# EFFECTS OF EXTRACTION AND DETECTION METHOD ON THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS) VALUES IN RAW OR HEATED BEEF AND PORK LONGISSIMUS MUSCLES

Sasaki K.[1], Mitsumoto M.[1], Yatabe T.[2], Chikuni K.[1]

[1] Department of Animal Products, National Institute of Livestock and Grassland Sciences, Tsukuba, Ibaraki 305-0901, Japan

[2] Swine Research Center, Ibaraki Prefectural Livestock Institute, Edosaki, Ibaraki 300-0508, Japan

## Background

Thiobarbituric acid reactive substances (TBARS) have been used as an index of lipid peroxidation in meat, meat products and other kind of foodstuffs. The products of lipid peroxidation developed in meat and meat products during storage are the source of warmed-over flavor[1)], and are cytotoxic and mutagenic compounds[2)]. Thus, lipid peroxidation has been considered an inferior reaction for the quality of meat and meat products. Estimation of TBARS values is important for evaluating and maintaining the quality of meat and meat products.

On the other hand, many factors, not only TBARS, must be determined at the same time for research and evaluation of meat quality. An improvement of sample preparation method has been needed for determination of TBARS and other different measurements related to meat quality in the same pre-treatment.

In addition, most of consumers heat meat for their meal. TBARS values in heated meat are important for meat quality, such as sensory property and human health. The difference of TBARS values between raw and heated meat must be compared for improvement of meat quality evaluation.

Throughout of these investigations, we intend to improve the determination method of TBARS values in muscle food samples.

## Objectives

In the present study, we investigated the effect of extraction and detection method on estimated TBARS values in raw and heated meat.

At present, we have been used homogenization with 20% trichloroacetic acid (TCA) for extraction of TBARS from muscle samples[3)]. This extraction method is very simple and easy to use[4)], but is not fit for extraction and determination of other quality related substances, such as nucleic acids and oligopeptides. We tried to prepare homogenate of meat samples with water before TBARS extraction with TCA for application of the homogenate for determination not only TBARS values but also other measurements.

On the other hand, spectrophotometric analysis was generally used for the determination of reacted products of lipid peroxide and thiobarbituric acid (TBA), but is not suitable for detection of TBARS at low concentration. We compared the TBARS values estimated by spectrophotometry and fluorophotometry in the same samples.

## Methods

*Samples*: Beef longissimus muscles were obtained from six Japanese Black cattle. Pork longissimus muscles were sampled from Landrace (n=3), Duroc (n=3) and crossbred pigs (Landrace×Large White×Duroc, n=4). These samples were stored at –20°C for beef or -30°C for pork in vacuum-package, and applied to experiments immediately after they were thawed.

*Experimental Design*: Each muscle sample was divided into two parts, one was for determination in raw form and another was for analysis in heated form. Raw and heated samples were additionally divided into two parts for two extraction methods as indicated in *TBARS extraction* section.

*Heat Treatment*: Samples were formed into rectangular prism with a base 4 cm × 4 cm and a thickness of 2 cm. The formed muscles were put into PVC bags and incubated in the water bath at 70□ for 30 minutes after reaching 70°C of the inner temperature of meat samples.

*TBARS extraction:* Two methods were used for TBARS extraction. (1) *TCA method*:[4,5)] 10 grams of raw and heated samples were homogenized with 50 ml of 20% TCA reagent and made up to 100 ml by distilled water. The homogenate was filtered with ADVANTEC No.131 filter paper, and the filtrate was applied to TBA reaction. (2) *Water-TCA method*: 10 grams of raw and heated samples were homogenized with 50 ml of distilled water and made up to 100 ml. The homogenate was picked up and was admixed with equal volume of 20% TCA solution. The mixture was filtered as similar as *TCA method*, and the filtrate was applied to TBA reaction. Extracts prepared as the previous two methods were admixed with equal volume of 5 mM TBA solution. These mixtures were incubated at room temperature in the dark for 16 hours.

*TBARS detection*: TBARS was detected by the following: (1) Spectrophotometric detection at 532 nm, (2) Fluorophotometric detection; Ex. 515 nm and Em. 553 nm. 1,1,3,3-Tetraethoxypropane was used as the standard.

## Results and discussion

Mean TBARS values in raw or heated beef and pork extracted and determined with different methods were indicated in Table 1.

In raw samples, TBARS values in *Water-TCA method* were differ from the values in *TCA method*. Witte *et al.*[4] developed the TCA extraction (*TCA method* in the present study) and compared with distillation method for the determination of TBARS values in beef and pork, and indicated that the extraction efficiency was different in differ extraction method. Therefore, the difference of TBARS values between *TCA method* and *Water-TCA* method was considered as a natural result.

Witte *et al.*[4] also indicated that TBARS values determined by TCA extraction method were highly correlated (r = 0.845) with the values assessed by distillation method in pork, and considered the TCA extraction method as a substitute for distillation method. In the present study, the correlation coefficient of TBARS values between *TCA method* and *Water-TCA method* was not high in raw pork (r = 0.478 by spectrophotometric assay, r = 0.785 by fluorophotometric assay), but was high in raw beef (r = 0.965 by spectrophotometric assay, r = 0.987 by fluorophotometric assay). In raw beef, *Water-TCA method* is suitable for evaluation of relative TBARS values as similar to *TCA method*. But in raw pork, further investigation was needed for TBARS determination by *Water-TCA method*.

In heated samples, TBARS values assessed by *Water-TCA method* were not significantly correlated (P>0.05) with the values assesed by *TCA method* in both beef and pork. In addition, TBARS values in *Water-TCA method* were higher than that of *TCA method* (approximately 3.8- fold for beef and 6.0- fold for pork), differ from the results in raw samples. These results suggested that heat-treatment increased water- and 5% TCA-soluble TBA reactive components in beef and pork. Lipid peroxidatioin has been generally considered to be accelerated by heat treatment. But in heated samples, TBARS values in *Water-TCA method* may include TBA reactive aldehydes other than lipid peroxidation products. Changes in TBA reactive aldehydes during heat-treatment must be examined in both beef and pork.

TBARS values in fluorometric assay were not similar to those in spectrophotometric assay. But the values in fluorophotometric assay were highly correlated with the values in spectrophotometric assay (r = 0.850 – 0.999, P<0.01). We considered that fluorophotometric assay was suitable as similar to spectrophotometric assay, especially at low TBARS concentration.

## Conclusions

*Water-TCA method* was suitable for TBARS extraction in raw beef, but not in raw pork and heated samples. In addition, fluorophotometry fitted for the detection of TBARS as similar to spectrophotometry at low concentration.

## Pertinent literature

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Table 1. TBARS values in raw or heated beef and pork longissimus muscles determined by different extraction and detection methods

| | Raw samples | | | | Heated samples | | | |
|---|---|---|---|---|---|---|---|---|
| Extraction | *TCA method* | | *Water-TCA method* | | *TCA method* | | *Water-TCA method* | |
| Detection | *S* | *F* | *S* | *F* | *S* | *F* | *S* | *F* |
| Species | | | | | | | | |
| **Beef** | 2.10 | 2.13 | 1.82 | 2.64 | 3.21 | 4.07 | 12.33 | 15.19 |
| **Pork** | 0.50 | 0.84 | 0.24 | 1.35 | 1.30 | 1.52 | 7.81 | 9.35 |

Values are indicated as least-squares means of nanomoles malondialdehyde equivalents per gram of sample.
Outlines of heat-treatment and extraction were indicated in **Methods** section.
Detection; *S* means spectrophotometry, and *F* means fluorophotometry.