Selection of Optimal Conditions of Inulin Extraction from Jerusalem Artichoke (Helianthus Tuberosus L.) Tubers by using Ultrasonic Water Bath

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ABSTRACT

Ultrasonic extraction is an inexpensive, simple and efficient alternative to conventional extraction techniques, as compared with other novel extraction techniques such as microwave-assisted extraction & supercritical fluid extraction techniques, the ultrasound apparatus is cheaper and its operation is easier. Ultrasound assisted extraction has risen rapidly in the latest decade, and for most applications it has proven to be effective compared to traditional extraction techniques. In this paper, a method of ultrasonic-assisted extraction was used to extract Inulin from tubers of Jerusalem artichoke, which have been reported to have several medicinal properties and uses. Inulin is a storage carbohydrate found in many plants especially in chicory root, Jerusalem artichoke and dahlia tuber. In this study, the effect of time, temperature, pH and solid to liquid ratio on Inulin extraction from Jerusalem artichoke tubers by using ultrasonic water bath. The highest yield of Inulin were investigated from Jerusalem artichoke tuber was (99.47%) at temperature of 70°C, pH=7, 60 min and ratio of solid to solvent was (10gm/100ml). Then, The UV detector by colorimetric method with vanillin–sulfuric acid was used for the quantification of Inulin.

Key words: jerusalem artichoke, extraction conditions, inulin, ultrasonic bath.

اختيار افضل الظروف لاستخلاص الانولين من درنات نبات الالمازة باستخدام حمام مائي بالموجات الفوق الصوتية

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الخلاصة

الاستخلاص باستخدام الموجات الفوق الصوتية تقنية غير غالية وبسيطة وذات كفاءة عالية في تحول الاستخلاص مقارنة مع تقنيات الاستخلاص التقليدية الاخرى مثل تقنيات الاستخلاص بالموجات المساعدة و الاستخلاص بالمائع الحرج، يعتبر الاستخلاص بالموجات الفوق الصوتية رخيص وسهل التشغيل وبدا استخدامها تدريجيا في اغلب التطبيقات واثبتت كفائتها مقارنة مع تقنيات الاستخلاص المعروفة. في هذا البحث استخدمت طريقة الاستخلاص بالموجات الفوق الصوتية لاستخلاص الانولين من درنات نبات الالمازة الذي يستخدم في العديد من الاستخدامات الطبية.الانولين خازن للكاربوهدرات موجود في العديد من النباتات وخاصة الخرشوف والالمازة ودرنات الداليا. حيث تم دراسة تاثير الوقت ودرجة الحرارة ودرجة الحموضة ونسبة الصلب الى السائل على استخلاص الانولين من درنات نبات الالمازة باستخدام الحمام المائي بالموجات الفوق الصوتية . اعلى نسبة انولين تم الحصول عليها عند درجة حرارة 70

مئوية وفي وسط متعادل وبمدة 60 دقيقة وباستخدام 10 غرامات من قطع درنات الالمازة في 100 مل ماء مقطر ثم تم تحليل المستخلص بجهاز UV بالطريقة اللونية باستخدام الفنلين مع حامض الكبريتيك وذلك لمعرفة نسبة الانولين في المستخلص.

الكلمات الرئيسية: الألمازة , ظروف الاستخلاص , الانولين , حمام الموجات الفوق الصوتية.

1. INTRODUCTION

Inulin is a polysaccharide found in more than 30,000 species, which composed of a chain of fructose units with a terminal glucose unit**, Toneli, et al., 2008 and Costa, et al., 2013.** It is a natural carbohydrate source found mainly in roots and tubers of many plants, such as chicory artichoke (*Cynara scolymnus* L.), viper's grass (*Scorzonera hispanica* L.), and Jerusalem artichoke (*Helianthus tuberosus* L.) , and has been reported to have an Inulin content of 14-19% (wet weight) **, Saengkanuk, et al., 2011, and Abou Arab, et al., 2011.** Inulin is increasingly used in processed foods for its unusually adaptable characteristics. It can be used to replace sugar, fat and flour. The Inulin has minimal increasing impact on blood sugar and is not insulemic. Therefore, it is suitable for diabetics and potentially helpful in managing blood sugar-related illnesses**, Panchev, et al., 2011.** Inulin is particularly suitable for fat replacement in low fat or fat free products, such as chocolate, confectionery, cheese and ice cream dressing, because it has a fat creamy form, gelling capacity and good body, texture and mouthfeel**, Nogueira, et al., 2007.** In addition, Inulin has health-promoting properties such as prebiotic activity, enhancement of calcium absorption, and weight control through the promotion of satiety, **Ripoll, et al., 2010**. Inulin is water soluble, which depends on the temperature. At 10°C its solubility is about 6% where as at 90°C it is about 35 % **, Winarti, et al., 2011, Leite, et al., 2004 and Gaafar, et al., 2010.** From various extraction methods used to separate the active principles from plants tissues, e.g. solvent extraction, steam extraction, supercritical fluid extraction, pressurized liquid extraction, ultrasound-assisted extraction, micro wave assisted extraction etc., Conventional extraction with hot solvent under stirring and ultrasound-assisted extraction are the most common techniques to obtain Inulin. Extraction yield and quality of Inulin are depending on the extraction technique, temperature and pH of extraction medium, operating time and solid/solvent ratio**, Dobre, et al., 2010.** There are many demands on new extraction techniques with shortened extraction time, reduced organic solvent consumption and efficient extraction of target components in plant matrixes. Recently, there have been numerous reports on the application of high intensity or power ultrasound in the extraction of various photochemical such as alkaloids, flavonoids, polysaccharides, proteins and essential oils from various parts of plant and plant seeds**, Firdaus, et al., 2010.** It has been successfully applied in the static mode for extraction of formaldehyde from cosmetics and shiitake mushroom**, Chen, et al., 2008.** Ultrasound assisted extraction depends on the destructive effects of ultrasonic waves. The possible advantages of ultrasound in extraction are intensification in mass transfer, cell disruption, enhanced penetration and capillary effects**, abdul razak, 2009 and Glibowski, and Bukowska, 2011.** Ultrasound can be considered as a useful alternative for solid sample pretreatment because the energy imparted facilitates and accelerates some steps, such as dissolution, fusion, and leaching, among others**, Jalbani, et al., 2006.** Ultrasound extraction has two main principles that constitute its advantage over other leaching techniques. These are cavitation phenomena and the mechanical mixing effect, both of which increase the extraction efficiency and reduce the extraction time**, Ince, et al., 2013.** Ultrasound-assisted leaching is an expeditious, inexpensive and efficient alternative to conventional extraction techniques and, in some cases, even to supercritical fluid and microwave-assisted extraction**, Li, and Zhou, 2008.** Ultrasonication is a potential alternative technology: this is the application of high-intensity, high-frequency sound waves and their interaction with material. The propagation and interaction of sound waves alters the physical and chemical properties of materials that are subjected to ultrasound. High-intensity ultrasonication can accelerate heat and mass transport in a variety of food process operations and has been successfully used to improve drying, mixing, homogenization and extraction**, Li, et al., 2004.** Ultrasound-assisted extraction has been proven to significantly decreased extraction time and increased extraction yields in many vegetables materials**, Plaza, et al., 2008.** Is this study, this technique is used for the extraction of Inulin from Jerusalem artichoke tubers and the important parameters such as temperature, pH and ratio solid to liquid are studied.

2.EXPERIMENTAL

2.1 Material

Sodium hydroxide (Reachim 98%), hydrochloric acid 35.4% (1.18) (AR), vanillin (4-hydroxyl-3 methoxybenzal dehyde) (BHD), concentrated sulfuric acid (98%), standerd Inulin (Sigma Aldrich), deionized water , Jerusalem artichoke tubers (Helianthus tuberosus) were purchased from a commercial market in Baghdad, Iraq .

2.2 Equipment

Ultrasonic heater bath (Grant Laboratories Ltd F5200b , 240 volt, 15 Amp) as shown in **Fig.1**, thermometer, pH meter (CRISON, made in EU), hot plate magnetic stirrer (Model L-81), digital balance (KERN(ABS)), volumetric flask (150 ml), filter paper, UV- visible spectrophotometer (Model 80 D, made in UK).

2.3 Procedures

The samples of Jerusalem artichoke tubers were cleaned with tap water to remove sand and other undesirable materials. Cleaned tubers were peeled and cut into small pieces and used immediately. An extractor equipped with an ultrasonic water bath transducer working at 20 kHz frequency with amplitude, range and sample temperature being monitored up to 70°C. Ten grams of previously prepared tubers were used for extraction in 100 ml of deionized water heated to (30,50,70°C) by magnetic stirrer in a 150 ml conical flask then placed into the ultrasound assisted extractors at room temperature. The ultrasonic bath were filled with water and heated to the temperatures required then the conical flask containing the mixture was submerged in the ultrasonic water bath and samples were taken periodically each 10 min for 1 hour, then extract filtered through filter paper and in refrigerator stored for further analysis using UV/Vis spectrophotometer.

2.4 The standard curve

The standard curve was done by preparing stock solution using vanillin, in presence of concentrated sulfuric acid, forms with Inulin a deep red color. The colorimetric method with vanillin– sulfuric acid is used for the quantification of Inulin**, Dobre, et al., 2008.** This has been reported to be a simple, quick and accurate method. 1.52 gm. Vanillin was dissolved in 200 ml of sulfuric acid then 4 ml of this solution was added to 4 ml of extracted samples. Inulin standard stock of solution (1000 ppm) was prepared. (20, 50, 100,150, 200 ppm) different concentrators were prepared. The protocol requires that the colorimetric reaction is done for 15 min to obtain maximum color development; complex that yields a characteristic adsorption spectrum with a peek at 520 nm. The absorbance will be scan using UV/Vis spectrophotometer in the range of 200-700 nm. The absorbance at Vis 520 nm will be determined with a glass cell of 1 cm. Standard curve was drawn by measuring the absorbance of the samples containing Inulin standard is shown in **Fig.2** and the Chemical structure of inulin compounds is shown in **Fig. 3.**

3. RESULTS and DISCUSSIONS

3.1 Effect of temperature

The effect of ultrasonic duration at selected temperature on the recovery of Inulin from the tubers of Jerusalem artichoke is shown in **Fig. 4**. Results illustrated that the ultrasonic extraction was more efficient in obtaining Inulin by hot water extraction. The recovery rate was found to increase gradually during the first 40 minutes of experiences time then reached a constant value until 60 min. In contrast, the recovery rates of Inulin at 30°C was found to be (Inulin < 800 mg/ml) whereas at 50°C was 1220 mg/ml which corresponds to an extraction efficiency of (64.2%).the highest recovery rate of 1859.91 mg/ml corresponding to extraction efficiency (97.89%) of Inulin was obtained at 70°C after 40 min. then, increased to (99.47%) after 60 min. The mechanism of ultrasonic extraction involves two physical processes: the dissolution of the extracted Inulin near the particle surface (rinsing) and the diffusion of Inulin to the liquid extract (slow extraction)**, Yang, et al., 2008.** Increasing the ultrasonic time could enhance these two activities, resulting in a high extraction recovery rate, which might account for the increase, observed in the recovery rate of Inulin as the treatment time was extended from 40 to 60 min. At high ultrasonic temperature, the liquid viscosity and density decreased resulting in a more rapid mass transfer. Furthermore, high ultrasonic temperature can lead to an increase in the number of cavitations within these tissues and surface contact areas .Thus, application of high ultrasonic temperature resulted in enhanced extraction efficiency.

$$extraction\ efficiency\ \% = \frac{conc.of\ inulin\ in\ the\ sample}{conc.of\ inulin\ in\ the\ Jerusalem\ artichoke} \times 100\% \tag{1}$$

$$Conc.of\ inulin\ in\ the\ Jerusalem\ artichoke\ \left(\frac{\mu g}{ml}\right) = Inulin\ content\ in\ the\ Jerusalem\ artichoke\ of\ 19\% \times wet\ weight \tag{2}$$

3.2 Effect of Solid to Liquid Ratio

From **Fig. 5** it can be seen that the highest extraction rate of Inulin is 1889.93 mg/ml at a solid to liquid ratio 10 wt/vol %. While a lower value of ratio 20 wt/vol % is 1600 mg/ml. The lowest value of was obtained 1400mg/ml at 5 wt/vol %.

The reason for the low rates at the latter two concentrations may be that at 5 wt/vol % concentration, the solute is not sufficient (dilute solution) and that at 20 wt/vol %, the solution is not sufficient. And there is an optimum concentration in between of 10 wt/vol %.

3.3 Effect of pH

From **Fig. 6** it can be seen that the highest extraction rate was obtained at pH equal to 7. It was noted that for the acidic media of pH = 3 (adding few drops of hydrochloric acid) for the significant decrease in Inulin recovery rate from tubers while alkaline medium (adding few drops of sodium hydroxide solution) at pH value of 11, the extraction was more efficient as compared to the acidic extraction. It is clearly seen that lowest rate of Inulin recovery was exhibited in the acidic extraction media 390 mg/ml (20.5%) after 60 min; while in the alkaline extraction media the recovery rate was 1422.86 mg/ml (74.84%) after 60 min. The highest extraction rate at pH = 7 was 1889.93 mg/l after 60 min. These results are in agreement with previous studies**, Glibowski, and Bukowska, 2011.**

3.4 Effect of Extraction Time

From the three figures presented above it can be seen that the extraction rate when used the ultrasonic water bath increases fast up to 40 minutes, then at slower rate after 40 to 60 minutes. When compared the best results obtained in these studied of extraction of inulin with extraction of inulin without using the ultrasonic water bath at the same condition (pH=7 & 10 wt/vol % at 70°C). In **Fig. 7** we seen the best extraction rate giving Inulin concentration of 987.52 mg/ml (51.97%) was obtained without using the ultrasonic water bath at 60 minutes.

4.CONCLUSIONS

Ultrasound method improves extractions of microalgae significantly, with higher efficiency, reduced extraction times and increased yields, as well as low to moderate costs and negligible added toxicity.

The best extraction rate giving Inulin concentration of 1889.93 mg/ml was obtained when using:

- A temperature of 70 ^{0}C.
- A solid to liquid ratio of 10 gm/100 ml (10 wt/vol %).
- A pH = 7.
- Operation time of 60 minutes.

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Figure 1. Schematic diagram of ultrasonic water bath.

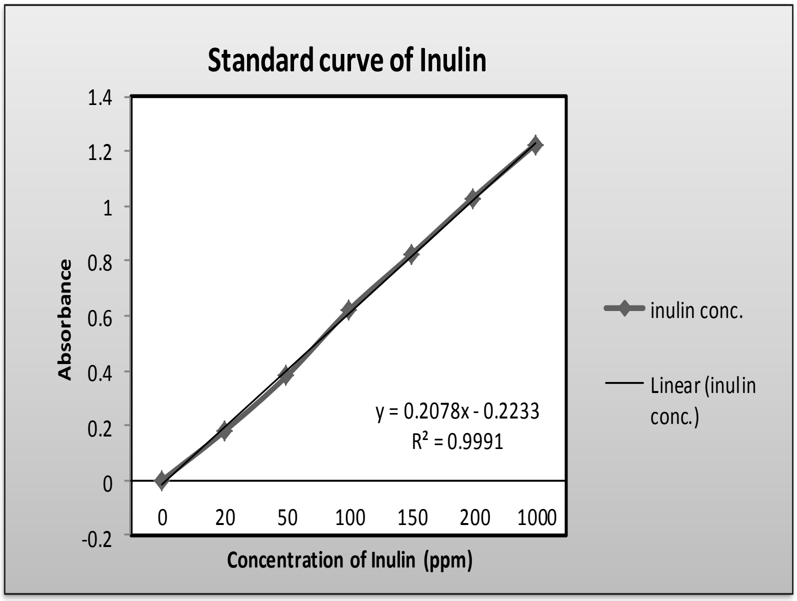


Figure 2. Standard curve of Inulin .

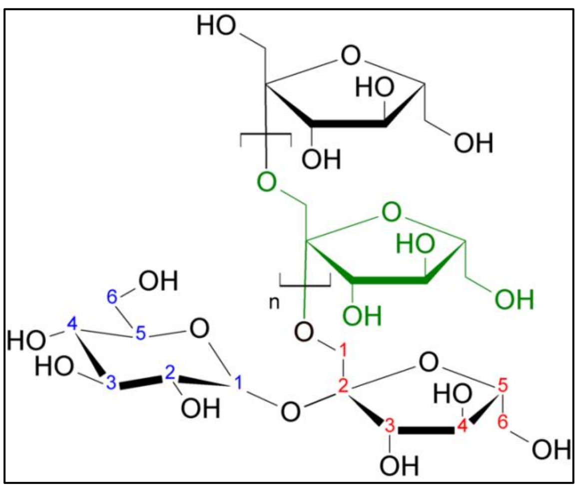
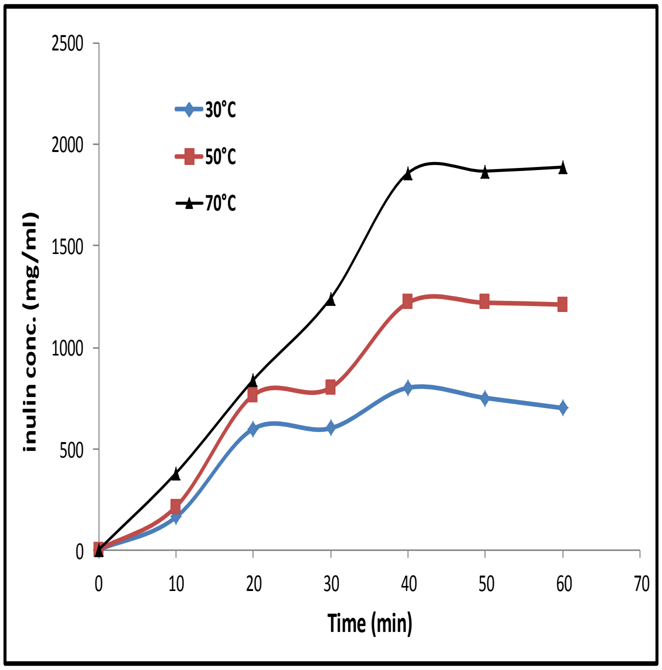


Figure 3. Chemical structure of Inulin compounds.



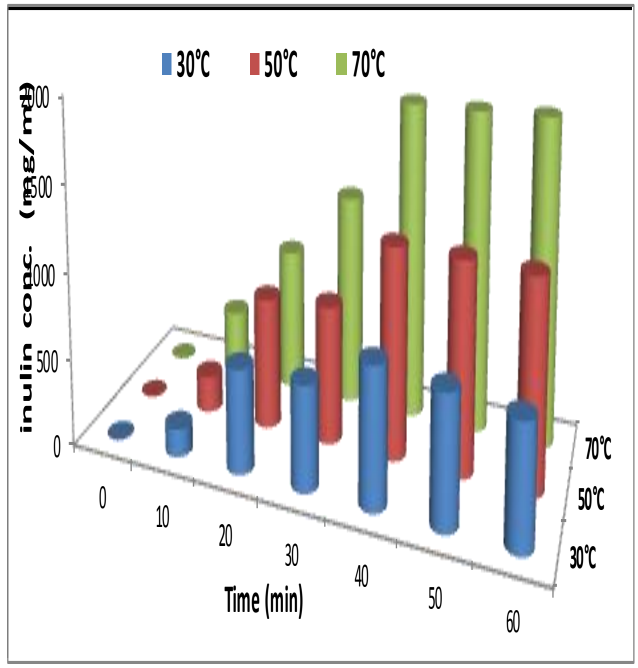
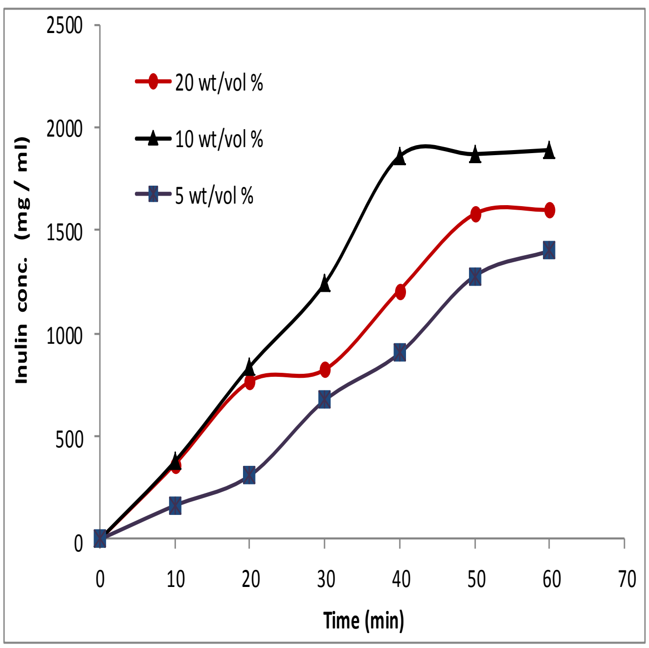


Figure 4. Effect of different temperature on Inulin concentration.



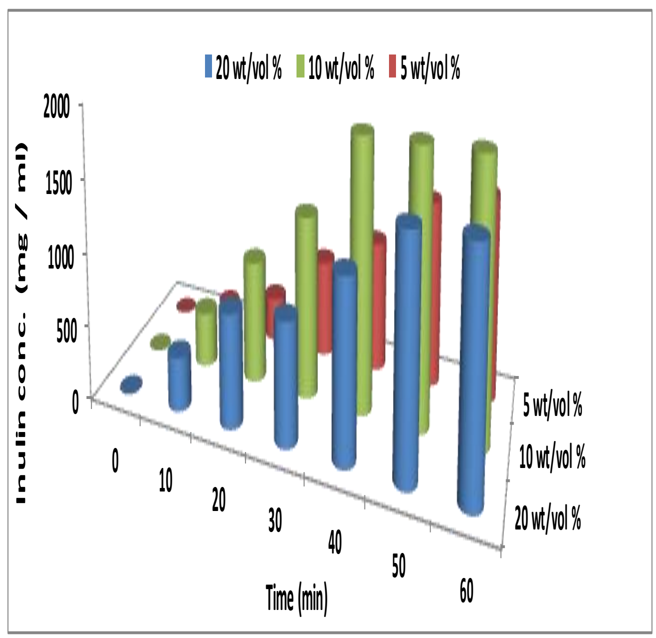


Figure 5. Effect of solid to liquid ratio on Inulin concentration (at pH=7 and at 70°C).

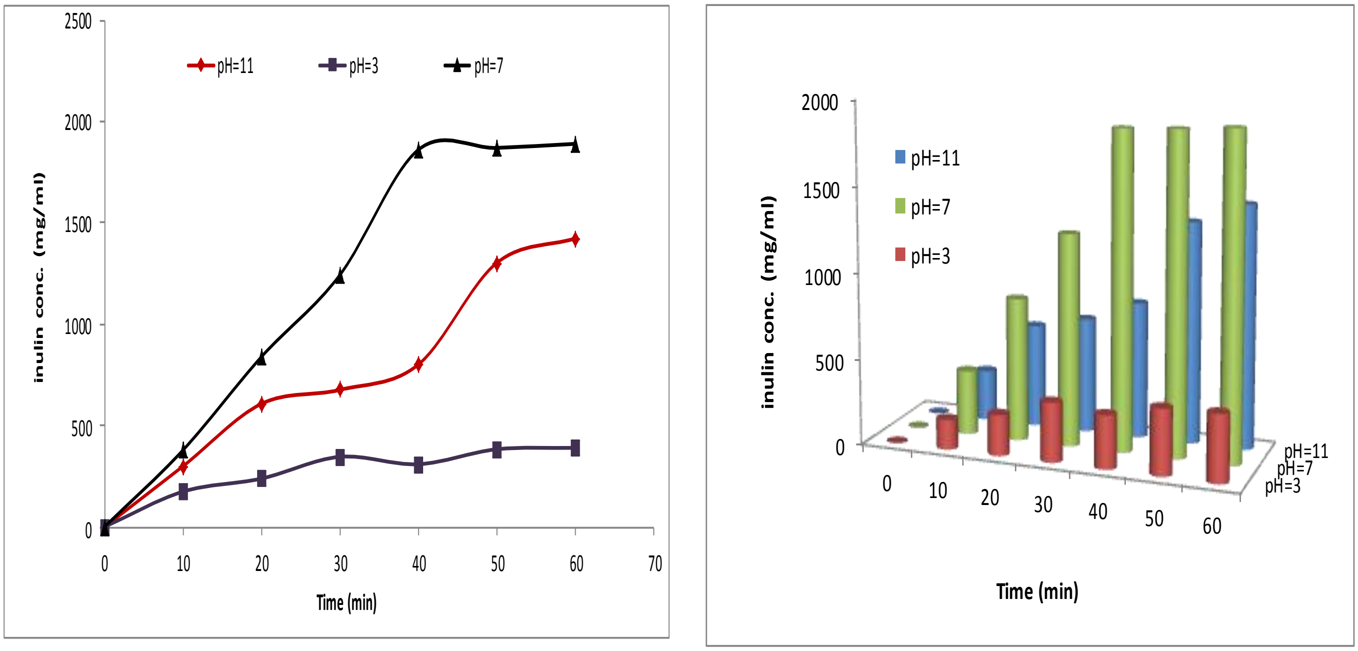


Figure 6. Effect of different pH on Inulin concentration (at 10 wt/vl % and at 70°C).

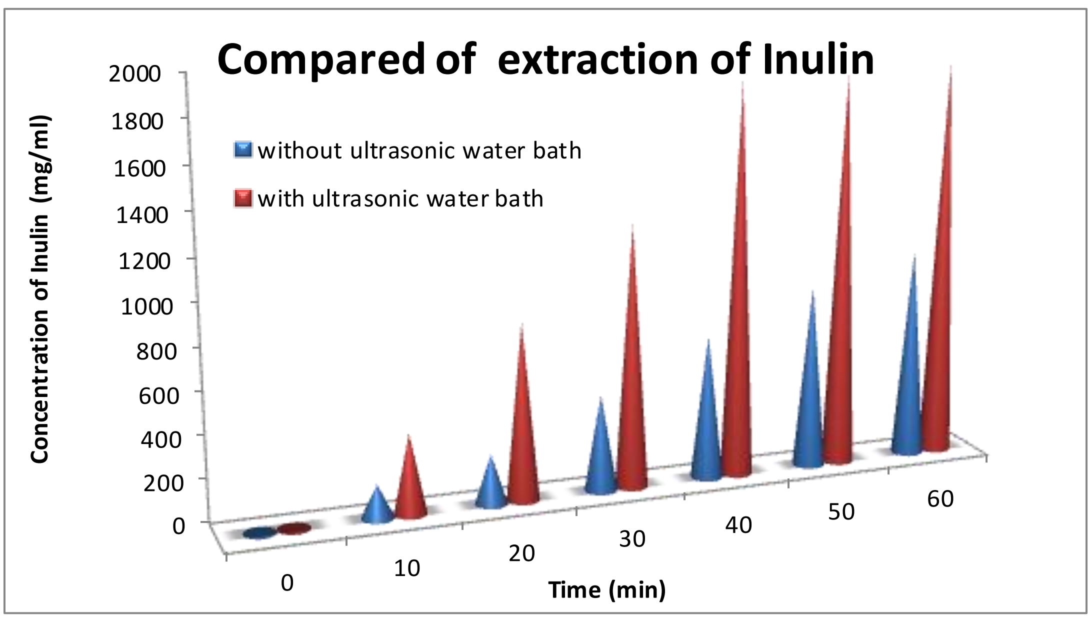


Figure 7. Compared of extraction of Inulin (at pH=7 & 10 wt/vol % and at 70°C).