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The Effect of Phytic Acid as Antioxidant in Meat Products

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DURING cooking and storage of meat, significant amounts of free iron were released. Apparently iron bound to negatively charged phospholipids and caused site specific oxidation that generated warmed over flavor (WOF) within 24 hr of refrigerated storage. Antioxidant and colour stability effects of phytic acid and wheat bran were compared in a fresh and cooked meat samples. Both compounds inhibited metmyoglobin formation in raw samples during storage, iron release from heme during cooking and lipid peroxidation in raw and cooked samples. These actions of phytic acid and/or wheat bran indicate that they may be useful as additives for meat products to prevent warmed over flavor (WOF) formation and increase shelf life of these products.

Phytic acid (myo-inositol hexaphosphate, IP6) is widely found in cereals, nuts, legumes, oil seeds, pollen and spores, constituting 1-5% (Graf & Eaton, 1990; Peterson, 2001 and Carneiro *et al.*, 2002). Phytic acid is historically considered to be an antinutrient. Structurally, phytic acid contains phosphorus, which binds minerals such as calcium, iron and zinc, causing a decrease of their bioaviability in human and animal models (Reddy *et al.*, 1989). However, recently, phytic acid has been reported to be an antioxidant (Ahn *et al.*, 2004; Barretto *et al.*, 2003; Graf & Eaton, 1990 and Midorikawa *et al.*, 2001). Lipid peroxidation products and free radicals are involved in the oxidation of oxymyoglobin to metmyoglobin and associated with brown discoloration (Renerre and Labas, 1987). Lipid peroxidation is a major cause of quality deterioration in restructured or precooked meats (Akamittath *et al.*, 1990). Transition metals such as Fe^{2+} and Cu^{2+} as well as heme compounds are important in lipid peroxidation in skeletal muscle (Decker and Welch, 1990). However, the role of heme proteins or nonheme iron in lipid peroxidation in muscle tissues is uncertain (Love and Pearson, 1974), hemoproteins are prooxidants of muscle tissues (Love, 1983). Phytic acid can tightly bind to metal ions and the metal phytate complexes are highly insoluble over a wide pH range (Graf & Eaton, 1990 and Lee & Hendricks, 1995). Therefore, phytate has been hypothesized to interfere with mineral bioavailability in humans and animals (Ellis *et al.*, 1982). Recently, phytic acid has been reported to be an anticarcinogenic (Shamsuddin *et al.*, 1997) and hypoglycemic or hypolipidemic (Rickard & Thompson, 1997 and Jenab & Thompson, 1998). Phytic acid is considered to be an antioxidant, because it is a potent inhibitor of iron-catalyzed hydroxyl radical formation by

chelating the free iron and then blocking its coordination site (Graf & Eaton, 1990 and Ahn *et al.*, 2004).

Our objective was to study the ability of phytic acid to inhibit lipid peroxidation and formation of metmyoglobin in raw and cooked beef meat samples.

Material and Methods

Materials

All reagents, solvents and chemicals used in this study were of analytical grade. Phytic acid, 2-thiobarbituric acid (TBA), trichloro acetic acid (TCA), sodium hydroxide, potassium phosphate (monobasic anhydrous), nitric acid, acetone hydrogen peroxide, ascorbic acid, ferrozine [3-(2-pyridyl)-5, 6-bis (4-phenylsulfonic acid)-1, 2, 4-triazine] and ammonium acetate, were obtained from Food Technology Department (Sigma Chemical Co.), 5 kg beef meat (round cuts) were purchased from a local market in Ismailia city and transferred in an ice to the laboratory.

Sample preparations

Antioxidant effects of phytic acid in a model beef system

Meat samples (2 kg) were cut into small pieces (visible connective tissues were removed and ground using a food processor. Treatment groups included: (1) control (0.0 phytic acid), (2) 0.1% phytic acid, (3) 0.3% phytic acid and (4) 0.5% phytic acid. The samples were divided into two parts, the first part was stored covered in Petri plates for 7 days at 4°C. The second part was transferred into 50 ml polypropylene test tubes and cooked to 70± 2°C (internal temperature) in a water bath, then cooled in an ice bath. The meat samples were chopped into very fine pieces and stored covered in Petri dish for 7 days at 4°C.

Application field (use of wheat bran as natural source of phytic acid in beef burger)

Fresh low fat meat (3 kg) was minced by a meat mincer and divided into six parts. Beefburger blends were prepared by adding wheat bran at (0.0, 2.0, 4.0, 6.0, 8.0 and 10%), 0.56% spices mixture (Red pepper 11.5%; all spice 10.0%; black pepper 45.0%; coriander 12.0%; nutmeg 2.5%; cummin 15.0% and clove 4.0%), onion 0.3%, garlic 0.3% and 1% sodium chloride. After mixing, the samples were divided into small lots (100 g) to prepare burger patties. All samples pre and after cooking were stored covered in Petri dishes for 7 days at 4°C.

Analytical Methods

1- Total iron

Total iron concentration was determined in wet-ashed samples using the ferrozine assay (Stookey, 1970). Beef muscle (0.2 – 0.3 g) in a test tube was digested with concentrated nitric acid and 30% hydrogen peroxide on a hot plate until a white ash was formed. The ash was dissolved in 0.2 ml of 1.0 N HCl and diluted with 0.8 ml deionized water. Ascorbic acid (1ml, 1%) was added and the

tube contents were mixed. After 20 min, 1 ml 10% ammonium acetate buffer and 1 ml of 1 mM ferrozine color reagent were added and the mixture was mixed well. The mixture was allowed to stand at room temperature for 45 min, then the absorbance was determined at 562 nm using Baush and Lamb spectrophotometer. The concentration of iron was determined from a standard curve made with standard iron solution (Sigma, St. Louis, Mo).

Heme iron

Heme iron was measured according to the method of Lee *et al.* (1998). Beef (2 g) was transferred into a 50 ml polypropylene tube, and 9 ml of acid acetone (90% acetone + 8% deionized water + 2% HCl) was added. The mixture was then macerated with a glass rod and allowed to stand for 1 hr in a dark cabinet at room temperature. The extract was filtered through Whatman filter paper 42 and the absorbance was read at 640 nm against the acid acetone blank.

Total pigments as acid hematin, were calculated using the formula:

Total pigments (ppm) = A_{640} x 680

and heme iron was calculated as follows (Clark *et al.*, 1997) :

heme iron (ppm) = total pigment (ppm) x 8.82/100

Non-heme iron

The concentration of non heme iron in samples was calculated using the formula:

Non-heme iron (ppm) = total iron – heme iron

Metmyoglobin

Percent metmyoglobin was determined using the formula by Krzywicki (1982) :

% MetMb = 1.395 – $[(A_{572}-A_{700})/(A_{525}-A_{700})]$ x 100

Minced meat (5 g) was placed into a 50 ml polypropylene centrifuge tube and 25 ml ice-cold phosphate buffer (pH 6.8, 40 mM) was added. The mixture was homogenized for 10 s at 10,000 rpm with an Ultra-Turrax T50 (Jank & Kunkel Gmbh, Staufen Germany). The homogenized sample was allowed to stand for 1 hr at 4°C and centrifuged at 5000 rpm for 30 min at 4°C. The supernatant was filtered through whatman (1) filter paper and the absorbance was read at 700, 572 and 525 nm with spectrophotometer.

TBA values

Thiobarbituric acid was determined as described by Pearson (1981).

Warmed-over flavor (WOF)

Sensory evaluation was carried out after 48 hr storage of the cooked samples at 4°C. All experimental samples were reheated in boiling water to an approximate internal temperature of 70°C and served while hot. The panel

scoring system was as follows: 1 = very pronounced WOF; 2 pronounced WOF; 3 = moderate WOF; 4 = slight WOF and 5 no WOF (John *et al.*, 1979).

Statistical analysis

Analysis of variance was conducted for the data in accordance with procedures described by Ott (1984).

Results and Discussion

Metmyoglobin

Effect of phytic acid on metmyoglobin formation

Metmyoglobin formation was time dependent when meat samples were stored for 7 days at 4°C (Tables 1 and 2). Treatment with phytic acid or wheat bran inhibited metmyoglobin formation. The effectiveness of phytic acid or wheat bran was more pronounced with increasing storage time. Meat samples treated with phytic acid or wheat bran exhibited a colour difference from that of the control.

TABLE 1. Effect of phytic acid on metmyoglobin (%) formation in raw beef models system during storage at 4°C.

Treatments	Metmyoglobin (%) at phytic acid concentration of			
Storage days	0 %	0.1 %	0.3 %	0.5 %
0	24.6a	24.5a	24.7a	24.3a
1	33.2a	29.7b	27.6c	27.1c
3	48.5a	32.6b	33.0b	30.5c
5	60.8a	44.0b	41.3c	36.2d
7	98.3a	53.7b	49.8c	42.7d

Means in the same row with different superscripts are significant at $p< 0.05$

TABLE 2. Effect of wheat bran on metmyoglobin (%) formation in beef burger during storage at 4°C.

Treatments	Metmyoglobin (%) at wheat bran of					
Storage days	0 %	2 %	4 %	6 %	8 %	10 %
0	26.0a	25.8b	25.8b	25.6b	25.7b	25.7b
1	32.7a	30.8b	28.7c	27.2d	26.9f	26.5f
3	47.9a	45.1b	36.5c	36.4c	33.7d	32.8d
5	61.1a	59.0b	48.1c	46.9d	43.5f	39.7g
7	99.2a	73.9b	61.0c	55.3d	51.2f	45.2g

Mean in the same row with different superscripts are significantly different at $p< 0.05$ % phytic acid in wheat bran 1.14

Results in Tables 1 and 2 show that increasing the concentration of phytic acid or wheat bran resulted in a decrease in metmyoglobin formation at any given time of cold storage, while control samples showed the highest metmyoglobin content. This could be attributed to the inhibitory effect of added phytic acid or wheat bran as antioxidant in reducing the metmyoglobin formation (Ahn *et al.*, 2004 and Lee *et al.*, 1998).

The colour stability of meat is directly related to shelf life. The bright red colour of oxymyoglobin indicates high quality fresh meat, which is attractive to consumers. The influence of biological factors on meat discoloration depends largely on the nature of fibers. Colour stability is muscle-dependent (Lee *et al.*, 1998 and Reddy & Carpenter, 1991). Although many factors can influence meat color stability, myoglobin oxidation by free radicals or lipid peroxidation products (Gray *et al.*, 1996). Wheat bran had a colour protecting effect on beef burger during cold storage and its effectiveness was very similar that of phytic acid. Lee *et al.* (1998) reported that the colour stability effect of phytic acid may be the result of their ability to chelate transition metals involved in free radical generation and/or free radical scavenging, thereby delaying the oxidation of oxymyoglobin to metmyoglobin. A similar conclusion was reported by Midorikawa *et al.* (2001).

Lipid peroxidation in raw beef and beef burger during storage at 4°C for 7 days

Changes in lipid peroxidation rates, determined as changes in TBA values, in raw beef and beef burger samples as affected by phytic acid or wheat bran are shown in Tables 3 and 4.

TABLE 3. Effect of phytic acid on TBA number (mg malonaldehyde/kg) in raw beef models system during storage at 4°C.

Treatments	TBA (mg/kg) at phytic acid concentration of			
Storage days	0 %	0.1 %	0.3 %	0.5 %
0	0.18a	0.16a	0.14a	0.14a
1	0.36a	0.21b	0.19b	0.15b
3	0.59a	0.52a	0.42b	0.20c
5	1.30a	0.94b	0.61c	0.39d
7	1.89a	1.01b	0.82b	0.43c

Means in the same row with different superscripts are significant at $p < 0.05$
% fat on dry weight basis 19.80

TABLE 4. Effect of wheat bran on TBA number (mg malonaldehyde/kg) in beef burger (uncooked) during storage at 4°C.

Treatments	TBA (mg/kg) at wheat bran of					
Storage days	0 %	2 %	4 %	6 %	8 %	10 %
0	0.17a	0.15a	0.13b	0.13 b	0.13b	0.13b
1	0.39a	0.19b	0.18b	0.18 b	0.15b	0.16b
3	0.61a	0.60a	0.54b	0.46 c	0.41c	0.23d
5	1.29a	0.98b	0.73c	0.65 d	0.61d	0.45d
7	1.85a	1.36b	0.96c	0.91 c	0.81c	0.51d
% fat*	19.81	19.50	19.22	18.91	18.52	18.1

Mean in the same row with different superscripts are different at $p < 0.05$
* on dry weight basis
% phytic acid in wheat bran 1.04

In raw beef samples (Table 3) it was obvious that addition of phytic acid reduced the TBA values of treated samples from 0.18 for the control sample to 0.1, 0.3 and 0.3 mg malonaldehyde/kg, when samples treated with 0.1, 0.3 and 0.5% of phytic acid, respectively. From the same table it could be noticed that the TBA values of the treated samples during storage for 7 days at 4°C were lower than those of untreated samples. For example addition of 0.5% phytic acid reduced TBA formation by 77.2 % after 7 days of storage. As also shown in Table 4, the addition of wheat bran to beef burger gave the same inhibitory effect in lipid peroxidation displayed as a reduction in TBA values during all storage periods in all samples. This effect is expected due to the phytic acid from wheat bran. However, the phytic acid was more effective than wheat bran so that 0.5% phytic acid inhibited TBA formation by 77.2% compared with 10% wheat bran which inhibited only by 72.4% after storage for 7 days at 4°C.

The development of off-flavor in meat results from lipid peroxidation during storage (Lee *et al.*, 1998). Transition metals such as iron and copper and heme moiety are important in the reaction (Kanner *et al.*, 1988). Phytic acid can chelate the transition metal ions inhibiting metal-catalyzed lipid peroxidation or free radical formation from the fenton reaction (Chan *et al.*, 1994 and Lee *et al.*, 1998). Such actions of phytic acid may be involved in the inhibition of lipid peroxidation. Lee and Hendricks (1995) reported that phytic acid inhibited lipid peroxidation effectively dependently in beef homogenates.

Lipid peroxidation in cooked meat and beef burger during storage at 4°C for 7 days

Lipid peroxidation as expressed by TBA number is increased with cooking and with increasing storage time after cooking. When meat is cooked iron liberated from myoglobin interacts with phospholipids to catalyze lipid oxidation. Peroxidation thereby increase the development of WOF (Lee and Hendricks, 1995). Both phytic acid and wheat bran inhibited TBA formation and the effect was dose-dependent. The inhibitory effect by wheat bran was nearly similar to by phytic acid. Both nonheme and heme iron in beef muscle can catalyze lipid peroxidation (Kanner *et al.*, 1988 and Monahan *et al.*, 1993). However, Chen *et al.* (1984) reported that iron was released from heme pigments during cooking and proposed that the resultant increase in nonheme iron was responsible for lipid peroxidation. Baking and microwaving ground beef increases the concentration of nonheme (Schricker and Miller, 1983). Temperature also affects the release of iron ferritin (Decker and Welsh, 1990).

There was negative relationship between heme iron content (Fig. 1) and TBA number (Fig. 2) of cooked beef. The TBA value in control sample was 6.00 mg/kg after 7 days of cold storage. When samples were treated with 0.5% phytic acid, however, the TBA value dropped to 0.92 mg/kg the same phenomenon observed in beef burger samples (Fig. 3). The antioxidant effect of phytic acid may be due to the inhibition of non heme iron activity.

Lee and Hendricks (1995) reported that the action of phytic acid as antioxidant may be due to maintaining the ferric ion state and chelating with ferric ion, which inhibits the initiation and propagation of lipid peroxidation. A similar conclusion was observed by Barretto *et al.* (2003) and Oatway *et al* (2001).

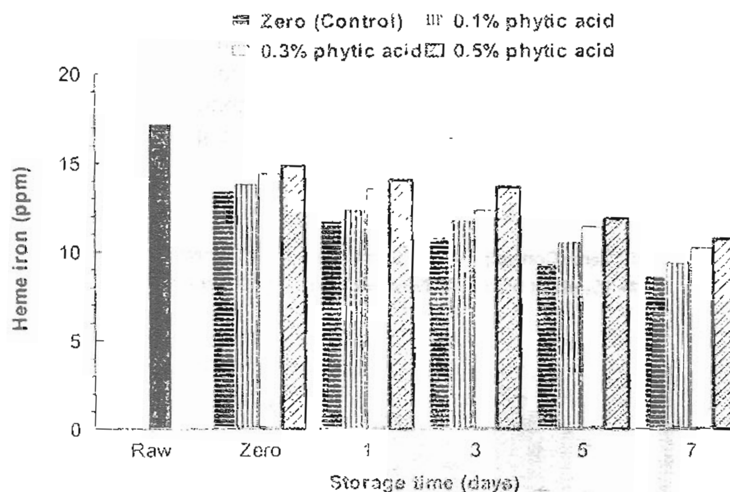

Fig. 1. Effect of phytic acid on heme iron content in cooked beef burger during storage at 4°C .

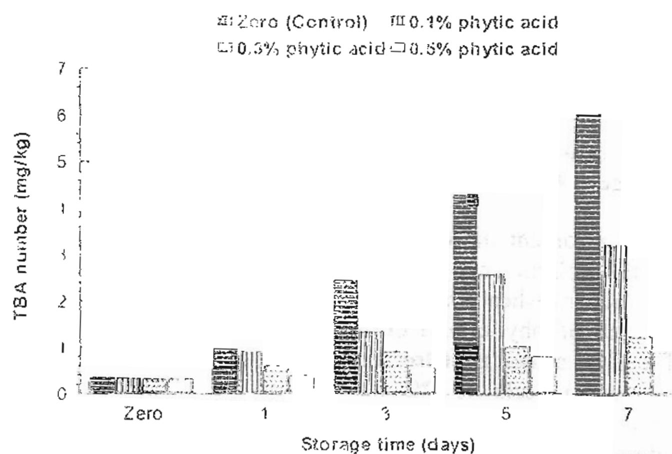

Fig. 2. Effect of phytic acid on TBA number (mg malonaldehyde/kg) in cooked beef burger during storage at 4°C .

Heme iron content during cold storage of cooked samples

Muscle tissue contains considerable iron bound to proteins. Myoglobin is the most abundant hemoprotein in muscle tissue. Our total iron and heme iron content of ground beef were 25.1 and 17.2 µg/g wet weight, respectively. The heme iron content in ground beef was decreased with cooking (Fig. 1, 4). Treatment with phytic acid or wheat bran decreased the nonheme iron release by cooking. In control samples, cooking increased non heme iron release by 4.1 µg/g sample, corresponding to decrease in heme iron by 23.8%. Cooking destroys the porphyrin rings of heme pigments resulting in non heme iron release from heme pigments (Lee *et al.*, 1992) The release of nonheme iron depends on temperature, time, type (slow or fast) and method of cooking boiling, baking, and microwaving (Schricker *et al.*, 1982).

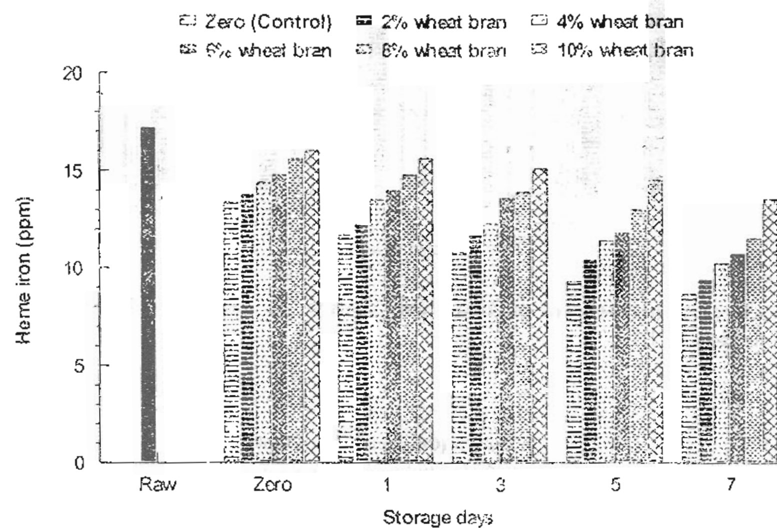

Fig. 3. Effect of wheat bran on heme iron content in cooked beef burger during storage at 4°C .

Heme iron content in cooked beef decreased with increasing storage time (Fig. 1,4). Both phytic acid and wheat bran inhibited nonheme iron release. The inhibitory effect of wheat bran on iron release from degradation of pigments was similar to that of phytic acid during storage for 7 days. Harel *et al.* (1988) reported that the amounts of free iron increased almost four fold during storage of turkey dark muscle at 4°C for 7 days. Phytate was more effective for inhibition of iron release from heme during cooking. Therefore, phytate as a meat additive may not result in a decrease in total iron absorption during digestion in the small intestine (Lee *et al.*, 1998). Phytate is known to inhibit nonheme iron absorption but it dose not affect heme iron absorption (Carpenter and Mahoney, 1992). Rogov *et al.* (1989) reported that the higher amounts of

dietary fibers in the presence of cysteine (meat factor) caused lower binding of iron to the dietary fibers (increased iron solubility).

Results demonstrate that non-heme iron is the major prooxidant of lipid autoxidation in cooked meat and meat products. These results confirm the reports of Sato & Hegarty (1971) and Love & Pearson (1974), that nonheme iron is the principal prooxidant in cooked meat. Results also demonstrated that nonheme iron is released from the heme pigments due to cooking.

Warmed-over flavor (WOF) evaluations

During cooking and storage of beef burger significant quantities of free iron were released. Apparently iron bound to negatively charged phospholipids and caused site-specific oxidation that generated warmed over flavor (WOF) within 24 hr of refrigerated storage (Graf and Panter, 1991).

WOF generation in beef burger can be successfully inhibited by iron sequestration. Figure 4 demonstrates the large effect of wheat bran on TBA formation in cooked beef burger. The aroma of beef burger treated with phytic acid (wheat bran) remained pleasant for 7 days.

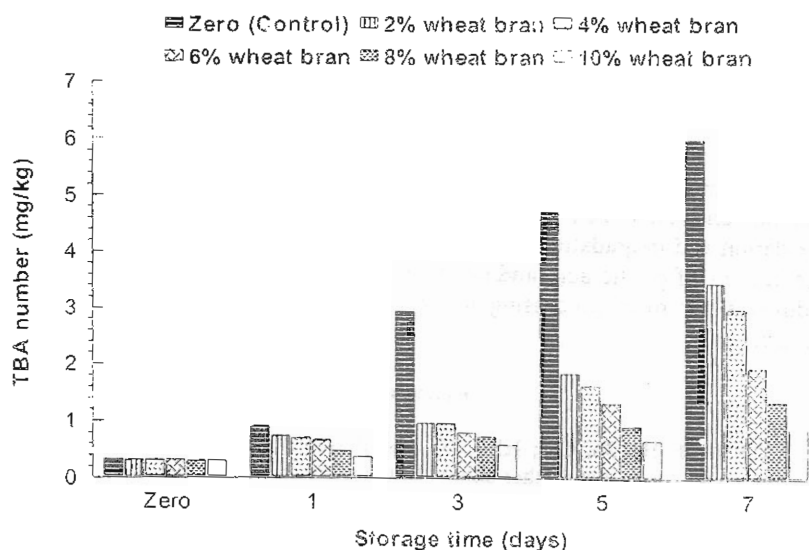

Fig. 4. Effect of wheat bran on TBA number (mg malonaldehyde/kg) in cooked beef burger during storage at 4°C .

As noted TBA are a useful chemical index for WOF in refrigerated precooked meat. When oxidative damage occurs, water soluble low molecular weight aldehydes such as malondialdehyde (MDA), hexanal and heptanal are formed as degradation products of lipid hydroperoxides. The characteristic warmed-over flavor in precooked meat is caused by the accumulation of these lipid degradation products.

To test the effect of phytic acid (wheat bran) on WOF formation in cooked beef burger samples kept under possible consumer home refrigerator abuse conditions, samples were stored uncovered on a plate at 4°C. after 1 day the TBA values for control (beef burger) rose to 5.48 and for the test sample containing 10% wheat bran to 0.87 mg MDA/kg. Therefore, the inhibitory effects of wheat bran on MDA generation became even more pronounced under these detrimental storage conditions. Thus, our results demonstrated that treatment with wheat bran successfully delayed onset of WOF in precooked beef burger (Table 5).

TABLE 5. Sensory scores in cooked beef burger during storage at 4°C.

Treatments	Wheat bran (%)					
Storage days	0	2	4	6	8	10
0	4.35 a	4.61 a	4.61 a	4.60 a	4.61 a	4.61 a
1	3.50 b	3.82 b	3.95 b	4.32 a	4.40 a	4.40 a
3	2.3 a	3.60 b	3.72 b	4.10 b	4.10 b	4.23 b
5	1.91 a	3.42 b	3.50 c	3.93 c	3.97 c	4.00 d
7	1.32 b	2.67 c	3.15 c	3.70 c	3.71 a	3.92 a

Means in the same row with different superscripts at $p < 0.05$.

Conclusions

Phytic acid and/or wheat bran as antioxidant inhibited metmyoglobin formation and stabilized red meat colour. In addition, it inhibited lipid peroxidation and degradation of heme pigments caused by cooking and storage. These actions of phytic acid and/or wheat bran indicate that they may be useful as additives for meat processing to prevent off-flavor formation and increase shelf life.

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تأثير حامض الفيتك كمضاد اكسدة

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قسم الصناعات الغذائية – كلية الزراعة – جامعة قناة السويس –
الاسماعيلية – مصر

يهدف هذا البحث الى دراسة الدور الوظيفى لحمض الفيتك كمضاد للاكسدة وامكانية التطبيق العملى خصوصا فى منتجات اللحوم لذلك تم دراسة تاثير كل من حمض الفيتك النقى على اكسدة الدهن والصبغات فى اللحم الطازج والمطبوخ وكذلك استخدام ردة القمح كمصدر طبيعى لحمض الفيتك ودراسة تاثيره على جودة البرجر كاحد منتجات اللحوم ، وذلك مقارنة بحمض الفيتك النقى. وقد اوضحت النتائج ان كلا من المادتين له تاثير مثبط على تكوين مركب ميتميوجلوبين فى العينات الخام وعلى تحرر الحديد من صبغات الهيم واكسدة الدهن فى العينات الخام والمطبوخة. وبناء على ذلك يمكن استخدام حامض الفيتك النقى او ردة القمح كمصدر طبيعى ومتوفر لحامض الفيتك كاحد الاضافات فى صناعة اللحوم لتقليل تكوين النكهات الغير مرغوبة وزيادة فترة صلاحية المنتجات.