STUDY ON THE PREPARATION OF DATE SEEDS FOR ANIMAL FEEDING

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ABSTRACT

Chemical constituent of date seeds, germinated date seeds and the methods of removing steroid contents were studied. The germination was done on different lengths of radical until the appearance of plumule, while the date seeds were treated by hexane and diethyl ether (ether) to remove the steroid compounds. The results revealed that steroid content of date seeds was decreased by germination (at the end of seeds germination) and decreased after treating with by hexane. Progesterone hormone was disappeared by germination until 8 millimeter length of radical and in the ungerminated seeds after treating by water. Osteriol hormone was decreased gradually by germination to 0.028 mg/100 gm, disappeared in the ungerminated seeds, decreased to 0.083 mg/100 g of hexane-treated seeds and decreased to 0.014 mg/100 g of ether-treated ones.

Introduction

Egypt is the second important countries in date world production which produced 710000 tons as reported by **FAO, (1997)**. The Egyptian dates represented about 17% of the total world production.

Date seeds represented 10-15% of date fruits. At present time there are 16 factories for date processing and some other will be build in the future **(GOI, 1999)**. All these factories have a mass production of date seed which comes as waste product during date processing. If these seeds were exploited well, it could play a good role in the national income.

Some works, have been done on date seeds, **Sumianah *et al.*, (1984)** who studied the effect of germination at 35-36ºC for 22 and 52 days on three cultivars (Razaz, Khalas and Beshi). They found that crude protein, fats, total carbohydrates and starch decreased by germination but crude fiber, ash, total soluble carbohydrate and reducing sugars increased

during germination. Also, the germination for 52 days was useful as a pretreatment of date seeds for animal feeding.

Buckaeve *et al*., (1976) found that Zahdi date seeds contained estradiole (0.857 mg/kg) and estrone (1.030 mg/kg) as well as other hormones such as progesterone and testosterone.

Barreveld (1993) mentioned that a growth stimulating hormones were found in date seeds such as estrone (1.9 mg/kg), the synthetically produced sisters of this female sex hormone have been used in chemical caponization of young cocks, however are more known for their growth promoting effect in animals. The use of these hormones in most countries is strictly regulated or totally forbidden for fear of the continuing effects of these hormone on human consumed animal products fed on these substances**.**

The remaining steroids hormones in different parts of chicken were studied by **Mohammad, (1987)** who found that the concentration of these hormones more than double the normal contents (feeding on diet free of hormones), the increase of these hormones may have an important role in uterus and breast cancers.

So, this study aimed to lowering and remove the steroids hormones contents of date seeds for feeding purposes.

MATERIALS AND METHODS

Date seeds germination:

Siwi date seeds were collected, washed, dried by air oven then wetted by water covered by a wet cloth and then left at ambient temperature for 40 days keeping the cloth in wet nature. The germinated seeds were collected and fractionated to six fractions depending on level of radical lengths as follow: appearance of radical length 0.1-0.4, 0.5-1.9, 2-4, 5-8, >8 centimeter and appearance of plumule.

The germinated seeds with the same characteristics were washed, dried by fan oven, then crushed and grinded to powder. Every fraction was placed in a jar and stored in deep freezer (-18ºC) until chemical analysis.

Date seeds treatment:

Powdered date seeds (100 gm)was treated by hexane or ether (250 ml) then filtered and dried for analysis.

Methods:

Moisture and crude protein were determined according to **A.O.A.C., (1990).**

Reducing sugars were extracted by ethanol 80% and determined by arsinomolybdates and Somogi Cupper reagent as described by **Somogi, (1952) and Nelson, (1974).**

Total free amino acids were determined by formal titration as recommended by **Kirk and Sawyar, (1991)**

Total free phenols were determined by using Folin-Denis reagent as described by **Swain and Hillis, (1959)**

Starch was measured as reported by **Ranganna, (1977).** Lignin was determined according to the method published by Tanaka et al. (1985).

Amino acids were fractionated by high performance amino acid analyzer Model Beckman System7300 and Data system 7000, column No/A/B/D 25 / cm length.

Steroids:

Oil fraction of date seeds was extracted by hexane (three times), sodium hydroxide (20%) was added for saponifaction. The unsaponifible matters were extracted by benzene (three time) and sodium sulfate anhydrous was used to remove any traces of moisture in benzene. Filtrated benzene was transferred to small bottle and removed the solvent by air dryer. The remaining compound until fractionation of steroids by HPLC **(Harborne, 1973).**

Fractionation of steroids by HPLC.

High pressure liquid chromatography was used (Hewel H Packed 1050). LC. equipped with a reversed phase column (C18) 12× and U.V detector adjusted at 254 nm. The solvent mixture used was methanol/water (63-27) with a flow rate of 1ml/min for the separation of different steroids.

RESULTS AND DISCUSSION

By evaluation of date seeds for feeding usage it was found that the chemical composition of dried and germinated date seeds were as follows:

The moisture content of date seeds after air drying ranged from 9.00 to 10.15%.

Reducing sugars:

Table (1) showed that reducing sugars of germinated seeds increased gradually until the appearance of plumule. This increase might be resulted from the effect of specific enzymes of special substances (amylase on starch and invertase on sucrose), these results are in agreement with the findings of **Sumianah *et al.*, (1984).** The increase in reducing sugars was pronounced in ingeminated seeds by respiration and there was no activation of polysaccharides hydrolysis, this might be related to the enzymatic growth inactivation of the ingeminated seeds.

Starch:

Dried date seeds contained high amount of starch, this component decreased gradually by germination until the appearance of plumule. The decrease of starch might be related to the activity of starch enzyme (amylase) which in turn led to the increase in reducing sugars (Table, 1). Previous results are obtained by similar to the results of **Sumianah *et al*., (1984).**

Free amino acids:

From Table (2) it could be noticed that free amino acids were increased by germination of date seeds until the appearance of plumule, this increase might be resulted from the hydrolysis of protein, the same results were found by **Sumianah *et al*., (1984).**

Protein:

The crude protein of dried Siwi date seeds was 7.37% (Table, 2), nearly similar to values of on Ruzeiz date seed (**Sawaya *et al.*, 1984**).

A decrease in protein contents during first stage of seeds germination (0.1-0.4 cm radical length) until the radical reached to 4 cm

length, was noticed then protein content increased until the appearance of plumule.

Total free phenols:

Total free phenols (Table, 2) were decreased clearly after appearance of radical (0.1-0.4 cm length) which might be related to consumption of simple phenols through the formation of other complicated and high molecular weight compounds, which have a good role in the new parts of seed growth during germination (radical and plumule).

Total free phenols showed a slight increase during growth of radical from 0.1 to >8 cm length and slight decrease was observed after appearance of plumule.

Lignin:

The data in Table (2), showed a gradually decrease in lignin contents by germination until radical length reached to 2-4 cm, then increased from radical length 5-8 cm until appearance of plumule.

The first decrease might be attributed to the hydrolysis of lignin at the beginning of germination, on the other hand lignin was increased until appearance of plumule resulted from the formation of new parts (radical and plumule) rich in lignin. The lignin in ingeminated date seeds was higher than dried date seeds, this increase might be related to a decrease of total solids such as (sugars, starch, protein, total free phenols) which led to increasing of lignin, parentage.

Anthocyanidin:

By measuring anthocyanidin compounds flavonoids, tannins are another compounds all are palyphenol (Barreveld, 1993), it was observed (Table, 2) that dried date seeds content of anthocyanidin was dropped from 1.19 to 0.57% by germination to radical length 0.1-0.4 cm, then component increased gradually until radical length >8 cm. The appearance of plumule led to exhausting more than half of anthocyanidin content.

Fractionation of amino acids:

By measuring the fractionated amino acids (Table, 3), it was found that threonine content of date seeds was more than human requirement, this amino acid decreased by germination to 3.08 g/100g protein of

radical length 5-8 cm. and after appearance of plumule decreased to 1.47 g/100g protein.

Valine increased by germination to be near the requirements, on the other hand cysteine and methionine together were more than the requirements during germination until radical length reached 8 cm, but after appearance of plumule these two amino acids decreased to 1.58 g/100g protein. Isoleucine content was more than half of the requirements in dried date seeds and during germination until appearance of plumule.

Leucine increased by germination and slight decrease was observed after appearance of plumule. The leucine, (tyrosine and phenylalanine) and lysine contents in dried and germinated date seed represented more than 70, 66 and 70% of requirements (FAO/WHO, 1973).

Generally, glutamic, aspartic and arginine the non essential amino acids represented the maximal percentage of total protein content in all samples of seeds (Table, 3).

Steroids:

Steroids specially the hormones represented one of the most problem for beneficial uses of the date seeds specially in nutrition purposes (Mohammad, 1987).

In this study fractionation of steroids content of dried and germinated date seeds samples was done by HPLC and the results are shown in Table (4).

Steroids as progestrone of every germinated phase, of dried seeds decreased gradually by germination until appearance of plumule, treated grind dried date seeds by hexane or ether led to decrease the steroids in addition to steroid decrease in ingeminated date seeds.

The status of individual hormones were as follows:

Ostriol hormone decreased from 0.24 mg/100g of dried date seeds to 0.149, 0.09, 0.07, 0.059, 0.055 and 0.028 mg/100g of germinated date seeds at radical length 0.1-0.4, 0.5-1.9, 2-4, 5-8 and >8 centimeter and appearance of plumule; respectively; On the other hand it decreased to 0.083 and 0.044 mg/100g of treated seeds by hexane or and by ether respectively, but disappeared from ingeminated date seeds.

Progesterone hormone decreased by germination from 0.505 mg/100g to 0.182, 0.132, 0.054 and 00 mg/100g of germinated date seeds at 0.1-0.4, 0.5-1.9, 2-4, 5-8 cm and the other germinated stages; respectively.

Estrone 3-methyl ether hormone decreased from 0.814 mg/100g of dried date seeds to 0.70, 0.63, 0.18, 00, 00 and 0.063 mg/100g of germinated date seeds with radical length 0.1-0.4, 0.5-1.9, 2-4, 5-8, >8 cm and appearance of plumule; respectively. Moreover this hormone was disappeared in ingeminated date seeds (Table, 4), but increased from 0.814 mg/100g of dried date seeds to 0.906 and 1.25 mg/100gm of treated date seeds by hexane and by ether. No, estrone found in Siwi date seed.

So, it is very clear that reducing sugars and free amino acids increased by germination until appearance of plumule but starch, total free phenols and anthocyanidin were decreased at first stage of germination then showed an increase.

The total steroids were decreased by germination and by solvent (hexane and ether) treatments. Ostriol was decreased by germination and by solvent treatments. Progesterone was reduced to zero by germination, but decreased to 00 and 0.047 mg/100gm after treating by hexane and ether; respectively. Estrone 3-methyl ether was decreased from 0.814 mg/100gm of dried date seeds until 0.00 of radical length 5-8 and >8 cm but after appearance of plumule, hormone reached to 0.063, while treating by solvents led to increase this hormone.

Table (1): Total solids, reducing sugars, starch and lignin of different germinated stages of date seeds *.

stages	Total Solids %	Reducing sugars %	Starch %	Lignin %
Dried date seeds	90.62	5.46	17.98	7.20
Appearance of radical length 0.1-0.4 cm	91.00	5.60	17.84	6.45
Radical length 0.5-1.9 cm	89.85	5.75	17.05	5.20
Radical length 2-4 cm	90.60	6.05	16.61	4.80
Radical length 5-8 cm	90.15	6.32	15.08	4.80
Radical length >8 cm	90.18	6.32	14.35	5.60
Appearance of plumule	90.65	6.99	10.98	8.20
Ingeminated seeds	90.00	4.28	14.56	7.40

* All values were calculated on dry weight basis.

Table (2): Total free phenols, free amino acids, protein and anthocyandin of different germinated stages of date seeds *anthocyanidin.

stages	Total free phenols %	Free amino acids %	Protein %	Anthoc-yandin %
Dried date seeds	3.66	0.79	7.37	1.19
Appearance of radical length (0.1-0.4 cm)	2.55	1.01	7.14	0.57
Radical length 0.5-1.9 cm	2.28	1.04	7.00	0.60
Radical length 2-4 cm	2.42	1.05	6.65	0.64
Radical length 5-8 cm	2.70	1.06	6.95	0.79
Radical length >8 cm	2.99	1.09	7.00	1.06
Appearance of plumule	2.91	1.49	7.30	0.50
Ingeminated seeds	3.37	1.03	7.03	1.13

* All values were calculated on dry weight basis.

Table (3): Amino acid composition (g\100 g) of dried and germinated date seed protein.

Amino acid	Fresh dried seeds	Radical length 0.5-1.9 cm	Radical length 5-8 cm	Appear-ance of plumule	FAO/WHO 1973
Essential amino acids					
Lysine	4.55	4.87	5.09	4.87	5.5
Threonine	5.16	2.99	3.09	1.47	4.0
Valine	3.64	4.65	4.73	4.08	5.0
Methionine	2.57	1.77	1.77	0.56	3.5
Cysteine	3.03	2.54	2.13	1.02	3.5
Isoleucine	2.73	2.77	2.96	2.94	4.0
Leucine	5.61	5.87	6.16	6.12	7.0
Phenylalanine	3.64	5.76	3.90	3.85	6.0
Tyrosine	0.60	0.55	0.59	0.56	6.0
Tryptophan	--	--	--	--	--
Non essential amino acids					
Asparatic acid	10.78	12.19	13.50	8.27	--
Serine	5.61	3.54	3.31	1.81	--
Glutamic acid	25.79	26.49	32.70	29.25	--
Proline	--	--	--	--	--
Glysine	5.16	4.76	5.09	4.98	--
Alanine	4.24	4.54	4.97	5.10	--
Histidine	1.94	1.99	2.13	2.04	--
Arginine	10.77	16.60	7.81	23.01	--

Table (4): Steroids and other unknown compounds (mg/100g) of different levels of date seeds HPLC analyse.

Steroids retention time	**1.4 ***	**1.826 ***	**2.13 ***	**2.444 ostriol**	**2.601 progesterone**	**2.732 ***	**3.19 ***	**3.514 estron-3-methyl ether**	**3.95 ***	**4.780 ***	**4.91 ***	**5.280 ***	**5.43 ***	**5.90 ***	**6.46 ***	**6.891 ***	**7.149 ***	**7.49 ***
Dried date seeds		0.307	000	0.24	0.505	0000	0.104	0.814	0.354	0.066	0.044	0.045	0.062	0000	0.114	0.444	0.178	0.240
Appearance of radical length 0.1-0.4 cm	0000 0000	0.175	.031	0.149	0.182	0000	0000	0.7	0000	0000	0.050	0000	0000	0000	0.070	0.200	0000	0000
Radical length 0.5-1.9 cm		0.161	.031	0.090	0.132	0000	0000	0. 63	0000	0000	0.055	0000	0000	0000	0.064	0.140	0000	0000
Radical length 2-4 cm	0000	0000	000	0.070	0.054	0.039	0000	0. 18	0000	0.094	0.062	0000	0000	0000	0.059	0000	0000	0000
Radical length 5-8 cm	0.036	0.045	000	0.059	0000	0.052	0000	0000	0000	0000	0.090	0000	0000	0000	0000	0000	0000	0000
Radical length >8 cm	0.031	0000	000	0.055	0000	0.036	0000	0000	0000	0000	0.067	0000	0000	0000	0000	0000	0000	0000
Appearance of plumule	0.028 0.022	0000	000	0.028	0000	0000	0000	0.063	0000	0000	0.057	0000	0000	0000	0000	0000	0000	0000
Ingeminated seed	0.028	0.026		0000	0000	0000	000	0000	0.032	0.04	0.074			0.324		0.42		
Seeds treated by hexane	0.024	0000	000	0.083	0000	0.025	.062	0.906	000	000	000	000	000	0000	000	000	000	000
Seeds treated by ether	0000	0.045	000 000	0.044	0.047	0000	000	1.25	000	000	000	000 000	000 000	0.047	000 000	000	000 000	000 000

*** Unknown compounds**

All compounds measured as progesterone .

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دراسة علي إعداد نوى التمر للاستخدامات الغذائية

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الملخص

نظرا لما تتميز به مصر من الإنتاج المرتفع من التمور وكذلك تكدس مصانع تعبئـة وتصنيع التمور بالنوى الناتج منها لذلك تم دراسة التركيب الكيماوي لنـوى التمـر وكيفيـة التخلص من الهرمونات والمواد الاستيرويدية حتى يمكن استخدامها بصورة غذائيـة أفـضل. وبالدراسة الكيميائية لمحتوى النوى في مراحل إنبات البذور فقد وجد الآتي:

ولقد حدث انخفاض في محتوى النوى من المواد الإستيرويدية بالإنبات واستمر هـذا الانخفاض حتى الوصول إلى مرحلة ما قبل ظهور الريشة مباشـرة أيـضا عنـد المعاملـة بالهكسان حدث انخفاض في هذه المواد ، كذلك وجد أن هرمون البروجستيرون قد تم التخلص منه كليةً عند الإنبات ووصول الجذير إلى طول 8 سم وكذلك العينات المعرضة للمـاء ولـم تنبت، أما هرمون الاستريول فقد انخفض تدريجيا في العينات المنبتة حتـى مرحلـة ظهـور الريشة حيث وصل إلى 0.028 ملجم /100 جم بينما انخفض تركيزه إلى الصفر في العينات التي لم تنبت كذلك انخفض إلى 0.083 ملجم /100 جم في العينـات المعاملـة بالهكـسان و 0.044 ملجم/100 جم عند المعاملة بالأثير.