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Cholesterol content of fried-shredded pork extracted by supercritical carbon dioxide

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Abstract

To produce reduced cholesterol fried-shredded pork with minimum changes in sensory attributes, cholesterol of fried-shredded pork was extracted using supercritical CO_2 extraction at pressures of 73, 204, and 340 atm and temperatures of 50, 100 and 150°C, and was quantified by HPLC. Cholesterol concentrations of the extracts increased sharply as pressure increased from 73 to 340 atm at all temperatures, which resulted in large decreases in cholesterol contents in the extracted products. Changes in extraction temperature did not affect cholesterol extracted at higher pressures of 204 or 340 atm. No significant difference in sensory attributes was observed between 340 atm/50°C extracted product and unextracted control. The extraction condition of 340 atm/50°C was suggested for producing reduced-cholesterol fried-shredded pork.

1. Introduction

Fried-shredded pork is a dried, shredded Taiwanese style meat product with low moisture (4%) and high fat (<43%) and protein (>32%) contents (Chen, 1987). Due to its good nutritive value and excellent sweet and salty tastes, the product has been a favorite breakfast dish and one of the major ingredients of baked foods in Taiwan. Reduction in fat and cholesterol in the diet continues to be recommended for improving human health. Thus, the meat and poultry industry is placing substantial emphasis on fat and cholesterol reduction in processed products. Reducing the cholesterol level, to provide a more healthy fried-shredded pork product, while retaining the positive flavor, would be advantageous.

Recent studies using supercritical CO_2 (SC–CO_2) extraction for sample preparation, including determinations of ground meat carbamate (Argauer, Eller, Ibrahim, & Brown, 1995), polychlorinated benzene (PCB) of seafood (Hale & Gaylor, 1995) and oil seeds (Seidel & Lindner, 1995), nitrosamines in hams (Pensabene, Fiddle, Maxwell, Lightfield, & Hampson, 1995), vitamins A and E from tablets (Scalia, Ruberto, & Bonina, 1995), and sulfamethazine in chicken eggs (Pensabene, Fiddler, & Donoghue, 1998) have demonstrated that SC–CO_2 extraction can be comparable to conventional solvent extraction in extraction efficiency.

Application of supercritical fluid to extract cholesterol from meat products has been reported and the results are varied. In the study of cholesterol extraction from fresh ground beef, Chao, Mulvaney, Bailey, and Fernando (1991) observed a higher cholesterol extraction rate of 39.8% at 172 atm/50°C as compared with 36.9% at the higher pressure of 310 atm/50°C, while Wehling, Froning, Cuppett, and Niemann (1992) observed up to 85–87% extraction rate of cholesterol from dehydrated cooked powdered and chunked beef at 381 atm/55°C. Although no significant difference ($P > 0.05$) in cholesterol extraction was detected between those two beef types, Froning, Fieman, Wehling, Cuppett, and Niemann (1994) reported that the chunk type of dehydrated cooked chicken meat was superior to the powdered type product regarding the amount of cholesterol reduction at 299 atm/45°C, but no difference between those two types was observed at 381 atm/55°C. A higher cholesterol level was also detected in the extract at the higher pressure of 381 atm/55°C than 299 atm/45°C, regardless of the meat type, which was consistent with the previous studies of dehydrated dairy products, reported by Arul,

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Boudreau, Makhlouf, Tardif, and Sahasrabudhe, (1987) and Bradley (1989).

Extraction pressure and temperature have considerable effects on the density and solvating power of supercritical fluids, which in turn can influence the selectivity of extraction. In a study of fatty acid compositions of beef fat, Merkle and Larick (1995) reported that more saturated fatty acids were extracted at lower pressures and, as pressures and density of fluid increased, the amount of unsaturated compounds and degree of unsaturation increased. Similar results on extractions of dairy fatty acids were also observed by Arul et al. (1987) and Bradley (1989).

Our study was conducted to determine the effect of various SC–CO_2 conditions on cholesterol extraction of this shredded type pork product. The cholesterol level of the extract was analyzed and sensory characteristics of the resulting fried-shredded pork products were also evaluated.

2. Materials and methods

Two kilograms fresh tenderloin, obtained from a local supermarket, was cut into 5 cm^3 chunks. After cooking at 100°C for 20 min in a water bath, the chunk meat was shredded with a meat hammer. The shredded cooked meats were mixed with the seasoning of 3% soy sauce, 2% salt, 7% cane sugar, and 0.6% monosodium glutamate, and stir-fried at 80°C for 10 min. After another 10 min stir-frying with addition of 3% lard at 180°C, the products were held at −31°C for 3 days in the vacuum containers before being subjected to SC–CO_2.

For each SC–CO_2 extraction, fried-shredded pork (7 g) collected from a homogeneous sample (300 g) was loaded into a 10 ml extraction vessel, and the lipid fraction was extracted by SC–CO_2 at 73, 204, and 340 atm and 50, 100, 150°C using a Supelco SFE-400 Supercritical fluid extractor (Supelco Inc., Bellefonte, PA). The flow rate of CO_2 was maintained at 10 l/min for a 20 min extraction period. The extract was collected in a vial containing 8 ml hexane/2-propanol (93:7) for further extraction and quantification of cholesterol.

2.1. Cholesterol extraction

Following SC–CO_2 extraction, the lipid extract was flushed with a stream of nitrogen in a 55°C water bath until dry. The residue was then saponified with 10 ml 15% potassium hydroxide in methanol in a 75°C water bath for 20 min. Distilled water (5 ml) was added after cooling and cholesterol was extracted twice with 10 ml hexane (Park, Kouassi, & Chin, 1991). The mixture of two hexane extracts was then concentrated under a stream of nitrogen at room temperature and redissolved in 0.5 ml 7% 2-propanol in hexane (v/v). After filtration through a 0.45 μm membrane filter, cholesterol content of the extract was quantified by HPLC.

2.2. Quantitative determination of cholesterol

Cholesterol extracted from fried-shredded pork was quantified by high-pressure liquid chromatography using a 10 μm μPorasil column (3.9 mm i.d.×30 cm, Waters Associates, Milford, MA) (Park & Addis, 1985), a Jasco 870-UV detector (Tokyo, Japan) set at 214 nm; a Rheodyne 7125 injector (Cotati, CA), a Shimadzu LC-6A delivery pump (Tokyo, Japan) and a Shimadzu CR-3A integrator. A flow rate of 0.9 ml/min of 7% 2-propanol in hexane (v/v) eluent was maintained. The injection volumes of cholesterol extract and standard (Sigma Chemical Co., St. Louis, MO) were 2 μl. The cholesterol eluted at 5.2 min was identified by comparing the retention time with the cholesterol standard (Sigma Chemical Co., St. Louis, MO). The height of cholesterol peak was calculated as mg cholesterol/g pork.

2.3. Cholesterol extracted by solvent method

Lipid of fried-shredded pork was extracted using a conventional solvent extraction method (Park et al., 1991). Fried-shredded pork (5 g) was mixed with 100 ml of chloroform-methanol (2:1, v/v). After homogenizing in a Nihon Seiki 6083 universal homogenizer (Nihon Seiki Seisakusho Co., Tokyo, Japan) for 3 min at #4 setting, the homogenate was centrifuged at 500×*g* for 10 min in a Hsiangtai CN-3600 centrifuge (Hsiangtai Corp., Taipei, Taiwan). The lipid of the supernatant was then saponified and cholesterol was extracted and quantified by the same procedures as described above.

2.4. Hunter color determination

The Hunter color of fried-shredded pork was measured using a Nippon Denshoku Color Difference Meter TD model 1001 DP with a ND-1 sensor (Nippon Denshoku, Kogyo Co., LTD., Tokyo, Japan) adjusted with a white standard. Measurements of *L, a*, and *b* were performed on both SC–CO_2-treated and untreated samples.

2.5. Sensory evaluation

A trained, 15-member sensory panel, familiar with sensory properties of fried-shredded pork, evaluated the 340 atm/50°C-extracted product and the untreated control using a triangle test (Larmond, 1982). Panelists were selected from the faculty, staff, and student population of the school of Agriculture, Chinese Culture University. The panel, composed of eight female and seven male individuals, received a 1 h training session with six brands of commercial products from of local supermarket. Panelists were instructed to evaluate the specific visual attributes of orange–red color, degree of whiteness/darkness, and shred size and the specific sweet and salty tastes of those products. Sensory sessions were

held once every day. During each session, three samples (50 g/sample), labeled with 3-digit codes, were randomly presented in a white paper dish and served to the panelists. Panelists were asked to identify the odd sample from the three according to the appearance and taste. Unsalted crackers and water were given between samples. Sensory evaluations were conducted under fluorescent light on a black lab benchtop before lunch hour each day. The triangle test was performed in three replications.

2.6. Crude fat and moisture analyses

Crude fat content was determined by extracting 5 g fried-shredded pork with 50 ml hexane for 16 h in a Selecta DET-GRAS solvent recovery extractor (J.P. Selecta s.a., Barcelona, Spain) according to the method published by Chen (1987). Moisture content was measured by heating 2 g sample at 110°C for 2 h in an Ohaus MB-200 analytical electrical balance (Ohaus Corp., Florham Park, NJ) and was quantified by determining the percent weight loss relative to original sample weight (Chen, 1987)

2.7. Statistical analysis

All data were subjected to analysis of variance and Duncan Multiple Range Test using SAS (SAS Institute, 1986) at a significance level of 0.05. Each treatment was performed in three replications.

3. Results and discussion

3.1. Cholesterol extraction

Cholesterol contents of the extracts increased significantly ($P < 0.05$) with increased extraction pressure at all temperatures (Table 1). The highest extraction pressure of 340 atm resulted in the largest yield of cholesterol (0.405–0.562 mg/g pork) due to increase in solvating power of SC–CO_2 at higher pressure. These results were consistent with those obtained for chicken meat (Froning et al., 1994). Increased extraction temperature from 50 to 150°C at 204 and 340 atm did not change the yield of cholesterol ($P > 0.05$). This indicated that pressure rather than temperature was the predominant factor in determining solvating power for extracting cholesterol from fried-shredded pork. The lowest pressure of 73 atm resulted in the smallest amount of cholesterol extracted at 150°C and an undetected level at 50 and 100°C. While the cholesterol content of unextracted fried-shredded pork was 0.802 mg/g pork, the contents after 340 atm/150, 100, and 50°C extractions were 0.291, 0.222, and 0.397 mg/g pork, respectively, i.e. 50.5–70.1% reductions of cholesterol contents from the unextracted control. Increase in SC–CO_2 pressure appeared to be effective in reducing cholesterol levels of fried-shredded pork and the extraction condition of 340 atm/50–150°C was suggested.

3.2. Hunter color determination

Sharp increases in L values of fried-shredded pork from 45.2 to 67.5–72.2 were observed as temperature increased from 50 to 100–150°C at 340 atm indicating an increase in whiteness at higher extraction temperature due to the partial removal of the added soy sauce contributing to the darkness of the products. Since the fried-shredded pork is medium dark orange–red in color and the increase in degree of whiteness was regarded as poor quality, a temperature above 100°C at 340 atm was not suggested for producing the reduced cholesterol product. A slight increase in the degree of orange–red color was observed in the 340 atm/50°C extracted product due to the concentrating effect, as indicated by the higher ($P < 0.05$) Hunter a and b values compared with the control sample (Table 2). No significant difference ($P > 0.05$) in the degree of whiteness was observed between these two samples, suggesting that the extraction condition of 340 atm/50°C for 20 min did not alter the degree of whiteness of the product.

3.3. Sensory evaluation

Comparing 340 atm/50°C-extracted product and the unextracted control by the triangle test, eleven out of 15

Table 1
Effect of SC–CO_2 pressures and temperatures on quantity (mg cholesterol/g pork) of cholesterol extracted from fried-shredded pork

Temperature (°C)	Pressure (atm)		
	340	204	73
150	0.485ax[a,b]	0.059ay	0.010az
100	0.562ax	0.089ay	0bz
50	0.405ax	0.120ay	0bz

[a] a, b: Means with different letters in same column are significantly different ($P < 0.05$).

[b] x, y, z: Means with different letters in same row are significantly different ($P < 0.05$).

Table 2
Hunter L, a and b values of SC–CO_2 treated[a] and untreated fried-shredded pork

Fried-shredded pork	Hunter color		
	L	a	b
Untreated	43.9a[b]	13.9a	19.3a
SC–CO_2-treated[a]	45.2a	15.7b	20.2b

[a] 340 atm, 50°C, 20 min.

[b] a, b: Means in same column with different letters are significantly different ($P < 0.05$).

panelists were unable to identify the odd sample using both the appearance and taste. This was an indication of no significant difference ($P > 0.05$) in sensory characteristics between those two samples (Larmond, 1982). The result confirmed the reports by Froning et al. (1994) and Wehling et al. (1992), in which those authors showed that cholesterol and other lipids could be extracted from dehydrated chicken and beef without significantly changing the sensory properties.

3.4. Crude fat and moisture analyses

The crude fat content of fried-shredded pork after extraction at 340 atm/50°C for 20 min was 11.46%, a 30.7% reduction from untreated control of 16.53%. This large decrease in fat content did not affect the sensory notes, as described above, suggesting that positive sensory attributes could be retained in the product after the extraction. No significant difference ($P > 0.05$) in moisture content was observed between 340 atm/50°C-extracted sample and untreated control, indicating that moisture was also retainable after the extraction.

In conclusion, the highest extraction pressure of 340 atm resulted in the largest cholesterol yield in the extracts and lowest cholesterol content in the extracted products. While up to 30.7% fat was extracted, the lowest extraction temperature of 50°C combined with 340 atm did not deteriorate the sensory quality of the product. Therefore, the extraction condition of 340 atm/50°C was suggested for producing reduced-cholesterol fried-shredded pork. Future commercial application of SC–CO_2 extraction is feasible with healthy pork products using a well-designed large scale SC–CO_2 extraction system.

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