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Influence of high pressure on the color and microbial quality of beef meat

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Abstract

An experimental design was used to study the effect of various pressure values (50–600 MPa) and holding times (20–300 s) on color and microbiological quality of bovine muscle (*Biceps femoris*). The experiments were conducted at 10°C. The pressure intensity is more significant than holding time for redness, total color difference and metmyoglobin content. Pressure higher than 300 MPa induces modifications of meat color parameters such a decrease of the total color difference (ΔE), a decrease in the total flora and a 1 week delay before microbial growth (520 MPa, 260 s). During the first 3 days of storage (4°C), the increase in redness is maintained for the 130 MPa samples while the redness of the 520 MPa samples decreases gradually, in relation to the increase of metmyoglobin. The cooking (1 h at 65°C) done after pressurization led to the disappearance of color differences observed between the nontreated and pressurized samples.

Keywords: High pressure; Beef meat; Color; Microbiology; Experimental design

1. Introduction

High-pressure treatment appeared in the 1970s with the works of MacFarlane (1973) and MacFarlane and Morton (1978) reporting the improvement of pressurized meat tenderness. After this pioneer research, the effect of pressure on meat and meat products was investigated in numerous studies that dealt with the effect of this treatment on several meat characteristics (Cheftel & Culioli, 1997; Ledward, 1998). It has been reported that the pressurization of post-mortem beef meat could modify the enzymatic system (Homma, Ikeuchi, & Suzuki, 1994; Jung, de Lamballerie-Anton, & Ghoul, 2000a), the texture and ultrastructure (Bouton, Ford, Harris, Macfarlane, & O'Shea, 1977; MacFarlane & Morton, 1978; MacFarlane, 1985; Suzuki, Kim, Homma, Ikeuchi, & Saito, 1992; Jung, de Lamballerie-Anton, Taylor, & Ghoul, 2000b), the gelation properties of myofibrillar proteins (Ikeuchi, Tanji, Kim, & Suzuki, 1992) and the microbiological quality (Cheftel & Culioli, 1997) of meat. Besides, high-pressure treatment has started to emerge as a technology with high interest because of its capacity to preserve the essential functional and nutritional characteristics of the food products.

In fresh meat, color is the most important attribute that consumers use as purchase criterion (Faustman & Cassens, 1990). The modification induced by high-pressure treatment on meat color is related to the color criteria such as lightness (L^*), redness (a^*) or yellowness (b^*). Thus in some conditions, the lightness of meat could be heightened by high-pressure treatment and the redness increased or decreased. The increase in L^* values begins from 200 MPa and becomes stabilized for pressures around 300–400 MPa, as shown by Carlez, Veciana-Nogues, and Cheftel (1995) in beef mince and Shigehisa, Ohmori, Saito, Taji, and Hayashi (1991) in pork slurries. The lighter appearance of meat could be due to globin denaturation and heme displacement or release (Carlez, 1994), an increase in drip losses leading to changes of water content of the meat (Mussa, 1999), or a damage of porphyric ring and protein coagulation (Goutefongea, Rampon, Nicolas, & Dumont, 1995). A

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decrease in redness has been reported in minced beef meat after a 500 MPa treatment for 10 min at 10°C (Carlez et al., 1995) or on pork chops after a treatment at 350 MPa, 10–20 min at 25°C (Mussa, 1999). This decrease could be related to the increase in metmyoglobin (Fe^{3+}) and resulted in a brown coloration of meat which is undesirable and responsible for the meat reject by consumers (Renerre, 1990). Cheah and Ledward (1997) reported a redness increase of a 2-day-old beef *L. dorsi* muscle treated at 80–100 MPa for 20 min at 25°C, and an intensification of the redness increase during 12 days of storage. These authors correlated this fact with a lower accumulation of metmyoglobin during pressurized sample storage compared to the control sample (25% vs. 35% after 12 days). The modification of the meat color after pressurization could be a brake of the commercialization of the pressurized products, and the cooking of the samples could be a solution to these modifications. However, no data related to the color of pressurized meat after cooking has been found in the literature.

Under specific conditions, high-pressure treatment can inactivate microorganisms in meat products (Shigehisa et al., 1991; O'Brien & Marshall, 1996; Carlez, Rosec, Richard, & Cheftel, 1993). Consequently pressure treatment may be a suitable method to extend the shelf-life of fresh meat without any additives.

Although some studies have reported the effect of pressure treatment immediately after its application, few studies have monitored pressurized meat color stability during storage. Besides, the effect of a very short time of treatment has never been characterized on the color and microbiological in the same time, after treatment and during chilled storage. This study examines the effect of pressure level (50–600 MPa) and duration (20 and 300 s) using an experimental design on color parameters and total flora of pressurized meat, and the evolution of these characteristics during a refrigerated storage (4°C). Moreover, the effect of cooking on pressurized meat color is evaluated.

2. Material and methods

2.1. Animal and muscle preparation

Biceps femoris muscles were obtained from 6-year-old Holstein dairy cows ($n = 3$, around 400 kg). Animals were slaughtered at a local abattoir and samples of 2.5 cm × 2.5 cm × 2.5 cm (height × width × length) were cut parallel to the muscle axis at 24 h post mortem. Meat samples were then vacuum packed in polyethylene pouches (La Bovida, Nantes, France) and treated 48 h after slaughter. Samples were kept at 4°C until treatment.

2.2. Pressure treatment

For the color measurements, treatment conditions (pressure and holding time) were determined according to the experimental design described below. For the microbiological analysis, two pressure levels were applied to the meat samples: 130 and 520 MPa during 260 s. The pressure vessel was a 3 L cylinder (diameter: 120 mm, length: 300 mm, ACB Pressure System, Nantes, France). The pressurization fluid was distilled water. The treatment temperature was maintained at 10°C (±3°C). The kinetic of pressurization was 3 MPa/s, so 130 MPa (or 520 MPa) was reached within 43 s (or 173 s). The kinetic of depressurization was 5 MPa/s, so it required only 26 s from 130 MPa, and 104 s from 520 MPa.

2.3. Cooking

After pressure treatment, vacuum-packed samples were cooked in pouches in a water-bath for 1 h to a core temperature of 65°C. After cooking, pouches were opened, samples wiped (in order to remove the water present on meat surface) and color measurements were carried out without flattening of the meat, and at the middle of the samples.

2.4. Color and myoglobin measurements

After 3–5 h, the samples were opened and let to reoxygenate, covered by an aluminum foil for 30 min, before that the color was determined at the center of the samples, without previous refreshing. Indeed the dimensions of the samples were chosen to avoid any further preparation of the samples. For the storage, the samples were repacked in commercial oxygen-permeable film used for selling of meat (La Bovida, France). Reflectance spectra (360–760 nm) were determined on raw meat at 0 (day of treatment), 1, 3, 4 and 7 days of storage and from cooked meat, with an Uvikon 860 (Kontron) spectrophotometer. Color coordinates were determined in the CIE-LAB system and the results were expressed as lightness (L^*), redness (a^*) and yellowness (b^*). A numerical total color difference (E^*) was calculated by

$$\Delta E^* = [(L^* - L^*_{ref})^2 + (a^* - a^*_{ref})^2 + (b^* - b^*_{ref})^2]^{1/2} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}.$$

The color values of unpressurized samples were used as a reference in the ΔE calculation.

The percentage of metmyoglobin at the meat surface was determined by the method Krzywicki (1979). This method may be invalidated by protein denaturation. Each value represented the means of six measurements. Measurements were carried out in triplicate on two samples for three animals.

Table 1
Regression model fitted for redness values (a^*), metmyoglobin percentage and total color difference (ΔE) of pressurized *Biceps femoris* beef samples

	a^* values		Metmyoglobin		ΔE	
	RC	SL	RC	SL	RC	SL
Constant	8.81		31.52		−6.74	
Linear: P	0.04	<0.01	-8.5×10^{-2}	<0.01	0.06	<0.01
Linear: T	0.03	0.37	-7.5×10^{-2}	0.77	0.05	0.14
Interaction: $P\times T$	-2.56×10^{-5}	<0.01	7.1×10^{-5}	<0.01	3.84×10^{-7}	0.99
Quadratic: PP	-4.69×10^{-5}	<0.01	1.3×10^{-4}	<0.01	-5.3×10^{-5}	0.06
Quadratic: TT	-6.48×10^{-5}	<0.01	1.7×10^{-4}	<0.01	-1.3×10^{-4}	0.14

P: Pressure, T: Time, RC: regression coefficient, SL: significant level.

2.5. Microbiological determination

Vacuum-sealed samples were aged for 1, 2, 3 or 4 weeks at 4°C. Meat samples were placed in an aseptic Stomacher bag with peptone (1/2, w/v) and blended with a Stomacher 400 (Grosseron, St.-Herblain, France) for 3 min. The mixed samples were then diluted and the different dilutions were placed, in triplicate, on Plate Count Agar medium. The total flora was determined after incubation for 72 h at 25°C.

2.6. Experimental design and data analysis

Response Surface Methodology was adopted to study the simultaneous effect of pressure and treatment time. For the time, the results are expressed as pressure holding time, which does not take into consideration the time to reach the desired pressure and to return to the ambient pressure. For example, a sample treated at 600 MPa at a pressure holding time of 2 min is submitted in reality at the pressure during a total of 7.33 min. The experiments were based on a central composite rotatable design (Cochran & Cox, 1957). Five levels of pressure and time were chosen following the principles of the central composite design: 50, 130, 325, 520, 600 MPa and 20, 60, 160, 260, 300 s. The total number of observations was 12, which included three center points. The data were analysed using Statgraphics software (version 2.1, Rockville, USA).

Apart from the experimental design, Fisher's least significant difference procedure was used to discriminate among the means.

3. Results and discussion

3.1. Effect of pressure and time on color and metmyoglobin content of meat

Analysis of variance of regression models from experimental designs showed that model was significant for a^* values, total color difference (ΔE) and metmyoglobin content, with a coefficient of correlation of respectively 0.89, 0.84 and 0.88. The influence of pressure and time on these responses is shown in Table 1 and in Figs. 1–3. For a^* values and metmyoglobin form, all the parameters (P, $P\times T$, $P\times P$ and $T\times T$) were significant ($P<0.05$) except the time (T). In the case of ΔE, only the pressure had a significant effect ($P<0.05$). The increase in pressure led to the increase in a^* values until around 350 MPa, and then the decrease (Fig. 1). For pressurized minced meat Carlez et al. (1995) obtained an important decrease of a^* values between 300 and 500 MPa. This evolution of a^* values supposed that some modifications occurred during the treatment on the content of myoglobin pigments and particularly on the metmyoglobin form. Effectively, the evolution of metmyoglobin with pressure and holding time shows that up to around 300 MPa the treatment led to a decrease in metmyoglobin and for higher pressure there is an increase (Fig. 2). So the comparison between Figs. 1 and 2 illustrates the reverse evolution of a^* values and metmyoglobin: the increase in metmyoglobin causes the decrease in meat redness. These results suggest that even after pressure treatment, there is always a relationship between a^* values and metmyoglobin content. Moreover, it appears that the treatment

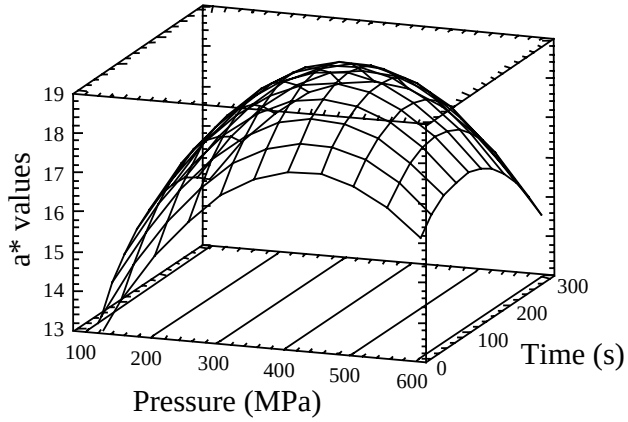

Fig. 1. Response surface for redness values of *Biceps femoris* beef samples pressurized between 0 and 520 MPa during 0–260 s (10°C).

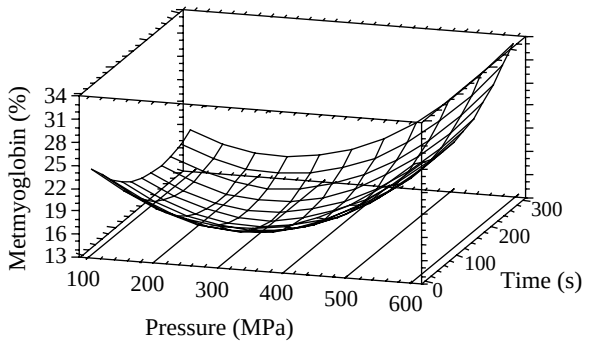

Fig. 2. Response surface of metmyoglobin content of *Biceps femoris* beef samples pressurized between 0 and 520 MPa during 0–260 s (10°C).

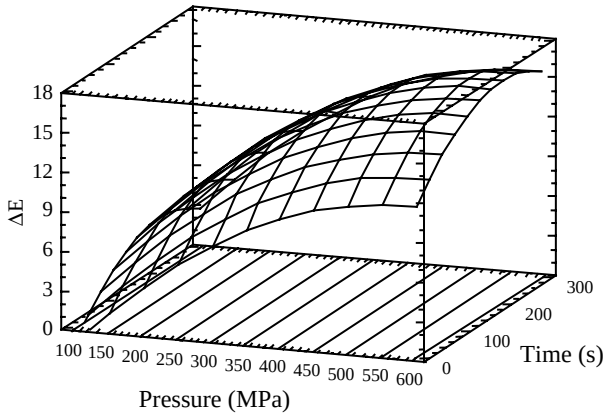

Fig. 3. Response surface on total color difference (ΔE) values of *Biceps femoris* beef samples pressurized between 0 and 520 MPa during 0–260 s (10°C).

is responsible for two types of behavior in the systems implied in the metmyoglobin formation. For moderate pressure, the enzymatic system implicated in reduction of metmyoglobin could be activated and lead to the decrease of metmyoglobin. At higher pressure, the enzymatic system and the reactions involved in the formation of metmyoglobin could be disturbed by changes in the enzymatic system itself or the environment of the enzymes.

The determination of the total color difference (ΔE) which take into account the evolution of the three color parameters (a^*, b^* and L^*) shows that the increase in pressure intensity is accompanied by an increase in ΔE (Fig. 3). This increase indicates a greater total color difference between pressurized and nontreated meat. This modification is only due to pressure level, as time does not have a significant effect (Table 1). If a rise of 10 units of ΔE is considered to modify significantly the appearance of color meat, Fig. 3 illustrates that this threshold was reached for pressure around 300 MPa. This figure also illustrates that even if a short time is used (20 s) the total color difference is equal to ten for pressure higher than 300 MPa.

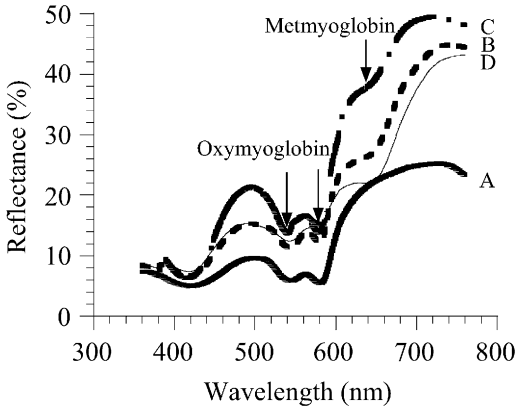

Fig. 4. Reflectance spectra of raw (A), cooked (B), pressurized (C, 520 MPa, 10°C, 260 s) and pressurized then cooked (D, 1 h at 65°C, 520 MPa, 10°C, 260 s) *Biceps femoris* beef samples.

Fig. 4 presents the reflectance spectra of control and pressure treated meat sample (520 MPa, 260 s), raw and cooked. The peaks at 540 and 580 nm are representative of oxymyoglobin. The curve appearance of control and pressurized meat is similar in this zone but the raw pressurized samples have higher reflectance values than the raw control, meaning that the lightness of samples becomes more significant. As suggested by Goutefongea et al. (1995), color modifications and particularly modifications of lightness could be a consequence of protein modifications. Severe protein denaturations were reported for treatment above 300–400 MPa by Cheah and Ledward (1996). Changes in myofibrillar and sarcoplasmic proteins due to pressure could induce meat surface changes and consequently color modifications.

After treatment at 520 MPa, 260 s samples changed intensity between 600 and 620 nm, suggesting the appearance of a metmyoglobin-like pigment. This evolution confirms the CIE values, which indicated that pressurized beef changed from a bright cherry-red to a dark red or brown color. The similarity between the spectra of the cooked control samples and pressurized raw sample could also be observed. This suggested that high-pressure treatment could induce the same kind of myoglobin modification than cooking, such as the denaturation of metmyoglobin and displacement towards the ferric state of the hematin iron.

Meat discoloration could be a problem for marketing pressurized raw meat, as meat color is one of the most important criterions for the consumers. The statistical analysis of the total color difference (ΔE) shows clearly that no color difference can be observed when both the control and the pressurized sample are further subjected to cooking (results not shown). This fact is illustrated in Fig. 4 by the similarity between the spectrum of the cooked control samples and pressurized cooked samples.

3.2. Color evolution of control and pressurized meat samples during storage

Figs. 5 and 6 show the evolution during storage of a^* values and metmyoglobin content, respectively, for the control and after treatment at 130 and 520 MPa, 260 s, 10°C. The package used for the storage is oxygen permeable, as it would be in the commerce. This fact certainly influenced the evolution of the color parameter during the storage, but no experiments were done to determine the oxygen permeability package effect on the samples. After a treatment at 130 MPa, the redness of meat remains significantly higher compared to the control during the third first days of storage. After a treatment at 520 MPa there is a significant decrease in a^* values between the 1st and 7th days of storage. Evolutions of a^* values could be correlated to the accumulation of metmyoglobin in the samples. Fig. 6 shows that the decreasing order classification for metmyoglobin content after 1 day of storage is 520 MPa samples, control samples and 130 MPa samples. This order is in agreement with the results obtained for the a^* values. Besides during storage, the content of metmyoglobin was constant for control samples, increased drastically between the 4th and 7th days of storage for 130 MPa samples and increased gradually for 520 MPa samples. The result obtained after a 130 MPa treatment is in accordance with those of Cheah and Ledward (1997) who followed the color evolution of different muscles after treatment at 80–100 MPa, 20 min, 25°C: in the case of *Psoas major* muscle, which is as *Biceps femoris* a color unstable muscle, the maintaining of the increase in a^* values until 4 days of storage was observed. The comparison between our results and those of Cheah and Ledward (1997) show that with a pressure of 130 MPa and a temperature of 10°C, a shorter time (260 s vs. 20 min) leads to comparable increase and maintaining of the redness of pressurized samples. Our results also show that for higher pressure, the treatment induces meat discoloration. From an enzymatic point of view these results could suggest that up to 3 days of storage the 130 MPa treatment leads to an activation of the system involved in the reduction of metmyoglobin. After treatment at 520 MPa, the enzymatic systems could be disturbed and this could explain the progressive accumulation of metmyoglobin content during storage. In order to test these hypotheses, the metmyoglobin-reducing activity could be estimated, according to Renerre and Labas (1987). After 1 week of storage, pressurized raw meat presents a less attractive color than the control.

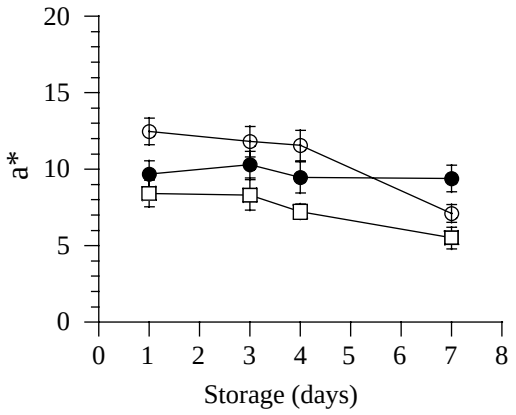

Fig. 5. Evolution of a^* values for control and pressurized *Biceps femoris* beef samples during a storage at 4°C. Control samples (●), samples pressurized at 130 MPa, 260 s (○) and 520 MPa, 260 s (□), 10°C. The error bars represent the 95% confidence intervals.

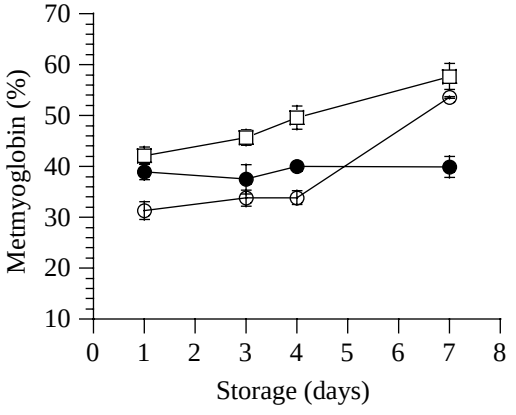

Fig. 6. Metmyoglobin evolution of control and pressurized *Biceps femoris* beef samples during a storage at 4°C. Control samples (●), samples pressurized at 130 MPa, 260 s (○) and 520 MPa, 260 s (□), 10°C. The error bars represent the 95% confidence intervals.

3.3. Total flora evolution of control and pressurized meat samples during storage

The total flora of the control samples (Fig. 7) is in the same range as those obtained by Venugopal, Ingham, McCurdy, and Jones (1993). After processing at 130 MPa total flora of the samples remained similar to the control. In contrast, a treatment at 520 MPa led to a decrease of 2.5 log. This shows that a moderate pressure (130 MPa) applied for 260 s at 10°C did not improve the microbiological quality of meat. This result is in agreement with the studies of Carlez, Rosec, Richard, and Cheftel (1994) and O'Brien and Marshall (1996), and confirms that the higher intensity of pressure led to the greater reduction in meat bacteria (Shigehisa et al., 1991). The effect of high pressure on microorganisms is dependent on the type of microorganisms present, and the composition of food (Hoover, Metrick, Papineau, Farkas, & Knorr, 1989).

During storage the growth of the samples treated at 130 MPa was comparable to the control. There is an increase in the total flora over 1 week of storage before

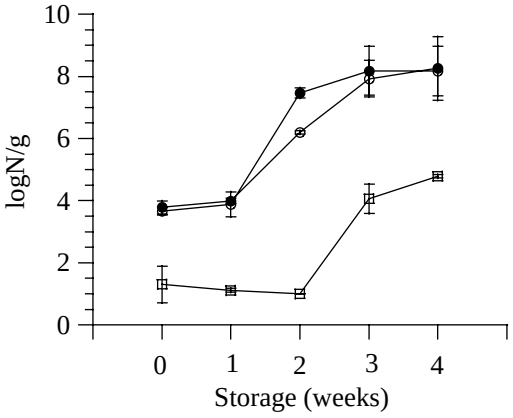

Fig. 7. Total flora evolution of control and pressurized *Biceps femoris* beef samples during a storage at 4°C. Control samples (●), samples pressurized at 130 MPa, 260 s (○) and 520 MPa, 260 s (□), 10°C. The error bars represent the 95% confidence intervals.

equilibrium was achieved. In some studies the use of moderate pressure, when it induced some bacterial inactivation, also caused a delay in bacterial growth during chilled storage (O'Brien & Marshall, 1996; Carlez et al., 1994b). Our results did not show such a delay for a pressure of 130 MPa, but after a treatment at 520 MPa a delay of 1 week appeared. On another hand, after 4 weeks of storage the samples treated at 520 MPa had the same total flora as the control the day of treatment. This shows the effectiveness of application of a 520 MPa pressure even if applied during a short time, and leads to the conclusion of an improvement in the microbiological quality of pressurized meat.

4. Conclusion

Application of high intensity of pressure for a short time leads to a decrease in meat total flora and a delay of growth of 1 week. This delay could be used to let the maturation of the meat occurr longer, which could improve the meat tenderness. However, this beneficial effect of treatment is accompanied by a discoloration of meat for pressure values higher than 325 MPa. The effect of pressure is more significant than that of the holding time on the color parameters and metmyoglobin content. Moderate pressures (130 MPa) cause an improvement in meat color with an increase in redness, which is maintained during few days of storage. However, this intensity of pressure is not sufficient to modify the microbiology of meat. Consequently, a choice must be taken between a color improvement and a microbiological improvement, depending of the final meat form commercialization. Indeed, 130 MPa will be chosen to sell fresh meat, but higher pressure level may be used to sanitize meat in case it is used in catering trade.

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