مجلة دجلة • المجلد ٦ ،العدد ٣ (٢٠٢٣)، ص: ٤٢ ـ ٥١
ISSN: 2222-6583

# Elimination of rhuvnose family sugars from some legumes using *S. cerevisiae* yeast producing the enzyme α-galactosidase

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## Abstract

Rhaphinose family sugars found in legumes cause lower abdominal bloating, and to reduce or reduce these sugars there are many ways to get rid of these sugars, including soaking and removing the crust or soaking with high pressure. And ***yeast S.*** cerevisiae under different conditions of temperature, pH and duration of soaking using the HPLC device located in the laboratories of the Department of Environment and Water \ Ministry of Science and Technology and we obtained that the treatment of soaking chickpea seeds with the use of baking yeast at a concentration of 15 g \ 100 g by reducing the soaking time and for a period ranging between (2-4) hours at a temperature ranging between 35-40 ° C and with a pH range of 4 and 4.5 and the complete removal of all RFOs sugars by 100% bean seed soaking treatment with the use of baking yeast at a concentration of 10 g / 100 g by reducing the soaking time and for a period ranging between (2-4) hours and at a temperature of 40 m and with a pH range of 4.5 to 4, to obtain complete removal of all RFOs with a loss rate of 100%. The use of bread yeast, because it is abundant in the local markets, cheap and nutritionally safe for the consumer and its ability to produce the enzyme α-gahactosidase, which proved its ability to analyze RFOs sugars in the current study.

## المستخلص

سكريات عائلة الرافينوزالموجودة في البقوليات تسبب انتفاخ أسفل البطن، و للتقليل او الحد من هذه السكريات توجد طرائق كثيرة للتخلص من هذه السكريات منها النقع و إزالة القشرة او النقع مع الضغط العالي .أجريت هذه الدراسة على نوعين من البقوليات هما الحمص و الفاصوليا من أسواق بغداد المحلية من نوع (VIP) التركية المنشأ، قدرت كمية سكريات RFOs للبذور بحالتها الخام و كذلك للعينات المعاملة و خميرة *S. cerevisiae* تحت ظروف مختلفة من حرارة ورقم هيدروجيني ومدة نقع باستخدام جهاز HPLC الموجود في مختبرات دائرة البيئة و المياه \ وزارة العلوم و التكنولوجيا وحصلنا ان معاملة نقع بذور الحمص مع استعمال خميرة الخبز عند تركيز ١٥غم\١٠٠غم بتقليل وقت النقع و لمدة تراوحت بين (٢-٤) ساعة عند حرارة تراوحت ما بين ٣٥-٤٠ م° وبمدى رقم هيدروجيني ٤ و ٤.٥ وكانت الإزالة تامة لجميع سكريات RFOs

بنسبة قد بلغت ١٠٠% معاملة نقع بذور الفاصوليا مع استعمال خميرة الخبز بتركيز 10غم\١٠٠غم بتقليل وقت النقع و لمدة تراوحت ما بين ( ٢-٤ )ساعة و عند حرارة 40 م و بمدى رقم هيدروجيني تراوح ما بين ٤.٥ و ٤ ، للحصول على إزالة تامة لجميع سكريات(RFOs ) وبنسبة فقد ١٠٠%. استخدام خميرة الخبز؛ لأنها متوفرة بكثرة في الأسواق المحلية و رخيصة الثمن وآمنة غذائيا للمستهلك و قدرتها على إنتاج إنزيم α-gahactosidase و الذي أثبت قدرته على تحليل سكريات RFOs في الدراسة الحالية

## Introduction

Leguminosae is a high-end flowering plant that contains folic acid, a vitamin necessary for the production of red blood cells, and folic acid also helps tissue growth, increases appetite and promotes digestive juices (Shakur, 2020). Chickpeas contain the percentage of protein in dry seeds, which may reach (31.5%) as well as its cheap price as well as low antinutrients and then high percentage of protein digestion Ali and others. (1990). Dry beans are vegetable crops rich in carbohydrates and their content of protein, calcium, vitamin, thymine and rabioflavin, and in general beans are a good source of calcium, which increases in green pods, Hassan. (2002) Most common legumes contain many compounds and these compounds are divided into two types: one that works to reduce the nutritional quality of pulses, and the second type negatively affects the health of the human who consumes them. However, their widespread acceptance is negatively affected by the presence of the a-galactosides bond which bind to polysaccharides molecules,. RFOs (Paleo leap LLC, 2019 ). which acts as the enzyme a-galactosidaes produced from the yeast *S. cerevisiae* Verma et al. 2022).These sugars are estimated using HPLC technology, which is the most important technology in chemical separation techniques between substances, and the most common in various industries and different fields of research. Their types are differentiated, including the mobile phase and stationary phase.THIS TECHNIQUE IS ALSO USED FOR THE DETERMINATION AND ANALYSIS OF MANY SUGARS AND PHARMACEUTICALS (RAGHAD I. AL SOUQI 2023) AS WELL AS THE DETERMINATION OF PHENOLIC COMPOUNDS IN APPLES (YOUSIF, 2023 & A. HASSAN). And the scientific basis for the work of the device is based on a liquid moving medium, so it is called by this name (high-performance liquid chromatography) (Hussein A. F. Kaluf Y. Ahmed B. 2020)**.**. The current study aimed to remove the semi-complex group of ravennose sugars, using soaking with water with *Saccharomyces cerevisiae* baking yeast by adopting several variables, the most important of which is the use of a pure enzyme α-galactosidase; . Using different pH media suitable for the action of the enzyme α-galactosidase. Incubation of samples at different temperatures and for different durations. Estimating

the amount of semi-complex sugars from the RFOs family. Finding the best conditions to eliminate the largest amount of RFOs sugars .

## Research Methodology

### Conditions of the experiment :-

- **Soaking seeds with theuse of a pure enzymeAlpha-galactosidase :-**
- **Concentration**: Use the enzyme in two concentrations (10 and 20) mg / 100 g
- **pH**:- Adjust the soaking water to pH 4
- **Soaking temperature**: - Threedifferent temperatures (30, 35 and 40) °C have been used.

**period**: - Soaked seeds for different periods (2, 4, 6 and 8) hours.

**How it works**

**Sample collection :-** Samples of legumes under study were collected from the local markets of the city of Baghdad by two types of legume crops, beans (Turkish VIP and chickpeas (Turkish) when carrying out the necessary tests for these crops, which are raw in the HPLC device to know the amount of RFOs sugars. This is done by grinding the sample, extracting and deriving it, and then injecting it with the HPLC device and the results appear using standard sugars (Ravennose, Stackuse and Verpascose) to infer them in the samples.

**Determination of RFOs in raw samples**: - Rhaphinose stachiose and ferbiscose sugars were detected and quantified using HPLC device

**Enzyme_**use **:-** g for beans and chickpeas was taken and placed in a flask containing 300 ml of water (pH 4). ) and the pure enzyme was added to each model at concentrations (10 and 20) mg 100 / g each x separately and the samples were placed in a water bath at temperatures (-30 degrees).35-40 °C°, then 25 g ofenzyme samples were withdrawn for periods (2 and 4 and6 and 8) hours per temperature, then the withdrawn samples were dried and analyzed by HPLC to detect and estimate RFOs ..

The **use ofyeast: -_**The weight of 100 g of chickpeas and beans was taken and placed in a flask containing 300 ml of water with two treatments, one pH 4 and the other 4.5, and yeast was added to each treatment in two concentrations (10and15 g / 100g model each separately, then placed the decanters in a water bath at temperatures (-30) 35-40 °C°, then withdrew 25 g per treatment for periods of (2-4 -6-8) hours from the time of start of the transaction and the samples were examined with HPLC .

**Sample Testing with HPLC** Device**:-**

I followed the method described by ) Agilent for the year 2005, and approved in the laboratory of the Ministry of Science and Technology \ Department of Environment and Water by preparing the models with the extraction and derivation processes, as follows:

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A. **Extraction**: **:-** The sugars were extracted from the legumes under study by taking 5 g of the dry sample and grinding well. After that, 100 ml of ethanol solution and water (30: 70) with 0.1 were added. Gm phosphoric acid. After that, the mixture was placed in a rocking water bath at a temperature of 50 ° C for an hour, then the mixture was placed in a centrifuge at a rotation of 1000rpm for 10 minutes. Take the clear and put it in the rotary evaporator to concentrate it and then filter the sample with a filter of 0.45, .

.B **Derivation:-** Taking 100µl of the extracted sample and dissolved well with 80 µL of distilled water and phosphoric acid in a ratio of (7:7)80 µL of $NaBH_3CN$ ( (0.1M is added to the mixture, and the mixture is then heated at 80 °C for 30 minutes and refrigerated to laboratory temperature and injected into HPLC device .

**HPLC Sample Analysis :-**

10ppm was injected for each of the extracted samples into the HPLC injector for the purpose of examining and estimating the amount of RFOs in them, and standard sugars (rafinozo stachiosis and ferbscose) were used as evidence of their presence in the samples under study, as these samples are transmitted after injection with the injector and move with 20% ACN : $H_2O$(Moving phase) With the help of a pump that operates at very high pressure, it works to pump the sample with the moving phase to the separation column containing $NH_2$ (Column 250 x 4.6 Id) mm and (partical size 5mm)) (the fixed phase) that works to separate the sugars from each other, and then these sugars go to the reagent (Flow Rate (0.6 ml/min, uv=210nm); to detect the identity of these sugars, and converts the amount of each of them into electrical signals and directs them to the concentrated processing unit (computer), and appears in the form of curves with peaks of different heights representing each peak of a specific sugar type (Ravennose, Stachiosis and Ferbscose) in the model as shown in Figure (1).

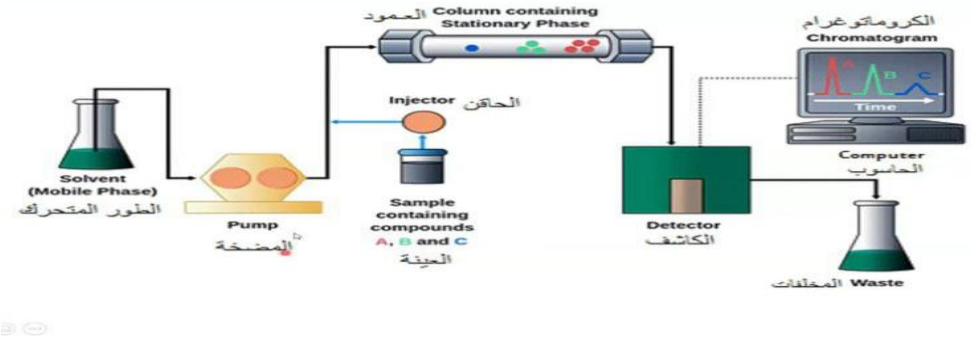


Figure (2) The shape of the curves containing vertices and each representing a certain sugar in the analytical program of the HPLC device

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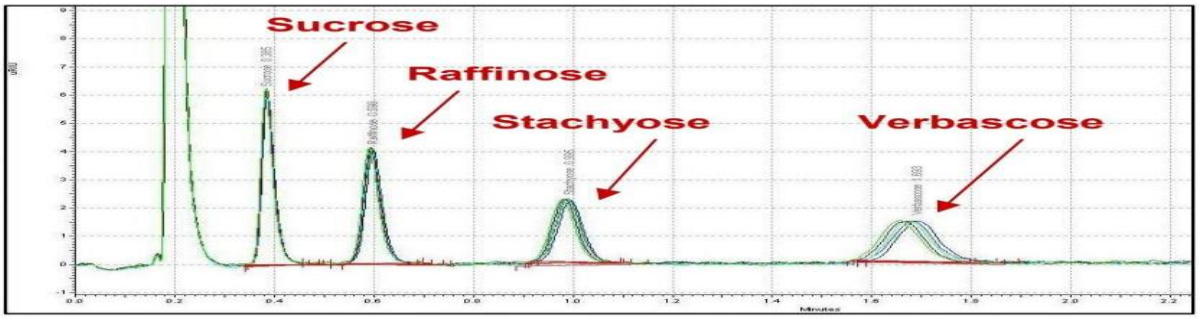


| RI Result Peak # | Name | Retention Time | Area | Theo. Plates | Asymmetry |
|---|---|---|---|---|---|
| 1 | Sucrose | 0.39 | 147997 | 1074 | 1.41 |
| 2 | Raffinose | 0.6 | 131200 | 1462 | 1.18 |
| 3 | Stachyose | 1.04 | 108780 | 1731 | 1.02 |
| 4 | Verbascose | 1.69 | 116696 | 1907 | 1.03 |

## Results & Discussion

### Optimal conditions for removing RFOs sugars :-

Table (1) collected the best conditions used in soaking chickpea seeds and beans once using enzyme and once using baking yeast.

Table (1) data indicate a decrease in the amount of RFOs in chickpea seeds, as the amount of ravennose decreased from 0.054 g / 100 g in the case of raw chickpeas to 0 g / 100 g when soaked using pure enzyme at a concentration of 20 mg / 100 g and a temperature of 30 °C and a pH of 4 for 8 hours, as well as the yeast used was able to give the same result in reducing the rhofinose from 0.054 g / 100 g to 0 g / 100 g at a concentration of 15 g / 100 g and a temperature of 40 °C and pH It ranges between (4 and 4.5) and for 2 hours with a 100% loss rate.

As for the Stachiose sugar, it is noted that it decreased from 0.314 g / 100 g in raw chickpeas to 0.003 g / 100 g after treatment with pure enzyme at a concentration of 20 mg / 100 g and a temperature of 30 ° C and a pH number of 4 and for 4 hours if we got a percentage of 99%. While a complete disappearance of stachiose is observed from 0.314 g / 100 g in raw chickpeas to 0 g / 100 g after treatment with yeast at a concentration of 15 g / 100 g and a temperature of 35 °C and a pH of 4.5 for 4 hours, If we get the highest percentage, it reached 100%. As well as for verbiscose sugar, which decreased from 0.238 g / 100 g in raw chickpeas to 0.001 g / 100 g after soaking using pure enzyme at a concentration of 20 mg / 100 g and at a temperature of 30 °C and pH 4 and for 8 hours, as the loss rate of this sugar reached 99.6% Panama The loss rate was 100% when soaking chickpeas using yeast at a concentration of 15 g / 100 g and a temperature of 35 m and a pH (4 and 4.5) for two hours as the ferbuscose decreased from 0.238 g / 100 g in Raw chickpeas to 0g/100g after treatment. As for the bean seeds, we note from Table (4-14) that they needed a higher soaking heat than in chickpeas in the case of using pure enzyme with soaking water at a concentration of 20 mg / 100 g and pH 4, as we note that the rhavinus sugar from 0.067 g / 100 g for raw beans to 0.001 g / 100 g after 8 hours of soaking and by 98.5%. Either Stakioz sugar has decreased from 0.399 g / 100 g in raw beans to 0.007

g / 100 g after two hours of soaking and the loss rate of Stackoz 98.2%, while we note a complete removal of ferbiscose sugar as it decreased from 0.256 g / 100 g in raw beans to 0 g / 100 g after 8 hours soaking and a loss rate of 100% as shown in Figure (1) and (2). Table (1) also shows that the use of yeast achieved complete elimination of all RFOs under study, as the loss rate in ravinnose sugar was 100% when using yeast by 15 g / 100 g and a temperature of 30 m and a pH of 4.5 for 4 hours, as the amount of ravinnose decreased from 0.067 g / 100 g to 0 g / 100 g. Either stachiose sugar decreased from 0.399 g / 100 g in raw beans to 0 g / 100 g when using yeast at a concentration of 15 g / 100 g and a temperature of 35 °C and at pH (4 and 4.5) for two hours. As for verpescose sugar, it required yeast at a concentration of 10 g / 100 g with a soaking temperature of 40 °C and a pH of 4.5 for 4 hours to obtain a complete removal of sugar from 0.256 g / 100 g in raw beans to 0 g / 100 g after treatment and with a loss rate of 100%, as shown in Figure (4-3) and (4-4). These results show that yeast was more efficient than pure enzyme, which may be due to the fact that yeast is a living organism that has worked to ferment the sugars found in chickpeas and beans and produce alcohols and gases (d.Nima Helou Jassim et al. 2010 (S. *cerevisiae* bread yeast was used because it is widespread in nature as well as its great effectiveness in the decomposition of complex sugars. *Saccharomyces cerevisiae* bread yeast is medium thermophilic and the best temperature for its growth ̊30 °C and yeast has a certain pH range ranging between (3.5-6) while (4-4.5) is optimal for it (AL eqabi, h, 2004). Therefore, these conditions are optimal for yeast to reduce or completely eliminate the RFOs family.

**Table (1) Quantity** of **RFOs Sugars and Percentage of Loss at Optimal Soaking Conditions**

| Seed type | Enzyme Source | Polysaccharides RFOs | Raw quantity (g/100g) | Conditions of the experiment | | | | Amount of remaining sugar (g/100g) | Loss % |
|---|---|---|---|---|---|---|---|---|---|
| | | | | concentration | Temperature m° | Ph | Duration (hours) | | |
| Hummus | Pure Enzyme | Raffinose | 0.054 | 20mg / 100gm | 30 | 4 | 8 | 0 | 100 |
| | | Stachyose | 0.314 | 20mg / 100gm | 30 | 4 | 4 | 0.003 | 99 |
| | | Verbascose | 0.238 | 20mg / 100gm | 30 | 4 | 8 | 0.001 | 99.6 |
| | Yeast bread | Raffinose | 0.054 | 15gm / 100gm | 40 | 4 and 4.5 | 2 | 0 | 100 |
| | | Stachyose | 0.314 | 15gm / 100gm | 35 | 4.5 | 4 | 0 | 100 |
| | | Verbascose | 0.238 | 15gm / 100gm | 35 | 4 and 4.5 | 2 | 0 | 100 |
| Beans | Pure Enzyme | Raffinose | 0.067 | 20mg / 100gm | 40 | 4 | 8 | 0.001 | 98.5 |
| | | Stachyose | 0.399 | 20mg / 100gm | 40 | 4 | 2 | 0.007 | 98.2 |
| | | Verbascose | 0.256 | 20mg / 100gm | 40 | 4 | 4 | 0 | 100 |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Yeast bread | Raffinose | 0.067 | 15gm / 100gm | 30 | 4.5 | 4 | 0 | 100 |
| | | Stachyose | 0.399 | 15gm / 100gm | 35 | 4 and 4.5 | 2 | 0 | 100 |
| | | Verbascose | 0.256 | 10gm / 100gm | 40 | 4.5 | 4 | 0 | 100 |

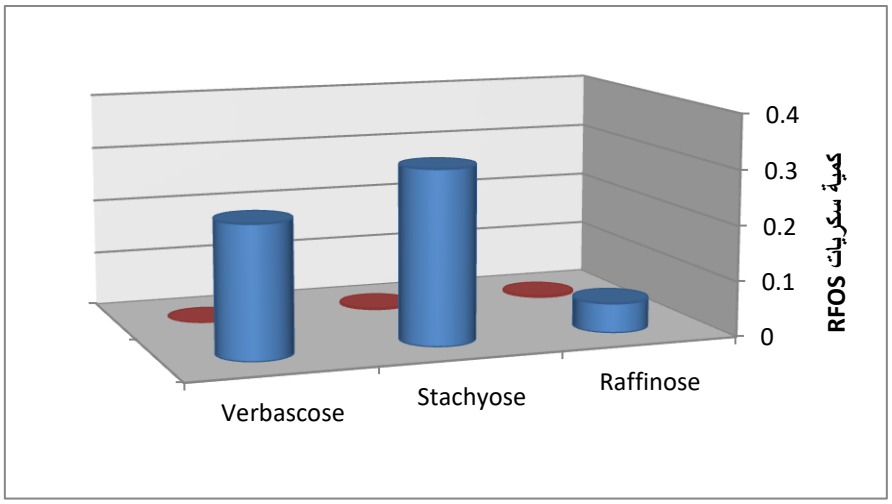


Figure (3) shows the amount of raw RFOs sugars \ the amount of sugars after treatment with pure enzyme at a temperature of 30 °C for a period of soaking ranging between (4-8) hours and pH (4) and a concentration of 20 mg / 100 g in chickpea seeds

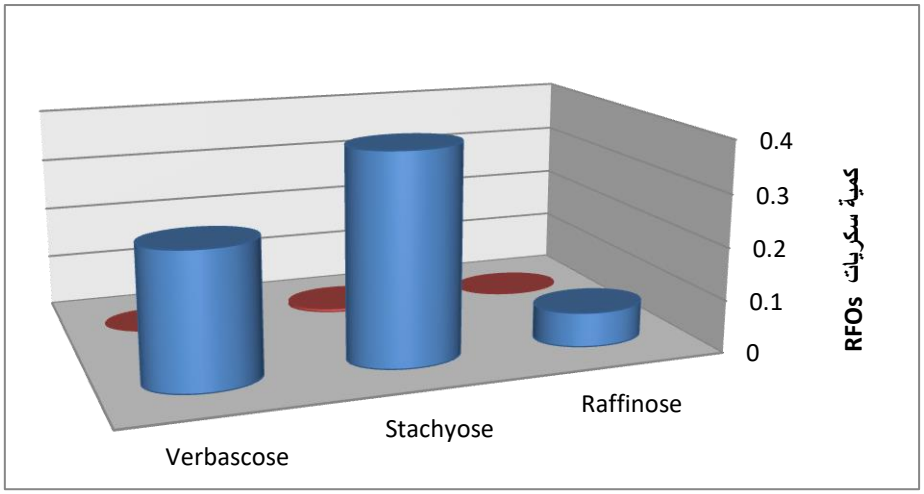

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Figure (4) shows the amount of raw RFOs sugars and the amount of sugars after treatment with pure enzyme at a temperature of 40 °C for a period of soaking ranging between (2-8) hours and pH (4) and a concentration of 20 mg / 100 g in bean seeds.

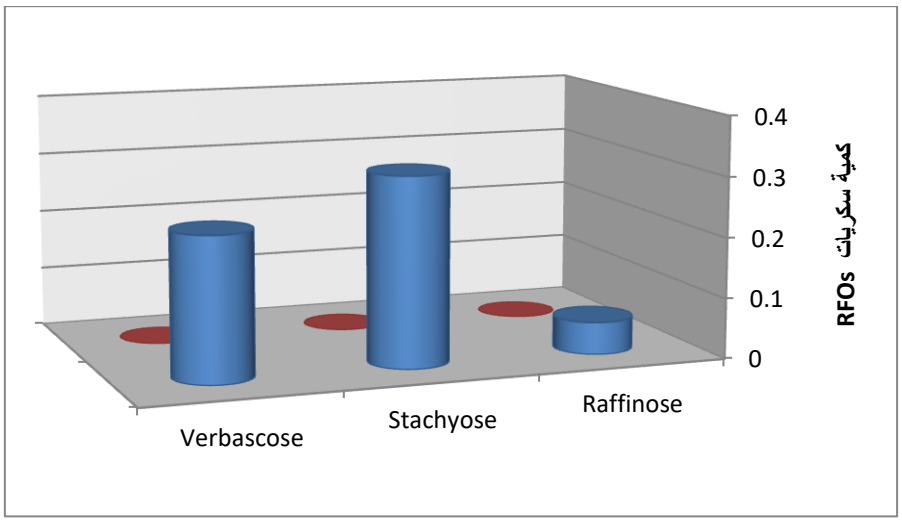


Figure (5) shows the amount of raw sugars RFOs The amount of sugars after treatment with yeast at a temperature ranging between (35-40) C and for a period of soaking between (5) hours and a pH (4.5 and 4) and a concentration of 15 g / 100 g in chickpea seeds

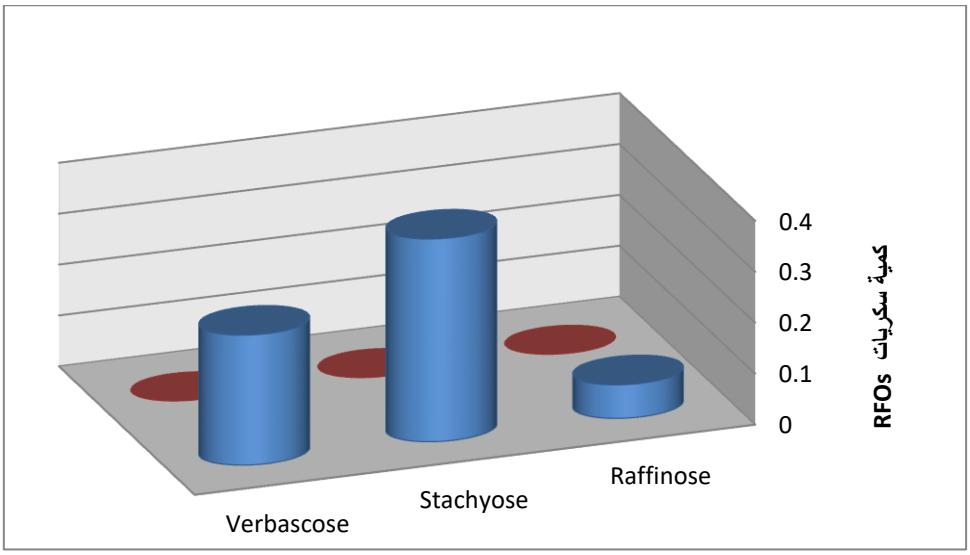


Figure (6) The amount of raw RFOs sugars shows the amount of sugars after treatment with yeast at a temperature ranging between (30-40) C and for a period of soaking ranging between (2-4) hours and a pH (4.5 and 4) and a concentration of 15 g / 100 g in bean seeds

**Conclusions:-**

The results obtained when using *S. cerevisiae* baking yeast are the complete removal of RFOs as they have been shown to be effective in the breakdown of large and complex sugars, namely oligosaccharides, including the sugars of the ravennose family by producing the enzyme α-galactosidase.. The best conditions under which the yeast worked were at a temperature between (35-4) °C for a period ranging between (2-4) hours, and a pH (4 and 4.5) and the yeast concentration was 15 g / 100 in chickpeas and the loss rate was 100%. ) at a temperature ranging between (30-4) °C for (2-4) hours, and the yeast concentration was 15 g / 100 g in the bean seeds. The use of regular bread yeast was more economically efficient because of its cheap price and in terms of its work to remove sugars as well.

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