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Improving the quality of citric acid and calcium chloride marinated culled cow meat

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Abstract

The objective of this research was to evaluate the effects of immersion marination with citric acid, calcium chloride and combined mixture of citric acid and calcium chloride solutions on Semimembranosus meat quality traits. Meat cuts obtained from culled cows were marinated with 0.05 M citric acid, 0.2 M calcium chloride or the combined mixture of these two compounds for 24 hr. Non- marinated meat served as control. Application of calcium chloride to the marination (either solely or in combination) improved water holding capacity (WHC) thus reduced cooking loss and shear force values. Meat marinated in citric acid solution showed high exudative during cooking and was not significantly different in shear force value compared to the unmarinated samples. Lightness was higher (paler) in all marinated meat samples. The a^* values showed that marinated meat with citric acid solution had less red intensity. Lower of yellow intensity (b^* value) appeared in the meat samples marinated with calcium chloride and the combined mixture. The sensory attribute evaluation showed that the highest scores for juiciness and tenderness were for calcium chloride and the combined mixture. The lowest flavor score was found in the meat marinated with citric acid solution and reported as sour. Sample treated with calcium chloride revealed no detrimental effect on meat flavor and odour. The evidence in the present study indicates that calcium chloride marinate solution increases juiciness and tenderness of meat from culled cow.

Introduction

Tenderness is considered to be the most important trait of meat acceptability by the consumers (Miller *et al*., 1995). Meat tenderness is related principally to the two structural elements: the connective tissue and the myofibrillar protein components of muscle (Lawrie, 1998). Basically, the mechanism responsible for increased meat tenderness is connected with the weakening of connective tissue and the weakening of myofibrillar protein component (Takahashi, 1996).

Marination involves the immersion of meat in organic acid or salt solutions are a practical method of altering meat tenderness for many years (Gault, 1991). Marination process not only improves tenderness but also increases juiciness of meat, due to the retention of added water (Offer and Trinick, 1983). Thus, the marinades with a tenderising capacity are important means for applications involving in low-value of meat cut or muscle with rich in connective tissue.

Citric acid, a food acidulant is often used in meat marination to improve the water holding capacity (WHC) and tenderness of beef muscle (Ke *et al*., 2009). The mechanism of the tenderising action of acidic marinades is believed to involve in the weakening of structures due to swelling of the meat and increased conversion of collagen to gelatin at low pH during cooking (Offer and Knight, 1988; Berge *et al*., 2001).

Calcium chloride has been identified as a means for increasing beef tenderness (Koohmaraie *et al*., 1990; Morgan *et al*., 1991). Benefit of marinating beef cuts with calcium chloride to increase tenderness is due to the enhancing calcium activated proteolysis (endogenons calpain proteinase system) which is responsible for the breakdown of myofibrillar proteins (Whipple and Koohmaraie, 1992). However, some of the sensory properties such as colour and flavour can be altered by calcium chloride treatment (Wheeler *et al*., 1993; Eilers *et al*., 1994).

Numerous experiments have demonstrated the use of organic acid and salts marinade solution improve meat tenderness. However, most of the investigations have been documented with growing finishing animals where rather tender beef could be expected anyway. In Thailand, beef originating from culling cattle is prevalent in local markets with most

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of them being *Bos indicus* origin (Jaturasitha *et al*., 2004). Meat from *Bos indicus* cattle is known to be more degree of toughness than beef from *Bos tauras* origin (Lansdell *et al*., 1995). Limited work has been done on marinated beef from culling *Bos indicus* cow.

Therefore, the objective of this study was to compare the effects of citric acid, calcium chloride and the combined mixture of citric acid and calcium chloride marinate on the sensory attributes such as marination mass gain, cooking loss, tenderness, colour and flavor of meat from culling cows.

Materials and Methods

Meat samples

The meat samples used in this research were obtained from six culling Thai native cows (about 7 years of age) reared in Tak province, Thailand. After slaughter, the samples of muscles from topside of hind legs (*M. semimembranosus* muscles) were collected and the visual connective tissues were manually removed as much as possible. The muscles were then stored at 4^{o}C over night.

Sample marination

Meat samples were cut parallel to the muscle fiber direction (a cutting thickness of 5 mm) using a meat slicer (Sirman snc. Padova, Italy). The meat strips from each cow were allocated randomly submitted to the four treatments as follow: 1) control (Co, without marination) 2) 0.05 M citric acid marination (Cc) 3) 0.2 M calcium chloride marination (Ca) and 4) combined mixture in equal volume of 0.05 M citric acid and 0.2 M calcium chloride (Cc + Ca).

Meat strips were dipped in marinade solutions at a ratio of 1:1 (meat: liquid) in plastic boxes and keep in refrigerator at 4^{o}C for 24 hr. The untreated samples (control) were only kept in the boxed without addition.

pH measurement

After marinating, meat samples (3 g) were cut into small pieces, made up to 30 ml with 5 mM Na-iodoacetate in 150 mM KCl and then homogenized. Slurry was then filtrated through Whatman paper no. 4. The pHs of filtrates were determined using a glass electrode pH meter (Sartorius AG, Gottingen, Germany).

Uptake of marinade

Following marination, excess marinade was removed by applying paper towel lightly to samples surface. %.uptake of marinade was taken by the difference between the sample weights before and after marinating.

Color measurement

Meat color was measured using a colorimeter (Hunter Lab, Hunter Associates Laboratory, Inc., USA) in the CIE L^*, a^*, b^* mode. Before each measurement, the apparatus was standardized against black and white plate.

Cooking loss

The meat samples were cooked by placing in plastic bags and immersing in a water bath at 80^{o}C until an internal temperature of 70^{o}C was reached (about 1 hr). Cooked meat strips were cooled to room temperature, blotted and reweighed. Cooking loss was calculated by the different weight before and after cooking.

Shear force determination

Three cores (1.3 cm diameters) were removed from each cooked meat samples. Each core was sheared once perpendicular with the muscle fiber (the longitudinal axis of the sample) using a computer controlled Texture Analyser (TA-XT plus, Stable Micro Systems, UK) equipped with a Warner-Bratzler type shear blade. The crosshead speed of 200 mm/min with a 5 kN load cell was used.

Sensory evaluation

For sensory characteristics assessment, the cooked meat samples were randomly served warm to a 50-member sensory panel in individual booth. Panelists assessed meat sample for juiciness tenderness, odor, flavor and general acceptability in an eight point scale, where 8 = extremely desirable juicy, tender, intense in meat flavor and very good appraisal and a score of 1 = extremely dry, tough, devoid of meat flavor and extremely undesirable.

Statistical analysis

The experiment was conducted according to completely randomized blocks design with six replicates. Analysis of variance was used in analyzing all data (Steel and Torrie, 1980). Significant means were separated using Tukey's w procedure test at $P < 0.05$.

Results and Discussion

Effect of marination on pH

Low pH (4.5) was observed in the meat samples marinated with citric acid and the combined mixture of citric acid and calcium chloride solutions (Table 1). Citric acid marinades decreased meat pH to below the isoelectric point (about 5.2-5.3 in red meat). In

Table 1. Effect of marinated solution treatments on pH, marinade uptake and cooking loss of the *Semimembranosus* muscle from culled cows

Item	Co[1]	Cc	Ca	Cc + Ca	SEM[2]
pH	5.5[a]	4.5[b]	5.5[a]	4.5[b]	0.06
Marinade uptake (%)	-6.5[a]	6.6[b]	9.1[b]	2.8[c]	1.20
Cooking loss (%)	36.5[a]	42.9[b]	36.9[a]	35.1[a]	1.05

[a,b,c] Values in a row with different letters are significantly different (P<0.05).
[1]Co, control (unmarinated); Cc, 0.05 M citric acid; Ca, 0.2 M Calcium Chloride; Cc + Ca, combined mixture of 0.05 M citric acid and 0.2 M calcium chloride . [2]Standard error of means

calcium chloride marinated meat, the pH revealed no different from the unmarinated control meat (pH 5.5) (P>0.05) (Table 1). Koohmariae *et al*. (1989) reported the application of meat with calcium chloride do not alter the meat pH.

Marinade uptake

Uptake of marinade after immersion in the treatment marinade solutions are shown in Table 1. It was found that the meat samples marinated with citric acid solution and calcium chloride solution revealed higher uptake than the samples treated with combined mixture of citric acid and calcium chloride solution(P<0.05). In the citric acid treatment, the high marinade uptake could be attributed to the strong effect of lower pH on protein as indicated by the results reported by Rao *et al.* (1989). The addition of acid below the isoelectric point of meat proteins led to the protonation of negatively charged (–COO-) groups on protein molecules. The increase in net positive charges on myofibrillar protein resulted in repulsive forces among protein groups of similar charge in the myofibrils. This repulsive force push the thick and thin filaments apart laterally and creating space for the immobilization of extra water (Ke *et al*., 2009). Previous study also reported the immersion of meat in acid marinade solution increased marinade absorption (Burke and Monahan, 2003).

In the calcium chloride medium, the high marinade uptake may be explained by the combination of 2 phenomena: (1) degradation of the cytoskeleton in muscle cell. During the marination, calcium ion activates endogenous calpain proteinases (μ- and m- calpains) which their activities specific degraded the proteins include those involved in inter- (eg. desmin and vinculin) and intra- myofibril (eg. titin and nebulin). The function of these proteins is to maintain the structural integrity of myofibrils (Pearce *et al.*, 2001). Thus, the proteolytic degradation of these proteins causes a weakening of myofibrillar and then creates the space available for water within the muscle cell. (2) osmotic pressure created by the difference in the free ions concentration. During the marination, the anions (Cl^-) of the calcium chloride could form ionised salts with the available cationic side groups ($R\text{-}NH_3^+$) on the protein molecules. This results in an unequal distribution of free ions between the myofibrillar protein and the external solution. This could induce the difference in osmotic pressure. The difference in osmotic pressure lead the flow of water into the protein structure results in a reduction of this difference.

When the mixture of citric acid and calcium chloride was applied to meat, the lower of marinade uptake was found in comparison with the treatments where two compounds were used separately. Goli *et al.* (2014) also found the limitation of marinade uptake when salt was applied to the acid marinate solution in turkey breast meat. Their experiment demonstrated the presence of salt in water acid marination considerably reduced the swelling observed upon the fall in pH compared with the swelling that would have been observed in a water acid solution. This phenomenon could be explained by the reduction in osmotic difference as described by Medynski *et al*. (2000). Addition of salt with acid at the same time increases the amount of free anions in the external solution. This could reduce the difference in anion concentration between the protein structure and external solution, hence reduction in the osmotic difference. Therefore, the swelling potential of protein fibers is reduced.

Cooking loss

Differences in the cooking loss in the meat samples have been seen in Table 1. Cooking loss was lower in the calcium chloride and in the mixture of citric and calcium chloride treatments than in the citric acid marinate solution (P<0.05). The difference may be due to conformational changes at the protein water interface. Lawrence *et al*. (2003) reported the addition of a salt (e.g. a calcium salt) to a solution increased ionic strength, thereby increasing the number of hydrophilic protein interactions which caused an increase in the binding of free water. Thus, calcium chloride when applied solely or added to

Table 2. Effect of marinated solution treatments on hunter colour and shear force of the *Semimembranosus* muscle from culled cows

Item	Co[1]	Cc	Ca	Cc + Ca	SEM[2]
Colour					
L*	48.5[a]	56.3[b]	57.5[b]	52.9[ab]	1.25
a*	9.5[a]	5.7[b]	7.7[ab]	7.5[ab]	0.62
b*	16.9[a]	15.7[ab]	14.7[b]	14.4[b]	0.49
Shear force (N)	78.0[a]	74.1[a]	62.8[b]	60.6[b]	3.50

[a,b] Values in a row with different letters are significantly different (P<0.05).
[1]Co, control (unmarinated); Cc, 0.05 M citric acid; Ca, 0.2 M Calcium Chloride; Cc + Ca, combined mixture of 0.05 M citric acid and 0.2 M calcium chloride .
[2]Standard error of means

acid water marinade solution may alter the affinity of the proteins to bind more extent of the free water molecules leading lower in cooking loss.

On the contrary, meat sample marinated with citric acid exhibit a high exudation when cooked. Previous researches have reported the acid treatment decreased cooking loss of beef cores (Oreskovich *et al.*, 1992; Önenc *et al.*, 2004). The contrast result may be due to the difference in types of connective tissue. Meat samples in this present study derived from the old cows (about 7 yrs of age) which the maturation of collagen fibers could be expected. At maturity, collagen developed the cross links result in the formation of rigid three dimensional architecture collagen fibers of considerable tensile strength. Such structure probably prevented the unfold at the protein water interface within the collagen fibers, thus reduce the number of hydrophilic protein interaction. Meat present with this type of collagen, which has retained its integrity, contributes to the exudation through its shrinkage when cooked, which compress the muscle fibers. Miller *et al.* (1983) also reported that collagenous tissue with more cross linkages would be more resistant to swelling and have a lower water holding capacity.

Hunter colour values

The effects of marinating meat on hunter colour values are showed in Table 2. It was found that the L* value (lightness) was higher or paler in marinate treated meat samples than in control (P<0.05) which agreed with results published previously (Pérez *et al.*, 1998; Önenc *et al.*, 2004). The increase in L* values could be due to the water uptake of meat during marination. Aktas and Kaya (2001) reported that the amount of water dispersed among the muscle fibers could affect the reflectance ability of meat. Our resulted supported to this with more extend of marinate uptake, the paler of meat subject.

Marinating meat with citric acid solution had a significant effect on a* value which was less red colour compared to the control (Table 2). Similar result was obtained by Aktas and Kaya (2001). Arganosa and Marriot (1989) concluded that the acid treatment appeared to enhance the conversion of myoglobin to metmyoglobin via oxidative reaction which turned red colour to brown and lower colour intensity. Shikama (1998) also reported that the formation of the oxidized forms of hemeproteins (metmyoglobin) might contribute from the rapid oxidation of muscle at acidic pH values. Thus, the lower pH in citric acid marinate solution could provide the oxidant effect condition for metmyoglobin formation.

In general, the lower the pH values, the stronger the prooxidant effect. However, it could be noticed that the meat samples in the combination of citric acid and calcium chloride solution treatment revealed no difference in a* value from the control (P>0.05), eventhough the lower pH has been seen. This could be assumed that the myoglobin in this medium was less susceptible to oxidation. It seems to us that calcium chloride addition might be responsible for decreasing the oxidatant effect of citric acid under low pH. Citric acid has been reported as strong metal chelators (Decker, 1998). Thus, the combination of citric acid and calcium chloride solutions could inhibit oxidation by binding the metal via bonds formed between the metal and the carbonyl or hydroxyl groups of the citric acid molecule as has been described by Francis *et al.* (1992).

Meat marinated with calcium chloride solely or combine with the acid revealed lower b* values (P<0.05) (less yellow) than the control. Calcium chloride treated meat demonstrated less in yellow has also been reported by Jaturasith *et al.* (2004) and Bunmee *et al.* (2014). They proposed that the discolouration of meat in Ca salt application could be due to the continuous action of oxygen which is facilitated by the catalyzing properties of the salts. However, the mechanism in detail did not elucidate.

Shear value

Tenderness, an important attribute of meat, was evaluated by a shear force. The data obtained here demonstrated that there were significant reductions in shear force for meat marinated with calcium chloride

Table 3. Effect of marinated solution treatments on sensory panel scores of the *Semimembranosus* muscle from culled cows

Item	Co[1]	Cc	Ca	Cc + Ca	SEM[2]
Juiciness	4.7[ab]	4.2[a]	5.0[bc]	5.4[c]	0.22
Tenderness	4.5[a]	4.3[a]	5.4[b]	5.8[b]	0.23
Flavour	4.6[a]	3.7[b]	4.2[a]	4.2[a]	0.23
Odour	5.0	4.5	4.6	4.8	0.23
Acceptance	4.6[a]	3.9[b]	4.7[a]	4.8[a]	0.22

[a,b,c] Values in a row with different letters are significantly different (P<0.05).
[1]Co, control (unmarinated); Cc, 0.05 M citric acid; Ca, 0.2 M Calcium Chloride; Cc + Ca, combined mixture of 0.05 M citric acid and 0.2 M calcium chloride. [2]Standard error of means

either solely or in the combination with acid compared to the control (Table 2). Previous studies (Koohmaraie and Shackelford, 1991; Morgan *et al.*, 1991; Whipple and Koohmaraie, 1992) supported the effectiveness of calcium chloride in decreasing shear force values. The reduction in shear force with calcium chloride salt applied could be attributed to the weakening of the myofibrillar structure and the increased water content of the samples. Calcium chloride application into meat increases intracellular calcium concentration sufficiently to activate the calcium dependent calpain system. The enzyme degrades the protein around Z-disk of myofibril results in myofibrils fragmenting into smaller segment (Koohmaraie *et al.*, 1986). The fragmentation of myofibrils leads to the structural weakening of myofibrils thus, increased tenderness. Furthermore, the application of calcium chloride promoted myofibril swelling that could retain as much added water upon cooking as evidenced by low cooking loss of the treatments seen in this study. The ability of meat to retain water is related to tenderness by dilute the load bearing material due to the swelling of the myofibrils across their main axis (Gault, 1985; Rao and Gault, 1990).

On the contrary, the shear force value of meat marinated with citric acid showed no statistical difference to the control (P>0.05) (Table 2). This indicated that citric acid marinade did not improve meat tenderness. Previous studies reported the achievement of meat tenderization in acid marination was due to the swelling of muscle fiber under low pH medium (Burke and Monahan, 2003; Aktas *et al.*, 2007). In this study, the citric acid marinated meat revealed high exudation during cooking. This indicated the low ability in water retention of the meat sample. It is suggested that the swelling of muscle was restricted to the citric acid at this concentration.

Sensory panel evaluations

After cooking, all the treatments samples were rated for sensory attributes and the results are shown in Table 3. Among the marinated samples, juiciness scores were significantly higher (P<0.05) for the calcium chloride and the combined mixture compared to the citric acid treatments. Since juiciness reflects WHC (Pearce *et al.*, 2011), therefore the increase in water binding ability of the meat marinated with calcium chloride (either solely or in combination with acid) could be corresponding to this situation.

Meat samples were more tender when calcium chloride was applied and showed a highly significant difference between untreated and meat samples marinated with citric acid (P<0.05). This result was fully corresponding to the shear values (Table 2) and consistent with the study by Whipple and Koohmarai (1992) who found that marination with calcium chloride solution improved tenderness.

When the samples were rated for flavor, the citric acid marinated meat was described as sour by the panelists and was judged as off flavor with the lowest score (Table 3). Similar result was also reported for beef treated with citric acid (Aktas and Kaya, 2001). No difference in odour scores was detected among treatments. For this point of view, previous reports of calcium chloride marination caused undesiable meat odour and flavor (metallic, livery, astringent and bitter tastes) (Morgan *et al.*, 1991; Wheeler *et al.*, 1993; Lawrence *et al.*, 2003). However, results in the present study found no difference in flavor and odour with respect to the unmarinated meat. This contrast results are probably due to the difference in concentration of calcium salt solution and marination duration. It could be stated that the potentially adverse effect on flavor may have been increased with increasing calcium chloride concentration and long marination duration. For example, Morris *et al.* (1997) reported the 0.3 M of calcium chloride treatments resulted in higher off flavor intensity scores than 0.1 or 0.2 M treatments. Furthermore, Gonzalez *et al.* (2001) reported the calcium marinade procedure applied longer than 24 hr. produced bitter flavor.

Concerning the overall acceptability attribute, the scores that rated with the calcium chloride and the combination revealed no difference from the unmarinated meat, even though tenderness score suggested the better acceptation. This could be due to the fact that the meat samples from culled cow *Semimembranosus* muscle was not tender which the mean shear force value was recorded as 78 N. According to Young and Gregoey (2001), the meat samples with shear force values of 58.8 N. (6 kgF) or below could have an acceptable tenderness to most consumers. Jones *et al.* (2001) reported an average shear force value of around 45 N for beef *Semimembranosus* muscle. In our study, marination with calcium chloride was able to reduce shear force values of the meat sample to about 60 N (Table 2), but the value was still above the critical value for the acceptable tenderness. Thus, in this situation, the panel rating score for tenderness did not correspond to the values of overall acceptability.

Conclusion

The results of this study showed that calcium chloride treatment was effective in increasing WHC of meat, hence reducing both cooking loss and shear force. On the contrary, immersion meat with citric acid solution failed to improve cooking yield and shear force. No synergistic effect on meat quality was observed with the combination of citric acid and calcium chloride marination. Even though meat marinated with calcium chloride was rated more tender than unmarinated meat, but the values on shear force and total acceptability suggested that the marinated meat remained under the tough category. Therefore, further studies are needed to achieve an acceptable level of tenderness from meat of culled cow.

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