Refrigerated broiler breast meat lipid oxidation inhibition by purified corn germ phytic acid

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Abstract— **The aim of this work was to observe *in vitro* the inhibition of purified phytic acid (PA) from corn germ in this lab on lipid peroxidation and the effect of its addition on broiler breast meat lipid oxidation stored for 6 days at 6°C. The maximum in vitro lipid peroxidation inhibition induced by the Fe was 78.32% and 78.62% under PA concentration of 12mg mL^{-1} both standard PA (Sigma) and from corn germ, respectively. Soon after the antioxidants addition the maximum activity in the 10mM concentration of PA from corn germ and PA standard was 45.78±0.58 and 40.73±0.17%, respectively. On the 4th and 6th day of storage this antioxidant activity of both PA from corn germ (10mM) and standard PA (5 mM) was decreased and did not show significant difference (P<0.05). Moreover, evaluating the meat warmed over flavour, the maximum antioxidant activity of 48.55±0.66% and 48.64±0.13% occurred when the standard PA was applied at the concentration of 10mM and 5mM, respectively and of 45.16 ± 0.58% also occurred when the germ corn PA of 10mM was applied. On the 2nd and 4th days of storage the PA standard (5mM) and the PA from corn germ (10mM) did not show significant results on their activity (P<0.05). Finally the 5mM addition of purified PA stabilized the meat metamyoglobin formation (P<0.05) from the 2nd day of refrigeration.**

Keywords— **antioxidant activity, warmed over flavour, metmyoglobin oxidation, chicken meat.**

I. INTRODUCTION

Phytic acid (myo-inositol hexaphosphate) shows negative charge in a wide pH levels and possesses 12 dissociable hydrogens. These characteristics provide a chelant property to phytic acid, which can form complexes with polyvalent metals, especially with di and trications [1]. Until the 1990s, phytic acid was considered an anti-nutrient. However, in the last 20 years, its chelant property has been recognized for its advantageous effects due to antioxidant and anticarcinogenic functions [1,2,3,4]. Many studies have shown that the high antioxidant potential against lipid peroxidation induced by iron was due to the combination of the metal chelant property and its ability to scavenge free radicals [2,3,5,6,7]. Phytic acid is a unique and versatile compound utilized as a food additive because of its antioxidant and chelant qualities [8]. Phytic acid was recognized in 1997 as GRAS (Generally Recognized as Safe) by the FDA (Food and Drug Administration) and in the *Codex Alimentarius,* phytic acid was revised as an antioxidant by the INS (System for Food Additives) number 391 [9].

Many studies have confirmed the antioxidant potential of phytic acid, primarily in meat products including in a prevention of Warmed-over flavour (WOF) or in model systems [2,3,6.10].Considering the function of phytic acid as an antioxidant and its elevated concentration in corn germ, this work evaluated *in vitro* the inhibition of purified phytic acid (PA) from corn germ in this lab on lipid peroxidation and the effect of its addition on broiler breast meat lipid oxidation stored for 6 days at 6°.

II. MATERIALS AND METHODS

A. Materials

Phytic acid from corn germ was purified, defatted and exhibited a final analytical grade of 85.41± 0.95%, according to descriptions by Filgueiras et al. [7]. The standard phytic acid was dodecasodium phytate ($Na_{12}C_6H_6O_{24}P_6$; PM= 923.8, 90% analytical purity, Sigma).

B. Phytic acid in the in vitro *inhibition of lipid peroxidation induced by iron*

The *in vitro* inhibition of lipid peroxidation induced by iron from standard or purified phytic acid from corn germ was evaluated by the decrease in the formation of thiobarbituric acid reactive substances (TBARS), in accordance to the procedures of Buege and Aust [11] and modified by Casagrande et al [12]. The results were shown as inhibition percentages of phytic acid in a lipid peroxidation assay induced by iron and were estimated as $(A_0 - A_a / A_0) \times 100$, where A_a is the absorbance of the sample and A_0 is the absorbance of the control (without phytic acid).

C. Effect of the phytic acid addition on refrigerated chicken meat lipid oxidation

The broiler chicken thighs and upper thighs meats without skin were ground by a grinder, and 500 g was homogenized with 50 mL of deionized water, with each portion containing 2.5 mM, 5 mM and 10 mM of standard phytic acid, corn germ phytic acid or 0.5% sodium erythorbate. Only deionized water was added to the control sample, without the antioxidant. The homogenates were collected in polystyrene trays covered by a permeable film and stored in a refrigerator at 6 ± 1ºC under a fluorescent light for 0, 2, 4 and 6 days. The analyses of antioxidant activity of the phytic acid, measured by TBARS assay, the WOF development and the formation of metmyoglobin were conducted in triplicate for each pre-established time period.

D. Antioxidant activity of phytic acid

Lipid oxidation was measured using the TBARS method according to the procedures described by Tarladgis et al [13] and modified by Crackel et al [14]. For the calibration curve, we used the aqueous solution of 1,1,3,3-tetraethoxypropane (TEP) in concentrations of 0.1 to 7.0 M. Lipid oxidation was shown in mg of TBARS kg^{-1} of the sample, and a percentage of the phytic acid antioxidant activity was estimated as follows: Antioxidant Activity (%) = $(TBARS_c – TBARS_s / TBARS_c) \times 100$, where $TBARS_c$ is the value of the TBARS control and $TBARS_s$ is the value of the TBARS sample.

E. Determination of the development of the WOF

WOF was evaluated according to the procedures described by Igene and Pearson [15]. The analyses of lipid oxidation in the TBARS assay and the percentage of the antioxidant activity of phytic acid was measured according to the previous description. The results of WOF development were shown in mg of TBARS kg^{-1} of the sample, and the percentage of antioxidant activity of phytic acid was estimated according to the previous description.

F. Determination of metmyogoblin formation

The content of metmyoglobin in 5.0 g of chicken homogenates containing antioxidants was determined by absorbance measurements at 700, 572 and 525 nm according to the procedures described by Krzywicki [16]. The percentage of metmyoglobin formed was determined as follows: MetMb (%) = $\{1{,}395 – [(A_{572}-A_{700}) / (A_{525}-A_{700})]\} \times 100$, where A_{572} = supernatant absorbance at 572 nm, A_{700} = supernatant absorbance at 700 nm and A_{525} = supernatant absorbance at 525 nm.

G. Statistical analysis

The concentration of phytic acid that inhibited the oxidative process by 50% (IC_{50}) was estimated by the GraphPad Prism software (Version 4.00) using a hyperbolic curve (one-site binding hyperbola). To evaluate the effect of the antioxidants addition to chicken homogenates, the data were analyzed using one-way ANOVA, followed by Tukey's multiple comparisons *t*-test ($p \leq 0.05$) (STATISTICA Program, Version 6.0)

III. RESULTS

Figure 1A shows that the maximum inhibition of lipid peroxidation was 78.32% at the concentration of 12.0 mg mL^{-1} of standard phytic acid. At the same concentration of corn germ purified phytic acid, the inhibition of lipid peroxidation was 78.62%, which was similar to the standard phytic acid (Figure 1B). The inhibition of 50% of the oxidative process, was 0.79 mg mL^{-1} for standard phytic acid and 1.66 mg mL^{-1} for purified phytic acid from corn germ.

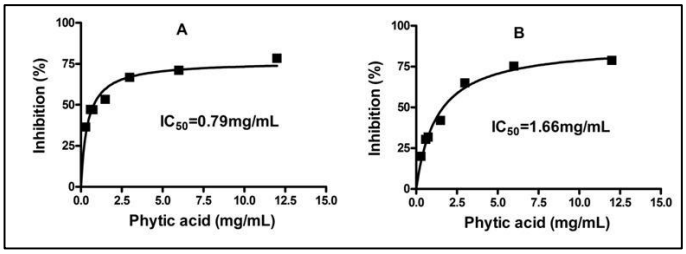

Figure 1 (A) Inhibition of lipid peroxidation *in vitro* induced by Fe^{2+} at different concentrations of standard phytic acid. Results are shown as the mean ± SD. (B) Inhibition of lipid peroxidation *in vitro* induced by Fe^{2+} in different concentrations of purified phytic acid from corn germ.

Tables 1 and 2 show the antioxidant activity of phytic acid measured by TBARS and WOF in chicken meat refrigerated at 6°C for six days, respectively. The sodium erythorbate exhibited higher antioxidant activity in all assays, compared with the corn germ purified phytic acid and standard phytic acid. There were significant differences in antioxidant activity measured by TBARS and WOF among all of the treated samples employing different antioxidants during the six-days storage. The antioxidant activity of corn germ purified phytic acid and standard phytic acid was significantly dependent on the concentrations used and on the storage time of the chicken meat at 6°C

Table 1 Antioxidant activity (%) of phytic acid measured by TBARS in chicken meat refrigerated at 6°C for six days

Treatment	Storage period (days)			
	0	2	4	6
AF-GM (2.5 mM)	28.22^{gA} ±0.23	15.06^{gB} ±0.55	13.64^{fC} ±0.15	12.88^{fD} ±0.63
AF-GM (5 mM)	39.31^{eA} ±0.04	34.74^{eB} ±0.08	28.82^{dC} ±0.97	25.22^{dD} ±0.45
AF-GM (10 mM)	40.73^{dA} ±0.17	37.47^{dB} ±0.78	31.56^{cC} ±0.99	28.69^{cD} ±0.13
AF Standard (2.5 mM)	34.52^{fA} ±0.06	33.31^{fB} ±0.97	25.08^{eC} ±0.11	18.17^{eD} ±0.73
AF Standard (5 mM)	44.31^{cA} ±0.77	40.30^{cB} ±0.42	31.42^{cC} ±0.01	28.83^{cD} ±0.40
AF Standard (10 mM)	45.78^{bA} ±0.58	43.17^{bB} ±0.15	36.25^{bC} ±0.25	31.11^{bD} ±0.47
Sodium erythorbate (0.5 %)	66.63^{aA} ±0.01	56.63^{aB} ±0.30	44.42^{aC} ±0.08	40.04^{aD} ±0.36

Values with different letters (a-g) in the same column and (A-D) in the same line differ significantly (P<0.05). Results shown as the mean ± SD. AF-GM: Phytic acid from corn germ.

Table 2 Antioxidant activity (%) of phytic acid measured by the development of WOF in chicken meat refrigerated at 6°C for six days.

Treatment	Storage period (days)			
	0	2	4	6
AF-GM (2.5 mM)	43.21^{eA} ±0.12	35.5^{fB} ±0.28	28.67^{fC} ±0.36	18.80^{gD} ±0.58
AF-GM (5 mM)	44.24^{dA} ±0.78	40.18^{dB} ±0.51	37.14^{dC} ±0.46	33.62^{eD} ±0.15
AF-GM (10 mM)	45.16^{cA} ±0.58	43.08^{cB} ±0.44	41.41^{cC} ±0.44	38.32^{cD} ±0.89
AF Standard (2.5 mM)	45.53^{cA} ±0.47	37.62^{eB} ±0.34	30.72^{eC} ±0.12	21.93^{fD} ±0.41
AF Standard (5 mM)	48.55^{bA} ±0.66	43.14^{cB} ±0.59	40.66^{cC} ±0.38	36.19^{dD} ±0.45
AF Standard (10 mM)	48.64^{bA} ±0.13	45.11^{bB} ±0.47	43.77^{bC} ±0.62	40.54^{bD} ±0.33
Sodium erythorbate (0.5 %)	77.51^{aA} ±0.33	65.23^{aB} ±0.11	60.60^{aC} ±0.49	52.03^{aD} ±0.22

Values with different letters (a-g) in the same column and (A-D) in the same line differ significantly (P<0.05). Results shown as the mean ± SD. AF-GM: Phytic acid from corn germ.

The addition of 5 mM corn germ purified phytic acid stabilized the chicken meat colour (P<0.05) through the 2nd day of storage at 6°C (Table 3).

Table 3 The percentage of metmyoglobin of phytic acid measured by the formation of metmyoglobin in chicken meat refrigerated at 6°C for six days

Treatment	Storage period (days)			
	0	2	4	6
AF-GM (2.5 mM)	17.42^{fA} ±0.16	16.48^{fB} ±0.98	15.37^{fC} ±0.99	15.11^{fC} ±0.54
AF-GM (5 mM)	19.11^{dA} ±0.20	17.28^{eB} ±0.36	17.03^{dB} ±0.23	17.00^{dB} ±0.33
AF-GM (10 mM)	20.46^{cA} ±0.21	19.66^{cB} ±0.09	18.37^{cC} ±0.61	18.19^{cC} ±0.37
AF Standard (2.5 mM)	18.13^{eA} ±0.33	17.59^{eA} ±0.16	16.06^{eB} ±0.01	15.97^{eB} ±0.29
AF Standard (5 mM)	18.98^{dA} ±0.19	18.30^{dB} ±0.94	16.19^{eC} ±0.72	16.05^{eC} ±0.66
AF Standard (10 mM)	24.61^{bA} ±0.89	20.66^{bB} ±0.62	20.03^{bC} ±0.29	20.00^{bC} ±0.56
Sodium erythorbate (0.5 %)	63.52^{aA} ±0.60	50.55^{aB} ±0.51	40.35^{aC} ±0.19	40.35^{aC} ±0.89

Values with different letters (a-g) in the same column and (A-C) in the same line differ significantly (P<0.05). Results shown as average ± SD. AF-GM: Phytic acid from corn germ

IV. DISCUSSION

The elevated inhibition of *in vitro* lipid peroxidation by phytic acid was likely caused by the combination of the chelant property of Fe^{+2} and its ability to scavenge the free radicals, such as peroxyl and alkoxyl radicals, which has also been observed in various studies [1,2,3,5,6,7]. Therefore, these results confirmed that the phytic acid from corn germ was efficient in the inhibition of lipid peroxidation *in vitro.*

Results of TBARS and WOF confirmed that corn germ purified phytic acid can be used in the inhibition of lipid oxidation in refrigerated chicken meats, corroborating the results obtained by the *in vitro* assay in the Fe^{+2}/ascorbic acid system or in the deoxyribose, bathophenanthroline and $DPPH^{\bullet}$ assays carried out by Filgueiras et al. [7].

The ability of phytic acid to chelate transition metal ions involved in the production of free radicals and/or scavenge of these radicals can reduce the oxidation velocity of oxymyoglobin to metmyoglobin [5]. The chelant activity of Fe^{2+} increased with an elevated concentration of corn germ purified phytic acid and inhibited the formation of metmyoglobin, maintaining the chicken original thigh colour.

V. CONCLUSIONS

The corn germ purified phytic acid inhibited lipid peroxidation induced by iron *in vitro* and metmyglobin formation, and this inhibition was dependent on the concentration of phytic acid and the storage time of the refrigerated chicken meat. The corn germ purified phytic showed a great potential to be used as an antioxidant for refrigerated chicken meat.

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