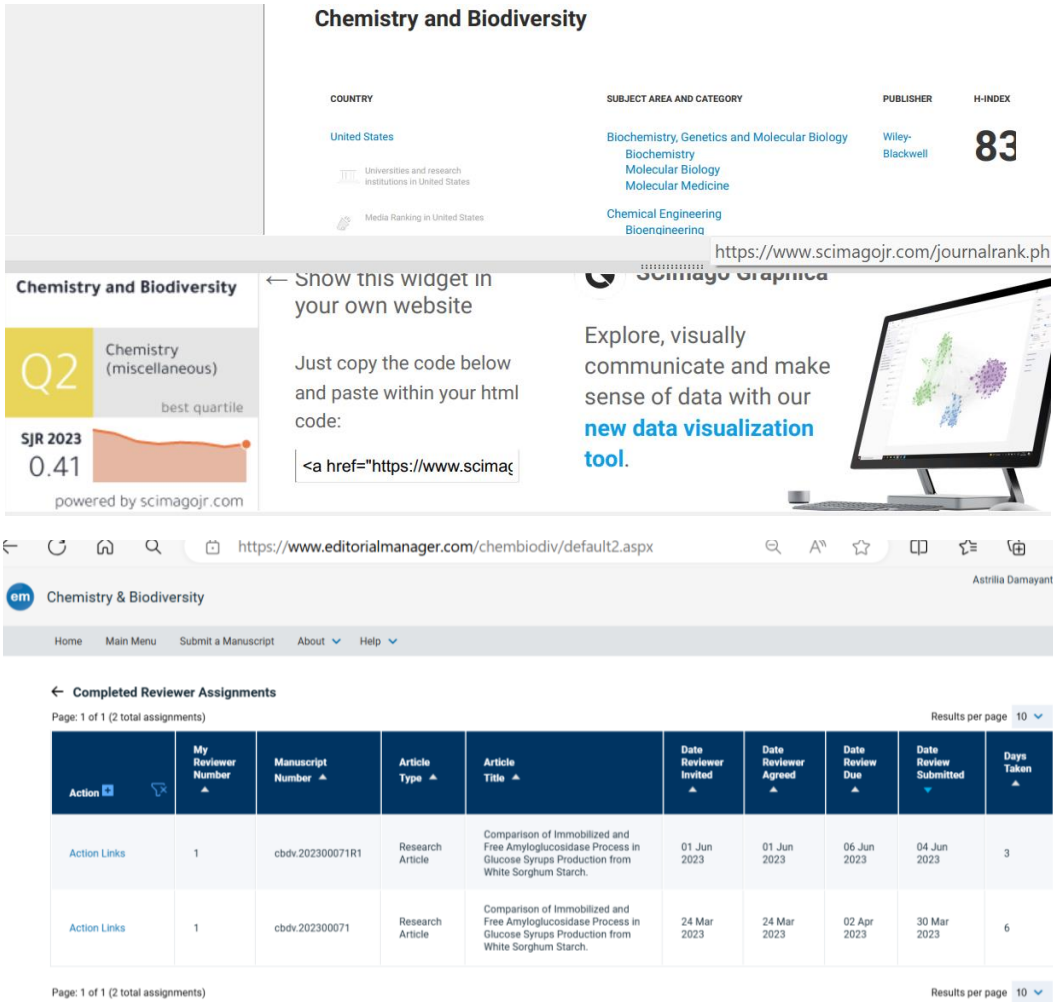
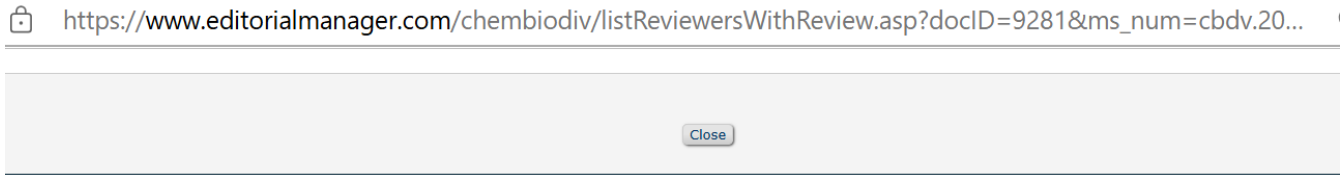


Reviewer Comment



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Note
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Chemistry and Biodiversity

Comparison of Immobilized and Free Amyloglucosidase Process in Glucose Syrups Production from White Sorghum Starch.

--Manuscript Draft--

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	Hakim Lounici
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Manuscript Classifications:	Catalysis; Materials Science: General; Sustainable Chemistry
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Comparison of Immobilized and Free Amyloglucosidase Process in Glucose Syrups Production from White Sorghum Starch.

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Abstract

Optimum conditions for production glucose syrups from white sorghum were studied through sequential liquefaction and saccharification processes. In liquefaction process, a maximum dextrose equivalent (DE) of 10.98% was achieved using 30% (w/v) of starch as using Termamyl α-amylase from *Bacillus licheniformis* and saccharification was done by free or immobilized amyloglucosidase from *Rhizopus mold* at 1% (w/v). DE values of 88.32% and 79.95% were obtained from 30% (w/v) of starch respectively with free and immobilized enzyme. The immobilized Amyloglucosidase in calcium alginate beads showed reusable capacity for up to 6 cycles with 46% of the original activity retained. The kinetic behaviour of immobilized and free enzyme gives K_m value of 22.13 and 16.55 mg mL^{-1} and V_{max} of 0.69 and 1.61 mg mL^{-1} min^{-1}, respectively. The hydrolysis yield using immobilized amyloglucosidase were lower than that of the free one. However, it relevant to reuse enzyme without losing activity in order to increase overall starch transformation into required products in industrial manufacturing. Hydrolysis of sorghum starch using immobilized amyloglucosidase can be a promising alternative towards the development of the glucose syrups production process and its utilization for several industries.

Keywords: Calcium alginate beads, Glucose syrups, Immobilization, Saccharification, White sorghum starch.

Introduction

Glucose syrup is a carbohydrate frequently utilized in food processing and other industries. It is considered as a major ingredient in confectionery products for their anti-crystallizing properties and in brewing for their sweetening capacity. [1] Also, it is useful in pharmaceutical and industrial fermentations. [2] Corn starch is the widespread raw material used to produce glucose syrup, which accounts for approximately 80% of global starch production. [3] However, due the increase demand for glucose syrup, other unconventional sources of starch such as cassava, sorghum has also been examined as a potential source of glucose syrup. [4, 5]

Over the last two decades, sorghum *(Sorghum bicolor (L.) Moench)* starch has received widespread attention due to its specific properties and the ability of sorghum to adapt to drought, disease and poor soil quality. Especially to its relevance as a food and industrial crop, particularly their prominent potential health benefits due to high starch content and its nutritional contribution at the agro-industrial level and processing applications. [6, 7] In hyper-arid regions of Algeria, Many landraces and domesticated sorghum were cultivated [8] and still undervalued although it may be an alternative source to the conventional crops. Previous studies [9] have revealed that white sorghum starch is an interesting source of glucose syrups production due to the starch contents in kernel around 60-70 % and interesting thermal and rheological properties influenced by their environmental and genotypic effects.[10, 11]

The industrial starch processing of syrup glucose may be realised by enzyme and acid hydrolysis or a combination of the two. Due to the disadvantages of using acid hydrolysis, the enzymatic hydrolysis is the most widely used methods because of its better control of the process and the resulting products. Using α-amylase for liquefaction starch to dextrins, subsequently, the sugar chains are further broken down by the amyloglucosidase during the saccharification process to produce simple glucose syrup with a high dextrose equivalent (DE). [12, 13]

Enzymes are of great interest in the field of biocatalysis in food. However, their cost and their limited stability over time are factors limiting their use. In order to overcome these drawbacks, a strategy was proposed: the immobilization of enzymes, which allows improving stability of the enzyme and convenience of their reuse in bioreactor systems. [14] The stability of the confined enzyme is determined by the intrinsic nature of the enzyme, the conditions of immobilization, the nature of the support material used and the conditions of reactions. [15]

Immobilization of amyloglucosidase has been used in the saccharification process for the continuous conversion of the starch in order to produce glucose syrups. Many interesting studies were focused on techniques, such as covalent adsorption, binding and cross-linking for entrapment of amyloglucosidase into insoluble supports [16] as non-porous polystyrene/poly (sodium styrene sulfonate) (PS/PNaSS), [17] Poly [(glycidyl methacrylate) Co(ethylene dimethacrylate)] [16, 18], polyglutaraldehyde-activated gelatin, chitosan and amberlite beads, [16, 19] magnetic nano-particles [20, 21] and alginate fibers, [22] but the most common supports for enzyme immobilization are calcium alginate beads. They are appreciated for their good biocompatibility, ease of preparation, low cost and easy availability. [23] For these benefits, the purpose of this research was to evaluate the performance of calcium alginate beads as a support material for *Rhizopus mold* amyloglucosidase entrapment and their subsequent utilisation in the hydrolysis of white sorghum starch. It involves the determination of optimum conditions of immobilization and glucose syrups production. Furthermore, liquefaction of white sorghum starch was examined and optimized using heat stable *Bacillus licheniformis* α-amylase. [9]

Results and Discussion

The achieved essays aimed to highlight performance efficiency of amyloglucosidase immobilization in alginate beads on the saccharification process to attain the best conditions for glucose syrups production from an interesting isolated white sorghum starch that grows in hyper-arid regions of Algeria.

Optimization of calcium alginate beads properties

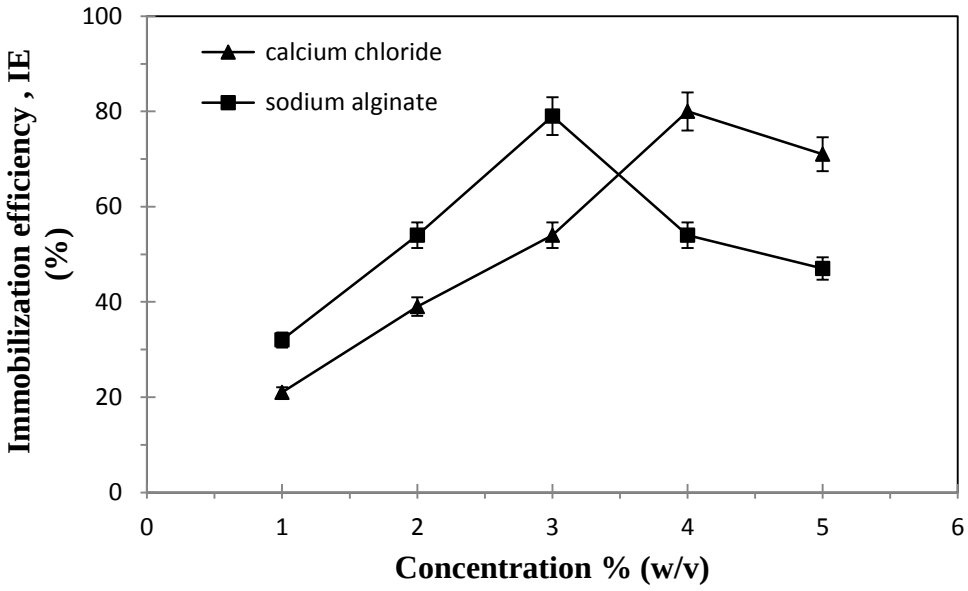
The calcium alginate beads are frequently used carriers in the encapsulation of biocatalyst due to their significant advantages such broad availability, cost effectiveness, good biocompatibility without toxicity and light gelation conditions. [24] In addition, this polysaccharide is adopted in several applications as the supporting material for release encapsulated cells and enzymes in the food and pharmaceutical industries. [25] The calcium chloride and sodium alginate are the most important components affecting the performance of amyloglucosidase immobilization in alginate. For this, the effect of their concentration was examined with the range of 1 to 5% (w/v) to attain an efficient immobilization of amyloglucosidase on alginate beads with desired mechanical strength.

The results revealed that amyloglucosidase was effectively confined using that interval of sodium alginate concentrations. However, the optimal concentration was 3% (w/v) as clearly seen in Figure1. It gave a maximum IE of 79 %, whereas, the lower IE of 32% was obtained with 1% (w/v). It might be due to less tightly cross-linked alginate gel and larger pore size of the beads [26] which allowed a greater enzyme leakage from beads. [27, 28] In the case of increasing concentrations of sodium alginate more than 3% (w/v), the IE decreased gradually due to the increased viscosity in the solution to form the beads, leading to smaller cavities size in the beads and diffusion limitation of the substrate to the active site of the enzyme. [27, 29]

Additionally, the effect of calcium chloride concentration (1-5 %, w/v) at 3% (w/v) constant sodium alginate concentration on immobilization efficiency was tested (*Figure 1*).

The results, as shown, in *Figure 1* revealed an increase of immobilization efficiency with increasing calcium chloride concentration up to 4% (w/v). It has been due probably to an increase in the cross-linking density of beads and the concomitant decrease in enzyme as explained by many authors as Konsoula et al. [23] and Priyanka et al.[29] Above 4% (w/v) of calcium chloride, enzyme activity decreases owing to a change in pH and its effect on the activity of confined enzyme. [30]

In order to investigate the conditions for the obtaining stable beads with an appreciable concentration of confined enzyme, the sodium alginate and calcium chloride concentrations were fixed at 3 and 4% (w/v), respectively. The beads were incubated for 30 to 120 min to determine the appropriate hardening time for improving the immobilization yield. The highest IE was obtained at the optimum hardening time of 90 minutes (*Figure 2*). Above 120 min, the activity of amyloglucosidase was decreased caused by leakage of amyloglucosidase from the beads as reported by Dey et al. [27]



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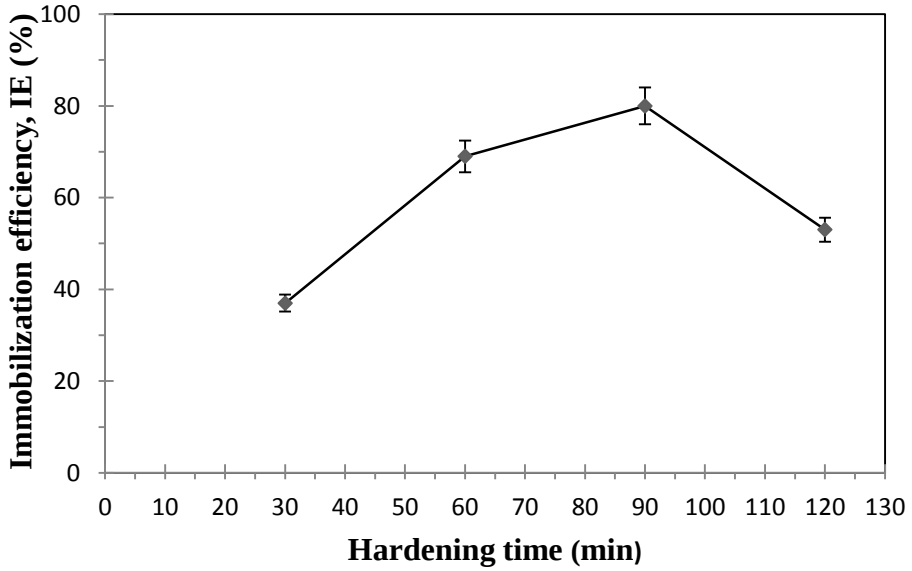
141 **Figure 1.** Effect of concentration of calcium chloride and sodium alginate
142 on immobilization efficiency, IE.



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144 **Figure 2.** Effect of hardening time on immobilization yield.

145 At the best immobilization conditions, regular and spherical shape of the calcium
146 alginate beads was obtained, as illustrated in *Figure 3*.

Figure 3. The Spherical shape of the calcium alginate beads (original, 2021).

Saccharification of liquefied white sorghum Starch

Monitoring of the saccharification process for 72 hours has been carried out to study the evolution of DE using, respectively, free and immobilized amyloglucosidase and to compare their efficiencies under the same conditions. *Figure 4* shows the results of continuously saccharification of liquefied white sorghum starch for free and immobilized amyloglucosidase.

A lower yield (79.95 ± 0.08%) of DE was produced by the immobilized enzyme compared to free enzyme (88.32 ± 0.25%). Both amyloglucosidase systems achieved their maximum DE yields within 48 to 54 hours. The saccharification (first run) with free amyloglucosidase was found more efficient with an increase of 8% approximately in the DE than that of immobilized amyloglucosidase. The contact between the free amyloglucosidase and its substrate is more efficient because both have a high degree of freedom, increasing the probability for one coming into close contact with the other. [31] However, as a result of immobilization, the yield of obtained DE decreased. It may be caused by the enzymes' restricted ability to reach substrate molecules at their active sites, their interaction with functional groups on the surface of beads, or their extensive surface contact with supports.

However, although the yield with immobilized amyloglucosidase was lower, the ability to reuse amyloglucosidase is of enormous relevance from operational and economic aspects, particularly due to the high cost of the enzymes which present the major duress in their industrial application.

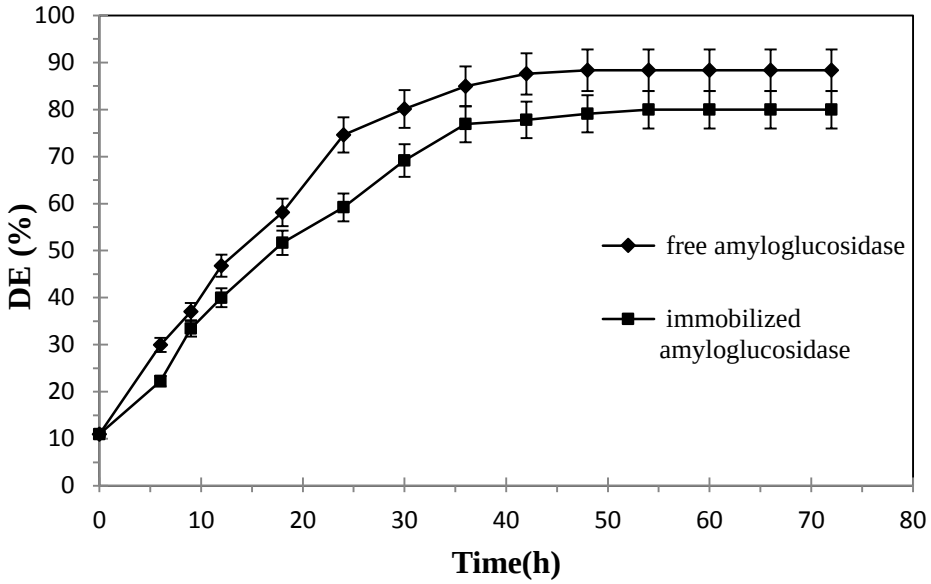

Figure 4. Kinetic of white sorghum starch saccharification at 30% (w/v) using free and immobilized amyloglucosidase.

Effect of amyloglucosidase immobilization on Kinetic hydrolysis

The kinetic constants V_{max} and K_m of both free and immobilized amyloglucosidase were determined using Lineweaver-Burk plots (*Figure 5*). A K_m of 16.55 and 22.13 mg mL^{-1} and a V_{max} of 1.61 and 0.69 mg $mL^{-1}min^{-1}$ were obtained, respectively, for free and immobilized enzyme as mentioned in *Table 1*. The results revealed that after immobilization of amyloglucosidase in calcium alginate beads, K_m was amplified by 5.58 mg mL^{-1}, while, V_{max} was reduced at 0.92 mg mL^{-1} min^{-1}. It might be due to the hindrance of the substrate to penetrate in beads for enzyme-substrate reaction. [32]

Table 1. Kinetic parameters of white sorghum starch hydrolysis using free and immobilized amyloglucosidase.

Enzyme	V_{max} (mg mL^{-1} min^{-1})	K_m (mg mL^{-1})
Free amyloglucosidase	1.61	16.55
Immobilized amyloglucosidase	0.69	22.13

Similar results have been reported about the increase in K_m and decrease in V_{max} of alpha-amylase, urease [33] and amyloglucosidase entrapped in the agar- agar matrix. [34] Pervez

et al. [35] have also obtained significantly larger K_m and lower V_{max} for immobilized amyloglucosidase compared to the free form. However, no change was found for the affinity of amylase towards soluble starch after it was confined in calcium agar tablets, whereas a higher augmentation was observed. [36]

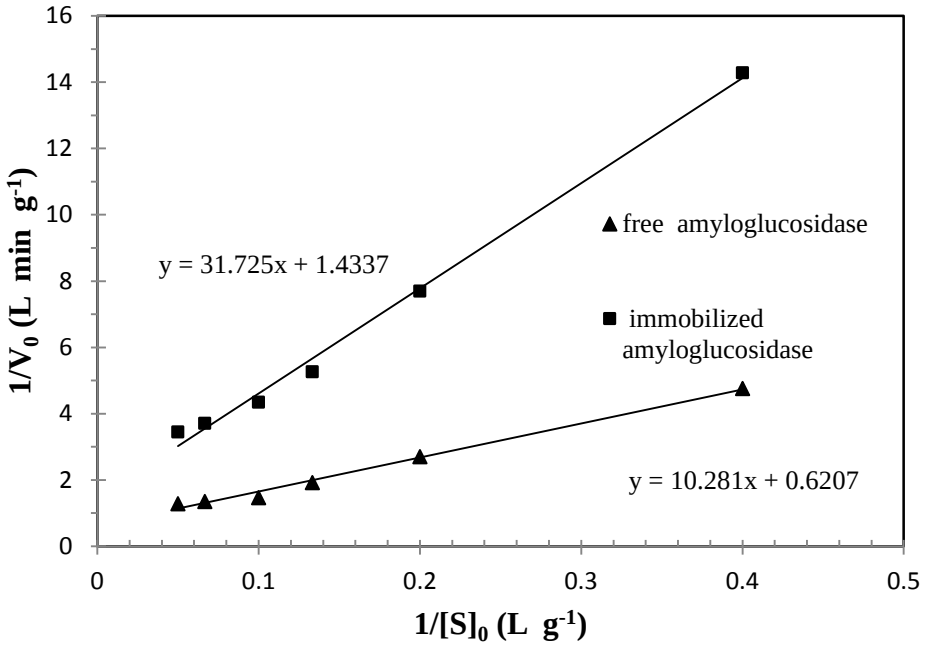

Figure 5. Lineweaver–Burk plots of white sorghum starch hydrolysis using free and immobilized amyloglucosidase.

Operational stability of Immobilized amyloglucosidase

One of the most significant advantages of the reusability of enzyme is its positive effect on the economic side by lower costs of glucose syrups production due to reducing consumption of enzyme. Therefore, the main goal of the current work was to patter an immobilization method that can retrieve amyloglucosidase from the reaction mixture in order to ensure its continued reuse for glucose syrup production.

The operational stability of enzymes is one of the most significant factors influencing performance of an immobilized enzyme system for utilisation in industrial bioprocess. For this reason, the reusability of amyloglucosidase entrapped was examined at 60 °C and the final DE concentration was determined over six cycles.

The results related to the reuse of confined enzyme were represented at the *Figure 6.* They showed that immobilized amyloglucosidase maintained its activity up to 81 and 46%

after the 2 and 6th recycles, respectively. [37] had reported that the glucoamylase from *Aspergillus niger* (50 U mg^{-1}) retained 88 % of its original activity after 20th cycles. Gupta et al.[16] obtained 43 successive reuse of immobilized amyloglucosidase obtained from solid state fermentation of *Aspergillus niger* with 35% residual activity and Wang et al. [38] reported that calcium-alginate entrapped enzyme can be reused up to10 cycles with the remaining activity of 56 %.

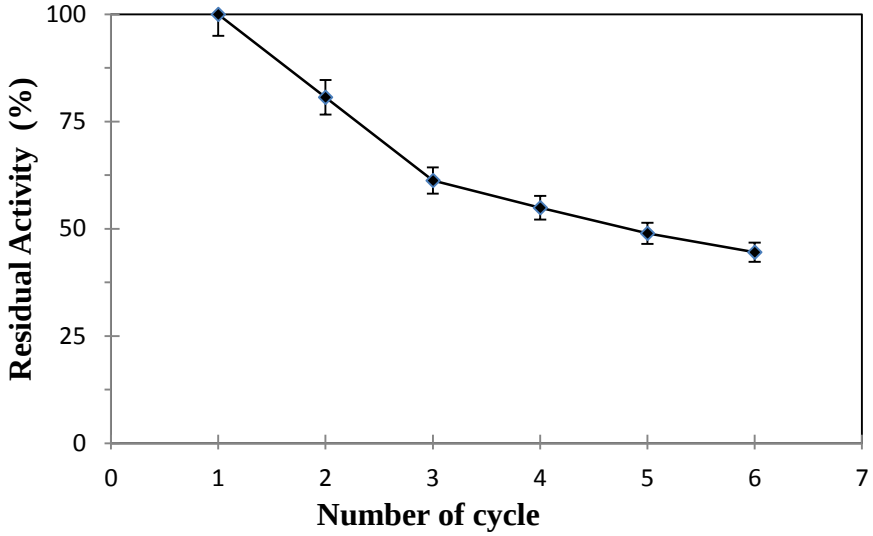

Figure 6. Reusability of immobilized amyloglucosidase.

The decrease in the amyloglucosidase activity could be due to repeated washing of the beads after each cycle and/or in increasing the size of pores after repeated uses, leading to increased leakage of the enzyme and activity reduction. [39, 40] The reduction in the activity observed after multiple cycles at the final batches is, probably also, a consequence to different factors as inherent wastes by the material transfer, but mainly due to the denaturation of the biocatalyst. [41]

Conclusions

The focus of this investigation revolves around the determination of optimum conditions for glucose syrups production from undervalued white sorghum starch with appreciable yield using enzymes in both liquefaction and saccharification processes. In addition, the optimization of the amyloglucosidase immobilization in calcium alginate was studied. The performances of confined and free amyloglucosidase were compared and it seems to be different as expected. An optimal initial concentration of 30% (w/v) of white sorghum starch and 0.1% (w/v) of free Termamyl α-amylase were used for liquefaction and

final DE of 10.98% was attained at the end of the saccharification step, DE increases until it reaches 79.95% and 88.32% using, respectively, immobilized and free amyloglucosidase and the immobilization efficiency was maintained until the sixth use.

However, the immobilized amyloglucosidase system may be an economically feasible glucose syrups production process for its easy separation from the substrate, allowing the reuse of the amyloglucosidase which could reduce the costs of the process, highlighting its potential for possible application in the production of glucose syrups. In addition, there is an opportunity to improve the glucose syrups yield from the starch isolated from Algeria white sorghum landrace cultivated in hyper-arid regions of with fewer costs by improvement of techniques for immobilization amyloglucosidase to enable the long-term re-usability of the enzyme and also the separation of catalyst more easily from the hydrolysates.

Experimental Section

Selected white sorghum landrace was cultivated in In Salah, a region of the Sahara of Algeria where high temperatures and very low rainfall were registered.

For hydrolysis process, *Bacillus licheniformis* α-amylase (Termamyl®300L Type DX, 300 KNU/g) and *Rhizopus mold* amyloglucosidase (23000 U/g) were used, respectively, for enzymatic liquefaction and saccharification. All chemical reagents were of analytical quality.

Isolation and purification of white sorghum starch

The alkaline method [42] was used to isolate and purify starch from grains of Algerian white sorghum. The different steps involve swelling in 0.20% (w/v) of NaOH, wet-milling, sieving (1000, 355, 50 µm), centrifugation (5000 rpm during 20 min) and lastly dried at 40°C. [10]

Amyloglucosidase activity determination

The activity of amyloglucosidase was measured by determination of starch hydrolysis rate. The liberated reducing sugar was determined by DNS acid method. [43] The enzymatic hydrolysis reactions were carried out using the Sigma method, [44] using white sorghum starch (1%, w/v) as a substrate in sodium acetate buffer (0.05 M, pH 4.5) at 40°C and glucose as the standard. This method consists of hydrolysing starch into reducing sugars. One unit of glucoamylase activity is defined as the amount of enzyme that produces 1.0 µmol of glucose from dissolubility starch per minute under the assay conditions. [37]

Immobilization of Amyloglucosidase onto the calcium alginate beads

The entrapment of amyloglucosidase in calcium alginate beads, was realized according to Roy et al. [45] by mixing aqueous sodium alginate (3%, w/v) and (1%, w/v) of *Rhizopus mold* amyloglucosidase in acetate buffer (0.05 M, pH 4.5). A volume of 50 mL of the mixture were drip-fed through a syringe into a calcium chloride solution (4%, w/v) under strict magnetic agitation. The produced beads were recovered by filtration, thoroughly rinsed with distilled water to remove excess calcium chloride and unbound enzyme molecules and then kept in sodium acetate buffer in a refrigerator until use.

Optimization of the calcium alginate beads properties

Bead properties were studied using beads of different permeability and rigidity in order to find the best conditions to have the best properties. The effect of sodium alginate concentration on the bead permeability was investigated using various concentrations of sodium alginate (1- 5%, w/v) and the effect of calcium chloride concentration on the rigidity of the beads was examined at different concentrations of calcium chloride solution (1- 5%, w/v). The effect of hardening time on the catalytic activity of immobilized amyloglucosidase was also determined using the optimal concentrations of alginate de sodium and calcium chloride solutions for times ranging between 30.0 and 120.0 min.

Determination of immobilization efficiency of the amyloglucosidase

The immobilization efficiency (IE) was calculated as the yield for confined amyloglucosidase in the calcium alginate beads according to the following equation (1):

$$\boldsymbol{IE}\ (\%) = \frac{Activity\ of\ immobilized\ Amyloglucosidase}{Activity\ of\ free\ Amyloglucosidase} \times 100 \quad (1)$$

Kinetic Parameters determination

The maximum initial velocity, V_{max}, and the concentration of substrate which permits the enzyme to achieve half maximum initial velocity, K_m of immobilized and free amyloglucosidase were determined from Lineweaver-Burk Plot using initial rates of hydrolysis catalyzed by free or immobilized enzyme using various concentrations of starch substrate (2.5 to 20.0 mg/mL).

Saccharification of Liquefied white sorghum Starch

The liquefaction of white sorghum starch was optimized in previous study using from heat stable *Bacillus licheniformis* α-amylase. [9] The liquefied starch was adjusted to pH 4.5 with 1 M HCl, then, 30 % (w/v) of liquefied sorghum starch was mixed with 1% (w/v) of free amyloglucosidase or equivalent units of alginate immobilized amyloglucosidase beads (about 2g of prepared beads under optimal conditions). The reaction mixture was incubated at 60°C for 72 h, to produce glucose syrup with high DE. During incubation, the samples were taken every 6 h until 72 h and the dextrose equivalent was determined. At the end of each run, sodium carbonate Na_2CO_3 solution was added to syrup glucose to remove the free acid and then activated carbon was used for purification of product produced by adsorption and vacuum filtration. Total refined glucose syrup was concentrated in Buchi Rotavapor model R-114.

Operational stability of Immobilized amyloglucosidase determination

Beads were removed from the end of saccharification process and mixture was by sifting, washed thoroughly with distilled water. The new medium using fresh substrate was added and the saccharification was continued. The same process was repeated until the 8th use. Percent remaining activity was determined by taking the enzyme activity of the first cycle as 100%.

Dextrose equivalent value estimation

Dextrose equivalent, DE, was estimated as the concentration of reducing sugars according to DNS acid method, as reported by Miller. [46] A calibration curve was previously established using glucose samples with known concentrations and using UV–Visible spectrophotometer (shimadzu UV-Vis 1605) at 540 nm. DE values were given using the following formula (2):

$$\boldsymbol{DE} = 100. \frac{total\ \ mass\ of\ released\ glucose}{initial\ mass\ of\ starch}\ .\ (162/180) \qquad (2)$$

Statistical analysis

All experiments were conducted in triplicate of each sample and results are notified as mean ± SD of the three values.

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Author Contribution Statement

Houria Taibi: Conceptualization, Methodology, Investigation, Writing - original draft. Nadia Boudries: Conceptualization, Investigation, Writing - review & editing, Supervision. Moufida Abdelhai: Writing - original draft. Hakim Lounici: review & editing, Supervision.

References

[1] N. Mohan, P. Singh, 'Sugar and Sugar Derivatives: Changing Consumer Preferences', **2020**, 1st ed. https://doi:10.1007/978-981-15-6663-9

[2] P. Hull, 'Glucose syrups: Technology and Application', Wiley Blackwell Publishers, **2010**.https://doi.org/10.1002/9781444314748

[3] R.T. Paraginski, R. Colussi, A.R. Dias, E. da Rosa Zavareze, M.C. Elias, N.L. Vanier, 'Physicochemical, pasting, crystallinity, and morphological properties of starches isolated from maize kernels exhibiting different types of defects', *Food chem.* **2019**, *274*, 330-336.

[4] I.K. Simpson, F.W.A. Owusu, M.E. Boakye-Gyasi, P. Entsie, M.T. Bayor, K. Ofori-Kwakye, 'Pharmaceutical Applications of Glucose Syrup from High Quality Cassava Flour in Oral Liquid Formulations', *Int. J. Food. Sci*. **2022**. https://doi.org/10.1155/2022/6869122.

[5] B.L. Tagliapietra, M.H.F. Felisberto, E.A. Sanches, P.H.Campelo, M.T.P.S. Clerici, 'Non-conventional starch sources', *Curr. Opin. Food Sci*. **2021**, *39*, 93-102. https://doi.org/10.1016/j.cofs.2020.11.011.

[6] L. De Morais Cardoso, S.S. Pinheiro, H.S. Martino, H.M. Pinheiro-Sant'Ana, 'Sorghum (*Sorghum bicolor* (L).): Nutrients, bioactive compounds, and potential impact on human health', *Crit. Rev. Food Sci. Nutr*. **2015**, *57*, 372-390. https://doi:10.1080/10408398.2014.887057.

[7] J.M. Awika, 'Sorghum: its unique nutritional and health-promoting attributes', gluten-free ancient grains. **2017**, 21-54. https://doi:10.1016/b978-0-08-100866-9.00003-0.

[8] M. Gast, J. Adrian, 'Millets and sorghum in Ahaggar: Ethnological and nutritional study. In: Memories of the Center of Anthropological, Prehistorical and Ethnological Researches', Paris: Graphic arts and trades. **1965**, 237-39.

[9] H. Taibi, N. Boudries, M. Abdelhai, H. Lounici, 'White sorghum landrace and corn starches in glucose syrups production using enzymatic and acid amylolysis', *J. Food Process Preserv*. **2020**, *45*. https://doi:10.1111/jfpp.15131.

[10] N. Boudries, N. Belhaneche, B. Nadjemi, C. Deroanne, M. Mathlouthi, B. Roger, M. Sindic, 'Physicochemical and functional properties of starches from sorghum cultivated in the Sahara of Algeria', *Carbohydr. Polym.* **2009**, *78*, 475-480. https://doi:10.1016/j.carbpol.2009.05.010.

[11] N. Boudries, B. Nadjemi, N. Belhaneche-Bensemra, M. Sindic, 'Morphological and Thermal Properties of Starches Isolated from White and Pigmented Sorghum Landraces Grown in Hyper Arid Regions', *J. Agric. Sci. Technol.* **2014**, *B 4*, 1939-1250. https://doi:10.17265/2161-6264/2014.08.009.

[12] R.N. Silva, F.P. Quintino. V.N. Monteiro , E.R. Asquieri, 'Production of glucose and fructose syrups from cassava (Manihot esculenta Crantz) starch using enzymes produced by microorganisms isolated from Brazilian Cerrado soil', *Food Sci. Technol.* **2010**, *30*, 213-217. https://doi:10.1590/s0101-20612010005000011.

[13] M. Lambri, R. Dordoni, A. Roda, D.M Faveri, 'Process development for maltodextrins and glucose syrup from cassava', *Chem. Eng. Trans*. **2014**, *38*, 469–74. https://doi:10.3303/CET1438079.

[14] S.D. Minteer,'Cell-Free Biotechnologies. Biotechnology for Biofuel Production and Optimization', 2016, 433-448. https://doi:10.1016/b978-0-444-63475-7.00016-9.

[15] M, Romero-Fernández, F, Paradisi, 'Protein immobilization technology for flow biocatalysis', *Curr. Opin. Chem. Biol.* **2020**, *55*, 1–8. https://doi:10.1016/j.cbpa.2019.11.008

[16] K. Gupta, A.K. Jana, S. Kumar, M. Maiti, 'Immobilization of amyloglucosidase from SSF of Aspergillus niger by crosslinked enzyme aggregate onto magnetic nanoparticles using minimum amount of carrier and characterizations', *J. Mol. Catal. B Enzym.* **2013**, *98*, 30-36. https://doi:10.1016/j.molcatb.2013.09.015

[17] J.T .Oh, J.H. Kim, 'Preparation and properties of immobilized amyloglucosidase on nonporous PS/PNaSS microspheres', *Enzyme Microb. Technol*. **2000**, *27*, 356-361. https://doi:10.1016/s0141-0229(00)00232-5.

[18] F. Švec, J. Kálal, I.I. Menyailova, L.A. Nakhapetyan, 'Immobilization of amyloglucosidase on poly [(glycidyl methacrylate) Co(ethylene dimethacrylate)] carrier and its derivatives', *Biotechnol. Bioeng*. **1978**, *20*, 1319-1328. https://doi:10.1002/bit.260200902

[19] A. Kumari, A. Kayastha, 'Immobilization of soybean (Glycine max) α-amylase onto Chitosan and Amberlite MB150 beads: Optimization and characterization', *J. Mol. Catal. B Enzym.* **2011**, *69*, 8-14. https://doi:10.1016/j.molcatb.2010.12.003

[20] G. Bayramoglu, B. F. Senkal, M.Y. Arica, 'Preparation of clay–poly(glycidyl methacrylate) composite support for immobilization of cellulase', *Appl. Clay. Sci.* **2013**, *85*, 88–95. https://doi:10.1016/j.clay.2013.09.010

[21] P.W. Tardioli, M.F. Vieira, A.M.S. Vieira, G.M. Zanin, L. Betancor, C. Mateo, J.M. 'Guisán, 'Immobilization–stabilization of glucoamylase: Chemical modification of the enzyme surface followed by covalent attachment on highly activated glyoxyl-agarose supports', *Process Biochem*. **2011**, *46*, 409-412. https://doi:10.1016/j.procbio.2010.08.011.

[22] A. Tanriseven, Y.B. Uludağ, Ş. Doğan, 'A novel method for the immobilization of glucoamylase to produce glucose from maltodextrin', *Enzyme Microb. Technol*. **2002**, *30*, 406–409. https://doi:10.1016/s0141-0229(02)00004-2

[23] Z. Konsoula, M. Liakopoulou-Kyriakides, 'Starch hydrolysis by the action of an entrapped in alginate capsules α-amylase from Bacillus subtilis', Process *Biochem.* **2006**, *41*, 343–349. https://doi:10.1016/j.procbio.2005.01.028

[24] W. Gombotz, 'Protein release from alginate matrices', *Adv. Drug Deliv. Rev.* **1998**, *31*, 267–285. . https:// doi:10.1016/s0169-409x(97)00124-5

[25] T.T. Mong Thu, W. Krasaekoopt, 'Encapsulation of protease from Aspergillus oryzae and lipase from Thermomyces lanuginoseus using alginate and different copolymer types', *Agric. Nat. Resour*. **2016**, *50*, 155-161. https://doi:10.1016/j.anres.2016.06.002

[26] M. Bilal, M. Asgher, H. M. N. Iqbal, H. Hu, X. Zhang, 'Delignification and fruit juice clarification properties of alginate-chitosan-immobilized ligninolytic cocktail', *LWT.* **2017**, *80*, 348–354. doi:10.1016/j.lwt.2017.02.040

[27] G. Dey, S. Bhupinder, R. Banerjee, 'Immobilization of alpha-amylase produced by Bacillus circulans GRS 313', *Braz. Arch. Biol.* Technol. **2003**, *46*, 167–176. https://doi:10.1590/s1516-89132003000200005

[28] D.R.N. Zusfahair, D. Kartika, M. Kurniasih, R. Nofiani, A. Fatoni, 'Improved Reuse and Affinity of Enzyme Using Immobilized Amylase on Alginate Matrix', *J. Phys. Conf. Ser.* **2020**, 1494, 012028. https://doi: 10.1088/1742-6596/1494/1/012028

[29] P. Priyanka, G.K. Kinsella, G.T. Henehan, B.J. Ryan, 'The Effect of Calcium Alginate Entrapment on the Stability of Novel Lipases from P. Reinekei and P. brenneri', *Trends Pept Protein Sci*. **2019**, *4*, 1-10 (e7).https://doi.org/10.22037/tpps.v4i0.26682

[30] A. Anwar, S.A. Qader, A. Raiz, S. Iqbal, A.Azhar, 'Calcium alginate: a support material for immobilization of proteases from newly isolated strain of *Bacillus subtilis* KIBGE-HAS', *World Appl. Sci. J*. **2009**, *7*, 1281-6.

[31] T. L. Ogeda, I. B. Silva, L. C. Fidale, O. A. El Seoud, D. F. S. Petri, 'Effect of cellulose physical characteristics, especially the water sorption value, on the efficiency of its hydrolysis catalyzed by free or immobilized cellulase', *J. Biotechnol.* **2012**, *157*, 246–252. doi:10.1016/j.jbiotec.2011.11.01

[32] M.A. Nawaz, H.U. Rehman, Z. Bibi, A. Aman, S.A.U Qader, 'Continuous degradation of maltose by enzyme entrapment technology using calcium alginate beads as a matrix', *Biochem. Biophys*. Rep. **2015**, *4*, 250–256. https://doi.org/10.1016/j.bbrep.2015.09.025

[33] S. Mulagalapalli, S. Kumar, R.C. Kalathur, A.M. Kayastha, 'Immobilization of urease from pigeonpea (*Cajanus cajan*) on agar tablets and its application in urea assay', *Appl. Biochem. Biotechnol*. **2007**, *142*, 291-297. https://doi:10.1007/s12010-007-0022-7

[34] S. Pervez, M.A. Nawaz, F. Shahid, A. Aman, I. Tauseef, S.A.U. Qader, 'Characterization of cross-linked amyloglucosidase aggregates from Aspergillus fumigatus KIBGE-IB33 for continuous production of glucose', *Int. J. Biol. Macromol*. **2018**, *135*, 1252-1260. https://doi:10.1016/j.ijbiomac.2018.11.097

[35] S.Pervez, N.N. Siddiqui, A. Ansari, A. Aman, S.A.U. Qader, 'Phenotypic and molecular characterization of *Aspergillu*s species for the production of starch-saccharifying amyloglucosidase', *Ann. Microbiol*. **2015**, *65*, 2287–2291. https://doi.org/10.1007/s13213-015-1070-9

[36] S. Pervez, M. A. Nawaz, M. Jamal, T. Jan, F. Maqbool, I. Shah, A. Aman, S. A. Ul Qader, 'Improvement of catalytic properties of starch hydrolyzing fungal amyloglucosidase: Utilization of agar-agar as an organic matrix for immobilization', *Carbohydr. Res.* **2019**, *486*, 107860. doi:10.1016/j.carres.2019.107860

[37] Y.X. Bai, Y.F. Li, M.T. Wang, 'Study on synthesis of a hydrophilic bead carrier containing epoxy groups and its properties for glucoamylase immobilization', *Enzyme Microb. Technol.* **2006**, *39*, 540–547. https://doi:10.1016/j.enzmictec.2005.08.0

[38] Y. Wang, H. Chen, J. Wang, L. Xing, 'Preparation of active corn peptides from zein through double enzymes immobilized with calcium alginate–chitosan beads', *Process Biochem*. **2014**, *49*, 1682–1690. https://doi:10.1016/j.procbio.2014.07.002

[39] M.L. Verma, M. Naebe, C.J. Barrow, M. Puri, 'Enzyme immobilisation on amino-functionalised multi-walled carbon nanotubes: structural and biocatalytic characterisation', *PLoS One*. **2013**, *8*, e73642. https://doi:10.1371/journal.pone.0073642

[40] L.J. Li, W.J. Xia, G.P. Ma, Y.L. Chen, Y.Y. Ma, 'A study on the enzymatic properties and reuse of cellulase immobilized with carbon nanotubes and sodium alginate', *AMB Express.* **2019**, *9*, 112. https://doi:10.1186/s13568-019-0835-0

[41] S.G. Valerio, J.S. Alves, M.P. Klein, R.C. Rodrigues, , PF. Hertz , 'High operational stability of invertase from *Saccharomyces cerevisiae* immobilized on chitosan nanoparticles', *Carbohydr. Polym*. **2013**, *92*, 462–468. https://doi:10.1016/j.carbpol.2012.09.001

[42] T. Beta, H. Corke, L.W. Rooney, J.R. Taylor, 'Starch properties as affected by sorghum grain chemistry'. *J. Sci. Food Agric.* **2000**, *81*, 245–251. https://doi:10.1002/1097-0010(20010115)81:2<245::aid-jsfa805>3.0.co;2-s

[43] G.L. Miller, 'Use of dinitrosalicylic acid reagent for determination of reducing sugar', *Anal. Chem*. **1959**, *31*, 426-428. https://doi:10.1021/ac60147a030

[44] Sigma method, 'Enzymatic Assay of α-Amylase (EC 3.2.1.1), 1-4'. 1997.

[45] J.J. Roy, S. Sumi, K. Sangeetha, T.E. Abraham, 'Chemical modification and immobilization of papain, *J. Chem. Technol. Biotechnol*. **2005**, *80*, 184–188. https://doi:10.1002/jctb.1177

[46] G.L. Miller, 'Use of dinitrosalicylic acid reagent for determination of reducing sugar', *Anal. Chem*. **1959**, *31*, 426-428. https://doi:10.1021/ac60147a030