Extraction and Assessment of Antioxidative Activity from Wheat Germ oil

Abathir Salih Aseer Alsafi **Ali Kudhair Jaber Al-Rikabi**
Food Science Department, College of Agriculture, University of Basrah, Iraq.

Abathir212@gmail.com

Abstract:

Iraqi wheat germ oil was extracted by Soxhlet extractor. The percentage of moisture, protein, fat, ash and carbohydrates was estimated in the Iraqi wheat germ. Extract the oil from the germ using a soxhlet extractor. The phenolic content in it was estimated. The antioxidant efficacy of the oil was assessed by estimation of its DPPH (1,1-diphenyl-2-picrylhydrazyl) radical reabsorption, and an estimate of its reduction capacity and its ability to bind to the ferrous ion. Estimate the oil content of vitamin E with High Performance Liquid Chromatography (HPLC) technology. The percentage of moisture, protein, fat, ash and carbohydrates in the wheat germ was 10.13, 26.82, 9.98, 5.12 and 47.95%, respectively. The total content of phenolic compounds and vitamin E in wheat germ oil was 50 g. ml and 310 mg. 100g, respectively, increases the ability of the oil to scavenging of radical DPPH, its reductive capacity and its ability to bind ferrous ion to increase the concentrations used.

Keywords: anti-oxidative activity, wheat germ oil, phenolic compounds.

استخلاص وتقييم الفعالية المضادة للأكسدة لزيت جنين الحنطة

أباذر صالح عسير الصافي علي خضير جابر الركابي

قسم علوم الاغذية ,كلية الزراعة , جامعة البصرة ,العراق

Abathir212@gmail.com

الخلاصة

استخلص زيت جنين الحنطة العراقية بطريقة السوكسليت . قدرت النسبة المئوية للرطوبة ، البروتين ،الدهن ، الرماد والكاربوهيدرات في جنين الحنطة العراقية. استخلص الزيت من الجنين بجهاز السوكسليت وقدر المحتوى الفينولي فيه .قيمت الفعالية المضادة للأكسدة للزيت بتقدير قابليته لاقتناص جذر DPPH وتقدير سعته الاختزالية وقدرته على ربط ايون الحديدوز.قدر محتوى الزيت من فيتامين E بتقنية HPLC . بلغت النسبة المئوية للرطوبة ، البروتين ،الدهن ، الرماد والكاربوهيدرات في جنين الحنطة (10.13 ، 26.82 ، 9.98 ،5.12 ،47.95) % على التوالي . بلغ المحتوى الكلي للمركبات الفينولية و فيتامين E في زيت جنين الحنطة 50 مايكرو غرام / مل و310 ملغم / 100غم على التوالي .أزدادات قابلية الزيت لاقتناص جذرDPPH وسعته الاختزالية وقدرته على ربط ايون الحديدوز بزيادة التراكيز المستعملة .

الكلمات المفتاحية : الفعالية المضادة للاكسدة , زيت جنين الحنطة , المركبات الفينولية

Introduction :

Wheat germ is a by-product obtained through the wheat flour production process [16]. The high nutritional value of wheat germ makes an important component in many nutritional foods[11]. It contains essential amino acids, minerals and fatty acids, about 34.92% protein and 10.74% ash. The most abundant minerals were potassium and phosphorous. Wheat germ is a good source of essential amino acids and essential fatty acids, unsaturated fatty acids represent about 80% of the total fatty acids[3].

 ISSN 2072-3875

Germ is about 2.5% of the weight of a grain of wheat, and is located at the bottom of the grain of wheat.

It consists of two main parts: the scutellum (First part), it is the layer that connects the embryo with the endosperm and consists of active cells rich in vitamins, especially thiamine, it also contains enzymes and mineral salts. The other part is the embryonic axis. The proportion of oil in the germ was high, reaching 16% in the embryonic axis and 32% in the scutellum. wheat germ contains many bioactive compounds, it was very important for the health of the body, used directly and some of it is added with other foods [8].

Wheat germ oil was a good source of fat-soluble vitamins, wheat germ oil contains a higher percentage of tocopherols compared to other vegetable oils, the proportion of tocopherol in wheat germ oil is 2500 mg. kg. It was the highest in vegetable oils [18]. Antioxidants were the first line of defense against free radical damage, and important to maintain optimum health. Also, regular consumption of vegetables and fruits contain antioxidants, reduce the risk of developing chronic diseases [7]. Antioxidants were a class of chemical compounds, which were ubiquitous in nature and have various working mechanisms, but the most important mechanism was their interaction with free radicals and the formation of stable and ineffective products [17]. Wheat germ oil has the ability to protect against oxidative stress, protect the liver from damage by generating free radicals, on weakening the endoplasmic reticulum. Altering the permeability of the mitochondrial membrane, leading to lipid accumulation, reduced protein synthesis and overproduction of oxidative stress.[21]. To eat wheat germ oil with some foods, protects the liver from acute damage, because of its ability to all reduce fat oxidation, preventing the oxidative stress that causes DNA damage [19].

In recent years many doubts have been raised about synthetic antioxidants such as BHT and BHA, because they may be carcinogens despite their high effectiveness as antioxidants, and since the embryo is one of the byproducts of flour manufacturing, because contains a good percentage of oil, therefore, the aim of the study is to estimate the chemical content of local wheat germ, extract oil from it, evaluate its effectiveness as an antioxidant, to estimate its content of phenolic compounds, and its content of vitamin E, due to its strong association with the antioxidant efficacy of this oil.

Material and Methods:

The source of wheat germ:

Wheat germ was obtained from the General Company for Mills, Dora Mill in Baghdad. The sample was cleaned of impurities, and it was placed in polyethylene bags and kept in the refrigerator at (4 ° C) until the study was conducted.

Chemical content of wheat germ:

1.Moisture determination:

Followed the method given in American Association of Cereal Chemists [1] , by taking 2 grams of germ powder, put it in a glass dish inside an electric oven at a temperature of 130 ° C for an hour and after weighing the model was returned to the oven for another hour to obtain a constant weight according to the following equation:

Moisture (%) = (weight of plate with sample - weight of empty plate) / sample weight x 100

2. Protein percentage determination:

Follow the Micro Keldhal method to determine the protein content of wheat germ, according to the American Association of Cereal Chemists [1] , by weighing 3 grams of wheat germ powder, and was digested using 15 ml of concentrated sulfuric acid to which Catalyst was added to aid in the digestive process, after the digestion process was completed, the distillation was done using the Keldhal device, the correction process was carried out with HCl acid (0.02 N), the percentage of total nitrogen was calculated according to the following equation:

ISSN 2072-3875

Nitrogen (%) = (HCL acid volume x N x 14) / sample weight x 100
The total protein percentage was calculated by (protein% = N% x 5.7).

3. Fat percentage determination:
Use the continuous extraction method using a Soxhlet extractor [1] . Take 20 grams of wheat germ powder, put in the device with the use of 300 ml of solvent hexane at a temperature of 40-60 ° C for 9 hours, transferred to the rotary evaporator, fat percentage was calculated through the following equation

Fat percentage (%) = (fat weight / sample weight) x 100

4. Ash percentage determination:
Ash percentage was estimated according to [1] method, 5 grams of wheat germ were taken, put in a ceramic jar, it was placed in an incineration oven at a temperature of 550 ° C, until the form turns gray to whitish, the ash ratio was calculated liking the following equation:
Ash (%) = (weight of the ceramic jar with the sample - the weight of the empty ceramic jar) / the weight of the sample x 100

5. Estimating the percentage of carbohydrates:
The percentage of carbohydrates was estimated in the wheat germ, by the difference between the components mentioned above, as explained by [15].

Second: Oil extraction:
Extract the oil according to the method mentioned in [1], Soxhlet continuous extraction machine, using hexane solvent at a temperature 40-60 C for 9 hours, then take the oil containing a small amount of hexane solvent into a rotary evaporator to get rid of the remaining solvent with the oil and get the pure oil.

Third: Determination of total phenolic compounds in wheat germ oil:
Total phenolic compounds in wheat germ oil were determined according to the Folin-Ciocalteu method and described by [20] . Whereas, dissolve 1 g of oil in 46 hexane solvent. add 1 ml of Folin-Ciocalteu reagent and mix well. After 3 minutes, 3 ml of Na_2Co_3 (2%) was added, leave the mixture for two hours with intermittent shaking, then absorbance was measured at a wavelength of 760 nm. The amount of phenols in the oil was calculated based on the graphical relationship between the acid concentration and the absorption at the wavelength of 760 nm, by using a standard solution of gallic acid at a concentration ranging 0-100 mg. ml. Prepare the standard solution by taking 0.1 g of gallic acid and dissolve in 100 ml of distilled water to make a backup solution of 1000 mg. ml, the required concentrations were prepared as shown in Figure (1).

 ISSN 2072-3875

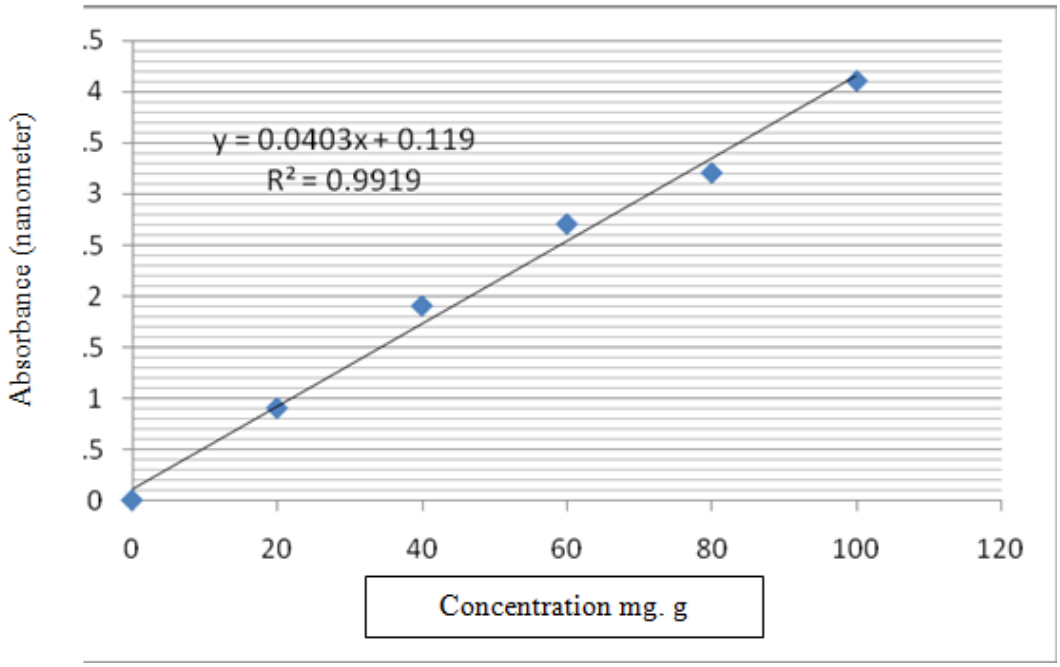

Figure 1: Standard curve for galic acid.

Fourth: Evaluation of the antioxidant efficacy of wheat germ oil

1. DPPH scavenging method:

Antioxidant activity was measured by DPPH, which was prepared by dissolving 0.005 in 100 ml of methanol and according to the method mentioned by [22], with some changes, 1 ml of wheat germ oil was taken from each concentration 2, 4, 6, 8 and 10 mg. ml, 2 ml of prepared DPPH solution were added to it and shaken well. Samples were incubated at room temperature for 30 minutes. The control sample was prepared by adding water instead of wheat germ oil, samples were measured at a wavelength of 517 nm and the efficacy was calculated from the following equation:

Antioxidant activity% = (A sample – A control / A control) 100

2. Reducing power:

Were in method [14] followed, which included mixing 2.5 ml of wheat germ oil prepared with concentrations 2, 4, 6, 8 and, 10 mg. ml, 10 mg. ml BHT prepared with 98% ethanol with 2.5 ml 0.2 M phosphate buffer solution, with a pH of 6.6 and 2.5 ml of Potassium Ferricyanide (1%) was added. Incubate the mixture at a temperature of 50 ° C for 20 minutes, then 2.5 ml of 1% trichloroacetic acid was added, the mixture was centrifuged at 3000 rpm for 10 minutes, then the organic layer of the solution was separated and 5 ml of distilled water and 1 ml of 1.0% ferric chloride were added to it. Absorbance was measured at a wavelength of 700 nm. The control sample was prepared by adding all the previous materials except for the addition of 2.5 ml of ethanol instead of wheat germ oil, apply the following equation to calculate the amount of reductive force of the oil :

Efficiency (%) = 100– (absorbance reading of the sample / absorption reading of the control sample) x 100

3. The ability to bind ferrous ion:

The ferrous ion binding of wheat germ oil was estimated according to the method reported by [6]. Where 0.4 g of wheat germ oil was mixed with different concentrations 2, 4, 6, 8 and 10 mg. ml, with 0.4 ml of 2 mmol ferrous chloride and 0.4 ml of vahydroxyquinoline at a concentration of 5 mmol (prepared with ethanol

ISSN 2072-3875

at a concentration of 98%). Incubate the mixture for 10 minutes at room temperature in a dark place, absorbance was measured at a wavelength of 562 nm. The ability of the ferrous ion to bind to the ethylene-dimine tetra-acetic acid disodium was also estimated in the same way for the purpose of comparison. The control sample was prepared in the same way with the exception of adding wheat germ oil The susceptibility was calculated by the following equation:

Efficiency (%)= 1 - (absorbance reading of the sample / absorption reading of a control sample) x 100

Fifthly: Vitamin E determination of wheat germ oil by HPLC tecchnique:

Were in method followed, where 0.5 g. ml of vitamin E was dissolved with 12.5 of hexane to obtain a concentration of 40 mg / ml of vitamin E, 5 concentrations of vitamin E have been prepared, which were 9, 12, 16, 20 and 24 mg. ml. It was injected into an HPLC device. The absorbance was measured at a wavelength of 290 nm, as shown in Figure (2).

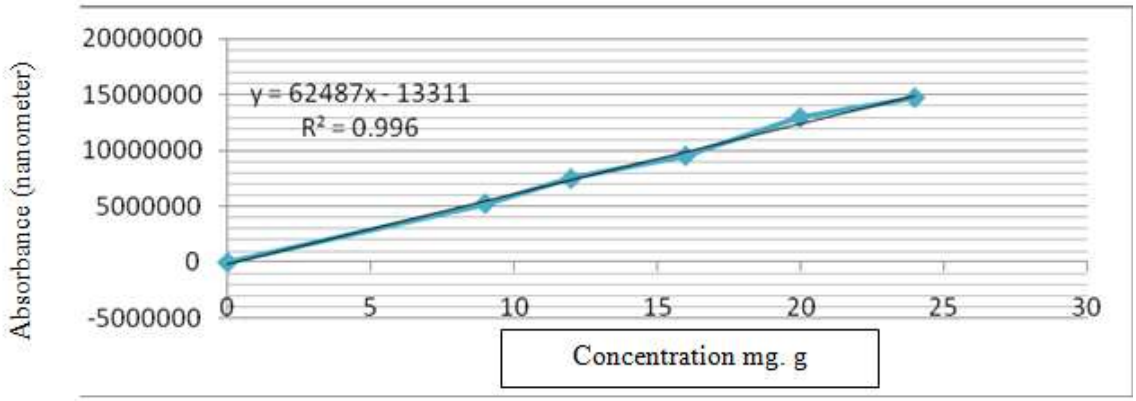

Figure (2) Standard curve for vitamin E determination.

The sample was prepared by taking 0.1 g of oil and dissolved in 10 ml of hexane and injected with the device, and it was measured at a wavelength of 290 nm.

Statistical analysis

Statistical analysis of the results was carried out using a complete randomized design (CRD) with one factor, the significant differences between the averages were compared with the LSD test at the level of 0.05, (SPSS version 13.0, 2012) were used.

Results and discussion :

1. The chemical content of wheat germ:

Table (1) shows the chemical content of the germ extracted from local wheat, the percentage of humidity reached 10.3, which was less than what was found by [3], the percentage of moisture found in wheat germ, was 12.46% and higher than that found by [9]. The humidity of wheat germ is 9.84%, as evidenced by the same percentage of wheat germ protein (26.82%), which was less than what was found by [3], where the protein content of wheat germ was 30.52%, as well as less than what [3] found, found that the proportion of protein in the wheat germ was 27.93%. The percentage of fat in wheat germ was 9.98%, this percentage was close to [3] found, where the percentage of fat in wheat was found 9.40%, was less than that found by [9]. The percentage of fat in the wheat germ was 10.18%, the ash percentage in wheat germ was 5.12%, which was higher than that found by [3]. As the percentage of ash was found in wheat germ was 4.52%, a higher than that by [9] found, where it was found that the ash in wheat germ was 4.06%. The wheat germ

ISSN 2072-3875

also showed a high carbohydrate content, with a rate of 47.95% higher than that found by[3]. where the percentage of carbohydrates found in wheat germ is 43.5%, which was close to what [9] found. The proportion of carbohydrates was 47.49%. The reason for the differences in chemical content is the model of wheat under study, to storage conditions for grains or embryos. Likewise, the state of the pills from which the fetus was taken, as well as the weather conditions, that affects the grains, the embryo, the method f extracting the embryo, and the wheat variety, the purity of the embryo is also affected by the fat extraction method and the type of solvent used for the extraction[10].

Table (1) the chemical content of wheat germ.

Content	Percentage (%)
Moisture	10.13
Protein	26.82
Fat	9.98
Ash	5.12
Carbohydrates	47.95

The numbers in the table represent the average of three replicates.

2. Total content of phenolic compounds:

The total phenols content in wheat germ oil was 50 µg. ml, it was less than the [2] found, where he found the percentage of total phenolic compounds in the wheat germ, completely 230 µg. ml. The reason for this difference is due to the estimation of the total phenolic compounds in the oil alone, after its extraction from the germ.

3. Vitamin E content in wheat germ oil:

The vitamin E concentration in wheat germ oil was 310 mg. 100 g, as its percentage was high compared to other vegetable oils, it was higher than what [9] found, was 240 mg. 100 g higher than [18] found, amounted to 250 mg. 100 g, was less than [13] found, reached 416 mg. 100 g in the germ oil that was extracted using CO_2. The reason for this difference in the amount of vitamin, refers to the type of grain from which the germ was extracted, as well as the method of extracting the embryo and the method of extracting the oil from the germ. Likewise, the prolonged storage period and the high temperature lead to a decrease in the concentration of vitamin E [5].

4. Antioxidant activity:

Figure (3) shows the antioxidant activity of wheat germ oil at concentrations 2, 4, 6, 8 and 10 mg. ml, compared to the industrial antioxidant DPPH at a concentration of 4 mg. ml. The figure shows that this effectiveness increases by increasing the concentrations used. The results of the statistical analysis revealed that there were significant differences in the activity values between the concentrations at a 0.05 level of significance. The reason for this effectiveness was that wheat germ oil contains many phenolic compounds, and it contains tocopherol (vitamin E). It was also found that the antioxidant efficacy of BHT was superior to all concentrations under study and at the same level of significance, this superiority was due to the fact that this antigen was a pure compound. While all prepared concentrations were due to crude wheat germ oil, which contains other compounds that reduce this effectiveness. The results were in agreement with [12]. was found that the effectiveness of wheat germ oil increased with the increase of the concentration used, during these results, it became possible to use wheat germ oil as a natural antioxidant in food products, to maintain the health of the body from oxidative stress instead of harmful industrial antioxidants, in addition to its high cost, the reason for this high efficiency is due to the oil's content of phenolic compounds, as well as its high content of tocopherols.

 ISSN 2072-3875

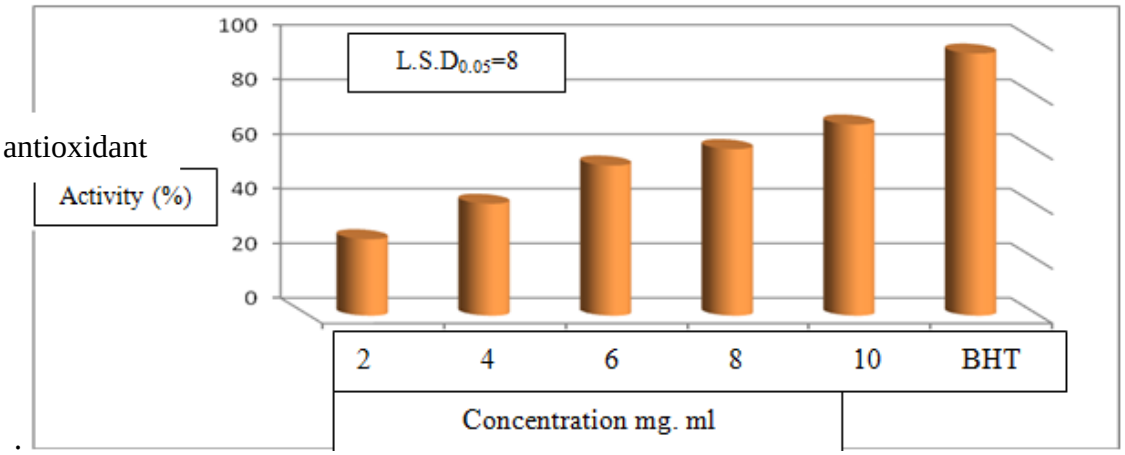

Figure (3) The antioxidant activity of wheat germ oil.

5. Capacity of the reductive power:

Figure (4) shows the reducing ability of wheat germ oil compared to the natural antioxidant (tocopherol). The higher the concentration of the oil, the greater the antioxidant activity, the highest efficacy was reached at concentration of 10 mg. ml (119%). While the reductionist force of the comparison model, with the same concentration, reached 175%. The results of the statistical analysis revealed that there were significant differences between the concentrations used, as well as between these concentrations and the natural antioxidant at the level of 0.05. The reason for the increased reducing power of fetal oil is due to the fact that it contains phenolic compounds, Alpha-Tocopherol and Beta-tocopherol

.

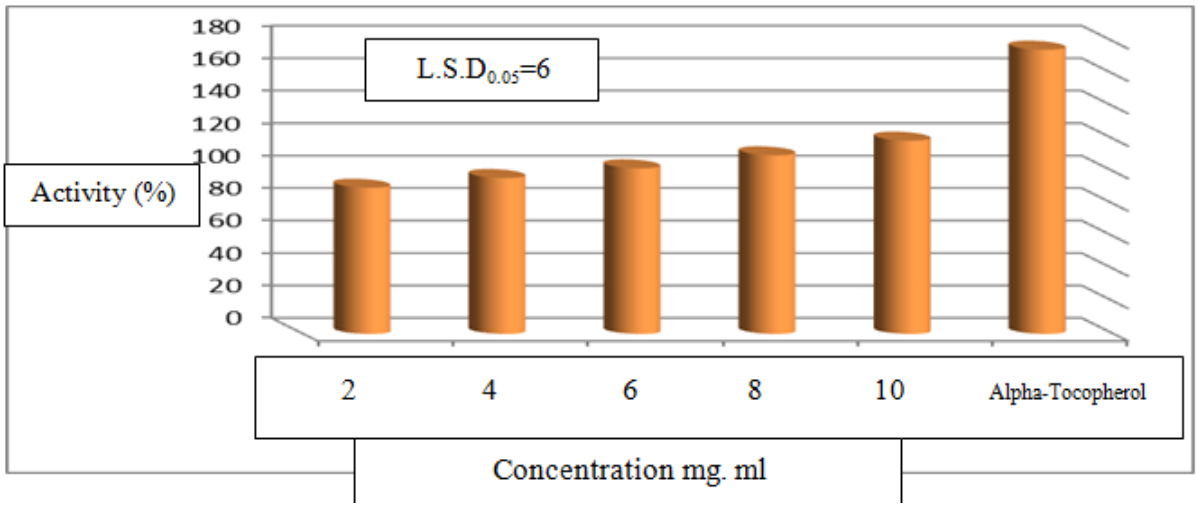

Figure (4) Capacity of reductive power of wheat germ oil at different concentrations and compared to tocopherol.

6. Ferrous ion binding:

Figure (5) shows the ability of wheat germ oil to bind to the ferrous ion at different concentrations, compared to ethylene diamine tetra acetic acid (EDTA). The figure shows that there was an increase in the binding ability to increase the concentrations used. The maximum efficacy was 89% at 10 mg. ml, the results of

ISSN 2072-3875

the statistical analysis show that there were significant differences between these concentrations at a 0.05 level of significance. The comparison model also significantly outperformed all the concentrations. Its efficacy was 91% at a concentration of 10 mg. ml. The effectiveness of binding the ferrous ion to the action of wheat germ oil because it contains many phenolic compounds, which has many hydroxyl (OH) groups, has the ability to catch these ions and thus delay the process of fat oxidation.

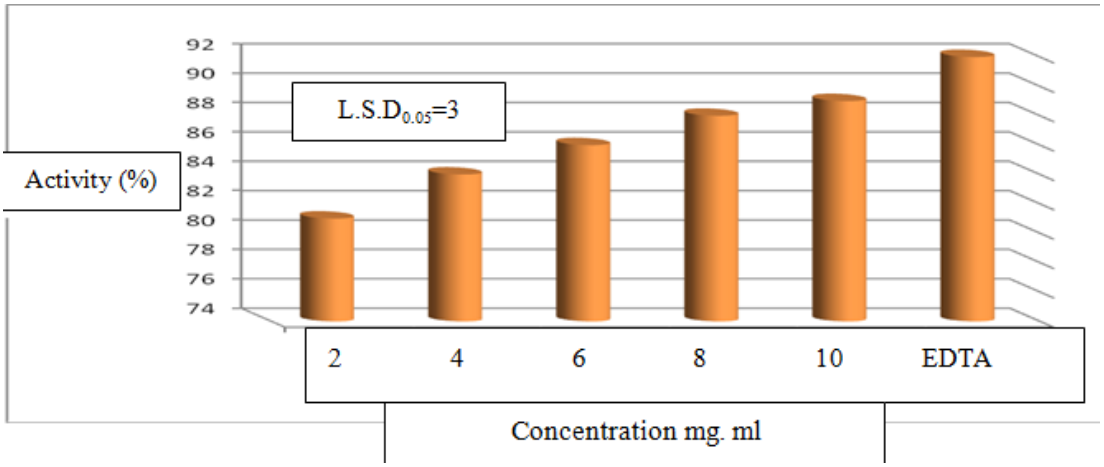

Figure (5) The ability to bind ferrous ion to wheat germ oil at different concentrations in comparison with EDTA.

Conclusions:

Iraqi wheat germ contains a good oil content with a high content of vitamin E and phenolic compounds. The possibility of using Iraqi wheat erm oil as a natural antioxidant, an alternative to the antioxidants industry in food systems.

Acknowledgement:

We thank the Deanship of the College of Agriculture, we also extend my thanks to the Department of Food Sciences and extend my thanks and gratitude to Prof. Dr. Diaa Faleh Abdullah Al-Fakiki for the opportunity to complete my research requirements in food research laboratories and consumer protection.

References

- A. A. C. C. (1976). Approved method. American Association of Cereal Chemists. Inc.Minnesota,U.S.A.1

https://doi.org/10.1016/j.jfoodeng.2007.11.005

2 - Al-Musafer, AMSM (2009). Extraction of antioxidants with the help of microwaves from the iron, wheat grain, germ and bran, and studying their effect on the rheological characteristics of the dough. College of Agriculture, University of Basrah. https://iqdr.iq/search?view=445f8f5e4c585a5b5017cdde446a5a41

3 - Attia, R. S., & Abou-Gharbia, H. A. (2011). Evaluation and stabilization of wheat germ and its oil characteristics. *Alexandria Journal of Food Science and Technology*, *8*.39-31 ,(2)

https://www.ajfs.journals.ekb.eg/jufile?ar_sfile=56427

4 - Brabcová, I.; Kovářová, L.; Šatínský, D.; Havlíková, L. & Solich, P. (2013). A fast HPLC method for determination of vitamin E acetate in dietary supplements using monolithic column. *Food Analytical* m*ethods, 6(2): 380-385.*

https://link.springer.com/article/10.1007/s12161-012-9452-0

5 - Capitani, M.; Mateo, C. M. & Nolasco, S. M. (2011). Effect of temperature and storage time of wheat germ on the oil tocopherol concentration. Brazilian

 ISSN 2072-3875

Journal of Chemical Engineering, 28(2): 243-250.
https://doi.org/10.1590/S0104-66322011000200008

6 - Decker, E. A. and Welch, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *Journal of Agricultural and food Chemistry, 38(3): 674-677.*
https://doi.org/10.1021/jf00093a019

7 - Dembinska-Kiec, A., Mykkänen, O., Kiec-Wilk, B., & Mykkänen, H. (2008). Antioxidant phytochemicals against type 2 diabetes. British *Journal of Nutrition, 99(1): 109-117.*

https://doi.org/10.1017/S000711450896579X

8 - Fardet, A. (2010). New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre*?. Nutrition research reviews, 23(1), 65-134*
https://doi.org/10.1017/S0954422410000041.

9 - Jarad, BB (2012). Study of the qualitative and storing properties of wheat germ oil and its introduction into the biscuit industry.The second scientific conference, Faculty of Agriculture, Karbala University: 1153-1162. https://iqdr.iq/search?view=443e3f34ba7477d14435d29e7d59403a

10 - Killilea, D. W., McQueen, R., & Abegania, J. R. (2020). Wheat germ agglutinin is a biomarker of whole grain content in wheat flour and pasta. *Journal of food science*, *85*(3), 808-815.
https://doi.org/10.1111/1750-3841.15040

11 - L banoğlu, E. (2002). Kinetic study on colour changes in wheat germ due to heat. *Journal of Food Engineering*, *51*(3), 209-213.
https://doi.org/10.1016/S0260-8774(01)00057-7

12 - Mahmoud, A. A.; Mohdaly, A. A. & Elneairy, N. A. (2015). Wheat germ: an overview on nutritional value, antioxidant potential and antibacterial characteristics. Food and Nutrition Sciences, 6(02): 265.
http://creativecommons.org/licenses/by/4.0/

13 - Molero Gomez,A. & Martinez de al Ossa,E.(2000).Quality of wheat germ oli Extracted b liquid and supercritical carbon dioxide .*Journal of the American Oil Chemists Societ ,77(9):969-74*
https://link.springer.com/content/pdf/10.1007/s11746-000-0153-y.pdf

14 - Oyaizu , M.(1986). Studies on products browning reaction : antioxidative activities of products of browning reaction prepared from glucosamine . jpn.J.Nutr., 44: 307- 315.
https://doi.org/10.5264/eiyogakuzashi.44.307

15 - Pearson, D. (1976). The Chemical Analysis of Foods. 7th ed. Edinburgh, New York: Churchill Livingstone. PP: 575.
https://trove.nla.gov.au/version/45250339

16 - Piras, A.,Rosa,A.,Falconieri,D.,Porcedda,S., De ssi, M.A. & Marongiu, B. (2009). Extraction of oil from wheat germ by supercritical CO2. Molecules, 14: 2573-2581.
https://doi.org/10.3390/molecules14072573

17 - Pokorńy, J. and Korczak , J. (2001). Preparation of natural antioxidant In: Pokorny , J . Yanishlieva , N ., Gordon , M , editors . Antioxidants in food: Practical application. Cambridge England: Wood head publishing Limited. P 41 – 311.
https://www.scirp.org/(S(351jmbntvnsjt1aadkposzje))/reference/ReferencesPapers.aspx?ReferenceID=1170177

18 - Schuler, P. (1990). Natural antioxidants exploited commercially. In *Food antioxidants* (pp. 99-170). Springer, Dordrecht.
https://link.springer.com/chapter/10.1007/978-94-009-0753-9_4

19 -Sliai, A. M. (2015). Protective Effects of Wheat Germ Oil on Doxorubicin-Induced Hepatotoxicity in Male

Mice. Intern. *J. Res. Stud. Bios, 3: 21-25.*

https://www.arcjournals.org/pdfs/ijrsb/v3-i6/5.pdf

20 - Slinkard, K. & Singleton, V. L. (1977). Total phenol analysis: automation and comparison with manual methods. *American journal of enology and viticulture, 28(1): 49-55.*

https://www.ajevonline.org/content/28/1/49.short

21 - Weber, L. W.; Boll, M.& Stampfl, A. (2003). Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Critical reviews in toxicology, 33(2): 105-136.

https://doi.org/10.1080/713611034

22 -Yang, J., Guo, J., & Yuan, J. (2008). In vitro antioxidant properties of rutin. *LWT-Food Science and Technology*, *41*.1066-1060 ,(6)
https://doi.org/10.1016/j.lwt.2007.06.010