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CHEMICAL AND TECHNOLOGICAL STUDIES ON SOME MEDICINAL PLANTS

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ABSTRACT

This study indicate the importance of some wild medicinal plants such as milk thistle seed and chicory seed. Therefore , chemical characteristies such as crude protein, ether extract, crude fiber, amino acids, fatty acids, vitamin C and minerals contents were determined.

Also ,the antioxidative properties such as phenolic compounds and flavonoid compounds and scavenging activity on DPPH free radicals were also determined. Moreover ,the possibility to use these seeds in biscuit production, where the utilization of milk thistle seed flour and chicory seed flour as replacers of wheat flour at different levels (5, 10, and 15%) to prepare biscuits rich in protein and minerals, as well as organoleptic evaluation of prepared biscuits and firmness values were also investigated.

Results indicated that milk thistle seed and chicory seed have high amount of protein, ether extract, crude fiber, minerals content, vitamin C, flavonoids, and phenolic compounds (antioxidants) which, could be used as alternative natural source for synthetic antioxidants in industry.. The major unsaturated fatty acids in milk thistle seed and chicory seed oil are oleic and linoleic. The essential and nonessential amino acids are more abundant in milk thistle seed and chicory seed protein.

Sensory evaluation of the obtained products indicated that successful replacement was 10% of both seeds . So , it could be recommended to use the investigated plant seeds in biscuit production for its nutritional concept and antioxidative effects.

For these reasons, we must take in consideration to cultivate these plants on a large scale to take their advantages of their therapeutic and nutritional .

INTRODUCTION

For centuries people have used plants for healing, most medicinal plants are collected from the wild. One of the important issues that the plant may be accepted as a safe herbal product, since no health hazard or side effects till now are known (Medical Economics Company, 2000).

Milk thistle is native to the Mediterranean basin and is now widespread throughout the world.

Seeds of milk thistle have been used for more than 2000 years to treat liver diseases (Kren and Walterova, 2005).

Milk thistle is usually found and widespread in Egypt on road sides, waste ground and in cereal crops as a weed, this stout thistle usually grows in dry, sunny areas . Milk thistle has a stem of 40-200 cm high, glabrous or slightly downy, erect and branched in the upper part of plant (Montemurro *et al.*, 2007).

Milk thistle (*S. marianum L. Gaertn.*) is cultivated as a medicinal plant but it can also be a troublesome weed (Khan *et al.*, 2009)

Milk thistle is an annual or biennial plant belongs to the family Asteraceae (Compositae), the plant is native to Mediterranean area and now has spread to other warm and dry regions (Li *et al.*, 2012).

Now *silybum marianum* is one of the most medicinal plants, the plant has many common names such as, bull thistle, heal thistle, holy thistle, lady's thistle, pig leaves, royal thistle, snake milk, so thistle, St.Mary's thistle, Venus thistle, Marian thistle, Mary thistle, mild thistle, milk ipecac, our lady's thistle (Corchete, 2008).

Seed flour is a primary by-product from seed oil production and is often discarded as waste, this flour may contain many health beneficial components such as natural antioxidant and protein (Parry *et al.*, 2008).

Silymarin content of milk thistle seed ranged from 4 to 6% based on dry weight (Khalil,2008).

Silymarin is the most commonly used herbal product for chronic liver disease (Freedman et al., 2011).

Abd Raboh, (2012) revealed that the seed of milk thistle contained high amounts of protein (27.54%), ether extract (29.68%) and crude fiber (29.95%) on dry weight basis, also the seed had antioxidant properties due to its content of phenolics, flavonoids, saponins, silymarin and ascorbic acid.

Chicory *(Cichorium intybus* L.*)* is a perennial plant with blue or white flowers that is easy to grow and can be used for many purposes.

It is perhaps best known for the roasted roots used as the traditional coffee substitute with no caffeine and less well known as grazed forage for ruminants (Barbara *et al.*, 2007).

All parts of this plant possess great medicinal importance due to the presence of a number of medicinally important compounds such as alkaloids, inulin, sesquiterpene lactones, coumarins, vitamins, chlorophyll pigments, unsaturated sterols, flavonoids, saponins and tannins (Muthusamy *et al.*, 2008 and Atta *et al.*, 2010).

Ying and Gui , (2012) revealed that two varities of seeds of *Cichorium intybus* L. contained substantial amounts of crude proteins (over 19 %), crude fat (over 22 %) and carbohydrate (over 31 %).

The essential fatty acid, linoleic was the predominant fatty acid accounted for over 76 % of the total fatty acids in the two chicory seeds, with lower saturated/unsaturated ratios (about 0.11) making them potentially a superior source of nutritional oil, compared with alfalfa.

Minerals analysis showed that chicory seeds possess high values of K, Ca, P, Mg, Cu, Zn and Mn.

All parts of chicory contain considerable amounts of phytochemicals and are good source of antioxidants (Shad *et al.*, 2013).

Accordingly, this study was designed to evaluate chemical properties of milk thistle seed and chicory seed, also evaluate the effect of adding these seeds at different ratio on the sensory and nutritional properties of biscuit .

MATERIALS AND METHODS

Materials:

Milk thistle seeds were collected from Agricultural research farms of Sakha at Kafr El-sheikh City. Chicory seeds were obtained from the local market at Kafr El-sheikh City, Egypt.

Wheat flour (72% extraction) were purchased from Delta Middle and West Milling Company, Tanta, Egypt.

The other ingredients such as sugar, egg, corn oil and baking powder were purchased from local market at Kafr El-sheikh City, Egypt.

Chemicals used in this study were purchased from El-Gomhoria Company for Chemicals and Drugs.Tanta , Egypt.

Methods:

Preparation of the samples

Preparation of milk thistle seed and chicory seed flour was done according to the method described by (Atta and Imaizumi, 2002) as follows:.

The seeds of milk thistle and chicory were washed with tap water followed by distilled water then dried at 60°C in an air drying oven, and grinded into fine powder using a laboratory electronic mill (Broun, Model 2001 DL, Germany) at speed 2 for 3 min to pass trough 40 mesh sieve. The powder was kept into polyethylene bags and stored at -20°C in the deep freezer for further analysis

Biscuit preparation:

Biscuit blends were processed using the method described by Hooda and Jood, (2005) as follows : Blends containing 5, 10, and 15% of milk thistle seed powder or chicory seed powder were used as a replacement of wheat flour (72% extraction). The basic ingredients were 500g of flour blends, 175g corn oil, 175g sugar, 110g of whole egg and 6 g of baking powder

Biscuit blends were prepared by mixing sugar powder and egg in a rotary mixer (Moulinex model Depose type 171) for 5 min until they creamed, then oil was added thoroughly for 2 min. The dry ingredients such as wheat flour or flour blends and baking powder were added. Milk thistle seeds powder and chicory seeds powder were substituted with wheat flour at different levels namely 5, 10 and 15%. The biscuits were baked in an oven (Freibol, FB Model) at 160 ◦C for 20 min. Then biscuits were allowed to cool at room temperature for 2 hours. After cooling, biscuit was kept in polyethylene bags at room temperature and protected from light, then divided into three lots; first lot, was subjected to sensory evaluation by a semi-trained panel of twenty members.

Biscuit was evaluated for appearance, color, flavor, texture and overall acceptability according to Peter, (2004). The second was used for the measurement of firmness values of biscuit and the third lot was used for chemical analysis and biological evaulation

Analytical methods:

Gross chemical composition

Moisture, crude protein, ether extract, crude fiber and ash contents of milk thistle seed ,chicory seed and wheat flour were determined according to the method described in the A.O.A.C (2000).

Total carbohydrates were calculated by difference.

Determination of minerals content.

Minerals content (Na , K ,Ca, Fe ,Cu , Zn ,Mn and P) were determined according to the methods described in A.O.A.O (2005) using atomic absorption spectrophotometer (Perkin Elmer Model 4100 ZL)

Determination of fatty acids composition

Esterification of fatty acids was performed as described by Atta and Imaizumi (1998).

Fatty acids composition of milk thistle seed oil and chicory seed oil were determined by Gas Liquid Chromatography (GLC) GC Model, Shimadzu-4CM (PFE), equipped with FID detector and glass column 2.5mX 3mm i.d, following the method of Radwan (1978).

Determination of amino acids composition

Amino acids composition was performed in National Research Center, Cairo , Egypt according to the method of Duranti and Cerletti (1979). The amino acids composition was determined by subjecting 0.1 g of sample to acid hydrolysis using 10 ml of 6 N hydrochloric acid with 0.1% mercaptoethanol in an evacuated tube at 110°C for 24 hours. After cooling at room temperature, the hydrolyzed samples were filtered through Whatman No. I filter paper and the filtrate was diluted with distilled water to 25 ml in a volumetric flask. Five ml of the diluted filtrate was dried in a vacuum desiccator in the presence of potassium hydroxide. The resultant dried residue was dissolved in 1 ml of sodium citrate buffer (pH 2.2) and stored at 4°C until analysis by using amino acid analyzer (Beckman amino acid analyzer, Model 119CL).

Determination of tryptophan:

Tryptophan content of samples was determined colorimetrically, in the alkaline hydrolyzate using P-dimethyl-amino-benzaldehyde (DMAB), following the method of Miller ,(1967).

Tryptophan content was secured by the means of standard curve prepared under the same conditions

Determination of total phenolic compounds:

Phenolic compounds were determined by the method described by Jindal and Singh (1975)

Determination of total flavonoid compounds

Total flavonoids of crude extract were determined following the method of Zhishen *et al.*, (1999) modified by Kim *et al.,* (2003)

Determination of Vitamin C

Vitamin C was determined through the HPLC method according to Romeu-Nadal , *et al* ., (2006).

Scavenging activity on DPPH free radical :

The ability of a compound to donate a hydrogen atom was assessed on the basis of the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical according to a procedure based on *Miliauskas et al.,* (2004) with slight modifications by *Lim and Quah* (2007)

Evaluation of firmness of biscuit

The firmness of biscuit was measured using the Texture Analyzer (Cometech, B type, Taiwan) according to Bourne (2003). The test speed of 1 mm/s was used for all tests. Three replicates of each formulation were conducted. Breaking strength of biscuit were determind using the three-point bending rig probe. The experimental conditions were: supports 50 mm apart, a 20 mm probe travel distance and a trigger force of 20g. The force at break (N) was measured

Statistical analysis:

Statistical analysis of data was carried out according to Steel and Torrie (1980) procedures using (Duncan's multiple range test DMRT).

RESULTS AND DISCUSSION

Chemical composition of raw materials

Data in Table (1) represent values of moisture, protein, ether extract , crude fiber and ash (on dry weight basis), for milk thistle seed , chicory seed and wheat flour. Total carbohydrates content was calculated by difference.

Table (1): Gross chemical composition of raw materials (g/100 g on dry weight basis).

Samples	Moisture	Crude protein	Ether extract	Ash content	Crude fiber	Total carbo-hydrates
milk thistle seed	9.80ab	23.56a	23.12a	4.98b	30.33b	48.34 c
Chicory seed	8.33b	21.53a	12.00b	10.19a	33.91a	56.28b
Wheat flour (72%)	11.12 a	11.15b	0.81 c	0.65 c	0.51 c	87.39a

Each value was an average of three determinations.

Total carbohydrates (crude fiber are included) were calculated by difference.

Values followed by the same letter in column are not significantly different at $P \leq 0.05$.

The obtained results from table (1) indicated that, milk thistle seed had the higher percentage of crude protein and ether extract which their values were 23.56 and 23.12 % respectively compared with 21.53 and 12% for chicory seed.

On the other hand, it could be also noticed that, chicory seed had higher content of ash and crude fiber (10.19 and 33.91%), respectively than milk thistle seed which their values were 4.98 and 30.33% , respectively,while wheat flour had higher content of moisture and total carbohydrates (11.12 and 87.39%) than those of milk thistle and chicory seeds

The significance of fiber in human nutrition is its role in lowering blood cholesterol with the attendant benefit of reducing the risk of developing diabetes, hypertensión, cancer and hyper-cholestroleamia (Singh *et al.*, 2002; Koulshon *et al.*, 2005; Oboh and Omofoma 2008).

The obtained results agree partially with those of Abu Jadayil *et al.* (1999) who pointed out that milk thistle seed contained 19.1%, 26.3%, 25.4%, and 4.8% for protein, lipids, crude fiber, and ash, respectively. Wallace *et al.*, (2005) reported that seed of milk thistle contained 25% w/w of oil.

Also, these results agree with those reported by Abd Raboh, (2012) who found that milk thistle seed is a rich source for protein, lipids and crude fiber. Since, crude protein, ether extract and crude fiber contents are 27.54%, 29.68% and 29.95%, respectively. Ash and total carbohydrate were 4.50 and 38.16% respectively.

Ying and Gui , (2012) found that chicory seeds contained substantial amounts of crude proteins (over 19 %), crude fat (over 22 %) and carbohydrates (over 31%).

The great discrepancies in the values of chemical composition of milk thistle and chicory seeds reported in literature, might be related to varieties and the soil structures.

Based on the value of crude protein, milk thistle seed and chicory seed can play vita role as supplementary nutrient source for cereals and other low protein seeds.

The high amounts of protein, ether extract, ash and crude fiber in milk thistle and chicory seeds qualified them to use as a novel source of plant oil and protein.

Also, it could be utilized them as suitable food ingredient in low fiber containing food such as meat products. The cultivation and utilization of these plants should be encouraged. They are good sources of nutrients and could be useful informulating balanced diet.

So, it is clear that addition of milk thistle seed flour or chicory seed flour to the bakery products lead to increase nutritive components of final products due to raise percentage of protein, ash and crude fiber, at the same time , the total carbohydrates decrease

Minerals content of raw materials

Table (2) gives the content of four major elements: Calcium (Ca); sodium (Na); potassium (K) and phosphorus (P) as well as four of trace elements; iron (Fe), copper (Cu), Zinc (Zn) and manganese (Mn) in milk thistle , chicory seeds and wheat flour .

Major elements:

Data listed in Table (2) reveal that seeds of milk thistle and chicory appear to be poor in sodium (40 and 60 mg/100 respectively, but very rich in calcium (880 and 780 mg/100 g) and phosphorus (750 and 780 mg/100 g). They contain considerable amounts of potassium (400 and 190 mg/100 g). The obtained results were in agreement with Ying and Gui (2012) who showed that chicory seeds possess high amounts of K, Ca, P, Mg, Cu, Zn and Mn elements that are essential for proper human nutrition. Also , it could be observed that wheat flour (72%) contains lower values in all determined elements compared to milk thistle seed and chicory seed

Micronutrients or trace elements as commonly termed are a group of nutrients supplied in the diet in micro-quantities. Trace elements content of diets is of great current interest to the nutrition community because of increasing evidence of marginal or inadequate intakes among segments of the population (Weaver *et al.,* 1981).

Table (2): Mineral contents of raw materials (mg/100 g)on dry weight basis

samples	Major elements			
	Phosphorus (P)	Potassium (K)	Sodium (Na)	Calcium (Ca)
MTSF	750	400	40	880
CSF	780	190	60	780
WF(72%)	139	140	25.89	37
Trace elements				
samples	Iron (Fe)	Zinc (Zn)	Copper (Cu)	Manganese (Mn)
MTSF	65.50	3.76	15.76	2.21
CSF	397.16	4.48	4.86	7.30
WF(72%)	0.73	0.46	0.13	0.70

MTSF=milk thistle seed flour
CSF=Chicory seed flour
WF =wheat flour

Trace elements

Content of four trace elements iron ,zinc , copper and manganese , was estimated in the milk thistle seeds , chicory seeds and wheat flour the results are listed in Table (2). Data reveal that, the chicory seed contained higher amounts of iron., zinc and manganese ((397.16, 4.48 and 7.30 mg/100 g), than those of milk thistle (65.50, 3.76 and 2.21mg/100 g respectively).

Wheat flour had the lowest contents of iron, zinc, copper and manganese compared to milk thistle seeds and chicory seeds. Therefore, milk thistle seed and chicory seed are consider as a good source for the minerals

Fatty acids composition of milk thistle and chicory seeds oil .

Data of Table (3) show fatty acid composition of crude fresh milk thistle seed oil and chicory seed oil. The major unsaturated fatty acids in milk thistle and chicory seeds oil are oleic C18:1 (23.32% and 29.71%) and linoleic C18:2 (51.62 % and 50.62 %) .

It is clear from these data that the predominant saturated fatty acids in milk thistle and chicory seed are palmatic C16:0 (7.70 %and 7.81%)and stearic C18:0 (8.99 %and 7.29%). However, the oil contained traces of Caproic C6:0 (0.04% and, 0.16 %), Caprylic C8:0 (0.03%and 0.06 %), Lauric C12: 0 (0.037 %, and 0.05 %), and Pentadecanoic C15:0 (0.09%, 0.12 %) as saturated fatty acids, and myristoleic C14:1(0.08% and 0.08 %), cis-10-Pentadecenoic C15:1 (0.12 % and 0.16%), palmitoleic C16:1 (0.05% and 0.16 %) and erucic C22:1 (1.89% and 0.10 %) as unsaturated fatty acids in the seeds of milk thistle seed and Chicory seed, respectively.

These results close to those reported by Khalil (2008) who found that milk thistle seed oil contained 25.98% oleic, 31.5% linoleic, 14.71% palmatic, and 8.9% stearic.

It is clear that the major unsaturated fatty acid in milk thistle seed oil are oleic and linoleic as well in sunflower oil .Therefore, the oil could be used as salad oil and for different applications such as hydrogenation and shortening industries.

Ying and Gui (2012) found that the saturated fatty acids palmitic and stearic were in low ratio (about 9.9 %) of the total fatty acids. However, on the percentagebasis, in each case, the essential fatty acid, linoleic acid was the predominant fatty acid, and it accounted for 77.25 % and 76.14 % of the total fatty acids in the seeds of Puna Chicory and Commander Chicory, respectively. Meanwhile, oleic acid (18:1n-9) was the second higher content (over 11.6 %) in the Puna and Commander Chicory. In virtue of the higher content and percentage of unsaturated fatty acids in the chicory seeds, the ratio of saturated to unsaturated fatty acids was very low, and it was only about 0.11 in the seeds of Puna Chicory and Commander Chicory, although the amount of linolenic acid (18:3n-3) was very low (0.18-0.20 mg/g on dry weight basis

Table (3): The common measured fatty acids (%) in milk thistle and chicory seeds oil

Acids	Symbol	Milk thistle seed oil	Chicory seed oil
Caproic Acid	C6:0	0.04	0.16
Caprylic Acid	C8:0	0.03	0.06
Lauric Acid	C12:0	0.04	0.06
Tridecanoic Acid	C13:0	0.18	0.25
Myristic	C14:0	0.29	0.38
Pentadecanoic Acid	C15:0	0.09	0.12
Palmatic	C16:0	7.70	7.8 1
Stearic	C18:0	8.99	7.29
Heneicosanoic Acid	21:0	1.33	0.39
Tricosanoic Acid	C23:0	0.52	1.63
Total Saturated	%	19.21	18.15
Myristoleic Acid	C14:1	0.08	0.08
cis-10-Pentadecenoic Acid	C15:1	0.12	0.16
Palmitoleic Acid	C16:1	0.05	0.16
Oleic Acid	C18:1	23.32	29.71
Linolelic acid	C18:2	51.62	50.83
cis-11-Ecosenoic Acid	C20:1	2.93	0.45
cis-11, 14, 17- Ecosatrienoic Acid	C20:3ω3	0.78	0.36
Erucic Acid	22:1	1.89	0.10
Total Unsaturated		80.79	81.85
Un sat/ sat ratio		4.21 :1	4.51 :1

Amino acids composition:

The nutritive value of food, especially protein mostly would dependnot only on its amino acids profile in general but also on the quantities of the essential amino acids content in particular (Afify *et al.*, 2012). Amino acids composition of milk thistle seed , chicory seed and wheat flour are given in Table (4).

The results show that protein of milk thistle seed considers as a poor source of proline (0.49 %) and methionen (0.66 %) , contrary to,glycine and threonine that present in high ratios (27.21and 22.08%). All these essential amino acids in milk thistle seed were higher than chicory seed flour in exception Arginine.

Taking up adequate essential amino acids are very important for health since they are building blocks of proteins, which carry functions of human body (Zhao, 2007).

From the data of Table (4), it could be noticed that, concentrations of amino acids in chicory seed flour are lower than those present in milk thistle seed flour and wheat flour .

Table (4): Amino acids composition of raw materials (g amino acid/100 g protein)

Type	Amino acids	Milk thistle seed	Chicory seed	Wheat flour (72%)
Essential amino acids	Lysine	7.00	3.35	5.59
	Isoleucine	5.84	3.93	3.89
	Leucine	8.80	6.55	7.78
	Phenylalanine	2.29	5.32	5.99
	Arginine	2.00	11.14	6.92
	Histidine	1.91	2.91	3.11
	Valine	5.34	5.10	5.63
	Threonine	22.08	3.35	5.18
	Tryptophan	0.77	1.60	1.17
	Methionen	0.66	2.04	4.72
	cystine	N.D	3.13	1.59
Total essential amino acids		56.69	48.42	51.57
Nonessential amino acids	Aspartic	9.98	10.50	7.74
	Serine	3.67	3.86	4.43
	Proline	0.49	4.22	9.40
	Glycine	27.21	5.83	7.32
	Tyrosine	1.96	3.28	2.53
	Gultamic	N.D	19.59	11.67
	Alanine	N.D	4.30	5.34
Total nonessential amino acids		43.31	51.58	48.43
E/N ratio		1.31 : 1	0.94:1	1.06:1

N.D =Not detected

The amino acids composition of chicory seed protein and wheat flour protein had low total concentration of essential amino acids (48.42% and 51.57 %) compared with that of the milk thistle seed protein (56.69) as shown in Table (4).

Tryptophan is used for synthesis of neurotransmitter serotonin and relief depression,tyrosine is for dopamine, norepinephrine and adrenalin synthesis. Isoleucine is necessary for the synthesis of hemoglobin in red blood cells.Leucine has beneficial effect for skin, bone and tissue wound healing and promotes growth hormone synthesis. Lysine and valine are essential for muscle proteins. All these essential amino acids can be found in plant foods (McDougall, 2002).

Abd Raboh (2012) reported that the essential and nonessential amino acids are more abundant in milk thistle seed protein when compared with sunflower seed protein. Also, it could be concluded that milk thistle seed protein which is rich in lysine but poor in methionine and cystine, can be used to complement cereal proteins.

Ying and Gui (2012) showed that chicory seeds are good source of most essential amino acids according to the WHO recommended pattern for an ideal dietary protein, and the two chicory seeds were rich in various amino acids, except for lysine (0.32 % of Commander Chicory).Therefore, for more efficient use of their nutritional values, they should be mixed with other edible materials containing high levels of lysine in an appropriate ratio.

.Phytochemical constituents of raw materials

The flavonoids and phenolic acids are known to possess antioxidant activities due to the presence of hydroxyl groups in their structures and their contribution to defence system against the oxidative damage due to endogenous free radicals that are extremelyimportant (Boskou, 2006).

Total phenolic compounds

Table (5) display that total phenolic compounds of 80% aqueous ethanolic extract for milk thistle seed is 31.44 mg/ g. This result is in normal range to those found by Parry (2006) who reported that milk thistle seed flour contained 25.02 mg/g total phenolic compounds and was higher than those obtained by Khalil (2008).

Abd Raboh (2012) found that total phenolic compounds of 80% aqueous ethanolic extract is 37.61 mg/ g.

It is well known that phenolic compounds, especially, polyphenols are oxygen-scavengers and act as natural antioxidants as well as they have antimicrobial activity (Kapoor and Kaur 2002).

Total phenolic compounds of 80%aqueous ethanolic extract for chicory seed was 13.38 mg/ g.

Table (5) Phytochemical compounds as mg/g of raw materials in ethanolic extract

Aqueous ethanolic extract	Phenolic acids	Flavonoids
Milk thistle seed	31.44	12.00
Chicory seed	13.38	0.55
Wheat flour (72%)	0.13	0.06

These results are at the base line of those reported by *Milala* et al (2009) who found that the richest in polyphenols (more than 10% of dry matter) turned out to be the extract obtained from chicory seeds. As shown in Table (5), total phenolic compounds of 80% aqueous ethanolic extract for wheat flour was 0.13mg/ g.

Total flavonoids

The biological function of flavonoids include protection against allergies, inflammation, platelets aggregation microbes, ulcer, vineses and tumour (Okwu and Okwu, 2004). Also, a positive correlation between dietary flavonoids (such as myricetin, quercetin, and isoflavones) intake and decreased mortality from coronary heart disease, partly due to the inhibition of LDL (low density lipoprotejn cholesterol) oxidation and reduced platelet aggregability by flavonoids (Plosch *et al.*, 2006). Data presented in Table (5) clearly indicate that total flavonoids content of milk thistle seed was 12.00 mg/g.

The obtained results agree partially with those of Abd Raboh (2012) who found that flavonoid content was 19.51 mg/g in 80% ethanolic extract.

Total flavonoids content of chicory seed was 0.55mg/g.Whereas, this result is lower than that reported by Shad et al., (2013) who found that the total flavonoids content was 0.6 mg/g in chicory seed. On the other hand, the lowest flavonoids was detected in wheat flour (0.06mg/g).

Ascorbic acid (vitamin C)

Vitamin C content of milk thistle was 2.40 mg/ g. whil, chicory seed contained 1.40 mg/g. Data of Table (6) indicate that, it is clear that wheat flour didn't contain vitamin C.

Ascorbic acid (vitamin C) is involved in many metabolic activities and is essential for collagen biosynthesis, it increases blood circulation and plays an important role as an antioxidant (George 2003) .

Table(6) Ascorbic acid content in raw materials (mg/g)

Sample	Ascorbic acid
Milk thistle seed	2.40
Chicory seed	1.43
wheat flour (72%)	0.00

Antioxidant of raw materials extracts

DPPH radical scavenging activity .

DPPH scavenging capacities of ethanolic extracts of milk thistle seed and chicory seed in terms of percent inhibition of DPPH at 100 ppm are shown in table (7).

The DPPH radical scavenging capacities of milk thistle seed and chicory seed were found to possess comparatively good free radicals scavenging capacity due to their high DPPH radical inhibition..

The oxidative stress caused by free radicals is delayed or even prevented by a special class of substances called as antioxidants. Results presented in Table (7) clearly indicate that milk thistle seed flour and chicory seed flour exhibited higher antioxidant activity with DPPH than the wheat flour.

Free radical scavenging is one of the known mechanisms of inhibition of lipid oxidation, in DPPH free radical scavenging assay, antiradical power of an antioxidant is measured as color changes from purple to yellow, it can be used to evaluate hydrogen-donating ability of the compound, it can be used to evaluate hydrogen donating ability of compound, it is a rapid method and most widely employed to characterize antioxidant activity of plant materials (Arnao, 2000).

Table (7) Antioxidant activity percentage using DPPH of raw materials

ethanolic Extract	Inhibition % of DPPH
Milk thistle seed	88.57
Chicory seed	88.29
Wheat flour (72%)	3.11

These results are in agreement with those reported by Abd Raboh (2012) who reported that scavenging radical activity indicated that milk thistle seed flour extract of 80% aqueous ethanolic extract had 80.10% inhibition at 50 ppm raised to 92.76% inhibition at 500 ppm.

Mehmood, *et al* ., (2012) reported that as the concentration of phenolic compounds or degree of hydroxylation of the phenolic compounds increases, DPPH scavenging activity increases, hence does the antioxidant activity chicory seeds extracts and fractions showed excellent radical scavenging activity.

Shad *et al.*, (2013) reported that, all parts of chicory contain considerable amounts of phytochemicals and are good source of antioxidants

Sensory evaluation of biscuit with milk thistle seed flour

Organoleptic properties of biscuit containing different substitutions of milk thistle seed flour are illustrated in Table (8). There was a significantly ($p< 0.05$) difference in score values of taste, color, texture, appearance and over all acceptability between control and all levels of substitutions. Also, it could be noticed that the augment in substitution levels of different milk thistle seed flour led to decrement in the mean value of most scores of control one.

The most significantly of organoleptic characteristic of biscuit altered was the taste, whereas, taste panelist's score decreased at 15% substitution level of milk thistle seed especially the moderately bitter taste which detected by panelists.

Sensory characteristics of samples prepared using milk thistle seed flour until 10% substitution ratio had nearly similar scores compared with those of control.

Using of milk thistle seed at levels more than 10% milk thistle seed led to decrease the scores for sensory characteristics of biscuits especially taste of sample contained 15% which recorded 4.70 compared to 7.00 in control.

The low overall acceptability of the biscuit of blends containing more than 10% milk thistle seed may be attributed, to darkening color.

Generally, all samples prepared with milk thistle seed are acceptable for the sensory evaluation.

Darkening of cookies is attributed to sugar caramelization and the Maillard reactions between sugars and amino acids (Alobo, 2001).

In the sameTable, it is clear that, the texture of the biscuit supplemented with milk thistle seed flour at level (5%; and 10%) had high scores compared to the control, this may be related to the presence of some seed coat of the milk thistle seeds that contain a high proportion of fiber.

Table(8): Organoleptic characteristics of biscuit supplemented with different levels of milk thistle seed flour

parameters	Replacememt %			
	Control 0%	Milk thistle seed 5%	Milk thistle seed 10%	Milk thistle seed 15%
Taste (7)	7.00 a	6.50 b	6.00c	4.70d
Color (7)	7.00 a	6.33ab	6.00b	4.33 c
Flavor (7)	7.00 a	6.30 b	6.00 b	4.27 c
Texture (7)	5.8 b	6.33 a	6.50 a	5.47 c
Appearance (7)	7.00a	6.10b	6.00b	3.80 c
Overall acceptability (7)	6.76 a	6.16ab	5.96b	4.23 c

.Values followed by the same letter in row are not significantly different at P ≤ 0.05.

Sensory evaluation of biscuit with chicory seed flour

It is clear from the results of Table (9) that addition of chicory seed flour to the biscuit enhanced significantly texture comparing with control.

The panalist's scores of taste, color, flavor appearance and overall acceptability of biscuit containing chicory seed flour are lower than those prepared without chicory seed flour.

Whereas, the lowest panalist's scores were given to the biscuit containing 15% chicory seed flour. The best panalist's scores are recorded to the control biscuit followed by biscuit involved 5% and 10% chicory seed flour.

Owon et al (2012) who showed that biscuits which prepared using different substitution levels (10, 15 and 20%) of some medicinal plants such as carrot, thyme, rosemary and mixtures of them had Sensorcally higher scores at replacement ratio of 10%

Table (9): Organoleptic properties of biscuit as affected by adding chicory seed flour at different ratios

Parameters	Replacememt %			
	Control 0%	Chicory seed flour 5%	Chicory seed flour 10%	Chicory seed flour15%
Taste (7)	7.00a	6.77 ab	6.63b	6.23 c
Color (7)	7.00a	6.24 b	6.00 b	5.03 c
Flavor (7)	7.00 a	6.70 ab	6.40 b	6.30 b
Texture (7)	5.90c	6.23 b	6.60 \a	6.70a
Appearance (7)	7.00a	6.22b	6.00 b	4.73 c
Overall acceptability (7)	6.78a	6.38 ab	6.29 ab	5.80b

.Values followed by the same letter in row are not significantly different at P ≤ 0.05

Influence of milk thistle and chicory seed flours on firmness of biscuits

Data of Table (10) display that the firmness of the biscuits decreased by addition of milk thistle or chicory seed flours. The obtained results showed that, the lowest value of firmness (3.95) was in biscuit with chicory seed flour, but the highest value 4.35 was in control, While there were non significant decrease in all values when compared to control .

The extensible and cohesive structure is contributed by sugar or water interaction with wheat protein thus forming gluten but with an increase in fat content and crude fiber the flour gets coated and this network gets interrupted thus properties of biscuits are changed and a less harder, at very high fat content the lubricating function is high thus less water is required and a softer texture is obtained

Table(10). Firmness values of biscuit with 0% and 10% milk thistle seed or chicory seed

Additive mixture	Firmness
Control	4.35 a
Biscuit with10% milk thistle seed	4.15 ab
Biscuit with10% chicory seed	3.95 b

CONCLUSION

The present study confirmed that milk thistle seed and chicory seed had high amounts of protein, ether extract and crude fiber. This qualified them to use as a novel source of plant oil and protein, in addition contain important antioxidant substances such as phenolic and flavonoid compounds and vitamin C. Therefore these seeds can be considered a good source of natural antioxidant in food additives concerns associated of the use synthetic antioxidants.

Also, milk thistle seed flour and chicory seed flour could be incorporated up to 10% level in the formulation of biscuit without adversely affecting overall quality and textural characteristics

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دراسات كيميائية وتكنولوجية على بعض النباتات الطبية.

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هذه الدراسة توضح الاهمية الغذائيه لبعض النباتات الطبية البرية مثل بذور شوك الجمل وبذور الشكوريا حيث تم تقدير محتواها من العناصر الغذائيه مثل البروتين الخام والمستخلص الاثيرى والالياف الخام والاملاح المعدنية والاحماض الامينيه والدهنيه والمركبات الفينوليه والفلافونيدات وفيتامين استخدامها فى صناعة البسكويت. ثم دراسة خصائص مضادات الاكسدة الكلية لمستخلص البذور. كذلك دراسة الخواص التكنولوجية و الحسية للبسكويت بعد اضافة مطحون البذور بنسب٥ ،١٠، ١٥%.

أوضحت النتائج أن بذورشوك الجمل وبذور الشيكوريا تحتوي على نسبة عالية من البروتين والمستخلص الاثيرى الالياف والمعادن والمركبات الفينولية والفلافونيدات وفيتامين C مما يجعلها ذات نشاط قوى كمضاد للاكسدة و يمكن استخدمها كبديل لمضادات الأكسدة الصناعية.

وكذلك تحتوى البذور على نسبة عالية من الاحماض الدهنية غير المشبعة (الاوليك واللينوليك) و من الا حماض الامينية الاساسية وغير الاساسية. ووجد ان أحسن نسب الاستبدال كانت ١٠ % من كليهما، لذلك نوصى باستخدام بذور شوك الجمل وبذور الشيكوريا فى تدعيم بعض الأغذية المختلفة لمحتواهما العالى من العناصر الغذائية و مضادات الأكسدة الطبيعية. والاهتمام بزراعة هذة النباتات على نطاق واسع للاستفادة منها غذائيا وعلاجيا