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HPLC ANALYSIS FOR DETERMINATION OF CHARACTERISTICS OF FRUCTO-OLIGOSACCHARIDE SYRUPS EXTRACTED FROM JERUSALEM ARTICHOKE

Sibel (YİĞİTARSLAN) YILDIZ[*], N. Suzan KINCAL[**]

[*] Chemistry Department, Süleyman Demirel University, 32260 Isparta, Turkey
e-mail: ysibel@metu.edu.tr, ysibel@fef.sdu.edu.tr
[**] Chemical Engineering Department, Middle East Technical University, 06531 Ankara, Turkey
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Abstract: This study was aimed to assess the feasibility of extracting fructo-oligosaccharides (degree of polymerization of 3-6) from jerusalem artichoke tubers by solvent extraction. Together with harvest date, storage time, and peeling of the raw material it was also investigated the influence of some process parameters on final dry matter, degree of polymerization and product profile of the extracts: time (10-60 min), temperature (40-80 °C), amount of solvent (30-50 ml) and citric acid concentration (0-39 mmol/L). HPLC analysis showed that the most functional extracts (2.33) were obtained with mid-February harvested, 20 day-stored whole jerusalem artichoke tubers extracted with 40 ml of water containing 26 mmol/L citric acid at 60 °C for 40 min. Under those conditions, syrups with 17g of dry matter and degree of polymerization of 6 were produced. The density, viscosity, darkness and color of the syrups were found as 0.98 g/ml, 1.1 mPa-s, 0.38, and 13.3, respectively.

Keywords: Fructo-oligosaccharides, HPLC, inulin, extraction, jerusalem artichoke

YER ELMASINDAN ÖZÜTLENEN FRÜKTO-OLİGOSAKKARİT ŞURUPLARININ KARAKTERİSTİK ÖZELLİKLERİNİN HPLC ANALİZİ İLE BELİRLENMESİ

Özet: Bu çalışmada, yer elmasından, çözücü özütleme yöntemiyle früktо-oligosakkaritlerin (polimerizasyon derecesi 3-6) özütlenebilirliğinin fizibilitesinin tayini amaçlanmıştır. Hasat zamanı, depolama süresi ve ham maddenin soyulmasının etkilerinin yanı sıra diğer bazı proses parametrelerinin (zaman (10-60 dak), sıcaklık (40-80°C), çözücü miktarı (30-50 ml) ve sitrik asit derişimi (0-39 mmol/L)) de sonuçtaki kuru madde, polimerizasyon derecesi ve özütteki ürün profili üzerindeki etkileri incelenmiştir. HPLC analizleri, en fonksiyonel özütün (2,33) Şubat ayı ortalarında hasat edilmiş, 20 gün depolanmış, soyulmamış yer elması yumrularının, 26 mmol/L sitrik asit içeren 40 ml suyla, 60 °C'de 40 dakika süreyle özütlenmesi sonucunda elde edildiğini göstermiştir. Bu koşullar altında polimerizasyon derecesi 6 ve kuru madde içeriği 17g olan şuruplar üretilmiştir. Şurubun yoğunluğu, viskozitesi, koyuluğu ve rengi sırasıyla 0.98 g/ml, 1.1 mPa-s, 0.38, ve 13.3 olarak belirlenmiştir.

Anahtar kelimeler: Frukto-oligosakkaritler, HPLC, inulin, ekstraksiyon, yerelması

INTRODUCTION

Both inulin and fructo-oligosaccharides (FOS) are fructose polymers consisting of *beta*-(2-1)-fructosyl-fructose links (ROBERFROID 1999). The terminal glucose moiety can be present but not necessary. The number of monomer units called degree of polymerization (DP) in FOS are up to 6 whereas in inulin up to 60 (ROBERFROID 1999). Because of *beta*-configuration in their structure, they are resistant to human digestive enzymes; thus they are prebiotics (GIBSON & ROBERFROID 1995). The industrial plant sources of inulin are jerusalem artichoke (*Helianthus tuberosus*) (JA) and chicory (*Cichorium intybus*) (D'EGIDIO et. al. 1998), the latter being preferred due to its characteristics and its wide availability. In contrast to bitter taste of chicory inulin, JA does not contain bitter taste compounds and because of the absence of interfering components they can be easily extracted and processed. JA is a low requirement plant grown in many countries including Turkey. JA is an alternate for the production of FOS syrups because especially of high inulin content (75-80%) (SCHORR-GALINDO & GUIRAUD 1997) and the ability of modifying DP via its native inulin synthesizing and degrading enzymes (inulinase E.C. 3.2.1.7 with optimal conditions: 55-56 °C and a pH of tubers 6-6.5) (BRICH & GREEN 1973). The usage of hydrolyzed form of inulin, that is FOS, is much more desirable since some of the properties are improved; for example, increased solubility and sweetness (FRANCK 2002). FOS have a sweetness of about 35% in comparison with sucrose and glucose, and unlike such sugars they can be used by diabetic people. The caloric value of FOS is 1.5 kcal/g which is nearly 38% of that of a digested hexose molecule (ROBERFROID 1999). Both inulin and FOS have Generally-Recognized-As-Safe (GRAS) status (FRANCK 2002). According to technological and functional aspect, the DP should be in between 3-6. The DP of the fructans vary according to the plant species, wheather and storage conditions, harvest date, extraction and post-extraction processes (D'EGIDIO et. al. 1995, CRITTENDEN & PLAYNE 1996).

FOS production is achieved either by enzymatic or acidic hydrolysis of inulin (KIM et. al. 1999, PANDEY et. al. 1999, KANG & KIM 1997) or synthetic synthesizing from sucrose (YUN & SONG 1993). Acid hydrolysis displays several disadvantages such as undesirable coloring of the hydrolysate (PANDEY et. al. 1999).

The extraction of JA have been studied by many researhers, but no study for the production of FOS from JA tubers was found in the literature. In the first study on the extraction of JA, a fructose syrup production was achieved by extracting the carbohydrates via normal diffusion at 80 °C during an hour (CONTI 1953). The highest total dry matter content was 18-26% in February harvested tubers, while the least value was found by April-harvested ones. A pH of the syrup was 4-4.4. In the study of Flemming et.al. (FLEMING & GROOTWASSINK 1979), an atmosphere of sulfur dioxide was utilized in order to lower the pH of the water to 1-2. Lower temperatures (up to 70 °C) and lower contact time (30 min) were used, the obtained extraction yield was higher (95%). In a more recent study (D'EGIDIO et. al. 1998), in which water extraction at 105 °C for 2 h was applied, after a preliminary extraction by ethanol at 96 °C, the achieved yield was 90%. For continuous preparation of fructose syrups from JA tubers, Wenling et al. (WENLING et. al. 1999) extracted 500g of tuber as dry powder with 2 L of water at 100 °C during 40 min. The maximum volumetric productivity was

obtained with inulin hydrolysis of 75%. In the latest study (LINGYUN et. al. 2006), the researchers optimized the conventional inulin extraction by using central composite design and response surface method for experimental design. The optimal conditions for maximizing inulin extraction yield (83.6%) were at neutral pH for 20 min at 76.65 °C and solvent to solid ratios of 10.56:1 (v/w).

The objective of this study was to produce high-yield, high-functional FOS syrups from JA with a DP range 3-6 by the action of its native inulinase enzyme. JA tubers has been reported to exhibit discoloration reactions during processing due to the action of polyphenoloxidases (PPO). In the research of Ziyan and Pekyardımcı (ZİYAN & PEKYARDIMCI 2003), 50% and 89 % remaining activity was found after addition of citric acid for skin and flesh PPO, respectively. Since colored compounds produced by PPO may be undesirable in food production, citric acid addition was included in the parameters investigated (extraction time and temperature, amount of solvent, harvest date, storage time, and peeling of JA tubers).

MATERIALS AND METHODS

Because of changing FOS content and average DP in the tubers, they brought from Beypazarı, Ankara (Turkey) to the laboratory in the same day as they harvested and fresh-case experiments were done. In other cases, they stored in a refrigerator at 4°C after wrapping with a paper towel to delay spoiling. All chemicals (citric acid, potassium sodium tartrate, sodium carbonate, sodium bicarbonate, sodium sulfate, copper-II-sulfate, ammonium molibdate, sodium arsenate dibasic heptahydrate, D-glucose, D-fructose, sucrose, 1-kestose, nystose, MRS broth, yeast extract, sodium acetate trihydrate) were of analytical grade supplied by Sigma-Aldrich (Steinheim, Germany) and Merck (Darmstadt, Germany). In all of the experiments, 10 g of JA tubers were grated by using an ordinary food processor (Arçelik) to obtain 2x6x40mm (average) particle size before extraction, since in the preliminary extractions the DP of tubers juice was found as 33 much higher than the desired range. *Lactobacillus plantarum* NCIMB 1193 was obtained from METU (Turkey), Food Engineering Department.

Investigated parameters were extraction time (10-60 min), temperature (40-80 °C), amount of solvent (30-50 ml) citric acid concentration (0-39 mmol/L), particle size, peeling, harvest date (January to May) and storage time (up to 20 days in the refrigerator) of the tubers. The extraction yield was determined by AOAC dry matter analysis (AOAC 2000). The DP of extracts were determined according to Somogyi (SOMOGYI 1993). Monosaccharide unit (MU), and the DP were calculated by equation 1 and 2, respectively.

$$\sum MU(mmol) = \left\{ \frac{Total \quad dry \quad matter \quad (mg)}{\left[(DP-1)x162+180\right]} \right\} xDP \tag{1}$$

$$DP = \left\{ \left[\frac{Total \quad dry \quad matter \quad (mg)}{RE \quad in \quad the \quad sample \quad (mgD-Glucose)} \right] x180-18 \right\} x \frac{1}{162} \tag{2}$$

The product profiles were determined by HPLC analysis using Aminex HPX-42C (Biorad) with refractive ındex detector. The column temperature was 80 °C, and the mobile phase was distilled water with flow rate of 0.6 ml/min. The compounds that have a DP up to four were accurately determined in these analysis, since the standarts of these compounds (fructose, glucose, sucrose, 1-kestose and nystose) were available. The amounts of sugars with DP of 5 and 6 giving distinct peaks were estimated by using the equation 3;

$$AxMWx10^{-6} = 47.006 \# F^2 - 112.25 \# F + 338.68 \quad (3)$$

The formula used was obtained by searching the relation between the calibration constants (A) calculated by area/concentration of the sugars to the number of fructose units.

The density, viscosity of the extracts were measured. The color was also measured utilizing the Hunter Lab system and the value of color difference between the standart (commercial apple juice) and syrup was calculated as it was applied in the study of Koca et.al. (KOCA et. al. 2003), while the darkness indicated the absorbance at 420nm.

The prebiotic property of the syrup obtained was analyzed by comparing the groeth of FOS-fermenter bacterium, *Lactobacillus plantarum* NCIMB 1193 on two liquid media. The first medium contained MRS basal (no carbohydrate source, only vitamins, minerals and some nucleic acids) added with 10ml of the syrup produced. The second medium was called as standart containing MRS basal and same concentration of glucose, fructose and sucrose mixture in the syrup obtained. Bacterium was activated twice by transferring them in 10ml of growth medium and incubated at 20 °C overnight. After that, 200µl of them were transferred into three replicates of growth media each of 200ml. Samples were analyzed in a spectrometer at 600nm. The control solution was the standart media with no bacteria in it.

The reproducibility of experiments was determined by extracting jerusalem artichoke tubers (that have the same harvest date, taken from the same field, and stored in a refrigerator during the same durations) with 40 ml of water containing 26mM citric acid five times in the same day to eliminate the effect of wheather and soil conditions.

Data were analyzed according to Student's t distribution test with probability level of $P<0.05$.

RESULTS

The content of raw material was characterized as 78.9% moisture, 19.8%. total carbohydrate and 1.3% ash. There were 1.6% difference in dry matter analysis and 2.1% difference in DP in reproducibility. The highest standart deviation in repeated HPLC analysis was ±0.0023. The difference in HPLC analysis were 0.59% in DP 1-2, 0.73% in DP 3-4, 0.82% in DP 3-6. Statistically different values were represented with different letters in the corresponding figures.

3.1 OPTIMIZATION OF EXTRACTION PARAMETERS

The syrup obtained with extraction of February 2005-harvested fresh jerusalem artichoke tubers with 40ml of water at 60 °C during 40min was found to have highest dry matter (15.8g) with lowest DP (6.6). This time duration was the same as the one in the study of Wenling et. al. (1999). The extraction time used in the study was lower than the studies of Conti (1953), and D'Edigio et.al. (1998).

Experiments done with October 2004-harvested fresh jerusalem artichoke tubers showed that as the DP decreases yield increases, because shorter compounds can easily be extracted from the tubers. The highest dry matter (nearly 15g) with lowest DP (7) was obtained at 60 °C. It was the lowest temperature for extraction processes in the literature. The decrease in yield and increase in DP was observed at 70 °C and 80 °C. It can be explained by the inactivity of the native inulinase enzyme, since these temperatures are so higher than the optimum temperature of the enzyme stated in the literature (Brich et.al. 1973). It may also result from the change that decreases the permeability of the tissue.

Extraction of December 2004-harvested fresh jerusalem artichoke tubers with 40 ml of water produce highest dry matter in syrups. Thus, constant solid to solvent ratio was chosen as 1/4 as in the study of Wenling et.al. (1999). Nearly 31% increase in yield, and 13% decrease in degree of polymerization was obtained with February 2005-harvested 10 day-stored whole tubers. Thus, it was concluded that the native inulinase enzyme is in or near to the shell of the tubers.

As a result, the optimum conditions for the extractions were found as 60 °C, 40 min, 40 ml of water, whole tubers usage. Under those conditions, depending on the harvest date, storage time, wheather and soil conditions, the syrups having 12 to 16g dry matter with a DP of 6 to 7 were obtained. Nearly same yields were obtained with extractions with reduced time and temperature that reduce the cost of the process. Extraction at 60 °C, near to the optimum temperature of the inulinase enzyme, supported the hydrolysis of inulin; thereby reduced the cost.

3.2 THE EFFECT OF CITRIC ACID ADDITION, HARVEST DATE AND STORAGE TIME

Highest dry matter contents with lowest DP were obtained with extractions in which 26mM citric acid added solvents, and those conditions were called as acidic conditions. The medium was found to behave as buffer, no change in DP was observed in both of the syrups obtained under non-acidic and acidic conditions following up to 90 min after extraction. The pH of the medium was found as 3.8 which was lower than Conti's study (1953) and higher than Flemming and coworkwer's one (1979). As a conclusion acid addition aiming color reduction may also prevent contamination as it was aimed also by Flemming et.al. (1979).

Since acid addition did not increase the dry matter extracted significantly, obtained dry matter values with the extraction of JA tubers of different harvest date and stored in a refrigerator during different time durations were represented in Figure 1.

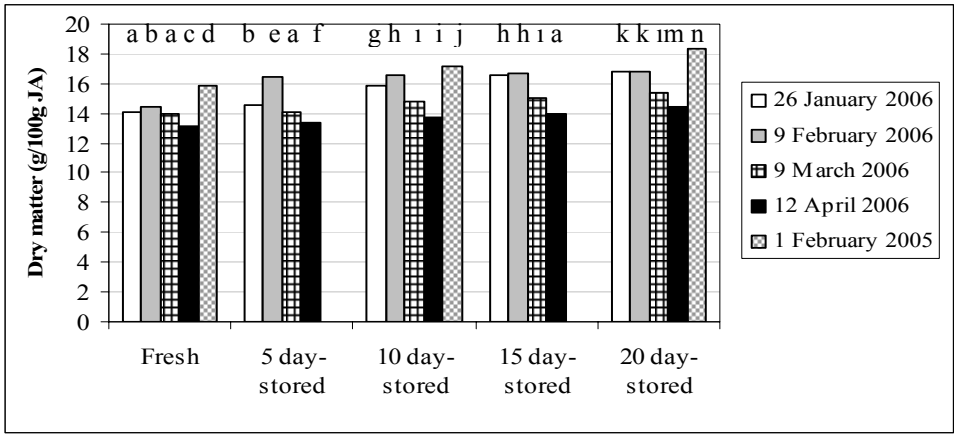

Figure 1. The effect of harvest date and storage time on dry matter of the extracts obtained by conventional water-bath extraction under non-acidic conditions of jerusalem artichoke tubers harvested in the years 2005 and 2006.

Their corresponding DP values were given in Figures 2 and 3 for non-acidic and acidic conditions, respectively. Comparing Figure 2 and 3, it was concluded that acid addition may produced the decrease in DP under some cases depending on the inulin content and average DP of the tubers which also depends on wheather and soil conditions. It was found that DP decreased and thus extracted dry matter increased as storage time increased under both conditions, since the required energy for respiration during storage met from the inulin breakdown. Highest dry matters with lowest DP were obtained by extracting February-harvested tubers in both of the years and both of the conditions. It was concluded that the year 2005 was a better year since higher dry matter and lower DP could be obtained.

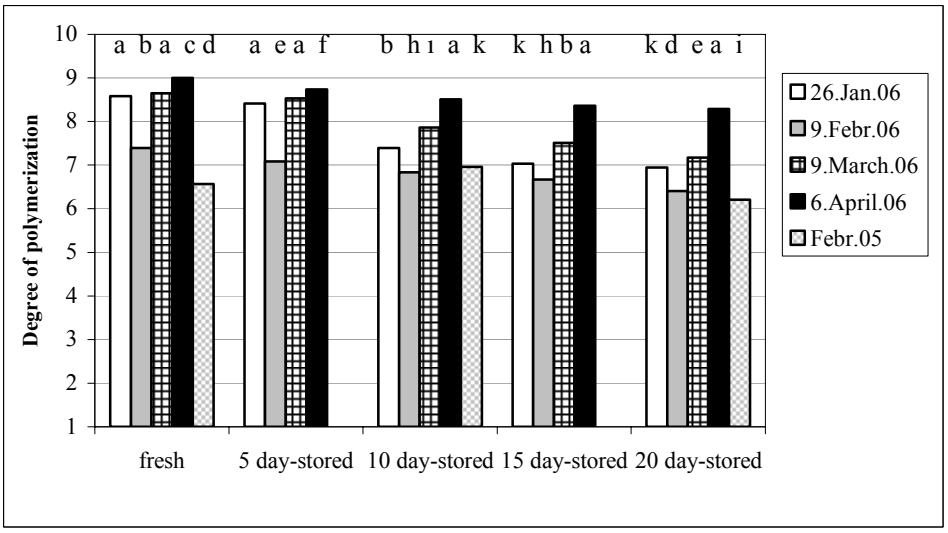

Figure 2. The effect of harvest date and storage time on degree of polymerization of the extracts obtained by conventional water-bath extraction under non-acidic conditions of jerusalem artichoke tubers harvested in the years 2005 and 2006.

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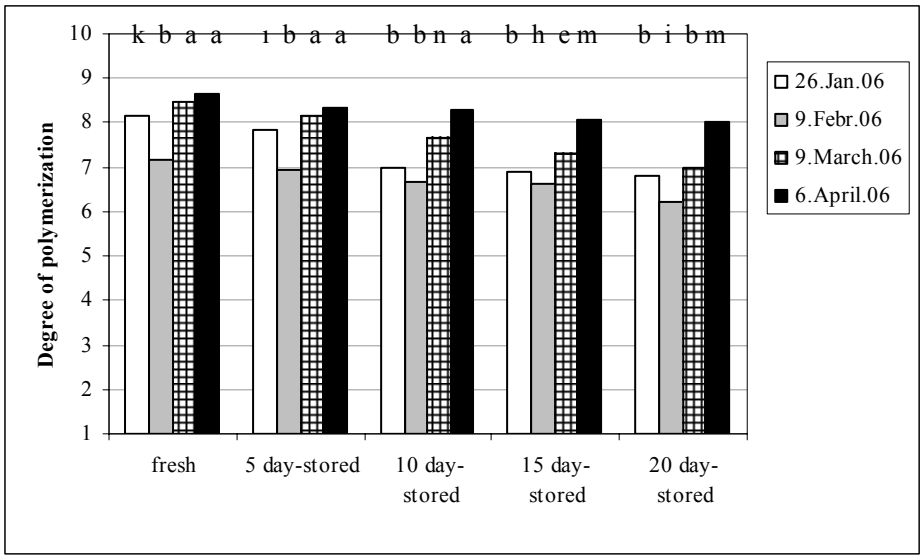

Figure 3. The effect of harvest date and storage time on degree of polymerization of the extracts obtained by conventional water-bath extraction under acidic conditions of jerusalem artichoke tubers harvested in the years 2005 and 2006.

Comparing the product profiles of syrups obtained by using February-harvested (most suitable harvest date) tubers (Figures 4 and 5), the amounts of sugars with DP 1-2 (regarded as waste according to the prebiotic point of view, since they will be digested by human enzymes and will not reach to the probiotic bacteria in the colon) and DP 3-6 (regarded as functional) were found flactuating due to the combined effects of storage time, wheather and soil conditions and acid addition. Under both of the conditions, 20 day storage produced the highest amount of functional and the lowest amount of waste sugars.

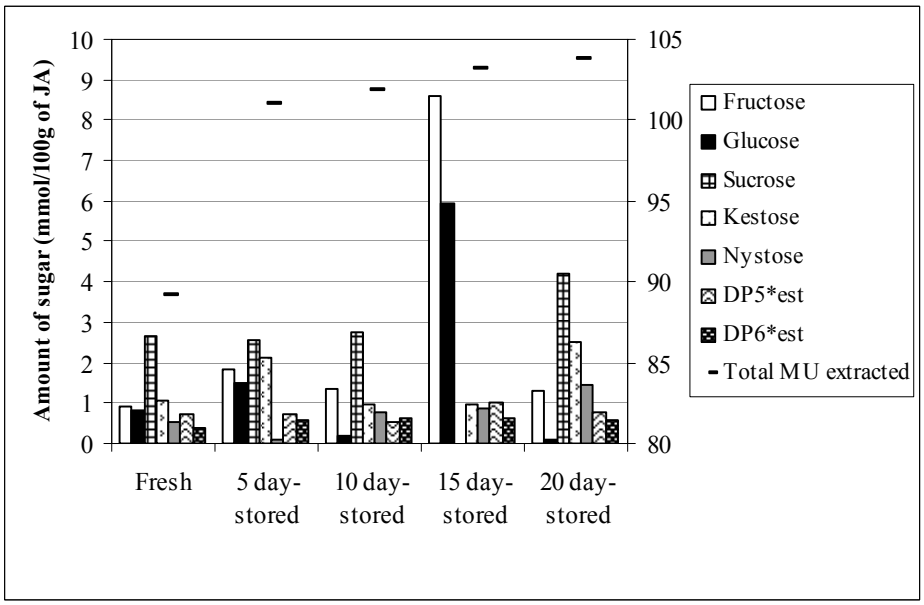

Figure 4. The effect of storage time on product profiles of the extracts obtained by conventional water-bath extraction under non-acidic conditions of February 2006-harvested jerusalem artichoke tubers.

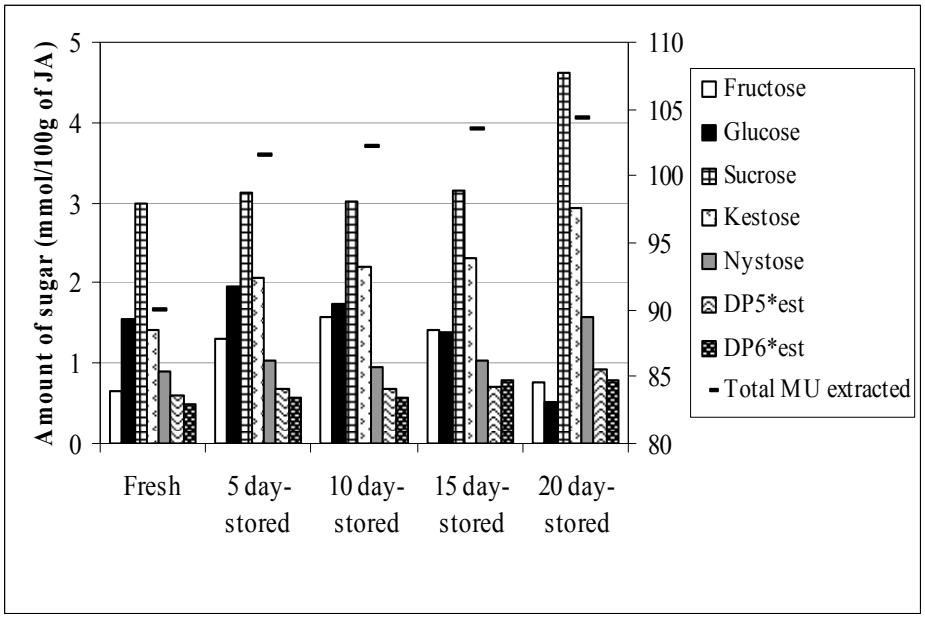

Figure 5. The effect of storage time on product profiles of the extracts obtained by conventional water-bath extraction under acidic conditions of February 2006-harvested jerusalem artichoke tubers.

In the analysis of physical properties, harvest date and storage ime were found unaffective any of them measured. Corresponding average values for density, viscosity, color and darkness of the syrups were found as 0.97 g/ml, 1.03 cp, 42.3, and 1.89; and 0.98 g/ml, 1.1 cp, 13.3, and 0.38 for non-acidic and acidic conditions, respectively. The darkness and color values are unitless because they were obtained from comparison with the control solution. It was found that acid addition decreased the color and darkness of the syrups nearly 70 and 80%, respectively, but it did not affect the density and viscosity.

Fluctuations were also observed in functionalities of the syrups (Figures 6 and 7) because of the same reasoning as it was stated before. Most functional sugars were obtained with mid-February-harvested 20 day-stored tubers, and its prebiotic property was verified by 1.26 times increase in growth rate (Figure 8). No change of time passing to the stationary phase was showed that the FOS in the syrup produced was as good substrate as simple sugars for the microorganism chosen.

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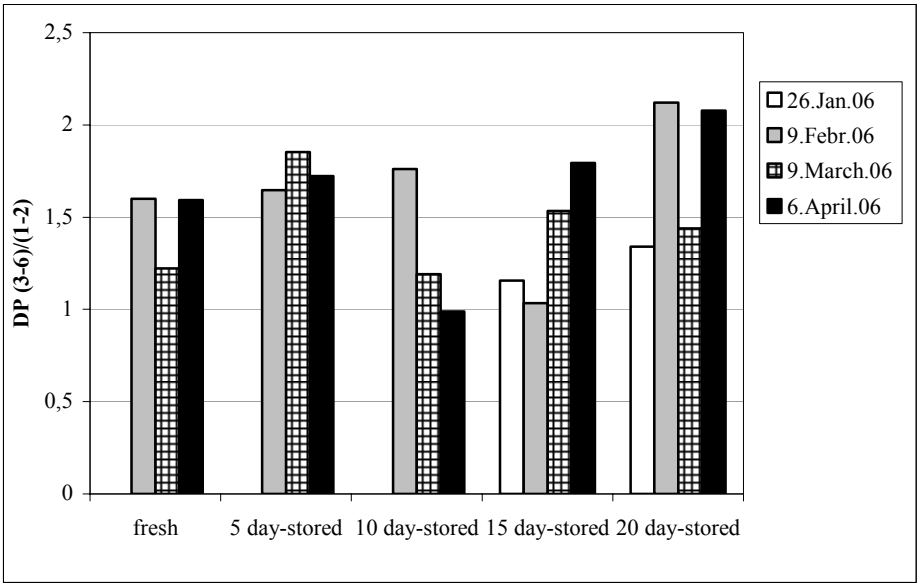

Figure 6. The effect of harvest date and storage time on functionality of the extracts obtained by conventional water-bath extraction under non-acidic conditions of 2006-harvested jerusalem artichoke tubers.

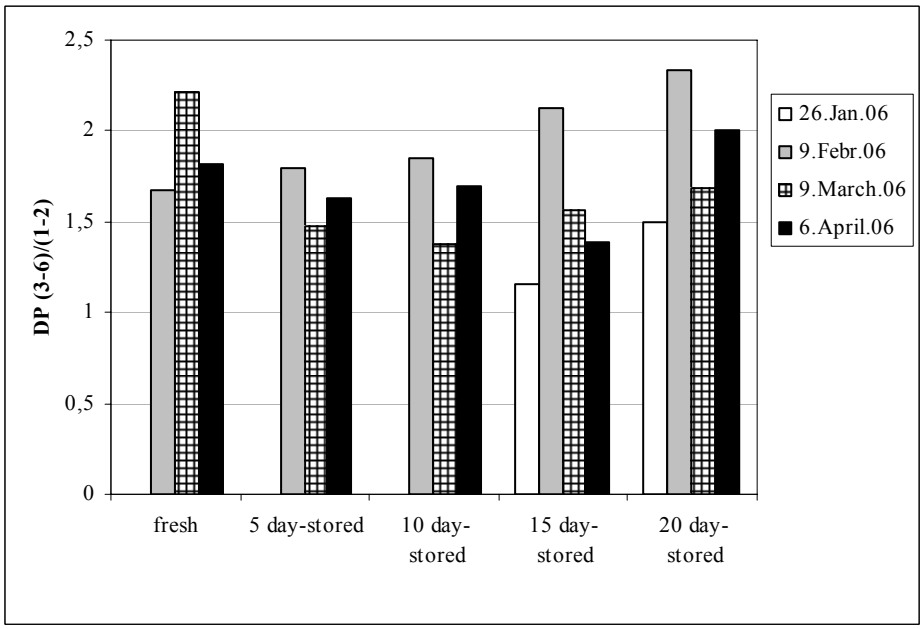

Figure 7. The effect of harvest date and storage time on functionality of the extracts obtained by conventional water-bath extraction under acidic conditions of 2006-harvested jerusalem artichoke tubers.

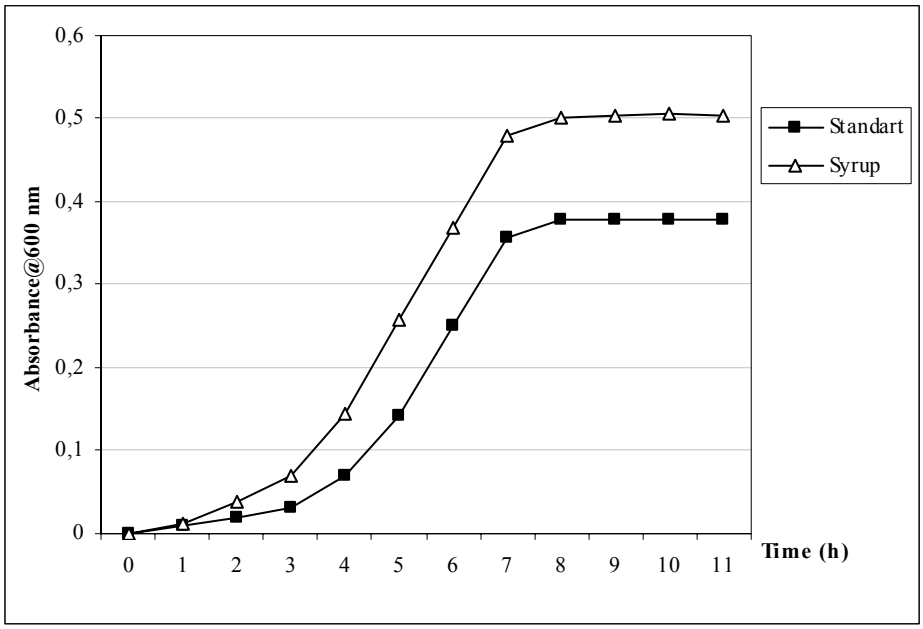

Figure 8. The verification of the prebiotic content of the syrup obtained by conventional water-bath extraction under acidic conditions of February 2006-harvested, 20 day-stored jerusalem artichoke tubers by a probiotic microorganism, *Lactobacillus plantarum* NCIMB 1193.

If the results of 26th of January-harvested 15 day-stored samples compared with those of 9th of February harvested fresh ones to determine whether storing under soil or in a refrigerator is better, higher dry matter (Figure 1) and lower DP (Figures 2 and 3) were obtained via storing in a refrigerator, but the functionality was also lowered (Figure 6 and 7). Thus, it was concluded that storing under soil up to mid-February was found better than storing in a refrigerator since the decrease in DP resulted from increase in the amounts of waste sugars.

DISCUSSION

In this study, Mid-February harvested whole tubers stored during 20 day at 4°C in a refrigerator and grated in rectangular prism and the extraction with 40 ml of water containing 26 mM citric acid during 40 min at 60°C were found to be the optimum conditions. It was concluded that these syrups can be produced from January-to-May of the same year with nearly 20% decrease in dry matter, and 15% decrease in functionality with increasing DP from 6 to 9. The physical properties of the syrups were measured as 0.97 g/ml for density, 1.1 cp for viscosity, 42.3 and 1.89 for color and darkness with no change because of the harvest date and storage time. Acid addition reduced the color and darkness of the syrups nearly 70 and 80%, respectively, which may be so important in the production of colorless foods. Harvest date and storage time were found strongly affected on the modification of the sugar content, thus dry matter, DP, and functionality of the syrups.

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NOMENCLATURE

FOS: Fructo-oligosaccharides
DP: Degree of polymerization
HPLC: High Performance Liquid Chromatography
JA: Jerusalem artichoke
AOAC: American Association of Analytical Chemists
PPO: Polyphenol oxidases
MRS: Basal medium containing (10g pepton from casein, 4g yeast extract, 2g di-potassium hydrogen phosphate, 1ml Tween-80, 2g di-ammonium hydrogen citrate, 8.3g sodium acetate trihydrate, 0.038g magnesium sulphate monohydrate dissolved in 1000ml of distilled water)
NCIMB: National Collections of Industrial and Marine Bacteria