Effects of Rigor, Salt, Freezing, Lyophilization and Storage Time on pH, Water-Holding Capacity and Soluble Protein Nitrogen in Beef Muscle

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ABSTRACT

The water-holding capacity (WHC) of frozen and reconstituted lyophilized (freeze-dried) beef (both pre- and post-rigor) increased (P<0.05) with the increase in salt levels (0, 2 and 4%). Freeze-dried and reconstituted beef had lower (P<0.05) WHC than the frozen control at all salt levels tested. The freeze-drying process may damage some of the beef muscle proteins. The WHC of the freeze-dried beef (both pre- and post-rigor) decreased (P<0.05) with the increase of storage time (10 weeks). Salt (2 and 4%) retarded the glycolysis process in the pre-rigor frozen and freeze-dried beef as indicated by higher (P<0.05) pH values than the post-rigor frozen and freeze-dried beef. The addition of salt (0, 2 and 4%) increased (P<0.05) the extractable soluble protein nitrogen content in the pre-rigor frozen beef and decreased (P<0.05) the soluble protein nitrogen content in the post-rigor frozen beef. The pre-rigor freeze-dried beef with 2% salt contained (P<0.05) more extractable soluble protein nitrogen than the other two pre-rigor freeze-dried groups (0 and 4% salt). The pre-rigor beef contained more (P<0.05) extractable soluble protein nitrogen than the post-rigor beef at the three different salt levels (0, 2 and 4%) during the 15 weeks of storage.

Ockerman (6) reported that addition of salt (NaCl) to meat lowers the isoelectric point toward the acid end of the pH scale. This is accomplished by the chloride ion binding with the positive charges of the muscle to a stronger degree than the sodium ion combines with the negative charges. Salt when added before rigor reduces the effectiveness of the glycolysis system, and a higher pH is observed at the completion of rigor thus increasing the water holding capacity of the tissue. Wierbicki et al. (11) reported that the pH shift toward alkalinity produced by addition of sodium chloride, decreased after freezing and thawing. However, Deatherage and Hamm (1) reported that pH values of meat were not signific-

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bleeding. Samples were quickly ground twice through a 3/8 in. (9.5 mm) plate (Stimpson Grinder Model 5412) into a cooler (3 ± 1°C) and the ground mixed samples were evenly divided into three groups. Each subgroup received one of three different levels of salt (0, 2 and 4%). Following addition of salt (approximately 55 min post bleeding), the ground beef was reground two additional times through the 3/8 in. plate to obtain a more uniform sample. To control the weight, shape and thickness of beef samples during freeze-drying, the salted and unsalted pre-rigor ground beef was made into uniform shaped 100-g patties using a Tastee Ring Burger Press plastic mold (Robinson Co., Inc.). The whole process for making the pre-rigor beef patties was completed within 1-1/2 h after obtaining the pre-rigor muscle. Each subgroup was further divided into 2 samples for freezing or freeze-drying.

The beef samples of the frozen group were vacuum packaged (0.6 kg/cm²) with LC Flex 90366 film (moisture and oxygen impermeable, Smith Co.) by Super Vac (Model GK/165, Smith Co.) and immediately frozen and stored at -29°C. The beef samples of the freeze-dried group were also quick-frozen in a freezer bag (LC Flex 90366 Film by Smith Co.) at -29°C for approximately 24 h and then freeze-dried (Model No. 10-145MR-BA, VirTis, Gardiner, NY) for 48 h (10 µ of vacuum, shelf temperature 22°C). After freeze-drying, the beef samples were quickly vacuum packaged (0.6 kg/cm² in Super Vac with LC Flex 90366 film by Smith Co.) and stored at 25°C.

The post-rigor beef muscle was obtained (cold-boned) from the beef sides stored at 3 ± 1°C for at least 48 h. The post-rigor groups (0, 2 and 4% salt) were also subdivided into two groups (frozen and freeze-dried). The procedures for preparing post-rigor frozen and freeze-dried beef samples and the storage conditions were the same as the pre-rigor group. Packages of freeze-dried and frozen beef samples were randomly assigned to be held for 0, 5, 10 and 15 weeks.

The frozen beef samples (pre- and post-rigor) were thawed at room temperature (approximately 4 h) and the freeze-dried samples were removed from storage before completing chemical analysis.

Water-holding capacity (WHC)

Approximately 0.5 g of meat was used to determine the WHC by the press method according to Ockerman (6).

pH

The pH of the samples was determined with a Fisher Accumet pH meter (Model 610A) according to Ockerman (6). Ten grams of meat sample and 100 ml of distilled water were blended for 1 min at low speed before determining the pH with the pH meter. The freeze-dried beef samples were rehydrated by adding excessive distilled water (4:1). Ten grams of the rehydrated meat was used to determine the pH values of freeze-dried beef.

Nitrogen compounds

Approximately 2 g of freeze-dried sample was used to determine the total soluble nitrogen, soluble non-protein nitrogen and soluble protein nitrogen according to a modification of the procedure of Regier and Tappel (7).

Soluble nitrogen. Two grams of freeze-dried beef and 2 g of powdered glass were placed in a mortar. Enough 0.5 N KCl was added to thoroughly wet the material. The mixture was ground, transferred to a 500-ml volumetric flask with 0.5 N KCl and brought to volume with this solution. The extraction was allowed to proceed with occasional shaking. At the end of 2 h, the solution was centrifuged at 2000 × g for 15 min. The total soluble nitrogen in 50 ml of the supernatant fluid was determined by the Kjeldahl method.

Non-protein nitrogen. The non-protein nitrogen was also determined using the KCl extract supernatant fluid. Ten ml of trichloroacetic acid (85 g/100 ml) was added to 50 ml of the supernatant fluid from the soluble nitrogen preparation. After mixing and letting stand for 15 min, the solution was centrifuged at 2000 × g for 15 min. The soluble non-protein nitrogen in 50 ml of this supernatant fluid was determined by the Kjeldahl method. This value was multiplied by 60/50 (necessary because of the TCA dilution). The soluble protein nitrogen was equal to the total soluble nitrogen minus the non-protein nitrogen.

The sample of frozen tissue was blended with 100 ml of 0.5 N KCl for 1 min at low speed, and the solution was diluted to a total volume of 200 ml with 0.5 N KCl and allowed to stand for 2 h. The procedures to determine the total soluble nitrogen, soluble non-protein nitrogen and soluble protein nitrogen for frozen meat were the same as for the freeze-dried meat except approximately 7-g (same weight equivalent) sample was used.

FIGURE 1 The water holding capacity (WHC) of beef (S.E. = 0.74) as affected by rigor state, salt level and storage method (effect of storage time absorbed).
and 5 weeks of storage, but at 10 and 15 weeks of storage, the differences between them were not significant. The higher WHC in pre-rigor samples is probably due to the higher pH values. Ockerman (6) reported the WHC of muscle tissues increases with the increase of pH values. The WHC in freeze-dried beef (both pre- and post-rigor) decreased (P<0.05) during the 10 weeks of storage. The WHC in frozen beef (both pre- and post-rigor) decreased during the 0-10 week storage period, and increased slightly at the 15 week storage period.

The pH values of beef were affected (P<0.01) by rigor state, salt level, storage method, and storage time. In addition, the interactions of rigor state × salt level × storage method and rigor state × storage period × storage time were also significant (P<0.01).

In Fig. 3, the pH values for both frozen and freeze-dried beef (pre-rigor) increased (P<0.05) with an increase in salt levels (0, 2 and 4%). This agrees with the work by Ockerman (6), who reported that the average pH value of pre-rigor muscle tissue normally increases with the increase in salt level. The pH values for both frozen and freeze-dried beef (post-rigor) were not affected by the addition of three different levels of salt (0, 2 and 4%).

With the addition of 2 and 4% salt (Fig. 3), the pre-rigor meat (frozen and freeze-dried) had higher (P<0.05) pH values than the post-rigor meat (frozen and freeze-dried). However, with no added salt, the pre-rigor meat (both frozen and freeze-dried) and the post-rigor meat (both frozen and freeze-dried) were not significantly different. This indicates that in pre-rigor meat without salt, to retard the glycolysis process, the pH values of this tissue dropped to approximately the same pH values as the post-rigor meat.

Figure 4 shows the pH values of beef as affected by rigor state, storage method and storage time. The pH values of the pre-rigor frozen beef (5.77-5.86) were higher (P<0.05) than those of the post-rigor frozen beef (5.52-5.56) at the various intervals of storage (0, 5, 10 and 15 weeks) at -29°C. The same trend was followed (P<0.05) by the pre-rigor freeze-dried beef (5.63-5.74) and the post-rigor freeze-dried beef (5.46-5.51) stored at 25°C. This suggests that glycolysis was retarded in frozen and freeze-dried beef during storage (15 weeks) at -29°C and 25°C, respectively, when the effect of salt levels is not considered.

The soluble protein nitrogen (soluble total nitrogen minus soluble non-protein nitrogen) of beef was affected (P<0.01) by rigor state, salt level and storage time, but not by storage method. However, the interactions of rigor state × salt level × storage methods, and rigor state × salt level × storage time, and rigor state × storage method × storage time and salt level × storage method × storage time were also significant.
Figure 5 shows the soluble protein nitrogen of beef as affected by rigor state, storage method and salt level. Ockerman (6) reported that the myofibrillar proteins were most soluble in concentrated salt solution (approximately 0.6 N KCl) and the sarcoplasmic proteins were soluble in a dilute salt (approximately 0.1 N KCl) solution. In this research, the salt solution (0.5 N KCl) was used to extract the salt-soluble protein, which comprised most of the beef myofibrillar proteins. The extractable soluble protein nitrogen in the pre-rigor frozen beef (Fig. 5) increased with the increase in salt levels (0, 2 and 4%). This is probably due to the increase of pH values by adding salt to the pre-rigor frozen beef (Fig. 3). Johnson and Henrickson (5) used different materials and found that addition of 1, 2 and 3% salt to pre-rigor pork muscle also increased the amount of extractable soluble protein. Saffle and Galbreath (8) reported that the soluble protein content (extracted with 3% saline solution) could be used as a measure of emulsifying capacity (the higher, the better). This suggests that the pre-rigor frozen beef with addition of 4% salt may be used as a pre-blend to manufacture sausages or other emulsified products. However, further research is probably needed to study the functional and organoleptic properties of sausages made by such a pre-blend.

In the pre-rigor freeze-dried beef (Fig. 5), the treatment with 2% salt contained a greater amount of extractable soluble protein nitrogen than the other treatments (0 and 4% salt). Addition of 4% salt to the pre-rigor freeze-dried beef decreased the amount of extractable soluble protein when compared to that of the 2% salt level. This phenomenon is different from that of pre-rigor frozen beef.

The increase in salt levels caused a decrease (<0.05) in solubility of the post-rigor frozen beef (Fig. 5). This indicates that to obtain the greatest amount of extractable soluble protein for sausage preparation, no salt should be added to post-rigor frozen beef. One explanation for these results is that the pH of the post-rigor frozen beef with three different salt levels (5.52-5.56) was very close to the isoelectric point of the beef muscle protein (5.0-5.4); thus the meat protein was in a less soluble state. Johnson and Henrickson (5) also found similar results by adding 1, 2 and 3% salt to post-rigor pork muscle. They explained this by stating that the post-rigor pork muscle protein was tied up in the actomyosin complex and that the pH of the post-rigor muscle was at the isoelectric point of pork muscle protein.

In the post-rigor freeze-dried beef (Fig. 5), the group with 4% salt contained less (P<0.05) extractable soluble protein nitrogen than the other two groups (0 and 2%). The amount of extractable soluble protein nitrogen was not significantly different between the group with no salt addition and the group with 2% salt.

More soluble protein nitrogen was extracted from the pre-rigor samples (both frozen and freeze-dried) at the three different levels of salt (Fig. 5). This probably was due to the higher pH of the pre-rigor frozen and freeze-dried samples. Saffle and Galbreath (8) reported that the amount of salt-soluble protein extracted is greater for pre-rigor frozen meat than for post-rigor frozen meat.

Figure 6 shows the soluble protein nitrogen as affected by rigor state, salt level and storage time. The effects of rigor state and salt level were discussed previously. The soluble protein nitrogen in pre-rigor meat (with three different levels of salt) decreased between 0-10 weeks of storage. However, between 10 and 15 weeks of storage, the soluble protein nitrogen in these treatments (except for the pre-rigor meat with 4% salt) increased slightly. The soluble protein nitrogen in post-rigor meat (with three different levels of salt) remained
very stable during the 10 weeks of storage. However, between 10 and 15 weeks of storage, the soluble protein nitrogen of the post-rigor meat also slightly increased in the pre-rigor meat. Regier and Tappel (7) reported that the KCl-soluble protein nitrogen of freeze-dried beef decreased during 16 d of storage at 54.4°C. The reason why the soluble protein nitrogen increased between 10 and 15 weeks of storage is not known, but is probably due to autolysis of the less soluble proteins into the soluble protein fraction during storage.

REFERENCES

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