Evaluation and Stabilization of Wheat Germ and Its Oil Characteristics

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ABSTRACT

Wheat germ (WG) was analysed for its proximate composition, amino acids and minerals. Physico-chemical parameters, lipid classes and fatty acid composition of wheat germ oil (WGO) were also determined. Wheat germ was subjected to different heat treatments in order to inactivate lipase. The results showed that WG contains high levels of protein (34.92%). Crude ether extract, crude fibers, and ash contents were 10.74, 4.89 and 5.17%, respectively. The most abundant minerals (mg/100g) were: Potassium (1567.6) and phosphorus (389.5). Moreover, wheat germ showed to be a good source for essential amino acids (35.81g/100g protein). Saponification, iodine and peroxide values of the WGO were 182.94, 112.54 and 0.35 (mEq O_2/kg), respectively. Also, the free fatty acids (as % oleic acid) and the unsaponifiable matters were 2.39% and 1.92%, respectively. The wheat germ oil was found to be an incredible source of ω_3 and ω_6 essential fatty acids. Furthermore, the unsaturated fatty acids represent around 80% of the total fatty acids. A slight difference was observed in the fatty acid composition before and after lipase inactivation. The TLC technique for lipid classes showed that the triacylglycerols fraction was considered as the major fraction. Also, phosphatidyl inositol, phosphatidyl choline and phosphatidyl ethanol amine were the major fractions of wheat germ phospholipids. The results also showed that the sample subjected to a heat treatment (100°C/ 30min) before storing at –18°C was more efficient to inactivate the enzyme activity as compared to the other four samples stored at different conditions.

Key words: wheat germ, wheat germ oil, lipase inactivation, fatty acids, amino acids, minerals.

INTRODUCTION

Wheat is a staple crop used for food production throughout the world. Wheat germ is a by-product obtained from commercial wheat flour production. The germ is the most nutritious part of the wheat grain, representing about 2-3% of the whole grain (Rao *et al*., 1980, Bidlack, *et al*., 2000, Piras, *et al*., 2009). Commercial germ fraction is not completely pure but it may contains variable portions of bran and endosperm. It can be used in foodstuffs but it must be stabilized against hydrolytic and oxidative rancidity. This may be partly achieved by drying the germ to 4.0% moisture content (Barnes, 1982). The germ contains a mostly 28% protein and it's a good source of essential amino acids as compared to other cereal products. The high nutritional value of wheat germ makes it a good enrichment component in many foods (Ibanoglu, 2002). On the other hand, a minor part of wheat germ of the annual world production (16 millions tons) is used for human consumption, but a major part of it is used for fodder (Appett, 1986).

The germ is usually removed from the endosperm during wheat milling process because of its unfavourable baking properties and susceptibility to oxidation. Differences in elasticity and friability between endosperm and outer layers are used to favour the separation of flour and semolina from bran in the milling process of wheat grain (Sjövall *et al*., 2000).

The beneficial effects of vegetable oils in human diet have been well known, basically due to their high content of unsaturated fatty acids and their high energy value. Wheat germ contains 10-12% oil consisting mainly of oleic, linoleic and linolenic acids (Mecham, 1978, Sjövall *et al*., 2000, Gomez & Ossa, 2002).

High lipase and lipooxygenase activities as well as a high content of unsaturated fatty acids are

characteristics of wheat germ (Sjövall *et al.*, 2000). Although wheat germ is the richest source of antioxidant glucosylated hydroquinones and tocopherols, a slight oxidation may cause destruction for essential fatty acids and vitamins (Appett, 1986, Zhokhov *et al*., 2010).

The poor stability of raw germ restricts its uses, this problem could be overcome by inactivating the enzymes by heat shock (Rao *et al*., 1980, Pinarli *et al.*, 2004) or by removing the oil fraction (by extraction) from the wheat germ (Karwowska & Kostrzewa, 1988) or combined techniques. Wheat germ also can be subjected to different heat processed using roasting, microwave heating, autoclaving or oven drying to improve the shelf-life of the wheat germ (Appett, 1986).

The present work has been done to study the physico- chemical properties and stabilization of wheat germ. Also, to study the identity characteristics, lipid classes and fatty acid composition of wheat germ oil.

MATERIALS AND METHODS

Materials

Five kilograms of fresh wheat germ (WG) were obtained from commercial mill (El Tahanon El Massreyon Company, 6th October City, Egypt). Wheat germ sample was packed in polyethylene bags and stored at –18°C for further study.

Methods

Chemical Methods

Proximate composition of wheat germ (WG) including moisture content, crude protein, crude ether extract, crude fibers and total ash were carried out according to the AOAC (1995) procedures. Minerals (Ca, Mg, Mn, Fe, Cu and Zn) were measured in ash solution using Perkin Elmer Atomic Absorption Spectrophotometer (Model 2380). Sodium and potassium were determined by using the flame photometer (Gallenkamp, FGA 330C). Total phosphorus was assayed colorimetrically at 630 nm using Carl Zeiss Specol Colorimeter as described by the AOAC (1995).

Amino acid composition was determined in the protein hydrolyzate by the method of Spackman *et al*. (1958) using Beckman amino acid analyzer (Model 119 CL). Tryptophan was quantified on the $Ba(OH)_2$ hydrolyzate by the colorimetric method of Miller (1967).

Physico-chemical characteristics of wheat germ oil

Wheat germ oil (WGO) was extracted by a solvent extraction technique using n-hexane (BP. 68°C). Refractive index at 25°C, iodine value, saponification value, peroxide value (as mEq O_2/kg oil), free fatty acids (as oleic acid %) and unsaponifiable matter (%) were determined as described in the AOAC (1995).

Preparation of fatty acid methyl esters from WGO were performed according to the procedure of Radwan (1978) using 1% sulphuric acid in absolute methanol. The fatty acid methyl esters obtained were separated by Shimadzu Gas Chromatograph (GG–4 CM-PFE) under the following conditions: Column, 10% DEGS on 80/100 chromosorb QIII, Detector, FID, column temp. 190-240°C, Detector temperature, 270°C flow rate, 20 ml/min, Gas flow, N_2 and chart speed, 5 mm/min. Crude oil extract was fractionated using a thin layer chromatography (TLC) technique according to the method of Mangold & Malins (1960) on glass plates (20×20 cm) precoated with 0.25 mm silica gel, G60. Neutral lipids were developed in petroleum ether: diethyl ether: glacial acetic acid (70:30:2, v/v/v) and separated spots were visualized by iodine vapour. Polar lipids (phospholipids) were separated by TLC using chloroform: methanol: water (65: 25: 5, v/v/v). The separated spots were visualized by iodine vapour. Also, triacylglycerols were fractionaed by TLC technique according to the method of Barrett *et al*. (1962). Triacylglycerols were developed using toluene: diethylether (96:4 v/v) and visualized by charring with 50% aqueous sulphuric acid.

Wheat germ stabilization

Pre-experiment was done to select the suitable time for wheat germ stabilization by heating in an oven as follows: Approximately 200g wheat germ were placed in pyrex petri dishes and heated in an oven at 100°C for different periods, 15, 30, 45, 60 min at the same conditions. Heated wheat germ samples were cooled at ambient temperature and incubated at 35°C for 1 week. Free fatty acids were determined and expressed as % oleic acid to compare among different applied heat treatments for wheat germ stabilization (complete inactivation of lipase in wheat germ was reached after 30 min of heating).

Heat treatment and storage condition

Approximately 2 kg from the wheat germ sam-

ple were divided into 5 parts. One part was heated in an oven at 100°C for 30 min, in the form of thin layer, cooled at ambient temperature and stored in polyethylene bag at –18°C for 4 weeks. The other four parts were stored (without heating) at different temperatures: –18, 5, 22, 35±2°C (Freezer, refrigerator, ambient and incubator for 4 weeks).

About 50g of each treatment of wheat germ were ground and extracted with petroleum ether (40–60°C). Oils extracted from each sample at weekly intervals (up to 4 weeks) were used for determination of free fatty acids as % oleic acid and were used for the fractionation to different classes by TLC as mentioned previously.

Statistical analysis

The standard deviation (SD) was calculated using the method described by Steel & Torrie (1980).

RESULTS AND DISCUSSION

Wheat germ

Proximate chemical composition

Table (1) presents the proximate composition of wheat germ (WG). The values of crude protein, crude ether extract, ash and crude fiber contents were 34.92, 10.74, 5.16 and 4.89%, on dry weight basis, respectively. Al-Kahtani (1989) mentioned that the moisture content varied from 6.95% in Saudi WG and 5.32% in the French WG due to subjecting to a heat treatment, which was very low as compared to our sample (12.46%). Still, the crude protein and crude fibers contents reported by Al-Kahtani (1989) were slightly lower (33.80 and 2.60%, respectively) as compared to our results (34.92 and 4.89%, respectively). Abou-Zaid (1978) and Pomeranz (1988) reported that moisture, crude protein, crude ether extract, crude fibers and ash contents of WG ranged from 5.3 to 13.30%, 27.0 to 33.8%, 6.0 to 14.0%, 1.33 to 5.5% and 3.96 to 5.18%, respectively. These differences may be attributed to the variety and cultivating conditions.

Arshad *et al*. (2007) reported that the crude fiber and ash contents were 5.35 and 4.52%, respectively for defatted wheat germ flour which are in agreement with the present results, although the protein content was higher in our study (34.92%) as compared with that reported by Arshad *et al*. (2007).

Minerals content

Table (2) represents the minerals content of wheat germ. Concentration of nutritive macro and micro elements of wheat germ are arranged in a descending order as follows: Potassium (1567.6), phosphorus (389.5), sodium (272.1), magnesium (224.3), calcium (153.0), iron (11.15), manganese (5.42), zinc (3.81) and copper (2.73) mg/100g. The most abundant mineral was potassium (1567.6 mg/100g). This result is comparable with that reported by Carcia *et al*. (1972), but lower than that reported by Arshad *et al*. (2007). Table (2) further shows that values for the most minerals (Mg, Ca, Na, Mn and Cu) were in good agreement with those reported by Carcia *et al*. (1972) for wheat germ.

Amino acid composition

Table (3) shows the amino acid composition of wheat germ as g/100g protein. The present results indicate that aspartic acid (12.67) and glutamic acid (14.64) were the major abundant amino acids in the WG. The total essential amino acids in wheat germ were 35.81g/100g protein. Threonine (5.01), me-

Table 1: Proximate chemical composition of wheat germ

Constituent	Value*(%)	
	On fresh weight basis	On dry weight basis
Moisture content	12.46±0.12	---
Crude protein	30.57±0.33	34.92±0.43
Crude ether extract	9.40±0.14	10.74±0.18
Ash	4.52±0.17	5.16±0.2
Crude fibers	4.28±0.20	4.89±0.23
Nitrogen free extract **	38.77±0.96	44.29±1.02

*Mean ± SD. ** Calculated by difference.

Table 2: Minerals content of wheat germ

Minerals	Values (mg/ 100g)
Potassium (K)	1567.6
Sodium (Na)	272.1
Magnesium (Mg)	224.3
Calcium (Ca)	153.0
Iron (Fe)	11.15
Manganese (Mn)	5.42
Zinc (Zn)	3.81
Copper (Cu)	2.73
Phosphorus (P)	389.5

Table 3: Amino acid composition of wheat germ

Amino acid	g/100g protein	
	Wheat germ	FAO pattern*
Isoleucine	1.90	2.80
Leucine	4.97	6.60
Lysine	5.70	5.80
Methionine	3.19	---
Cystine	0.12	---
Total sulfur amino acids	**3.31**	**2.50**
Tyrosine	2.37	---
Phenyl alanine	4.75	---
Total aromatic amino acids	**7.12**	**6.30**
Threonine	5.01	3.40
Tryptophan	0.76	1.10
Valine	3.46	3.50
Histidine	3.58	1.90
Total essential amino acids	**35.81**	**33.90**
Arginine	6.97	
Aspartic acid	12.76	
Glutamic acid	14.64	
Serine	6.85	
Proline	4.68	
Glycine	7.70	
Alanine	6.54	
Total non-essential amino acids	**60.14**	

* FAO/ WHO/ UNU (1985).

thionine (3.19), histidine (3.58) were much higher than the FAO/ WHO/ UNU (1985) reference values of 3.40, 2.5, 1.9g/100g protein, respectively. Only lysine (5.7) and valine (3.46) showed comparable values to the FAO, reference values being 5.8 and 3.5 g/100g protein, respectively. Isoleucine (1.90), leucine (4.97), and tyrptophan (0.76) showed lower values than the FAO reference values of 2.8, 6.6 and 1.1 g/100g protein, respectively. However, the present results indicate that wheat germ is still a good source of the essential amino acids and its protein is of high quality. The results are also in a good agreement with those reported by Yiqiang *et al*. (1999).

Wheat germ stabilizations

Figure (1) shows the effect of storage at different temperatures (–18°C, 5°C, 25°C and 35°C) on the enzymatic activity of wheat germ as compared with wheat germ sample stored at –18°C after subjecting to a heat treatment at 100°C for 30min. It could be seen from the figure that there was a decrease in hydrolysis activity for the wheat germ sample stored at –18°C after heat treatment due to lipase inactivation as compared to the other samples stored at different temperatures, which was also observed in the TLC for the total lipid classes (Fig. 2). Also, it can be observed from the figure the high content of free fatty acids produced during the entire storage period due to the high enzyme activity of the samples stored at room temperature (25°C) and in the incubator (35°C). Megahed (2011) found that the lipase activity was suppressed by exposure to a heat treatment of 70°C for 30 min to keep the majority of oil triacylglycerols in the intact condition. It can be mentioned that the best method to store the wheat germ, is to inactivate the enzymes first by heat treatment followed by storing in deep freezer at –18°C.

Wheat germ oil

Physico-chemical properties

Table (4) presents the physico- chemical characterization of wheat germ oil (WGO). The oil is liquid at ambient temperature (25°C), the iodine value (IV) was 112.54, being much higher than that reported by Taniguchi *et al.* (1985) being 107 and comparable to the results reported by Gomez & Ossa (2002) ranging from 107 to 115. On the other hand, Barnes (1983) mentioned that IV of WGO ranged from 120 to 130. The refractive Index (RI) of WGO was 1.4762. This result is in agreement with those mentioned by Gomez & Ossa (2002) being 1.472 and also with the results reported by Barnes (1983) which ranged from 1.470 to 1.480. Furthermore, saponification value (SV) was 182.94, which shows a slight difference with the results indicated by Barnes (1983) (184-185). Gomez & Ossa (2002) mentioned a wide great range of SV varied from 181 to 197 according to the method of extrac-

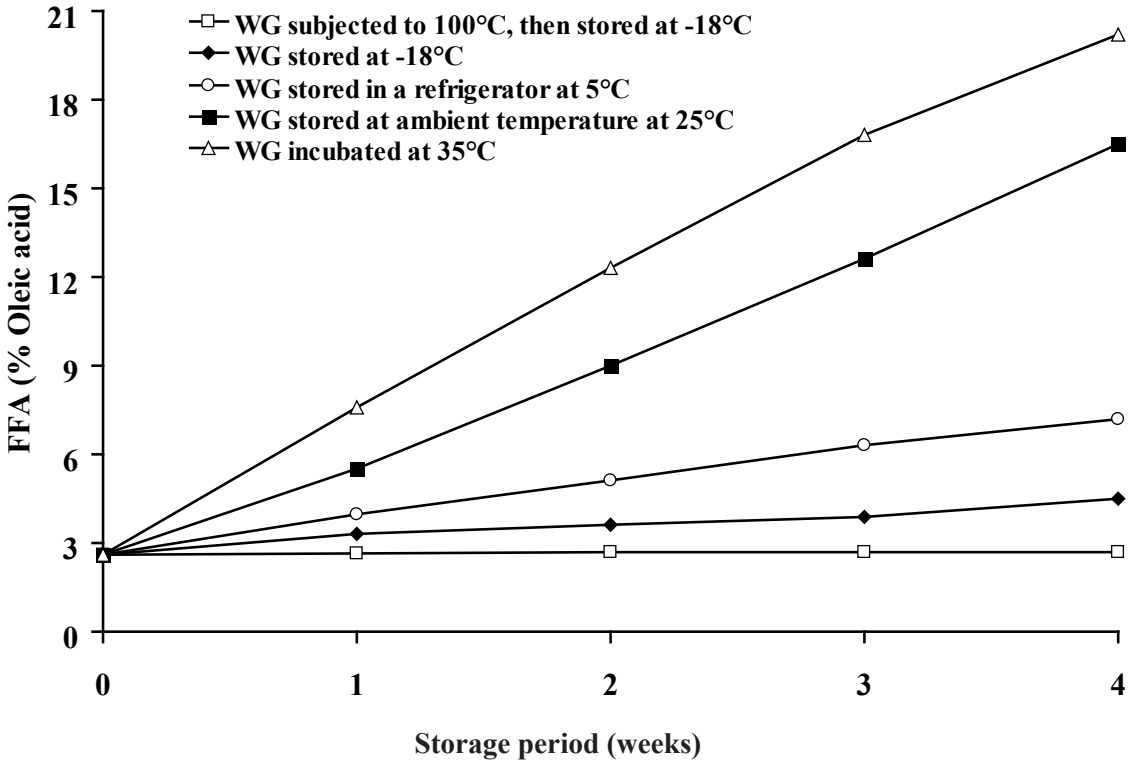

Fig. 1: FFA as % oleic acid for wheat germ stored at different storage conditions

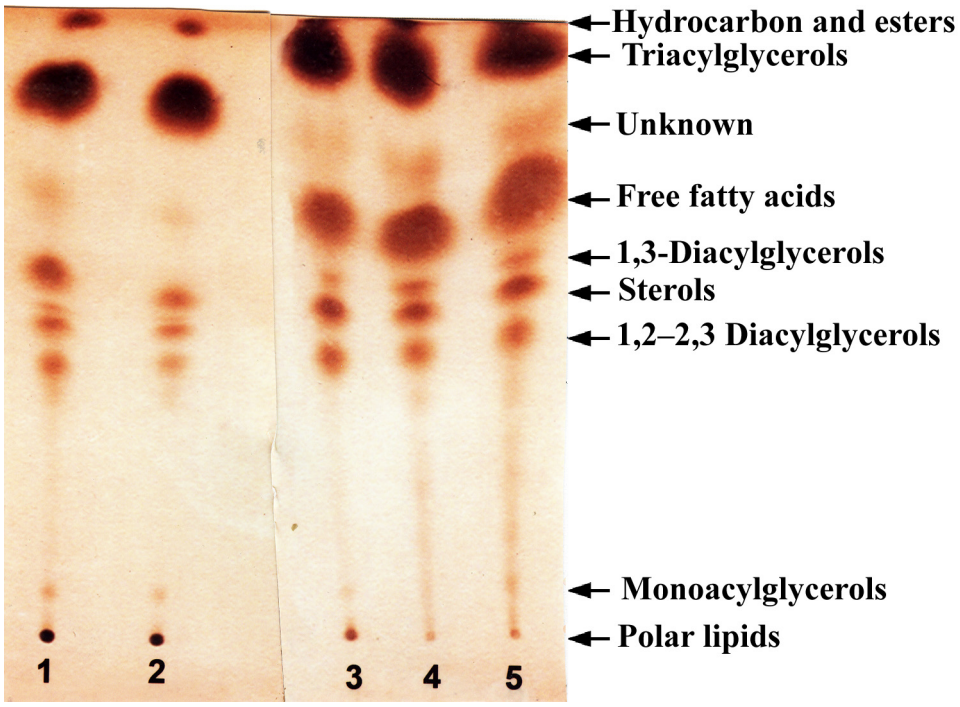

Fig. 2: TLC for lipid classes of wheat germ (WG) subjected to different storage conditions (4 weeks)

1- Raw WG, stored at –18°
2- WG subjected to 100°C, stored at –18°C
3- WG stored at 5°C
4- WG stored at ambient temperature
5- WG incubated at 35°C

Table 4: Physico-chemical properties of wheat germ oil

Property	Value*
Iodine value	112.54±0.83
Refractive index (25°C)	1.4762±0.00
Saponification value	182.94±1.12
Unsaponifiable matters (%)	1.92±0.24
Free fatty acids (as % oleic acid)	2.39±0.10
Peroxide value (mEq O_2/kg oil)	0.35±0.03

* Means±SD

tion. Table (4) also shows that the unsaponifiable matter value for WGO was 1.92%. Barnes (1983) mentioned that the range of unsaponifiable matter ranged from 1.5 to 7.8%. Furthermore, Saker *et al.* (1986) found that the unsaponifiable matter was 3.8%. Although, Koukoubains & Boskou (1984) mentioned a higher value for the unsaponifiable matter being 10.50%. The free fatty acid (FFA) content was 2.39%, which seems to be in the same range for the results mentioned by Barnes & Taylor (1980) for commercial wheat germ oil (0.7-7.7 g FFA/ 100g). Gomez & Ossa (2002) reported that the FFA ranged from 12.4 to 31.6 depending on the method of extraction and the storage conditions which seems to be very comparable to our results (2.39%). However, Wang & Johnson (2001) reported that wheat germ oil contained 15.7% FFA. As shown in Table (4), peroxide value (PV) of WGO was 0.35, whereas Tag El-Deen (2004) mentioned that the PV of WGO was found to be zero, this latter result was in agreement with that reported by Ibrahim *et al.* (1990) but differ from that reported by Wang & Johnson (2001). These differences may be attributed to the method of extraction, storage conditions, temperature, light and relative humidity, which affect the oil quality.

Fatty acid composition

Table (5) shows the fatty acid composition of WGO before and after lipase inactivation. The results indicated that WGO is rich in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) which represented around 80% of the total fatty acids. Linoleic acid was the major fatty acid, accounted for 58.61% of the total fatty acids, whereas linolenic and oleic acids represent 9.58 and 11.21% of the total fatty acids, respectively. Small amount of palmitoleic acid (0.23%) was also present. These results are in agreement with the results reported by Sjövall *et al.* (2000) for oleic (10.9%), linoleic (58.2%) and linolenic (9.3%) acids. However, Barnes (1982) mentioned that oleic, linoleic and linolenic acids represented 13.8, 59.3 and 11.4%, of the total fatty acids, respectively, of WGO. Also, Tag El-Deen (2004) mentioned that oleic, linoleic and linolenic acids of WGO accounted for 15.8, 54.7 and 6.33% of the total fatty acids, respectively, which was different from our results. Moreover, Piras *et al*., (2009) found that the PUFA were the main group of fatty acids in wheat germ oil, ranged from 57 to 60% and the main unsaturated fatty acids were oleic, linoleic and linolenic acids. The data in Table (5) indicate that most of the saturated fatty acids present were palmitic acid (17.92%), whereas myristic and stearic acids represented only 1.30 and 1.15%, respectively. Megahed (2011) reported that the palmitic acid makes more than 80% of the saturated fatty acids of wheat germ oil.

Table 5: Fatty acid composition of wheat germ oil before and after lipase inactivation

Fatty acid	%	
	Before inactivation	After inactivation
Myristic acid ($C_{14:0}$)	1.30	1.27
Palmitic acid ($C_{16:0}$)	17.92	15.81
Palmitoleic acid ($C_{16:1}$)	0.23	0.20
Stearic acid ($C_{18:0}$)	1.15	1.33
Oleic acid ($C_{18:1}$)	11.21	15.23
Linoleic acid ($C_{18:2}$)	58.61	54.50
Linolenic acid ($C_{18:3}$)	9.58	8.96
Total saturated (S)	20.37	21.11
Total unsaturated (U)	79.63	78.89
U/S ratio	3.91 : 1	3.74 : 1

A slight difference was also observed after enzyme inactivation (at 100°C/ 30min) for most of the fatty acids, whereas there was a considerable increase for oleic acid from 11.21 to 15.23%. On the other hand, as shown in Table (5) there was a considereable decrease from 58.61 to 54.5% for linoleic acid and a slight decrease from 9.58 to 8.96 for linolenic acid, due to the applied heat treatment which leads to a slight oxidation of PUFA.

Lipid classes, phospholipids and triacylglycerols

Figure (3a) shows the lipid classes fractions by TLC technique for raw and treated wheat germ.

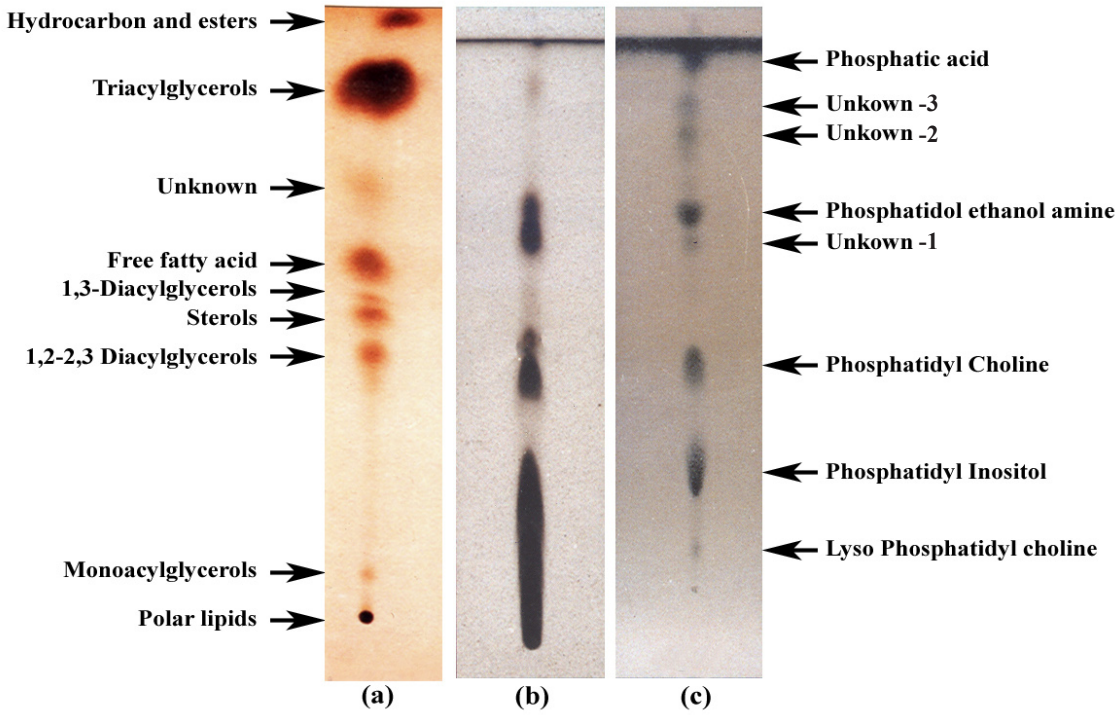

Fig. 3: TLC of the lipid classes (a), triacylglycerol (b) and phospholipid (c) fractions of wheat germ oil

The chromatogram revealed that the crude oil consisted of 8 fractions mainly acylglycerols and nonacylglycerols compounds in addition to polar lipids located on the base line. As it can be observed, triacylglcerols fraction is considered as the major fraction of lipid classes. Also, diacylglycerols (1,2-2,3 and 1,3) of wheat germ oil were the third major fractions after triacylglycerol and free fatty acids of the total lipids. On the other hand, polar lipids and monoacylglycerol represent a very small portion of the total lipids of wheat germ oil samples for all treatments. Moussa *et al*. (1980b) separated the lipid classes of French soft red wheat flour into 10 fractions. Three classes were not identified and the major fraction was triacylglycerols.

As illustrated in Fig. (3b) triacylglycerols of wheat germ oil were fractionated on silver nitrate impregnated TLC into fractions differing in their intensities and unsaturation degree. The unsaturated triacylglycerols which represent the major component were found near the base line, followed by lower unsaturation, where as the more saturated triacylglycerols could be observed near the front line and represent a small portion of the total triacylglycerols, which may be due to the fatty acid composition.

Figure (3c) represents the phospholipid fractions of wheat germ oil. Eight fractions could be observed, namely from the base line, lysophosphatidyl choline, phosphatidyl inositol, phosphatidyl choline, unknown-1, phosphatidyl ethanolamine, unknown-2, unknown-3 and phosphatidic acid. The major fractions of wheat germ phospholipids were phosphatidyl inositol, phosphatidyl choline and phosphatidyl ethanolamine. According to Moussa *et al*. (1980a), the phospholipid fractions of French soft red wheat flour were 10 fractions. Phosphatidyl choline and phosphatidyl ethanol amine were the major fractions.

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تقييم وثبات جنين القمح وخواص الزيت المستخلص منه

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تم تحليل جنين القمح لمعرفة التركيب الكيماوي، الأحماض الأمينية والعناصر المعدنية، وقدرت الخصائص الفيزوكيميائية، وأقسام الليبيدات وتركيب الأحماض الدهنية في زيت جنين القمح، كما أجريت بعض المعاملات الحرارية المختلفة على جنين القمح لتثبيط نشاط إنزيم الليبيز.

أوضحت النتائج أن جنين القمح الكامل يحتوي على مستوى مرتفع من البروتين الخام (٣٤,٩٢٪) وكان المحتوى من المستخلص الإثيري الخام والألياف الخام والرماد هو ١٠,٧٤، ٤,٨٩، ٥,١٧٪ على الترتيب، وجد أن العناصر المعدنية الأكثر تواجداً (ملجم/ ١٠٠جم) هي البوتاسيوم (١٥٦٧,٦) والفوسفور (٣٨٩,٥ ملجم/ ١٠٠جم)، إضافة إلى ذلك أظهرت النتائج أن جنين القمح كان مصدراً جيداً للأحماض الأمينية الضرورية (٣٥,٨١جم/١٠٠جم بروتين) وكانت قيم رقم التصبن والرقم اليودي ورقم البيروكسيد هي ١٨٢,٩٤، ١١٢,٥٤ و٠,٣٥ ملليمكافئ أكسجين/ كجم عينة على الترتيب.

وكانت قيم الأحماض الدهنية الحرة والمواد غير القابلة للتصبن هي ٢,٣٩٪ و١,٠٩٢٪ على الترتيب، ويعتبر زيت جنين القمح مصدراً ممتازاً للأحماض الدهنية الضرورية (ω-3، ω-6)، فضلاً عن وجود الأحماض الدهنية غير المشبعة بنسبة تصل إلى حوالي ٨٠٪ من مجموع الأحماض الدهنية الكلية، ولوحظ أيضاً وجود اختلافات طفيفة في تركيب الأحماض الدهنية قبل وبعد عملية تثبيط الإنزيمات، باستخدام تكنيك كروماتوجرافيا الطبقة الرقيقة تبين أن ثلاثي أسيل الجليسرول هو المكون الرئيسي، أيضاً وجد أن الفوسفاتيدايل أينوزيتول، وفوسفاتيدايل كولين وفوسفاتيدايل إيثانول أمين كانت هي المكونات الرئيسية لفوسفوليبيدات زيت جنين القمح. أوضحت النتائج أيضاً أن العينة التي أجرى لها معاملة حرارية (١٠٠°م/ ٣٠ دقيقة) قبل التخزين عند –١٨°م كانت أكثر كفاءة في تثبيط النشاط الإنزيمي مقارنة بالعينات الأربع الأخرى المخزنة عند ظروف مختلفة.