Comparison of Distillation and Extraction Methods in TBARS Determination of Cured Meat

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Thiobarbituric acid reactive substances, TBARS, has been well determined as an index of lipid oxidation in food. Addition of nitrite as a color development agent to cured meat results in a low TBARS value. Therefore, we examined whether nitrite suppresses lipid oxidation or if it interferes with color development during determination of TBARS. The relationship between nitrite concentration and TBARS value was compared under both extraction and distillation methods. TBARS values, whether determined by either extraction or distillation method, were depressed with concentrations of nitrite above 80 ppm. When adding either Orange I reagent, OI, as a coupling reagent with nitrite or sulfamic acid, SA, which decomposes nitrite, the decline in the TBARS value was restrained. Nitrite interference could be eliminated completely in the extraction method, which used SA, and this procedure was the most effective. However, these reagents were not effective under strongly acidic conditions. This observation suggests that TBARS reacts with nitrite resulting in a substance which does not develop any color with TBA. The extraction method is here recommended because of the high recovery of TBARS value and rapid operation compared with the distillation method.

Keywords: lipid oxidation, TBARS in cured meat, interference of nitrite, extraction, distillation

Introduction

Nitrite is an important ingredient in cured meats. Apart from producing the characteristic cured meat color, it acts as a potent antioxidant and plays a key role in prevention of warmed-over flavor on storage. It also has an important antimicrobial effect. The reaction between 2-thiobarbituric acid, TBA, and MA, a secondary oxidation product of polyunsaturated fatty acids, is widely used as a measure of rancidity development in meat and its products. The distillation method of Tarladgis et al. is commonly used, sometimes with modifications. Apart from distillation, extraction methods which are less time consuming have also been developed using the TBA reagent. However, the presence of nitrite in cured meats renders the TBA test inadequate for determination of oxidative deterioration in such products. Nitrite reacts with both TBA and MA leading to underestimation of the values obtained. Efforts have been made to eliminate the nitrite interference either by use of various reagents or

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Abbreviations

TBARS: Thiobarbituric acid reactive substances, SA: Sulfamic acid, OI: Orange I reagent, MA: Malondialdehyde.

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by chromatographic techniques\[1,10\]. Attempts have also been made to study the reactions taking place between nitrite, MA, and TBA. Results from these studies show that nitrite retards formation of carbonyl compounds when added before cooking and at low pH reacts completely with MA leading to the depressed values\[6\]. It has been shown that sulfanilamide can prevent the reaction between nitrite and MA\[6\].

In this study, an attempt was made to evaluate existing procedures to determine a suitable one for use in cured meats either by distillation or extraction. The effects of different reagents which can either form coupling reactions with nitrite or cause its degradation to give better TBARS of cured meats were studied.

Materials and Methods

Reagents
All reagents were analytical grade. TBA, trichloroacetic acid (TCA), sulfanilamide, naphthol and SA were from Wako Pure Chemicals; sulfanilic acid was from Isuzu Pharmaceutical Company.

Preparation of meat samples
Previously frozen meat was allowed to thaw overnight at 4°C. It was then trimmed of fibrous tissue and ground twice through 4mm diameter hole plate using a hand grinder. The meat was placed on plastic trays inside a polyethylene bag. The atmosphere of the bag was evacuated and then flushed with oxygen for approximately 5min. The trays were kept in an incubator at 34°C for 15 hr to stimulate reactivity development. The trays were transferred to a refrigerator and held at 4°C until used. Two hundred g of meat was homogenized with 1400ml distilled water at high speed in a Physequotron NS-50 homogenizer (Nitto Co Ltd). A part of the suspension was thoroughly mixed and used for all determinations.

Measurement of TBARS
The extent of lipid oxidation in all samples was evaluated by the TBA test using the distillation method of Tarladgis et al\[4\] as modified by Izumimoto et al\[10\] and the extraction method of Izumimoto et al\[10\]. The effect of nitrite on the TBARS was determined as follows: To each 80ml suspension, 10ml sodium nitrite solution containing 0 - 1.2 mg/ml, was added to correspond to a final concentration of 0 - 1250ppm nitrite on meat weight basis. The samples were held for 1 hr. Ten ml, 20% TCA was then added and the mixture held for a further 30min before distillation. The effect of SA and OI solution\[10\] on the TBARS in the presence of nitrite was determined as follows: to 80ml suspension, 10ml of nitrite solution containing 0.088 and 0.15mg/ml was then added to give a final concentration of 0, 80 and 150ppm on a meat basis. The samples were held for 1 hr.

The first treatment consisted of the addition of 2ml of 0 or 2% SA after treating with nitrite. The samples were allowed to stand for 30min after the addition of SA. Ten ml, 30% TCA was added before or after addition of SA to determine the effect of the sequence of addition on the TBA reaction with nitrite. The samples had a total volume of 160ml each and were held for 30min. This was followed by distillation for TBARS.

In the second treatment, samples were prepared similar to control. After addition of nitrite and holding for 1hr, 40ml OI solution was added. OI solution contains 0.14g naphthol and 0.5g sulfanilic acid in 14% acetic acid which enables it to form an azo dye with nitrite. Ten ml of 20% TCA was added before or after OI treatment. The addition of TCA was followed by a holding time of 30min. In each case, 50ml of distillate was collected for approximately 10min after boiling started.

Extraction procedure was also carried out to study the effect of nitrite on the TBARS. The samples were prepared in the same way as the
control. This was followed by treatment with or without SA to the suspension as described above. As color develops with OI treatment in the presence of nitrite, this treatment was not performed with the extraction method. Ten ml 20% TCA was added to the suspension. After 30 min, the samples were filtered using Toyo No. 2 filter paper. The filtrate collected was used for the test.

In both the distillation and extraction procedures, a 2ml aliquot of the collected sample was reacted with 2ml of the TBA reagent for the test. Extinction was measured at 532 nm using a Shimadzu UV-160 spectrophotometer.

Results and Discussion

The recovery of TBARS value decreased with increasing concentrations of nitrite. The results are shown in Fig. 1. The figure shows that at low nitrite levels up to 80 ppm, the TBARS value is depressed by 10% or less by distillation while the extraction procedure has a higher recovery. The addition of 150 ppm of nitrite caused the values to decrease by approximately 25%. However, at high concentrations of nitrite and the low pH required for the TBA test, nitration of the MA takes place leading to the excessive depression in the TBARS value observed8. The reactions taking place have not been fully elucidated, but depression of the TBARS value may be due to either coupling of the nitrite and the TBARS9 or the prevention of lipid oxidation from taking place. However, the experiment was carried out using meat model systems and there was a definite underestimation of TBARS in the presence of nitrite. Two reagents were tested to break up the complex formed between nitrite and TBARS

A method using an OI reagent was used to eliminate nitrite interference through complex formation with nitrite10. This is able to form an azo-dye with nitrite and thus remove it from the reaction. Yamashita and Araki11 reported that when they used this reagent they were able to eliminate nitrite interference completely. The results obtained are shown in Table 1. The OI solution had a recovery of 91% when it was added before TCA. The value decreased with increasing nitrite concentration from 0 - 150 ppm. In the absence of nitrite, the values were lower than for control. When added after addition of the TCA, the recovery dropped to 82%. The product formed between nitrite and TBARS could not be broken by OI once it was formed.

Zipser and Watts12 modified the test for cured meats by addition of sulfanilamide. This reacts with nitrite to form a diazonium salt thus freeing MA to take part in the TBA reaction. However, later work showed that when nitrite was absent or in low concentration, TBA numbers were lower for samples containing sulfanilamide13,14. This was thought to be due to the reaction of sulfanilamide with MA15,16. Though it has been reported that sulfanilamide is able to react with nitrite and thus enable oxidative deterioration to be determined in cured meats using the TBARS reaction15,16. This is also a coupling reaction similar to the OI reaction.
Tab 1 Effect of SA and Oil on TBARS value of meat in the presence of nitrite

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<thead>
<tr>
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<th>NaNO3 (ppm)</th>
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<tr>
<td></td>
<td>0</td>
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<tr>
<td>Extraction</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0.368</td>
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<tr>
<td></td>
<td>(100)</td>
</tr>
<tr>
<td>SA before TCA</td>
<td>0.377</td>
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<tr>
<td></td>
<td>(105)</td>
</tr>
<tr>
<td>after TCA</td>
<td>0.387</td>
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<td></td>
<td>(81)</td>
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<td>Distillation</td>
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<tr>
<td>control</td>
<td>0.312</td>
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<tr>
<td></td>
<td>(100)</td>
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<tr>
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<td>(92)</td>
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<tr>
<td>after TCA</td>
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<td>Oil before TCA</td>
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<td>(83)</td>
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<td>after TCA</td>
<td>0.296</td>
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Numbers in parentheses represent percentage TBARS recovery. SA or O1 was added to samples before or after addition of TCA.

SA was also evaluated. This compound is able to degrade nitrates and nitration compounds. It was therefore expected to be able to reduce TBARS from any compound it formed with nitrite. The results obtained are shown in Table 1. Both distillation and extraction procedures were carried out. Extraction procedure gave higher recovery particularly when SA was added before addition of TCA. The results also show that SA had slightly higher recovery values in the absence of nitrite. This may be attributed to interfering factors inherent in the meat itself though the reason remains unknown. SA showed a tendency to maintain constant and satisfactory recovery values even at high nitrite concentration when added before TCA. However, when added after the TCA, there was a decreasing tendency. These trends were observed for both distillation and extraction. This shows that though SA is able to prevent nitrite interference, it cannot degrade all of the TBARS-N0 complex after acidification. This observation suggests that TBARS react with nitrite resulting in a substance which does not develop any color with TBA.

Conclusion

In the presence of nitrite, TBARS values of cured meat were underestimated. However at 0 - 40 ppm nitrite the effect was low. The TBARS values determined by either extraction or distillation method were depressed at nitrite levels above 40 ppm. When adding SA, the decline of the TBARS value was restrained more than with oil. The interference of nitrite at 150 ppm could be eliminated completely in the extraction method which used SA. Furthermore, the extraction procedure was simpler and less time consuming than the distillation procedure.

References

塩漬肉の TBARS 測定における蒸留法と抽出法の比較

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（生産物利用学講座）

食品の黄変酸化の指標として TBA 反応物質（TBARS）がよく測定されている。肉製品には発色帯の
亜硝酸塩が添加されるが、これによって TBARS が低く測定される。そこで、亜硝酸塩が亜硝酸化を抑制す
るのか、TBARS 測定の呈色反応を阻害するのかを調査を行い、TBARS 測定値と亜硝酸塩濃度との関係につ
いて、抽出法と蒸留法について比較した。

抽出法および蒸留法ともに亜硝酸塩50 ppm以上になると TBARS 値の低下が認められた。亜硝酸塩との
カップリンを試験であるオレンジ試験（0）あるいは亜硝酸塩分解試薬をサルファミン酸（SA）を添加した
ところ、TBARS 値の低下が見られた。この亜硝酸塩の影響は SA を使った抽出法で制限でき、最も効果
的であった。これらの試験が試料を強酸性にした後に添加されると効果が認められなかったので、TBARS は
亜硝酸塩と反応して TBA 呈色物質になることが示唆された。