture was homogenised in a plastic tube (2.5 cm in diameter) at 20 °C with a Polytron homogeniser (IKA T18 basic, IKA-Werke GmbH & Co., Staufen, Germany) at 3500 rpm for 30 s and then centrifuged at approximately 3000 *g* for 10 min using a centrifuge at 4 °C. Aliquots of 0.5% NaCl (3 mL) were added to the test tubes, which were then vortexed at 800 rpm for 15 s and centrifuged for 10 min at 3000 *g* to separate the sample into two phases. The lower phase (5 mL) was collected and transferred to another 50 mL test tube, then 5 mL of ice cold chloroform:methanol (2:1, v/v), 25 μL of ammonium thiocyanate solution (30%, w/v) and 25 μL of ferrous chloride solution (0.4 g barium chloride and 0.5 g ferrous sulphate were each dissolved in 50 mL of water, mixed and centrifuged for 5 min at 2000 *g*) were added to the test tube. The colour reaction of the sample mixture was completed after 5 min at room temperature (about 20 °C). Then, the absorbance was measured at 500 nm. A standard curve was prepared using reduced iron. The lipid PV is expressed as meq/kg of lipid.

#### 2.3.2. Thiobarbituric acid-reactive substances

Thiobarbituric acid-reactive substances (TBARS) were evaluated according to Wang and Xiong (2005). The TBARS value was calculated using the following equation and expressed as mg of malonaldehyde/kg of dumpling filler:

$$\text{TBARS}(\text{mg/kg}) = (A_{532}/Ws) \times 9.48$$

where $A_{532}$ is the absorbance (532 nm) of the assay solution, *Ws* is the meat sample weight (g), and "9.48" is a constant derived from the dilution factor and the molar extinction coefficient (152,000 $M^{-1}$ $cm^{-1}$) of the red thiobarbituric acid reaction product.

#### 2.3.3. Carbonyl content

Myofibrillar protein (MP) was prepared from dumpling filler according to the procedure of Xia et al. (2009). The carbonyl groups were detected by reaction with DNPH to form protein hydrazones according to the method of Oliver, Ahn, Moerman, Golstein, and Stadtman (1987) with slight modifications. The MP solution was precipitated with 100 g/L TCA (w/v; final concentration). After centrifugation (10 min, 2000 *g*), the pellet was treated with 2 g/L DNPH in 2 M HCl for 1 h (agitated every 15 min) at room temperature in the dark. The fractions were then precipitated with 100 g/L TCA (final concentration) and centrifuged. The pellets were washed twice with 1 mL of ethyl acetate:ethanol (1:1 v/v), and the solution was precipitated with 100 g/L of TCA and centrifuged. The proteins were then dissolved in 2 mL of 6 M guanidine in a 20 mM sodium phosphate buffer at pH 6.5. The absorbance was measured for the DNPH-treated sample against a HCl control at 370 nm. The amount of carbonyl is expressed as nmol of DNPH fixed/mg of protein using an absorption coefficient of 22,000 $M^{-1}cm^{-1}$ for protein hydrazones.

### 2.4. Free fatty acids

#### 2.4.1. Total free fatty acid content

The total free fatty acid (FFA) content (mg/g) was determined according to Natseba, Lwalinda, Kakura, Muyanja, and Muyonga (2005) with some modifications. About 3 g of dumpling filler was homogenised in 20 mL of n-hexane for lipid extraction. The extracted lipids were dissolved in 50 mL of ethylether:alcohol (2:1 ratio, v/v); 0.1 mL of 1% phenolphthalein was added to the mixture, then it was titrated with 0.1 M NaOH and shaken constantly until a pink colour was formed and persisted for at least 15 s. The FFA content is expressed in Oleic acid equivalents:

$$\text{FFA}(\text{mg/g}) = N \times V \times M/W$$

where *N* is the standard concentration of NaOH (M), *V* is the consumed volume of NaOH (mL), *M* is the molecular weight of Oleic acid (280.2 g/mol) and *W* is the sample quantity (g).

#### 2.4.2. Free fatty acid composition

The FFA composition was determined according to the method of Ramírez, Morcuende, Estévez, and López (2005) with some modifications. The lipids in the dumpling filler were extracted with n-hexane and esterified with 0.4 M NaOH in $CH_3OH$ at room temperature (20–25 °C). The FFA methyl esters were analysed by gas