

The effect of phytic acid on oxidative stability of raw and cooked meat

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Received 3 October 2005; received in revised form 23 February 2006; accepted 23 February 2006

Abstract

The effects of phytic acid addition (0.1, 1 and 5 mM) to pork and beef homogenates on TBARS and metmyoglobin levels in raw meat, and TBARS and heme iron contents in cooked meat during 3 days of storage at 4 °C were investigated. Also, the role of inositol as a potential synergist of IP₆ (phytic acid) was examined. IP₆ effectively decreased the TBARS accumulation in raw and cooked meat homogenates. The metmyoglobin formation was inhibited in raw beef by phytic acid in a dose-dependent manner. The effect of IP₆ was more pronounced in cooked meat than in raw and in cooked beef homogenates more than pork. Inositol did not enhance antioxidant action of phytic acid in minced meat.

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Keywords: Phytic acid; Antioxidant; Lipid peroxidation; Metmyoglobin; Ground meat

1. Introduction

The oxidation of lipids begins at the moment of slaughter and then occurs during storage, processing, heating and further storage of meat. Being the basic cause of fat rancidity, it leads to deterioration of quality or even spoilage of raw materials and food products. Heating of meat, followed by a low temperature storage, always results in the development of warmed-over flavours (WOF).

The most important process in oxidation of lipids in meat is peroxidation of polyunsaturated fatty acids from cell membranes (Keller & Kinsella, 1973), the major catalysts of which are transition metal ions, such as Fe²⁺ and Cu⁺. Heme compounds of meat can also contribute to this process, but it is known that the participation of heme iron in accelerating lipid peroxidation is less significant than that of non-heme iron. Heme iron was found to initiate the oxidation of lipids in both raw and heated meat, while non-heme iron played a major role in facilitating the perox-

idation of lipids in heated meat (Decker & Welch, 1990; Kanner, Hazan, & Doll, 1988; Pikul, 1992). The main sources of non-heme iron in meat are chromoproteins (Pikul, 1992). Lipid peroxidation products and free radicals are involved in the oxidation of myoglobin to metmyoglobin, which leads to brown discoloration (Renner & Labas, 1987). For the consumer, colour is the major distinguishing feature of meat and slightly processed meat product quality.

Phytic acid is a common plant constituent, comprising 1–5% by weight of edible legumes, cereals, oil seeds, pollens and nuts (Graf, Empson, & Eaton, 1987). It exerts major antioxidant properties due to its relatively high binding affinity for iron (Graf & Eaton, 1990). Phytic acid is capable of forming an iron–phytate chelate that is totally inert in the Fenton reaction, because it occupies six coordination sites of iron ion and displaces all of the water molecules coordinated in the Fe³⁺–phytate complex (iron participation in hydroxyl radical formation requires at least one coordination site opened or occupied by water or other readily dissociable ligand). Moreover, phytic acid causes the rapid removal of the Fe²⁺ without the simultaneous production of hydroxyl radical. As for Fe³⁺, it was proven to be relatively inert, even when oxygen and polyunsatu-

Abbreviations: TBARS, thiobarbituric acid-reactive substances; IP₆, inositol hexaphosphate, phytic acid; MDA, malondialdehyde.

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rated lipids were present in the reaction medium (Graf et al., 1987).

Due to its antioxidative potential, phytic acid arouses great interest as a potential food preservative. Lee, Hendricks, and Cornforth (1998) found that phytic acid was effective for inhibition of the oxidative changes in a model beef system.

The aim of our work was to determine whether phytic acid solutions could be useful in extending the stability of beef and pork meat, both raw and cooked, stored at refrigerated temperatures 0–4 °C. We also examined whether inositol could act as the synergist of IP₆ when added to stored beef homogenates. Our work was conducted on meat after *rigor mortis* because only then is it suitable for preparing dishes from ground pork in the industry (Kołczak, personal information). Pork is most popular among consumers from the European Union, surpassing beef and poultry. Pork is preferably eaten in Poland, where in 2004 it amounted to about 56% of meat consumption with only 7% for beef (Cukierski & Wojda, 2005).

2. Materials and methods

2.1. General

The experimental pattern was based on the Lee et al. (1998) model, with some modifications.

The experiment was conducted on two kinds of meat: pork loin and beef entrecote (purchased from the local butcher), 48 h *postmortem*. First, visible fat was removed with a stainless steel knife and then the meat was minced twice. During this process, the meat was cooled with ice.

2.2. Preparing the homogenates

Ninety grams of minced meat were homogenated with 10 ml of either: 0.1, 1 or 5 mM IP₆, 5 mM inositol solution and the mixture of IP₆ and inositol (final concentration of both ingredients was 5 mM). All solutions were made with deionised water. For the control sample, 10 ml of deionised water were taken for the homogenation. Before preparing the homogenates, the pH of every solution was adjusted to the level measured in the meat after mincing.

2.3. The raw meat test

One half of every homogenate was placed on a Petri dish, tightly closed and sealed with laboratory film. Then samples were stored in refrigerator (at about 4 °C).

In the freshly prepared homogenate and in samples stored for 1 and 3 days, TBARS and metmyoglobin contents were determined.

2.4. The cooked meat test

One half of every homogenate was placed in a polypropylene tube, closed and stored for 6 h in a refrigerator (at

4 °C). Then, the samples were heated until the temperature of homogenate inside the tube reached 70 ± 2 °C, cooled in an ice bath and placed in the refrigerator. In samples, immediately after cooking and after 1 and 3 days of storage, the TBARS and heme iron levels were measured.

2.5. Analytical methods

TBARS were detected using the method described by Buege and Aust (1978) and expressed as μmol of malondialdehyde per kg. Heme iron (ppm) was estimated by the method given by Hornsey (1956). Metmyoglobin (percent in total heme components) was determined according to the formula of Krzywicki (1982). Total iron (mg/kg) was measured using the method described by Lee et al. (1998). All determinations was carried out in four replications.

The content of fat in meat was determined according to AOAC Official Method 991.36.

2.6. Statistic analysis

Data were analyzed using Statgraphics Plus for Windows. The results were statistically evaluated using a multifactor ANOVA test. To determine significant differences, the LSD test was used at $p < 0.05$.

3. Results and discussion

3.1. Experiment I

3.1.1. Raw meat test

The gradual increase of TBARS and metmyoglobin levels was measured in raw beef and pork homogenates stored for 3 days at 4 °C (Tables 1a, 1b, 2a and 2b). Similar results were obtained by Lee et al. (1998) during storage of beef homogenates. It is well known that disruption of the muscle membrane by homogenisation allows oxygen to penetrate inside the tissue, which makes methylene groups and unsaturated bonds more reactive, and, in turn, accelerates lipid peroxidation (Pikul, 1992). However, the intensity of oxidative changes obtained in this study was different for beef and pork homogenates. The TBARS values obtained in controls (samples prepared with deionized

Table 1a
Changes in TBARS value (μmole MDA/kg) in raw beef homogenate stored for 3 days at 4 °C

Treatment	Storage time			
	0 h	24 h	3 days	Mean
Control	0.0	28.0	63.1	30.4 b
0.1 mM IP ₆	0.0	27.5	68.4	32.0 b
1 mM IP ₆	0.0	27.2	74.9	34.0 b
5 mM IP ₆	0.0	14.4	29.0	14.5 a
Mean	0.0 A ^a	24.3 B	58.8 C	

^a Values with different letters (a–c) within a column and (A–C) within a row differ significantly ($p < 0.05$).

Table 1b
Changes in TBARS value ($\mu\text{mole MDA/kg}$) in raw pork homogenate stored for 3 days at 4 °C

Treatment	Storage time			Mean
	0 h	24 h	3 days	
Control	1.94	2.66	5.23	3.28 c ^a
0.1 mM IP ₆	1.94	2.51	4.52	2.99 ab
1 mM IP ₆	1.94	2.54	4.95	3.14 bc
5 mM IP ₆	1.94	2.39	4.29	2.86 a
Mean	1.94 A ^a	2.52 B	4.75 C	

^a Values with different letters (a–c) within a column and (A–C) within a row differ significantly ($p < 0.05$).

Table 2a
Changes in metmyoglobin (%) formation in raw beef homogenate stored for 3 days at 4 °C

Treatment	Storage time			Mean
	0 h	24 h	3 days	
Control	17.1	44.0	73.3	44.8 c ^a
0.1 mM IP ₆	17.1	52.5	49.2	39.6 b
1 mM IP ₆	17.1	53.0	46.1	38.7 ab
5 mM IP ₆	17.1	39.2	56.0	37.4 a
Mean	17.1 A ^a	46.4 B	56.1 C	

^a Values with different letters (a–c) within a column and (A–C) within a row differ significantly ($p < 0.05$).

Table 2b
Changes in metmyoglobin (%) formation in raw pork homogenate stored for 3 days at 4 °C

Treatment	Storage time			Mean
	0 h	24 h	3 days	
Control	49.5	52.1	82.5	61.4 ab ^a
0.1 mM IP ₆	49.5	62.1	84.7	65.4 b
1 mM IP ₆	49.5	60.7	85.8	65.3 b
5 mM IP ₆	49.5	48.7	80.9	59.7 a
Mean	49.5 A ^a	55.9 B	83.5 C	

^a Values with different letters (a–c) within a column and (A–C) within a row differ significantly ($p < 0.05$).

water) after 3 days of storage were 11-fold higher in beef than in pork (Tables 1a and 1b). This might possibly be explained by differences in general ‘antioxidant potential’ of both kinds of meat used in the experiment, which in the case of pork was higher. We do not know about the diets of animals that were sources of meat used in our test. But it is generally proven that it is possible to improve the antioxidant potential of a muscle tissue by enriching the animals diet in antioxidants (Janik & Barowicz, 1998; Mitsumoto, Cassens, Schaefer, Arnold, & Scheller, 1991). The example is vitamin E, which, when added to feeds, may be both built into the cell membranes and located in storage lipids that accompany muscles (Barowicz, 2000; Bartnikowska, 2004). Greater susceptibility of beef lipids to damage caused by oxygen free radicals than those from pork has been noted by other researchers (Kim, Nam, & Ahn, 2002; Park et al., 2004).

The addition of 5 mM IP₆ to beef and pork homogenates effectively decreased the TBARS accumulation dur-

ing storage as compared to the control treatments. This is in agreement with Lee et al. (1998) who found a positive correlation between the level of phytic acid and the degree of inhibition of lipid peroxidation in beef homogenates.

In the present study, the myoglobin oxidation patterns observed during storage of homogenates prepared with IP₆ were different for beef and pork (Tables 2a and 2b). In the case of pork treatments, phytic acid exerted no influence on the rate of metmyoglobin formation. On the other hand, in beef homogenates, significantly lower metmyoglobin levels were observed for all treatments prepared with IP₆ in comparison to the controls. These results agree well with those of Lee et al. (1998). Moreover, in our study, the inhibitory effect of phytic acid on the rate of myoglobin oxidation was dose-dependent. This was not previously reported for 3 days of storage. In the Lee et al. (1998) model, the addition of phytic acid to beef homogenates becomes more effective with increasing time of storage. This phenomenon was clearly observed in our study. After 24 h of storage, a lower level of metmyoglobin was measured in beef homogenates containing 5 mM IP₆, and after 3 days, also in treatments with lower doses of phytic acid (0.1 and 1 mM IP₆). The effect of phytic acid on beef homogenates may be associated with a higher initial level of the heme compounds, in beef than in pork homogenates. Total iron measured in our test was higher in beef samples – 17.5 mg (experiment I) and 12.1 mg (experiment II) than in pork ones – 9.99 mg/kg of meat. Heme moieties, together with transition metal ions, such as iron, are well known modulators of peroxidation of polyunsaturated fatty acids located in the membranes of muscle foods (Keller & Kinsella, 1973). As a consequence, beef can be more susceptible to oxidative damage than pork during storage, which most probably contributed strongly to the effect exerted by phytic acid on beef homogenates. Furthermore, the lower ‘antioxidant potential’ of beef could additionally increase the possibility of the protective action of IP₆.

3.1.2. Cooked meat test

The initial level of TBARS obtained in beef and pork homogenates, after heating to 70 °C (Tables 3a and 3b), was much higher than those in raw homogenates (Tables 1a and 1b). It is commonly known that food processing, such as heating, cooking, baking and freezing, causes breakdown of heme constituents and the release of sub-

Table 3a
Changes in TBARS value ($\mu\text{mole MDA/kg}$) in cooked beef homogenate stored for 3 days at 4 °C

Treatment	Storage time			Mean
	0 h	24 h	3 days	
Control	11.1	25.5	65.7	34.1 b ^a
0.1 mM IP ₆	9.05	16.7	71.3	32.3 b
1 mM IP ₆	9.68	16.9	49.8	25.5 b
5 mM IP ₆	6.16	7.03	13.8	8.97 a
Mean	8.99 A ^a	16.5 A	50.2 B	

^a Values with different letters (a–c) within a column and (A–C) within a row differ significantly ($p < 0.05$).

Table 3b
Changes in TBARS value ($\mu\text{mole MDA/kg}$) in cooked pork homogenate stored for 3 days at 4 °C

Treatment	Storage time			
	0 h	24 h	3 days	Mean
Control	7.30	25.7	55.4	29.4 c ^a
0.1 mM IP ₆	5.88	26.2	55.4	29.2 c
1 mM IP ₆	5.07	16.9	45.5	22.5 b
5 mM IP ₆	3.74	8.72	24.2	12.2 a
Mean	5.45 A ^a	19.4 B	45.1 C	

^a Values with different letters (a–c) within a column and (A–C) within a row differ significantly ($p < 0.05$).

stantial amounts of low-molecular-weight iron. This non-heme iron is an active catalyst, responsible for rapid oxidation of lipids in heated meat. The rate of iron-release from porphyrin rings depends on time, intensity and temperature of cooking (Pikul, 1992). The temperature considered as optimal for this process is 70 °C (Pikul, 1992) and it was applied in the present work. It is known that free non-heme iron could bind strongly to negatively charged phospholipids (Graf & Panter, 1991). Fe³⁺-phosphatidylethanolamine (PE) complexes catalyze rapid autooxidation of polyunsaturated fatty acyl components of PE, which in turn cause the increase of TBARS. A consequence is the development of WOF and meat rancidity (Decker & Welch, 1990).

During 3 days of storage, the accelerated rate of lipids oxidation in cooked homogenates, compared to raw ones, was found only in the case of pork (Tables 1b and 3b). This may be explained by the greater susceptibility of pork lipids to rancidity after cooking than those of beef, that has been shown by Wilson, Pearson, and Shorland (1976).

Phytic acid, at 5 mM, effectively inhibited the accumulation of TBARS occurring in cooked beef and pork homogenates stored for 3 days (Tables 3a and 3b). Lee and Hendricks (1995) found that phytic acid inhibited the peroxidation of lipids in beef homogenates heated to 37 °C for 60 min (a dose-dependent effect). Johnson, Addis, and Epley (1996) obtained lower levels of TBARS in precooked beef patties when wild rice was added, and explained this mainly by the influence of IP₆. Phytic acid displaces the iron ions from complexes with PE and forms reactively inert iron chelates, disrupting the generation of oxygen free radicals by the Fenton reaction. The result is the inhibition of WOF formation (Empson, Labuza, & Graf, 1991; Lee & Hendricks, 1995).

The effect of phytic acid obtained in our study was more pronounced in cooked meat than in raw one. pH applied in the experiment was within the range of 4.5–6.0. According to Pikul (1992), under such conditions, non-heme iron is of more importance in accelerating lipid peroxidation than is heme iron. This confirms the effective role of phytic acid as a chelator agent in the presence of free iron ions released during heating of meat.

The addition of IP₆ was more effective in cooked beef homogenates than in pork treatments. Wilson et al. (1976) showed that phospholipids are of major importance in developing of WOF. We suppose that it is connected

with the level of the phytic acid antioxidant activity in beef homogenates, as IP₆ inhibits metal-catalyzed phospholipid peroxidation. In the case of pork, the accumulation of WOF is caused mainly by peroxidation of triglycerides and not by phospholipids (Wilson et al., 1976).

During storage of cooked homogenates, no changes in heme iron levels were observed (Tables 4a and 4b), which is different from the data provided by Lee et al. (1998). However, phytic acid, at 0.1 and 5 mM, was more effective in maintaining the heme iron content in pork homogenates than were control treatments. This result confirms the hypothesis of Park et al. (2004) and Lee et al. (1998) that IP₆ can slow the iron ions release from heme moieties. This is very interesting in the context of phytic acid as a food additive for pork products. IP₆ can inhibit the non-heme iron absorption in the intestine but not the heme iron (Carpenter & Mahoney, 1992). As, at the same time, phytic acid maintains heme iron content in pork, its influence on the absorption of iron in the intestine, that occurs mainly in heme form, may be positive.

3.2. Experiment II

The effects of phytic acid, at the most effective level of 5 mM (experiment I), alone and combined with 5 mM inositol, in the raw and cooked beef homogenates were examined. The aim of the experiment was to determine whether inositol could act as the synergist of IP₆ in its inhibitory action on oxidation processes during storage of beef homogenates. It is known that phytic acid may be dephosphorylated to IP_{1–5} in living cells. Inositol, in turn, can undergo phosphorylation to higher inositol phosphates,

Table 4a
Changes in heme iron concentration (ppm) in cooked beef homogenate stored for 3 days at 4 °C

Treatment	Storage time			
	0 h	24 h	3 days	Mean
Control	17.1	17.6	17.7	17.4
0.1 mM IP ₆	17.2	17.9	16.7	17.2
1 mM IP ₆	17.6	17.7	17.1	17.5
5 mM IP ₆	17.1	17.2	16.7	17.0
Mean	17.3	17.6	17.0	

Table 4b
Changes in heme iron concentration (ppm) in cooked pork homogenate stored for 3 days at 4 °C

Treatment	Storage time			
	0 h	24 h	3 days	Mean
Control	2.48	2.48	2.23	2.40 a ^a
0.1 mM IP ₆	3.56	4.11	3.24	3.64 c
1 mM IP ₆	3.43	3.21	3.34	3.33 b
5 mM IP ₆	3.12	3.19	3.12	3.13 b
Mean	3.15	3.25	2.97	

^a Values with different letters (a–c) within a column differ significantly ($p < 0.05$).

Table 5
Effects of inositol, phytic acid and mixture of both on changes in raw and cooked beef homogenates stored for 3 days at 4 °C

	Storage time			Treatment			
	0 h	24 h	3 days	Control	5 mM inositol	5 mM inositol + 5 mM IP ₆	5 mM IP ₆
<i>Raw meat homogenate</i>							
TBARS (μmole MDA/kg)	2.70 A ^a	17.6 B	52.0 C	38.7 b	37.9 b	10.8 a	9.12 a
Metmyoglobin (%)	16.9 A	39.0 B	60.0 C	40.7 bc	44.7 c	38.7 b	30.5 a
<i>Cooked meat homogenate</i>							
TBARS (μmole MDA/kg)	12.7 A	25.4 A	86.3 B	87.0 c	45.2 b	24.4 ab	9.23 a
Heme iron (ppm)	14.1 A	13.7 B	12.1 C	12.7 a	13.2 b	13.5 cb	13.7 c

^a Values with different letters within a row differ significantly ($p < 0.05$).

of which the most significant is IP₃. The molecules of IP₃ are very important in signal transduction and can also participate in cell division (Shamsuddin, 2002). Therefore, according to Shamsuddin (2002), the addition of inositol to phytic acid solution could increase the pool of lower inositol phosphates, as phosphates released from IP₆ might be captured by inositol. Inositol (1,2,3)P₃, which has the phosphates in axial, equatorial, and axial positions, respectively, is as an effective chelator of iron ions as is phytic acid. It may also maintain all of the iron in its oxidized form, thus preventing the initiation of lipid hydroperoxides catalyzed by ferrous iron, and, in consequence, lipid peroxidation (Phillippy & Graf, 1997).

Our results show no significant effect of the addition of inositol to beef homogenates prepared with IP₆ as compared to the action of IP₆ alone during 3 days of storage of both raw and cooked beef homogenates (Table 5).

However, cells after *rigor mortis*, are no longer capable of catalyzing metabolic reactions other than catabolic ones (i.e., proteolysis). Results of our tests show that inositol cannot be effectively used as the synergist of phytic acid in meat products.

In conclusion, IP₆ may be recommended as a food additive that prolongs the stability of both raw and cooked meat, but particularly of cooked. Moreover, the inhibition of lipids peroxidation by 5 mM IP₆, observed in our work, was more pronounced in beef than in pork.

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