THE PROTEIN SOLUBILITY METHOD AS AN INDICATOR OF PORK QUALITY

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SUMMARY

Extraction time, extraction temperature and NaCl concentration were varied in an attempt to find the most suitable conditions for the use of protein solubility to predict pork quality. Six NaCl concentrations (0, 0.25, 0.50, 0.75, 1.0 and 1.25 M in 0.025 M sodium phosphate, pH 7.4) as well as 0.55 M KI, 0.05M potassium phosphate pH 7.4 were evaluated at each of two extraction times (1 h and 24 h) and two extraction temperatures (4 and 21 C) for their ability to solubilize protein in the anterior portions of ten longissimus muscles of each of three pork quality types (PSE, normal and DFD).

For DFD muscles KI was equal to and for normal and PSE muscles superior to all NaCl concentrations in its ability to solubilize protein. NaCl was most effective for solubilizing protein at a concentration of 0.5 to 0.75 M for DFD and normal muscles extracted for 24 h, and at a concentration approaching 1.25 M for PSE muscles and for normal muscles extracted at 1 h. KI was better able to distinguish PSE from normal pork, but less able to distinguish DFD from normal pork than was NaCl. Yet protein solubility was more highly correlated to subjective quality scores, reflectance and pH for certain NaCl concentrations than for KI. The most intensive extraction conditions (24 h at 21 C) gave the highest correlations.

Since extraction with 1.0 M NaCl for 24 h at 4 or 21 C yielded soluble protein values which correlated higher with quality traits and distinguished better between normal and DFD pork than did KI, this approach should be considered a viable alternative to either the very low salt or the KI extractions currently used as indicators of pork muscle quality.

Introduction

The protein solubility method, which is most commonly used as an indicator of pork muscle quality, is that of Barton-Gade (1984). This method involves the extraction of protein from homogenized muscle overnight at 4 C with 0.55 M KI, 0.05 M potassium phosphate, pH 7.4. The extraction of soluble protein in 1.1 M KI distinguishes between PSE (pale, soft, exudative) and normal quality types but low salt (0.03M potassium phosphate, pH 7.4) extraction distinguishes better between DFD (dark, firm, dry) and normal quality groups (Lopez-Bote et al., 1989).

Protein solubility of muscles is known to be affected by factors such as freezing, homogenization conditions, type and concentration of salt, pH, dilution ratio, centrifugation speed, etc. (Helander, 1957; Saffle and Galbreath, 1964; Morrissey et al., 1987; Richardson and Jones, 1987; Lan et al, 1993). Although the relationship of protein solubility to physiological state of pig muscle (Sayre and Briskey, 1963) has been documented for some time, little effort has been directed to the optimization of conditions for the solubilization of muscle protein in pig muscles of differing qualities. Additionally, since the amount of NaCl-soluble protein is a major determinant of the amount of fat that can be emulsified (Saffle and Galbreath, 1964), it would be useful to have a NaCl-based protein solubility method.

In the current study extraction time, extraction temperature and NaCl concentration were varied in an attempt to find the most suitable and convenient conditions for the use of protein solubility to predict pork quality.

Materials and Methods

On the day following slaughter at a commercial abattoir longissimus muscles were removed from wholesale pork backs. Approximately 15 min after this, a 15 cm piece was cut from the anterior end of the muscle

exposing a fresh cross-section. After an additional 15 min, muscle quality was assessed on the longitudinal and cross-sectional surfaces as a consensus of two experienced raters according to the Agriculture Canada Pork Quality Standards (Agriculture Canada 1984) as described by Murray and Johnson (1990). Ten muscles were assigned to each of the PSE, normal and DFD quality groups based on color and structure scores, such that PSE, normal and DFD muscles had color scores of ≤ 2, 3 and ≥ 4, respectively, and structure scores of ≤ 2, 3 and ≥ 4, respectively.

Approximately 20 min after exposing the cross-sectional surface, its CIE L*, a* and b* (Commission Internationale de l'Eclairage, 1978) light reflectance coordinates were measured in duplicate using a Minolta Chroma Meter II.

The 15 cm portion from the anterior end was ground two times through a #12 grinding plate (3 mm diameter pore size). The ground samples were frozen, stored up to 1 month at -20 C and thawed at 3 C for 24 h prior to analysis.

After thorough mixing of each ground sample, its pH was measured with a Fisher Model Accumet 825MP pH meter fitted with an Orion spear-type electrode. Then 1 g portions were weighed into test tubes on ice and to each was added 25 ml of one of six different NaCl solutions (0, 0.25, 0.50, 0.75, 1.0 and 1.25 M in 0.025 M sodium phosphate, pH 7.4) as well as 0.55 M KI, 0.05 M potassium phosphate pH 7.4 at 4 C and 21 C. Immediately these mixtures were homogenized for 15 s with a Omni 2000 homogenizer fitted with a 20 mm generator at a speed setting of 1. Superficial microscopic examination of several homogenized samples indicated that the majority of muscle fibres were broken to myofibrils and the visible fibers were of lengths which rarely exceed their diameters. The content of each tube was then subdivided into two portions which were maintained at either 4 or 21 C. At 1 h and 24 h after homogenization, the tube contents were well mixed and two 1.5 ml aliquots were withdrawn, and centrifuged at 15,000 xg for 5 min. This speed eliminated the turbidity which yields unreliable protein assays (Gumpen and Fretheim, 1983). The protein concentration in the supernatant was determined by the Biuret method of Gornall et al. (1949). Protein solubility was expressed as g. Kg^{-1} fresh muscle. The extraction with KI for 24 h at 4 C was essentially that used by Barton-Gade (1984).

Results And Discussion

The three muscle groups, PSE, normal and DFD are defined in Table 1 in terms of several objectively measured quality traits. Of the three reflectance measurements L* value was the only one that clearly distinguished between quality groups with no overlap in values between groups. This is not surprising since muscles were chosen to be typical of the quality groups. Protein solubility, measured by the Barton-Gade (1984) approach differed between quality groups, although the difference between PSE and normal muscle was much greater than the difference between DFD and normal muscle. Ultimate pH was able to distinguish well between normal and DFD pork but was not able to distinguish between normal and PSE muscles.

The effect of extraction time, extraction temperature and NaCl concentration on the solubility of longissimus muscle protein is presented in Figure 1 for each of the three quality groups. Maximum solubility was reached at approximately 0.5 M NaCl for DFD muscles under all extraction conditions, and solubility decreased only slightly at higher salt concentrations. For normal muscles extracted for 24 h, maximum solubility occurred at 0.5 M NaCl and decreased slightly at higher concentrations. An extraction time of 1 h caused solubility to be at a maximum at 0.75 M NaCl. Maximum protein solubility was attained at approximately 1.0 M NaCl for PSE muscles. Protein solubilities were lower with a 1 h extraction than a 24 h extraction. They tended also to be slightly lower at 4 C than at 21 C. Quality groups were distinguishable at all salt concentrations.

KI solubilized protein in normal and PSE pork more completely than any of the NaCl solutions (Figure 1). There was a great difference between protein solubilities of PSE and normal muscles. In agreement with (Lopez-Bote et al., 1989), protein solubility was found to be an excellent method for distinguishing PSE from normal pork. Yet the solubilities of normal and DFD muscles were more similar for KI than for certain concentrations of NaCl. Normal muscle has much poorer ability to hold water that does DFD muscle (Kauffman et al., 1986; Lopez-Bote et al., 1989). Therefore it is important that an appropriate method can distinguish both PSE and DFD from normal pork. KI extracts both myofibrillar and sarcoplasmic proteins (Sayre and Briskey, 1963). Lopez-Bote et al. (1989) suggest that the use of a low ionic strength solution, which extracts only sarcoplasmic proteins, provides a much more suitable method for distinguishing between normal and DFD pork.

In order to determine which extraction conditions were most effective for distinguishing both PSE and DFD from normal muscles, one way analyses of variance were conducted for each set of extraction conditions,

with just PSE and normal muscles included and with just DFD and normal muscles included. The F-value from the analysis is indicative of the ability to distinguish between quality groups. The data are summarized for NaCl in Figure 2. For the comparison of PSE and normal muscles, the highest F-values (77-79) were obtained at 1.25 M NaCl with a 1 h extraction at 4 C, at 0.75 M NaCl with a 24 h extraction at 21 C, and at 0.75-1.25 M NaCl with a 24 h extraction at 4 C. For the comparison of DFD and normal muscles, the highest F-values (26-31) were obtained at 1.25 M NaCl with a 24 h extraction at 21 C, at 1 M NaCl with a 24 h extraction at 4 C and at 1.25 M with a 1 h extraction at 21 C. The low salt extraction suggested by Lopez-Bote et al. (1989) was not effective in distinguishing between these quality groups. Based on F-values the difference of both PSE and DFD muscles from normal muscles was optimized by extraction in 1 M NaCl for 24 h at 4 C.

The highest F-values for KI were 201 and 10 for the comparison of PSE and DFD, respectively, to normal muscles. Both occurred with a 24 h extraction at 21 C and were superior to F-values of 172 and 8, respectively, obtained with the commonly-used 24 h extraction at 4 C.

The F-values comparing the protein solubilities of PSE and normal muscle were more than twice as great for KI compared to NaCl extraction. On the other hand, the F-values comparing the protein solubilities of DFD and normal muscle were more than twice as great for NaCl compared to KI extraction. KI was superior to NaCl for distinguishing between PSE and normal pork, but was inferior to NaCl for distinguishing between DFD and normal pork. Comparison of plots of L* value and protein solubility for the extraction of muscle for 24 h at 4 C in 1.0 M NaCl and in 0.55 M KI (Figure 3) further clarify this point. Under these conditions the KI was a much more powerful extractant than was NaCl and thus it shifted the PSE and normal quality groups were more toward the DFD group than was the case for NaCl. L* was more linearly related to protein solubility for NaCl than for KI extraction.

The relationships of reflectance and especially pH to protein solubility were not linear for all extraction conditions. Therefore quadratic regression analyses were conducted to investigate the degree of association between quality and solubility measurements. R^2 values presented in Figure 4 indicate the degree of correlation between L* value and protein solubility for various salt solutions and extraction conditions. At 1 - 1.25 M NaCl R^2 values were 0.87 - 0.89 with the exception of the 1 h extraction at 4 C. This was higher than the 0.82 - 0.84 obtained for KI.

R^2 values presented in Figure 5 indicate the degree of correlation between ultimate pH and protein solubility for various salt solutions and extraction conditions. At 0 - 0.25 M NaCl, R^2 values were 0.84 - 0.86. At 1 - 1.25 M NaCl R^2 values were between 0.81 and 0.87 with the exception of the 1 h extraction at 4 C. This was considerably higher than the 0.62 - 0.66 obtained for KI.

Based on R^2 values the correlation of both L* and pH to protein solubility is optimized by extraction in 1-1.25 M NaCl for 24 h at 21 C.

Conclusions

A preliminary study of the effect of extraction time, extraction temperature and salt concentration on the utility of the solubility of pig longissimus muscle protein as an indicator of muscle quality indicate that: 1) KI is superior to NaCl as an extractant for distinguishing between PSE and normal muscles, 2) a 1 M NaCl extraction distinguishes well between PSE and normal muscles and is superior to a KI extraction for distinguishing between DFD and normal muscles, 3) protein solubilities using a 1 M NaCl extraction correlate better with reflectance and pH than those using a KI extraction, 4) protein solubilities using a 1 M NaCl extraction are superior to those using a very low salt extraction for distinguishing between muscle quality traits.

This indicates that 1 M NaCl should be considered as an extractant in the protein solubility method for the evaluation of pork muscle quality.

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TABLE LEGENDS

Table 1. The relationship of subjective quality group to objective measures of longissimus muscle quality.

FIGURE LEGENDS

Figure 1. Effect of NaCl and KI on protein solubility of longissimus muscles of three different quality types, extracted for 1 (solid symbols) and 24 h (open symbols) at 4 (squares) and 21C (circles).

Figure 2. F-Values resulting from analysis of variance comparing protein solubility of PSE to normal muscles (solid symbols) and DFD to normal muscles (open symbols) for several NaCl concentrations and for KI. Extraction times/temperatures were: 1h/4C (squares); 1h/21C (circles); 24h/4C (triangles); 24h/21C (diamonds).

Figure 3. Relationship of L* value and protein solubility in 1.0 M NaCl (Figure 3a) and in 0.55 M KI (Figure 3b) after an extraction for 24 h at 4 C. PSE (squares), normal (circles), and DFD (triangles) quality types are indicated.

Figure 4. R^2 Values resulting from quadratic regression of L* value on protein solubility of pig muscle extracted for 1 (solid symbols) and 24 h (open symbols) at 4 (squares) and 21 C (circles).

Figure 5. R^2 Values resulting from quadratic regression of pH value on protein solubility of pig muscle extracted for 1 (solid symbols) and 24 h (open symbols) at 4 (squares) and 21 C (circles).