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Probiotics in Medicine and Methods with Electrochemical Detection

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Abstract: Preventing diseases from diarrhea to cancer, probiotics are used on different food products. Electronic tongues (e-tongues) were developed for complex matrices as food products. These new methods combine electrochemical devices (amperometric, potentiometric, impedimetric) and have as additional advantages to be fast and convenient for field an in situ analysis, being used with good qualitative and (semi)quantitative results also in the place were food products are (at the manufactures, in stores, in storage places).

Keywords: Probiotics; Prebiotics; Sensors; Biosensors; E-tongue; Electrochemical determination.

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Introduction

Probiotics (Greek: for life) are microorganisms being used for years now, preventing diseases from diarrhea to cancer [1]. Microbes were used unintentionally in dairy products or fermented vegetables. Recently, the beneficial aspect in health restoration and disease treatment was as important as improving food flavor. Probiotics are also known as “healthy”, “friendly” or “beneficial” bacteria.

The LAB (live microbes), a group of Gram-positive bacteria, include several species the genera *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Aerococcus, Bifidobacterium*, and *Weissela*. Lactobacillus is the most widely encountered for probiotics [2]; these include *L. acidophilus, L. bulgaricus, L. casei, L. fermentum, L. plantarum, L. reuteri, L. rhamnosus,* and *L. salivarius*.

The nutrient value of milk and the digestibility is being enhanced by the addition of specific lactic acid bacteria in milk used in the production of yogurt. The active cultures used in the fermentation process improve tolerance to milk sugar lactose, induce the denaturation of proteins therefore improving bioavailability and improve the absorption of calcium, magnesium and phosphorous [3].

Probiotics reduce the incidence of chronic diarrhea and acute gastroenteritis; they inhibit microbial colonization and translocation within the intestinal tract. Probiotic supplementation is used as prophylaxis and treatment in diarrhea, as well as for children or for immune-compromised patients [3].

The efficacy of probiotics was proved in many in vivo human clinical trials in treatment and prevention of several diarrheal diseases, like rotavirus diarrhea [4], travelers’ diarrhea [5], inflammatory bowel disease (IBD) [6,7], bacterial vaginosis in women [8], serious intestinal conditions like necrotizing enterocolitis (NEC) [6,9] and antibiotic-associated diarrhea (AAD) [10-12].

The use of antibiotics for eradication and killing of disease causing microorganisms is being favored by the existing clinical approach. Therefore, the preservation of the flora rather than its deletion provides a great clinical benefit. The probiotic therapy has no side effects and is preventive in comparison to the antibiotic therapy and is a natural way of non-invasive suppression of the growth of pathogens. Oral administration of probiotics is made through pharmaceuticals or/and food. A disadvantage for people with digestion related stomach disorders is that they cannot consume large quantities of probiotic food. Therefore pharmaceutical preparations for probiotics can overcome these limitations [13].

Adding prebiotics to probiotics enhances the efficacy. Prebiotics are nonviable food components that have a health benefit for the host, associated with modulation of the intestinal flora [14].

Electrochemical methods for food analysis

Electronic tongue

During the last years modern electronic tongues (e-tongues) were developed for food analysis [15], being used for complex matrices, using chemometric processing of signals produced by array of sensors that are in contact with the sample (Fig.1). E-tongues crudely mimic the human taste sensors (liquid and gas sensors) and their communication/connection with the human brain.

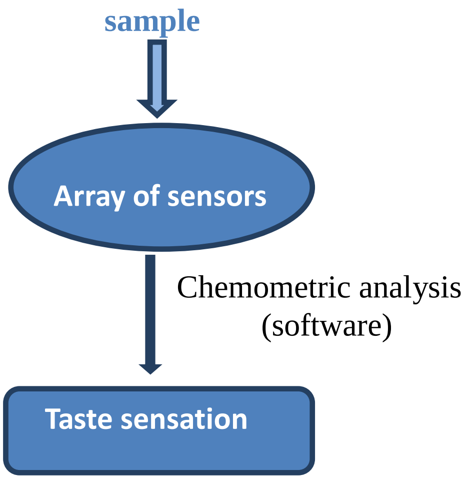

Fig. 1: *General schema of e-tongue.*

E-tongues are very promising for evaluation of food quality, replacing for some situations the usage of traditional methods. The advantages and disadvantages of traditional methods and e-tongues methods are presented in Table I. Regarding the sensor array used for e-tongues, different chemical sensors have been employed: potentiometric [15,16,17], voltammetric [18,19], amperometric [20,21], impedimetric [22], conductometric [23], optical [24], mass [25], and enzymatic-based sensors (biosensors) [26].

E-tongue combines signals of various sensors with pattern recognition. For amperometric sensors there are four categories including metal, phtalocyanine film, conducting polymer, and biosensors.

Table I: *The advantages and disadvantages of traditional methods and e-tongues methods*.

Method	Advantages	Disadvantages
Traditional	- Good precision. - Accurate. - Reliable.	- Destructive. - Time-consuming. - Require expensive equipment. - Are unsuitable for in situ or at site monitoring.
E-tongue	- Rapid. - Easy-to-use tools. - In places where foods are obtained/commercialized/stored. - Have shown good correlations with organoleptic scores provided by human panelists. - Can test samples that are unfit for human consumption. - Different types of sensing materials (liquid sensors), and liquid sensors possess higher selectivity and lower detection limits.	- Sometimes provide only qualitative or semi-quantitative information. - Is affected by the environment (temperature, humidity) which can cause sensor drift.

Three different detection modes are described: fixed potential preferred in flow systems (for biosensors) and also in step pulse potential and sweeping potential (batch systems). Amperometric screen-printed electrodes were coupled with microchip capillary electrophoresis [27]. E-tongues based on conducting polymers are recommended to evaluate bitterness [28], sweet, acid, bitter, salty, and astringent tastes [29]. One of the advantages of conducting polymers is considered the rapid adsorption/desorption process [27].

Phtalocyanine film electrodes have chemical properties that permit the coordination with transition metal. Films of phthalocyanine, naphthalocyanine and porphyrin showed cross-selectivity to some antioxidants like pyrogallol, banillic acid, ascorbic acid, and catechin. E-tongues containing films of phthalocyanine sensors may discriminate between model solutions of sweet, salty, bitter, and bitterness (in olive oils) [28].

Biosensors used for e-tongue are systems that use biochemical transducers. Oxidases (consume oxygen and produce hydrogen peroxide) or dehydrogenase (reduced form of β-nicotinamide adenine dinucleotide (phosphate) NAD(P)H) enzymes are frequently used as biorecognition elements. The performance of metal sensors, polymers with conductive properties or (bio)sensors is increased because of the size to nanodimensions that determine the detection limit decreasing [27].

A synthesis of e-tongue methods are presented in Table II, concentrating on the sensor type and the most important characteristics of the developing method.

Table II: *Characteristics of different sensors and their advantages/disadvantages.*

Sensor type	Method	Advantages/Disadvantages	Ref
Potentiometric	Simultaneous determination of ethanol, lactic acid, acetic acid and citric acid, acetaldehyde, diacetyl content in probiotic fermented milk. Sensor array (contains 7 non-specific, cross-sensitive sensors) used for food analysis, coupled with Ag/AgCl as reference electrode.	- Poor performance. - Low sensitivity.	[30]
Potentiometric	Allows differentiation between the five basic tastes, salty, bitter, acid and umami tastes, being more effective towards the recognition of the acid, salty and umami tastes. The multi-sensor system includes two sensor array sets together with a reference electrode Ag/AgCl with double junction.	- Fast (real time) analysis. - Economic procedure. - Could evaluate possible adulterations of goat raw milk with cow raw milk. However, in order to use the e-tongue as a routine methodology for caprine milk adulteration detection in the dairy industry, it is needed to improve the multi-sensor system by testing and including more sensible sensors to milk composition variations.	[31]
Amperometric	Amperometric biosensor for the determination of L-lactic acid in probiotic yogurts has been assembled using L-lactate dehydrogenase entrapped in 1 % (v/v) neutralized Nafion solution deposited on Variamine blue redox mediator modified screen-printed electrodes. The Variamine blue was previously covalently linked to oxidized single-walled carbon nanotubes and used for modifying screen-printed electrodes	- Fast (short period of time). - Easy to use. - Permit the analysis of a great number of samples in sequence.	[32]

Electrochemical sandwich assay

An electrochemical sandwich assay was developed to analyze attomoles (10^{-18} mol) of DNA and RNA from beer spoilage bacterial cells *Lactobacillus brevis*. The electrochemical sandwich DNA hybridization assay is exploiting enzymatic activity of lipase [33].

Electrochemical optical waveguide lightmode spectroscopy

Scientific literature indicated biosensing technique in order to evaluate the effect of different inhibitors on lactic acid bacteria. Electrochemical optical waveguide lightmode spectroscopy combines the processes of electrochemical control of adsorption at the surface with evanescent-field optical sensing [34]. A layer of indium tin oxide was used for optical sensing, serving as a high refractive index waveguide and as a conductive electrode.

Electrochemical optical waveguide lightmode spectroscopy measurements were performed and analyses were done in a flow-injection analyzer system. *Lactobacillus plantarum* 2142 suspended in Jerusalem artichoke syrup were used. The bacterial cells were adsorbed in native form without chemical binding on the surface of the sensor. The effect on bacteria cells in presence of different concentration of lactic acid/Jerusalem artichoke syrup, acetic acid/Jerusalem artichoke syrup, and hydrogen peroxide/Jerusalem artichoke syrup solution was analyzed. The experimental results were compared to the classic micro-assay procedure. Based on their conclusion, this new technique could be used in real-time application.

Conclusions

Probiotics are interesting agents with an important contribution in the field of preventive nutrition, and they should therefore have an important development. Positive effects have now clearly been demonstrated in specific fields with certain products. The electrochemical methods are developing fast, discovering easier, more efficient ways to detect, observe and monitor the probiotics. The use of e-tongue has shown great advantages in this field, being a reliable method. In conclusion, the electrochemical methods are very promising and more research in this area must be done to develop faster, easier ways to detect probiotics and other important substances.

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