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# THE EFFECT OF HEATING TEMPERATURE AND HEATING TIME ON TBARS, WATER SOLUBLE AND DIFFUSATE IRON IN BEEF AND PORK

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## BACKGROUND AND OBJECTIVE

Muscle phospholipids slowly oxidize during chill storage of raw meat. Heat treatment changes the oxidative stability of meat, and phospholipid oxidation proceeds at a much higher rate during chill storage of cooked meat. These oxidative changes are associated with the development of an off-flavor known as warmed-over flavor (WOF).

WOF is usually measured by the level of thiobarbituric-acid-reactive substances (TBARS). TBARS development during chill storage of cooked meat is affected by heating temperature. Increasing cooking temperatures below 100°C accelerate TBARS development [1], while higher heating temperatures retard TBARS development due to the formation of antioxidative Maillard-reaction products [2]. The acceleration of oxidative processes induced by heat treatment is normally explained by disintegration of sarcoplasmic membranes and production of prooxidants by denaturation of myoglobin and other iron-containing proteins. Denaturation might turn iron-containing proteins into prooxidants by exposing [3] or liberating [4] prooxidative iron.

Han *et al.* [5] examined how heating temperature affected various iron pools in beef and chicken meat. While the total iron content was unaffected by heating, the content of water soluble iron (WSI) and iron in heme proteins and ferritin decreased, and the content of water insoluble iron, diffusate iron (DI) and iron in hematin increased with increasing heating temperature.

The objective of this work was to study the changes in TBARS, WSI and DI after heat treatment of beef and pork meat. Heating temperatures in the range 60-70°C were studied in greater detail to correlate the different levels of TBARS developed at these temperatures with the changes in iron distribution.

## METHODS

*Sample preparation:* Beef *semitendinosus* and pork *longissimus* muscle were trimmed from visible fat and connective tissue, ground through a 4-mm plate and mixed thoroughly. Meat for iron and TBARS determinations was divided into 70 and 20 gram samples, respectively. The samples were vacuum packed, and samples for TBARS determinations were pressed into 4 mm thick plates. All samples were stored at -18°C for a maximum of four weeks. The samples were used in two experiments investigating the effects of heating temperature (HTEMP) and heating time (HTIME). Experiment HTEMP was performed on both types of meat, but only ground beef was used in experiment HTIME.

*Heat treatments:* Meat for iron determinations was thawed in tap water, and 10 grams were mixed with 10 ml deionized water in centrifuge tubes. Meat for TBARS determination was heated directly in the vacuum bags. Samples for experiment HTEMP were cooked for 30 minutes in a water bath preheated at 50, 55, 60, 62, 64, 66, 68, 70, 75 or 80°C, and samples for experiment HTIME were cooked for 0, 10, 20, 30, 40, 50 or 60 minutes in a water bath preheated at 70°C. Subsequently all samples were chilled in an ice/water bath for 10 minutes. Heating at 80°C caused essentially uniform temperatures within 2 minutes in samples for TBARS determinations, while temperature equilibration took 15 minutes in the samples for iron determinations.

*Thiobarbituric-acid-reactive substances (TBARS):* Samples for TBARS determinations were repacked in polyethylene bags and stored at 4°C for 2 days. TBARS were quantified on triplicate samples by an extraction procedure [6], and were expressed as µmoles malonaldehyde equivalents per kg dry matter to correct for differences in water loss after different heat treatments.

*Iron determinations:* WSI and DI were quantified as described by Han *et al.* [5] with minor modifications. Triplicate samples were homogenized, centrifuged for 20 minutes at 3000*g*, and the supernatant was filtered. The sediments were resuspended in 10 ml deionized water, homogenized, centrifuged and filtered three more times. The pooled filtrate was analyzed for WSI. 20 ml filtrate were dialysed against 100 ml deionized water for 1 day in a cellulose tube with a cut-off value of 12000 D. The diffusate was freeze dried, and the dried sample was dissolved in 10 ml 3 M HCL. This solution was analyzed for DI. DI was not quantified in experiment HTIME. Iron determinations were made on a Perkin-Elmer 5000 Atomic Absorption Spectrophotometer.

## RESULTS AND DISCUSSION

Observed values of TBARS, WSI and DI in ground beef and pork from experiment HTEMP are shown in Fig. 1. Standard deviations of TBARS, WSI and DI were 12, 1.7 and 0.7, respectively, in beef, and 3.4, 0.8 and 0.4, respectively, in pork.

Several papers have demonstrated that TBARS development during chill storage of various types of cooked meat is affected by the heating temperature. Heating at 70°C cause a significantly faster development of TBARS than heating at 60°C [1,7,8]. Increasing cooking temperatures in the range 70-100°C might slightly decrease [1], slightly increase [7] or have no effect on TBARS [8]. In this experiment the rate of TBARS development mainly increased as cooking temperatures was increased from 62 to 75°C.

Han *et al.* [5] showed that higher heating temperatures cause lower concentrations of WSI and higher concentrations of DI in beef and chicken thigh. The most pronounced changes were observed when the temperature was increased from 55 to 70°C. Fig. 1 shows similar decreases in WSI in beef and pork, but WSI in beef was gradually decreasing with increasing temperature in the range 60-80°C, while WSI in pork mainly decreased in the temperature range 55-66°C. WSI in beef *semitendinosus* muscle was approximately three times higher than WSI in pork *longissimus*. DI in beef and pork was not significantly affected by heating temperature due to a relatively high standard deviation.

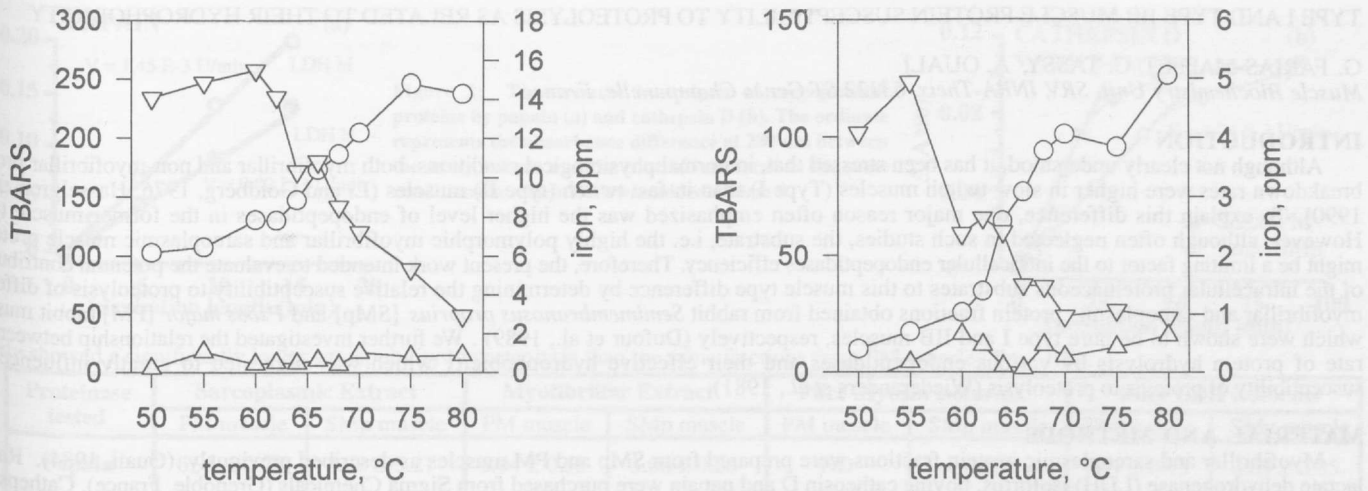


**Fig. 1** Development of TBARS (o) and content of water soluble (▽) and diffusate (△) iron in ground beef (left) and prok (right) heated at various temperatures.

Various forms of iron are important prooxidants in meats. The change in iron distribution after heat treatment shown by Han *et al.* [5] results in an increased prooxidative activity of the various forms of iron. Nonheme iron content is highly correlated to TBARS development in cooked beef [4,9]. In this study the correlation coefficient between TBARS and WSI was 0.95 in beef and 0.89 in pork. The slightly lower correlation in pork reflects that changes in WSI started at lower temperatures than changes in TBARS. These results support the assumption that heat induced changes in iron distribution increase the oxidative lability of meat.

The preceding results show that increasing heating temperatures cause almost parallel increases in TBARS and decreases in WSI. More intense heat treatments were obtained in experiment HTIME by longer heating times in stead of higher heating temperatures. Fig. 2 shows TBARS and WSI from experiment HTIME. Longer heating times caused higher levels of TBARS and lower amounts of WSI in the heated samples. But the changes were not parallel: TBARS were accelerated when the heating time increased from 10 to 50 minutes, while the decrease in WSI was manifest within the first 10 minutes. Furthermore, a higher level of TBARS was developed in raw samples than in samples heated for less than 40 minutes, while the main decrease in WSI took place in the beginning of the heat treatment. The correlation coefficient between TBARS and WSI was only 0.15. When observations from the raw meat were excluded, the correlation increased to 0.64. The very low correlation obtained when raw samples are included seems to suggest that other factors than insoluble forms of iron are responsible for oxidation in raw meat. The faster loss of WSI than increase in TBARS development might indicate that further degradation processes are needed to convert heat denatured iron-containing proteins to prooxidants.

Temperature equilibration was rather slow in samples for WSI measurement, and it was probably not obtained in the samples heated for 10 minutes. This does not explain the low correlation obtained in experiment HTIME, because the different heat transfer rates should cause a slower decrease in WSI than increase in TBARS.

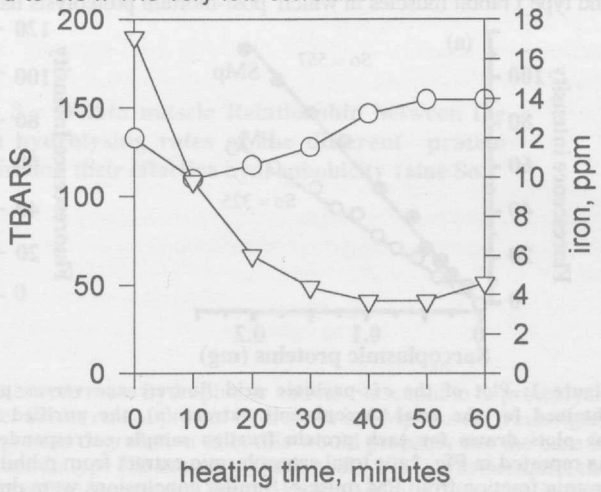


**Fig. 2** Development of TBARS (o) and amount of water soluble iron (▽) in ground beef heated at 70°C for 0 to 60 minutes.

## LITERATURE


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