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Short communication

An approach to potentiometric sensing of sugars: Baker's yeast assisted pH electrode

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ABSTRACT

Rapid development of "electronic tongue" technology requires novel sensors with response toward various classes of taste substances. Potentiometric sensors gained wide acceptance for these purposes, however, they cannot provide for sensitivity toward numerous sugars, since typical carbohydrates are normally not ionized under measurement conditions. We report on feasibility study of an approach to potentiometric sensing of sugars using *Saccharomyces cerevisiae* (baker's yeast) as a sample modulator. The ability of the yeast to ferment sugars and proportionally increase sample acidity can be tracked with ordinary pH glass electrode and can be related to the amount of sugar in a quantitative way. We optimized measurement conditions with yeasts and demonstrated the applicability of this simple approach to quantitative sucrose determination in apple juices.

1. Introduction

Sweetness is considered to be the most pleasant taste and/or odor for numerous animals, insects as well as for human beings. Typical sweet substances are numerous carbohydrates, certain amino acids, oligopeptides, proteins and other chemical compounds [1]. There is a strong interest in instrumental assessment of sweet taste intensity and character from food, beverage and pharmaceutical industries. Various multisensor systems (so called electronic tongues (ET)) are actively being suggested and studied in recent years [2]. The two most widely employed analytical platforms for ET are potentiometric and voltammetric, the first one being preferable for the simplicity reasons [3]. While there is a certain progress with potentiometric sensor development for sweeteners which can produce ionic species in a sample medium [1,4,5], determination of carbohydrate sugars by simple methods is still challenging. The substances like sucrose, fructose, and glucose are not ionized in the sample under normal conditions and thus cannot induce potentiometric response.

There are numerous instrumental methods described in literature for sugar determination. Classical analytical techniques like chromatography, capillary electrophoresis, etc. require quite expensive equipment, tedious sampling pretreatment procedures and long analysis time, thus they are poorly compatible with a concept of a sensor as simple, fast and inexpensive device. Enzyme or receptor based sensors are promising alternatives, but procedures for biological sensing element purification are quite complicated. Moreover resulted sensors are typically quite capricious, possess low stability, short life time and require pre-adjusted parameters in a sample for proper functioning [6]. Instead of using enzymes it is possible to use whole microorganisms, cells or living tissues, containing analyte-specific enzymes [7]. Microorganisms like bacteria and yeast are easy to cultivate, they do not require expensive purification, they have a great potential for detection of a wide range of chemical compounds and allow for multienzyme sensing schemes [8–10]. Three general types of microbial biosensors are known: electrochemical, optical and microbial fuel cells [8,10–14]. The electrochemical platform is very popular due to typically high sensitivity, fast response, ease of miniaturization, and ability to deal with dispersed heterogeneous media. Nevertheless, the most of suggested microbial biosensors employ immobilized microorganisms [12,15] which leads to certain drawbacks, like the need in proper immobilization method and limited life time of immobilized microorganism under varying environmental conditions.

The idea of this study was to develop very simple potentiometric method for sugar quantification which will be deprived of the drawbacks listed above and can be potentially employed in potentiometric electronic tongues. For this purpose we employed

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Saccharomyces cerevisiae (baker's yeast) culture as a sample modulator. In the course of sugar fermentation in a sample by yeast pH values are decreasing and this can be monitored with pH glass sensor and related to the sugar concentration. The applicability of this approach was demonstrated with quantitative sucrose determination in apple juices. Unlike in other biosensor methods the suggested approach does not require immobilization of bioactive transducer and thus significantly simplifies all handling procedures.

2. Materials and methods

2.1. Materials

Yeast extract was from "Fluka Analytical" (Germany), peptone (Bacto™ Tryptone) was from "BD Diagnostic Systems" (United States). D(+)Glucose, D(−)Fructose, sucrose Na_2HPO_4, KH_2PO_4, NaCl, KCl, $K_3[Fe(CN)_4]$ and NaOH were from "Vekton" (St. Petersburg, Russia). Methylene blue and HCl were from "Reaktiv" (St. Petersburg, Russia). All the chemicals were of analytical grade. Four apple juices of different brands were purchased from the local retail store.

2.2. Baker's yeast cultivation and preparation for analysis

Active dry baker's yeast *S. cerevisiae* employed in this study was from "SAF NEVA" (Russian Federation) and was purchased from the local retail store. Yeast cultivation was performed at aerobic conditions. Standard liquid medium for yeast cultivation was used, YPD which was used (yeast–peptone–dextrose, 1% yeast extract, 2% peptone and 2% glucose, pH 6.5–6.6). 1 g of dry yeast was added into 100 ml sterile YPD medium and was cultivated under stirring at 100 rpm at 30 °C for 1.5 h. After that the yeast cells were harvested by centrifugation at 4000 × *g* for 5 min at 4 °C and were washed once with PBS (phosphate buffered saline solution, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.3–7.6) and then centrifuged once again under the same conditions. After that the yeast cells were re-suspended in 5 ml PBS buffer and were stored in the fridge at 4 °C during 1 week.

2.3. Potentiometric analysis

Commercial glass pH electrode (ES-106017) and Ag/AgCl reference electrode (ESR Ag/AgCl 10103) were from Izmeritelnaya Tehnika (Moscow, Russia). Potentiometric measurements were performed with 0.1 mV precision using digital high input impedance mV-meter HAN-32 (Sensor Systems LLC, St. Petersburg, Russia) connected to PC for data acquisition and processing. Measurements were performed in thermostated (Julabo FP-50, Seelbach, Germany) electrochemical cell at 30 °C. Yeast assisted analysis was performed in 0.9% NaCl solution. The amount of yeast employed for each measurement was 50–55 μl of yeast cells suspension in 25 ml of 0.9% NaCl solution which corresponds to optical density of 0.5 at 540 nm (measured with photocolorimeter "KFK-2" from Zagorsk optical and mechanical plant (Yekaterinburg, Russia)). It was found that higher densities lead to the long establishment of steady state in potentiometric response (longer than 10–15 min). A sample to be analyzed was introduced directly into the described NaCl solution containing yeast culture and pH measurements were performed therein. The changes in metabolic activity of yeast were recorded by pH sensor during 5 min. The value ΔpH_5 which is the difference between $pH_{steady\text{-}state}$ and pH_5 was used for calibration purposes (Fig. 1). The observed pH changes are associated with general excretion of different organic acids and CO_2.

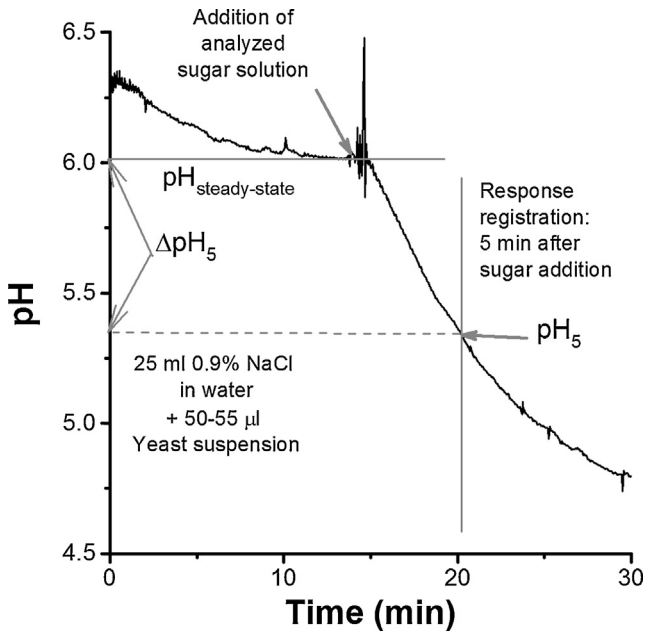

Fig. 1. Potentiometric response employed for yeast assisted sugar sensor development.

2.4. Titrimetric analysis

Sugar concentration in juice samples was determined with standard ferricyanide titrimetric method according to [16,17]. Briefly, the analysis was performed in three stages: (1) determination of the standard ratio between ferricyanide and glucose; (2) determination of total concentration of glucose and fructose; and (3) determination of sucrose (inverted sugars). 1.6% D(+)Glucose solution was used for titration of saline potassium ferricyanide solution (0.8% $K_3[Fe(CN)_4]$, 2% NaOH) with analyzed sample. Methylene blue was used as an indicator. Since sucrose cannot be determined directly using the same method, this sugar was inverted to glucose and fructose first by acidic hydrolysis.

3. Results and discussion

3.1. Optimization of measurement conditions

First it was necessary to optimize measurements conditions in the yeast-containing system to provide for robust and reproducible results.

3.2. Calibration measurements

Recorded calibration curves for D(+)Glucose, D(−)Fructose and sucrose in logarithmic scale were sigmoid (Fig. 2A). These data were obtained with standard solutions of sugars prepared from accurately weighed portions in 0.9% NaCl. Certain differences in the shape of the curves was observed, which obviously correspond to the differences in metabolism of these carbohydrates [18]. Since the yeasts employed for each cultivation were not from standard culture, but were from the local retail store and of different production batches and packs, the reproducibility of the response was studied. It was found that the yeasts from different packs show similar response to glucose (Fig. 2B), and only minor deviations (typically below 20%, which is negligible from the point of view of microbiology) can be observed in linear calibration region (0.18–1.3 mM). The same results were obtained for fructose and sucrose (data are