EXTRACTION AND ACID HYDROLYSES OF FRESH JERUSALEM ARTICHOKE INULIN FOR FRUCTOOLIGOSACCHARIDE PRODUCTION

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ABSTRACT

The objective of this study was to produce fructooligosaccharide (FOS) from fresh jerusalem artichoke (JAT) inulin which was extracted by conventional method (hot water extraction) at two different temperature (80˚C & 90˚C\ 90 min). JAT slices were mixed with distilled water at (1 : 5) (w : v) ratio, the obtained extract purified and concentrated to 32˚Brix, then mixed with acetone (1 : 3) (v : v), kept at 4˚C for 24 h., centrifuged at 10000g/ 15 min., and the precipitate dried at 55˚C. Acid hydrolysis of the dried inulin was carried out at three different pH values being (1.5, 2.5 & 3.0) at 85˚C using citric acid (10 %), aliquot of inulin hydrolysate were taken after (5, 10, 15, 20, 25 & 30) min. for qualitative analysis using TLC and RP-HPLC. The results revealed that there were no significant differences in the yield of extracted inulin (33.86, 33.67 %) and extraction efficiency (80.62, 80.17 %) between the extraction at 80˚C and 90˚C\ 90 min. respectively. The optimum condition for acid hydrolysis of the obtained inulin was at pH 2.5 after 15 min at 85˚C. The qualitative analysis for hydrolysate using TLC and RP-HPLC showed that the degree of polymerization (DP) of inulin ranged from (2 - 35) unit and for FOS ranged from (2 – 9) units.

Keywords: hydrolysate, FOS, JAT, citric acid, TLC and RP-HPLC.
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استخلاص والتحلل الحامضي للانيولين من درنات الالمازة الطازجة لانتاج سكريات الفركتوز القصيرة

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المستخلص:

هدفت الدراسة الحالية الى انتاج سكريات الفركتوز القصيرة من الانيولين المستخلص من درنات الالمازة الطازجة بالطريقة التقليدية (الاستخلاص المائي) بحرارة (80 مْ و 90 مْ لمدة 90 دقيقة). إذ تم مزج شرائح درنات الالمازة الطازجة مع الماء المقطر بنسبة خلط (1 : 5) (وزن : حجم) واجريت عملية الاستخلاص للمزيج لمدة 90 دقيقة وبعد تنقية الانيولين من الشوائب تم تركيز المستخلص الناتج الى 32 بركس تلتها عملية المزج مع الاسيتون بنسبة خلط (1 : 3) (محلول استخلاص : اسيتون) وترك المزيج على حرارة 4 مْ لمدة 24 ساعة, ثم نبذ مركزياً بسرعة 10000g / 15 دقيقة وجفف الراسب الناتج عند حرارة 55 مْ. اجريت عملية التحلل الجزئي الحامضي للانيولين المنقى باستخدام حامض الستريك (10 %) عند ثلاثة ارقام هيدروجينية مختلفة (1.5, 2.5 و 3.0) بدرجة حرارة 85 مْ بمدد زمنية بلغت (5, 10, 15, 20, 25 و 30) دقيقة, وتم تشخيص الانيولين المستخلص ونواتج التحلل الجزئي للانيولين بأستخدام تقنية RP–HPLC و TLC. اشارت النتائج المستحصل عليها الى عدم وجود فروق معنوية في الحصيلة (33.86, 33.67 %) وفي كفاءة الاستخلاص (80.62, 80.17 %) بين الاستخلاص عند درجة حرارة 80 مْ و 90 مْ/ 90 دقيقة, كما بينت النتائج ان الظروف المثلى لانتاج سكريات الفركتوز القصيرة بالتحلل الجزئي الحامضي كانت عند استخدام الاس الهيدروجيني 2.5 / 85 مْ ووقت تحلل 15 دقيقة. بينت نتائج التحلل الكمي والنوعي بتقنيتي RP–HPLC و TLC ان درجة البلمرة للانيولين تراوحت مابين (2 – 35) وحدة ولسكريات الفركتوز القصيرة تراوحت مابين (2 – 9) وحدة.

الكلمات الافتتاحية: متحللات, FOS, JAT, حامض الستريك, RP–HPLC و TLC.
***البحث مستل من أطروحة دكتوراه للباحث الاول**

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INTRODUCTION

Inulin, a non-digestible polysaccharides, consisting of several fructose residues linked by β (2→1) glycosidic bond and eventually a terminal glucose unit molecule (α, D-glucopyranose) linked to fructose by an α-(1→2) glycosidic linkage (12). In many members of the *Asteraceae* family such as *Helianthus tuberosus* (Jerusalem artichoke) and *Cichorium intybus* (chicory) inulin serves as main storage carbohydrate. Puttha *et al*. (26) reported that the Jerusalem artichoke tubers (JAT) is containing about 14 % to 19% of inulin (from fresh weight) so it can be a valuable source for inulin. Inulin extracted from JAT is characterized by a degree of polymerization (DP) ranging (2 - 60) units. The oligosaccharides of inulin with DP less than 10 units called of fructooligosaccharide (FOS) is one of the bestknown prebiotics (4). FOS can be obtained through partial hydrolysis of inulin, with different (DP) (ranging from 2 - 9 units), which has a relative sweetness ranging (10 - 40) % of sucrose sweetness (19). Inulin and FOS are considered as functional food that is beneficial to human health through decrease the risk of some diseases like intestinal infections, colon cancer, diabetes, constipation, obesity and increase intestinal absorption of calcium and magnesium in small intestine (15, 37). Several methods for inulin extraction from JAT have been adopted by researcher. Li *et al*. (18) used hot water for inulin extraction (at 70 - 100 ˚C for 60 - 90 min.) after pretreatment of fresh JAT (peeling & polyphenol oxidases inhibition), and then followed by a purification step to remove the impurities (25). The current study was aimed to extract inulin from fresh JAT and partially acid hydrolysis of purified inulin for producing FOS using citric acid at different pH values (1.5, 2.5 & 3.0)/ 85˚C. The hydrolysis product analyzed using RP-HPLC and TLC technique.

MATERIALS AND METHODS

Chemicals and reagents:

Standard chicory inulin, glucose, fructose, sucrose, raffinose and 1-kestose were obtained from (Sigma, Germany). All chemicals and reagents used were of analytical reagent grade and were used as received without any further purification.

Determination of inulin content in fresh JAT:

Saengkanuk *et al*. (30) method was adopted to determine Inulin content in JAT according to the following equation:

Inulin content (%) = *k* (Total fructose – Free fructose)

(*k* is correction factor = 0.995)

Preparation of JAT:

The fresh Jerusalem artichoke tubers (locally cultivated) were purchased from local markets in Baghdad, Iraq; it was harvested in November to December (2018). The samples of JAT were prepared according to the method described by Abou-Arab *et al*. (1).

Extraction of inulin from JAT

Inulin was extracted from fresh JAT by hot distilled water at 80˚C/ 90 min. and 90˚C/ 90 min., and the mixing ratio was (1 : 5) (w : v). the pH of extraction solution adjusted to 7 (10). The impurities (protein, pectin, and cell wall materials) from extraction solution were removed according to method described by Paseephol *et al*. (25). Then the extract was concentrated to 32°brix and precipitated according to Ku *et al*. (17) method, and Inulin yield was calculated according to the equation described by Terkmane *et al*. (35).

Y = (mI/mJAT) x100

Where, mI is the inulin mass obtained from the extraction and mJAT is the mass of artichoke tubers taken for the extraction.

Analytical methods

Phenol-sulphuric acid method was adopted to determine total carbohydrate (8), dinitrosalicylic acid method to determine of reducing sugar (20), resorcinol reagent method for free fructose determination (5) and the moisture contents was determined by the method described by AOAC, (3).

Inulin hydrolysis for producing FOS

Purified Inulin solution (5 %) at different pH values (1.5, 2.5 & 3.0) were prepared using citric acid solution (10 %), these solution subjected to partial acid hydrolysis at 85˚C for (5, 10, 15, 20, 25 & 30) min. The pH of inulin hydrolysates were adjusted to 7 using calcium hydroxide (0.6%), then clarified (the salts precipitated) using Heding *et al*. (13) method. The supernatant was concentrated to 32 brix°, FOS and inulin precipitated according to Ku *et al*. (17) method, then they obtained inulin and

FOS were dried (at 55˚C/ 12 h) using the method described by Sangeetha, (32). The percentage of (FOS & inulin) remaining after acid hydrolysis of inulin was calculated according to Ngampanya *et al*. (23) method, using the following equation:

(%) FOS & Inulin = Total carbohydrate – reducing sugar

HPLC analysis

Sample analysis was performed using RP-HPLC model LC-2010 a HT. UV- detector 190 nm. Equipped with a quaternary pump and empower software. An nucleodur NH2 columns (column temperature 40°C, mobile phase of acetonitrile : water (75:25) (v/v), flow rate of 1.4 ml/min and the sample injection volume 10 µl). Using (Fructose, glucose, sucrose, raffinose, 1-Kestose and Inulin from chicory root) as standards (22).

Thin-layer chromatography identification

The qualitative identification of inulin and FOS was performed by (TLC) before and after hydrolysis of inulin according to the method described by Reiffova & Nemcova, (28).

Statistical analysis

The result was statistically analyzed by the (LSD) value at 0.05 probability level as described in Al-Juthery *et al*. (2).

RESULTS AND DISCUSSION

Chemical composition of JAT:

Table 1 shows the chemical composition of locally cultivated fresh JAT which included (moisture, total solid, total carbohydrates, inulin and reducing sugar). The total carbohydrate percentage was 14.20 %. This result is conforms to Barta & Patkai, (6) findings who reported that the total carbohydrate content in fresh JAT was 14.97%. The percentage of inulin in experimental JAT was 9.66 %. This is in line with Sahar, (31) who stated that inulin content in fresh JAT was 9.60%. The high content of inulin makes JAT a potential source for the production of inulin and FOS at the commercial scale. The results in table 1 also indicate that the moisture content of JAT was 77.0 %; this is consistent with Munim *et al*. (21) finding who stated that the moisture content in fresh JAT was 78.01%. Finally, the percentage of reducing sugar in fresh JAT was 0.49 %. Our finding was lower than that mentioned by Rubel *et al*. (29) who reported that fresh JAT contained 0.68 % of reducing sugar.

Table 1. Chemical composition of locally cultivated JAT

Constituents	(%) Fresh JAT
Moisture	77.00
Total solid	23.00
Total carbohydrates	14.20
Inulin	9.66
Reducing sugars	0.49

Extractable inulin yield from JAT

Data presented in Fig. 1 shows the inulin recovery from fresh and fermented JAT which was extracted at 80˚C and 90˚C/ 90 min. The percentages of extractable inulin were (33.86 % & 33.67 %) respectively. with no significant differences between them (P<0.05). Fig. 1 shows that the yield of inulin extracted from the fermented JAT was 29.71 %. The low inulin yield in fermented JAT attributed to the consumption of inulin by microorganism as carbon sources besides the hydrolysis of inulin to fructose units by exo-inulinase bacteria. Yokoi *et al*. (38) found that during the fermentation of JAT slices for 10 days in brine (sodium chloride), LAB bacteria such (L. *mesenteroides*, L. *lactis*, L. *plantarum*) was dominant in fermented product. Concerning the extraction efficiency (EF), Fig. 1 demonstrates that the highest EF (80.62 %) achieved at 80˚C\ 90min., followed by 90˚C\ 90 min (80.17 %). Sahar, (31) found that the highest EF of inulin was achieved at 80˚C\ 90 min using conventional extraction method.

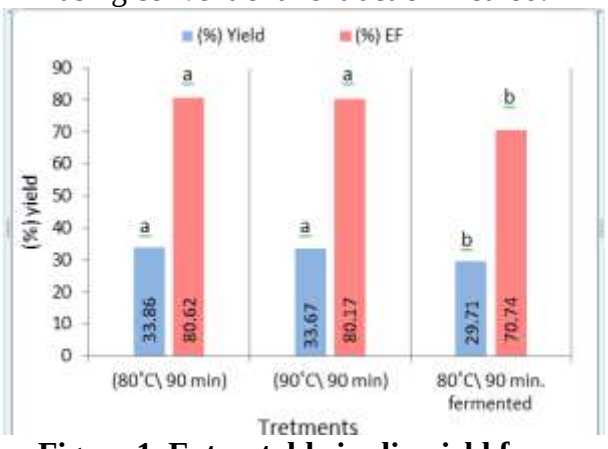

Figure 1. Extractable inulin yield from fresh & fermented JAT at 80˚C & 90˚C for 90 min

Characterization of extracted JAT inulin

The chromatographic profile of extracted JAT inulin was determined by RP-HPLC method as shown in Fig. 2. In comparison with native chicory inulin, the DP profile of inulin showed that the DP of inulin ranging from (2 - 35)

unit. These results are consistent with Panchev *et al*. (24) who stated that the DP for inulin extracted from JAT ranging from (2 - 33) units. Based on the generally accepted presumption that the retention time of a inulin units increased as the degree of polymerization increased, and that each sequential peaks appeared in chromatographic pattern, represents an inulin moiety which had a fructose more than that of the previous peak (7). Additionally as expected the well resolved peaks suggesting that inulin and FOS were linear units. It has been noticed the DP for FOS ranging between (2 - 9) units, started to appear after the sucrose peak. The percentages of FOS in inulin purified extracted reached 16.30 %. This result was in agreement with Judprasong *et al*. (14) who stated that the content of FOS in inulin extracted from fresh JAT was 19.18 %, finally, the percentage of reducing sugar reached to 3.22%. Our results are in agreement with those of Khuenpet *et al*. (16) who found that the reducing sugar content in inulin extracted from fresh JAT was 3.45 %. The percentage of remaining carbohydrates represents the pure inulin with DP more than 10 units.

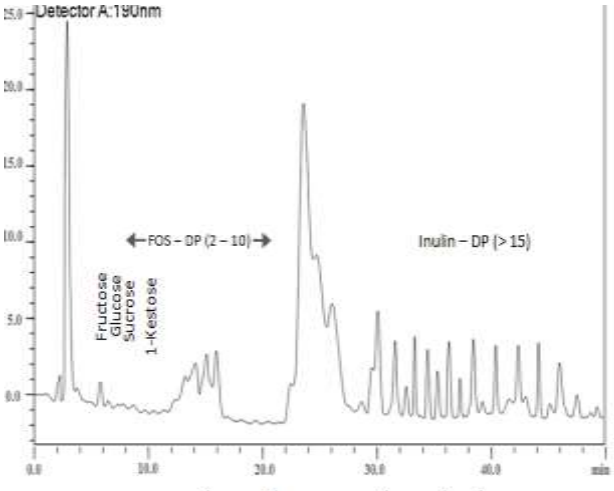

Figure 2. High-performance liquid chromato-graphic analysis of fresh JAT inulin.

Qualitative analysis (TLC) of inulin extracted from fresh JAT :

TLC analysis of fresh JAT inulin (Fig. 3), shows a polydisperse inulin moiety with different DP ranging from (2 - 9) units. The analysis of a TLC chromatogram of a homologous series of carbohydrates is based on the generally accepted assumption that each spots represents an inulin units with a certain DP value and that a higher DP leads to a decrease in the retention factor (RF) (33). Therefore, it is assumed that each spot has one more fructose unit than the previous spot. Results of TLC corresponded with RP-HPLC analysis in the inulin units separation. Then due to increasing of molecular weight, this debars their mobillity with the mobile phase and remains close to the baseline. Our results are in correspond with those of Walz *et al*. (36) who found the first spots on TLC in line belongs to monosaccharides (fructose or glucose) with the lowest molecular weight, then sucrose and gradually other components of FOS or polysaccharide polymer chain with increasing molecular weight

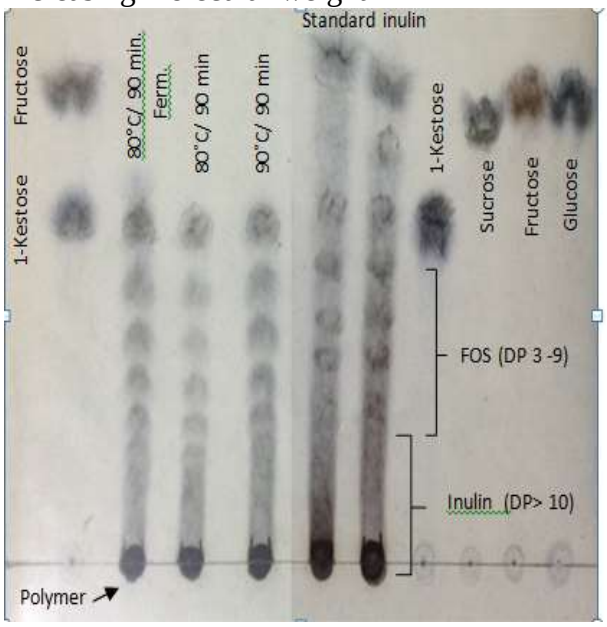

Figure 3. Thin layer chromatography pattern of inulin extracted from fresh JAT

Optimization of inulin hydrolysis to produce FOS and chromatographic characterization:=The FOS was excluded from crude inulin extract before acid partial hydrolysis of inulin as shown in fig. 4; the objective of this step was to identify the percentages of FOS produced due to the partial hydrolysis. The FOS content in purified inulin became 3 % while it was 16.30 % in crud inulin.

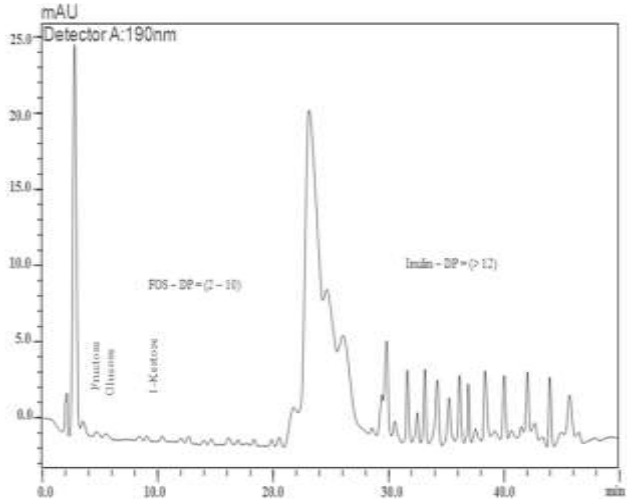

Figure 4. High-performance liquid chromato-graphy pattern of inulin purified from FOS.

Partial hydrolysis of inulin was carried out using different pH values and different hydrolysis period at 85˚C (Fig. 5, 6 & 7). Fig. 5 shows that the percentage of (remaining inulin and produced FOS mixture) significantly ($p<0.05$) decreased from (63.19% to 14.97%) during reaction time (5 - 30) min. respectively. Fig. 5 also illustrate that the percentage of free fructose was significantly ($p<0.05$) increased from (33.13% to 66.60%) at the hydrolysis time (5 - 30) min. respectively, Razmovski *et al*. (27) stated that using low pH values ranged from (1 - 2) and temperature higher than 85˚C accelerated hydrolysis of the glycosidic bond, resulting in high percentage of free fructose

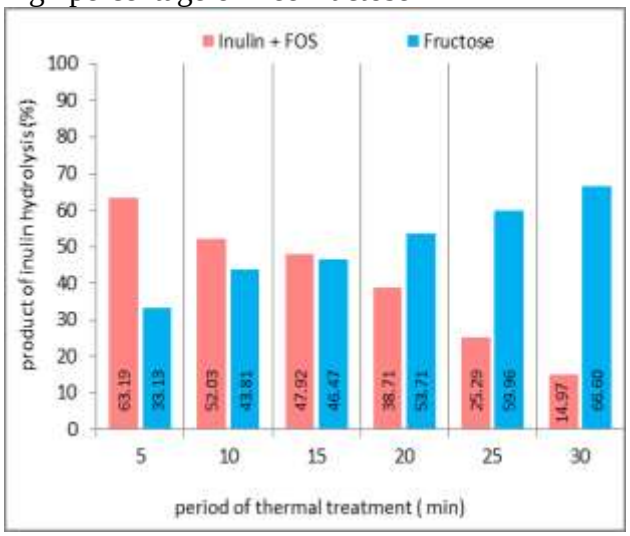

Figure 5. Percentages of (remaining inulin & produced FOS mixture) & free fructose during acid hydrolysis of inulin (pH 1.5\ 85˚C)

Fig. 6 shows that the percentage of (remaining inulin and produced FOS mixture) upon the hydrolysis at pH 2.5 were (88.47, 83.19, 77.72, 66.82, 59.40 & 48.02) % at (5, 10, 15, 20, 25 & 30) min. respectively. While, percentage of free fructose was increased as the hydrolysis time increased. After 5 min. of hydrolysis the percentage of free fructose was (8.03 %). While after 30 min. was (44.92 %). Our results agreed with that of Glibowski *et al*. (11) who found that the high temperature and low pH value enhance the acid hydrolysis of inulin, as well as agree with Szambelan & Nowak, (34).

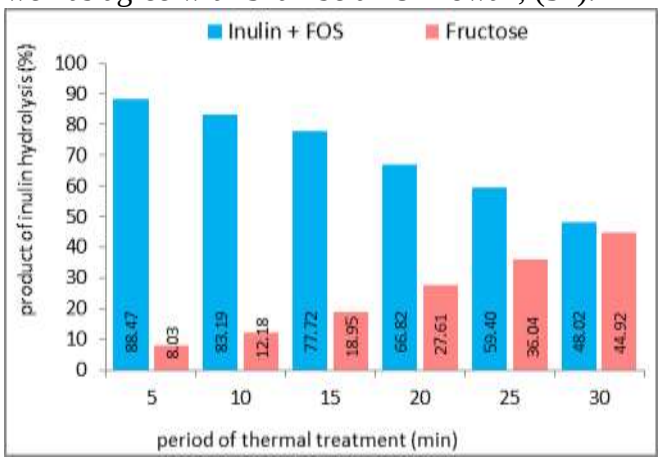

Figure 6. Percentages of (remaining inulin & produced FOS mixture), free fructose during acid hydrolysis of inulin (pH 2.5\ 85˚C)

Fig. 7 shows inulin hydrolysate products (pH 3) the depolymerization were slow, the percentage of (remaining inulin and produced FOS mixture) was (89.31, 83.91, 78.73, 71.07, 65.44 & 59.94) % at (5, 10, 15, 20, 25 & 30) min. hydrolysis respectively. The free fructose percentage were increased from (6.75 % to 31.48 %) at (5 - 30) min. hydrolysis respectively.

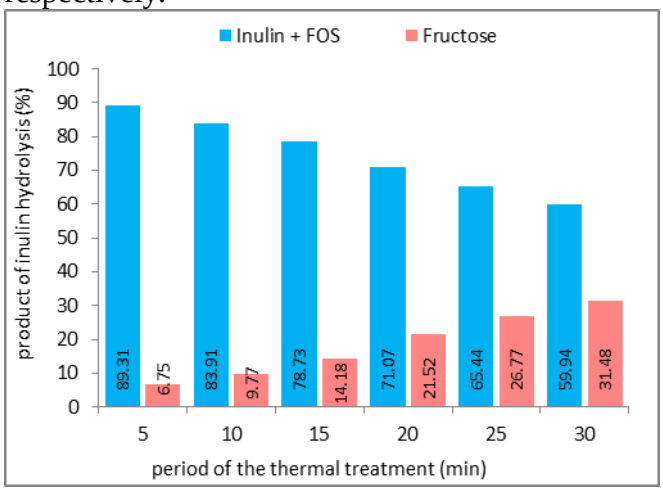

Figure 7. Percentages of (remaining inulin & produced FOS mixture), free fructose during acid hydrolysis of inulin (pH 3.0\ 85˚C)

Fig. 8 shows fructose free FOS products (DP, 2–9) in inulin hydrolysates which produced using citric acid at pH, 2.5\ 85˚C for (5, 10, 15, 20, 25 & 30) min. designated (A, B, C, D, E & F) respectively. The percentages of FOS were increased through hydrolysis time from 5 to 15 min., and then the percentages of FOS were decreases with hydrolysis time. The percentages of FOS were (23.26, 31.65, 40.37, 32.56, 27.89 & 20.80 %) at hydrolysis time (5, 10, 15, 20, 25 & 30) min respectively. The qualitative profiles clearly indicate the dominancy of FOS (up to 15 min hydrolysis), then free fructose started to increases with hydrolysis time (up to 30 min.). Fontana *et al*. (9) stated that the best conditions for acid hydrolysis of inulin were with citric acid (pH 2.5), temperature 85˚C and reaction time 15 min, which resulted in high amount of FOS with DP ranging from (2 - 9) units. Fig. 9 shows that the degree of hydrolysis of inulin increases with hydrolysis time (5 – 30 min) at pH 1.5. Consequently, the amount of free fructose increased, so these conditions can be applied for producing high fructose syrup. It has been noticed that pH 1.5 was more effective in inulin hydrolysis than pH 2.5. The hydrolysis at pH 2.5 gave the highest percentage of FOS with low amount of free fructose through (5 - 20) min. The qualitative analyses indicate an increase in the amount of FOS during the first 15 min of hydrolysis; this was coincides with an increase in free fructose (up to 30 min) and decreases in DP of inulin. At pH 3.0 the hydrolysis of inulin was less effective in deploymerization of inulin as compared to that at pH 1.5 and 2.5. In conclusion the JAT inulin was extracted at (80˚C\ 90 min) and the yield of purified inulin was (33.86 %) with extraction efficiency up to (80.62%). FOS was produced by partial acid hydrolysis of the purified inulin at pH (1.5, 2.5 & 3.0). This inulin polymer was partially depolymerized in aqueous citric acid solution (pH 2.5\ 85˚C) to release FOS, with a degree of polymerization ranging from (2 – 9). There is no need for catalyst removal in most industrial applications of the FOS syrups that are produced with citric acid.

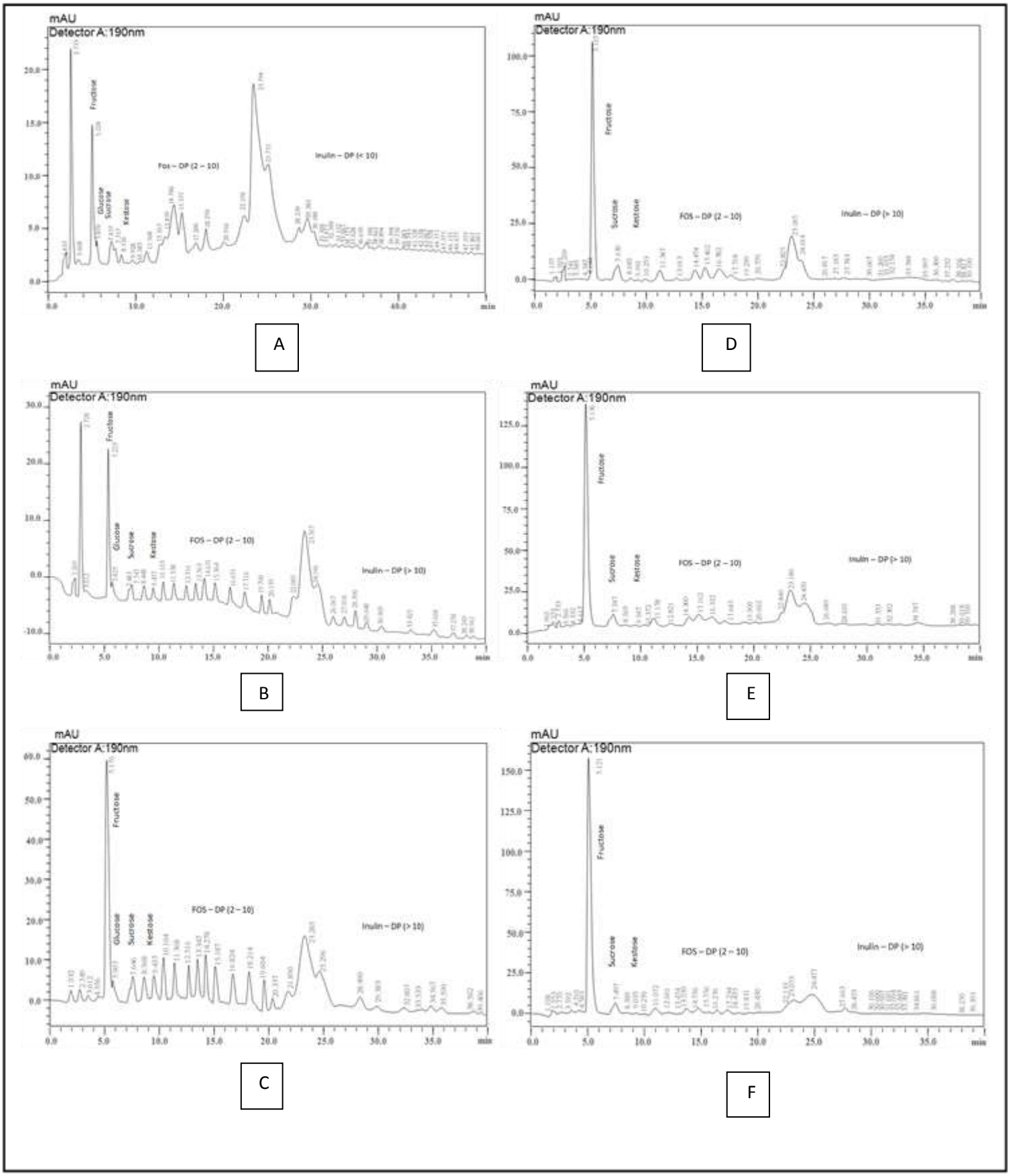

Figure 8. RP- HPLC analysis of (FOS) produced at pH of 2.5\ 85°C, for (5, 10, 15, 20, 25 & 30) min. referred to (A, B, C, D, E & F) respectively

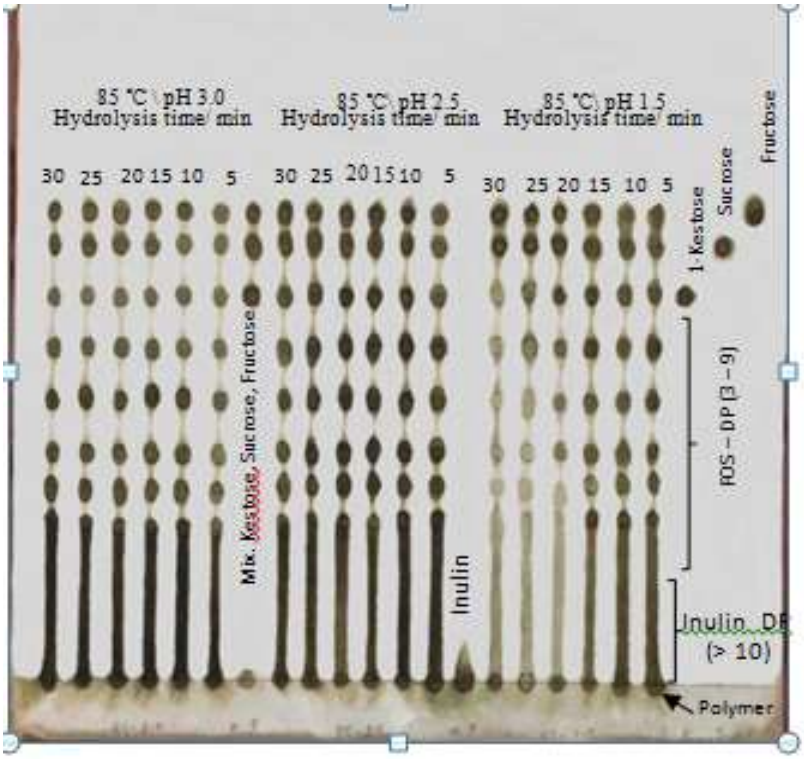

Figure 9. TLC analysis of fresh JAT inulin hydrolysis produced at pH (1.5, 2.5 & 3.0)\ 85˚C after (5, 10, 15, 20, 25, 30) min. hydrolysis

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