EXTRACTION OF NATURAL SWEETENER FROM LICORICE ROOTS

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ABSTRACT

Glycyrrhizin is a natural sweetener extracted from licorice roots. It is considered natural flavoring compound, as well as non-caloric sweetener, for this reason it can be used for dietetic and diabetic foods. Chemical composition and glycyrrhizic acid content of licorice roots were studied in this investigation. Results indicated that licorice roots contained high amounts of protein (5.27%), ash (6.3%) and fiber (20.3 %). On the other hand they contained little amounts of reducing, non-reducing and total sugars, which were 1.02, 0.05 and 1.07 %, respectively.

Extraction of glycyrrhizin was studied in this investigation using three treatments, i. e.: -

1- Extraction by citric acid using 0.5, 1.0,1.5 and 2% concentrations at room temperature (25 °C ±1) and 70 °C for 1.5,2 and 2.5 hr .
2- Extraction by ammonia using 20,40,60 and 80% concentration at room temperature for 20,30,40,50 and 60 min.
3- Extraction by water at room temperature (25 °C ±1), 60 °C and boiling point for 0.5, 1.5, 2 and 2.5 hr.

Results indicated that the highest amount of glycyrrhizin (10.5%) was extracted by hot water at 60 °C for two hours. While the best treatment to obtain the glycyrrhizin in light color was the extraction by citric acid 1.5 % at room temperature after two hours, it was 9.9% . After crystallization and purification by 95% alcohol, charcool and chlorine solutions were used to obtain more light color of glycyrrhizin .

Solubility of glycyrrhizin was also studied at pH range from 1 to 10 and temperature degrees from 10 to 100 °C. Results indicated that the optimum pH for maximum solubility of glycyrrhizin is 4 , and the optimum temperature for maximum solubility is 60 °C to 70 °C .

KEY WORDS: -
Licorice roots, Natural sweeteners and Glycyrrhizin

INTRODUCTION

Licorice, well known for centuries and widely used, is obtained from the roots of *Glycyrrhiza glabra L.*, a small shrub, grown and hand harvested in Middle Egypt at El. Tahrir Province and El- Wadi El-Gideed and small areas are cultivated in El-Fayoum .

Licorice leaves are used as a fodder especially in desert zones. Roots of the plants are utilized in the preparation of the summer drink consumed by common people who think much for its medicinal virtues. The main water soluble constituent of licorice is glycyrrhizin(**El-shaarkawy , 1967**)

Non calorie sweetener consumption has increased from 2.9 Ib/person in 1950 to 7.1 Ib/person in 1978 .Consumption,as measured by the USDA for 1984 , was recorded as 15.8 Ib/person **(Iecos,1985)** .

The Calorie Control Council (1985) reports that in a recent consumer survey, more than 68 million Americans, age 18 and over, use low or free – calorie foods and beverages.

Glycyrrhizin is a non-nutritive sweetener or non-calorie component **(Beck, 1980, Abou-Zaid 2000 and Zena 2000)**. It is nontoxic to human and also it is generally recognized as safe (GRAS) within the Food and Drug Administration (FDA) list of natural flavoring compounds **(Pomeranz, 1986)**.

Glycyrrhizin, known for its flavor –enhancing and sweetening attributes, is a triterpene glycoside extracted from the licorice roots. Consisting of the potassium, calcium, and magnesium salts of Glycyrrhizic acid (**Dziezak , 1986**) .

The present work was undertaken with the following objectives:

1- To study the chemical composition and glycyrrhizic acid content of licorice roots.

2-To extract the glycyrrhizin from licorice roots by three treaments i.e. by citric acid, ammonia and water in different times and temperatures.

3- To choose the best treatment for extraction of glycyrrhizin in high quantity and white crystals as possible .

4- To study the effect of pH and heat on the solubility of glycyrrhizin .

MATERIALS AND METHODS

1- Materials :-

Licorice (*Glycyrrhiza glabra L.*, Fam . *Leguminosae*) .

The roots of licorice were obtained from the local markets at Alexandria Governerate .

2- Methods :

A- Analytical methods :-

- Gross chemical composition i.e.moisture, protein, fat, ash, fiber, sugars (reducing, non reducing and total sugars) and acidity (as citric acid) were determined as recommended by **A.O.A.C. (1990).**
- Total soluble solids % were determined by refractometer HSR.500.
- Solubility was determined as recommended by **Dziezak 1986**
- Glycyrrhizin was determined as described by **El-Shaarkawy (1967) .**

B- Technological methods :-

The licorice roots were dried to 10 % moisture, then shredded into ¼ to ½ in. lengths. Those were subjected to extraction as follows: -

1- Citric acid extraction :

Hundred gram samples of licorice roots were extracted with 600 ml solution of 0.5, 1.0, 1.5 and 2 % citric acid. These concentrations of citric acid were utilized in the extraction at two temperatures i.e. room temperature (25 °C ±1) and 70 °C for 1.5, 2 and 2.5 hours.

2- Ammonia extraction :-

Hundred grams of licorice roots were extracted with 500 ml solution of ammonia 20, 40, 60 and 80% for 20, 30, 40, 50 and 60 min. at room temperature (25 °C ±1).

3- Water extraction :-

Hundred gram samples were extracted in 200ml distilled water. The extraction was carried out for 0.5, 1.0, 1.5, 2.0 and 2.5 hrs. at room temperature , 60 °C and boiling point (100 °C) in covered glass container.

The obtained extractions were concentrated with vacuum, precipitated with concentrated sulfuric acid and crystallized with 95% alcohol. Crystallization was repeated twice .

Charcool was used and glycyrrhizin was chlorinated by 1 % chlorine solution to remove the dark color .

C. Sensory methods :-

The purified glycyrrhizin was subjected to the sensory evaluation as described by **king , *et al* , (1995)** , Overall acceptability includes color, taste

, order and texture which were assessed by ten panelists working in Food Technology laboratory in Sabahia Horticultural Research station , Alexandria . A score from 100 to 40 was applied for this evaluation where from 40 to less than 50 is poor, from 50 to less than 70 is fair , from 70 to less than 80 is good and from 80 to 100 is excellent .

RESULTS AND DISCUSSION

A- Chemical composition

The chemical analysis of licorice roots is shown in Table (1) In light of the obtained results it is obvious that licorice roots were found to contain high amounts of protein (5.27%) ,ash (6.3%) and fiber (20.31%). On the other hand it contains little amounts of reducing, non-reducing and total sugars, which were 1.02 , 0.05 and 1.07 % , respectivly , such results mean that licorice is considered dietetic and diabetic food . These results are in agreement with those reported by **Abou-Zaid (2000)** .

Table (1) Chemical composition of licorice roots (for 100 g on dry weight basis)

Chemical components %	Licorice roots
Moisture	12.30
Protein	5 27
Fat	1.62
Ash	6.30
Fiber	20.31
Reducing sugars	1.02
Non-reducing sugars	0.05
Total sugars	1.07
Acidity (as citric acid)	1.40
Glycyrrhizic acid	10.50
Total soluble solids	28.30

The glycyrrhizic acid content of 10.5 % found in this present work is relatively high in comparison with the 8.47% found by **El-Shaarkawy (1967).** While such result is in good agreement with that reported by **Zena (2000),** who mentioned that glycyrrhizic acid accounts for 6 - 14 % of licorice roots on dry weight

B- Extraction: -

1- Citric acid extraction :-

As noted in Table (2) the glycyrrhizic acid of licorice citric acid extraction is obtained in high amount (9.9 %) in the treatment of 1.5 % citric acid at room temperature after 2 hr. The results show a decrease in glycyrrhizic acid content when 70 °C was used for the extraction, this could be due to the evaporation of citric acid as a result of high temperature used in the extraction (**Gala *et al*. 1983**) .

Table (2) Glycyrrhizic acid content of licorice as extracted by citric acid at different concentrations, times and temperatures (for 100 g licorice roots on dry weight basis)

Temperature	Room temperature (25 °C ±1)			70 °C		
Time (hr.) / Citric acid (con.)	1.5	2	2.5	1.5	2	2.5
0.5 %	6.2	6.9	6.7	5.8	5.6	4.2
1.0 %	8.0	8.5	8.2	6.2	6.0	4.5
1.5 %	8.1	9.9	9.8	6.5	6.2	5.0
2.5 %	7.0	7.1	7.0	6.9	6.5	5.3

Results also indicate that the smallest content of glycyrrhizic acid was observed when the extraction was carried out at 70 °C at low concentration of citric acid .

2- Ammonia extraction :-

Results in Table (3) show the glycyrrhizic acid content in the ammonia extracted licorice. Data indicate that 50 min. and 60 % ammonia were sufficient to reach the maximum glycyrrhizic acid in the ammonia extract, which was 8.1 % .

The results in Table 2 and 3 also show that there was a slight decrease in the Glycyrrhizic acid precipitated in the licorice ammonia extract comparing with its content in the licorice citric acid extract. Our results are in fair agreement with those reported by **Dziezak , (1986)** who found that glycyrrhizic account for 6-14 % of licorice roots dry weigh after extraction with aqueous ammonia .

Table (3) Glycyrrhizic acid content of licorice extracted by ammonia at different concentrations and times at room temperature(25 °C±1). (for100g licorice roots on dry weight basis)

Times (min.) / Concentration (%)	20	30	40	50	60
20	5.2	6.1	6.2	6.3	6.8
40	5.4	6.0	6.3	6.8	6.8
60	6.6	6.3	7.2	8.1	8.0
80	6.3	6.4	7.1	8.0	8.0

3- Water extraction: -

Data presented in Table (4) reveal that the best treatment for obtaining high amount of glycyrrhizic acid, is the extraction by water at 60 °C for two hours which yielded 10.5 %..

Table (4) Glycyrrhizic acid content of licorice extracted by water at different times and temperatures (for 100 g licorice roots on dry weight basis)

Temperature (°C) / Times (hr.)	Room temperature (25 °C ±1)	Hot water (60 °C)	Boiling point (100 °C)
0.5	1.2	6.0	7.5
1.0	2.5	7.5	5.1
1.5	2.3	8.2	4.7
2.0	6.4	10.5	2.3
2.5	6.8	10.2	1.0

Results in the same Table (4) also indicate that the amounts of glycyrrhizic acid extracted at 60 °C was much higher than those obtained at the boiling point . On the other hand results obtained by **El-shaarkawy (1967)** showed that extraction at 60 °C yielded an extract with soluble solids content slightly higher than at the boiling temperature .

Data in the same Table (4) also reveal that boiling for 30 min. resulted in an extract higher in its glycyrrhizic acid content than that obtained by boiling for 2.5 hr. this may be due to the destruction of glycyrrhizin by boiling. For the same reason it is observed that the extraction of glycyrrhizin by hot water at 60 °C is preferable than the extraction by boiling water. The results in Table (4) also indicate that the extraction by water at room temperature yielded small amounts of glycyrrhizic acid in the extract comparing with that obtained by the extraction at 60 °C or at boiling point. Our results are in agreement with those obtained by **Mac-Andrews and Forbes co. (1976)** who found that the glycyrrhizin has poor solubility in water at room temperature .

It could be concluded that extraction by water at 60 °C for two hours, was the best tested extraction treatment for obtaining the highest amount of glycyrrhizic acid (see Tables 2,3 and 4)

C- Sensory evaluation

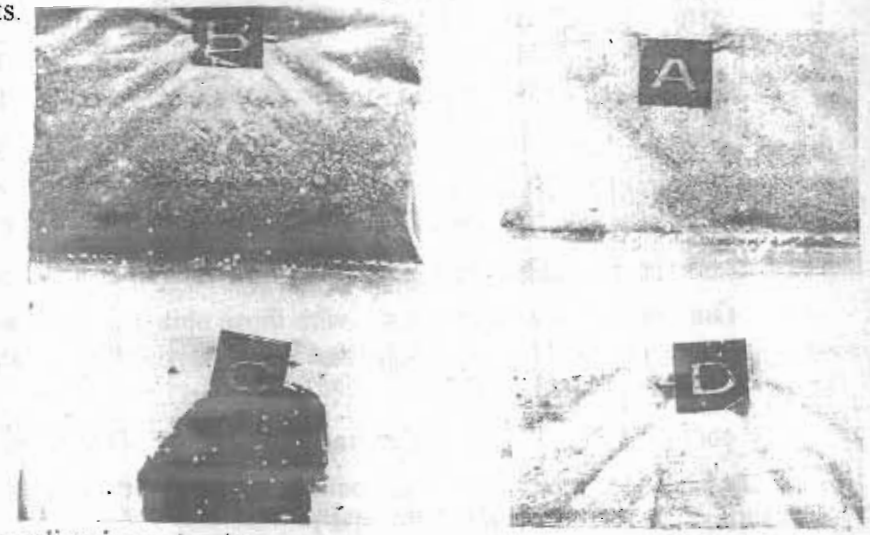
Results presented in Table(5)and Fig(1) show the sensory evaluation of the glycyrrhizin extracted by three treatments i.e. citric acid, ammonia and water.

Table(5) Sensory evaluation of glycyrrhizic acid extracted by different treatments

Extracts / Parameters	1.5 % citric acid for 2 hr. at room temp.	60 % ammonia for 50 min. at room temp.	Water for 2 hr. at 60 °C
Color (25)	22	10	10
Taste (25)	20	10	11
Oder (25)	20	5	20
Texture (25)	18	16	10
Overall acceptability100	80 (Excellent)	41 (poor)	51 (Fair)

Among these treatments citric acid extract resulted in the best color of glycyrrhizin. It is lighter than the color obtained when water or ammonia were used for extracting. This is due to the ability of citric acid to convert the dark soluble glycyrrhizin to the light insoluble glycyrrhizic acid.

Fig (1): sensory evaluation of the glycyrrhizic acid from the three licorice extracts.

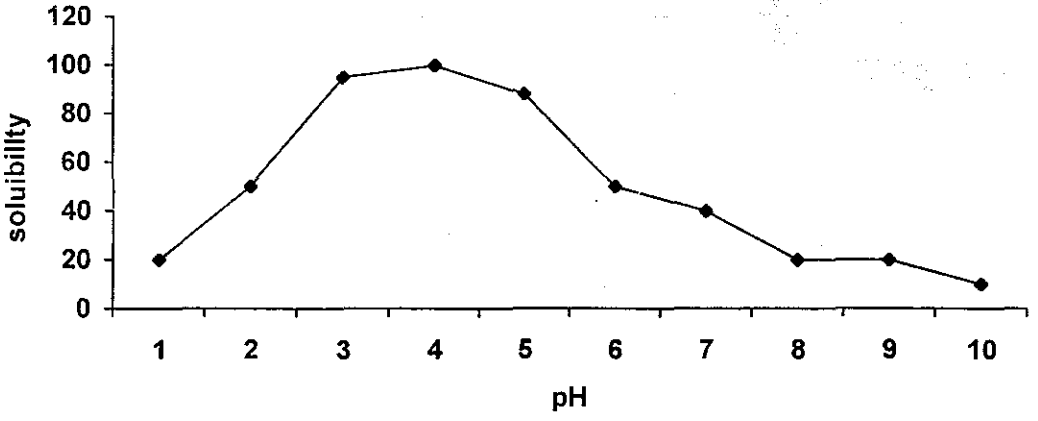


The three licorice extracts:
A: Citric acid extract
B: Ammonia extracts
C: Water extract
D: After whitening citric acid extract

Results in Table (5) also show that ammonia extraction led to the highest adverse effect on overall acceptability. Fig (1) show that , glycyrrhizin extracted by ammonia had a dark color , lingering licorice – like after taste , making it used in some special foods only to enhance the licorice flavor and it had ammonia oder , which results ammoniated glycyrrhizin contains more than one mole of ammonia **(Zena , 2000).** On the other hand it had a good powdery texture . The second acceptable extraction by water produced glycyrrhizic acid with fair rank of overall acceptability. It had dark color, licorice – like after taste and pasty texture. Results of citric acid extract from the same Fig (1) indicate that it produced glycyrrhizic acid with lighter color because of the action of citric acid as mentioned before. It had good powdery texture, good taste and in the same time there was no unfavorable effect on the flavor of glycyrrhizic acid.

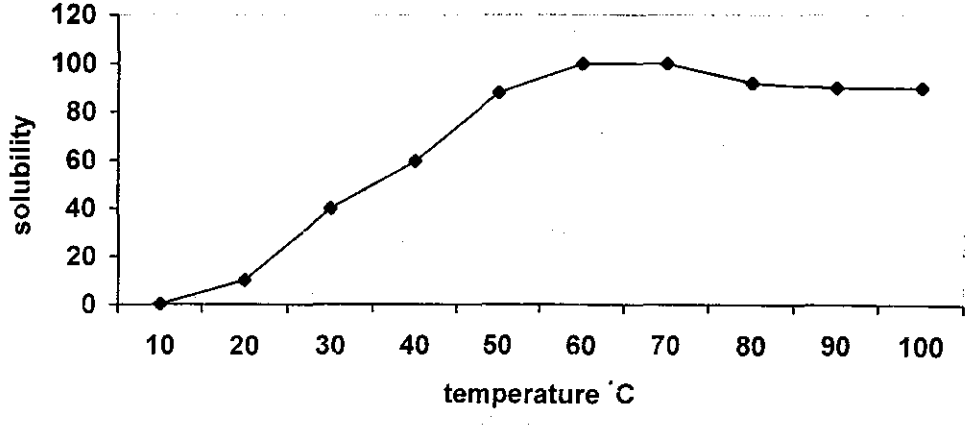
D- The solubility of glycyrrhizic acid: -

Fig (2) The effect of pH on the solubility of glycyrrhizic acid:



Results shown in fig (2) indicate that the solubility of glycyrrhizic acid is best within the range of 3-5. While the maximum solubility of the glycyrrhizic acid is attained at pH 4, it means that glycyrrhizic acid can be used as a sweetener in high – acid preparations.

Fig (3) The effect of temperature on the solubility of glycyrrhizic acid .



From Fig (3) it is clear that glycyrrhizic acid has poor solubility in cold water or at room temperature, it can not be used as sweetener for cold drink While it readily dissolves in hot or boiling water with maximum solubility at 60 to 70 C. It can be therefore applied in squash processing and can be also used in jam processing providing that its addition will be in the last 20 min. to avoid the damage of licorice extract if heated for too long time .

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J. Agric. Res. Tanta Univ., 28(4) 2002

الملخص العربى

" استخلاص مُحلّى طبيعى من جذور نبات العرقسوس "

فاطمة الزهراء على عبد العال
قسم تصنيع الحاصلات البستانية – معهد بحوث تكنولوجيا الأغذية – مركز البحوث الزراعية

مادة الجلسرين هى محلى طبيعى مستخلص من جذور نبات العرقسوس و تعتبر من مركبات النكهة الطبيعية كما أنها من المواد المحلية الطبيعية التى ليس لها سعرات حرارية لذلك تستعمل فى الأغذية منخفضة السعرات لراغبى الرجيم أو الأغذية الخاصة بمرضى السكر .

فى هذه الدراسة تم دراسة التركيب الكيماوى لجذور بنات العرقسوس و محتواها من مادة الجلسريزين و كانت النتائج المتحصل عليها تشير إلى أن جذور العرقسوس تحتوى على كميات كبيرة من البروتين (٥,٢٧%) و الرماد (٦,٣ %) و الألياف (٢٠,٣%) و من جهة أخرى وجد أنها تحتوى على نسبة بسيطة من السكريات المختزلة و الغير مختزلة و كذلك السكريات الكلية حيث كانت نسبتها على التوالى ١,٠٢ ، ٠,٠٥ ، ١,٠٧ % كما تم أيضا استخلاص مادة الجلسرين بواسطة ثلاث معاملات ألا و هى :-

١- الاستخلاص بواسطة حمض الستريك بتركيز ٠,٥ ، ١ ، ١,٥ ، ٢ % على درجة حرارة الغرفة و درجة ٧٠م لمدة ١,٥ ، ٢ ، ٢,٥ ساعة .

٢- الاستخلاص بواسطة الأمونيا بتركيز ٢٠ ، ٤٠ ، ٦٠ ، ٨٠ % على درجة حرارة الغرفة لمدة ٢٠ ، ٣٠ ، ٤٠ ، ٥٠ ، ٦٠ دقيقة .

٣- الاستخلاص بواسطة الماء على درجة حرارة الغرفة و درجة حرارة ٦٠° م و درجة الغليان لمدة ٠,٥ ، ١ ، ٢ ، ٢,٥ ساعة .

كانت النتائج المتحصل عليها من هذه الدراسة أن افضل استخلاص للحصول على أعلى كمية من الجلسريزين (١٠,٥%) هو الاستخلاص بواسطة الماء الساخن على درجة ٦٠ ° م لمدة ٢ ساعة بينما كان أفضل معاملة للحصول على الجلسريزين بلون أفتح هو الاستخلاص بواسطة حمض الستريك ١,٥ % على درجة حرارة الغرفة بعد ساعتين و كانت نسبته ٩,٩% بعد إجراء البلورة و التنقية بواسطة الكحول ٩٥% ، استخدم الفحم النشط و محلول الكلور للحصول على لون أفتح لمادة الجلسريزين .

فى هذا البحث تم أيضا دراسة درجة ذوبان الجلسريزين و تأثرها بدرجة pH لذلك استخدم درجات pH من ١ إلى ١٠ و أيضا درجة الحرارة حيث استخدم درجات حرارة من ١٠ إلى ١٠٠ ° م و كانت النتائج تشير إلى أن درجة pH المثالية لذوبان لمادة الجلسريزين هو ٤ بينما كانت درجة الحرارة المثالية لأقصى ذوبان لمادة الجلسريزين هى ٦٠° إلى ٧٠ ° م أى أن الجلسريزين يصلح لتحلية المشروبات الحامضية الخفيفة التى تقدم ساخنة و المربات بشرط إضافته قبل نهاية التصنيع بعشرين دقيقة تلافيا لتلف مادة الجلسريزين لطول مدة التعرض الحرارة العالية .