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The effect of processing temperature and addition of mono- and di-valent salts on the heme- nonheme-iron ratio in meat

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Abstract

Heme iron from meat has a superior bioavailability compared to non-heme iron and, from a nutritional point of view, processing of meat should be optimised to maintain high levels of heme iron. The effects of heat treatment and addition of NaCl and other ionic species, on the heme-iron/nonheme-iron ratio (H/NH) in meat, have been studied by measuring heme and non-heme iron in minced, vacuum-packed pork. Heating temperature has a gross effect on H/NH with a decrease in heme iron content of 62% after heating at 80°C for 2 h. The correlation (r^2) between heme and non-heme iron determinations was −0.92. NaCl increases H/NH in cooked meat by preventing the heme molecule from liberating iron, probably by an increase in the ionic strength of the meat. Calcium ions have a gross negative effect on H/NH during cooking of meat. These effects of sodium and calcium on H/NH in heat treated meat have not been previously reported.

Keywords: Heme iron; Non-heme iron; Myoglobin; Meat; Bioavailabillity; Processing; Heat treatment; Lipid oxidation; Warmed-over flavour; NaCl; Calcium; Salt

1. Introduction

Although iron is one of the most abundant minerals on earth, iron deficiency in humans is one of the most common nutritional problems in both the developing and developed countries (Monsen, 1999). Iron deficiency is mainly a problem for women of fertile age. The occurrence of depleted iron stores in young Danish women has been found to be 14 times higher than in men of the same age (Milmann & Kirchoff, 1992). Depleted ironstores in child-bearing women can lead to a severe iron deficiency if no iron supplementation is taken during pregnancy (Milmann, Agger & Nielsen, 1991). The long-term consequence of low iron stores is not known, but it has been shown that memory and learning performances are reduced (Bruner, Joffe, Duggan, Casella & Brandt, 1996).

Heme iron from meat is easily absorbed by humans while non-heme iron from both meat and other foods is absorbed to a lesser extent (Carpenter & Mahoney, 1992; Garcia, Martinez-Torres, Leets, Tropper, Ramirez & Layrisse, 1996; Hunt & Roughead, 2000) and the absorption of non-heme iron is influenced by the individual iron status and dietary factors (Carpenter & Mahoney, 1992; Hallberg, Sandstrøm & Aggett, 1993). In a recent study, it was found that 22–26% of the heme iron content was absorbed from a meat containing diet whereas only 2–3% of the non-heme iron content was absorbed from the diet (Hunt & Roughead, 2000). Thus, heme iron from meat has a superior bioavailability compared to non-heme iron. Previous work has found a decrease in heme iron content during heat treatment of meat and meat products, accompanied by an increase in non-heme iron content (Buchowski, Mahoney, Carpenter & Cornforth, 1988; Carpenter & Clark, 1995; Han et al., 1993) and it is believed that iron is liberated from the heme complex due to denaturation of myoglobin, followed by a release and degradation of the heme molecule (Awad & Deranleau, 1968; Kristensen & Andersen, 1997; Ledward, 1974, 1978). Sodium chloride is a commonly used additive in heat-treated meat products which increases the water-holding capacity and protein solubility by an increase in the ionic strength. It has been shown that the ionic

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strength in both meat (Lytras, Geileskey, King & Ledward, 1999) and model systems (Ahn & Maurer, 1989; Tomicki, Jackman & Stanley, 1996) influences the temperature at which denaturation of myoglobin occurs. However, the extent to which NaCl affects the heme-iron/nonheme-iron ratio (H/NH) in meat after heat treatment has not previously been given much attention.

The objective of the present work was to obtain basic information about how the stability of heme-iron in meat is influenced by processing temperature and addition of NaCl. This objective was achieved by studying the effect of NaCl concentration on the H/NH at various temperatures. To elucidate whether the effect of NaCl was an ion-specific effect or a general effect of the ionic strength, the effect on H/NH of several mono- and di-valent salts were also tested.

2. Materials and methods

2.1. Chemicals

Ferrozine [3-(pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine], neocuproine [2,9-dimethyl-1,10-phenanthroline], ascorbic acid and $FeCl_2.4H_2O$ were obtained from Sigma. All chemicals used were of analytical grade, and double deionized water was used throughout. Iron standard stock solution (100 ppm) was prepared by dissolving 0.356 g of $FeCl_2.4H_2O$ in 1 / 0.1 M HCl. Ferrozine colour reagent was prepared by mixing 75 mg ferrozine and 75 mg neucoproine in 25 ml water containing 1 ml concentrated HCl and stored at 4°C. Ascorbic acid solution and acidified acetone were prepared 24 h before use and stored at 4 and 25°C, respectively.

2.2. Meat preparation

Fourteen pork *longissimus* muscles were obtained from a local slaughter house 24 h post mortem. The meat was trimmed from visible fat and connective tissue, coarsely ground through stainless steel kidney-shaped knives (hole size: 3.7×1.6 cm) in two batches. Each batch was mixed thoroughly, vacuum packed in 500 g portions and stored at−20°C. The water content of the meat was 74.80%, as determined by heating at 105°C to constant weight. Before use, the meat was thawed at room temperature for 2 h.

2.2.1. Effect of heat treatment and addition of mono-valent salts

Meat (100 g) was mixed with salts, either 1.5 g or 3.0 g NaCl, 3.8 g KCl, 7.7 g NaI or 8.5 g KI. The meat was blended in a kitchen blender (KRUPS Type 708A) and vacuum packed. To enhance the heat transfer during cooking, the vacuum bag was squeezed with a block of plastic to a thickness of 3 mm and afterwards stored at 4°C overnight. Heat treatment of the meat was performed in thermostatted circulating water baths for 2 h, followed immediately by cooling in ice-water. The heat-treated meat was stored at−20°C overnight and iron determinations carried out the next day.

2.2.2. Effect of temperature and divalent salts

To overcome the decreasing meat pH, which occurs when specific divalent salts are added to meat (Table 1), a buffer was used to stabilize meat pH. Twenty millilitres of 2.5 M MES buffer [2-(N-Morpholino)ethanesulfonic acid], pH 5.9, were added to 8.2 mmol divalent salt or 24.6 mmol NaCl and mixed with 50 g of meat. The concentrations of the buffer and the divalent salt in the aqueous phase of the meat were 0.87 and 0.14 M, respectively. The concentration of NaCl was 0.42 M which, in terms of ionic strength, is equal to 0.14 M divalent salt. The meat was vacuum packed and heat-treated as described above.

2.3. Iron determination

2.3.1. Non-heme iron

Non-heme iron was determined using the method of Ahn, Wolfe and Sim (1993) and Carter (1971) with minor modifications. Meat (5 g) or 3.74 ml iron standard solution (0, 1, 2, 3 or 4 ppm) was transferred to a 50 ml poly-propylene centrifuge tube and 15.0 ml of 0.10 M citrate-phosphate buffer at pH 5.50 was added. The suspension was homogenised for 30 s at 13 500 rpm using an Ultra Turrax T25 with a stainless steel dispersing element mounted with an SR 18 rotor. Before use, the dispersing element was rinsed twice with 250 ml citrate-phosphate buffer for 3 min at 13 500 rpm, to minimise release of iron during homogenisation of the

Table 1
pH of meat with different ionic species added

Salt	Without MES[a]	With MES[b]
NaCl	5.50	5.91
KCl	5.65	–
NaI	5.82	–
KI	5.93	–
$CaCl_2$	4.81	5.74
$MgCl_2$	5.15	5.85
Na_2SO_4	5.62	5.97
K_2SO_4	5.70	5.96
Control	5.59	5.91

[a] All salts were added to a final ionic strength of 0.686 in the aqueous phase of the meat, which is equal to addition of 3.0% NaCl to meat.

[b] Concentration of MES, di-valent and mono-valent salts in the aqueous phase are 0.87, 0.14 and 0.42 M, respectively. monovalent salt (0.42 M) is equal to 0.14 M divalent salt in terms of ionic strength. "Control" indicates meat with MES buffer but without added salt.

samples. Homogenate (1.5 g) was transferred to a 5 ml sample tube, treated with 0.50 ml of 2% ascorbic acid in 0.2 M HCl and incubated at room temperature for 15 min. One millilitre of 11.3% trichloroacetic acid was then added to the sample and mixed thoroughly, then 2 ml was transferred to a 2.2-ml centrifuge tube and centrifuged for 10 min at 20 000 *g*. One millilitre of the clear supernatant was mixed with 0.40 ml of 10% ammonium acetate and 0.10 ml of ferrozine colour reagent. The mixture was filtered through a Minisart RC-15 filter (Satorius AG, 37070 Göttingen, Germany) of 0.2-μm pore size, transferred to a disposable semi-micro cuvette and absorbance read at 562 nm. The concentration of non-heme iron was calculated from a standard curve.

2.3.2. Heme iron

Heme iron was determined using the acidified acetone extraction method of Hornsey (1956) with modifications. Meat (5 g) was transferred to a 50 ml poly-propylene centrifuge tube and 10.0 ml of acidified acetone was added (95.7% acetone; 2.4% HCl). The suspension was homogenised for 30 s at 13 500 rpm, using an Ultra Turrax T25 which afterwards was rinsed 3 times with 3.00 ml of acidified acetone. The final concentration in the soluble phase of the suspension was 80% acetone and 2.0% HCl. The suspension was briefly mixed and stored on ice for 1 h. Insoluble substances were precipitated by centrifugation (1 h, 0°C, 10 000×*g*) and 5 ml supernatant was filtered through a Minisart RC 15 filter. The absorbance of the filtrate at 640 nm was measured, and the heme iron content was calculated using a molar extinction coefficient of 4800 M^{-1} cm^{-1} (Hornsey, 1956). All filtered samples were visually examined for turbidity before measurement. Turbid samples were filtered once more before measurement.

All results were corrected for addition of salt and buffer to the meat, and the results are presented as ppm iron in pure meat without any additions.

2.4. Statistical analysis

Duncan's Multiple Range Test (Montgomery, 1991) was used to determine statistically significant differences between treatment means.

3. Results and discussion

3.1. Effect of processing temperature on H/NH

The content of heme iron in meat decreases only slightly after heat treatment for 2 h at 55°C compared to a raw control which contains 2.7 ppm heme iron (Fig. 1). From 60°C to 80°C the heme iron content gradually decreases to 1.7 ppm after heat treatment at 80°C for 2 h. The decrease in heme iron content is followed by an increase in non-heme iron content of the meat from 2.0 ppm in the raw meat to 2.9 ppm in meat processed at 80°C. The correlation coefficient (r^2) between the means of heme and non-heme iron determinations at the seven temperatures shown in Fig. 1 is −0.92. It is therefore statistically reasonable to follow changes in the content of both heme and non-heme iron in meat during processing by measuring only one of the two iron forms.

The inverse relationship between heme and non-heme iron contents, as a function of heating temperature, is in agreement with previous work where both heme and non-heme iron were measured in meat heat-treated at different temperatures (Buchowski et al., 1988; Carpenter & Clark, 1995; Han et al., 1993).

Han et al. (1993) measured heme iron content in beef and chicken as a function of heating temperature and showed no significant differences between a raw unheated control and meat heated to 55°C. However, between 55 and 70°C a large decrease in heme iron content was observed, which is in accordance with our results. The major part of heme iron in meat is located in myoglobin and the thermal stability of myoglobin is highly dependent on an intact heme molecule (Chanthai, Ogawa, Tamiya & Tsuchiy, 1996a, b; Hargrove & Olson, 1996). A destruction of the heme molecule will therefore easily lead to denaturation of myoglobin. Thus, loss of iron from the heme molecule will not occur

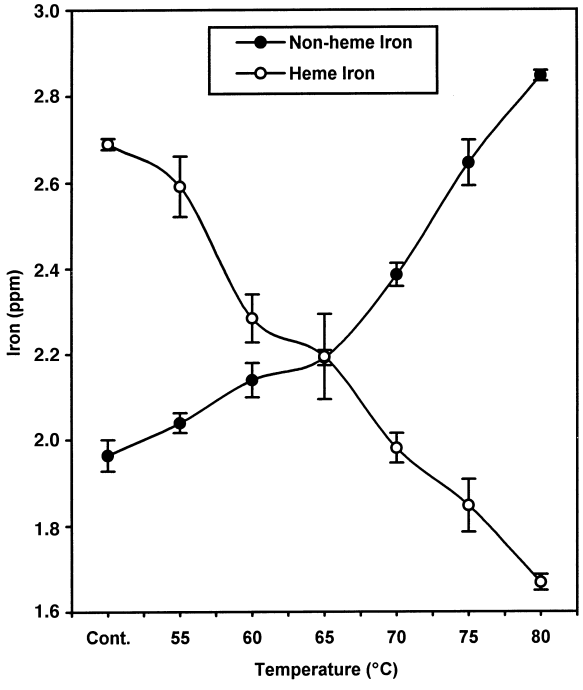

Fig. 1. Effect of heating temperature on heme and non-heme iron contents of meat. Meat was heated for 2 h. "Control" indicates raw (unheated) meat. Three replicates were made at each temperature in non-heme iron determinations and four replicates in heme iron determinations. Bars indicate standard error of the mean. The correlation (r^2) between heme and non-heme iron determinations was −0.92.

in heat treated meat without denaturation of the myoglobin molecule. Geileskey, King, Corte, Pinto and Ledward (1998) have investigated the kinetics of myoglobin denaturation in meat and found a very low denaturation rate at 55°C. At temperatures above 55°C, a large increase in denaturation rate was observed. Our results showed only small differences in both heme and non-heme iron content between raw unheated meat and meat heated at 55°C for 2 h (Fig. 1). An explanation for this could be that, at 55°C, the major part of myoglobin is not denatured to a degree where the iron from heme can be liberated, which is the case at higher temperatures. It was also found that the non-heme iron content increased in a linear manner ($r^2 = 0.97$) for up to 2 h of heat treatment when samples were taken out at 15 min intervals at 80°C (results not shown), which is in agreement with several related studies (Hamdaoui, Esseghaier, Hedhili, Doghri & Tritar, 1992; Schricker & Miller, 1983; Wang & Lin, 1994).

3.2. *Effect of processing temperature and NaCl addition on non-heme iron content*

Addition of NaCl to meat followed by heat treatment, is a procedure very often used in the meat and catering industries, and also in small-scale meal preparations in private kitchens and restaurants.

The non-heme iron content in meat, without added NaCl, increases from 1.6 ppm in the raw meat to 2.6 ppm in meat processed at 80°C for 2 h (Fig. 2; results presented in Fig. 1 and in Figs. 2–5 are from two different batches of meat). This increase in non-heme iron content is suppressed when 1.5 or 3.0% NaCl is added to the meat before processing, whereas non-heme iron only increases to 2.3 and 2.1 ppm, respectively, after processing at 80°C. Thus, addition of NaCl seems to protect the heme molecule from heat-induced destruction with a subsequent liberation of iron.

To test that the effect of NaCl on non-heme iron content in processed meat was a true effect and not caused by a NaCl-induced error in the method for determining non-heme iron, the heme iron content was determined in meat processed at 80°C with and without 3.0% NaCl (Fig. 2 insert). The heme iron determination showed the same results as the non-heme iron determinations, i.e. meat processed with NaCl had a higher amount of heme iron than meat processed without NaCl. Thus, the observed effect of NaCl on the non-heme iron content was not caused by an induced error in the method.

The effect of NaCl on non-heme iron content in heat treated meat has not previously been studied in any detail. However, Ahn et al. (1993) observed an increase in non-heme iron content in meat mixed with 2% NaCl before heat treatment in an electrical oven at 350°C for 30 min, compared to meat heat treated without NaCl. This result contradicts our observations. However, Ahn et al. (1993) discarded drippings from the heat-treated meat before measuring non-heme iron. Drippings from heat-treated meat have been shown to contain up to 20% of the meat's total iron content, which has leaked out of the meat during heating (Buchowski et al., 1988). Addition of NaCl decreases cooking loss from meat (Hamm, 1986) and thereby decreases the total amount

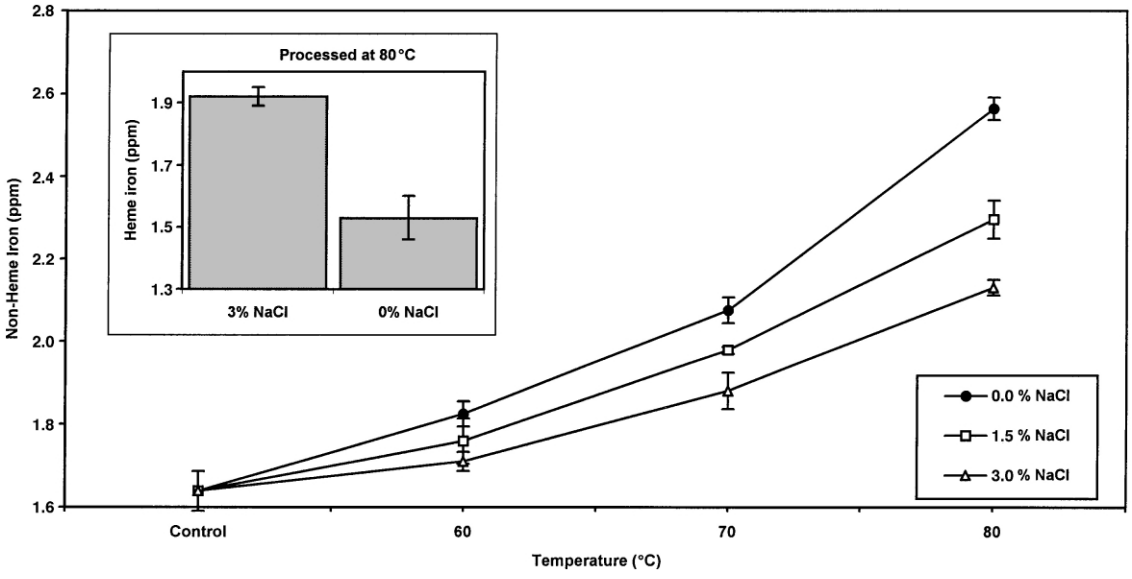

Fig. 2. Effect of NaCl addition and heating temperature on non-heme iron content of meat. Meat was heated for 2 h. "Control" indicates raw (unheated) meat. Three replicates were made at each temperature and NaCl level. Bars indicate standard error of the mean. Insert: heme iron content of meat with and without NaCl processed at 80°C for 2 h (Four replicates).

of iron which can leak out during heat treatment. The differences in non-heme iron content which Ahn et al. (1993) observed were therefore most probably caused by a decreased cooking loss in the NaCl treated meat, which results in a higher content of iron compared to meat without NaCl.

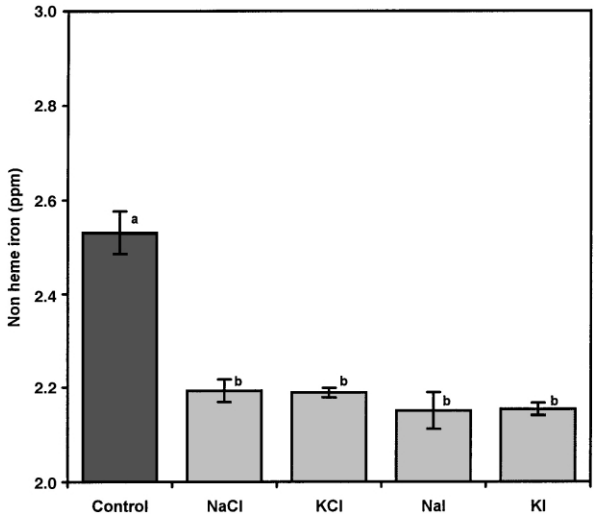

Fig. 3. Effect of monovalent salts on the non-heme iron content of meat after heating for 2 h at 80°C. The concentration of salt in the meat was 0.513 mmol/g, which equals the NaCl concentration in meat with 3.0% (w/w) NaCl added. ''Control'' indicates heat treated meat without added salt. Three replicates were made. Bars indicate standard error of the mean. Different subscripts indicate significant differences at the 5% level.

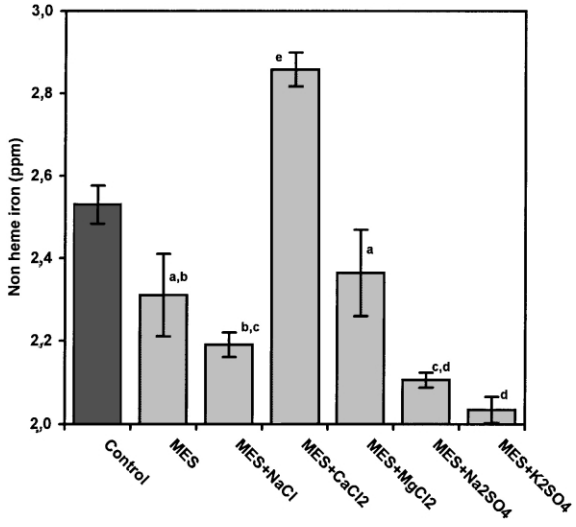

Fig. 4. Effect of divalent salts on non-heme iron content of meat after heating for 2 h at 80°C. All MES containing samples had a MES concentration of 0.87 M in the aqueous phase. The ionic strength added by $CaCl_2$, $MgCl_2$, Na_2SO_4, K_2SO_4 or NaCl was 0.42 in the aqueous phase. ''Control'' indicates heat treated meat without MES and salt. Three replicates were made. Bars indicate standard error of the mean. Different subscripts indicate significant differences at the 5% level.

3.3. Effect of heat treatment and addition of mono-valent salts

The protecting effect of NaCl on heme-iron during heat treatment can be caused by several mechanisms. The following study was done in order to test whether it is an ion-specific effect of NaCl or a general effect which also can be achieved by using other mono-valent salts. Addition of either NaCl, KCl, NaI or KI had a highly significant effect on the non-heme iron content in meat processed at 80°C for 2 h compared to meat processed without salt addition (Fig. 3). No significant differences in non-heme iron content were observed between meats treated with the four mono-valent salts. Thus, KCl, NaI and KI have the same protecting effect on the heme molecule as does NaCl. One possible explanation for this protecting effect on the heme molecule is the increase in ionic strength which occurs when salts are added to meat. The four mono-valent salts were all added to give an increase in the ionic strength of 0.686 in the aqueous phase of the meat which is equal to the addition of 3.0% NaCl. Changes in ionic strength are known to influence the rate of chemical reactions (Espenson, 1995). However, the mechanism and reaction order of iron liberation from heme in meat during heat treatment are not known. Consequently, our explanation for the protecting effect of mono valent salts cannot be verified at present.

The contents of non-heme iron in meat with and without NaCl were 2.2 and 2.5 ppm, respectively. These non-heme iron contents are not significantly different (Student's t-test, $\alpha = 5\%$) from the result presented in Fig. 2 with the same NaCl level (3%). The addition of

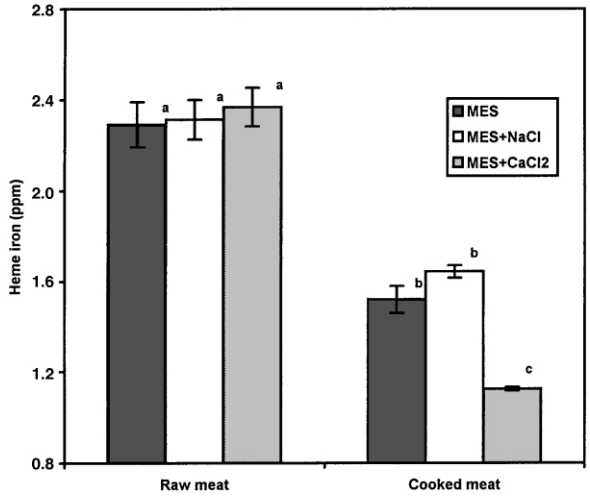

Fig. 5. Effect of $CaCl_2$ or NaCl on heme iron content of meat before and after heating for 2 h at 80°C. All samples had a MES buffer concentration of 0.87 M in the aqueous phase. The ionic strength added by $CaCl_2$ or NaCl was 0.42 in the aqueous phase of the meat. ''Control'' indicate meat with MES buffer but without added salt. Four replicates were made. Bars indicate standard error of the mean. Different subscripts indicate significant differences at the 5% level.

mono-valent salts had only a minor effect on the pH of the meat (Table 1).

In conclusion, the results presented above indicate that addition of NaCl or other mono-valent salts to meat before heat treatment increases H/NH in heat-treated meat by protecting the heme molecule from destruction and, thereby, release of iron. This implies that the nutritional value of processed meat could be optimised, in terms of iron bioavailability, by manipulating the processing parameters, such as temperature and time, in connection with increased ionic strength. In the production of cured meat products, small amounts of nitrite are added to the meat, together with a relatively large amount of NaCl. Nitrite has been reported to stabilise the heme molecule in meat products during processing (Chen, Pearson, Gray, Fooladi & Ku, 1984; Igene, Yamauchi, Pearson & Gray 1985; Schricker & Miller, 1983). However, to what extent the NaCl influences this effect of nitrite is not known.

3.4. Effect of heat treatment and addition of di-valent salts

In order to test that the protecting effect of monovalent salts also applies for divalent salts, four divalent salts were tested in comparison with NaCl.

Addition of $CaCl_2$ and $MgCl_2$ to meat without added buffer had a gross effect on pH of the raw meat (Table 1). The two salts changed pH from 5.59 in untreated meat to 4.81 and 5.15, respectively, after addition of the two salts. Geesink, Smulders and van Lack (1994) observed the same effect on meat pH by addition of $CaCl_2$ and especially by addition of $ZnCl_2$. To overcome this salt induced effect on meat pH, a buffer (MES) was added to the meat. Addition of buffer to a large extent kept pH of the meat fairly constant (Table 1). After addition of buffer, the pH of the meat ranged from 5.74 in $CaCl_2$-treated meat to 5.97 in Na_2SO_4 treated meat.

The effects of di-valent salts on non-heme iron content in meat processed at 80°C for 2 h are presented in Fig. 4. There are no significant differences between MES- and MES + NaCl-treated meat, and MES and MES + $MgCl_2$. No significant differences were observed between MES + NaCl and MES + Na_2SO_4, or between MES + Na_2SO_4 and MES + K_2SO_4. However, meat with added MES + $CaCl_2$ was very significantly different in non-heme iron content from all other treatments.

To test that the effect of $CaCl_2$ on non-heme iron content in processed meat was a true effect and not caused by a $CaCl_2$-induced error in the method for determining non-heme iron, the heme iron content was determined in meat processed for 2 h at 80°C with added $CaCl_2$ or added NaCl (Fig. 5). The heme iron determination showed the same results as the non-heme iron determinations did, i.e. meat processed with $CaCl_2$ had a significantly lower amount of heme iron than meat processed with NaCl or without salt. Thus, the effect of $CaCl_2$ on non-heme iron content was not caused by an induced error in the method for determining non-heme iron.

The results presented in Fig. 5 also show that there are no differences in heme iron content in the raw meat after addition of NaCl or $CaCl_2$. This indicates that the effect of $CaCl_2$ is stimulated by heating.

The lack of significant differences in non-heme iron content between meat treated only with MES and meat treated with MES + NaCl (Figs. 4 and 5) are in contradiction to the results presented in Figs. 2 and 3, where a highly significant difference were observed between NaCl treated and non-NaCl treated meat. However, in Figs. 4 and 5, all samples contained MES to a concentration in the aqueous phase of 0.87 M, which is twice the concentration of NaCl (0.42 M). How much of the MES that is dissociated in meat is not known, but it seems reasonable to assume that addition of 0.87 M MES increases the ionic strength in the meat. This MES-induced increase in ionic strength most probably masks the effect of afterwards increasing the ionic strength with NaCl, which was suggested in connection with Fig. 3. This explanation is partly confirmed in Fig. 4 where it is shown that MES addition decreases the non-heme iron content compared to the control without added MES.

The four divalent salts were all added to give an increase in the ionic strength of 0.42 in the aqueous phase of the meat. Since there are highly significant differences in non-heme iron content between meat treated with the four salts, our suggestion that the increase in ionic strength might explain the effect of monovalent salts does not solely apply to the effect of divalent salts. It could be argued that the small differences in pH which exist between meats with the four divalent salts added (Table 1) can explain the different non-heme iron contents in the meat. However, these pH differences are smaller than the pH differences which occurred by adding monovalent salts to meat and there were no differences in non-heme iron contents in these samples (Fig. 3). Consequently there does not seem to be an effect of pH on non-heme iron content of meat in the pH range presented in Table 1.

The pH of meat has an effect on the ionic strength by changing the degree of MES-dissociation. However, the small changes in ionic strength caused by this process cannot explain the gross differences in non-heme iron contents between meats with added $CaCl_2$ and meat with added $MgCl_2$, Na_2SO_4, K_2SO_4 or NaCl. It is therefore supposed that $CaCl_2$ has a specific destabilising effect on the heme molecule in meat. Since $MgCl_2$, NaCl or KCl do not have the same effect as $CaCl_2$ it is most probably the calcium ion which is the active component of $CaCl_2$ in destabilising the heme molecule in meat during heat treatment.

The mechanism by which the calcium ion destabilises the heme molecule is not known. More experimental work will be needed to elucidate this mechanism.

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