# Evaluation of nutritional, biological and microbiological properties of jam sweetened by date "debs"

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### **Abstract**

One of the important products of date palms is date syrup. Date syrup locally known as "debs" is probably the most common derived date product. The present work was done to evaluate the antioxidant capacity as well as the biological effects of date syrup locally known as "debs" on streptozotocin-induced diabetic rats and to investigate the microbiological changes occurring during the storage of jam containing debs replacing sucrose. The results of phytochemical screening showed that "debs" contains different constituents such as flavonoids, phenols, steroids, and saponins that could be effective as antioxidant and antimicrobial substances. The results also revealed that "debs" has high antioxidant capacity. For the biological study thirty six healthy adult Albino male rats were used. The data collected for blood glucose levels indicated the antidiabetic effect of debs. The results of the microbiological study revealed that the addition of date debs instead of sucrose resulted in the decrease of microbial load in jam upon storage up to three months.

Keywords: dates; debs; diabetes; streptozotocin; phytochemical; antioxidant; antibacterial

# Introduction

Date palm (*Phoenix dactyliferaL.*) has been cultivated since thousands of years in Egypt. According to the Food and Agriculture Organization, there are 14 million palm trees, occupying 30,934 ha, which represents

6.32 % of the fruit cultivated area in Egypt representing about 20 % of the total world production (Al-Khayri et al., 2015). Date fruits are good sources of many important elements such as K, Na, Ca, Mg, Fe and Zn as well as source of amino acids and vitamins A, B1 and B2 (*Wafaa et al., 2016*).

One of the important products of date palms is date syrup. Date syrup locally known as "debs" is probably the most common derived date product (*Al-Khayri et al., 2015*). It is a natural extract of dates, without any additives, colors or preservatives (*Doma et al., 2016*). Date syrup contains various components such as carbohydrates, proteins, lipids, pectin, salts and minerals (*Gabsi et al., 2013*). The date syrup is directly consumed or used as an ingredient in some food formulation such as ice cream products, beverage, confectionery, bakery products, sesame paste/date syrup blends, jam and butter (*Abbès et al., 2013*). Date syrup is a rich source of phenolic compounds which are known potent radical scavengers, and also it has antibacterial activity attributed to its bioactive components including plant-derived phenolic molecules (*Taleb et al., 2016*).

Diabetes mellitus (DM) is rapidly spreading disease worldwide; it is associated with long-term complications especially with increased oxidative stress that results in alteration of several cellular biomolecules (*Hasan and Mohieldein, 2016*). It has been demonstrated that, reactive oxygen species (ROS) could play a key event in the development of diabetes complications such as hepatic injury (*Hussein et al., 2015*).

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Over the years, many conventional medicines were used to control diabetes. Natural products are a good remedy as they are inexpensive and easy to access without any complications. Dates and their constituents show a role in diseases prevention through anti-oxidant, anti-inflammatory, anti-bacterial activity (*Rahmani et al., 2014*). Some studies have proven that date palm extracts have a hypoglycaemic effect in alloxan-induced diabetic rats and were highly effective in managing the complications of diabetes mellitus such as hyperlipidemia and weight loss (*Mard et al., 2010*).

In the present study, the objectives of the work are to evaluate the antioxidant capacity as well as the biological effects of date syrup on streptozotocin-induced diabetic rats and microbiological changes occurring during the storage of jam containing date syrup instead of sucrose.

# Materials and Methods

Date syrup was purchased from local supermarket in Egypt. Two types of treatments were done to the date syrup, in the first treatment date syrup was dried completely (syrup A) and in the second treatment it was semi dried (syrup B).

#### Preparation of jam

Jam preparation was done as described by *Habiba and Mehaia, (2007)* with some modifications. To prepare the jam samples, apples purchased from local market, washed, peeled and cut into cubes. The cubes were then cooked in stainless steel container. The apple cubes were mixed with sugar in ratio 45% to 55%, gently heated and when the total soluble solids (TSS) reached 65%, pectin was added (5 g/kg of apple: sugar mixture) followed by the addition of citric acid (3 g/kg of added sugar). Finally, the resultant jam was hot filled in clean dry jars. Jam pH was 4.The cooked fruit was mixed with "date debs" dried or semi dried instead of sugar and a control jam was made by adding sugar. The cooking of the jam was continued and then jam samples were cooled and placed in glass jars.

#### **NUTRITIONALEVALUATION**

#### 1- INVITRO:

#### **TOTAL ANTIOXIDANT ACTIVITY (TAA)**

The total antioxidant activity of syrups A and B was determined using the phosphomolybdenum method according to the procedure described by *Prieto et al.*, (1999). The antioxidant activity was calculated using a standard curve of ascorbic acid.

# **TOTAL PHENOLIC CONTENTS (TPC)**

Contents of total phenolics of the syrups were estimated by spectrophotometer using the Folin–Ciocalteu assay (*Singleton et al., 1999*). A standard curve was plotted using different concentrations of Gallic acid. The absorbance obtained was converted to gallic acid equivalent in mg per gm of dry material [mg GAE/g] using gallic acid standard curve.

#### **TOTAL FLAVONOIDS (TF)**

Total flavonoids content was determined as described by the method of *Willet (2002)*. Total flavonoids content results were reported as equivalents to querectin used as standard.

#### PHYTOCHEMICAL SCREENING

Date syrup samples were tested for the presence of different classes of secondary metabolites using previously described methods of *Harbone*, (1973).

#### 2- IN VIVO BIOLOGICAL EVALUATION

A total number of 36 healthy adult Albino male rats between 2 and 3 months of age and weighing about 180–200 g were used for the study. The rats were housed in cages with 12/12 hrs light/dark cycles at 22-23°C. The rats were maintained in this condition for a period of three days to acclimatize them prior to experimental uses. The animals were fed with standard rat diet AIN-76 and water *ad libitum* according to *NRC*, (1995).

#### **INDUCTION OF DIABETES MELLITUS**

Diabetes was induced in overnight fasted adult Albino male rats weighing 180-200g by a single intraperitoneal injection of 50 mg/kg body weight streptozotocin (Sigma Aldrich, Germany) in 0.1 M citrate buffer of pH 4.4.Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection.

#### **EXPERIMENTAL DESIGN**

Total 36 rats were included and were divided into 6 groups, each groups consist of six rats as follows: 6 rats on basal diet and left as negative control and the rest were diabetic.

Group I: Normal control (control -)

Group II:Diabetic control (control +streptozotocin)

**Group III:**Diabetic with syrup A (5%) **Group IV:**Diabetic with syrup A (10%)

Group V: Diabetic with syrup B (5%)

Group VI: Diabetic with syrup B (10%)

#### **BLOOD SAMPLING**

Blood samples for glucose analysis were taken after overnight fasting from orbital flexus nervous by using fine capillary tube. Blood samples were obtained at 0,4,6,8 and 10 weeks and were tested using blood glucose meter (Merck Microlab 200).

#### **BACTERIOLOGICAL EXAMINATIONS**

Jam samples were stored for three months and bacteriological tests were carried out at different storage intervals. Total bacterial count was carried out according to *Berrang et al., (2001).* Faecal coliform counts were carried out according to *Mercuri and Cox (1997).* Total yeasts and molds were carried out according to *NMKL (1999).* 

Isolation of *E.coli* was carried out according to *Collins et al., (1998). E.coli* colonies are green metallic sheen on Eosin methylene blue (EMB) agar medium. *E. coli* identification attempts were made using the criteria described by *Kreig and Holt (1984)* using the following tests: growth on TSI, urea, indole, M.R, V. P and sugar fermentation.

Isolation of *Staphylococcus aureus* was carried out according to *Gouda (2000)*. The isolation of *Staphylococcus aureus* is based on the appearance of black, convex, shiny colonies surrounded by a yellow zone on Vojel Johnson agar medium. Isolation of *Clostridium perfringens* was carried out according to FAO (1992),. *Cl. Perfringens* colonies are black on clostridium perfringens selective agar base supplemented with tryptone- sulf-cycloserine (TSC) supplement.

#### STATISTICAL ANALYSIS

Statistical data was carried out with the use of Microsoft Excel 2010 computer program. Results are expressed as mean ±SD. Comparisons between groups were made using Student's t-test and p-values<0.05 were considered as significant.

# Results and Discussion

#### Phytochemical constituents of date syrup

In the present study, the phytochemical screening of date syrup indicated the presence of different constituents such as flavonoids, phenols, steroids, and saponins (Table 1). The phytochemical screening confirmed the presence of various classes of biologically active plant metabolites that could be effective as antioxidant and antimicrobial substances. Phytochemical constituents are highly responsible for many protective health benefits. So based on the phytochemical screening results "debs" could offer many advantages for human health.

Table(1)
Phytochemicals of date syrups

Sample	Tannins	Flavonoids	Saponins	Alkaloids	Steroids	Phenols	Terpenoids
Syrup A	+	+	+	-	+	+	-
Syrup B	+	+	+	-	+	+	-

Positive sign (+) indicates presence, negative sign (-) indicates absence

#### Antioxidant properties of date dibs

In the present study, the antioxidant properties of date syrups are presented in Table (2). The results show higher antioxidant capacity for Syrup B compared to Syrup A. Antioxidant activity is recognized due to the wide range of phenolic compounds present in dates including p-coumaric, ferulic, and sinapic acids, flavonoids, and procyanidins (*Rahmani et al.*, 2014).

The total phenolic compounds as well as the total flavonoids were also found in elevated amounts in Syrup B compared to Syrup A. Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells (*Saeed et al., 2012*). Phenolic compounds and flavonoids are powerful antioxidants and act in a structure-dependent manner; they can scavenge reactive oxygen species (ROS), and chelate transition metals which play vital roles in the initiation of deleterious free radical reactions (*Wang et al.,2010*).

Numerous in vitro studies have suggested that antioxidant potential and other useful biological actions of medicinal plants could be attributed to their high phenolic contents. The phenolic compounds by virtue of their reducing properties can absorb and neutralize free radicals, quench singlet and triplet oxygen, or decompose peroxides to act as antioxidant (*Khan et al., 2016*). Previous studies concerning the composition of date syrup have identified significant antioxidant capacity which may imply to the scientific basis of date fruit and date syrup's traditional medicinal application (*Taleb et al.,2016*).

Table (2)

Total antioxidant capacity, total phenols and flavonoids of date syrups

Sample	Total antioxidant capacity	Total phenolic content	Total flavonoids (mg/100g	
Sample	(mg/100g ascorbic acid equivalent)	(mg/100g gallic acid equivalent)	querectin acid equivalent)	
Syrup A	122.43	582.95	15.60	
Syrup B	141.59	1205.15	23.05	

#### Effect of date syrup on blood glucose of diabetic rats

The results showing the effect of replacing sucrose with date syrup on blood glucose of diabetic rats are given in Table (3). There were no significant differences (p>0.05) between all groups at the start of the experiment as all groups were normal rats taking same diet. After 4 weeks, Group I recorded significantly (p<0.05) lower blood glucose compared to all other groups injected with streptozotocin. It is known that streptozotocin (STZ) is used as an agent to induce diabetes mellitucreatic injury and by pan selective cytotoxicity effect on pancreatic beta cells which leads to an increase in blood glucose level (Shirwaikar et al., 2004; .Hasan and Mohieldein, 2016).

During the experimental period from week 6 to the end of experiment (week 10), it was observed that the groups (III, IV, V and VI) consuming date syrup showed significantly (p<0.05) lower blood glucose levels as compared to the positive control group II, nevertheless the glucose level of these groups was still significantly higher than that of the healthy controls (group I). This means that the consumption of date syrup decreased the blood glucose level of diabetic rats but did not completely treat diabetes as the rats did not retain their normal blood glucose levels. It is also worthy to note that the rats in group VI recorded the significantly (p<0.05) lowest blood glucose value when compared to other treated groups and to the positive control group. This signifies that the semi dried date syrup at a level of 10% was the best treatment in the present work.

Previous studies conducted on diabetic rats showed that continuous administration of aqueous extract of Saudi date seed significantly reduced the blood glucose concentration in STZ induced diabetic rats for 8 weeks. Also, the hypoglycemic effect of date seed extract along with insulin treatment as well as the endogenous stimulation of Insulin production has been reported (*Hasan and Mohieldein, 2016*). The antidiabetic effect of date syrup could be due to the effect of active flavonoids, phenols, steroids, and saponins; these compounds may scavenge free radicals in diabetic rats. Hypoglycaemic effects have been reported for some plants that contain flavonoids (*Rahmani et al., 2014*).

Table (3)
Effect of dibs on blood glucose levels in experimental rats

	1 <sup>st</sup> week	4 weeks	6 weeks	8 weeks	10 weeks
Group I	107.50°±12.99	108.00 <sup>e</sup> ±2.58	101.50 <sup>f</sup> ±1.98	102.75 <sup>f</sup> ±0.90	108.75 <sup>f</sup> ±2.37
Group II	113.33° ±3.54	300.00°±13.31	305.00°±12.97	310.00°±13.40	310.00°±14.34
Group III	115.00° ±3.84	260.00 <sup>b</sup> ±8.04	230.00°±5.98	215.00° ±9.43	200.00°±5.00
Group IV	117.50°±2.38	260.00 <sup>b</sup> ± 6.36	200.00 <sup>d</sup> ±1.92	170.00 <sup>d</sup> ±11.70	140.00 <sup>d</sup> ±3.62
Group V	113.75°± 1.92	250.00°± 1.87	250.00 <sup>b</sup> ±4.39	235.00 <sup>b</sup> ±6.58	224.00 <sup>b</sup> ±8.08
Group VI	106.00°± 1.63	230.00 <sup>d</sup> ±6.30	180.00°±0.79	160.00°±7.32	133.00°±0.61

a,b,c.....f: different superscripts within the same column indicate significant differences (p<0.05)

# Microbiological study

The effect of replacing sucrose by date syrup on the microbiological and properties of this jam was evaluated through storage. In this respect the total bacterial count (TBC), faecal coliform (FC), and total fungi (TF) were measured in jam samples containing sucrose, syrup A or syrup B at different preservation time (zero, one month, two months and three months). The microbiological results are shown in Tables (4 - 7). It is observed that during every single storage period the total bacterial count and yeasts were significantly (p<0.05) lower in the jam containing syrup B followed by that one containing syrup A and the highest microbial count was found in the jam with sucrose. Also it was found that faecal coliform and total fungi were not present in any of the samples throughout storage period up to two months.

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After two months of storage (Table 6) there was a notable decrease in the total bacterial count of jam samples containing syrup A and syrup B and no coliform were detected. After three months of storage (Table 7), it was noticed that the total bacterial counts of syrup A and syrup B jams were significantly lower than that of sucrose jam.

Table (4)
Microbiological analysis of jam made by sucrose and debs (semi and dry) at zero time

Treatments		(	Count		Isolation			
rreatments	T.B.C	F.C	T.F	Yeasts	E.coli	Staph. aureus	CI. perfringens	
Sucrose	6x10 <sup>5a</sup>	0	0	2x10 <sup>5a</sup>	0	0	0	
Syrup A	5x10 <sup>4b</sup>	0	0	6x10 <sup>3b</sup>	0	0	0	
Syrup B	3x10 <sup>3c</sup>	0	0	2x10 <sup>3b</sup>	0	0	0	

a,b,c: different superscripts within the same column indicate significant differences (p<0.05) T.B.C: total bacterial count; T.C: total coliform; F.C: feacal coliform; T.F: total fungi

Treatments		Count			Isolation			
rreatments	T.B.C	F.C	T.F	Yeasts	E.coli	Staph. aureus	Cl. perfringens	
Sucrose	6x105a	0	0	9x105a	0	0	0	
Syrup A	4x103b	0	0	8x103b	0	0	0	
Syrup B	2x102c	0	0	6x103b	0	0	0	

a,b,c: different superscripts within the same column indicate significant differences (p<0.05) T.B.C: total bacterial count; F.C: feacal coliform; T.F: total fungi

Table (6 )

Microbiological analysis of jam made by sucrose and debs (semi and dry) after two months of storage

T		Co	ount		Isolation			
Treatments	T.B.C	F.C	T.F	Yeasts	E.coli	Staph. aureus	CI. perfringens	
Sucrose	7x104a	0	0	3x105a	0	0	0	
Syrup A	4x103b	0	0	4x103b	0	0	0	
Syrup B	2x102c	0	0	7x102c	0	0	0	

a,b,c: different superscripts within the same column indicate significant differences (p<0.05) T.B.C: total bacterial count; F.C: feacal coliform; T.F: total fungi

Table (7)

by sucrose and debs (semi and dry) after three

Microbiological analysis of jam made by sucrose and debs (semi and dry) after three months of storage

		С	ount		Isolation			
Treatments	T.B.C	F.C	T.F	Yeasts	E.coli	Staph. aureus	CI. perfringens	
Sucrose	8x104a	0	0	2x106a	0	0	0	
Syrup A	3x103b	0	0	6x105b	0	0	0	
Syrup B	2x102c	0	0	4x104c	0	0	0	

a,b,c: different superscripts within the same column indicate significant differences (p<0.05) T.B.C: total bacterial count; F.C: feacal coliform; T.F: total fungi

The results of the microbiological study revealed that the addition of date syrup instead of sucrose resulted in the decrease of microbial load in jam upon storage up to three months of storage. The antimicrobial effect of date syrup could be attributed to its phenolic content. Previous work of *Taleb et al.* (2016) reported that date syrup polyphenols, the most abundant bioactive constituent in date syrup, have antibacterial activity.

# **Conclusion**

Date syrup (debs) contains several bioactive components which makes it good antioxidant and antibacterial component in food. Debs consumption could be beneficial for maintaining blood glucose levels around normal levels. Further studies must be continued to investigate the effect of long term consumption of debs and its effect on diabetic rats.

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# تقييم الخصائص التغذوية والبيولوجية والميكروبية للمربى المحلاه بدبس التمر د. حنان مجدالغندور , د. أحمد فريدعبدالسلام ,د. غديرعلي الشغبي المركز الإقليمي للأغذية والأعلاف – مركز البحوث الزراعية العربى الملخص

تم إجراء هذا البحث لتقييم القدرة المضادة للأكسدة وكذلك التأثيرات البيولوجية لشراب التمر المعروف محليا بإسم "الدبس "علي الجرذان المصابة بداء السكري المستحدث بالستربتوزوتوسين وللتحقق من التغيرات الميكروبية التي تحدث أثناء تخزين المربي المحتوية علي "الدبس " كبديل للسكروز. أظهرت النتائج الفحص الكيميائي النباتي أن "الدبس " يحتي علي مكونات مختلفة مثل الفلافونويد، الفينول، الستيرويدات والصابونين التي يمكن أن تكون فعالة كمواد مضاد للأكسدة ومضاد للميكروبات. وكشفت النتائج أيضا أن "الدبس " لدية قدرة عالية مضادة للأكسدة. بالنسبة للدراسة البيولوجية، تم إستخدام سته وثلاثين فأر بالغ (ذكور البينو). وتشير البيانات التي تم جمعها لمستويات السكر في الدم إلي التأثير المضاد لمرض السكر لدى الجرذان التي تناولت الدبس. وكشفت نتائج الدراسة الميكروبيولوجية أن إضافة دبس التمر بدلا من السكروز أدي إلي انخفاض الحمل الميكروبي في المربى عند التخزين لمدة تصل إلى ثلاثة شهور.

الكلمات الدالة: دبس التمر -ستربتوزوتوسين – عرض السكرى – مكونات فيتوكيميائية – مضادات أكسدة – مضادات بكتريا