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Effect of Cytochrome c on Pork Fat Oxidation Measured by TBA Test

Moo-Ha Lee and R.G. Cassens*

Korea Advanced Institute of Science and Technology, Seoul
**Muscle Biology Laboratory, University of Wisconsin-Madison, U.S.A.*

Cytochrome c가 돼지지방산화에 미치는 영향

이무하 · 알 지 캐센스*

한국 과학기술원, *미국 위스컨신 주립대학교 근육생물학연구실

Abstract

The effect of cytochrome on pork fat oxidation was studied either in the absence or in the presence of nitrite and/or ascorbate. Results showed that the back-fat oxidation measured by TBA test increased with increasing concentration of cytochrome c but the increment decreased with increasing concentration. The addition of ascorbate alone to cytochrome c did not prevent the oxidation. The same result was obtained with the addition of nitrite alone to cytochrome c. However, the backfat oxidation was prevented by the addition of nitrite and ascorbate together. With the rendered fat, the trends were more obvious than with backpat.

Introduction

It is generally accepted that heme proteins accelerate lipid oxidation in meat. Igene et al.[1] reported that the increased rate of lipid oxidation due to heme pigments in cooked meat was due to non-heme iron released from heme pigments during cooking. Ferrous ion, which possesses a high catalytic activity in hydroperoxide formation at pH 5.8 might initiate the oxidation of phospholipids by forming free radicals.[2] Cytochrome c is one of heme proteins present in all organisms that contain a mitochondrial-respiratory chain. Phospholipids which are in close proximity with the heme catalysts of the mitochondria could take the primary responsibility for rancidity development in meat.[3,4,5]

Cytochrome c has shown to catalyze the oxidation of lipid.[6,7,8] However, Banks et al.[8] reported that cytochrome c had an antioxygenic effect at higher concentration ($3.5\times10^{-5}M$) when a suspension of linoleic acid was used as a lipid source. It was due to the decomposition of lipid peroxide by cytochrome c and the denaturation of cytochrome c.[8,9]

Heart is rich in cytochrome c. It has 16-20 *μmoles/kg* comparing to 4*μmoles* in skeletal muscle.[10] Many cured meat products showed the inclusion of heart ranging from 5 to 20% in their formulations.[11] Therefore, cytochrome c due to the addition of heart to cured meat could be suspected to affect the degree of lipid oxidation in meat products.

In this study, the effect of cytochrome c on lipid oxidation was investigated in the absence or the presence of ascorbate or/and nitrite.

Materials and Methods

Horse heart cytochrome c type III (Sigma Chemical Co.) was used to study the effect of cytochrome c on pork fat oxidation. Pork backfat was obtained from pork carcases without considering the storage time. Rendered fat was prepared by heating adipose tissue and centrifuging to remove residues.

About 0.2*g* lipid or solidified rendered fat was weighed exactly up to mg range into a 25 *ml*-Kimax test tube with a screw cap. Sodium cacodylate buffer at pH 5.8 was added to the sample with or without cytochrome c and then vortexed for 5 seconds. When sodium nitrite (2.2 *mM*) or/and soidum ascorbate (2.8 *mM*) were used, the mixture was vortexed with them again. After vortexing, the mixture was heated at 70°C for 30 minutes to accelerate the oxidation. Blanks were always run with treatments. Total volume of the mixture was 2-3 *ml* depending on the experiment. After heating at 70°C, the

mixture was cooled to room temperature and 1 *ml* of 0.05*M* EDTA at pH 4.0 was added. The TBA values of samples were measured. For measurements of TBA, the method from Ke and Woyewoda[12] was used with following modifications: The working TBA solution contained 0.1 mM of BHA. After heating in a boiling water bath and cooling to the room temperature by tap water, 5 *ml* of 50% TCA was added to the mixture and the mixture was centrifuged for 15 minutes in a clinical centrifuge. Absorbance of the upper aqueous layer was measured at 534 *nm*.

To compensate the effect of nitrite and sodium ascorbate on TBA measurement, the mixture was prepared with the known amount of malonaldehyde bis (dimethyl sorbances was made to compensate the difference between the sample with and that without nitrite or/and ascorbate. Statistical analysis was done by Newman-Keuls method according to Snedecor and Cochran.[13]

Results and Discussion

Cytochrome c increased the rate of lipid oxidation with increasing concentration.[6] However, high concentration ($3.5 \times 10^{-5}M$) showed the delaying effect on the oxidation.[8] In fact, high concentration of cytochrome c did not stop the oxidation at the level in which the sample does not contain cytochrome c. Fig. 1 shows that TBA values of backfat increased with increasing concentration of cytochrome c but the increment decreased with increasing concentration.

Fig. 2 illustrated the effect of reduced cytochrome c

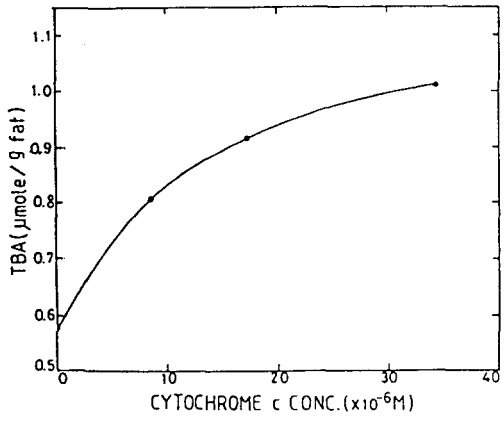

Fig. 1. Effect of cytochrome c concentration on backfat oxidation (n = 11)

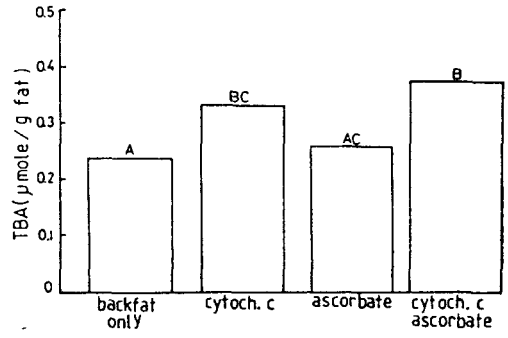

Fig. 2. Effects of cytochrome c(17.1×10^{-6}M) or/and ascorbate (2.8×10^{-3}M) on backfat oxidation (n = 6)
Bars not marked by the same letter are different ($P<0.05$).

on the lipid oxidation. The addition of commerical cytochrome c, which is in the oxidized form, without ascorbate caused more oxidaton than in control as expected. With the addition of ascorbate, the effect of cytochrome c was not much different from that of cytochrome c without ascorbate. It has been reported that heme compounds are catalysts for the oxidation of unsaturated fatty acids regardless of iron state.[14,15] The effect of ascorbate alone on fat oxidation shown in the literature is inconclusive. Benedict et al.[16] reported that ascorbate at the level of 50 ppm increased TBA value in lean beef ground with beef fat and pork fat. Sato and Hegarty[17] showed that concentration of ascorbic acid up to 500 ppm exhibited pro-oxidant properties but at 10,000 ppm it acted as an antioxidant. However, Greene et al.[18] reported that ascorbic acid at 500 ppm provided protection to lipids from oxidation. In this study, ascorbate did not show much effect on fat oxidation in the absence of cytochrome c. It might be because this system had lipids alone whereas other studies used lean meat.

The addition of nitrite to cytochrome c did not prevent the oxidation but the oxidation was inhibited by the addition of nitrite in the presence of ascorbate (Fig. 3). When ferricytochrome c (oxidized form) reacted with nitric oxide at acidic pH, Compound I ($Fe^{++} - NO^{+}$) was rapidly formed.[19,20] The amount of Compound I formed in the absence of ascorbate was dependent on pH and the amount formed was very small at pH above 5.0.[21,22] The presence of ascorbate significantly enhanced the amount formed in the pH range 5.0-6.0.[22] According to Orii and Shimada,[20] in the presence of reductants Compound I was transformed immediately into Compound II ($Fe^{++} - NO$). On the other hand, ferrocytochrome c (reduced form) reacted with nitric oxide to form Compound II.

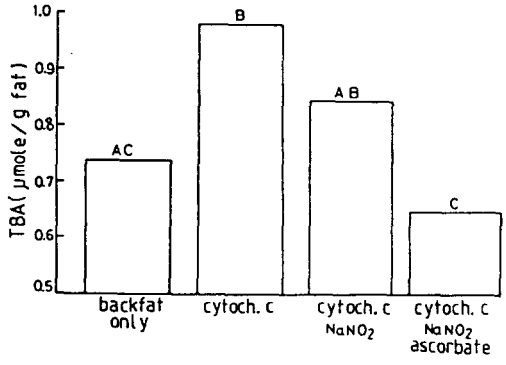

Fig. 3. Effects of cytochrome c($17.2 \times 10^{-3}M$), $NaNO_2$ ($2.3 \times 10^{-3}M$) and ascorbate ($2.8 \times 10^{-3}M$) on backfat oxidation (n = 6)
Bars not marked by the same letter are different ($p<0.05$).

Then Compound II released nitric oxide to return to ferrocytochrome c. However, if the reductant is in a large excess over nitrite, Compound II will be the end product. Therefore, this result can be interpreted as when oxidized cytochrome c(ferrycytochrome) reacts with nitrite, Compound I is formed which has lack of one electron, therefore, it cannot prevent the oxidation. However, in the presence of excess ascorbic acid, compound II would be the final product which is electro-chemically stable. It is well known that the formation of nitrosyl hemochrome, in which nitric oxide is bound to the reduced state of iron, is the main reason for prevention of oxidation in meat by nitrite.

With the rendered fat, the effects of cytochrome c, ascorbate and nitrite on the oxidation were more obvious than with backfat (Fig. 4 and 5). It is presumably because backfat, which is adipose tissue, has residual blood and

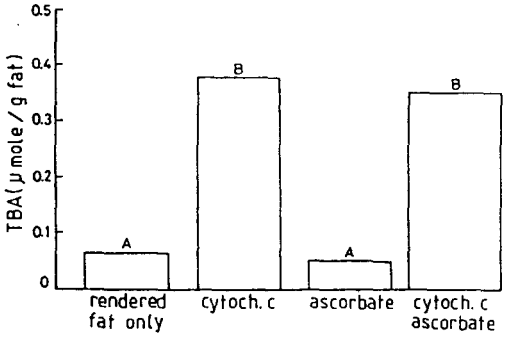

Fig. 4. Effects of cytochrome c(12.7×10^{-6}M) or/and ascorbate (2.8×10^{-3}M) on rendered fat oxidation (n = 4)
Bars not marked by the same letter are different ($p<0.05$).

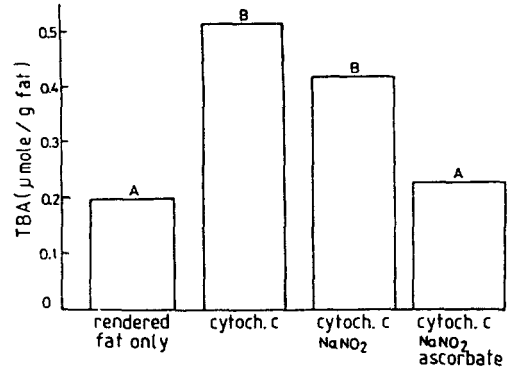

Fig 5. Effects of cytochrome c($11.6 \times 10^{-6}M$), $NaNO_2$ ($2.2 \times 10^{-3}M$) and ascorbate ($2.8 \times 10^{-3}M$) on rendered fat oxidation (n = 5)
Bars not marked by the same letter are different ($p<0.05$).

other tissue residues such as collagen fibers, vascular bed, fibroblastic connective tissues, leucocytes and macrophages.[23] On the other hand the rendered fat is void of heme pigments such as residual blood which can catalyze the oxidation.[1,5]

Since meat has lots of natural reducing substances in addition to the added ascorbate for curing, it can be concluded that the use of heart in cured meat products does not impose any threat to shelf life of products as long as reductatnts are in excess over nitrite. Lozano and Cassens[24] reported that when an extract of heart (up to 10%) was added to bologna preparation, it did not influence the TBA values of the products.

요 약

Cytochrome C가 돼지지방의 산화에 미치는 영향을 조사하고자 돼지등 지방, cytochrome c, 아스콜빈산 및 아질산염을 사용하여 pH 5.8에서 지방산화도를 TBA방법으로 측정하였다. Cytochrome c첨가량이 증가할수록 지방산화는 증가하였고, 산화증가정도는 Cytochrome c 첨가량이 증가할수록 감소하였다. 아스콜빈산이나 아질산염은 Cytochrome c의 지방산화촉진효과를 억제하지 못하였으나, 두가지를 동시에 첨가하였을때는 지방산화 촉진을 억제하였다. 결과적으로 가공육제품에 Cytochrome 가 다량함유된 염통을 첨가하여도 육제품 품질에 나쁜 영향을 주지 않을 것으로 사료된다.

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