Review article

Lactate biosensors for food industry

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Abstract: *Lactic acid and lactate fermentation play important role in food and beverages production, control and quality. Analysis of lactate by standard methods is time, work consuming, and cannot be implemented at the production site. The alternative is the use of biosensors for lactate. The article reviews the biosensors for D- and L-lactic assay, their types, construction and application in food analysis area. The special emphasis is given to the commercial biosensor for lactate. The market survey indicates that there is a lack of lactate biosensors addressed to food industry except wine production. QUALI_JUICE project was the practical attempt to use some of the commercial biosensors in production and quality control in fruit juice industry. The results of the project indicates that commercial biosensors can be used after some minor functional adaptation in fruit juice production control. The application of these biosensors can be broadened to the other sectors of food and beverage industry.*

Keywords: *lactate assay; lactate biosensor, fermented food; food spoilage; process control.*

Introduction

Lactic acid is the end product of glycolysis in anaerobic conditions. There are two optically active stereoisomers of lactic acid: L(+)-lactic acid and D(-)-lactic acid. L-lactic acid is produced in muscles during anaerobic glicolysis and by most of lactic acid bacteria [1]. D-lactic acid is produced by some bacteria, algae and plants [2]. Many lactic acid bacteria are producing racemate mixture of lactic acids [3, 4]. Lactic acid and lactate assay is very important in many areas. Among them are:

- Medicine – indicator of ischemia for critical care, indicator for some inflammations,
- Sport medicine – testing and monitoring of training intensity and recovery,
- Food quality analysis – fermented food, marker for bacteria spoilage of food and beverages,
- Oenology – monitoring malolactic fermentation in wine,
- Agriculture – indicator of bacteria mammary infection (mastitis),
- Veterinary – blood lactate control in animal diseases and training (racing horses or dogs),
- Process control in biotechnology – production of lactic acid.

Lactic acid fermentation is one of the key processes in the food industry. The production of fermented food is one of the oldest food processing technologies used by mankind. Fermentation of milk, meat and vegetables by lactic acid bacteria is known from 6000 BC [3]. The main goal of the use of this fermentation in food processing is a preservation effect. This process helps also to provide products with right taste, aroma and texture, improves the nutritional value of food, can eliminate some toxins [5-7] and prevent growth of undesired microorganisms like Gram-negative bacteria or moulds [8, 9]. The lactic acid fermentation contributes also to some probiotic properties of final product [10].

L-lactic acid (E270) is used in food industry to decontaminate meat or poultry and prevent bacteria growth on them [11-13], to inhibit spoilage yeast grow [14] and also as antibrowning agent [15]. About 50% of lactic acid is used as emulsifying agent in bakery production [16]. It is used also as acidulant, pH buffering agent and flavouring in variety of foods and beverages like candy, jams, jellies, soft drinks and many others [17]. L-lactic acid can be produced either by chemical methods or by lactic acid fermentation [17]. Lactic acid is also used in the chemical industry to produce biodegradable polymers [18], in cosmetics, pharmaceutical, leather and textile industries [17].

On other hand sometimes lactic acid fermentation is undesired in food and is leading to its spoilage. The increased level of L-lactate in egg is an indicator of spoilage by contamination or incubation [19]. Also in canned fruits and vegetables, UHT milk the increase of L-lactate is the marker of spoilage [20]. Contamination of fruit juices with lactic acid bacteria during production and storage often remained unnoticed for a longer time. Lactic acid fermentation of juices is leading to alteration of organoleptic properties of them, not acceptable for human consumption and economic loses for the producer and environmental problems [19, 21]. The Code of Practice of the Association of the Industries of Juice and Nectars from Fruits and Vegetables of the European Economics Community (AIJN) [22] established the maximum permissible concentration of sum of both isomers of lactic acid in fruit juices as 0.5 g L^{-1}. Lactic acid bacteria are also often causing spoilage of beer [23].

D-lactic acid is produced by some bacteria (*Staphylococcus sp.*, *Enterobacter sp.*) and its increased level is observed in urine and blood in some bacteria infections [24]. The presence of D-lactic acid is an indicator of food spoilage especially raw meat [25], tomato and products from them [26]. D-lactic acid is not utilized in humans so its consumption must be controlled. WHO recommend a daily intake of this isomer in man to be less than 100 mg kg^{-1} body weight [27] and do not recommend its consumption by infants and young children [28].

In wine and cider production very important is malolactic fermentation carried out by lactic acid bacteria (mainly *Oenococus oeni*) after main alcoholic fermentation. L-malic acid is converted to L-lactic acid reducing the acidity of wine and leading to improvement of taste and flavour [4, 29]. Some strains of lactic acid bacteria can carry out fermentation producing D-lactic acid causing „piqûre

lactique" taste [30], which is undesired and lowers wine quality. The presence of D-lactic acid in wine and cider is used as an indicator of their spoilage [4].

The concentration of lactic acid (sum of both isomers) is typically measured using HPLC with UV/VIS or conductometric detection [21, 31, 32] or capillary zone electrophoresis [33]. Attempts were made for chiral resolution of D- and L-lactic acid by capillary electrophoresis to assay them in food products [34]. The lactic acid stereoisomers can be assayed independently by enzyme kits with stereospecific NAD^+ dependent dehydrogenases [2, 35]. Such kits are produced for example by Boehringer, (Germany) or Megazyme, (Ireland). Other possibility of lactic acid assay (sum of both isomers) is the use of colorimetric test strips and Reflectoquant® supplied by Merck (Germany). Chromatographic methods and enzyme ones are expensive, time consuming and need well trained laboratory stuff. They need also intensive sample pre-treatment and cannot be used in food industry at line or on line. The alternative for these methods is the use of biosensors for lactic acid assay. Biosensors provide rapid, simple and direct measurement, with no need of sample preparation [36, 37].

What is a Biosensor ?

According to definition given by IUPAC, a biosensor is an integrated receptor-transducer device, which is capable of providing selective quantitative or semi-quantitative analytical information using a biological recognition element in direct spatial contact with transducer element [38]. Biosensors can be classified according to the transducer element into [39]:

- Electrochemical (amperometric, potentiometric, coulometric, conductometric),
- Optical (absorbance, fluorescence, chemiluminescence, surface plasmon resonance),
- Piezoelectric (quartz crystals, surface acoustic wave),
- Calorimetric,
- Semiconductors (field effect transistors, light addressable potentiometric sensors),
- Others.

The first biosensor for lactic acid (L-lactate) was reported in 1970 by Wiliams et al. [40]. It was amperometric electrode with cytochrome b_2 as biological sensing element with $Fe(CN)_6^{3-}$ as redox mediator.

Biological Recognition Elements in Lactate Biosensors

As the most important part of every biosensor is the biological recognition element, the review of possible biological receptors for lactate (both isomers) is given below.

The most common biological receptor for L-lactic acid is L-lactate oxidase (LOD) which catalyzes the reaction:

$$L\text{-}lactate + O_2 \xrightarrow{L-lactate\ \ oxidase} pyruvate + H_2O_2 \qquad (1)$$

L-lactate content can be assayed by measuring consumption of oxygen by means of oxygen electrode [41, 42] or electrooxidation of H_2O_2 produced in reaction (1)

on platinum electrode. In this case, the current produced by electrochemical reaction is directly proportional to the lactate concentration [42-44]. Because ascorbate, uric acid and paracetamol react at the electrode at nearly the same potential as H_2O_2, their presence in the samples can interfere with lactate assay. To eliminate interferences the additional layers can be added covering the immobilized LOD. Typically Nafion® is used as the anti-interference layer [45], aromatic polyamines like polyaniline or polytyramine [46] or overoxidized polypyrrole [47]. The elimination of interferences influence could be achieved also by lowering the potential of H_2O_2 oxidation by modification of electrode surface by phtalocyanine [48] or Prussian blue [49].

LOD is the enzyme that accepts artificial redox mediators instead oxygen:

$$L\text{-}lactate + Med_{ox} \xrightarrow{L-lactate\ \ oxidase} pyruvate + Med_{red} \tag{2}$$

The reduced mediator is oxidized at electrode surface generating current which is proportional to the lactate concentration.

$$Med_{red} - z\bar{e} \rightarrow Med_{ox} \tag{3}$$

Typical redox mediators used in lactate biosensors are transition metal complexes like $Fe(CN)_6^{3-}$ [50], ferrocene and its derivatives [51-53], Prussian blue [54] or organic dyes like Meldoda blue [55]. Application of osmium complex conjugated with polymer leads to "wiring" of the mediator to LOD and the direct exchange of electrons between active centre of the enzyme and electrode could be achieved giving the electrode response at the absence of oxygen [56].

L-lactate oxidase was used also in construction of electrochemilumenescent biosensors, in which oxidation of luminol by H_2O_2 produced in reaction (1) is followed by measuring either current or intensity of produced light [57]. Oxidation of luminol by H_2O_2 produced in reaction (1), catalysed by peroxidase is accompanied by chemiluminescence. This phenomenon is the basis of chemiluminescent optical biosensor for lactate [58, 59].

The other biological receptors for L-lactate are:

- Lactate monooxygenase (specific for L-lactate) which was used in optical sensor [60]

$$L-lactate + O_2 \xrightarrow{lactate\ \ monooxygenase} CH_3COOH + CO_2 + H_2O \tag{4}$$

- Cytochrome b_2 from yeast catalyzing reaction [40]

$$L-lactate + Med_{ox} \xrightarrow{cytochrome\ \ b_2} pyruvate + Med_{red} \tag{5}$$

- PQQ (pyrroloquinoline quinone) dependent L-lactate dehydrogenase [61],
- Microorganisms like *Saccharomyces cerevisiae* [62], *Escherichia coli* [63], *Shewanella putrefaciens* [64], *Acetobacter pasteurianus* [65],
- Porcine kidney tissue used in chemiluminescent biosensor [66].

Unless cytochrome b_2 the other biological receptors mentioned above are rarely used and their practical importance is negligible.

As it was mentioned earlier, there are two stereoisomers of lactic acid. All biological receptors described before are specific for L-lactic acid but sometimes there is a need to assay D-lactic acid alone or sum of both isomers. There are two stereospecific NAD^+ dependent lactate dehydrogenases (L-LDH and D-LDH):

$$L-lactate + NAD^+ \xrightarrow{L-lactate\ \ dehydrogenase} pyruvate + NADH + H^+ \quad (6)$$

$$D-lactate + NAD^+ \xrightarrow{D-lactate\ \ dehydrogenase} pyruvate + NADH + H^+ \quad (7)$$

The lactate content can be in this case measured amperometricaly by electrooxidation of NADH [67-69] or optically by absorbance [70] or fluorescence of NADH [71]. The application of lactate dehydrogenases in biosensor construction is connected with many problems:

- The electrooxidation of NADH is not fully reversible and is leading partially to a dimer production which is enzymatically inactive,
- The electrooxidation of NADH needs high overpotential,
- The reactions (6) and (7) are reversible. Because of that L-LDH is used sometimes to assay pyruvate in reverse reaction at low pH,
- Reaction rate of reactions (6) and (7) is strongly pH-dependent and is high enough at pH greater than 7.

To overcome the problems connected with electrooxidation of NADH the surface of the electrode was modified by redox mediators like Meldoda blue [30, 72, 73] or polyaniline [74].

The other possibility is co-immobilization of diaphorase – the enzyme catalysing reaction:

$$NADH + Med_{ox} \xrightarrow{diaphorase} NAD^+ + Med_{red} \quad (8)$$

The reduced form of synthetic redox mediator is then oxidised on the electrode according to reaction (3). Typical redox mediator used in such approach is $Fe(CN)_6^{3-}$ [75, 76].

NADH can be also oxidised by specific oxidase:

$$NADH + O_2 + H^+ \xrightarrow{NADH\ \ oxidase} NAD^+ + H_2O_2 \quad (9)$$

When lactate dehydrogenase and NADH oxidase are coimmobilized on electrode surface, lactate concentration can be evaluated either by oxygen consumption or H_2O_2 production or with the use of redox mediator $Fe(CN)_6^{3-}$ [77, 78].

L-LDH and LOD can be immobilized together yielding enzyme electrode with much greater sensitivity as compared with electrode with each enzyme alone due to the substrate recycling [79, 80]. This combination of enzymes with additional catalase was used in enthalpy meter for lactate [81] in which L-lactate was assayed by measuring temperature increase. By coimmobilzation of D-LDH, LOD and horseradish peroxidase on oxygen electrode the biosensor for total lactate was constructed [82].

The equilibrium of reactions (6) and (7) can be shifted to the products by consumption of pyruvate, as it is done in enzyme kits, by application of additional enzyme L-alanine aminotransferase (glutamic-pyruvic transaminase) catalysing reaction:

$$pyruvate + glutamate \xrightarrow{L\text{-}alanine\ \ aminotransferase} L-alanine + 2-oxoglutarate \quad (10)$$

Such approach was used in some constructions of lactate electrodes [74]. The same sequence of enzyme reactions connected with *Vibrio sp.* luciferase was used in bioluminescent biosensor for L- and D-lactate [83].

Specific D-lactate cytochrome c oxidoreductase (D-LCR) isolated from *Saccharomyces cerevisiae* catalysing reaction:

$$D-lactate + 2\ ferricytochrome\ c \xrightarrow{D\text{-}LCR} pyruvate + 2\ ferrocytochrome\ c + 2\ H^{+} \quad (11)$$

was used with phenazine methosulphate as the mediator in amperometric screen-printed electrode for D-lactate [84].

Key Factors in Lactate Biosensor Aplication

Practically only L-lactate oxidase and both lactate dehydrogenases have real importance in lactate biosensors construction. Most of the lactate biosensors described in literature are amperometric ones and are reviewed up to 2007 by Nikolaus & Strehlitz [19] and only they found application in commercial biosensors for lactic acid. The main trends in construction of amperometric electrodes are: miniaturization to obtain implantable probes, incorporation in flow system, production by screen-printing technique allowing for cheap and mass production of biosensors.

The key factor in developing of a biosensor is the immobilization of the enzyme at the transducer surface. The performance factors (lifetime, sensitivity, selectivity, stability, response time and linear range) depend strongly on the method used to immobilize the enzyme [19]. Typical methods of immobilization are physical adsorption, entrapment in polymer matrix or inorganic gel, crosslinking by bi-functional reagents, covalent binding and enclosing by semi-permeable membrane [38]. Immobilization method influences the enzyme properties like thermal stability, pH optimum, Michaelis constant [52, 85, 86]. Very promising strategy in production of enzyme electrodes is screen-printing using inks composed of powdered carbon or graphite, enzyme, mediator and polymer as binder to obtain working electrode [55, 71, 84, 86, 87], Such approach enables miniaturisation of the biosensor, which can be used as disposable one.

The important parameters of biosensor operation are sensitivity, linear range and lower detection limit. These parameters are connected together and depend on the biological receptor, method of immobilization and construction of the biosensor [19]. For most of the lactate electrodes with LOD or LDH the upper limit of linear range is between 1 and 2 $mM \cdot L^{-1}$, when the biosensor is used in batch mode. The linear range can be extended considerably by adding covering

membrane limiting the substrate diffusion to the biosensor surface [43]. The response time of lactate biosensors is typically less then 5 minutes reaching in some cases even 10 seconds [50].

One of the important problems in practical application is the shelf and operational lifetime of the biosensor determined greatly by the stability of the enzyme but also by the immobilization method. The activity of the enzyme immobilized on the biosensor is decreasing in time, what causes the necessity of recalibration during prolonged use of the biosensor. This problem can be omitted by the use of disposable biosensors if the shelf life is long enough. Such approach is used in some commercial amperometric biosensors for lactate. The data in literature about operational stability of lactate biosensors are hardly comparable, because this parameter was examined in too many different ways [19]. Generally, the described operational lifetimes are not longer than 2 weeks. The shelf life (storage stability) of lactate biosensors is much longer reaching 6 months for biosensor kept in refrigerator [88].

The electrochemical lactate biosensor can work in batch mode or in flow system. The incorporation of the biosensor into flow system allows automatic measurements, less work and time, with extended linear range [47, 89].

Application of Lactate Biosensors in Food Analysis

Most of the applications of lactate biosensors described in literature are in the area of clinical analysis and sport medicine – L-lactate assay in blood and serum but also in saliva and tears [90]. Apart typical medical applications lactate biosensors were used to assay lactate content in food and beverages. Most of biosensors proposed in literature to assay lactate in different food samples are amperometric enzyme electrodes with L-lactate oxidase or with L- or D- NAD^+ dependent dehydrogenases [19].

Although other types of sensor (potentiometric, field effect transistors, (electro)chemiluminescent) are used to construct lactate biosensor [90], only few of them were applied to assay lactate in real food or beverage samples. Nguyen-Bosse et al. described conductometric biosensor with L-lactate oxidase and horseradish peroxidase immobilized in bovine serum albumin gel [91]. They claimed that LOD lost after immobilization its stereospecifity and applied with success proposed biosensor to assay both stereoisomers of lactic acid in yoghurt samples. Fluorimetric biosensor with L-LDH was used in flow injection system to assay L-lactate in milk drink through increase of NADH fluorescence [71]. Girotti et al. described a flow system with D- or L-LHD, luciferase from *Virio sp.*, (NADH:FMN) oxidoreductase and L-alanine aminotransferase immobilized on the tip of optical fibre measuring D- or L-lactate in beer samples by bioluminescence [83]. Luminol oxidation by H_2O_2 formed in reaction (1) catalysed by peroxidase accompanied with chemiluminescence was used to assay L-lactate in milk [66] and yoghurt [59]. Electrochemiluminescence accompanying luminol electrochemical oxidation coupled with reaction (1) was used in biosensor for assay of L-lactate in whey [57].

Amperometric lactate biosensors were used to assay L-lactate in milk and milk products like yogurts [53, 92-95], cream or cheese [96, 97] without special pre-treatment of samples only with appropriate dilution. The concentrations of L-lactate measured by the biosensors were in good agreement with the results obtained by standard enzyme spectrophotometric methods. The other area of lactate biosensor application in dairy industry is following the fermentation of milk [43, 46, 98] or mozzarella cheese production and ripening [99]. There were attempts to use lactate biosensor to assay traces of antibiotics in milk through inhibition of LOD [100]. L-lactate biosensor was also applied to detect bacterial contaminations in UHT milk leading to its spoilage [20]. Other important area of lactate biosensors application is wine production. Wine contains L-lactate as the result of malo-lactic fermentation but can contain also D-lactate in case of spoilage [76]. Because of that, for wine quality control, both types of biosensor for L- and D-isomers were developed [30, 72, 73, 75, 85, 101]. Mazzei et al. proposed a multienzyme biosensor with LOD and D-LDH for assay the sum of D-and L-lactic acid in wine [82]. L-lactate biosensors were used with success to follow the malo-lactic fermentation in wine[29, 102]. The polyphenolic compounds present in wine, especially in red ones, give some matrix effect to the biosensor response [53] but this problem can be solved by dilution of samples. Lactate biosensors were used also for quantification of L-lactate in other beverages like cider [74], beer [83, 103] or kvass [104]. There are only few examples of lactate biosensor application in other food sectors. Lactic acid was assayed by biosensors in tomatoes and products from them [26, 47] or in meat extracts [105]. Lactate biosensor was also used to assay L-lactate in fermented food like olive and cabbage brine [95], kimchi [106], sport drinks [107].

Assay of L-lactate concentration by lactate biosensor can be used to monitor fermentation processes [31, 79]. L-lactate biosensors can be helpful in veterinary and agriculture for example to detect cattle mastitis [108] because L-lactic acid is present in milk during cow milking in case of udder inflammation. Additionally lactate biosensors can be used in veterinary in similar cases as in human (sport) medicine.

Commercial biosensors for lactate

Most of the commercial biosensors for lactate available on the market utilize L-lactate oxidase as biosensing element and are devoted to assay L-lactate in blood and serum in emergency rooms in hospitals, in clinical laboratories or for personal use for athletes, as the blood lactate level during exercise and after it is the indicators of training status and recovery. Depending on application, the apparatus measuring L-lactate with biosensor could be a laboratory stand, portable or hand held device. Biosensors used in L-lactate analysers could be reusable or disposable. The last approach is typical for hand held devices in sport medicine for personal use. Some devices present on the market can be applied for bioprocess control and food analysis. The commercial biosensor for lactate are summarised in Table 1.

Table 1. Commercial models of lactate biosensors, their producers and application

Producer	Model	Purpose
YSI Inc (Ohio, USA)	YSI 2300 STAT™ Plus Glucose & Lactate Analyzer	Clinical analysis
	YSI 2700 SELECT™ Biochemistry Analyzer	Bioprocess control, analysis of food and beverages
	YSI 7100 Multiparameter Bioanalytical System	Bioanalysis
	YSI 1500 SPORT™ Lactate Analyzer	Sport medicine
Nova Biomedical (Massachusetts, USA)	Stat Profile® Critical Care Xpress Stat Profile® pHOx	Clinical analysis
	BioProfile® Analalyzers	Bioprocess control
	Lactate Plus	Personal use (sport medicine)
Abbot Laboratories (Illinois, USA)	i-STAT®	Clinical analysis (point of care and emergency room)
EKF Diagnostic GmbH (Germany)	Biosen C_Line Biosen S_Line	Clinical analysis
Sens Lab (Germany)	Lactate SCOUT	Personal use (sport medicine)
ARKRAY (Japan)	Lactate Pro LT-1710	Personal use (sport medicine)
Roche Diagnostic (Germany)	Accutrend® Plus	Clinical analysis (point of care)
	Accutred® Lactate	Personal use (sport medicine)
DiaSys GmbH (Germany)	SensoStar	Clinical analysis
Med-Tronik GmbH (Germany)	Powerlact®	Personal use (sport medicine)
ApexBio (Taiwan)	The Edge™	Personal use (sport medicine)
Bio Sensor Technology GmbH (Germany)	LABTREND	Clinical analysis
	LAC^{PRO}	Clinical analysis (portable)
	LactatProfi 3000	Clinical analysis (portable)
Sensolytics GmbH (Germany)	OLGA (On Line General Analyser)	Bioprocess control
Trace GmbH	Labo TRACE	Clinical analysis
	TRACE C2 Process TRACE Multi TRACE	Bioprocess control
BioFutura s.r.l. (Italy)	Wine checker “Per Bacco”	Wine analysis
Tectronik s.r.l. (Italy)	Senzytec 1	Wine analysis

Only YSI Inc. offers the biosensor for food and beverages analysis purpose. In Italy, which is a great wine producer, two little companies (BioFutura and Tectronik) developed amperometric biosensors for D- and L-lactic acid assay, while concentrations of both of them are important wine quality parameters. These two devices use disposable biochips with immobilized lactate dehydrogenases. Application of both of them can be broadened to other analytes like glucose, ethanol, L-malate and others using appropriate electrodes [75].

Very near to the concept of biosensor is SIRE® Biosensor 101e produced by Chemel AB (Sweden). It consists of small reaction chamber with electrodes. For measurement, a small aliquot of L-lactate oxidase is injected to this chamber and the electric current accompanying reduction of H_2O_2 produced in reaction (1), which is proportional to L-lactate concentration, is measured. SIRE technology was used to measure L-lactate in tomato paste and baby food [109].

QUALI_JUICE project

The aim of QUALI_JUICE project "Quality assurance and development of an early warning system for microbial spoilage for the European fruit juice industry", contract number COLL-CT-2005-012461 was the application of lactate biosensor at juice producing plants (especially from apples) to control the production process and quality of the final product. Small and medium fruit juice producers face the problem of contamination with lactic acid bacteria, provoking undesired fermentation processes leading to juice spoilage and causing financial losses for the affected companies and environmental problems. To reach the goals of the project it was divided into few work packages:

- Definition of the problem assumption (risk of contamination by the lactic acid bacteria, producers requirements, state of art in area of lactate biosensors),
- Laboratory validation of chosen biosensors (stability, sensitivity, limits of detection, accuracy, precision, reproducibility, interferences),
- Functional adaptation of chosen biosensors to measure lactate in fruit juices (preparation of samples, elimination of interferants, changes in measurement protocols),
- Practical application (lactate assay in different juice samples and comparison of the results with other methods – enzyme kits and chromatography, measurements of lactate level in fruit juices at the production plant by the producer personnel, evaluation of the biosensors by juice producers, installation of early warning system with the biosensor on-line),
- Training of the juice producers in the use of the biosensors,
- Dissemination (information campaign in press, at exhibitions and conferences and by internet).

The consortium involved in the project decided to use commercial biosensors as the control instruments. The chosen ones were: Biosen C_Line sport (EKF, Germany), LactatProfi 3000 (ABT, Germany), OLGA (Sensolytics, Germany) and Senzytec 1 (Tectronik, Italy). The first two devices are devoted to assay

L-lactate in blood and serum samples. While the chemical composition and pH of fruit juice is completely different from blood both biosensors needed detailed validation [110]. The obtained results indicated that they can be used to assay L-lactate in juice samples after some modifications in operational protocols. The reliable results were obtained when the samples were purified by polyamide 6 instead polyvinylopirrolidone. This result was adopted by Tectronik in the new version of Senzytec device. In case of LactatProfi 3000 crucial point is the calibration of the device. When L-lactate is measured in fruit juices the calibration must be done before every measurement not every 8 hours as it is stated in the producer instruction. The biosensors were used to assay of D-lactate (only Senzytec 1) and L-lactate in samples of apple pulp, apple must and concentrates from the production site [110, 111] , commercial apple juices [110], other fruit juices and traditional fermented food. The results were compared with those obtained by enzyme kits. The results obtained with three tested biosensors and standard enzyme kit method were in good agreement. The biosensors were used to follow the laboratory fermentations of apple juice and apple pulp by lactic acid bacteria and the results were comparable with those obtained by chromatographic method [31, 32]. The use of the biosensors to assay L-lactate in connection with bacteriological analysis allowed to find the critical point at the production plant where the secondary microbiological contamination of pasteurised juice could happened [111]. Biosen C_Line sport, LactatProfi 3000 and Senzytec 1 were tested by some juice producing companies. The evaluation by the end users indicates that from their point of view the best choice would be Biosen C_line sport because of its accuracy, user's friendly interface and simplicity of measurement. The limiting factor of its use is the economical impact (the price of the device is too high for small and medium enterprises).

Because the increasing level of L-lactate in juice is the indicator of the begging of lactic acid bacteria fermentation [111] lactate biosensor can be a part of early warning system on-line. For this purpose OLGA device was chosen while it is working in stopped flow mode. It was integrated with alarming device and connected with a sampling system at the critical point at the production plant. The functional test of the whole system lasted 4 weeks and showed that continuous control of L-lactate concentration at the production line is possible.

The results of QUALI_JUICE project indicates that commercial biosensors for L-lactate can be used in juice production industry to control the production process and quality of final product. The final choice of the device (biosensor) by the future user (juice producing company) would be defined by its particular demands (simplicity of the measurements, possibility of usage at line) and economical impact (price of the device and consumables).

Conclusions

After more than forty years from the first described lactate electrode the application of lactate biosensors in areas different from medicine (including sport medicine) is still very limited. In food and beverage production the assay of lactic

acid is very important to control lactic acid fermentation processes (also undesired leading to the spoilage) and product quality. The use of the biosensors for this purpose could be the alternative for standard methods like enzyme kits and chromatography. The main advantages of the lactate biosensor use are as follows:

- Shortening the time of analysis,
- The assay can be made by unskilled personnel after short training,
- The measurements can be done at the production site,
- The cost of analysis is lower as compared with standard methods.

The disadvantages of the biosensor use in food and beverage industry are: the fact that the measurement with biosensors is not included in standards and legislation and the lack of commercial biosensors devoted for lactate assay in food except YSI 2700 SELECT™ Biochemistry Analyzer (YSI Inc., USA). Only for the wine industry, the market offers specific biosensors for complex analysis of wine production and quality.

Application of lactate biosensors in food and beverage production described in literature is still limited mainly to dairy products and wine. The results of the QUALI_JUICE project proved that commercial biosensors dedicated for assay of L-lactate in blood could be used without any changes in construction for analysis of fruit juices and other products. There is only a need of some simple sample preparation (absorption of interferants by polyamide 6) and some changes in operation protocols (frequency of calibration). These results open the opportunity for much broader use of lactate biosensors in food and beverage industry.

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