EXTRACTION , PURIFICATION AND PARTIAL CHARACTERIZATION OF PHYTIC ACID FROM DEFATTED SESAME OIL CAKE

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ABSTRACT

In this study phytic acid (PA) extracted from defatted sesame oil cake using three different solutions (distilled water , HCl 0.5 M , TCA 3%) , then it was purified by removing of protein and minerals, and tested for the prevention of browning in apple slices and color degradation of anthocyanin solution during storage time at room temperature(25°C). The antioxidant activities of irradiated and non- irradiated phytic acid were tested on DPPH radical. The results revealed that the highest phytic acid percentage was observed with TCA 3% after 6 hr extraction with (2:100 w/v) solid: liquid ratio, and the lowest values were noticed with distilled water at all mixing ratios. It has been noticed that the purified PA was effective in preventing color degradation for anthocyanin solution when used at 0.025% and 0.05% over 11 days storage at room temperature, and it was also effective in preventing apple slice browning when apple samples immersed for 10 min in phytic acid solutions (0.5 % & 1.0 %) and stored in polyethylene bags (vacuumed) at 7°C for 37 days. The antioxidant capacity of the purified phytic assay showed that the phytic acid irradiated at 22kGy gave the highest antioxidant activity whereas the non- irradiated phytic had no antioxidant activity on DPPH radical.

Key words : antioxidant, sesame, browning reaction, phytic acid.
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استخلاص وتنقية وتوصيف جزئي لحامض الفايتيك من مخلفات السمسم مزالة الزيت

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المستخلص

تضمنت الدراسة الحالية استخلاص حامض الفايتيك من مخلفات السمسم المزالة الدهن باستخدام ثلاثة محاليل استخلاص مختلفة (الماء المقطر،حامض الهيدروكلوريك 0.5 مولار، ثلاثي كلورو حامض الخليك3%)، ثم اجريت له عملية تنقية بازالة البروتينات والمعادن وتم اختبار قابليتهِ في منع الاسمرا ر في شرائح التفاح المعاملة به والمُخزنة على حرارة 7 °م وفي منع تحلل صبغة الانثوسيانين اثناء خزنها لمدة 11 يوم على درجة حرارة الغرفة 25 °م. كما تم أختبار قابلية حامض الفايتك (المعامل بالاشعاع وغير المعامل) كمضاد للأكسدة باستخدام الـ DPPH . اشارات النتائج المستحصل عليها الى ان اعلى نسبة مئوية من حامض الفايتيك المستخلص كانت في محلول ثلاثي كلورو حامض الخليك وبنسبة خلط 2:100 و/ح ومدة استخلاص 6 ساعات وان اقل القيم ظهرت في مستخلص الماء المقطر وفي كافة نسب الخلط المستعملة. واشارت نتائج فاعلية حامض الفايتك في حفظ الصبغات الى ان معاملة محلول صبغة الانثوسيانين بتركيزين من حامض الفايتيك (0.025 و0.05 ملغم/مل) أسهم في اطالة مدة حفظ صبغة الانثوسيانين خلال 11 يوم خزن على درجة حرارة الغرفة 25 °م. كما لوحظ ان حامض الفايتيك المنقى كان فعالاً في منع الاسمرار لشرائح التفاح التي غمرت في محاليل مختلفة التركيز من حامض الفايتيك (1%و0.5%) ولمدة 10 دقائق ثم حفظت في اكياس من البولي اثيلين بعد ازالة الهواء مدة 37 يوم وعلى حرارة 7°م واظهر اختبار قابلية حامض الفايتيك كمضاد للأكسدة ان حامض الفايتيك المنقى والمعامل بأشعة كاما (22KGy) اعطى اعلى نسبة مئوية للفعالية عند استخدام DPPH في حين لم يظهر حامض الفايتيك المنقى غير المشعع اي فعالية مضادة للاكسدة اتجاه DPPH.

الكلمات المفتاحية: مضادات الأكسدة، السمسم،التفاعل البني،حامض الفايتيك.
جزء من رسالة ماجستير للباحث الأول.

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INTRODUCTION

Phytic acid a naturally occurring plant acid ,exists in a large amount (1-5%) in edible plant seeds. Two structures were proposed for phytic acid, one by Neuberg(1908) being $C_6H_{24}O_{27}P_6$ with 18 acid hydrogen about an inositol phosphate nucleus, the second structure was proposed by Anderson (1914) $C_6H_{18}O_{24}P_6$ with 12 acid hydrogen. Some authers favored the Neuberg formula (4) and others agreed with Anderson model (5). Phytic acid is historically known to be an anti-nutrient due to its ability to chelate minerals such as Ca^{+2},$Fe^{+3}$and Zn^{+2} , protein and starch causing a decrease in thier bioavailability in human and animal models (13,15). However many researchers were reported that the phytic acid has an antioxidant, anticancer , hypoglycemic and hypolipidemic function (11). Moreover ,other researchers mentioned to phytic acid effects on an enzymatic and non-enzymatic browning, milliard reaction and the formation of acrylamide (6,17) . Phytic acid is a strong antioxidant due to its ability to chelate (Fe^{+2}) , so it is a potent inhibitor of the iron-driven formation of reactive oxygen species that adversely affect the production and storage of various forms of food. Although phytic acid is not affirmed GRAS (Generally Recognized As Safe) by the United State Food and Drug Administration ,it is used widely as food additives in several countries , phytic acid produced by Tusno has been approved as GRAS by FDA in the U.S. It is used as an acidulate for pH adjustment and for prevention of color degradation, besides its strong antioxidant action. In view to the important role of phytic acid in the natural and artificial preservation oxidizable material and perhaps, in prevention of a number of the undesirable changes in food this study conducted to find out the optimum condition for phytic acid extraction, form sesame oil cake and examine some of its properties.

MATERIAL AND METHODES

Sample preparation

Sesame oil cake was obtained from local factory of Baghdad - Iraq; cleaned ,defatted using diethyl ether according to Borchani *et al.* (2) then dried at room temperature and kept in same temp.

Phytic acid extraction and determination

Different extraction solutions were used being distilled water , HCl (0.5M) , and TCA(3%) in this study . Defatted sesame oil cake mixed at different ratios (2,5,10:100) with above solutions and held at room temperature (25°C) for 12 hr. The phytic acid content of each extract after 1,2,4,6,8,10,12 hr of extraction time was determined to find out the optimum condition for phytic acid extraction. The extracted phytic acid was purified according to Canan *et al.* (3) as described in following diagram:

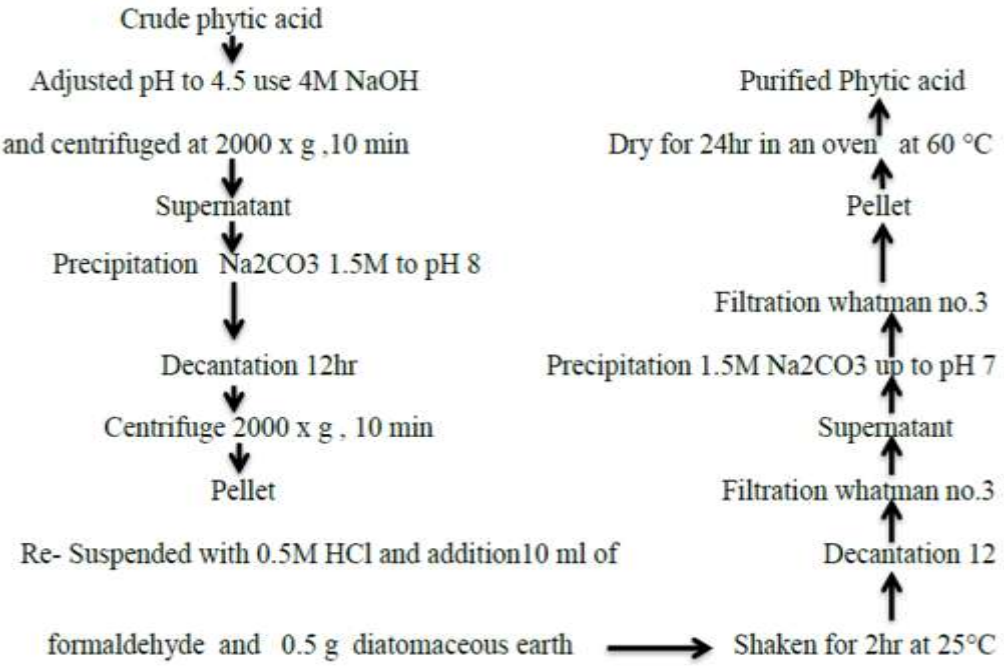

Diagram for extraction and purification of sesame oil cake phytic acid (PA).

Standard curve preparation

A series of standard sodium phytate (Sigma) solution (0.02-0.2 mg/ml) were prepared . Aliquot of each (3 ml) concentration was mixed with (1 ml) of wade reagent (0.03% $FeCl_3.6H_2O$ + 0.3% sulfsalicylic acid) and vortexed for 5 second the absorbance of the mixture measured at 500 nm using spectrophotometer type (Optima SP-300, Japan).

Phytic acid determination

The phytic acid content was determined following Latta and Eskin (9).1ml of extracted (PA) diluted to 25ml with distilled water , 3ml of diluted sample was mixed with 1ml of wade reagent and vortexed for 5 second the absorbance of the mixture measured at 500 nm.

Anthocyanin preservation

Anthocyanin pigment solution prepared according to Ranganna (14) , 20 ml of prepared solution diluted to 200 ml and 20 ml of diluted solution was transferred in 3 containers (each containing 20 ml) , two of the container mixed with a certain amount of phytic acid solution to achieve (0.025 and 0.05 mg/ml) phytic acid , and the third container mixed with 1 ml of distilled water (control) . All containers kept at room temperature (25°C) for 11 days . The degree of anthocyanin degradation was determined by measuring the absorbance at 535 nm as well as by naked eye .

Prevention of browning reaction

The obtained phytic acid was used to prevent the browning reaction in apple slices .The apple slice (40 mm) soaked in phytic acid solution (0.5 and 1%) at pH 3 for 10 min , Then the slices were removed and packed in polyethylene bag under vacuum and stored at 7°C. The color changes determined by naked eyes. Ccontrol sample was apple slices soaked in distilled water under the same conditions

Antioxidant capacity of phytic acid

DPPH radical scavenging activity:- The free radicals scavenging activity of phytic acid extracted from sesame oil cake was determined according to the method described by Shimada *et al.* (16). Phytic acid solution with different concentrations (0.05% ,0.075% ,1.5%) were used. 1.5ml of each concentration was mixed with 1.5ml of 1mM DPPH (Sigma) ethanol solution. The mixture was shaken and kept at 25°C for 30 min in dark place and then the absorbance of the mixture was measured at 517nm against a blank . The DPPH radical – scavenging capacity was estimated from differences between the absorbance with or without samples and expressed as a percentage of DPPH- scavenging activity.

RESULTS AND DISCUSSION

Phytic acid was extracted using different solutions as summarized in Table 1. The most satisfactory level of extraction was gained using 3% TCA with a yield of 7.2% phytic acid during a period of extraction for 6 hr, followed by using 0.5M HCl which gave a yield of 6.56% of phytic acid during one hour extraction. Meanwhile using distilled water was not efficient for the extraction of phytic acid compared to other solutions, since it was used for 4 hr with a yield of 2.75% phytic acid. These findings were higher than that reported by Norazalina *et al.*(12) when HCl 3%+TCA10% were used. While El-Sayed (7) stated that the higher value of PA (6.5%) was achieved when HCl (2.4%) was used as extraction solution .These differences in PA yield may be attributed to the extraction condition , determination method and the source of the raw material. According to the above observations 0.5M HCl was chosen as the most satisfactory solution for phytic acid extraction and that agreed with most of the earlier reports which showed that HCl is a highly sensitive solution for small quantities of phytic acid specially with immature seeds (10).

Table 1. Optimization of extraction condition of phytic acid from sesame oil cake using distilled water , 0.5M HCl and 3% TCA with different solid:liquid mixing ratio

Time(hr) Phytic acid%								
12	10	8	6	4	2	1	Mixing ratio	Extraction solutions
2.17	2.2	1.61	2.34	2.75	1.75	1.3	100:2	
1.32	1.66	0.64	0.36	0.55	0.44	0.25	100:5	Distilled water
0.65	0.84	0.93	0.26	0.28	0.22	0.02	100:10	
5.42	5.91	6.04	5.86	6.17	6.12	6.56	100:2	
1.17	2.19	2.33	2.53	3.35	3.2	2.94	100:5	0.5 M HCl
4.98	4.72	4.74	5.08	4.85	4.89	4.8	100:10	
6.08	6.5	6.7	7.2	6.8	6.7	6.22	100:2	
2.58	2.76	2.9	2.9	4.55	6.3	3.8	100:5	3% TCA
5.1	4	4.46	4.8	5	3.7	4.1	100:10	

Anthocyanin was incorporated into the human diet for many years due to its great impact on health as a suppressor to cell cancer by scavenging free radicals, controlling blood pressure, working as antimicrobial and anti-inflammatory agent, improving eyesight, protecting against liver injuries and many other demonstrable benefits. The addition of phytic acid to prevent the degradation of anthocyanin was presented in Fig 1 and Table 2. The degradation of anthocyanin was decreased by increasing phytic acid concentration. Using 0.05mg/ml of phytic acid retained about 0.309 (mg/100g) of anthocyanin compared to 0.298 (mg/100g) by using 0.025mg/ml of phytic acid. While the control retained 0.198 (mg/100g) after 5 days of storage under 25°C. This observation is similar to the findings of many related studies in this area (8). They concluded that using phytic acid in food beverages has a great impact to prevent color degradation including anthocyanin.

Table 2. Effect of phytic acid concentration (0.025, 0.05 mg/ml) on Anthocyanin degradation during 11 days storage at 25°C.

	Control	0.025mg/ml	0.05mg/ml
0 Day	0.61	0.61	0.59
3 Day	0.31	0.39	0.41
4 Day	0.246	0.35	0.37
5 Day	0.198	0.298	0.309
7 Day	0.143	0.246	0.252
11 Day	0.067	0.162	0.171

Figure 1. Prevention of anthocyanin degradation during 11 days storage at 25°C using different concentration of phytic acid (A:0.05, B:0.025 mg/ml, C: Control) .

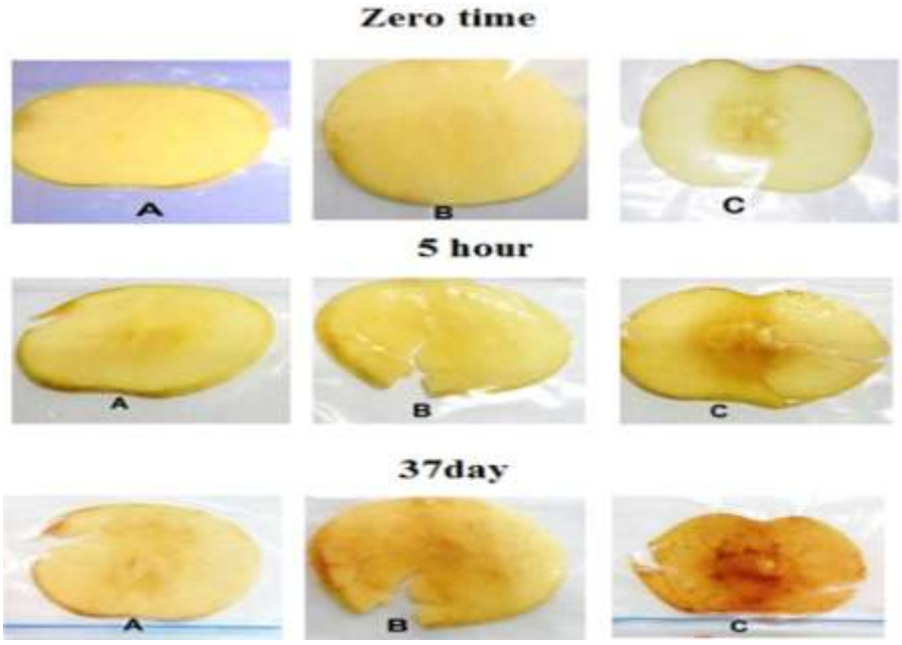

Figure 2. Effect of phytic acid concentrations (A:1%PA, B:0.5%PA, C: 0%PA) in prevention the Browning reaction in Apple slices during different storage time at 7°C

An experiment was conducted to investigate the ability of phytic acid to prevent browning reactions of apple slices during storage under 7 °C. Results in Figs 2 showed a rapid changing in the color of apple slices submerged in water only (C) as Compared with those submerged with 0.5% (B) of phytic acid which had slow browning but using 1% (A) of phytic acid solution had very slow change in their color. Prevention of color changing in apple slices increased by increasing the concentration of phytic acid solutions. These results agreed with Du *et al.* (6) who found that phytic acid by virtue of its ability to bind with polyphenol oxidase and chelate Fe, shows inhibition of browning reactions. For that phytic acid considered as a potent inhibitor of the reaction of iron driven formation of reactive oxygen species that affects the storage of food products. Table 3. shows the antioxidant activity of different concentration of irradiated phytic acid(0.05,0.075,1.5%) toward DPPH (2,2 diphenyl -1- picryl hydrazyl). It has been noticed that the non- irradiated phytic acid solution was not able to show DPPH radical – scavenging activity regardless of its concentration, however the irradiated phytic acid showed DPPH radicals – scavenging activity with a maximum value of 45%, when 1.5% phytic acid solution irradiated at 22KGy .Moreover, the radical scavenging activity was positively proportional to irradiation dose and to phytic acid concentration as well. This finding was in agreement with that reported by Ahn *et al.* (1) as he noticed that the irradiation dose was positively correlated with DPPH radical – scavenging activity in his study. Phytic acid function as antioxidant could be attributed to its ability in chelating iron and suppressing the iron catalyzed oxidative reaction (8). Ahn *et al.* (1) stated that gamma irradiation induces the radiolysis of phytic acid in aqueous solution and increasing the free radical – scavenging activity of degraded phytic acid, hence irradiation process reduces the levels of phytic acid and increases the antioxidant activity.

Table 3. Antioxidant activity of irradiated phytic acid on DPPH

scavenging activity%	Irradiation dose (KGy)	PA%
7	10	0.05
12.3	22	0.05
21.1	10	0.075
25.4	22	0.075
38.7	10	1.5
45	22	1.5

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