

ISOLATION AND IDENTIFICATION OF GUAR SEED  
(CYAMPOSIS TETRAGONOLOBA) GUM

BY

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ABSTRACT

Guar polysaccharide was extracted from its grounded seeds by using hot water, alkali and acetic acid treatments. The highest yield of this polymer (11.55%) was obtained with hot water and its  $(\alpha)_D^{25}$  in water was  $(+74^\circ)$  while cupperammonium complex reached  $(-224^\circ)$ .

Silyl derivatives on G.L.C. technique showed that the monomer residues were identified as D (+) galactopyranose and D (+) mannopyranose with molar ratio 1:2, respectively.

I.R., spectrum of the polysaccharide indicated that the linkages between the units were of B-type.

The average molecular weight of guar gum was determined physically and chemically the first value was  $22 \times 10^3$ , while the second was  $166 \times 10^3$ .

Periodate oxidation technique indicated that the guar polysaccharide has (1 ---> 4) glycosidic linkages and a branched structure with Ca 16.7% of the units in terminal positions.

The proposed structure of guar polysaccharide might consist of 171 repeating segments, each segment composed of a main chain of four manno-opyranose units jointed by (1 ---> 4) glycosidic linkages. Each segment is also jointed with two units of galactopyranose through B (1 ---> 6) glycosidic bonds.

The technological and jelling examination of guar gum proved that it has high jelling quality and a good capacity (stability) for preservation.

## INTRODUCTION

Guar belongs to leguminosae family (Gyamopsis tetragonoloba L. tab.), known as (Cyamopsis psaralioides Dc.). It was originally grown in India and recently in other many countries (Khater, 1977). Sandford and Baird (1983), reported that the total world production of guar gum reached to 90,000 tonnes (1980) since the gum has a wide range of functional characteristics exhibited by its polysaccharide. Khater (1977), stated that the guar polysaccharide has 5-8 times the thickening power of starch and it is used as a filter aid in the mining industry, textile industry, pharmaceuticals as a thickener in cosmetics and as a strengthening agent in paper industry. Also, Ibrahim et al. (1983), found that guar gum can be used as a pectin substitute in jam making and preservation.

In Egypt, nowadays several attempts for planting guar were successfully carried out, its yield amounted to 1.2 tonne per feddan (Khater, 1977)

Rees (1972), reported that galactomannans occur as reserve polysaccharide in seeds of many leguminous plants and guar powdered seeds contain large amounts of these galactomannans.

Smith and Montgomery (1959), McCleary et al. (1976), and Dey (1978), have confirmed that galactomannans are composed of mannopyranose residues. These residues are joined through B- (1 ---> 4) glycosidic linkages. On the other hand, the galactopyranose units were present as branches and linked through (1 ---> 6) glycosidic bonds.

McCleary (1979, 1983) concluded that D-gal-p-groups in locust bean, guar and other galactomannans of similar galactose content could be randomly distributed.

Tewari et al. (1984), have extracted a galactomannan polysaccharide from Cassia carymbosa (Leguminosae) seeds with cold, acidulated water and purified it to give a water soluble product having  $(\alpha)_D^{25} + 72$ , ash content 0.2% and containing D-galactose and mannose in molar ratio 4: 7. Acid catalyzed fragmentation, periodate oxidation and methylation showed that the seed gum has a branched structure consisting of a linear chain of B- (1 ---> 4) linked manno-pyranosyl units, some of which are substituted at 0-6 by two -D-(1 ---> 6) galactopyranosyl units mutually linked glycosidically.

The aim of the present investigation is to study the polysaccharide content of guar gum in a trial to elucidate the skeletal of this polymer accompanied with some technological examination of this substance for better use in industrial aspects.

#### MATERIALS AND METHODS

The seeds of guar gum were supplied by Agricultural Research Centre at Giza.

Guar polysaccharide was isolated by three different methods, i.e. hot water; Manzi *et al.* (1984), aqueous alkali, Metry (1977) and acetic acid (1.5%) as recommended by Tewari *et al.*, (1984). The highest yield of guar polysaccharide was obtained with hot water (11.55%) on dry weight basis while extraction with alkali and acid gave lower values, 9.77% and 7.11%, respectively.

Purification of the obtained polysaccharide was accomplished via the formation of its cupperammonium complex as mentioned by Saad (1980). This complex gave  $(\alpha)_D^{25} -224$  (0.01% in water) for different samples. The polysaccharide was recovered from the cupperammonium complex with N. Hcl. The product gave  $(\alpha)_D^{25} + 74$  (0.01% W/W in water).

The following tests were applied on the obtained polysaccharide according to A.O.A.C. (1980). Molish test (+), Fehling's test (-), Benedict's test (-), Furfural test (-), Iodine test (-) and Lassaigne's test (Nitrogen (-), sulphur (-), phosphorous (-) and Halogens (-)).

After acid hydrolysis, the hydrolyzate product gave positive result with reducing tests.

Moisture and ash contents were determined according to the method of A.O.A.C. (1980), while estimation of cations were carried out as reported by Saad (1985), by using a Pye Unicam atomic absorption spectrophotometer (Sp 1900).

The hydrolysis of the polysaccharide was accomplished as described by Saad (1980) and the ions were removed from the hydrolyzate using ion exchange chromatography as reported by Guindi *et al.* (1977), cation resin: IR- 120; anion resin: Dowex IX- 400. Sugars identification of the hydrolyzate were achieved using the method reported by Farag *et al.* (1985), using G.L.C. (model sp 2100) and silylation of the soluble sugars. The G.L.C. was set up under the conditions of flow rates of the nitrogen, hydrogen and air 25,

30 and 300 ml/min, the chart speed was one cm/min; temperatures for injector, column and detector were 220, 190 and 220 respectively.

Peak identification was performed by comparison of the relative retention time for each peak with those of standard chromatogram.

The relation of the individual sugars were therefore, obtained by determination the partial areas relative to the total area.

Periodate oxidation was carried out according to Whistler (1965) and applied by Attia (1983), a blank experiment was run at the same time under the same conditions. The amount of periodate consumed by each mole of polysaccharide was calculated in moles per gram corresponding to each mole of anhydroglucose units.

Estimation of the liberated formaldehyde was carried out using the method mentioned by Metry (1977).

The I.R. spectrum of guar gum was recorded on pressed disc of potassium bromide (100-150 mg) using Pye Unicam (Sp 1000) spectro-photometer.

The average molecular weight was determined as described by Christensen (1954) and modified by Sarhan (1975).

Determination of technological and jelling properties of guar gum was carried out according to the method described by Kertesz (1951). The prepared jellies were compared with jelly of a standard pectin sample which was supplied from the United Kingdom (Bulmer Limited company). Gel strength of guar gum was also evaluated comparing with Egyptian Standards as mentioned by Wasef (1979).

## RESULTS AND DISCUSSIONS

### Analysis of Seeds:

Different constituents of guar (Cyanopsis tetragonoloba L. tab.) seeds were determined. The results are shown in table (1).

Table (1): Different components of guar seeds (G/100 gm of guar seeds).

Moisture	10.43	
Ash	4.93	5.51*
Fats	3.92	4.38*
Total carbohydrates	36.09	40.30*
Proteins	43.80	48.91*

\* These values were calculated on dry weight basis.

The values in table (1) illustrate that the seeds of guar, which are grown in Egypt, contain a high protein content i.e. 48.91%. Consequently, the cake after removing the gum might be used as a rich source of proteins. This result is in agreement with that obtained by Misra *et al.* (1984). As mentioned before the isolation of this polymer from guar seeds using hot water gave the highest amount (11.55%) while the extraction with acid gave the lowest amount (7.11%).

This observation might be attributed to the partial degradation of this polysaccharide, resulting from both acid hydrolysis and alkali treatments. Alkali treatment leads to the conversion of aldehydic terminal unit to ketose rearrangement, followed by saccharinic acid formation (Aspinall, 1982), this process mostly occurred for the polysaccharides containing 3-linked main chains and for 4-linked glycans. Also, the side chain units remotely attached at C-6, might be degraded as for the linear glycan forming 0-glycosylsaccharic acids.

Such alkaline degradation causes the reduction of the polysaccharide yield. Similar explanation have been reported by Aspinall (1982), with the galactomannan of guaran (4-linked B-D-mannan chain). Also acidic conditions cleavage of glycosidic bonds can be occurred during the extraction of this polysaccharide.

#### Preliminary tests and properties of the guar gum (guaran):

The extracted guar gum was free from starch, reducing sugars, proteins and contains traces of halogens (chloride), its  $(a)_D^{25}$  was + 74 (in water). This result indicates that there is a homogeneity of this polysaccharide. Such results were obtained by Tewari *et al.*, (1984).

The precipitate of copper complex with the polysaccharide gave  $(a)_D^{25}$  -224. This observation has been shown by Reeves (1979), these reactions involving the hydroxyl groups occur at C<sub>2</sub> and C<sub>3</sub> of the type galactopyranoside

units. This shift of rotation sign to levorotatory was also observed in the cases of starch and glycogen (Reeves, 1951).

The ash content of guaran polysaccharide was 0.25% and its individual mineral constituents are shown in table (2). In general, the content of elements (specially heavy metals) in all samples investigated was lower than that found in some American commercial polysaccharides, according to Joseph (1955).

**Table (2): Analysis of guar gum ash (mg/100 gm seeds).**

Type	Ca <sup>++</sup>	Mg <sup>++</sup>	K <sup>+</sup>	Na <sup>+</sup>	Fe <sup>+3</sup>	Zn <sup>+2</sup>	Cu <sup>+2</sup>
Guar gum extracted with water	23.02	5.42	42.2	76.0	2.4	3.40	5.30
Guar gum extracted with 0.2% NaOH	--	16.30	39.5	68.09	7.4	--	4.90
Guar gum extracted with 1.5% acetic acid.	19.38	12.90	86.0	67.00	3.3	4.02	4.48

#### **Determination of molecular weight:**

The molecular weight of guar gum was calculated as described by Christensen (1954), using an Ostwald-Cannon Fenske pipette and the sample was compared with standard citrus pectin. The results of viscosity measurements for molecular weight calculation are shown in table (3) and Fig. (1). From this figure, the intrinsic viscosities ( $\eta_i$ ) of standard citrus pectin and guar gum were obtained, table (4). The average molecular weight of guar gum is almost in agreement with that stated by Hoyt (1966).

#### **Identification of sugar components in the hydrolyzate:**

The pure polysaccharide of the seeds was hydrolyzed using N. sulphuric acid then the cations and anions were removed using the cationic and anionic resins. G.L.C. technique was utilized to fractionate the silyl derivatives of the simple sugars of the hydrolyzate. The results indicated that this polysaccharide consists of two components. The two simple sugars were identified as D(+) galactose, and D(+) mannose, respectively, comparing with the standard authentic sugars, Fig. (2,a). The ratio of galactose residue to mannose was 1:2, according to the total area of the peaks and the area of each of each peak

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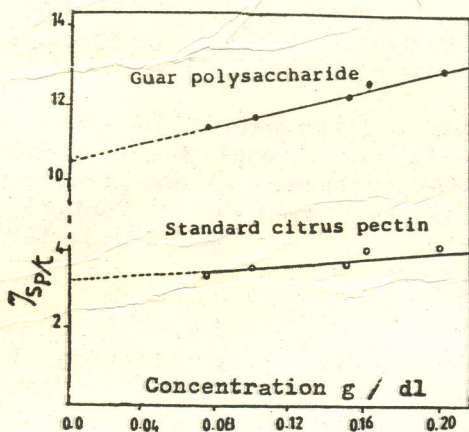


Fig. (1) Intrinsic viscosities of guar polysaccharide and standard citrus pectin.

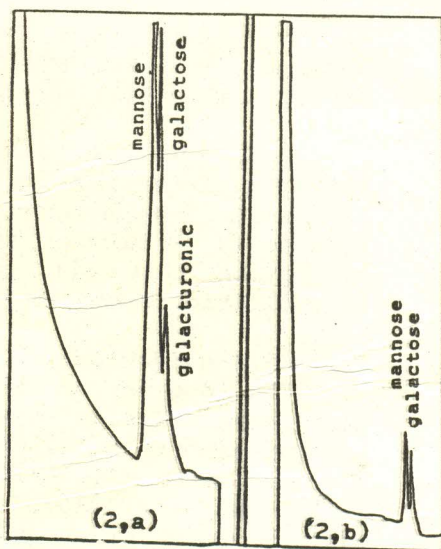


Fig. (2) G.L.C. chromatgram

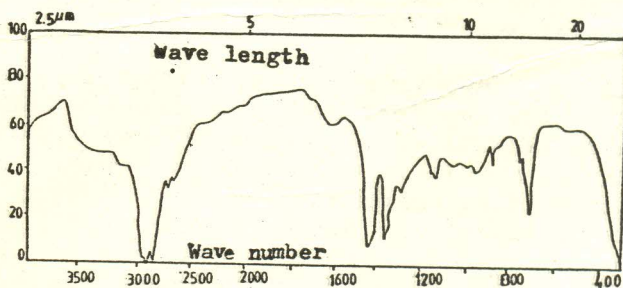


Fig. (3) I.R. spectrum of guar polysaccharide

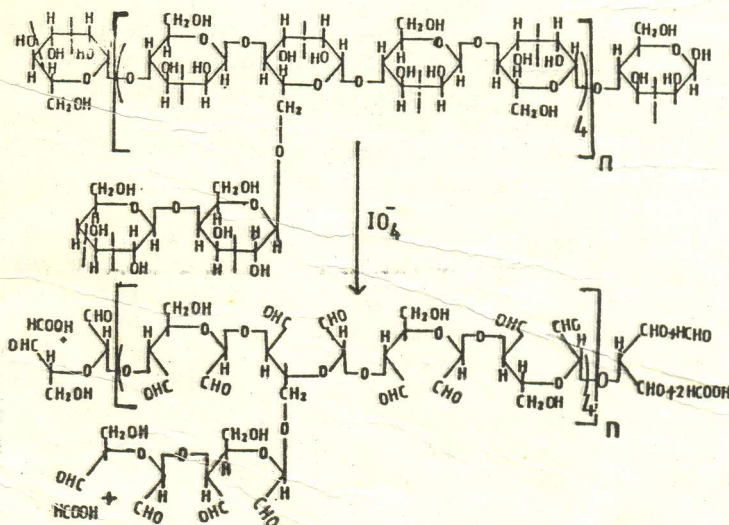


Fig. (4) Proposed structure of guar seed polysaccharide



in Fig. (2,b). Such results are in agreement with those obtained by Smith and Montgomery (1959), McCleary *et al.*, (1976); Dey (1978) and Tewari *et al.*, (1984).

I.R. spectrum of guar polysaccharide is shown in Fig. (3). The appearance of the vibrational bands at  $885\text{ cm}^{-1}$  is due to B-type glycosidic linkage (Attia, 1983) and the absence of a linkage at  $810\text{-}840\text{ cm}^{-1}$  gives support to the assumption that all linkages in the polysaccharide under investigation are of the B-type.

#### Periodate oxidation:

The degree of simple sugar polymerization (Dp) of the polysaccharide molecule was calculated on the basis of the amount of liberated formaldehyde for each anhydroglycose unit ( $0.05/162$ ). This amount equals  $0.000972$  ( $0.000003/0.0003080$ ), consequently the number of anhydroglycose units which produce one mole of formaldehyde with periodate oxidation equals  $1029$  ( $1/0.000972$ ) aldose units. According to these results the guaran polysaccharide has a molecular weight  $166,650$ .

It is important to note that the molecular weight given by the physical method (viscosity method) was higher than that determined by the latter chemical method (periodate oxidation) the ratio of molecular weight for both methods was  $1.33: 1.0$ , respectively. Such results were obtained by Tawfik (1978), in native potato starch and corn starch. The amount of formaldehyde released is proportional to the number of reducing end, while the molecular weight determined by the physical method (intrinsic viscosity-molecular weight relation) is an indication of molecular size of the whole polysaccharide. This difference in molecular weight values could be interpreted on the fact that the polysaccharide molecular consists of a mixture of a wide range of different molecular weights. Each fragment contains a reducing end which releases a molecule of formaldehyde by periodate oxidation and leads to a certain decrease in the molecular weight.

The total amount of formic acid which was liberated from a molecule of guaran polysaccharide equals about  $172$  moles ( $166,650/162 \times 0.167$ ) and the consumed moles of periodate per anhydroglycose unit ( $0.05/162$ ) was calculated. This result indicated that each mole of anhydroglycosyl unit consumed  $1.168$  mole of sodium metaperiodate. Hence, formic acid molecules are liberated from terminal units, besides, each mole of formic acid is produced per non reducing terminal unit in case of  $(1 \rightarrow 4)$  glycosidic

Table (3): Viscosity measurements for standard citrus pectin (C.P.) and guar polysaccharide (G.P.) at pH 6.5 and temperature 25 (in phosphate buffer).

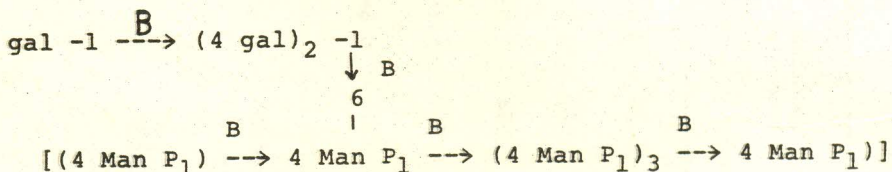
Concentration g/dil. litre	to solvent flowtime (seconds)	t		$\eta_r$ relative viscosity		$\eta_{r/c}$		$\eta_{sp}$ specific viscosity		$\eta_{sp/c}$ intrinsic viscosity	
		C.P.	G.P.	C.P.	G.P.	C.P.	G.P.	C.P.	G.P.	C.P.	G.P.
0.20	34	61.8	122.5	1.82	3.60	9.09	18.00	0.818	2.603	4.09	13.01
0.16	34	55.9	103.7	1.64	3.04	10.28	19.00	0.643	2.050	4.02	12.81
0.15	34	53.5	97.5	1.57	2.87	10.49	19.13	0.574	1.86	3.82	12.45
0.1	34	46.65	74.5	1.37	2.19	13.72	21.91	0.372	1.191	3.72	11.91
0.075	34	43.5	64.0	1.28	1.88	17.06	25.07	0.274	0.882	3.65	11.76

Table (4) : The intrinsic viscosities and the average molecular weight of standard citrus pectin and guar gum :

Sample	Intrinsic viscosity ( $\eta_i$ )	Average molecular weight
Standard citrus pectin	3.35	$71.276 \times 10^3$
Guar gum	10.38	$220.853 \times 10^3$

linkages. Consequently, guar polysaccharide has a branched structure with  $C_4$  16.7% of the units in terminal positions. Besides, the ratio of consumed periodate to an anhydroglycosyl unit was 1.168: 1, which shows that the majority of the mannopyranose or galactopyranosyl were linked through  $C_1$  and  $C_4$ .

According to the results obtained from acid hydrolysis, infrared spectroscopy, cupperammonium complex and periodate oxidation, the polysaccharide probably consists of repeating segments, as shown in structure (1).



Structure (1): Proposed repeating formula of guar polysaccharide.

However, the proposed repeating formula in structure (1) is in agreement with the general structure element of all galactomannans which was confirmed by Smith and Montgomery (195), McCleary *et al.*, (1976) and Dey (1978). Since the molecular weight of guar gum was equivalent to 1028 glycosyl units, therefore, this polysaccharide contains about 171 repeating segments. Fig. (4) illustrates the proposed structure formula for the guar polysaccharide which fits well with the molar ratio of galactose and mannose (1:2) and the obtained results of periodate oxidation and other analysis.

#### Technological and gelling properties of guar gum:

Standard pectin and guar gum were compared for further utilization of guar as pectin substitute in jam making. The results are shown in table (5).

Table (5): Technological and gelling properties of guar gum and citrus pectin.

Property	Guar gum	Pectin
Colour	white	creamy
Gel strength	++	++
pH (1% solution)	5.85	2.80
Ability to form gel at room temp.	++++	++
Ability to form gel at high temp. then the gel was colled	++	++++
Viscosity (0.2% in phosphate buffer	3.60	1.82
Stability capacity for preservation of juices	++++	—

The results indicate that pH of pectin solution is lower than that of guar solution, which is probably due to the presence of free carboxylic groups of galacturonic acid, in pectin molecules as major compounds while guar polysaccharide is free from this acid. In addition the main backbone and branches of guar polysaccharide is composed of galactose and mannose i.e. neutral residues.

Guar polysaccharide was found to be suitable to form firm gel when compared with standard pectin, i.e. the ability of guar to form gel at room temperature is higher than that of pectin. On the other hand, the ability of gel formation for pectin at high temperature (100°C) is higher than that of guar polysaccharide. Ibrahim *et al.* (1983), stated that the guar polysaccharide can be used as pectin substitute in jam making, in relatively small proportion (0.067 g/100 g sugar), while pectin was used in higher proportion (1 g/100 g sugar), i.e. the amount of pectin equals fifteen times that of guar gum to obtain the same gel strength in jam making. This point seems to be important from the economical stand point of view, however, such point needs to be more investigated to come to conclusive results.

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## الفصل والتعرف على صمغ الجوار

عزت رزق جندى      منير عبد العظيم تركي      صلاح مصطفى محمود  
حسن عبد المعطى قرشم

يهدف البحث الى دراسة التركيب الكيميائي لصمغ بذور الجوار وكذلك دراسة خواصة التكنولوجيا والجيلية بهدف استخدامة كبديل لمادة البكتين المستخدم فى صناعات الأغذية وكذلك الاعراض الطبية .

تم استخلاص صمغ الجوار باستخدام الماء الساخن والقلوي المخفف والحامض المخفف وقد أعطى الاستخلاص بالماء الساخن أعلى ناتج (٥٥/١١٠) بينما الاستخلاص بحمض الخليك المخفف أقل ناتج (١١/٠٧) وقد اجريت التنقية لهذا السكر العديد عن طريق معقود النحاس وقد أوضحت التجارب والتحليل الأولية وجود تجانس بين السكريات وصمغ الجوار المستخلص بالطرق المختلفة السابقة .

استخدم الكروماتوجرافى الغازى لتفريد مشتقات السليل للسكريات الأحادية الناتجة من التحليل المائى الحامض وقد أوضحت النتائج أن ذلك السكر العديد يحتوى على وحدات م (+) جلكتوبيراتوز و م (+) مانوبيرانوز بنسبة جزئية ١:٢ وقد تم الاستدلال على أن الرابطة الجليكيوسيدية بين وحدات السكر الاندريه المكونه له من النوع بيتا باستخدام طيف تحت الحمراء .

قدر متوسط الوزن الجزئى طبيعيا باستخدام قياسات اللزوجة ووجد أنه يساوي ٢٢٠٠٠٠ وقد قدر أيضاً كيمائياً باستخدام الأكسدة بواسطة فوق الايودات ووجد أنه يساوي ١٦٦٠٠٠ وقد فسر ذلك الاختلاف الجزئى المقدر كيمائياً وطبيعياً على أساس أن السكريات العديدة من مخلوط ذا مدى واسع من الأوزان الجزئية .

أوضحت الأكسدة بالفوق ايودات أن السكر العديد للجواز يحتوى على الرابطة (١١ — ٤) وأن حوالى ١٦٧/٠ من وحدات السكر الاندريدي توجد كنهايات طرفية غير مختزلة .

من النتائج المتحصل عليها من التحليل المائى الحامض والدوران الضوئى لمتراكب النحاس والدورات الضوئى للسكر العديد والأكسدة الفوق الايودات وطيف الأشعة الحمراء من المحتمل أن هذا السكر العديد يتكون من حوالى ١٧١ مقطع (متوسط) مكرر وكل مقطع يتكون من سلسلة رئيسية من وحدات المانوريرانوز الاندريديه (٤ وحدات) متملة بالرابطة الجليكويزيدية بيتا (١ — ٤) بينما وحدات الجلكتويرانوز الاندريديه (وحدتين) مرتبطة بالسلسلة الرئيسية على صورة فرع بالرابطة بيتا (١ — ٦) . أما الرابطة بين الوحدات الجلكتوبيراتوز من نوع بيتا (١ — ٤) .

وقد اجريت بعض الخواص التكنولوجية والجيلية لجل الجوار وقورنت بعين بكتين قياسية مستوردة من المملكة المتحدة . وأوضحت النتائج المتخصص عليها أنه يمكن استخدام صمغ الجوار كبديل لمادة البكتين حيث أنه يعطى جل ثابت وأوضحت النتائج أيضاً أن لصمغ الجوار سعة عالية لحفظ العصائر فى صورة مجنسة لفترة طويلة .