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LEVELS OF SOME SECONDARY METABOLITES AND EFFECT OF SOME PROCESSING TREATMENTS IN SOME LOCAL VARIETIES OF LUPINE (*Lupinus termis,* L) SEEDS

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ABSTRACT

In the present study, six different groups of secondary metabolites (SMs) in the seeds of *L. termis*, L were extracted using six different solvents; these solvents are either polar solvent or non-polar ones. The seed extracts obtained were examined for qualitative detection. Seed extracts of *L. termis L.* contain flavonoids, saponins, alkaloids, tannins, terpens and resins. Total alkaloids (TAs), tannins and total phenolic compounds (TPCs) in seed samples were determined. The polar solvent distilled-H_2O is more efficient in extracting three secondary metabolites but only two solvents are capable for alkaloids extraction these are petroleum ether and distilled-H_2O. Three solvents are not suitable for saponins extraction. The polar solvent acetone is more efficient in extracting all secondary metabolites except alkaloids, terpens and resins. The proportion of seed coats (hulls) of four varieties of lupine seeds ranged from 18 to 25%. The proportions of seed coats (hulls) in bitter lupine Giza-1 (21-25%) are higher than those determined in sweet lupines. The weight of 100-seeds of four varieties of lupine seeds had the highest lupine bitter Giza-3 (37.13 g) and had the lowest lupines sweet Giza-1 (33.1 g), lupines and lupines bitter Giza-2 (35.97 g) bitter Giza-1 (33.12 g).

Three processing treatments (germination, soaking and cooking) were undertaken to reduce the levels of antinutritional factors (ANFs) in lupine seeds and TAs and TPCs were determined in germinated and non-germinated lupine see44ds, non-germinated seeds contain higher levels than those reported in germinated ones. These results indicate

that germination is effective and cheap tool to reduce bitterness from lupine seeds, whereas TAs (%) and TPCs sharply decreased in all treatments and all varieties. A better understanding of the effect of different traditional processing methods; soaking for 24, 48, 72 h), cooking (2-3 h) and soaking for 24h. TAs contents were reduced under the various processing methods, the treatments are more efficient in extracting total alkaloid in sweet lupine is (soaking for 24 h+ cooking + soaking for 24h). The treatment are more efficient in extracting total alkaloid in bitter lupines (soaking for 72 h + cooking + soaking for 24h) These treatments are useful process to improve the nutritional value of lupine seeds, may lead to a wider use of this legume.

Key words: Alkaloids, antinutritional factors (ANFs) bitterness, germination, lupine, *Lupinus termis*, saponins, secondary metabolites, soaking, tannins.

INTRODUCTION

Lupine (*Lupinus termis, L*.) is cultivated in a wide range of environments across Egypt. Its seed has a nutritional quality similar to soybean seed and superior to other legumes seed (Raza and Jrnsgard, 2005), and could be an important source of protein and oil. In fact, lupine seeds have been used for human consumption and as a medicinal plant in Egypt (Kattab, 1986; ARC, 1994) and other countries for thousands of years. Lupine is one of the oldest agricultural crops widely used in the world as a protein source in fodder production and for soil improvement (Maknickiene, 2001). Lupines are good source of proteins and lipids and have no lectins and very low content of protease inhibitors (Australia New Zealand Food Authority, 2001).

In general, lupines are used for many purposes; these include pasture improvement, ornamentation, and erosion control and soil stabilization. It has also been used as a green manure and for fixing atmospheric nitrogen to the soil. Furthermore; it can be mixed in the soil during the flowering period in green houses to control some pests due to its alkaloids (Cowling *et al*., 1998).

Lupine like other legumes has some anti-nutritional factors which inhibits its consumption. Mainly the presence of alkaloids (i.e. quinolizidine alkaloids) hinders its consumption without processing to remove them. Raffinose family oligosaccharides, phytates, tannins are also the other antinutritional factors found in the raw seed. However; it has limitations for continuous consumption by the society. Primarily, anti-nutritional factors like quinolizidine alkaloids limit its direct consumption (Mahamed, *et al*., 1994, Jimenez-Martinez *et al*., 2003, Sujak *et al*., 2006, Gulewicz *et al*, 2014).

Soaking has been documented to be an effective treatment to remove

antinutrional factors, which can be eliminated with the discarded soaking solution, but some metabolic reactions can take place during soaking, affecting the content of some compounds (Vidal-Valverde *et al*., 1992). In general, soaking treatment reduced antinational factors and improved *in-vitro* protein digestibility, but the effects varied with legume cultivars and soaking conditions such as type of soaking solutions, soaking period and temperature (El-Baltegy, 1996). The flour or the meal of soaked legume seeds is used in several dishes and as supplement in weaning food mixes, bread, biscuits and other products (El-Adawy, 1986, Sobihah, 1998). The nutritional quality of legume seeds can be improved through removal or elimination of such compounds by soaking (El-Baltegy, 1996), germination (Rahma *et al*., 1987, El-Baltegy 1996) and cooking (Mansour and El-Adawy, 1994, El-Baltegy, 1996).

Germination process is a cheap and effective way to enhance the nutritional value and quality of legume. The antioxidant activities in raw legume can be further boost up after germination (López-Amorós *et al*., 2006). In order to improve the nutritive value and digestibility, and to reduce antinutritional factors, a number of methods can be applied and include soaking, dehulling, and germination (Sripriya *et al*., 1997). The use of *Lupinus* in food process applications requires reduction of content of non-nutritional ingredients such as tannins, alkaloids and oligosaccharides, undesirable compounds that must be removed before consumption (Ballester *et al*., 1980; Jimenez *et al*., 2001).

The main objectives of the present investigation were: (1) determining the levels of secondary metabolites (2) studying the effect of processing treatments such as germination, soaking and cooking on the levels of TAs and TPCs in lupine seeds.

MATERAL AND METHODS

Samples:-

Four dry beans of lupine (*Lupinus spp.* L.) varieties were provided from Agricultural Research Centre, Giza, Egypt. Three varieties were bitter samples and one variety was sweet sample. These samples were kindly provided by Prof. Dr. Ahmed Mekheimar, professor of agricultural crops, in Agricultural Research center, Giza.

Detection of saponins:-

To detect saponins, 10 ml of distilled water were mixed with alcoholic extract of plant seeds and the agitated for 15 min. Formation of thick foam and survival for a long time indicated the presence of saponins (Harborne, 1973).

Extraction and determination of saponins

Saponins were extracted according to the procedure described by Obadoni and Ochuko, (2001). Using double solvent extraction method, 20g of the finely ground sample was weighed out into a conical flask. One hundred mL of 20% aqueous ethanol was added into the

sample, it was heated over a water bath for 4 h with continuous stirring at about 55ºC. The mixture was filtered and the residue re- extracted with another 100 mL of 20% ethanol. The combined extract was reduced to about 40 mL over a water bath at 90ºC, and the concentrate transferred into 250 mL separating funnel and 40 mL of pet-ether were added and vigorously shacked. Separation was done by partition during which the ether layer was discarded and the aqueous layer reserved. The saponin was extracted with 60 mL of *n*-Butanol. The combined extracts were washed with 5% aqueous NaCl solution, and evaporated to dryness in a pre-weighed beaker (W1) and was dried at 60ºC in the oven and reweighed (W2). The saponin content was determined by difference and calculated as a percentage of the original sample.

Detection and determination of total alkaloid:-

Mayer's' Reagent was used to detect alkaloids in lupine extracts. The appearance of a white precipitate is an indication on the presence of alkaloids in the case of the use of Meyer detector (Atherder, 1969). Determination of alkaloids was done by the alkaline precipitation gravimetric method described by Harborne, (1973). Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed. The absorbance was taken at 565 nm against a blank. The experiment was carried out in triplicate.

Detection of terpens:-

Terpens existence was detected by mixing 1 ml of the plant extract with 2 ml of chloroform and added a drop of anhydrous acetic acid and a drop of sulfuric acid (Al-Masry, 1999). Appearance of brown color indicated the presence of terpens.

Detection of resin:-

The blending 10 g of lupine seed powder with 50 ml of ethyl alcohol (95%) were mixed for two minutes in boiling water bath. Resin and then nominated the solution and 100 ml of acidified water hydrochloric acid (4%) were added (Harborne, 1984).

Preparation of extracts:-

The seeds of lupine were dried in shade under normal environmental conditions and homogenized to coarse powder and stored in opaque screw tight jars until use. Powdered material was charged into Soxhlet apparatus and extraction was carried out with following solvents successively. (1) Petroleum ether (40-60^{o}C), (2) Chloroform, (3) Ethyl acetate, (4) Acetone, (5) Methanol and (6) distilled water. Each time before employing the solvent of higher polarity was dried.

Qualitative examination of SMs in *L. termis* .

Determination of tannins:-

Tannins content was determined following the methodology proposed by Burns (1971). Briefly, a chromogenic agent was prepared by mixing equal parts of an 8% HCl/methanol solution with a 4% vanillin/methanol solution. Immediately thereafter, a standard curve was run by preparing a catechin/methanol solution containing

100 mg catechin in 5 mL methanol. Four solution dilutions were prepared in duplicate from the catechin solution by diluting at a 1:10 ratio each time. Then, 5 mL chromogenic solution were added to each tube, the tubes agitated in a vortex and transmittance measured at 500 nm in a spectrophotometer. The chromogenic agent was used as a blank.

Detection of total flavonoid (TF):-

Detection of flavonoids was carried out according to the method described by Jaffer *et al*., (1983).

Extraction and determination of total flavonoid (TF):-

Oven-dried sample seeds (30 g) are extracted in a Soxhlet extractor with 100 ml distilled ethanol for 1 h and the extract filtered. A known volume of extract was placed in 10 ml volumetric flasks. Distilled water is added make 5 ml and 0.3 ml $NaNO_2$ (1:20) were added. 3 ml $AlCl_3$ (1:10) were added 5 min later. After 6 min, 2 ml 1 mol $litre^{-1}$ NaOH was added and the total was made up to 10 ml with distilled water. The solution was mixed well again and the absorbance was measured against a blank at 510 nm with a Spectrophotometer (Taizhou Radio Factory) and flavonoid content was expressed as mg of quercetin equivalents (QE)/g of dried seeds (Zhishen *et al*., 1999).

Extraction and determination of total phenolic compounds (TPCs):-

TPCs were extracted from lupine sample (1.0g) by refluxing with 30 ml of methanol containing 1 % HCl for 10 min, the extract was centrifuged at 8.000 r.p.m. for 10 min. The concentrations of total phenolic compounds in the methanolic extracts were determined by the method described by Singleton and Rossi (1965**)** with some modifications. One milliliter of sample was mixed with 1 ml of Folin and Ciocalten's phenol reagent. After 3 min. 1 ml of saturated Na_2CO_3 (35 %) was added to the mixture and this was made up to 10 ml by adding distilled water. The reaction was kept in the dark for 90 min, after which its absorbance was read at 725 nm. A calibration curve was constructed with different concentrations of gallic acid (0.01–1 mM) as standard.

Extraction and determination of phytic acid content:-

Phytic acid (PA) was extracted from 4 lupine samples by the method described by Ellis *et al*. (1977). Phytic acid of row and processed samples was determined according to the method described by Wheeler and Ferrel (1971).

Extraction and determination of carotenoids (TCs) :-

Carotenoids content in lupine seed flours was extracted by first placing one g flour in a glass, adding 50 ml acetone and agitating for 12 h to extract the pigments. The glass was covered during extraction to prevent contact with light and consequent pigment degradation. The extract was filtered and its absorbance read at 472 and 508 nm in a spectrophotometer (Spectronic, Genesys 5, La Joya, USA). Carotenoids concentration was reported for the red and yellow isochromic fractions and the

concentration of each calculated using the absorbance values and the following formulas (Hornero-Mendez and Minguez-Mosquera, 2001):

$$C_Y = \frac{(A508)(2144.0) - (A472)(403.3)\ \mu g / ml}{270.9}$$

$$C_R = \frac{(A472)(1724.3) - (A508)(2450.1)\ \mu g / ml}{270.9}$$

Where: C_R = Red isochromic fraction, C_Y = Yellow isochromic fraction
A_{508} = Absorbance at 508 nm, A_{472} = Absorbance at 472 nm

Experiment germination :-

Germination process of lupine seeds.

Samples were washed and cleaned with tap water before soaked for 6 hours at room temperature (28°C). After 6 hours, samples were put under wet muslin cloth and left germinated for 48 hours at room temperature (28°C) without direct contact with sun light (Yasmin *et al*., 2008).

Processing treatments and cooking methods:-

Effect of soaking and cooking and then soaking on total alkaloid contents in *L. termis* was done. All samples were cleaned manually to remove foreign matters, immature and damaged seeds. Samples were washed and cleaned with tap water before soaked at room temperature (28°C). Then the following main traditional processes were performed (Kaur and Kapoor, 1990). Soaking**:** lupines sample was soaked in tap water at ratio 1:10 (w/v) at room temperature (28 C°) for 24, 48 and 72 h before cooking. The soaked seeds were washed twice with ordinary water followed by rinsing with distilled water. Cooking: The soaked seeds (24, 48 and 72 h in tap water) were cooked in beakers with a seed to water ratio of 1:5 (w/v) for soaked seeds. The water was allowed to boil before the addition of seeds. The seeds were cooked until (about 2-3 h for soaked seeds). Soaking: The seeds soaked for 24 h and then dried in an oven at 30-60°C to a constant weight Dried samples were ground, stored in an airtight plastic container for further analysis.

Determination of proportion of seed coats (hulls**)**

The proportion of seed coats to whole seed of four varieties of lupine seeds (3 bitter lupine + one sweet lupine) was determined. The amount of seed coats varied inversely with the weight of the seed.

RESULTS AND DISCUSSION

Qualitative examination of secondary metabolites in *L. termis* seeds

Six different extracts of secondary metabolites (SMs) in the seeds of *Lupinus termis* were obtained using six different solvents, have different polarity. The seed extracts obtained were examined for qualitative detection and the results are given in Table (1). The polar solvent distilled-H_2O is more efficient in extracting four secondary metabolites. Two solvents are capable for alkaloids extraction these are petroleum ether and distilled-H_2O. Three solvents are not suitable for saponins extraction.

Acetone is more efficient in extracting 3 secondary metabolites. The lupine seed extracts are free from terpens and resins.

Table (1): Qualitative examination of SMs in lupine seeds

Extractant	Flavonoids	Saponins	Alkaloids	Tannins	Terpens	Resins
Chloroform	+	-	-	-	-	-
Petroleum ether	-	+	+	-	-	-
Acetone	+	+	-	+	-	-
Ethyl acetate	+	-	-	-	-	-
Methyl alcohol	+	-	-	+	-	-
Distilled-H_2O	+	+	+	+	-	-

Effect of solvent polarity on the extraction yield from *Lupinus* seeds**:-**

Results given in Table (2) show that the yield (g/kg) are different when the dried homogenized powdered of lupine seeds treated with six different solvents successively as following (**1**) Petroleum ether (40-60^oC), (**2**) Chloroform, (**3**) Ethyl acetate, (**4**) Acetone, (**5**) Methanol (**6**) Distilled-H_2O were used. The highest yield (250 g/kg) is recorded when the non-polar organic solvent, Petroleum ether (40-60^oC) was applied while the lowest yield (55 g/kg) is recorded when polar solvent (Distilled-H_2O) was used.

Table (2): Effect of solvent polarity on the extraction % from lupine seeds

Extractant	Weight (g/kg)	Extraction (%)
Petroleum ether (40-60^oC)	250	10±0.4
Acetone	180	5±0.5
Chloroform	145	4±0.2
Ethyl acetate	100	2±0.1
Methanol	81	9±0.3
Distilled-H_2O	55	8±0.3

The preparation of extracts from plants using organic solvents, with emphasis on common problems encountered and methods for their reduction or elimination. In addition to generally applicable extraction protocols, methods are suggested for selectively extracting specific classes of plant-derived compounds, and phytochemical procedures are presented for the detection of classes of compounds encountered commonly during extraction, including selected groups of secondary metabolites and interfering compounds. Successful extraction begins with careful selection and preparation of plant samples. During the extraction of plant material, it is important to minimize interference from compounds that may co-extract with the target compounds, and to avoid contamination of the extract, as

well as to prevent decomposition of important metabolites or artifact formation as a result of extraction conditions or solvent impurities (Jones and Kinghorn 2013).

Quantitative determinations of SMs in lupine seeds:-

Total phenolic compounds TPCs:-

Results of TPCs extracted from four different cultivars of lupines are given in Fig. (1). These results show that TPCs in the whole seeds of bitter lupines is 3-folds higher than those found in the seed sweet lupine (Giza-1 SL) (0.7310 mg/100g). The levels of TPCs in bitter lupines are always higher than those determined in seeds sweet lupines. The levels of TPCs in bitter lupines Giza-1 are (2.119 mg/100g) and bitter lupines Giza-2 (2.192 mg/100g) and bitter lupines Giza-3 (2.029 mg/100g) and sweet lupines (0.7310 mg/100g).

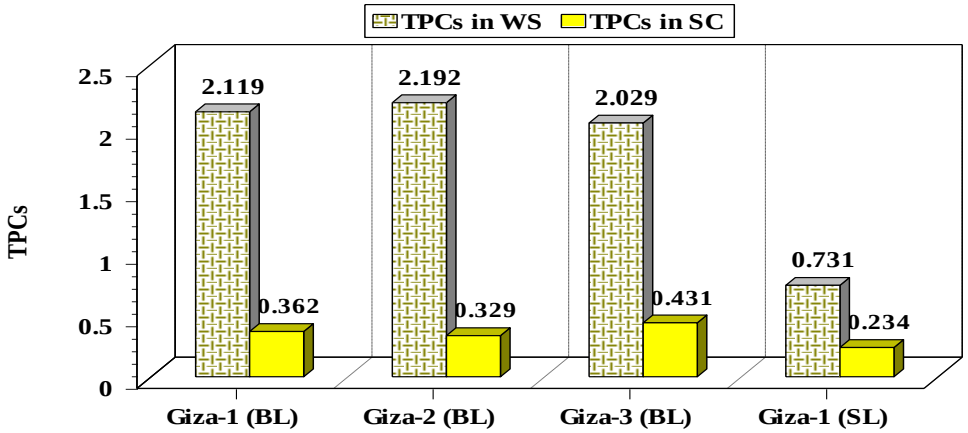

Fig. (1): Levels of TPCs in lupine whole seeds (WS) and seed coats (SC)

Results of TPCs of seed coats determined in four different varieties of lupines are given in Table (5). These results show that TPCs concentrations in the seed coats of bitter lupines are higher than those found in the seed coats sweet lupine sample. The levels of TPCs in the seed cots of bitter lupine Giza-1 (0.362 g/100g) and bitter lupine Giza-2 (0.329 g/100g) and bitter lupine Giza-3 (0.431 g/100g) and sweet lupine (0.234 g/100g). Results of TPCs of seed coats determined in four different varieties of lupines are given in Table (5). These results show that TPCs concentrations in the seed coats of bitter lupines are higher than those found in the seed coats sweet lupine sample. The levels of TPCs in the seed cots of bitter lupine Giza-1 (0.362 g/100g) and bitter lupine Giza-2 (0.329 g/100g) and bitter lupine Giza-3 (0.431 g/100g) and sweet lupine (0.234 g/100g).TPCs are designated as defensive compounds located in many parts of legumes and play an important role against store pests (Fouad and

Abd El-Naem 2000; Franco *et al*., 2002). Phenols or phenolics are widely distributed organic compounds in the plant kingdom, which help in defence against predators and pathogens; they have been reported to be active against a wide range of organisms (Upadhyay, 2011). On another hand phenolic compounds act as natural ideal antioxidants and have medical, pharmaceutical and health properties. Duthie *et al*., (2000**)** studied plant polyphenols and their roles in cancer and heart disease and implications as nutritional antioxidants.

Several studies on the possible roles of phenolic compounds have been conducted and indicated that the seed coats and/or whole seed of most legumes contain high levels of phenolic compounds such field bean (Abd El-Naem and Azazz 2009); kidney bean (El-Moris *et al*., 2013) and recently on soybean (Gamal-Fakhry *et al*., 2016).

Total flavonoid**:-**

Results of total flavonoid (TF) extracted from four different cultivars of lupine are given in Fig. (2). These results show that TF levels in the bitter lupines are higher than those found in the seed of sweet lupine samples. The levels of TF when expressed as quercetin equivalents ranged from 1251 µg/100g to 860 µg/100g and the highest level is determined in bitter lupine Giza-2 and the lowest one (860 µg/100g) is for Giza-1 sweet lupine seeds. The color of seed coats of lupine samples is a result of TF existence and the roles of their fractions are antioxidants, defensive compounds and source of some glycosides (Duenas *et al*., 2009). The higher levels of flavonoids are a positive indication of plant extracts under because the higher flavonoids content is the higher antioxidant potential and higher cancer preventive function (Zhishen *et al.,* 1999 and Duenas *et al*., 2009)

Flavonoids are the most second abundant polyphenols in our diets. They can be divided into flavones, flavonols, isoflavones, anthocyanins, flavanols, proanthocyanidins and flavanones. Other dietary polyphenols are not well-defined chemical entities and result from the oxidative polymerization of flavonoids and phenolic acids. This may occur during ripening or food processing (grinding, fermentation, storage, cooking and other processes) (Santos-Buelga and Scalbert, 2000).

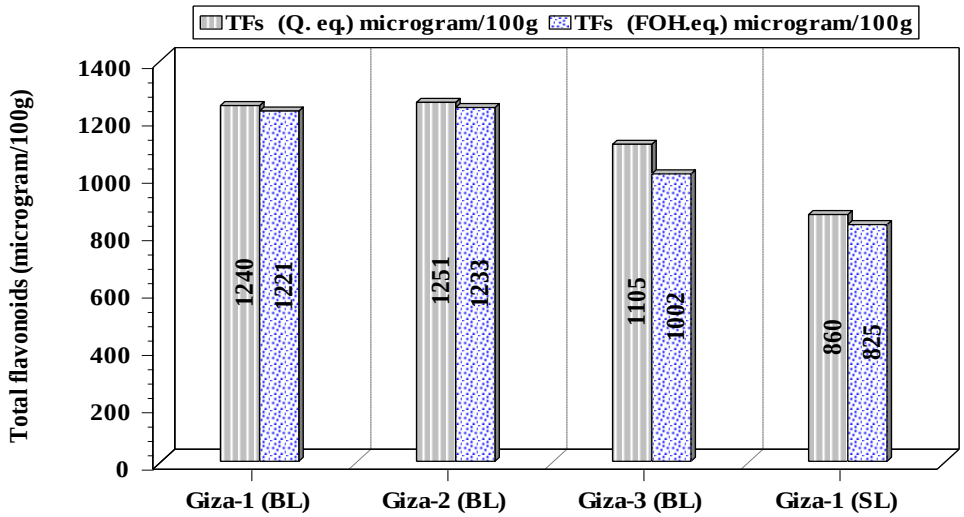

Fig. (2): levels of TFs in bitter lupines and sweet lupine seeds

Several investigators concluded that pharmacological effects of flavonoids are correlated with their antioxidants activities. Moreover, it is suggested that the overall antioxidant effect of flavonoids on lipid peroxidation may be related to their $^{\cdot}OH$ and $^{\cdot}O_2$ scavenging properties and their reaction with peroxy radicals (Husain *et al.*, 1987, Zhishen *et al.*, 1999 and Duenas *et al*., 2009).

Total alkaloid (TA):-

Total alkaloid in samples of lupine seed was determined and the results are given in Fig. (3) and Table (5). These results show that total alkaloid in bitter lupines Giza-1 (1.365%) are higher than those determined in sweet lupine (0.074%). The levels of TA in bitter lupine are always higher than those determined in seeds sweet lupines. Results also show that bitter lupines always have the highest contents of the determined secondary metabolites.

The biochemical and pharmacological properties of quinolizidine alkaloids (QAs) have a wide variety of biological activities. Quinolizidine alkaloids are toxic or inhibitors for most organisms. The relatively activities of sparteine and lupinine in vertebrates, especially rat and man isolated QA can be antiarrhythmic and oxytocic (sparteine, lupaine), hypoglycemic (lupinine), cytotoxic and antipyretic (matrine), hallucinogenic drug (cytosine, N-methylcytisine, teratogenic (anagyrine). Sparteine is used therapeutically as an antiarrhythmic drug and in obstetrics, whereas lupinine and matrine have been in use in folk medicine in Eastern Asia. Most of the over 150 QA have not been tested at all in any of the above-mentioned areas and the mechanism of their action in living systems has to be elucidated in most instances (García-López *et al*., 2004, Kubo *et al*., 2006).

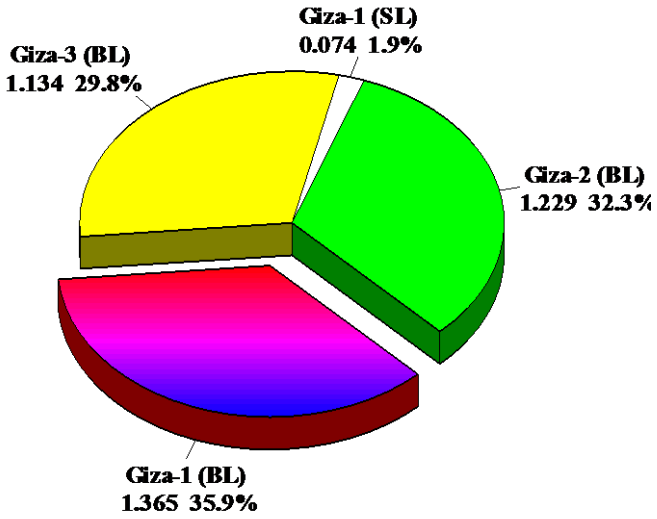

Fig. (3): Levels of TA in bitter lupines and sweet lupine seeds

One of the major safety issues of lupines-based foods is the presence of quinolizidine alkaloids (QAs), bitter compounds produced by lupines plants as a defense mechanism against predators. In mammals, QA intoxication is characterized by trembling, shaking, excitation, and convulsion. Lupanine and sparteine, the most common QAs, show acute oral toxicity due to neurological effects leading to the loss of motor co-ordination and muscular control (Resta *et al*., 2008)

The levels of alkaloids in seeds or meal can be reduced through a de-bittering process involving soaking or washing with water. This is commonly practised in Europe where high alkaloid lupins, so-called 'bitter lupins', are grown. The level of alkaloids in these lupins after the de-bittering process is reported to be approximately 500 mg/kg. In Australia, lupine varieties with low alkaloid content, so-called 'sweet lupins', have been developed through plant-breeding programs (Zamora *et al*., 2008).

Sparteine was used in the treatment of cardiac arrhythmias and to induce uterine contractions. It has also been shown to depress the central nervous system and to have hypotensive, diuretic and anti-inflammatory activities (Szczawinska *et al*., 1994, Schmeller and Wink, 1998). The QA's lupanine, 13-hydroxylupanine and multiflorine have been reported to have pharmacological activities such as anticonvulsant, antipyretic and hypoglycemic (Hatzold *et al*., 1983, García-López *et al*., 2004). Lupanine, 13-hydroxylupanine, angustifoline and sparteine were shown to have bactericide-like activity

against *Staphylococcus aureus*, *Bacillus subtilis*, and *Bacillus thuringiensis* (De la Vega *et al*., 1996). Lupanine and lupin alkaloid extracts have shown to have herbicidal activity, and the capacity to inhibit the growth of *Fusarium avenaceum*, *Fusarium solani*, *Pythium aphanidermatum*, *Botrytis cinerea*, *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum.* Lupin alkaloids also have feeding deterrence effects on the red legged earth mite *Halotydeus destructor* (De la Cuadra *et al*., 1994; Muzquiz *et al*., 1994; Maknickiene, 2001; Zamora *et al*., 2005; and 2008).

Concentrations of lupine seed saponins:-

The concentrations of saponins in samples of lupine seeds were determined and the results are given in Fig. (4) and Table (5). These results show that saponins in bitter lupines Giza-1 (0.0738 %) are higher than those found in both bitter lupine Giza-2 and Giza-3 and much higher than those found in sweet lupines.

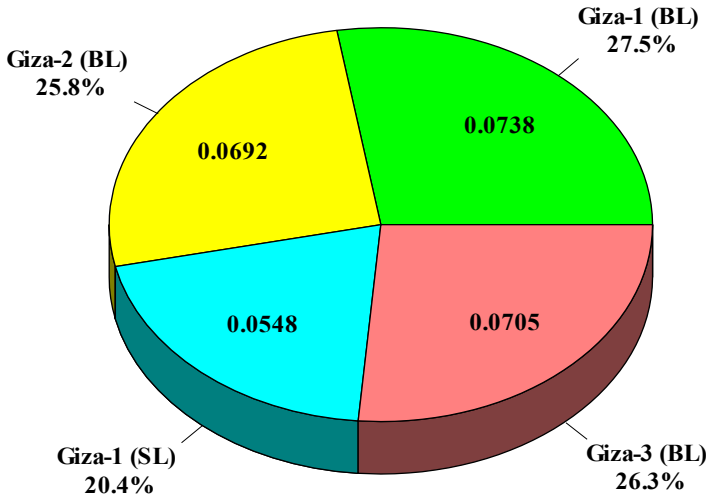

Fig. (4): Levels of saponins in bitter and sweet lupine seeds

Many legumes contain saponins, such as lupine, soybeans, beans and peas. Saponins possess clear insecticidal activities: they exert a strong and rapid-working action against a broad range of pest insects that is different from neurotoxicity. The most observed effects are increased mortality, lowered food intake, weight reduction, retardation in development and decreased reproduction. According to the main hypotheses in literature, saponins exert a repellent/deterrent activity, bear digestive problems, provoke insect moulting defects or cause cellular toxicity effects. As a consequence these interesting plant components open new strategies to protect crops in modern agriculture and horticulture with integrated pest management (IPM) programs against pest insects,

either by spraying, or by selecting high-saponin varieties of commercial crops (De Geyter *et al*., 2007 and Afrose *et al*., 2014).

The Egyptian plant breeders must targeting the saponin contents in field crops as tool in breeding programs to get resistant cultivars for both harmful insects and pathogens attacks.

Total carotenoid (TC) :-

Concentrations of TC in samples of lupine seeds were assayed and the results are given in Fig. (5) and Table (5). These results show that carotenoids isochromic yellow in bitter lupines Giza-1 (0.468 µg/ml extract) are higher than those determined in seeds lupines Giza-2, Giza-3 and sweet lupines. These results show that carotenoids isochromic red in sweet lupines, Giza-1 (0.261 µg/ml) are higher than those determined in seeds bitter lupines.

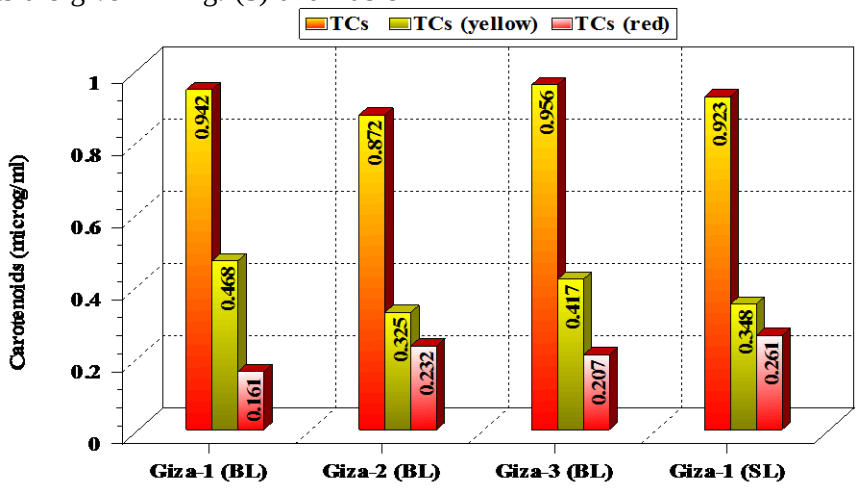

Fig. (5): Levels of carotenoids in bitter lupines and sweet lupine seeds.

Total carotenoid in samples of lupines seed, were determined and the results are given in Table (3). Total carotenoid concentrations ranged from 0.872 µg/ml to 0.956 µg/ml and the highest concentration is determined in extracts of bitter lupines Giza-3 and the lowest one in bitter lupine Giza-2.

The results in Table (3) show that the highest crude lipids (12.42) is corresponding highest total carotenoids (0.956) for bitter lupine Giza-3 and the lowest level of TCs is found in bitter lupine Giza-2. Consumption of lupine seeds with colored seed-coats with yellow, orange and red is preferred and recommended than that non-colored

Epidemiology studies showed that carotenoids can prevent the development of some chronic diseases in humans, including cancers and cardiovascular diseases, in addition to other biological activities, including antioxidant activity, influences on the

immune system, control of cell growth and differentiation and stimulant effects on gap junction communication (Wang *et al*., 2008).

Table (3): Crude lipids % and total carotenoids ratios in lupine samples.

Component	Lupine cultivars			
	BL Giza-1	BL Giza-2	BL Giza-3	SL Giza-1
Crude lipid CL	11.13	11.82	12.42	9.99
Total Carotenoids µg/ml	0.942	0.872	0.956	0.923

In the human diet, carotenoids have been shown to have antioxidant activity which may help to prevent certain kinds of cancers, arthritis and atherosclerosis. β-Carotene is a precursor of vitamin A (retinal) which is biosynthesized *via* the action of β-carotene 15,15'-monooxygenase. There are nearly 600 carotenoids in nature. In humans, four carotenoids (β-carotene, α-carotene, γ-carotene, and β-cryptoxanthin) have vitamin A activity, and they can be converted to retinal (Ngkok, and Solcha, 1991).

Levels of phytic acid (PA):-

Phytic acid levels in lupine seed samples were determined and the results are given in Table (4). These results show that phytic acid content ranged from 1.20 to 1.56 g/100g in extracts of Giza-3, Giza-2, and lupines sweet and Giza-1 respectively and the results also, showed that the phosphorus content ranged from 0.68 to 0.75% for the varieties and the highest concentration is determined in extracts of lupines bitter Giza-1.

Table (4): Phytic acid and phosphorus percent in lupine seeds

Constituents	Giza-1 (bitter)	Giza-2 (bitter)	Giza-3 (bitter)	Giza-1 (sweet)
Phytic acid	1.56	1.38	1.20	1.44
Phosphorus %	0.75	0.71	0.68	0.74
PA/P	2.08	1.94	0.816	1.95

Phytic acid (myoinositol, 1, 2,3,4,5, 6 hexakis-dihydrogen phosphate) and phytate are widespread in plant seed grains (also including cereals), roots and tubers (Graf, 1986; Lasztity and Lasztity 1990). Phytic acid is generally regarded as the primary storage form of both phosphate and inositol in seeds. Phytic acid phosphorus constitutes the major portion of total phosphorus in several seeds and grain. It accounts for 50–80% of the total phosphorus in different cereals. The phytic acid content in influenced by cultivar, climatic conditions and year. The accumulation site of phytic acid in monocotyledonous seeds (wheat, barley, rice, etc.) is the aleurone layer, particularly the aleurone grain.

Aleurone grain contains two types of inclusions: (a) globoids containing high amount of phytates, and (b) protein carbohydrate bodies. Corn differs from other cereals as more than 80% of phytic acid is concentrated in germ. Phytic acid content of cereals varies from 0.5 to 2.0%. Because most of the phytic acid is located in the outer parts of the kernel the different products of milling contain different levels of phytates. Bran is the product having a high phytic acid content, low extraction white flours contain low phytic acid quantities. If protein concentrates or isolates are prepared from cereals or other raw materials, such products contain also phytic acid in quantities depending on the raw material and method of processing (Hidvegi and Lasztity 2002).

Generally it is notice the varieties having high levels of phytic acid are favorable for human nutrition for many reasons. Phytic acid has hypo cholesterolemic, antioxidative, anticarcinogenic, and hypolipidemic effects and has been suggested to have a role in the prevention of caries and platelet aggregation in the treatment of hypercalciuria and kidney stones (Thompson, 1994, Potter, 1995). Phytic acid also has physiological effects similar to those of high-fiber diets and such as may be partly responsible for some of the health benefits. The function of phytic acid is not only for energy storage and antioxidation of fats of seeds but also for protecting seed from fungal invasion (Dayi *et al*. 1995).

In opposite manner, many other scientists (Coelho *et al*., 2002) have reported that phytic acid is the major storage form of phosphorus in seeds of legumes and cereals. Since phytate can form complexes with proteins and minerals thereby reducing the digestive availability of these nutrients, it is usually regarded as an antinutrient, although recent work indicates that it has important beneficial roles as an antioxidant and anticarcinogen. Therefore, there is an interest in the assessment and manipulation of phytate contents in important food grains such as beans.

PA is an important component of seedling germination because phytase hydrolyzes some of the PA-P (PA phosphorus) in order to release Pi, which is utilized by soybean seedlings for energy and nutrients (Urbano *et al*., 2000). The PA-P in mature soybean seeds, which are fed to livestock and humans, is unavailable for use by nonruminant livestock because phytase enzymes are not present in the digestive tract for breakdown of PA and release of Pi (Pedersen *et al*., 2002 and Jennifer *et al*., 2014).

Concentrations of tannins:-

Tannins content in lupine seed samples were determined and the results are given in Fig. (6) and Table (5). These results show that tannins in sweet lupines Giza-1 are higher than those determined in bitter lupines Giza-1, Giza-2 and Giza-3 lupines. Total and condensed tannin (responsible for negative effect in protein binding) levels in *Lupinus* were reported to be approximately

0.29% and 1.01%, respectively (Petterson *et al*., 1997). Tannins content in the present study resulted higher than the reported for *L. mutabilis* (Guemes-Vera *et al*., 2008), but lower than for *L. rotundiflorus, L. simulans* or *L. madrensis* (Ruiz and Sotelo, 2001). Tannins are a fraction from phenolic compounds which bind with protein resulting low digestibility (Guemes-Vera *et al*., 2012).

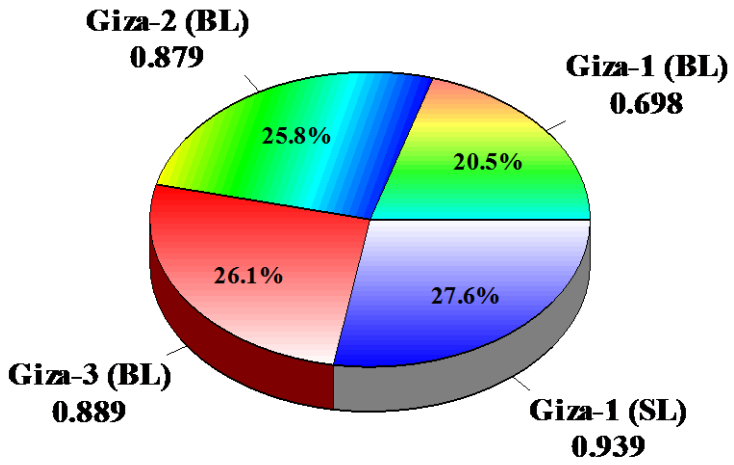

Fig. (6): Levels of tannins in bitter and sweet lupines seeds.

One of the most important of secondary metabolites, is tannins. Tannins are bound to proteins that form insoluble or soluble tannin-protein complexes. They have been closely associated with plant defence mechanisms towards mammalian herbivores and insect. They are found in many plant species such as *Acacia spp, Sericea lespedeza* as well as pasture species such as *Lotus spp*. In recent years many researchers demonstrated that tannins have positive effects on animals by antimicrobial, anthelmintic, protein bypassed effects in ruminants (Hassanpour *et al*., 2011 and Guemes-Vera *et al*., 2012)

Determination proportion of seeds coats in *L. termis* seeds

Results given in Table (6) show that the proportion of seed coats (hulls) of four varieties of lupine seeds ranged from 18 to 25%. The amount of hulls varied inversely with the weight of the seed, within each variety. These results show that proportion of seed coats (hulls) in bitter lupines Giza-1 (21-25%) are higher than those determined in seeds bitter lupines Giza-3 (18-23%) and sweet lupines Giza-1 (19-25%) and bitter lupines Giza-2 (19-24%). Edwin (2006) found that the proportion of seed coats (hulls) of four varieties of sweet lupines seeds ranged

from 19 to 25%. The amount of hull varied inversely with the weight of the seed, within each variety. All the measurements of analyzed samples were made in triplicate. Results given in Table (6) show that the weight 100 seeds of four varieties of lupines seeds had the highest bitter lupines Giza-3 (37.13 g) and had the lowest lupines sweet Giza-1 (33.1 g), bitter lupines Giza-1 (33.12 g) and bitter lupines Giza-2 (35.97 g).

Table (5): Quantitative analysis of SMs in *L. termis* seed and seed coats.

Constituents	Giza-1 (bitter)	Giza-2 (bitter)	Giza-3 (bitter)	Giza-1 (sweet)
TPCs mg/100g WS	2.119±0.21	2.192±0.21	2.029±0.20	0.731±0.07
TPCs mg/100g SC	0.362±0.03	0.329±0.03	0.431±0.04	0.234±0.02
TFs □g/100g	1239.77±12	1251.46±12	1105.26±11	859.65±8
Total alkaloids TA	1.365±0.136	1.229±0.122	1.134±0.113	0.074±0.008
Saponins (%)	0.0738±0.007	0.0692±0.006	0.0705±0.007	0.0548±0.005
(a)-TCs-yellow µg/ml	0.468±0.04	0.325±0.03	0.417±0.04	0.348±0.03
(b)-TCs-red µg/ml	0.161±0.01	0.232±0.02	0.207±0.02	0.261±0.02
(e)-Carotenoids (mix)	0.313	0.315	0.332	0.359
Total carotenoids µg/ml	0.942	0.872	0.956	0.923
Phytic acid g/100g	1.56	1.38	1.20	1.44
Tannins (%)	0.698	0.879	0.889	0.939

WS= whole seeds, SC= Seed coats

Table (6): Determination proportion of seeds coats in *Lupinus termis* seeds

Varieties	seed coats (hulls)	kernels	Average weight of 100 seeds
BL Giza-1	21-25%	75-79%	33.12 g
BL Giza-2	19-24%	76-81%	35.97 g
BL Giza-3	18-23%	77-82%	37.13 g
SL Giza-1	19-25%	75-81%	33.10 g

Experiment germination :-

Contents of total alkaloid and total phenolic compounds were analyzed in germinated and non-germinated lupine seeds and the results are shown in Figs. (7 and 8). From the results obtained, non-germinated lupine seeds contain the highest content of total alkaloid and total phenolic compounds followed by germinated lupine seeds, non-germinated seeds total alkaloid (%) and total phenolic compounds (mg/100g) significantly decreased in

all treatments by compared with control treatment. Germination process doesn't remove all the quantities of TPCs and TA until the third day. The remaining levels of TPCs and TAs exceeds 40%, these results are in a good agreement with those reported by Khandelwal *et al.* (2010) and Megat and Azrina (2012).

Results given in Figs. (7 and 8) show that the germination treatments obtained in the evaluation of the TPCs content and TA of both lupine samples can be deduced that germination caused a decrease in total phenolic content and total alkaloid. It is concluded that germination is a useful process to improve the nutritional value of lupines seeds. Khandelwal *et al.* (2010) reported that total phenolics and tannin content were reduced significantly in germinated green gram compared to Bengal gram, red gram and lentil. It is expected that total phenolics and tannin content changed differently in different legumes. Loss of total phenolics and tannin contents could be as high as 96% in germinated kidney bean as shown by Shimelis and Rakshit (2007). Germination process was shown to reduce the total phenolic, tannin and phytic acid contents. The reduction of antinutrients may improve the nutritional quality of legumes as shown by Megat and Azrina (2012). Moreover, it was shown that germination caused a decrease of alkaloids and RFOs content (Chilomer *et al*., 2010). Accordingly; the order of removal of the alkaloids follow roasting, boiling, and germination (Jimenez-Martinez *et al*., 2003). Lupine is toxic because of its alkaloid content, sparteine and lupanine in particular. In order to improve the nutritive value and digestibility, and to reduce antinutritional factors, methods such as soaking, dehulling, germination, fermentation, cooking, heat treatment and irradiation can be applied (Muzquiz *et al*. 2009).

Germination is a natural process occurred during growth period of seeds in which they meet the minimum condition for growth and development (Sangronis *et al.*, 2006). During this period, reserve materials are degraded, commonly used for respiration and synthesis of new cells prior to developing embryo (Vidal-Valverde *et al*., 2002). The process starts with the uptake of water (imbibitions) by the quiescent dry seed and terminates with the emergence of the embryonic axis, usually the radical (Bewley and Black, 1994).

Experiment soaking + cooking + soaking:-

From the result obtained and given in Table (7), non-treated lupine seeds contain the highest content of alkaloid, followed by treated lupine seeds. Total alkaloid% decreased in all treatments by compared with control treatment. Alkaloids are water soluble, but there is little information available on the effect of heating or cooking on the stability of lupine alkaloids. The levels of alkaloids in seeds can be reduced through a de-bittering process involving soaking or washing with water. This is commonly practiced in Europe where high alkaloid lupines,

so-called bitter lupines are grown (Australia New Zealand food authority, 2001).

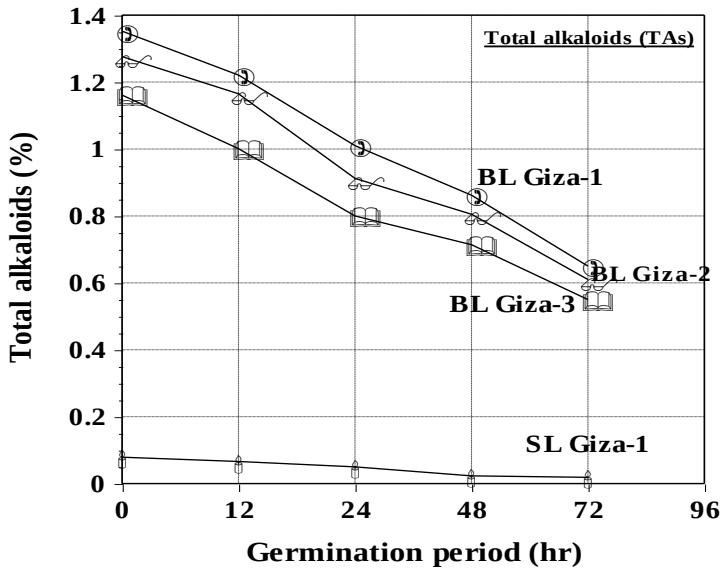

Fig. (7): Total alkaloids at different germination periods of lupine seeds.

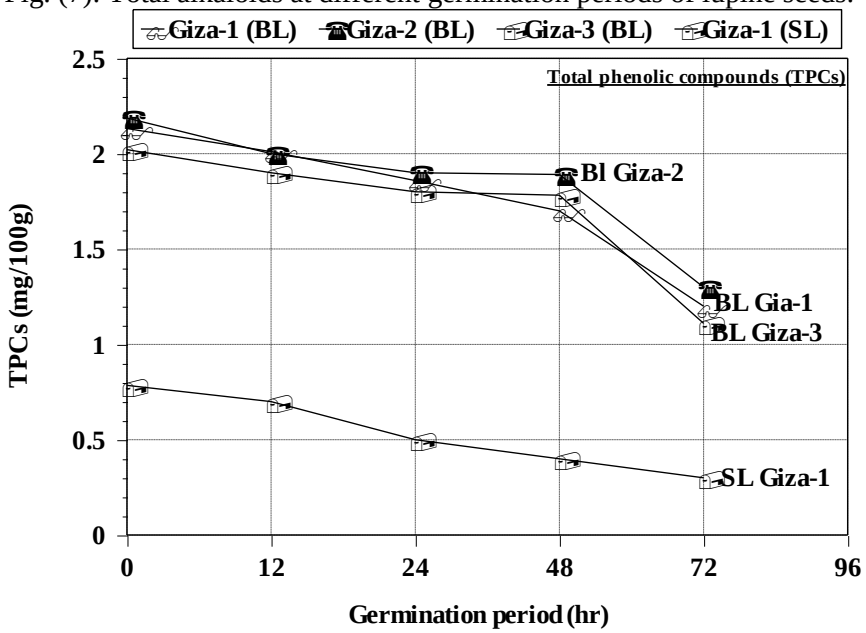

Fig. (8): Total phenolic content at different germination periods in lupine **seeds**.

Table (7): Effect of soaking and cooking and soaking on TA in lupine seeds.

Treatments	Total alkaloid in Cultivars			
	BL Giza-1	BL Giza-2	BL Giza-3	SL Giza-1
Control	1.377	1.239	1.141	0.075
Soaking for 24 h + cooking + soaking for 24h	0.615	0.532	0.492	0.012
Soaking for 48 h+ cooking + soaking for 24h	0.098	0.097	0.084	0.000
Soaking for 72 h+ cooking + soaking for 24h	0.000	0.000	0.000	0.000

Results given in Table (7) show that the treatment soaked for 24 h+ cooking + soaking for 24h was effective treatment in removal alkaloids from sweet lupine Giza-1. While the treatment soaked for 72h+ cooking + soaked for 24h was more efficient in de-bettering alkaloids from bitter lupines. The presence of bitter compounds in lupine seeds considers important limitation to the consumption of lupine. Similar results on white lupine (*Lupinus albus*) were found by Kurzbaum, *et al*., (2008).

It is concluded that germination is a useful process to improve the nutritional value of lupines seeds. Since alkaloids are water soluble, soaking in water can easily remove them from the whole seed. However; this depends on the type of soaking solution and permeability of the cell wall of the hull (Jimenez-Martinez, *et al*., 2003). In addition to genetic selection for low alkaloid containing lupine seeds, there are some physical and chemical treatments with acids and alkalis for eliminating these anti-nutritional factors. Arslan and Seker, (2002) reported that an elaborate cooking process is necessary to remove toxic alkaloids in the seeds.

Since soaking is usually done before dehulling and cooking grains, investigation of water absorption characteristics of different seeds during soaking has been considered by researchers. Grains in different soaking conditions, show different water absorption rates and water absorption capacities (Bello *et al*., 2010; Shittu *et al*., 2012; Montanuci *et al*., 2013 Shafaei and Masoumi, 2013).

CONCLUSIONS

It could be concluded that legumes especially lupine seeds are good sources of crude protein, carbohydrates, higher soluble dietary fiber and the consumption of these seeds has many health benefits such as antioxidant activities and antimutagenic effects of natural phenolic compounds concentrated in seed coats and cotyledons of beans. Further resolution of the genes controlling flavonoid and tannin formation, along with knowledge of the antioxidant activity of these compounds, may enable plant breeders to select bean varieties that have a range of antioxidant activities and also, perhaps, balance the positive effects of

antioxidant activity in diets with antinutritional effects.

RECOMMENDATION

The Egyptian plant breeders must targeting the saponin contents in field crops as tool in breeding programs to get resistant cultivars for both harmful insects and pathogens attacks.

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مستويات نواتج التمثيل الغذائي الثانوي و تأثير بعض المعاملات علي بذور بعض اصناف الترمس المحلية

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في هذه الدراسة تم استخلاص ستة مجموعات مختلفة من نواتج التمثيل الغذائي الثانوي من بذور الترمس و تم الاستخلاص باستخدام ستة مذيبات مختلفة و هذه المذيبات منها ما هو قطبي و غير قطبي . خضعت هذه المستخلصات المتحصل عليها من البذور للتحليل الوصفي. مستخلصات بذور الترمس تَحتوي علي الفلافونيدات flavonoids و الصابونين saponins و القلويدات alkaloids و التانينات tannins و التربينات terpens و الراتينجات resins. تم التقدير الكمي للقلويدات الكلية و التانينات و المركبات الفينولية الكلية في عينات البذرة.

أوضحت النتائج ان الماء المقطر أكثر المذيبات كفاءة في استخلاص كل نواتج التمثيل الغذائي الثانوي ولكن أثنان فقط من المذيبات قادر على استخلاص القلويدات الكلية هما الأثير البترولي و الماء المقطر. ثلاثة مذيبات لَيست قادرة على استخلاص الصابونين saponin . الأسيتون أكثر كفاءة في استخلاص كل نواتج التمثيل الغذائي الثانوي ماعدا القلويدات الكلية، و التربينات terpens و الراتينجات .

تم تقدير نسبة قصرة البذور الي البذور الكاملة في أربعة من اصناف الترمس و تراوحت النسبة من 18 إلى 25% . ان نسب قشور البذرة (هياكل) في نبات التّرمس المرة جيزة –1 (21–25 %) أعلى من تلك المقدرة في نبات التّرمس الحلوة .ايضا تم تقدير وزن الـ 100 بذرة في الأربعة الاصناف و كانت نباتات الترمس المر الأعلى جيزة –3 (37.134 جم) و كانت بذور نباتات الترمس الحلوة هي الأقل جيزة –1 (33.097جم) و بذور نباتات الترمس المر جيزة –2 (35.970 جم) و بذور الترمس المر جيزة –1 (33.115جم) .

تطرقت الدراسة إلي دراسة تأثير ثلاثة معاملات (الانبات والنقع والطبخ) علي مستويات مضادات التغذية (ANFs) في بذور الترمس وهى القلويدات الكلية (TAs) والمركبات الفينولية الكلية (TPCs) و قدرت في البذورِ المنبتة و الغيرِ المنبتة في بذور الترمس ، تحتوي البذور الغير المنبتة علي نسبة أعلى من تلك المقدرة في البذور المنبتة. هذه النتائجِ تبين بان الانبات أداة فعالة ورخيصةُ لتخفيض المرارة في بذورِ الترمس ، بينما النسبة المئوية للقلويدات الكلية (TAs) والمركبات الفينولية الكلية (TPCs) (مليجرام/100جرام) نقصت بشدة في كل المعاملات وكل الاصناف. أفضل طرق المعاملة التقليدية وهى النقع لـ (24، 48، 72 ساعة)، الطبخ (2–3 ساعة) و النقع (24 ساعة). انخفضت محتويات القلويدات الكلية (TAs) تحت طرق المعاملة المختلفة وكانت المعاملة الأكثر كفاءة في التخلص من القلويدات في بذور نبات الترمس الحلوة هي النقع لـ24 ساعة + الطبخ + النقع لـ 24 ساعة اما المعاملة الأكثر كفاءة في التخلص من القلويدات الكلية في نباتِ الترمس المر هي النقع لـ 72 ساعة + الطبخ + النقع لـ 24

ساعة. هذه المعاملات مفيدة لتحسين القيمة الغذائية للبذور الترمس و يودى استعمال هذه المعاملات إلى استخدام أوسع لقرن النبات.