Impact of citric acid and calcium ions on acid solubilization of mechanically separated turkey meat: Effect on lipid and pigment content

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ABSTRACT Increased demand for poultry products has resulted in an increased availability of by-products, such as the neck, back, and frame, that can be processed into mechanically separated poultry meat. The major problems with mechanically separated poultry meat are its high lipid content, color instability, and high susceptibility to lipid oxidation. The present work was undertaken to determine the effect of different concentrations of citric acid and calcium ions on protein yield, color characteristics, and lipid removal from protein isolates prepared using an acid-aided extraction process. Six levels of citric acid (0, 2, 4, 6, 8, and 10 mmol/L) and 2 levels of calcium chloride (0 and 8 mmol/L) were examined. The entire experiment was replicated 3 times, resulting in 36 extractions (3 × 6 × 2). The highest (P < 0.05) protein yield was found for the treatment with 6 mmol/L of citric acid. In general, all the combinations removed an average of 90.8% of the total lipids from mechanically separated turkey meat, ranging from 86.2 to 94.7%. The lowest amount (1.14%) of total lipids obtained was for samples treated with 4 mmol/L of citric acid. Maximum removal of neutral lipids (96.5%) and polar lipids (96.4%) was attained with the addition of 6 and 2 mmol/L of citric acid, respectively. Polar lipid content was found to be significantly (P = 0.0045) affected by the presence of calcium chloride. The isolated proteins were less (P < 0.05) susceptible to lipid oxidation compared with raw mechanically separated turkey meat. The most efficient removal of total heme pigment was obtained with the addition of 6 or 8 mmol/L of citric acid. Addition of calcium chloride had a negative effect on total pigment content. The study revealed that acid extractions with the addition of citric acid resulted in substantial removal of lipids and pigments from mechanically separated turkey meat, improved stability of the recovered proteins against lipid oxidation, and appreciable protein recovery yields.

Key words: mechanically separated turkey meat, citric acid, calcium chloride, lipid oxidation, protein isolates

INTRODUCTION

Consumption of poultry meat has increased greatly over the past decades. In particular, consumer tastes have begun to shift from a preference for whole carcasses toward cut-up parts (breast, thighs, wings) and processed poultry products. The increased demand for these types of products has caused an increased availability of neck, back, and frame supplies that can be processed into mechanically separated poultry meat (MSPM). The latter is widely used for the production of frankfurters, sausages, and restructured meat products (Dhillon and Maurer, 1975). The mechanical deboning process includes grinding meat and bone together and forcing the mix through a fine screen to remove bone particles (Froning, 1981). During this process, in addition to the extreme mechanical stress, extraction of considerable amounts of lipids and heme components (hemoglobin and myoglobin) from the bone marrow and aerated conditions result in problems with lipid oxidation and color instability of the final product. Hemoglobin and myoglobin are known to be the main pro-oxidants in muscle foods (Richards et al., 1998); their oxidation is usually associated with color problems in muscle foods. Therefore, removing pigments from MSPM could have a beneficial effect on color intensity and fast lipid rancidity, which in turn would extend the shelf-life stability of raw and cooked MSPM-based products.

Membrane polar lipids, being rich in polyunsaturated fatty acids, are considered the primary substrates for lipid oxidation, as compared with neutral lipids (triaclylglycerols). Because the phospholipid fraction (the major part of polar lipids) of MSPM is highly unsatu-
rated (Gomes et al., 2003), it is desirable to remove as much phospholipid content as possible, which in turn would greatly increase the stability of proteins against lipid oxidation. Although the hydrophobic triacylglycerols are easy to separate from minced muscles, the membrane lipids are relatively difficult to remove because of their amphiphilic nature (Gehring et al., 2009). To overcome the problems resulting from mechanical deboning, Japanese researchers have developed a process involving water washing of fish muscle minces that results in a functional protein ingredient called surimis. However, low processing yield, inefficient removal of membrane lipids, and excessively large volumes of water are factors that limit the use of this process (Hultin and Kelleher, 2000b).

A new approach to extract functional proteins from underused muscle protein sources was introduced by Hultin and Kelleher (1999). This process is based on pH-dependent solubility of muscle proteins for their separation and recovery from the undesirable components, such as oxidatively unstable lipids in cellular membranes (Kristinsson and Hultin, 2003). This process involves solubilizing the protein in acid or alkaline conditions and recovering the solubilized proteins by precipitation at their isoelectric point. Hultin and Kelleher (2000a) reported removal of 37 and 51% of phospholipids from chicken breast and thigh muscles, respectively, at pH 2.8. A study by Undeland et al. (2002) on the recovery of functional proteins from herring muscle achieved 20 to 30% phospholipid removal by an acid-solubilization process. To improve the stability of extracted protein against lipid oxidation, Liang and Hultin (2005a) also examined the effect of acid solubilization of fish proteins. They reported that treatment with citric acid and calcium ions could aid in the removal of membrane lipids from cod muscle homogenates at pH 3.0. The authors suggested that citric acid and calcium ions are able to disconnect the linkages between membranes and cytoskeletal proteins, which are then further separated via high-speed centrifugation.

Earlier studies showed that calcium ions are able to facilitate aggregation or fusion of membrane-phospholipid vesicles (Fraley et al., 1980; Wilschut et al., 1980). The efficiency of calcium ions in precipitating the phospholipoproteins of cheese whey was also reported (Maubois et al., 1987). However, no work has been carried out on the effect of citric acid and calcium chloride during protein extraction from mechanically separated turkey meat (MSTM). Hence, the objective of the present study was to investigate the effects of citric acid and calcium chloride in improving the efficiency of protein recovery from MSTM. An additional objective was to optimize the concentrations of citric acid and calcium chloride required to obtain high protein yields along with lipid and pigment removal. The effect of these compounds in improving the oxidative stability of proteins recovered from MSPM was also assessed.

### MATERIALS AND METHODS

#### Materials

Mechanically separated turkey meat was obtained from Lilydale Inc. (Edmonton, Alberta, Canada). Polyethylene bags were filled with MSTM (250 g) and stored at −20°C until use. Before extraction, samples were thawed overnight at 4°C. All chemicals used were of reagent grade and were obtained from Fisher Scientific (Waltham, MA).

#### Extraction Procedures

Acid-aided protein recovery from MSTM was done according to the method of Liang and Hultin (2003), with several modifications (Betti and Fletcher, 2005). A cold (1 to 3°C) distilled water-ice mixture and 200 g of MSTM were mixed at a 1:5 ratio (meat:water-ice, wt/vol), followed by addition of the respective concentration(s) of citric acid, calcium chloride, or both. The extraction steps were performed at low temperature (4°C). The mixture was homogenized using a 900-W Food Processor (Wolfgang Puck WPMFP15, WP Appliances Inc., Hollywood, FL) for 15 min. After homogenization, 1,200 mL of the meat slurry was transferred to a beaker and held at 4°C for 30 min. Thereafter, the pH of the homogenate was adjusted to 2.5 by drop-wise addition of 2 N hydrochloric acid to achieve the maximum protein solubility. This solubilization pH was chosen based on a solubility study conducted during our previous work (Hrynets et al., 2010), in which the highest value for MSTM proteins at acidic conditions was obtained at pH 2.5. The pH-adjusted homogenate was centrifuged using an Avanti J-E refrigerated centrifuge (Beckman Coulter Inc., Palo Alto, CA) at 25,900 × g for 20 min at 4°C. Centrifugation resulted in the formation of 3 phases: an upper phase of MSTM neutral lipids, a middle phase of water-soluble proteins, and a bottom phase of water-insoluble proteins and membrane lipids. The soluble protein fraction (middle layer) was collected and pH was adjusted to 5.2 to precipitate proteins isoelectrically. The precipitated proteins were further centrifuged at 25,900 × g for 20 min at 4°C. The precipitate was resuspended in a water-ice mixture (350 mL of water/350 g of ice) by homogenization for 10 min, and the pH of the homogenate was adjusted to 6.2. The proteins were finally collected via centrifugation at 25,900 × g for 20 min at 4°C. The isolated proteins were stored at −20°C until analysis.

#### Protein Yield

Protein yield was estimated according to the method described by Omana et al. (2010). Protein yield was calculated based on a difference in total protein content...
between isolates and raw MSTM and was expressed as a percentage. Protein content was estimated by the biuret procedure as described by Gornall et al. (1949) and Torten and Whitaker (1964). Bovine serum albumin (HyClone, Logan, UT) was used as a standard.

**Total Lipid Extraction**

Total lipids were extracted from 10.0 g of processed meat and 5.0 g of raw meat separately with 120 mL of a chloroform:methanol solution (2:1, vol/vol) by homogenization (Power Gen 1000S1, Fisher Scientific) at setting 1 for 10 min according to the method of Folch et al. (1957). After 30 min, the homogenates were filtered through Whatman No. 1 filter paper (Whatman International, Maidstone, UK). To allow clear phase separation, 40 mL of a 0.88% (vol/vol) sodium chloride solution was added and the mixture was carefully transferred to a separation funnel. After separation, the chloroform phase was filtered through anhydrous sodium sulfate (Fisher Scientific) placed on Whatman No. 1 filter paper. This filtration was done to remove any traces of water that might have been present in the chloroform phase. After filtration, the chloroform phase was transferred into a preweighed round-bottomed flask. The upper phase was discarded because it was rich in nonlipid components. To remove the solvent, lipid extracts were dried in a rotary evaporator (RE 121, Rotavapor, Büchi Labortechnik AG, Flawil, Switzerland) under temperatures not exceeding 40°C. The flasks were then placed in a hot-air oven at 60°C for 30 min and weighed accurately after desiccation for 30 min. To allow for further analysis of lipid classes, the total lipid extract was dissolved in 10 mL of chloroform, transferred into preweighed vials, and frozen at −20°C. Lipid reduction was calculated from the difference in lipid content between raw and treated materials and is expressed as a percentage.

**Fractionation of the Main Lipid Classes**

The total lipids extracted from the MSTM were further separated into neutral and polar lipid fractions by using the method described by Ramadan and Morasel (2003). The separation of these 2 lipid classes was carried out using a glass column (30 cm height × 2 cm diameter) packed with a slurry of silica gel (70-230 mesh, Whatman International) that was prepared by suspending silica gel in chloroform (1:5, wt/vol). The total lipid solution (9 mL) obtained from the lipid extraction was allowed to pass through the column. Chloroform (60 mL) was used initially as eluant to separate the neutral lipids (triacylglycerols). After removal of these triacylglycerols, 60 mL of methanol was applied to the column to elute the polar lipids.

The fractions obtained were completely evaporated to dryness and kept in a hot-air oven at 60°C for 30 min. The final weight of the flasks was taken after desiccating for 30 min. Both the neutral and polar lipid parts were determined gravimetrically and expressed as a percentage.

**Determination of TBA Reactive Substances**

Thiobarbituric acid reactive substances (TBARS) were determined in triplicate for each extraction group and also the raw material by using a modification of the procedure described by Kornbrust and Mavis (1980). Meat samples (3 g) were homogenized in 27 mL of 1.15% potassium chloride with a Power Gen 1000S1 homogenizer (Fisher Scientific) for 1 min operated at setting 3. A 1,000-μL quantity of 80 mM Tris-maleic buffer (pH 7.4), 400 μL of 2.5 mM ascorbic acid, and 400 μL of 50 mM ferrous sulfate were added to a 200-μL aliquot of the homogenate and incubated for 0, 30, 60, 100, and 150 min using a 37°C water bath. After incubation, 4 mL of a TBA-trichloroacetic acid-hydrochloric acid mixture (26 μM TBA, 0.92 μM trichloroacetic acid, and 0.8 mM hydrochloric acid) was added to the sample, and the test tubes were held in boiling water for 15 min. The samples were then cooled to room temperature and the absorbance of the solution was read at 532 nm against a blank containing all the reagents except the homogenate. The readings were taken using a spectrophotometer (V-530, Jasco Corporation, Tokyo, Japan). The TBARS concentration was calculated using the extinction coefficient of $E_{532} = 1.56 \times 10^5 \text{M}^{-1} \text{cm}^{-1}$. The extent of lipid oxidation was expressed as nanomoles of malondialdehyde per gram of meat.

**Total Pigment Determination**

The estimation of total pigment content was done by direct spectrophotometric measurement according to the method described by Fraqueza et al. (2006), with slight modifications. The pigments were extracted from 10 g of meat sample with a mixture of 40 mL of acetone, 1 mL of hydrochloric acid, and 1 mL of water. The mixture was vortexed for 3 min and allowed to stand for 1 h at room temperature. After the extract was filtered through Whatman No. 1 filter paper, the absorbance was recorded at 640 nm against an acid-acetone blank using a UV-visible spectrophotometer (V-530, Jasco Corporation). The absorbance value was multiplied by a coefficient of 17.18, and the concentration of total heme pigments was expressed in milligrams of myoglobin per gram of meat.

**Experimental Design and Statistical Analysis**

The experimental design is shown in Figure 1. The extraction range of variables and their respective levels were chosen based on data from the literature (Liang
and Hultin, 2005a). The experiment was conducted as a $6 \times 2$ factorial arrangement with 6 levels of citric acid (0, 2, 4, 6, 8, and 10 mmol/L) and 2 levels of calcium chloride (0 and 8 mmol/L) and was replicated 3 times, resulting in 36 extractions. Analysis of variance was performed with SAS software (SAS Institute Inc., Cary, NC) to determine the significance of the effects of these variables. The model tested the main effects for both citric acid and calcium chloride. The interactions were determined using residual error:

$$ Y = \mu + [CA] + [CaCl_2] + ([CA] \times [CaCl_2]) + e, $$

where $Y$ is the dependent variable, $\mu$ is the treatment average, $[CA]$ is the concentration of citric acid, $[CaCl_2]$ is the concentration of calcium chloride, and $e$ is residual error. Means were separated using Tukey’s adjustment. Differences were considered to be significant based on a 0.05 level of probability. Results are expressed as mean value ± SD.

**RESULTS AND DISCUSSION**

**Protein Yield**

A previous study by our group (Hrynets et al., 2010) on the solubility of MSTM proteins revealed the highest protein solubility at pH 2.5 among the different acidic conditions investigated; this pH was used for the current study because a high recovery yield was expected. Moreover, the efficiency of acid extraction of protein might be a result of additional recovery of sarcoplasmic proteins along with the myofibrillar protein fraction because the low pH facilitated protein solubilization, resulting in higher protein yields (Hrynets et al., 2010). This result is in agreement with the basic principle of the pH-shifting process, which indicates an additional recovery of sarcoplasmic proteins during the extraction process (Ingadottir, 2004). A high protein yield is very important during industrial extraction of proteins for economic reasons. Results for protein yield are shown in Table 1. No significant interaction was found between the effects of citric acid and calcium chloride ($P = 0.6545$). At the level of main effects, only citric acid significantly affected protein yield ($P < 0.0001$). The maximum protein yield (85.6%) was achieved when 6 mmol/L of citric acid was added to the MSTM homogenate. Further increases in citric acid concentration to 8 and 10 mmol/L caused a reduction in recovery yield. In general, the protein yield tended to be slightly higher when extractions were carried out at lower citric acid concentrations rather than at higher ones. Even though the protein yield was slightly improved by adding citric acid, the values were not significantly different from the control values, indicating a small influence of citric acid on protein yield during the acid extraction process. The protein yield obtained from the acid extraction process depends on 3 main factors: the solubility of the protein during exposure to low or high pH, the size of the sediments after centrifugation, and the solubility at the precipitation pH (Nolsoe and Undeland, 2009).

**Total Lipid Content**

Lipids in meat are classified as neutral lipids (triacylglycerols) and polar lipids (phospholipids) (Kono and Colowick, 1961). Neutral lipids are stored in connective tissue in relatively large deposits, whereas polar lipids are integrated into and widely distributed throughout the muscle tissues. To increase utilization of the extracted proteins, the total lipids from MSTM must be reduced. Total lipid contents of protein isolates prepared as a function of citric acid and calcium chloride concentration are shown in Table 1. A significant interaction was found between citric acid and calcium chloride ($P < 0.0001$). Within these treatments, the maximum removal of total lipid from MSTM (94.7%) was achieved with the addition of 4 mmol/L of citric acid (Figure 2). The most evident effect from the combination of citric acid and calcium chloride was observed when 8 mmol/L of calcium chloride was used in combination with 10 mmol/L of citric acid, which in

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**Figure 1.** Experimental design of the protein extraction process.
Turn decreased the total lipid to only 42.6% compared with the effect of citric acid without the addition of calcium chloride. This suggests that the addition of 8 mmol/L of calcium chloride may diminish the effect of citric acid at higher concentrations. In general, all the combinations removed on average 90.8% of the total lipids from MSTM (for the comparison between initial material and extracted proteins, the composition of raw MSTM is presented in Table 2). Statistical analyses also showed that the main effect for citric acid ($P < 0.0001$) was on the total lipid content of the protein isolates; as in the case of the interaction, the highest removal of total lipids from MSTM was achieved with the addition of 4 mmol/L of citric acid. However, no significant ($P = 0.5032$) difference was attained for total lipid content among the treatments when the main effect of calcium chloride was analyzed.

### Table 2. Properties of raw mechanically separated turkey meat (MSTM)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Raw MSTM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipid content (%)</td>
<td>21.7 ± 2.2</td>
</tr>
<tr>
<td>Neutral lipid content (%)</td>
<td>14.1 ± 1.6</td>
</tr>
<tr>
<td>Polar lipid content (%)</td>
<td>13.9 ± 1.4</td>
</tr>
<tr>
<td>Total hemoglobin (mg/L of meat)</td>
<td>4.1 ± 0.8</td>
</tr>
</tbody>
</table>

*Mean ± SD (n = 3).*
significant \( (P = 0.8584) \) influence. However, when the main effect of citric acid on neutral lipid content was evaluated, a significant \( (P = 0.017) \) effect was found. Addition of 6 mmol/L of citric acid resulted in maximum (96.5%) removal of the neutral lipid fraction from MSTM. The lipid content in this treatment was found to be 2.2 times lower than that in the control sample. Increasing the citric acid concentration to 8 and 10 mmol/L or decreasing it to 4 and 2 mmol/L resulted in an increase in neutral lipid content. The results showed that the addition of different levels of citric acid improved the removal (93.3 to 96.5%) of neutral lipids from MSTM.

### Polar Lipid Content

Although polar lipids are less predominant in muscle tissues, they are considered more susceptible to oxidative changes compared with neutral lipids. This is attributed to the high content of unsaturated fatty acids, the close contact with catalysts of lipid oxidation such as reactive oxygen species, and the large surface area exposed to the aqueous phase (Gandemer, 1999). Thus, the removal of phospholipids is highly desirable in terms of improving the stability of extracted proteins against lipid oxidation. The results for polar lipid fraction content are reported in Table 1. The interaction between citric acid and calcium chloride was found to have a significant \( (P = 0.0045) \) effect on polar lipid content (Figure 3). Maximum polar lipid removal was attained for the treatment with the addition of 2 mmol/L of citric acid; at these conditions, 96.4% of polar lipids were removed from MSTM, to reach a final content of 0.22%. With the addition of 2 mmol/L of citric acid, the polar lipid content was 3.1 times lower than that of the control sample, indicating an influential effect of citric acid on polar lipid removal from MSTM. Further increases in citric acid concentration to 4, 6, and 10 mmol/L resulted in less efficient removal of polar lipids. With the addition of 8 mmol/L of calcium chloride to 6 and 10 mmol/L of citric acid, significant removal of polar lipids was observed; however, addition of calcium chloride to treatments with 2, 4, and 8 mmol/L of citric acid decreased the efficiency of polar lipid removal. Removal of polar lipids was considerably high, with a range from 86.6 to 96.4% when different concentrations of citric acid were incorporated in the MSTM protein homogenate. The high efficiency of citric acid for polar lipid removal may be due to its ability to disconnect the linkages between cytoskeletal proteins and membrane lipids, linked together via electrostatic interaction. Citric acid might play a role as a binding agent for the basic amino acid residues of cytoskeletal proteins competing with the acidic phospholipids of membranes (Liepinia et al., 2003). As a result, membranes released from the cytoskeletal proteins might aggregate because of the low pH achieved by the addition of citric acid and could then be removed by centrifugation (Liang and Hultin, 2005a). Removal of up to 90% of the phospholipids was achieved with the addition of 5 mmol/L of citric acid along with 8 mmol/L of calcium chloride in a study on the acid solubilization of fish muscle proteins (Liang and Hultin, 2005a). In previous studies on the removal of membrane lipids from fish muscles, the addition of citric acid along with calcium chloride has been used successfully (Liang and Hultin, 2005a; 2005b). However, the present study revealed that the addition of 8 mmol/L of calcium chloride to a starting material already rich in calcium ions showed no effect on polar lipid reduction.

### Extent of Lipid Oxidation (TBARS)

Lipid oxidation is a complex process by which unsaturated fatty acids react with molecular oxygen via free radicals and form peroxides or other oxidation products (Gray, 1978). Secondary oxidation products, such as aldehydes, ketones, and esters, are responsible for increased deterioration and a rancid flavor (Ladikos and Lougovois, 1990). Spectrophotometric detection of these compounds by a TBARS test has been widely used to estimate the effects of oxidative stress on lipids (Gray, 1978).

The changes in TBARS values for raw MSTM and protein isolated under different extraction conditions are presented in Figure 4. Because the addition of calcium chloride did not have a significant effect on the removal of polar lipids from MSTM, the rate of lipid oxidation was tested only for samples treated with 0, 2, 4, and 6 mol/L of calcium chloride.
mmol/L of citric acid. Increasing the concentration of citric acid to 8 and 10 mmol/L also did not result in a significant improvement in polar lipid removal; therefore, lipid oxidation tests were not conducted for these treatments. Thiobarbituric acid reactive substances values at 0 min of incubation time were significantly ($P < 0.05$) lower for samples extracted with the addition of 2 mmol/L of citric acid. When the incubation time reached 150 min, the same samples tended to be significantly ($P = 0.0559$) lower compared with control samples and samples in the other treatments. The lowest level of lipid oxidation in this treatment is probably due to the efficient removal of the majority of phospholipids, as revealed by phospholipid analysis (Figure 3). Pikul et al. (1984) reported that 90% of TBARS formation was contributed by polar lipids in chicken meat. The highest malondialdehyde value, regardless of incubation time, was found for raw MSTMs, and the values were significantly ($P < 0.05$) higher compared with extracted samples. This study revealed that the addition of 2 mmol/L of citric acid might act as a protection against lipid oxidation by the removal of polar lipids. This effect might also be attributed to its ability to chelate pro-oxidants, such as iron and heme proteins, via bonds formed between the metal and carbonyl or hydroxyl groups of the citric acid molecule (Ke et al., 2009). Citric acid is often used as an antioxidant to stabilize fish muscle during frozen storage (Pokorny, 1990) and is included among the antioxidants that are generally permitted in foods (E330; Mikova, 2001). Our results are in agreement with those of Vareltzis et al. (2008), who reported that low pH treatments improved the oxidative stability of protein isolates from cod muscle, whereas calcium chloride alone did not.

### Total Heme Pigments

Color is an important factor that affects the consumer’s perception of product quality and influences purchasing decisions. It is also one of the key variables used when comparing different processing treatments. Overall, the market is most interested in protein isolates that are as white as possible (Tabilo-Munizaga and Barbosa-Canovas, 2004). The 2 major pigments responsible for the color of MSTMs are myoglobin and hemoglobin (Hernandez et al., 1986), which are also known as catalyzers of lipid oxidation in meat (Richards et al., 2005). Therefore, the effective removal of these pigments might not only improve the color characteristics of protein isolates, but also increase their stability against oxidative deterioration. The effects of citric acid and calcium chloride in removing total pigments from MSTMs during acid solubilization are shown in Table 1. The interaction between citric acid and calcium chloride was found to have a significant ($P < 0.0001$) influence on total pigment content of protein isolated from MSTMs (Figure 5). The lowest total pigment content was observed for treatments with the addition of 6 or 8 mmol/L of citric acid during extraction (0.23 and 0.25 mg/g of meat, respectively). The values were approximately one-half the value of the control sample. In general, treatments with the combination of citric acid and calcium chloride removed between 72.2 and 95.3% of total heme pigments from MSTMs. The present study also revealed that addition of calcium chloride to protein homogenates decreased the effectiveness of pigment removal from MSTMs. It has been reported that calcium chloride increases the size of the aggregates caused by increased protein-protein and decreased protein-water interactions, by occupying negative charges on polypeptide chains (Maltais et al., 2005). Hence, it may lead to the precipitation of heme

![Figure 5.](https://academic.oup.com/compsoc/article-abstract/90/2/458/1534609/figure-5)
pigments to the bottom sediment during centrifugation after isoelectric precipitation.

**Conclusions**

Citric acid significantly influenced protein yield and lipid and pigment removal during extraction of proteins from MSTM. The optimal concentration of citric acid for the maximum protein yield was found at 6 mmol/L. However, 2 mmol/L of citric acid was the most efficient for removing phospholipids. This resulted in greater stability of isolated proteins against lipid oxidation compared with raw MSTM. Citric acid also significantly affected total pigment content of the protein isolates, which is directly related to the color of extracted meat. An increase in protein yield with efficient lipid removal during extraction will benefit industry members using protein isolates from MSTM for the production of further processed products to improve their functionality. Therefore, the addition of citric acid during the acid solubilization technique is a highly appealing alternative for extracting proteins from MSTM to help overcome its compositional problems.

**ACKNOWLEDGMENTS**

The authors thank the Alberta Livestock and Meat Agency (Edmonton, Alberta, Canada), Alberta Chicken Producers (Edmonton, Alberta, Canada), Alberta Turkey Producers (Edmonton, Alberta, Canada), and Poultry Research Centre (Edmonton, Alberta, Canada) for funding this project. The meat supply from Lilydale (Edmonton, Alberta, Canada) is gratefully acknowledged. The authors extend appreciation to Graham Plastow (University of Alberta) for his valuable suggestions.

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