Extraction Conditions of Inulin from Jerusalem Artichoke Tubers and its Effects on Blood Glucose and Lipid Profile in Diabetic Rats

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Abstract: This study aimed to analyze Jerusalem artichoke tubers to identify its contents and to optimize conventional extraction of inulin, various time extract, temperature, and solvent ratio were used. 30 male albino rats divided into 5 groups (6 rats) were used to evaluated the extricated inulin as Hypoglycemic agents. It could be concluded that, the highest yield of inulin was recovered from Jerusalem artichoke tuber by using the following condition, sample to solvent ratio was 1: 5 w/v at 80°C for 90 minutes. The crude inulin extracted from Jerusalem artichoke tubers were used for production of orange juice and chocolate and estimated by aid of 10 panelists. The reduction of glucose was observed after one week of feeding till the end of experimental period, also, high level of inulin 15% led to amore reduction of blood glucose level comparing with the low level especially in the end of experimental period. The crude inulin extracted from Jerusalem artichoke tubers were used in diet for diabetic rats on different levels of inulin (10 and 15%) had significantly lower in total cholesterol, triglyceride and total lipids in comparing to positive diabetic rats fed on control diet. Meanwhile, HDL level was increased significantly after fed on 10 and 15% inulin. On the other hand, LDL and VLDL levels were decreased significantly after fed on (10 and 15%) inulin in comparing to positive group rats fed on control diet. [Journal of American Science 2010;6(5):36-43]. (ISSN: 1545-1003).

Keywords: Jerusalem artichoke, Extraction Conditions, inulin, blood glucose and lipid profile

1. Introduction

Today, the industrialized countries are facing, among others, three major challenges. Firstly, to control the cost of health care, secondly, to offer to their aging population a real opportunity to live, not only longer, but also better and thirdly, to provide to more and more. Jobs, consumers, a choice of healthy processed or ready to eat foods **(Roberfroid, 1999)**. Busy life styles and the increasing demand from consumers for meals and snacks that are quick sources of good nutrition have prompted the food industry to develop foods like nutrition bars that combine convenience and nutrition **(Izzo and Niness, 2001)**.

Inulin is a storage polysaccharide consisting of a chain of fructose molecules with a terminal glucose molecule. **Silva (1996)**, inulin is water soluble, the solubility being temperature dependent. At 10°C its solubility is about 6% whereas at 90°C it is about 35%. Inulin type fructans, are the best-documented oligosaccharides for their effect on intestinal bifidobacteria and are considered important prebiotic substrates **(Vos *et al.*, 2006)**. Jerusalem artichoke tubers with 14–19% inulin can be a valuable source of inulin **(Vanloo, et al., 1995)**.
Several methods for inulin extraction from Jerusalem artichoke have been developed. A pretreatment step involving boiling water-extracting for 10. 15 min of the ground tubers had been used **(Laurenzo *et al.*, 1999)**.

Initially, the application of inulin in the food industry was restricted to the production of a drink similar to coffee, due to its bitter taste. However, it was found that inulin could substitute sugar and fat with the advantage of exhibiting low caloric value **(Applied Technology, 1993)**. The application of inulin as a fat substitute is associated with its capacity of producing a cream-like substance, similar to fat dissolved in water, which can act as a rheological modifier **(Cândido and Campos, 1995 and Silva, 1996)**. Inulin shows interesting technological properties, as a low-calorie sweetener, as a fat substitute, or it can be used to modify texture **(Tungland and Meyer, 2002)**. These properties are linked to the degree of chain polymerisation. Inulin are added during cheese processing to decrease its fat percentage without losing its organoleptic characteristics, such as texture and flavour. One of the interesting functions of inulin and oligofructose in human nutrition is related to their prebiotic effect, i.e. the specific stimulation of growth and/or activity of a limited number of colonic bacteria beneficial to the host, as well as the growth inhibition of pathogens and harmful microorganisms **(Roberfroid, 2007**).

The combination of prebiotics and probiotics has given rise to the so-called 'synbiotics', with promising healthy properties (**Buritiet al., 2007; Pool- Zobel and Sauer, 2007**).

The functional effects of inulin on humans and experimental animals include relief of constipation lower blood glucose levels, improved absorption of calcium, reduced fasting triglycerides, LDL cholesterol, and inhibition of the growth of various kinds of tumors **(Kaur *and Gupta* 2002)**. **Marchetti (1993)** reported that, inulin is a natural polymer that not hydrolysable by the intestinal enzymes, because it has β (2-1) link which is not be hydrolyzed. So it could be considered a calorie free fiber, although some calories may occur due to the digestible fermentation of these by products in the colon. Inulin is such carbohydrates have a high potential nutritional advantage as low energy dietary supplements. It can be used as a source of carbohydrates for diabetic patients and more generally as dietary fiber. During 4 to 6 weeks improves glucose tolerance, decreases glycemia, and partially restores insulin secretion **(Cani *et al.*, 2005)**. Moreover, an improvement of glucose/insulin ratio has also been observed in rats receiving Oligofructose added in a high fructose diet (inulin) **(Busserolles *et al.*, 2003)**. In the case of low-calorie chocolates and derivatives, fibre compounds such as inulin and oligofructose are used as sugar substitutes **(Gonze and Van der Schueren, 1997)**. This study was carried out to investigate studying the best conditions to obtain inulin from Jerusalem artichoke for production of new foods and beverages with high biological value and studying the health benefits of inulin as functional food on diabetic and hyperlipidemic rats.

2. Material and Methods

Jerusalem artichoke tubers (*Helianthus tuberosus*) were purchased from the experimental station, Agriculture Research Center, Cairo, Egypt. inulin was isolated from the tubers of Jerusalem artichoke. Cholesterol, casein, bile acids, kits and cholin cholerid were purchased from El-Gomhoreria Co.

Technological methods:-

Preparation of Jerusalem artichoke tubers:

The samples of Jerusalem artichoke tubers were cleaned with tap water to remove dust and other undesirable materials. The cleaned tubers were cut into small pieces and used immediately.

Extraction of inulin from Jerusalem artichoke by different methods:

Jerusalem artichoke tubers were mixed with different volumes of hot water according to **Yamazaki (1994)** (sample to water ratio were 1 : 1, 1 : 3, 1 : 5 and 1 : 7 w/v) tuber : water. The effect of using different temperature (70, 80, 90 and 100°C) as will as different times (60, 70, 80 and 90 minutes) were also done to choose the best condition of extraction of inulin.

Preparation of juice and chocolate: Orange juice and Chocolate were prepared according to the method of **El-Gendy (1970).**

Sensory evaluation:

Juice and chocolate:

Orange juice, chocolate, juice and chocolate supplemented by different levels of inulin (10, 15, 20 and 25%) estimated by aid of 10 panelists. The following hedonic scale was used **Kramer and Twigg (1970)** for juice **and Kramer and Twigg (1970)** for chocolate.

Analytical methods:

The prepared samples were subjected to the chemical analysis moisture, protein, fat and ash by the methods described by **A.O.A.C. (1990)**. Crude fiber was measured using the method described by **Kirk and Sawyer (1991)**. Total carbohydrates were determined using the method described by **James (1995)**. Determination of inulin was recommended by **Prosky and Hoebregs (1999).**

Biological experiments:

Animals:

30 adult male albino rats weighting between 115 to 120 g were divided to 5 groups and (each group consisted of 6 rats), each rat housed individually in wire cages with wire bottoms.

Preparation of diabetic rats:

Pure fine was used for induction diabetes in normal healthy adult male albino rats by intraperitoneal injection of alloxan (150mg/kg body weight) according to the method described by **(Desia and Bhide, 1985)**, fasting blood samples obtained from retro orbital plexus (Superficial blood sample) to determined serum glucose. Diabetic rates were divided into 4 groups, 6 rats in each group fed on certain diets for 28 days as follows: First group was fed on basal diet only and used as control negative group. Second group diabetic rats were fed on basal diet and used as the control positive group. Third and fourth group diabetic rats were fed on basal diet containing 10 and 15% inulin.

Biological Evaluation:

At the end of trials, the animals were sacrificed, under ether anaesthetized and blood

samples were collected in clean dry centrifuge tube from hepatic portal vein. Serum was separated by centrifugation at 4000 r.p.m. for 10 minutes at room temperature then kept in plastic vials at-20c until analysis.

Biochemical Analysis:

Glucose was determined by enzymatic methods using kits according to **Trinder (1969)**. Determination of triglycerides in serum was determined calorimetrically according to **(Fossatip and Prancipel, 1982)**. Total cholesterol was determined by colorimetric method according to **Allain(1974)**. HDL Cholesterol was determined according to **Lopez (1977)**. Total lipid was determined by colorimetric method according to **Schimit (1964)**. Calculation of LDL and VLDL in mg/dl according to **Lee and Nieman (1996)**.

LDL cholesterol = Total Cholesterol - (HDL + T.G / 5) mg / dl.

VLDL cholesterol = Triglycerides / 5

Statistical analysis:

The results expressed as mean ± SD, arid performing using student (t) test. The oplained results will be analyzed to determine the degree of significances between different groups ($p \leq 0.05$) using one way analyzing of various (ANOVA) **(SAS, 1988)**.

3. Results and Discussion

Chemical composition of Jerusalem artichoke and extracted inulin:

Chemical composition of Jerusalem artichoke tubers percentages were calculated as dry weight (Table 1). Data obtained from this table showed that, Jerusalem artichoke had a low level of moisture content, their was 6.36% apparent also, from the same table that Jerusalem artichoke tubers seems to have inulin content (72.99%). Also, total carbohydrate content of Jerusalem artichoke were 78.03%, our results are in line with those of **Sahar (2003)** who reported that chemical composition of Jerusalem artichoke, Moisture, total carbohydrate, inulin, crude protein, crude fiber and ash were 6.50, 86.21, 71.78, 7.40, 7.52 and 5.30 g / 100 g, respectively. Also, these results are slightly with those of **Fleming and Groot-Wassink (1979); Guiraud *et al.* (1981) and Rashwan (1996)**, who reported that, Jerusalem artichoke tubers contained 85. 95% carbohydrates that were recovered mainly in the form of inulin. From the previous results, it could be concluded that, Jerusalem artichoke tubers have level of inulin high enough to be utilized commercially.

Meanwhile, Data in Table (1) showed chemical composition of extracted inulin from Jerusalem artichoke tuber. As shown the mean value of moisture, ether extract, crude protein, ash, inulin and crude fiber after chemical analysis of extracted inulin their were 4.57, 0.35, 0.49, 0.75, 96.87 and 1.54, respectively. our findings are in harmony with those of **Shalaby (2000)** who found that, inulin isolated from Jerusalem artichoke tubers was characterized by high value of inulin 96.25%.

Effect of solvent ratio, temperatures and extraction time on extractable inulin (%) from Jerusalem artichoke tuber:

Data obtained from Table (2) showed the effect of solvent ratio to samples, different temperatures and extraction time on extractable inulin (%) from Jerusalem artichoke. The optimum ratio to recover the highest yield of inulin was 1: 5 sample to solvent. This may be due to the presence of enough amounts of water required to dissolve and separate the maximum amounts of inulin found in the cell. From the same data it colud be noticed that increasing sample to solvent ratio from (1 : 5) to (1 : 7) had no significant effect. Data obtained from the same Table showed the influence of using different temperatures on the extractable inulin (%), as shown the mean values of extractable inulin after using different temperatures (70, 80, 90 and 100°C), their were 88.55, 92.46, 92.81 and 93.36, respectively, and these results were in agreement with the results recorded by **Margaritis and Bajpai (1982)** who extracted inulin from artichoke chips with water at temperatures ranged from 70 to 100°C.

Data recorded in Table (2) indicated that extractable inulin was increased with increasing the extraction time. Finally, it could be concluded that, the highest yield of inulin was recovered from Jerusalem artichoke tuber by using the following condition, sample to solvent ratio was 1 : 5 w/v at 80°C for 90 minutes. Our results are in agreement with the data obtained from **Sahar (2003)** who reported nearly the same condition.

Organoleptic evaluation:

Juice:

Organoleptic evaluation of the different manufactured orange juice is presented in Table (3). The data indicated that, orange juice prepared without inulin (control) had the highest values of taste, odor, color, texture and overall acceptability comparing to those prepared by adding different levels of inulin as judged by a group of panelists from nutrition and food science department, in addition, it should be noted from obtained data that, there were no significant differences between control and some levels of inulin (10 and 15%) on values of taste, odor, color, texture and overall acceptability.

On the other hand, using (20 and 25%) of inulin as a percent of substitution had a significant differences in comparingto control and those prepared with 10 and 15% inulin.

Chocolate:

Sensorial results of the chocolate studied did not indicate any significant differences in preference between control sample and different levels of inulin 10 and 15%, even though control sample was considered the most preferred trial of chocolate studied (Table 4). On the other hand, different levels of inulin 20 and 25% had a significant difference in comparing to control sample and samples prepared with 10 and 15% inulin. The appearance of the chocolates with partial fat replacement was similar to the appearance of conventional chocolate which is important because appearance in one of the four main sensory characteristics that plays a role in sensory acceptability **(Jones, 1996)**. **Thompson *et al.* (2004)** reported that, *Cocoa aroma* is a major driver influencing acceptability of chocolate milks.

Biological experiments:

Effect of different levels of inulin (10 and 15%) on glucose levels of diabetic rats:

Data presented in Table (5) showed the effect of using different levels of inulin 10 and 15% on serum glucose levels of diabetic rats. As shown the mean values of serum glucose levels for control negative group after first week, second week and fourth week were 78.63, 81.96 and 84.46, respectively. With regard to the mean values of glucose levels for control positive group after first, second and fourth week was recorded 231.00, 244.30 and 220.00, respectively. Apparent, also from the same table that the mean values of serum glucose levels of diabetic rats after receiving different levels of inulin (10 and 15%) first, second and fourth weeks were (224.70, 195.30 and 173.90) for positive group rats receiving 10% inulin and (226.40, 183.73 and 136.86) for positive group of rats receiving 15% inulin. It could be concluded that feeding on different, levels of inulin (10 and 15%) led to significant decrease in serum glucose level of positive group rats when compared with diabetic rats receiving standard diet. The reduction of serum glucose was observed after one week of feeding till the end of experimental period, also, high level of inulin 15% led to a more reduction of blood glucose level comparing with the 10% inulin level especially at the end of experimental period.

Our results are in agreement with those of **Alles *et al.* (1999) and Niness (1999)** who recorded that, inulin and oligofructose play an active role in reducing the caloric value and they do not lead to a rise in serum glucose or stimulate insulin secretion. However, **Molis *et al.* (2002)** reported that aforementioned action to the possible beneficial effects of inulin on blood glucose. However, there are some evidences that, inulin may decreased fasting blood sugar in type 2 diabetic that, can be explained as follow: inulin may delay gastric emptying and shorten small intestinal transit time, propionate may inhibit gluconeogensis. This can be done by the metabolic conversion to methyl malonyl-Co. A and succinyl-Co. **Roberfroid and Delzenne (1998)** reported that, supplementation with Jerusalem artichoke significantly decreased blood glucose levels in non-insulin dependent diabetes. Also, **Giacco *et al.* (2002)** found that, dietary fiber in particular the soluble fiber fraction plays the important role in controlling glucose concentration in serum and other risk factors associated with diabetes.

Effect of different levels of inulin (10 and 15%) on TC, TG and T. lipids of diabetic rats:

Data in Table (6) show the mean value of total cholesterol TC after receiving 10 and 15% inulin their were 148.40 and 123.06 while the mean value of TC for positive and negative control groups were 160.06 and 111.60, respectively. Apparent, also from this table the level of triglyceride TG of rats received different levels of inulin (10 and 15%) ranged from 195.63 for 10% and 165.83 for 15% inulin and ranged from 134.26 to 215.70 for negative and positive control groups. Data in this table indicated that total lipids for diabetic rates received 10 and 15% inulin ranged from 545.16 for 10% and 495.00 for 15% inulin, in comparing to positive and negative control groups which ranged from 650.96 for control positive and 421.86 for control negative. Finally, it could be concluded that, diabetic rats received different levels of inulin (10 and 15%) had a significant decreased in total cholesterol, triglyceride and total lipids when compared to positive diabetic rats received standard diet. Our findings are in agreement with those of **Levrat *et al.* (1991)** who found that, dietary inulin played active role in reducing serum cholesterol concentration in rats fed on diet contains inulin for 3 weeks. **Pushparaj *et al.* (2007)** reported that, administration of inulin extract of *Cichorium intybus* produced a significant reduction in serum glucose, triglycerides and total cholesterol in diabetic rats.

Effect of different levels of inulin (10 and 15%) on HDL, LDL and VLDL of diabetic rats:

Data in Table (7) show the effect of different levels of inulin (10 and 15%) on HDL, LDL and VLDL in serum of diabetic rats. It is noticed that HDL level was increased significantly after received

10 and 15% inulin by the means of 57.90 and 57.63 in comparing to control positive group rats which was 50.13. Data from the same table showed no significant difference between 10 and 15% inulin on HDL level of diabetic rats. On the other hand, LDL level was decreased significantly after fed a diet supplemented with 10 and 15% inulin by the means of 51.36 and 32.26 inulin in comparing to positive rats group which received the basal diet their was (66.83). In the same table, VLDL level was decreased significantly after received different levels of inulin by the means of 39.13 and 33.16 in comparing to positive rats group which fed the basal diet (43.10). In conclusion, data presented in this table indicated that, addition of inulin in different levels (10 and 15%) had significantly higher serum HDL in comparing to rats (control positive group) fed the basal diets, also, significantly lower in serum LDL and VLDL. Propionate, a product that yields from inulin fermentation in colon may inhibit hydroxymethylglutaryl-Co. A reductase which is considered the ratlimiting step in cholesterol biosynthesis **(Deleznne and Kok, 2001)**.

Table (1). Chemical composition of Jerusalem artichoke tuber and extracted inulin (as dry weight).

Constituents	Jerusalem artichoke tuber	Inulin
Moisture	6.36±0.97	4.57±0.56
Ether extract	1.40± 0.10	0.35± 0.16
Crude protein	7.55± 0.34	0.49± 0.11
Ash	5.72± 0.21	0.75± 0.15
Crude fiber	6.51± 0.17	1.45± 0.12
*Inulin	72.99± 2.34	95.36± 2.96
Total carbohydrates	78.03± 1.35	95.51± 2.45

* Inulin from total carbohydrates

Table 2: Effect of extraction time, solvent ratio and temperatures on extractable inulin (%) from Jerusalem artichoke tuber.

Time of extraction (min)	Extractable inulin (%)	Sample to solvent ratio	Extractable inulin (%)	Temperate of extraction (°C)	Extractable inulin (%)
60	$85.37^{b} + 2.12$	1:1	$77.61^{c} \pm 0.66$	70	$88.55^{b} \pm 0.80$
70	$87.92^{b} + 1.38$	1:3	$86.64^{b} \pm 0.71$	80	$92.46^{a} \pm 0.79$
80	$92.53^{a} + 1.21$	1:5	$92.26^{a} \pm 0.93$	90	$92.81^{a} \pm 0.57$
90	$93.12^{a} + 1.42$	1:7	$92.64^{a} \pm 0.83$	100	$93.36^{a} \pm 0.89$

Mean[1] in the same column with different letters are significantly different ($p \leq 0.05$).

Table (3). Organoleptic evaluation of orange juices substituted their sucrose with different levels of extracted inulin.

Type of juices	Quality index				
	Taste	Odor	Color	Texture	Overall acceptability
Control	$8.92^{a} \pm 0.13$	$8.82^{a} \pm 0.17$	$8.94^{a} \pm 0.13$	$8.84^{a} \pm 0.20$	$8.94^{a} \pm 0.02$
10% inulin	$8.88^{a} \pm 0.13$	$8.84^{a} \pm 0.15$	$8.74^{a} \pm 0.26$	$8.80^{a} \pm 0.21$	$8.74^{a} \pm 0.20$
15% inulin	$8.80^{a} \pm 0.15$	$8.74^{a} \pm 0.20$	$8.74^{a} \pm 0.25$	$8.68^{a} \pm 0.28$	$8.68^{a} \pm 0.34$
20% inulin	$7.66^{b} \pm 0.34$	$7.56^{b} \pm 0.38$	$7.52^{b} \pm 0.31$	$7.64^{b} \pm 0.30$	$7.54^{b} \pm 0.25$
25% inulin	$6.74^{c} \pm 0.45$	$6.80^{c} \pm 0.43$	$6.84^{c} \pm 0.45$	$6.74^{c} \pm 0.36$	$6.68^{c} \pm 0.27$

Means in the same row with different superscript letters are significantly different ($p \leq 0.05$).

Table (4). Organoleptic evaluation of chocolate substituted their sucrose with different levels of extracted inulin.

Type of chocolate	Quality index				
	Appearance	Texture	Flavour	Aroma	Overall acceptability
Control	8.42^{a}±0.10	8.42^{a}±0.07	8.46^{a}±0.08	8.36^{a}±0.05	8.44^{a}±0.05
10% inulin	8.36^{a}±0.05	8.40^{a}±0.04	8.38^{a}±0.05	8.32^{a}±0.04	8.36^{a}±0.05
15% inulin	8.24^{a}±0.05	8.40^{a}±0.12	8.36^{a}±0.13	8.28^{a}±0.04	8.34^{a}±0.05
20% inulin	7.62^{b}±0.13	7.48^{b}±0.08	7.46^{b}±0.05	7.48^{b}±0.08	7.34^{b}±0.11
25% inulin	6.78^{c}±0.27	6.42^{c}±0.08	6.52^{c}±0.19	6.40^{c}±0.15	6.38^{c}±0.13

Mean[1] in the same column with different letters are significantly different ($p \leq 0.05$).

Table (5). Effect of 10 and 15% inulin on (glucose level in blood) of diabetic rats.

Period	Groups				Mean [1]
	Control (-)	Control (+)	10 % Inulin	15 % Inulin	
0	78.63±1.11	231.6±2.31	224.7± 2.60	226.4± 4.84	**190.3^{c}± 67.4**
7 days	81.96±1.64	224.3±3.45	195.3± 2.35	183.7± 2.44	**171.31^{b}± 56.0**
14 days	84.46±2.10	220.0±1.62	173.9± 1.91	136.8± 4.76	**153.79^{a}± 82.0**
Mean [2]	**81.68^{a}±2.9**	**225.3^{d}± 5.5**	**197.9^{c}±22.16**	**182.3^{b}± 38.95**	

Mean[1] in the same column with different letters are significantly different ($p \leq 0.05$), LSD = 2.230
Mean[2] in the same row with different letters are significantly different ($p \leq 0.05$), LSD = 3.841

Table (6). Effect of different levels of inulin (10 and 15%) on TC, TG and TL of diabetic rats.

Groups / Variables	Control (-)	Control (+)	10 % Inulin	15 % Inulin	LSD
Total cholesterol (mg/dl)	111.6^{a} ± 1.56	160.06^{d} ± 2.11	148.4^{c}± 2.75	123.06^{b} ± 1.94	4.02
Triglyceride (g/dl)	134.26^{a}± 1.44	215.7^{d}± 5.55	195.63^{c}± 3.7	165.83^{b}± 1.89	6.67
Total lipids	421.86^{a}± 15.8	650.96^{d}± 16.3	545.16^{c}± 22.06	495.0^{b}± 6.84	30.52

Means in the same row with different superscript letters are significantly different ($p \leq 0.05$).
Each value in the table is the average of six replicates.

Table (7). Effect of different levels of inulin (10 and 15%) on HDL, LDL and VLDL of diabetic rats.

Groups / Variables	Control (-)	Control (+)	10 % Inulin	15 % Inulin	LSD
HDL (mg/dl)	63.96^{a}±1.75	50.13^{c}±1.61	57.9^{b}±1.66	57.63^{b}±1.05	2.91
LDL (mg/dl)	20.80^{a}± 0.88	66.83^{d}±1.33	51.36^{c}±2.40	32.26^{b}±1.4	3.04
VLDL (mg/dl)	26.83^{a}± 0.28	43.10^{d}±1.1	39.13^{c}±0.76	33.16^{b}±0.37	1.33

Means in the same row with different superscript letters are significantly different ($p \leq 0.05$).
Each value in the table is the average of six replicates.

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