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Effects of a humidity-stabilizing sheet on the color and *K* value of beef stored at cold temperatures

K. Saito [a], A. Ahhmed [b], H. Takeda [b], S. Kawahara [b], M. Irie [b], M. Muguruma [b,*]

[a] *Kawasaki Research Laboratory, Showa Denko Plastic Products Co. Ltd. 5-1,Ookawa-Cho, Kawasaki-Ku, Kawasaki-Shi, 210-0858,Kanagawa, Japan*
[b] *Laboratory of Food Science and Nutrition, Department of Biochemistry and Applied Biosciences, Faculty of Agriculture, University of Miyazaki, 1-1, Gakuen Kibanadai Nishi, Miyazaki-Shi, 889-2192 Miyazaki, Japan*

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Abstract

The effects of a humidity-stabilizing sheet containing glycerol, on the color and *K* value of beef stored at cold temperatures were investigated in this study. A drip-absorbing sheet seems to be effective for the preservation of meat quality, while a humidity-stabilizing sheet containing glycerol prevents the increase of metmyoglobin in cold stored beef. Maintaining samples wrapped in humidity-stabilizing sheets containing glycerol at low temperature for 7 days was a functional method for conserving the concentration of inosine monophosphate. Beef samples wrapped in sheets containing glycerol had lower *K* values than samples wrapped in sheets not containing glycerol. When the surface of the meat starts to desiccate, the humidity-stabilizing sheet releases moisture that has been absorbed from the beef in the early stages of storage. Thus, glycerol could potentially play a role as a humidity-stabilizing controller and color preservative. This research suggests that a humidity-stabilizing sheet containing glycerol is a useful moisture controller and prevents deterioration of meat quality, discoloration, and desiccation.

Keywords: Beef; Metmyoglobin; Humidity; IMP; *K* value; Meat freshness

1. Introduction

Meat manufacturing has many crucial factors. Those most valued by consumers include the meat's color and its components (lightness, redness, and yellowness), in addition to packaging type. Ultimately, meat color is closely correlated with packaging type, and color conversion is an important element of meat quality. When the meat's color turns, under uncontrolled circumstances, from bright red to brown, this suggests some degradation in meat quality. The incidence of such meat quality depreciation depends on the change of oxymyoglobin into metmyoglobin. This phenomenon either desensitizes or intensifies consumers' appetite for meat consumption. In the case of beef, two important visual clues that determine perceived quality are color and packaging (Issanchou, 1996).

Although nutritional value, texture, taste, and smell are important properties of meat products, the most important property valued by consumers remains the bright red color. Carpenter, Cornforth, and Whittier (2001) reported that appearance determines how consumers primarily perceive quality, which significantly influences purchasing decisions. Meat color is an extremely important sensory characteristic by which consumers make judgments of meat quality (Kinsman, Kotula, & Breidenstein, 1994). The color of meat depends on pigmentation. Forrest, Aberle, Hedreck, Judge, and Merkel (1975) reported that the pigment in meat largely consists of two proteins: hemoglobin, the pigment of the blood, and myoglobin, the pigment of the muscles. Furthermore, Kinsman et al. (1994) mentioned that cuts of meat with too much metmyoglobin are viewed as old and undesirable for consumption. Metmyoglobin is

* Corresponding author. Tel./fax: +81 985 58 7203.
E-mail address: muguruma@cc.miyazaki-u.ac.jp (M. Muguruma).

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formed as an intermediate in the conversion of oxymyoglobin to myoglobin under anaerobic conditions (Echevarne, Renerre, & Labas, 1990). It has been reported and illustrated by Sammel, Hunt, Krope, Hachmeistres, and Johnson (2002) that aerobic reducing ability correlated best with color stability over display, and appeared to be the best current method for measuring reducing ability. In this study, the percentage of metmyoglobin present is used as an index for meat browning.

The coloration of beef is important in distinguishing the meat's quality, in addition to indicating its age. Meat ageing, especially concerning beef, is a meticulous process in which the preferred color for the meat is achieved over time. The ageing of any meat must be done very cautiously under sterile conditions, to avoid unfavorable reactions such as lipid auto-oxidation, rancidity, color deterioration, and desiccation of the meat surface. O'Keeffe and Hood (1981) reported that meat aged for less than 1 week demonstrated greater color stability than meat aged for its normal ageing period.

Reducing the temperature of post-mortem beef to near freezing can inhibit negative effects such as reactions catalyzed by the enzymes in the tissues. Consequently, it is considered desirable to reduce muscle tissue temperature after death as quickly as possible, to minimize the protein denaturation that occurs during this period, and to inhibit the proliferation of microorganisms (Forrest et al., 1975). Strict temperature control is required to maintain high humidity without condensation.

When beef is not wrapped in waterproof packaging during chilling or while being processed in an air-conditioned area, the meat loses some of its moisture. Moisture on the surface and within the surface layer of the food can evaporate into the air (Lovatt & Merts, 1999). In addition, Lanier, Carpenter, and Toledo (1977) found that a relative humidity of nearly 90%, air velocity nearly 0.5 m per s, and near freezing temperatures appeared to be the best environment for beef color maintenance. To maintain the color and freshness of beef stored at near-freezing temperatures, the atmosphere around the meat must be kept stable throughout the storage period. Perhaps one of the most important factors in stabilizing the atmosphere around the beef is maintaining the humidity. This is intrinsically linked to maintaining the redness of the beef. Under low temperatures, maintaining high humidity without condensation in the absence of air circulation is difficult, and requires expensive technology such as complex instrumentation to regulate the air circulation system.

This research focuses on how humidity-stabilizing sheets containing glycerol affect the percentage of metmyoglobin, and *K* values. Lawrie (1991) mentioned that, when meat is removed from chilled storage, moisture tends to condense on the cool surface, especially when the relative humidity of the atmosphere is high. This phenomenon is known as 'sweating'.

K values were determined as a freshness index, while inosine monophosphate (IMP) was measured as a tastiness indicator of beef. In the 1950s, Saito, Arai, and Matsuyoshi (1959) proposed a new concept called the '*K* value', which is still used as the most effective indicator of the freshness of fish. Some researchers have stated that *K* values could also be used to indicate the freshness of chicken and pork. In this research, we measured the *K* values of beef samples wrapped in humidity-stabilizing sheets containing glycerol in an attempt to determine the freshness of cold stored beef, and how this type of sheet could potentially improve the freshness, color, and moisture content of beef. IMP is an ATP-related compound that is related to the *K* value. In this study, IMP was measured to quantify the effect of the humidity-stabilizing sheets on the taste of cold stored beef. Analogous to the theoretical work of Saito and others who used the *K* value for fish, we used it for beef. One of the substances affecting taste is considered to be IMP. ATP is decomposed to adenosine diphosphate (ADP), and ADP is further degraded to adenosine monophosphate (AMP), and from there to IMP. Honikel (2004) summarized that the IMP, again, is associated with favorable flavor.

Recently, humidity-stabilizing sheets containing glycerol have been developed to assist in maintaining high-quality beef products. Commercially, these sheets are used in restaurant kitchens and meat processing areas in supermarkets in Japan. The purpose of this study was to illustrate the commercial applicability and uses of humidity-stabilizing sheets containing glycerol pertaining to the maintenance of color stability, moisture content, and freshness of beef stored at low temperatures.

2. Materials and methods

2.1. Materials

The cut of meat used in this study was the biceps femoris muscle from Japanese Black Cattle; the meat was obtained from Minami Kyushu Chikusan Kogyo Ltd., Kagoshima, Japan. It was vacuum-packaged and stored for 4 days in a chilled refrigerator after slaughter before being used in this research. Humidity-stabilizing sheets containing a stabilizing agent (glycerol) at different levels (0 g/cm^2, 10 g/m^2, and 40 g/cm^2) were applied to the muscle tissue. In addition, sheets without glycerol were used simply as drip-absorbing sheets. The humidity-stabilizing sheets containing glycerol were obtained from Showa Denko Plastic Products Co. Ltd., Tokyo, Japan. The biceps femoris muscles were wrapped in these sheets such that the meat was in contact with the non-woven side of the wrapping material. Fig. 1 shows a cross-section of the humidity-stabilizing sheet. The humidity-stabilizing agent is sprayed onto pulp containing an absorbing polymer; this pulp is located between a non-woven fabric sheet and polyethylene film. The glycerol present in the sheets has been used as intermediate moisture in meats. Muguruma, Nishimura, Umetsu, Goto, and Yamaguchi (1987) reported that intermediate-moisture meats have relatively

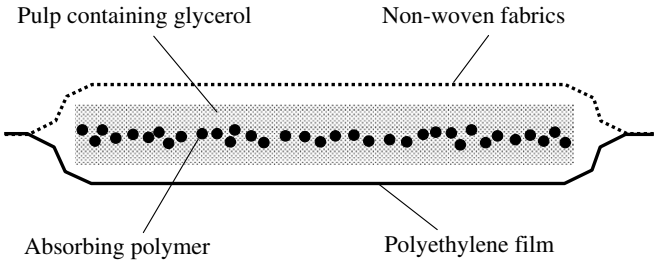

Fig. 1. Cross-sectional structure of humidity-stabilizing sheet.

long shelf-lives at normal room temperature due to the reduction in rawness brought about by the addition of various humectants. In another study, Muguruma, Katayama, Nakamura, and Yamaguchi (1987) reported that dehydration at low temperature (4 °C) with the use of a dehydrating sheet enabled the manufacture of intermediate-moisture meat of good quality. In terms of food safety, glycerol has been used in many applications and is reported to be safe; we therefore used it in beef preservation. However, Stecher, Windholz, and Leahy (1968) reported a lethal dosage of glycerol administered orally (31.5 g/kg) and intravenously (7.56 g/kg) in mice. The FAO/WHO Joint Expert Committee on Food Additives mentioned that the acceptable daily intake of glycerol is not specified. More information on this subject can be viewed at http://jecfa.ilsi.org/evaluation.cfm? chemical=GLYCEROL&keyword=GLYCEROL.

2.2. Sample preparation

The biceps femoris muscle used in this study was divided into five pieces of 200 g each. Each portion was wrapped in a humidity-stabilizing sheet containing glycerol, and placed in a clear plastic box (Tupperware) covered tightly with a lid. Samples wrapped in sheets without glycerol were also tested as a reference group. Each container was kept refrigerated for 7 days at 4 °C. After 7 days of refrigeration, a 2-cm^2 piece was cut from each sample of meat. The cuts were as deep as 2 mm into the surface of the meat. The weight of the extracted area was approximately 1 g. These samples were subjected to further analysis.

2.3. Relative humidity and temperature

Relative humidity and temperature are very closely associated; thus, the relative humidity must be measured to understand the functionality of the humidity-stabilizing sheets in mediating and adapting to the atmosphere around the meat at low temperatures. The relative humidity in this study was measured in relation to two different levels of glycerol (0 and 10 mg); also, measurements were made whenever the temperature dropped while the meat was in storage. The duration of the measurements was about 4.5 h. Measurements of humidity and from temperature pyrometers around the meat during storage were measured using a data logger, (Ondotori; T&D Co.). The measurements were repeated 275 times.

2.4. Hunter colorimeter measurements

Hunter color measurements of the samples reflect any changes that may have occurred as a result of using glycerol as a humidity-stabilizing agent. Colorimeter values were taken on the second day of storage from three different samples wrapped separately in sheets containing three different levels of glycerol. Additionally, data from the first day were recorded and used as a reference. The three main components of meat color (lightness, redness, and yellowness) were quantified to ensure that the humidity-stabilizing sheets containing glycerol had beneficial properties regarding the storage of meat, and also to establish a new packaging technique for meat manufacturing using glycerol. The measurements were repeated five times at different areas on each piece of meat. The measurements were conducted using a colorimeter (Minolta CM-1000; Tokyo, Japan).

2.5. Percentage of metmyoglobin

Myoglobin was extracted from the meat portions with cold (0 °C) water. One-gram samples with 15 ml of water were homogenized with a polytron homogenizer (Kinematica Co., Littau, Switzerland) twice for 1 min each with a 10-s interval at speed setting 5. The homogenates were centrifuged at 16,000 rpm for 20 min at 2 °C; finally, the supernatants were filtered through filter paper No. 5A (Advantec Toyo K. Ltd., Tokyo, Japan). Absorbance of the filtrates was measured at 525 nm, 572 nm, and 730 nm using a double-beam spectrophotometer (Model Bio Spec 1600; Shimadzu Co., Kyoto, Japan). The percentage of metmyoglobin was calculated using the following formula described by Krzywicki (1979).

$$\text{Metmyoglobin}(\%) = \left[1.395 - \left\{\frac{[\text{A572} - (\text{A730} \times 1.45)]}{[\text{A525} - (\text{A730} \times 1.73)]}\right\}\right] \times 100$$

2.6. ATP and ATP-related compounds

To test the freshness of the meat, the *K* value and its percentage were measured. The *K* value is considered to be a helpful index, emphasizing beef quality. To calculate the *K* value, ATP and ATP-related compounds (ARCs) must first be determined. In this study, ATP, ADP, AMP, IMP, inosine (RHx), and hypoxanthine (Hx) were determined by high-performance liquid chromatography (HPLC). ARCs were extracted from the meat protein with cold (0 °C) trichloroacetic acid (TCA). One gram of minced meat in 20 ml of 5% TCA was homogenized twice for 1 min with a 10-s interval using a polytron homogenizer at speed setting 5, and the homogenate was stored for 1 h at 4 °C. The homogenate was centrifuged at 16,000 rpm for 20 min at

2 °C, and the supernatant was filtered through filter paper. The precipitate was extracted again in 20 ml of 5% TCA. Two supernatants were combined and 5% TCA was added until the volume reached 50 ml. A volume of 5 ml of supernatant was extracted three times in 3 ml of diethylether. The diethylether was removed from aqueous layer with a rotary evaporator and then water was added until the volume reached 5 ml. A volume of 0.2 ml of the evaporated solution was diluted with eluent and was used for HPLC analysis. ARCs were determined by HPLC on a HAISIL ODS 100-C18-4D column (Showa Denko K.K., Tokyo, Japan). The composition of the eluent was methanol:20 mM KH_2PO_4–5 mM tetra-*n*-butyl-ammonium hydroxide (3:7). Afterwards, the absorbance of the eluent was measured at 254 nm. The *K* value was calculated using the following formula, as described by Saito et al. (1959). As the formula shows, fresh meat gives a small *K* value:

$$K(\%) = \frac{\mathrm{RHx} + \mathrm{Hx}}{\mathrm{ATP} + \mathrm{ADP} + \mathrm{AMP} + \mathrm{IMP} + \mathrm{RHx} + \mathrm{Hx}} \times 100$$

2.7. Statistical analysis

All data represent at least five independent experiments and are expressed as the mean ± SD unless otherwise indicated. Each experiment was analyzed as a complete factorial using ANOVA by means of the Tukey method.

3. Results and discussion

3.1. Evaluation of relative humidity and temperature

There is an inverse relationship between temperature and relative humidity during storage. As the temperature decreases, the relative humidity increases. The humidity increased rapidly, and condensate appeared in samples wrapped in sheets without glycerol (Fig. 2). In samples wrapped in sheets containing glycerol, the increase in relative humidity was slow and condensate did not appear. This results from the fact that the initial concentration of glycerol was high, and so moisture in the atmosphere and in the meat drippings, which were held by the pulp and non-woven fabric, was absorbed by the glycerol according to the principle of equalization. Moreover, the glycerol was spread on the pulp and non-woven fabric, and thus had a very large surface area. Such concentrations of glycerol enable rapid equalization of humidity, even under conditions of low temperature. As a result, the humidity was stabilized, maintaining high levels at low temperature without causing condensation.

3.2. Moisture movement and the glycerol mechanism inside the humidity-stabilizing sheet

Moisture behavior depends on the relationship between three components of the model: water constantly moves between meat, atmosphere (air) and glycerol (cover sheet). Moisture behavior in relation to air and glycerol is in accordance with the moisture absorption equilibrium. Meat desiccation occurs when the air humidity is low, and is prevented when the humidity remains high. Glycerol absorbs moisture from the air when the humidity is high, but when the atmospheric humidity is low, water vapor is exuded from the glycerol. Therefore, if a large amount of moisture is present in an enclosed area (inside a container), glycerol acts to maintain high humidity, while preventing the humidity from reaching 100% due to its absorptive characteristics. This mechanism enables glycerol to prevent condensation. During the early stage of storage, glycerol does not contain enough moisture, and thus meat desiccation may occur until the glycerol has absorbed sufficient moisture. This is somewhat mitigated through moisture exuded from the meat, whereby the glycerol can supply the meat with moisture, leading to only slight desiccation. Conversely, if there is no humidity-stabilizing sheet or a normal cover sheet is used under normal storage conditions, presuming the humidity is very high, desiccation might hardly occur but then condensation suddenly increases.

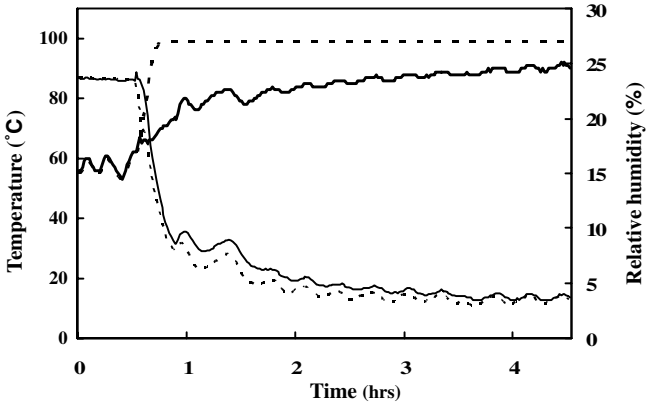

Fig. 2. Temperature and relative humidity during beef storage at 4 °C. Humidity of sample wrapped with sheet containing 0 g/m^2 (▪▪▪▪▪▪▪▪), 10 g/m^2 (▬▬▬). Temperature of sample wrapped with sheet containing 0 g/m^2 (· · · · · ·), 10 g/m^2(——).

3.3. Hunter colorimeter evaluations

Treatment did not have an effect on Hunter L^*, a^*, or b^* values. The three color components differed slightly related to the treatment (Fig. 3). The measurements were taken on the second day of storage, and the samples used in this experiment were: initial samples (day 0); samples having no sheets of any kind, considered as reference samples; and samples wrapped in humidity-stabilizing sheets containing glycerol at three different concentrations. The beef portions used in this study sustained stable values of L^*, a^*, and b^*. L^* increased in samples wrapped in sheets without glycerol, as well as in samples wrapped in sheets containing 10 g/m^2. The L^* values of samples wrapped in

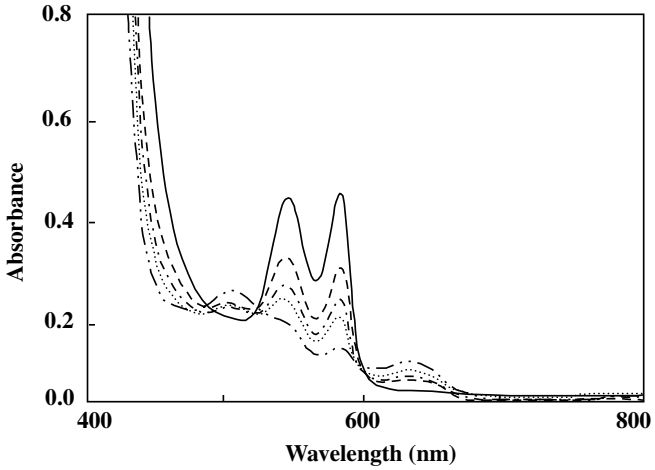

Fig. 3. Effect of glycerol on photo spectroscopy of beef. Meat samples were wrapped with sheets, placed in sealed plastic containers, and stored for 7 days in refrigerated storage at 4 °C. Sheet containing 0 g/m^2 (·····), 10 g/m^2 (–·–), 40 g/m^2 (----) of glycerol. Stored without sheet (–··–) and initial sample (——) also examined as reference.

sheets containing 40 g/m^2 were relatively good, and 40 g/m^2 of glycerol maintained L^* values similar to those of the initial samples. The humidity-stabilizing sheet containing glycerol maintained the three major color components at stable levels. This leads to the conclusion that the glycerol sheets can protect and preserve beef color, due to the function of glycerol as a non-conductor of oxygen.

3.4. Dripping and weight change evaluation

To test the effect of humidity-stabilizing sheets containing glycerol on the weight of beef samples, 15 single portions of beef were divided into three groups according to the glycerol level in the humidity-stabilizing sheet. The weight of samples was measured on day 0 and day 7 (data not shown). The weight of samples wrapped in sheets that did not contain glycerol changed, losing 1% of their total weight after 1 week. For sheets containing 10 g/m^2 glycerol, the weight of the samples decreased by 1.3% of total weight. Dripping increased by 1.8% of the total weight of samples wrapped in sheets containing 40 g/m^2 of glycerol. We observed that the weight of the samples changed according to the glycerol level, but that the dripping and weight loss that occurred due to temperature and duration of storage did not change drastically. Fresh meat that retains its moisture and tenderness is arguably one of the most important quality characteristics of raw products (Huff-Lonergan & Lonergan, 2005). Moreover, Offer and Knight (1988) mentioned that product weight losses due to purge can average as much as 1–3% in fresh retail cuts; similarly, Melody et al. (2004) reported weight loss of up to 10% in pale, soft, exudative products. However, 1.8% was the maximal ratio of weight decrease; this minimal change in weight is the result of using wrappings with glycerol to maintain beef weight.

3.5. Color stability

Myoglobin undergoes oxidization, causing beef to change color from bright red (oxymyoglobin) to brown (metmyoglobin). This surface discoloration is considered an undesirable reaction. Metmyoglobin is a byproduct of the oxidation of oxymyoglobin after beef is exposed directly to air. In the absence of oxygen, myoglobin has a dark red color that is almost purple. In the presence of oxygen, oxymyoglobin is bright red. The transformation to oxymyoglobin is seen a few minutes after cutting into the anaerobic center of a piece of meat. After prolonged exposure to the atmosphere, however, the iron atom of myoglobin may be converted to the ferric form, and brown metmyoglobin is formed (Swatland, 1994).

Whenever this phenomenon occurs, auto-oxidation of lipids begins, and 2-thiobarbituric acid (TBA) is formed. The relationship between TBA and color is based on the reaction of the ferric ion of oxymyoglobin in its conversion to metmyoglobin. The effect of oxygen exposure on beef is a shift of myoglobin to metmyoglobin in which the major event is the reaction involving the ferric ion, and whenever metmyoglobin increases lipid auto-oxidation also increases. The most critical time for meat product processing is when lipid auto-oxidation and color change start to occur, and these reactions can potentially appear whenever beef is being processed. Therefore, beef wrapping materials and refrigeration storage methods are of particular interest, as the handling and marketing of beef products is a fragile endeavor.

Fig. 4 shows the absorption spectra of water-soluble solutions extracted from meat portions stored for 7 days at 4 °C. The spectra of control samples showed two peaks of oxymyoglobin, at 544 nm and 581 nm (absorbance 0.423 and 0.435, respectively); we could not detect any peak of metmyoglobin. The spectra of samples stored without wrapping sheets showed two peaks of metmyoglobin, at 504 nm and 632 nm (absorbance 0.232 and 0.084, respectively); thus the peaks of oxymyoglobin were significantly reduced. The spectra of samples wrapped with drip-absorbing sheets (sheets containing 0 g/m^2 glycerol) showed two peaks of oxymyoglobin, at 544 nm and 581 nm (absorbance 0.217 and 0.179, respectively). However, the spectra for the same samples showed two peaks of metmyoglobin, at 504 nm and 632 nm (absorbance 0.203 and 0.065, respectively). The absorbance of oxymyoglobin peaks for samples that had been wrapped in sheets containing glycerol (10 g/m^2) at 544 nm and 581 nm were 0.243 and 0.214, respectively. Metmyoglobin peaks at 504 nm and 632 nm were reported from samples with sheets containing glycerol (10 g/m^2) (absorbance 0.196 and 0.056, respectively). The absorbance of oxymyoglobin peaks for samples wrapped in sheets containing glycerol (40 g/m^2) at 544 nm and 581 nm were 0.309 and 0.288, respectively. The absorbance of metmyoglobin peaks for the same samples at 504 nm and 632 nm were 0.212 and 0.056, respectively. Although the metmyoglobin content was reduced by wrapping sam-

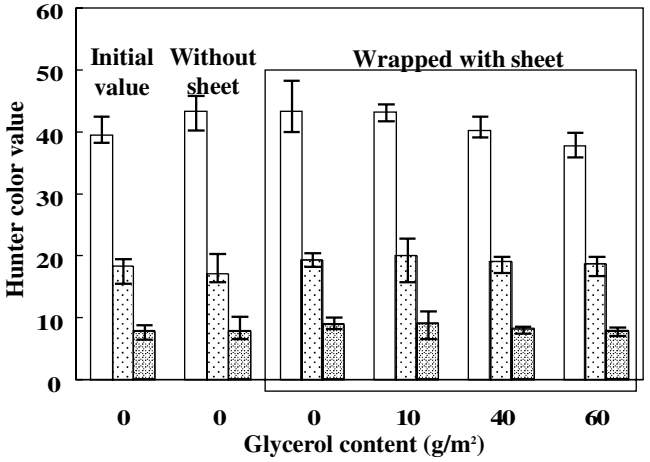

Fig. 4. Effect of glycerol on Hunter color value of cold stored beef. L^* value (□), a^* value (▨), b^* value (▨). All values of L^*, a^* and b^* were slightly affected, but there was no significant difference.

ples in sheets without glycerol, sheets with glycerol worked much better in keeping the metmyoglobin percentage low. Fig. 5 shows the percentage of metmyoglobin (met%) of sampled meat portions calculated from the absorption spectra. Samples stored without sheets gave a met% of 90%; samples stored in drip-absorbing sheets gave 60%. However, samples stored in sheets containing glycerol 10 g/m^2 and 40 g/m^2 gave 50% and 35%, respectively. The met% of samples wrapped in sheets containing 40 g/m^2 glycerol was reduced significantly compared with the met% of samples wrapped in sheets containing 0 g/m^2 glycerol. Moreover, a significant decrease of met% was detected in samples wrapped in sheets containing 10 g/m^2 glycerol compared with the met% of samples wrapped in sheets containing 0 g/m^2 glycerol. The data in Fig. 5 suggest that drip-absorbing sheets are effective and humidity-stabilizing sheets containing glycerol are even more effective in preventing the increase of metmyoglobin in cold stored beef. Beef products that appear in markets with a normal, perceived as preferable color are more likely to be purchased by consumers, as this coloration conveys the concept of freshness. Carpenter et al. (2001) reported that there is a close link between color preference and the decision to purchase, and that consumers prefer to purchase bright red beef rather than purple or brown beef.

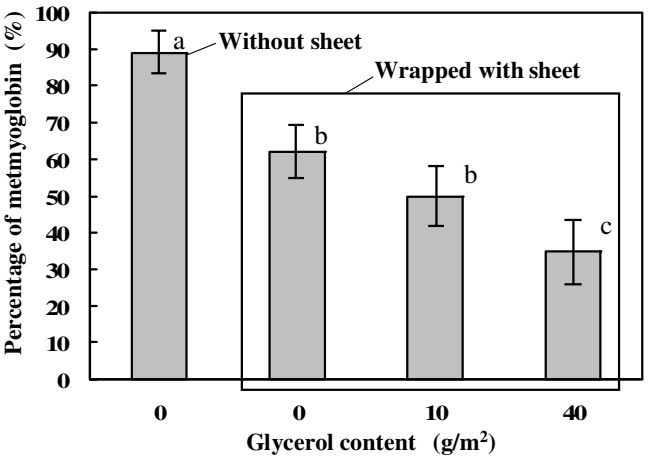

Fig. 5. Effect of glycerol on percentage of metmyoglobin in beef after 7 days in refrigerated storage at 4 °C. Values with different letter denote significant difference from each other at $p < 0.01$.

3.6. K value measurement

Fig. 6 shows ARC concentrations as μmol per gram of sampled meat. The ARC content of samples stored without sheets was 59 μmol/g. The ARC content of samples wrapped with drip-absorbing sheets was approximately 56 μmol/g, and the total quantity of ARCs in the initial samples was 75 μmol/g. Meanwhile, the ARC content of samples wrapped in humidity-stabilizing sheets containing 10 g/m^2 or 40 g/m^2 glycerol were 67 μmol/g and 70 μmol/g, respectively. Meat samples wrapped in drip-absorbing sheets and meat samples stored without sheets were additionally examined as reference samples. The ARC component ratio is considered as a freshness index for meats. ATP is metabolized into uric acid through ADP, AMP, IMP, RHx, and Hx. This metabolic process is considered as a factor that can improve meat freshness. However, the metabolism of ATP to IMP is a fast reaction. The ARCs found in this study were mostly IMP and Hx. Maintaining the samples wrapped in humidity-stabilizing sheets containing glycerol at low temperatures for 7 days seemed to be effective for maintaining the IMP concentration.

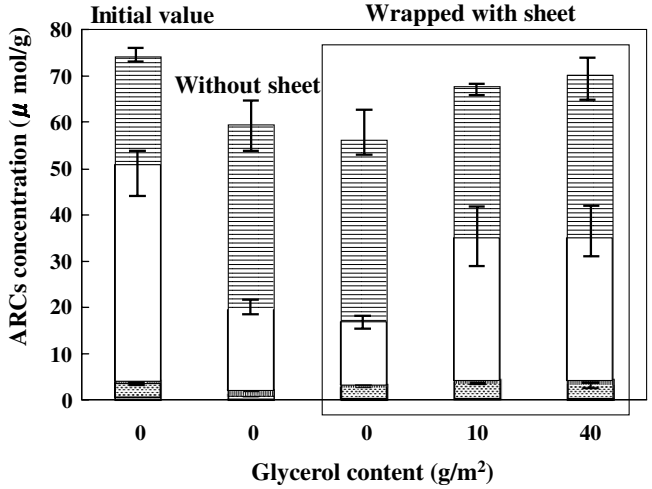

Fig. 6. Effect of glycerol on ARCs in beef after 7 days in refrigerated storage at 4 °C. ATP (▨), ADP (▨), AMP (▨), IMP (□), Hxs (RHx + Hx) (▨). ARCs determined by HPLC. Significant differences were observed at $p < 0.01$ as follows: ATP and ADP in samples without sheets versus samples wrapped in sheets containing 0, 10, and 40 g/m^2 glycerol; IMP in samples without sheets versus samples wrapped in sheets containing 0, 10, and 40 g/m^2 glycerol; Hxs in samples wrapped in sheets containing 10 g/m^2 glycerol versus samples without sheets and samples wrapped in sheets containing 0 g/m^2 glycerol.

Decomposition of Hx to uric acid may cause a reduction in the total ARC concentration in samples stored without glycerol-containing sheets. The IMP and Hx ratio of samples stored without glycerol also decreased. Jolley, Honikle, and Hamm (1981) reported that the ATP concentration at any time post-slaughter was the result of two factors: (a) the length of time during which the delay phase was operative, and (b) the subsequent rate of ATP depletion. It can be concluded that sheets with glycerol are effective in maintaining the *Umami* substance (IMP), as well as in maintaining the flavor of cold stored beef (*Umami* is a Japanese term for a fifth basic taste that is triggered by some amino acids, peptides, and their salts, notably monosodium glutamate), summarized by Pegg and Shahidi (2004). The results shown in Fig. 6 suggest that the ARC content was reduced by drip-absorbent sheets. Fig. 7 shows the percentage of *K* values for samples wrapped in sheets containing glycerol and sheets with no glycerol; the *K* value was calculated from the results of the ARC determination. The *K* value index of meat samples stored without wrapping sheets was 67%, whereas that of the samples wrapped in drip-absorbent sheets was 70%. The *K* value of samples wrapped with sheets containing glycerol at 10 g/m^2 was 48%, and the *K* value of samples wrapped in sheets containing glycerol at 40 g/m^2 was around 51%. Samples wrapped with sheets containing glycerol gave lower *K* values than all of the others. Glycerol is therefore considered to be an effective tool for maintaining the freshness of cold stored beef. *K* values were significantly reduced in samples wrapped in sheets containing glycerol at two concentrations of 10 g/m^2 and 40 g/m^2 compared with the *K* values of samples wrapped in sheets with no glycerol. The intended purpose of this research was to illustrate the important functions of humidity-stabilizing sheets in the storage of meat. In support of our proposed use of glycerol sheets for mitigating meat desiccation, Ledward (1981) mentioned that glycerol is a well-known reagent used in preparing intermediate-moisture meat.

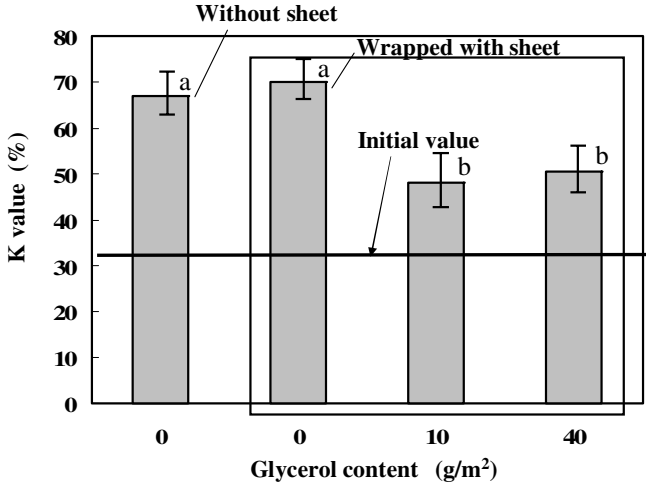

Fig. 7. Effect of glycerol on *K* value of cold stored beef after 7 days in refrigerated storage at 4 °C. Values with different letter denote significant difference from each other at $p < 0.01$.

Glycerol absorbs and transpires moisture quickly, according to the equilibrium hygroscopic curve, at low temperatures; it therefore protects meat from condensation and desiccation. The glycerol absorbs the moisture in the atmosphere around the meat until an absorption equalization state is reached. When the meat surface starts to become desiccated, humidity-stabilizing sheets containing glycerol release moisture that was absorbed from the meat during the early states of storage. The potential of glycerol as a humidity stabilizer could have many applications in the food industry. Use of this technique should be increased, as wrapping materials protect food from moisture loss, prevent color deterioration, and enhance food quality even after extended storage. Such humidity-stabilizing sheets are widely employed in beef packing in Japan.

4. Conclusion

The objective of this study was to elucidate the effects of humidity-stabilizing sheets on ARCs and metmyoglobin percentage in cold stored beef. Sheets containing a humidity-stabilizing agent (glycerol) can control color stability, inhibit the increase of metmyoglobin percentage, and prevent the increase of *K* value in cold stored beef. We therefore suggest that further experiments must be conducted to explore the effects of this sheet on lipid auto-oxidation. Such research could pioneer new, innovative packaging methods using wrappings treated with glycerol to maintain the freshness of beef. This research has suggested that humidity-stabilizing sheets containing glycerol are effective in preventing deterioration in the quality of meat.

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