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Invertase enzyme

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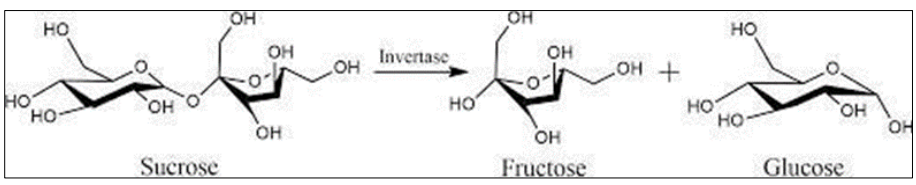
Abstract
The enzyme known as invertase catalysis the conversion of sucrose into glucose and fructose (EC 3.2.1.26). The best source of invertase is yeast. Internal and exterior in vertases are the two varieties found in S. cerevisiae. Invertase catalyses the hydrolysis of sucrose into fructose and glucose. Pharmaceuticals, analytical measuring probes, and yeast species detection ELISA kits all include this invertase. Immobilization of the invertase naturally is what is meant by this. Different levels of glycoprotein polymerization, glycosylation, and phosphorylation make bacteria invertase unique. A range of gram-positive and gram-negative organisms, including Zymomonas mobilis, and Bacillus strains, produce invertase. Comparing fungal growth to the time course of invertase synthesis by fungi, which ranged from 24 to 192 hours, was analysed. Twenty-four-hour intervals were used to measure the invertase activity. The biological characteristics that were looked at in this study included the amount of invertase-producing bacteria and soil invertase activity. Invertase is used to replace traditional acid hydrolysis.

Keywords: Invertase, hydrolysis, ELISA kits, biological

Introduction
Enzymes are globular proteins found in all cells that operate as a biocatalyst to speed up biochemical reactions in the body, both *in vivo* and *in vitro*. These biocatalysts were dubbed enzymes by Kuhan. Enzymes are derived from the Greek term 'enzumas,' which meaning 'bread brewing with yeast.' They are participating in a high-speed reaction without being drained. (Kumari N and Sethy K *et al*., 2020) [1].

The breakdown of sucrose into fructose and glucose is catalysed by invertase enzyme (EC 3.2.1.26). Inverted sugar is a glucose and fructose aqueous solution. Because the polarized light plane is tilted to the left, whereas light in a sucrose solution is tilted to the right. Because of the fructose, inverted sugar has a sweeter taste than sucrose. Invertase was the first carbohydrase to be explored in enzymology. Invertase enzyme activity was discovered in 1828 when baker's yeast was used to ferment an aqueous sucrose solution. Then it was discovered that when yeast degraded sucrose in a non-fermentative manner, invertase was not present in the cytoplasm. Berthelot isolated invertase from baker's yeast aqueous extract in 1860 by combining the yeast mass with moistened sand and precipitating it with ethanol. Yeast is the best source of invertase. There are two types of invertase in S. cerevisiae: internal and external. (Michele Vitolo *et al*., 2021) [7].

Sucrose is one of nature's most abundant products. Glucose and fructose involved in the signalling system in plants. Sucrose concentration acts as a crucial nutrition sensor in plants. Invertase also plays a role in cell formation and differentiation in this way. When animals, including humans, eat sucrose- containing diets, their genes do not call for this enzyme. To hydrolyze sucrose, they use a variety of enzymes, such as sucrose glucosidase. *Bacteroides thetaiotaomicron* and Bifidobacterium longum, two gut microbes found in humans, have invertase genes, implying that these organisms profit from human sucrose consumption.



(Luis E. Trujillo Toledo and Duniesky Martinez Garcia *et al*., 2019) [3]

Invertase PH is 4.5 and stability at 50 ℃. (Kumari N and Sethy K *et al*., 2020) [1]

Invertase is also used to make sucrose-based high-purity HFS. Protein adsorption is reduced in invertase membranes with higher hydrophobicity, a more negative surface, and a smoother surface. Invertase surface changes are a simple and adaptable way to increase the membrane's surface characteristics and thus its reusability. (J. Wang and J. Luo *et al*., 2020) [4]

Raw honey contains invertase, which is utilised for heating and timing purposes. (Huseyin Sahin and Sevgi Kolayli *et al*., 2020) [5] Fructofurunoside is another name for invertase. One of the advantages is that the sugar combination produced by this enzyme is colourless in comparison to the product produced by other methods. The Nelson method was used to determine the activity of invertase. (Orhun Hakkoymaz and Hidayet Mazi *et al*., 2020) [6]

Sucrose hydrolysis into fructose and glucose is catalysed by invertase. Inverted syrup is the result of this technique and is used as a sweetener in meals. This invertase can also be found in pharmaceuticals, analytical measurement probes, and yeast species detection ELISA kits. Due to the presence of invertase in the cell wall, yeast cells have been used to investigate sucrose hydrolysis. This is referred to as natural invertase immobilisation. (Michele Vitolo *et al*., 2021) [7]

Soy protein was also used to encapsulate the invertase enzyme using CLEA (cross linked enzyme aggregate). These immobilisation techniques cause enzyme release from the structure, resulting in decreased reusability (Agnes Cristina Oliveria Mafra and Maisa Bontorin Beltrame *et al*., 2018) [8]. Chemical techniques, on the other hand, result in more stable enzyme immobilisation. Invertase enzyme has been immobilised to improve stability, shelf life, and reusability. (illker Polatoglu and Aysenur Yardim *et al*., 2021) [9]

For sugar production, invertase has been immobilised on a variety of support materials using various ways. (Hesna Nursevin Oztop and Fatma Banu Catmaz *et al*., 2018) [10].

Sources

Sources of invertase distributed in different organisms including plants, animals, yeast and bacteria. Present in different isoforms in a different part of cell wall. (Nadeem and Rashid. M.H *et al*., 2015) [11].

Bacteria source

Single forms of all the bacterial invertase. (Barbosa and De, Morais *et al*., 2018) [12].

Extracellular and intracellular invertase are both produced by bacteria. Bacteria invertase are distinct in that they have varying degrees of glycoprotein polymerization, glycosylation, and phosphorylation. Invertase is produced by a variety of gram - positive and gram negative, such as Zymomonas monilis, Actinomycetales, and Bacillus strains. (Manoochehri H and Hoseini, N.F at al., 2020) [32]

Yeast source

Yeast is very good and common source of invertase enzyme. During extraction of invertase from S. cerevisiae specific invertase activity is 324 in Ro acetone cake. (Michele Vitolo at al., 2019) [25]

Plant source

Plants are the most abundant producers of the invertase enzyme. Invertase is split into isoenzymes based on its solubility in weak buffers, PH, iso electricity, and cell location. Invertase is found naturally in several plants. Plants are not chosen on industrial scale for invertase synthesis for a variety of reasons, including seasonal change, limited growth rate, time demanding, and expensive production costs. (Wan and Wu. L *et al*., 2017) [13].

Table 1: Plant source

Plant Species	KM in (mm)	References
Papaya	7.7	(Kumari N and Sethy K at al., 2020 [1]
Oat	4.58	
Maize	1.84	
Sugarcane	2.8	
Potato	16	

Production of invertase enzyme

By solid state fermentation

- At the University of Port Harcourt in Choba, Nigeria, soil samples were taken from decaying agricultural wastes in a waste disposal site. To extract invertase-producing fungi, 1 g composite soil sample was dissolved in 10 mL distilled water, yielding the 10-1 dilution, from which the 10-2, 10-3, 10-4, 10-5, and 10-6 dilutions were obtained. Volume of 0.1 mL from each of the 10-3, 10-4, and 10-5 dilutions was put onto a mineral salt agar with sucrose 20, yeast extract 10, (NH4) 2SO41.0, MgSO4 0.75, KH2PO4 3.5, agar 15, and pH 5.0 (in g/L of distilled water). To limit bacterial growth, the medium was adjusted with 0.1 percent (w / v) chloramphenicol.
- The plates were incubated at room temperature up to three (3) days and fungal growth was observed. On potato dextrose agar (PDA), fungal growths were purified and preserved in agar slants. Using the 50 percent sucrose-Cazpek liquid medium reported by Mehta and Duhan and Duhan *et al*., the fungi were tested for invertase production. Fehling's solution method was used to determine invertase production in the culture filtrate after the liquid medium was cultured for 10 days at 30 °C. Brown and green precipitate suggested positive results. Fehling's solution method was used to determine invertase production in the culture filtrate after the liquid medium was cultured for 10 days at 30 °C. Brown and green precipitate suggested positive results. In this investigation, the solid state fermentation (SSF) approach described by Malik *et al*. (2016) [38] was applied. In 250mL Erlenmeyer flask, ten grammes of each of the processed substrates (pineapple peels or potatoes peels) were added. With the flasks well agitated, the substrates were moistened with 10 mL of the basal medium (containing in g / L: sucrose 20 of yeast extract 10, (NH4) 2SO4 1.0, MgSO4 0.75, KH2PO4 3.5, and pH 5.0). The flasks were sterilized an autoclave at 121 °C (15 psi) for 15 minutes after being plugged with cotton wool. To inoculate each of the flasks containing the various substrates, a 10% (v / v) seed culture of the fungus was employed. The flasks were then incubated in a shaker incubator set at 150 rpm for 7 days at 35 °C. Francis Sopuruchukwu Ire and Vitalis Junior Aguguo, *et al*. (2018) [22].

Submerged fermentation for enzyme production

Submerged fermentation (SmF) was used to make invertase in Erlenmeyer flasks with 100 mL of medium containing KH2PO4 (1.0 g), K2HPO4 (6.27 g), MgSO4 (0.25 g), peptone (5.0 g), biotin (0.0005 mg), thiamine (0.005 mg), CaSO4 (0.005 mg), FeSO4 (0.5 mg),

As a substrate, pineapple peels (10.0 g) were used. The culture media was injected with 1106 spores/mL and cultured at 30 °C for 5 days after the pH was adjusted to 6.0. The cultures were filtered using the glass fiber filter paper (Whatman GF/A) after incubation, and the cell-free supernatants were utilised to calculate invertase activity. Triplicate fermentations were carried out. Olaoluwa and Oyedeji, *et al*. (2017) [23].

Invertase production by fungal strain *Aspergillus* spp

This investigation included five *Aspergillus* spp. isolates: *A. Niger*, A. *terreus*, *A. flavus*, *A. ochraceus*, and *A. parasiticus*. These strains were grown for four days at 28 °C on potato dextrose agar (Merck KGaA, Germany) and then stored at 4 °C. In the microbial genetics section, three isolates had previously been identified. A. ochraceus Egy2 LC360803, A. flavus Egy3 under accession number LC368455, and *A. Niger* Egy4 LC368456, as well as two isolates, were donated by the toxin section of the National Research Council.

The tests were carried out on a production media. Before sterilization at 121°C for 15 minutes, it was slightly changed by adding sucrose 30 g/l, yeast extract 2.0 g/l, NaNO32 g/l, MgSO4 0.05 g/l, and K2HPO4 0.5 g/l. Stock culture was inoculated into Erlenmeyer flasks (250 ml) containing 50 ml of the production medium and incubated for 48 hours at 30 °C with 150 rpm shaking.

- **Invertase activity** after 10 minutes at 50 °C incubation 0.2 Ml of enzyme solution with 1.8 mL 1 percent sucrose in 0.2M sodium acetate buffer (pH5) To measure reducing sugars, 1ml of dinitrosalicylic acid was added to boiling water for 10 minutes to stop the reaction. Finally, the absorbance was measured in a spectrophotometer at 540nm. The amount of enzymethatcatalyzed1mol of reducing sugar/min was defined as one unit of invertase.

Screening of some *Aspergillus* spp. for invertase enzyme production

After 48 hours of cultivation, A. Niger had the highest invertase production (6.9 U / ml), whereas A. parasitic us had the lowest invertase production (0.8 U / ml); invertase production of A. *terreus*, A. flavus, and A. ochraceus was 4.5, 6.6, and 4 U / ml, respectively.

Rasha G and Sali, *et al*.2021 [20].

Isolation and Screening of Invertase Production Bacteria by Escherichia coli

- Bacterial strains were isolated using the dilution plate method from agriculture soil in Al Ramadi city, west of Iraq. The generation of invertase enzyme was tested in the growth of Escherichia coli bacterial isolates
- The enzyme is produced in an immersed fermentation medium containing gmL of sucrose 10, yeast extract 6.0, KH2PO4 1.0, K2HPO4 1.0, and pH 7.2. Cultivation carried out in 250 mL Erlenmeyer flasks with fifty mL of sterile medium in each flask. After 48 hours incubation in a shaker incubator at 150 rpm, the inoculums achieved (1106 cfu / ml). Centrifugation at 6000 rpm for 20 minutes was used to extract the cells. The pellet was rinsed in 10 mM Tris-HCL buffer (pH 7.0) containing 1 mM EDTA before being resuspended in 10 ml of same buffer. A Branson Sonicator was used to destroy cells using ultrasonic waves. The supernatant was employed in the experiment an enzyme source. The sucrose in the media was replaced with Dates, Red carrot and Wheat bran as substrate.
- Enzyme activity was measured using a technique that included combining 0.1 ml of enzyme solution with 0.9 ml of sucrose 0.03 M acetate buffer (pH 5.5), adding 1 ml of dinitro salicylic acid reagent, and boiling at 100 °C for 5 minutes. Finally, the absorbance was measured at 540 nm using a spectrophotometer. Under the study conditions, 1 unit of enzyme (U) is defined as the quantity of invertase that releases 1 mole of glucose per minute/ml. Dhafer Fakhri Al-Rawi and Harith Kamel Buniy, *et al* (.2020) [21].

Invertase production by Analysis of soil biological, physical, and chemical properties

The quantity of invertase-producing bacteria and soil invertase enzyme activity were among the biological parameters examined in this study. The total plate count (TPC) approach was used to determine the number of invertase- producing bacteria using selective sucrose hydrolysis media. Soil Quantitative approaches were used to examine invertase activity.

Selected isolates were subjected to multiple phases of molecular identification. Isolation of bacterial genomic DNA using the PrestoTM gDNA Bacteria Mini Kit methodology (Geneaid). The purity of bacterial genomic DNA was determined using a Nano Drop Spectrophotometer, and the 16S rRNA gene was amplified from bacterial isolates using the My TaqTM Red Mix (Bioline) technique.

Electrophoresis on a 1% agarose gel was used to confirm the PCR results. Phylogenetic tree analysis with DNA sequencing BLAST-N, a tool accessible on the NCBI website, was used to match DNA sequences of invertase producing bacteria to DNA sequences of other bacterial strains in the database. The MEGA 6 programme is used for phylogenetic analysis. Ester raisa k. Lase1 and giyanto2, *et al*. (2021) [16].

Table 2: Isolation and Screening of Invertase Production Bacteria by Escherichia coli

Microorganisms strain	incubation & period	T °C	N source	pH	Inoculum size	C source	Media	Agitation rate rpm	Ref
Penicillium pp. And Trichoderma Viride Potato peel, wheat bran, apple peel, orange peel (1–10%w/v), carrot peel, beet peel, and sugar cane bagasse).	50 °Cfor30min 5 to 7 days	70 °C	wastes 20.0, yeast 10.0, ammonium sulfate 1.0, magnesium sulfate 0.75, and potassium dihydrogen phosphate 3.5	5.0	mm in diameter	.1ml buffer 0.9ml of 1% sucrose in 0.03M acetate acid	Fermentation media	8000rpm	Nehad E.A. and Sherien M.M. Atalla, *et al*.2020 [15].

Table 3: Invertase production by Analysis of soil biological, physical, and chemical properties

Microorganisms strain	Substrate	Yield	Optimum production, condition	Media	Ref
Fusarium solani	Molasses	9.90 U / mL	pH 5.0, 30 °C for four days	Submerged state fermentation	Wei Cheng Pang And Aizi Nor Mazila Ramli, *Et al*., 2019 [35].
A. Niger	Agro- Industrial wastes	154.27±9.38 µg-1	Tem 30 °C at 50% moisture for three days	Solid state fermentation	Ohara etal., (2015) [18]
A. Niger	Fruits peels	51 U / mL	pH5.0 at 30 °C for four days	Solid state fermentation	Raju *et al*., (2016) [17]
Unknown Bacteria isolated from sugarcane juice	Waste jiggery	2.63 U	pH6.0 at 37 °C	submerged fermentation	U Shah *et al*., (2016) [19]

Purification of invertase enzyme

The purification of invertase enzyme can be done in a variety of ways. An alternative method for enzyme purification is the three-phase partitioning method from Momordica charantia (bitter melon). Until complete dissolution of (NH4)2SO4, solid crystalline (NH4)2SO4 was added to crude extract until the appropriate saturation percentage was attained, and then tbutanol was added at various ratios after the (NH4)2SO4 was completely dissolved. To make easier separation stages, the mixture was allowed to remain for various amounts of time at room temperature before centrifuged at 4500 rpm for 10 minutes. Remove the upper phase and dissolve the interfacial precipitate with acetate buffer. Total protein content and total specific activity of invertase were evaluated after dialyzing samples against acetate buffer. Optimal conditions that resulted in higher invertase recovery in the purification procedure. (Nihan kubra Belligun and Burcu Saygideger Demir *et al*., 2019) [24]

Invertase enzyme in the presence of surfactants is the next approach. This autolyzate was combined with 0.12 sodium picrate at a 1:0.35 ratio. After that, the combination was allowed to sit at room temperature for five hours. After that, the precipitate was separated and discharged using centrifugation. Then, at a volumetric ratio of 3:1, acetone was added to the picric acid supernatant. The precipitate was then centrifuged and kept at 5 degrees Celsius until needed. Precipitation with acetone was done in the absence and presence of a nonionic surfactant blend consisting of TWEEN and SPAN at 1% weight by volume. The HLB interval ranged from 4.7 to 15.6. (Michele Vitolo *et al*., 2019) [25]

- In the next approach, the effect of centrifuge-time combination in partial purification is discussed. First, consider the influence of application duration, as well as the usage of a centrifuge to filter the enzyme produced at room temperature. Three alternative rotating speeds (1377, 6973, and 16,873 g) and application times (5, 10, and 15 minutes) were examined for this purpose. After that, the supernatants were tested for enzyme activity as well as protein content. Specific enzyme activity, yield increase, and purification coefficient were computed using the acquired data. The inulinase from the shaking incubator and bioreactors was partially purified using an ultrafiltration method. These are two distinct approaches. (Mustafa Germec And Irfan Turhan *et al*., 2020) [26]
- Invertase purification method based on raw extract purification, with raw extract purified by acetone precipitation. Slowly add cold solvent while stirring and cooling. The mixture was then chilled for 15 minutes before being centrifuged for 10 minutes at 2,025g and left to sit overnight at 4 degrees Celsius. Suspension was diazed for 12 hours with cellulose membrane and water replenished on a regular basis. The activity of soluble proteins and enzymes was then assessed. IEC purifies the dialyzed extract. In 2Ml eluates, soluble proteins and invertase activity were measured, then kept at -20 °C for subsequent electrophoresis. (Tariani Lemos Avila, Ricardo Peraca Torelles *et al*., 2021) [27]

The molecular weight of yeast invertase is 270kDa in this SDS-PAGE result of invertase purification from Saccharomyces cerevisiae. Invertase is a glycoprotein with a carbohydrate content of 50%. On SDS-PAGE, invertase bands have a "smeared" look and may be distinguished from other protein bands. Silver staining is a sensitive approach for detecting protein, even in nano gram quantities. As a result, the appearance of other bands may be influenced by the staining procedure. Biomacromolecules, such as enzymes and other protein types, can be purified using cryogel architecture, which has a number of advantages in addition to its innovative megaporous structure. (Kemal Cetin, Isik Percin *et al*., 2016) [28].

Table 4: Purification of cationic and anionic invertases (1 and 2)

Source of enzyme	Purification steps	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Yield (%)	Reference
Aspergillus terreus	Crude extract	170.3	22.1	7.7	1	100	(Maíra N. de Almeidaand ValeriaM. Guimarães*et al*.,2018) [29]
	ultrafiltration	155.7	6.9	22.6	2.9	91.4	
	Q-Sepharose						
	Invertase 1	15.7	0.8	19.6	2.5	9.2	
	Invertase 2	130.6	3.2	40.8	5.3	76.7	
	CM- Sepharose Invertase 1	0.21	0.01	21	2.7	0.12	
	SephacrylS300 Invertase 1	0.03	0.001	30	3.9	0.017	
	PhenylSepharose Invertase 2	49.9	0.18	277.2	36	29.3	
	SephacrylS300 Invertase 2	27.8	0.014	1985.7	257.8	16.3	

Table 5: Purification of mango soluble acid invertase by DEAE-Sepharose fast flow and SDSPAGE

Purification steps	Volume	Total protein (mg)	Total activity (U)	Specific Activity (U mg-1)	Fold Purification	Reference
Crude enzyme exract After ammonium sulphate precipitate and dialysis	1 L 160 mL	2300 (1780)	736 (338)	0.32 (0.19)	1 (0.59)	(Renjie Li and Xiaojun Liao *et al*.,2017) [30]
DEAE-Sepharose Fast Flow	40 mL	0.31	61.7	199	622	
Ultrafiltration 50 kDa	0.5 mL	0.26	53	203.85	637	

Candida utilis invertase purification

Table 6: Purification process of C. lanatus rind invertase from crude extract to gel filtration steps

Steps	Total protein (mg)	Activity (µM/min)	Total activity (µM/min)	Specific activity (µM/min/mg)	Purification fold	% Yield	reference
Crude	145.402	31.094	1554.682	10.692	1.000	100	
NH2SO4 ppt	51.006	14.757	737.826	14.465	47.46	47.46	(Rotimi Arise and Olalekan Olufemi et al.,2020) [31]
Dialyzed	3.305	7.686	384.303	116.293	24.72	24.72	
Gel filtration	0.718	7.211	360.546	501.880	23.19	23.19	

Applications

Invertase not only aids in the breakdown of carbohydrates, but it can also help to avoid human diseases such as those found in bee pollen. Access to the invertase enzyme declines with age, resulting in decreased extraction of the beneficial nutrition for human health. Honey is made by bees using the invertase enzyme, which means that sugar in natural nectar is used to make the honey.

- **Metabolism:** The metabolic activity of honey enzymes like invertase can also be examined. According to certain studies on Asparagus Officinalis, the top region of asparagus spears has a high level of metabolism, which may be associated to strong invertase activity. (Manoochehri H and Hosseini N F *et al*., 2020) [32]
- **Bioethanol production:** Through fermentation, invertase is also utilised to make alcoholic drinks, lactic acid, glycerol, and other products. The use liquid biofuels such as bioethanol and biodiesel in industrial applications has been promoted by global concerns about environmental safety, fuel efficiency, and energy security. Molasses, a viscous byproduct of sugarcane or sugar beet sugar refining, can be utilised as a substrate for bioethanol production by enzymatic hydrolysis of sucrose, fermentation of the generated sugars into ethanol, and distillation. In India, sugarcane molasses is the primary source of bioethanol, and ways to improve ethanol production have been investigated. Invertase and zymase enzymes in yeast may ferment sucrose to produce ethanol and carbon dioxide. (Ishita Malhotra and Seemi Farhat Basir *et al*., 2020) [33]
- **Natural respiratory support:** Invertase sources have been demonstrated to lessen colds, flu, and other respiratory disorders like bronchitis, asthma, and allergies. (Manoochehri H and Hosseini N F *et al*., 2020) [32]
- **Invertase Biosensor:** Biosensors are analytical instruments made up of immobilised biomolecules, primarily enzymes that interact precisely with an analyte and create easily observable physical, chemical, and electrical changes. The performance of biosensors is determined by the sensitivity and selectivity of biomolecules, as well as their stability. (Nadia Bashir, Monika Sood *et al*., 2020) [37] Because of their selectivity and sensitivity, enzyme-based biosensors have become extremely important in research. Biosensors based on enzymes are employed in a variety of fields and have a significant commercial value. Different enzymes are blocked or inhibited by inhibitor molecules, which is a unique feature that is exploited by various sectors for analytical purposes such as detecting the presence of different polluting substances such as heavy metals (e.g., lead, mercury) or other polluting agents. Because of their irreversible binding to thiol groups in enzymes, these heavy metals have a significant negative impact on enzyme activity. Enzyme-based biosensors are also available, which are based on the inhibition of enzymes by various inhibitory chemicals. Invertase-based biosensors have been widely used due to their excellent specificity and sensitivity to heavy metal pollution. Because of their low cost and stability, invertase-based biosensors have been favoured. The amount of sucrose in the solution was determined using immobilised invertase and glucose oxidase. The biosensor's working concept is based on the creation of glucose via invertase hydrolytic activity, followed by glucose molecule oxidation into glucose hydrogen per oxide, which is detected and quantified by an Ag / AgCl reference electrode.
- **Prostate cancer:** is a disease that affects a man's reproductive system and is becoming one of the most common malignancies in men. There is no effective treatment for prostate cancer that can extend a patient's life expectancy. Prostate cancer must, however, be detected early in order for patients' lives to be saved. The most effective biomarker in the detection and early diagnosis of prostate cancer in men is prostate specific antigen (PSA). The discovery of this biomarker is a crucial research topic. For the prostate-specific antigen, a very sensitive biosensor was designed (PSA). **(**Anam Aftab and Zia Ullah Khan *et al*., 2021) [34]

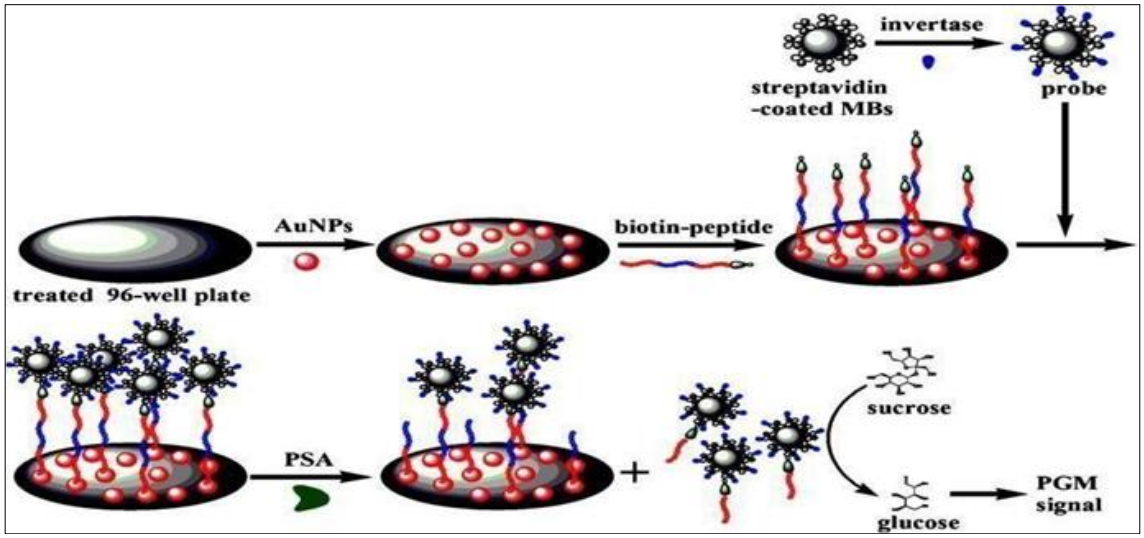


Fig 1: Schematic diagram of invertase based biosensor of prostate-specific antigen determination.

(Anam Aftab and Zia Ullah Khan *et al*., 2021) [34]

- **Invertase in Pharmaceutical industry:** Invertases, on the other hand, are used in the pharmaceutical business to make medications, cough syrup, digestive aid pills, nutraceuticals, and newborn meals. Furthermore, the discovery of invertase with transfructosylation activity, which creates FOS, has improved the commercial worth of invertase in the pharmaceutical business. FOS is a type of oligosaccharide made up of short fructose chains that are less sweet than sucrose and low in calories, making it a good choice for diabetics. FOS has also been shown to improve human health by acting as a prebiotic, assisting in the formation of short-chain fatty acids, preventing constipation and colon cancer, aiding in mineral absorption, and regulating sugar and lipid metabolism. **(**Wei Cheng and Pang AiziNor Mazila Ramli *et al*., 2019) [35]
- **Invertase in the food and beverage industries:** Invertase is utilised as a substitute for classical acid hydrolysis, which can result in unwanted byproducts, unfavourable colour, and harsh taste, as well as low conversion efficiencies and high ash content. Invert sugar is widely employed as a humectant in the creation of softcentered candies, fondants, calf feed preparation, jams, and chocolates due to its hygroscopic nature and low crystallisable qualities.

(Wei Cheng and Pang AiziNor Mazila Ramli *et al*., 2019) [35]

Soymilk Treatment

Carbohydrases that catalyse the hydrolysis of sucrose, raffinose, and other similar glycosides are known as invertases (EC 3.2.1.26). Using invertase to directly hydrolyze raffinose into melibiose and fructose is a straightforward technique to obtain melibiose in soymilk. Soymilk is made commercially by soaking and crushing soybeans in tap water. Following that, the crude slurry is filtered, cooked, and maintained at boiling temperature for 5–15 minutes. Finally, to obtain soymilk, the heated soymilk was immediately cooled to room temperature. To make soymilk processing easier, invertases added to soymilk are recommended to complete raffi nose hydrolysis at moderate or lower temperatures. Furthermore, because soymilk has a pH of 5.5-7.0 and is high in cations, it is a good source of calcium. The invertases used to make soymilk must have a high raffinose activity. **(**Liu J and Cheng J *et al*., 2021) [36]

Conflict of Interest

Not available

Financial Support

Not available

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