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Amperometric Determination of L-Lactate Based on Entrapment of Lactate Oxidase on a Transducer Surface with a Semi-Permeable Membrane Using a SIRE Technology Based Biosensor. Application: Tomato Paste and Baby Food

KIRSTIN KRIZ,[‡] LINDA KRAFT,[†] MARGARETA KROOK,[†] AND DARIO KRIZ*,[‡]

Chemel AB, Research Park IDEON, SE-223 70 Lund, Sweden, and Department of Pure and Applied Biochemistry, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden

Determination of dissolved L-lactate in tomato paste and baby food samples using a SIRE-based (sensors based on injection of the recognition element) biosensor is reported. The measuring principle is based on the use of a small amount of enzyme, which is injected into an internal delivery flow system and held in direct spatial contact with the amperometric transducer by the use of a semipermeable membrane. Measurements are based upon the reversible enzymatic conversion of L-lactate to pyruvate and hydrogen peroxide by lactate oxidase. Differential measurements are performed in which the samples are measured in the presence and absence of enzyme allowing for control over matrix interferences present in crude samples. The linear range investigated for the determination of L-lactate in tomato paste and baby food was 0−0.1 mM using a lactate oxidase concentration of 22 U/mL. Samples were diluted with buffer prior to biosensor measurements. The L-lactate concentrations of the tomato paste and baby food were determined to be 1.02 ± 0.02 mM and 2.51 ± 0.10 mM, respectively, using the standard addition method. The repeatability for tomato paste and baby food measurements was 2.5% (RSD, $n = 15$) and 4.0% (RSD, $n = 15$) and the reproducibility was 13.0% (RSD, $n = 45$) and 3.0% (RSD, $n = 45$), respectively. The concentration of dissolved L-lactate can be used as a measure of freshness in the food industry. All biosensor measurements were compared with measurements from an established spectrophotometric assay (Boehringer Mannheim). It was found that the biosensor had good correlation with the spectrophotometric method. The biosensor gave 12% higher values for the tomato paste measurements and 2.5% higher values for the baby food measurements. However, a distinct advantage of the biosensor is that it can perform L-lactate measurements within 3 minutes, whereas the spectrophotometric assay requires a 35-minute measurement time.

KEYWORDS: Biosensor; L-lactate; tomato paste; baby food; SIRE; differential measurements

INTRODUCTION

Food quality monitoring is one of the major concerns within the food industry. In particular, there is a growing need to develop analytical instruments which can provide quality monitoring for the entire food processing operation, including starting materials and final products (*1*). Biosensors are highly selective analytical instruments, due to the high selectivity of the biological recognition element employed, and have been applied in an array of disciplines including medicine, industry, environmental analysis, food technology, and military, among others (*2*).

Amperometric biosensors are self-contained integrated devices in which the biochemical receptor or biological recognition element is retained in direct spatial contact with an electrochemical transduction element (*3*). Furthermore, an effective biosensor requires intimate contact between its biocatalytic and its sensing components. The most conventional approach to this problem is to immobilize the receptor directly to the transducer surface, and such bioelectrodes have been used in the detection of lactic acid in tomatoes (*4*). Another way to create this closeness between the biocatalytic and sensing sites, and which has been applied in the detection of L-lactate in wine and dairy products, is the use of bulk modified bioelectrodes such as solid binding matrixes (*5*), graphite Teflon composite biosensors (*6*), and screen-printed biosensors coated in Nafion (*7*). Other approaches which have also been applied in the detection of L-lactate in dairy products involve entrapment of the biological recognition element in a nonconducting film such as poly (*o*-phenylendiamine) and overoxidized polypyrrole (*8*), polypyrrole−polyvinylsulfonate composite films (*9*), and glutaraldehyde

* To whom correspondence should be addressed. Phone: +46 46 286 2233. Fax: +46 46 286 2499. E-mail: Dario.Kriz@euris.org.
[†] Chemel AB.
[‡] Lund University.

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and overoxidized polypyrrole (*10*). Furthermore, there has been development within biosensor research to miniaturize the entire sensing system for application within the food industry using silicon sensor chip technology (*11*) and development of needle-type sensors (*12*).

Our group has suggested entrapment of the biological recognition element onto the transducer surface using a semi-permeable membrane for close intimate contact between all component parts in the use of sensors based on injection of the recognition element (SIRE) technology based biosensors (*13*). A flow-through amperometric microenzyme sensor and free enzyme has also been described for the detection of lactate (*14*). The SIRE biosensor is an integrated system in which the enzyme or biological recognition element is injected into an internal delivery system and uses a reaction chamber which is positioned in front of the electrochemical transducer. Once the enzyme has reached the reaction chamber, the flow is stopped, the enzyme is held in contact with the transducer using a semi-permeable membrane, and a quantitative electrochemical signal is obtained. A small amount of enzyme is introduced into the reaction chamber, and therefore, freshly prepared enzyme may be used for each measurement, circumventing problems normally associated with enzyme inactivation. Additionally, differential measurements are performed, where each sample is measured in the presence and absence of enzyme, allowing for compensation of matrix effects. Future development in the miniaturization and reconstruction of the biosensor probe will allow for clinical and industrial on-line possibilities. The SIRE biosensor has been employed to measure a wide range of analytes (*15*) and has been used in medical (*16*), industrial (*17*), and food technology (*18*) applications.

We report here a SIRE-technology-based amperometric biosensor for the detection of dissolved L-lactate in tomato paste and baby food samples. Measurements are based on the reversible enzymatic conversion of L-lactate to pyruvate and hydrogen peroxide by lactate oxidase. The linear range and sensitivity of the sensor were investigated in crude food samples. All biosensor measurements were compared with those from an established UV spectrophotometric technique. In addition, we have studied whether sample pretreatment would improve biosensor response. The concentration of dissolved L-lactate can be used as a measure of freshness in the food industry.

MATERIALS AND METHODS

Material. Lactate oxidase (31 U/mg solid, species *Aerococcus viridans*) was obtained from Genzyme (Kent, U. K.). Buffer A (sodium phosphate buffer, pH 7.4) and L-lactate standard were obtained from Chemel AB (Lund, Sweden). Tomato paste was purchased at ICA-handlarnas (Malmoe, Sweden) and baby food (potato and meat puree) was purchased from Findus (Helsingborg, Sweden). The UV analysis kit for L-lactate was obtained from Boehringer Mannheim GmbH (Darmstadt, Germany), and sodium hydroxide was obtained from Eka Chemicals AB (Bohus, Sweden). The SIRE P100 Biosensor probe−instrument combination 15:6, P100 922 0019 and 2:6, P100 922 0012 were also obtained from Chemel AB and the UV/Visible spectrophotometer was obtained from Amersham Pharmacia Biotech (Cambridge, U. K.).

Preparation of the Enzyme Solution. Lactate oxidase was dissolved in buffer A (sodium phosphate, pH 7.4). Final concentration was 22 U/mL.

Dilution of Tomato Paste for L-Lactate Measurements. All tomato paste samples were diluted with buffer A prior to biosensor measurements in order to facilitate proper stirring of the sample and reduce clogging of the biosensor membrane. Unspiked tomato paste samples and dilution media were prepared by diluting 10 mL of tomato paste in 90 mL of buffer A (total volume 100 mL). Spiked tomato paste samples were prepared by pipetting the desired volume of L-lactate standard into a 100-mL volumetric flask and subsequently adding dilution medium.

Dilution of Baby Food Samples for L-Lactate Measurements. Accordingly, 10 g of baby food was dissolved in 500 mL of buffer A in order to reduce the viscosity of the solution. For centrifuged samples, centrifugation was carried out at 5000 rpm for 10 min. Subsequently, baby food samples, which included centrifuged and noncentrifuged samples, were spiked with 1 M l-lactate standard to produce 1 mM stock solutions. These stock solutions were then added to centrifuged and noncentrifuged baby food samples, which had been diluted 1:50 with buffer A producing 0.025, 0.05, 0.75, and 0.1 mM spiked L-lactate samples. Unspiked samples contained baby food which was diluted 1:50 in buffer A.

Preparation of Tomato Paste and Baby Food Samples for UV/spectrophotometric Measurements. Tomato paste samples were filtered with a kitchen strainer overnight in a cold room. Thereafter, 60 mL of the filtrate was adjusted to pH 10 with 2.34 mL of 3 M NaOH. Baby food samples were diluted 1:50 with buffer A (10 g of baby food + 500 mL of buffer A). Thereafter, tomato paste and baby food samples were spiked with different concentrations of l-lactate standard. Calibration standards for the spectrophotometric assay were prepared by diluting the desired amount of L-lactate standard in buffer A. Subsequently, 100 μL (tomato paste) or 1 mL (baby food) of sample solution was placed in a cuvette containing L-lactate dehydrogenase, glutamate-pyruvate transaminase and NAD^+. Final volume of the cuvette was 2.24 mL and the absorbance of the samples was measured after 35 min at 340 nm. The spectrophotometric determination is based on the enzymatic reaction in which L-lactate dehydrogenase converts L-lactate to pyruvate and NAD^+ is reduced to NADH. Glutamate-pyruvate transaminase traps pyruvate by converting it to L-alanine, and thereby shifts the equilibrium from L-lactate to the production of pyruvate and NADH. Spectrophotometric measurements were performed according to the instructions provided with the L-lactic acid determination kit.

Biosensor Measuring Principle. The SIRE biosensor has an amperometric transducer, which is composed of a potentiostatic three-electrode configuration, with platinum wires acting as the working and auxiliary electrodes (each 0.5 mm in diameter) and an internal silver wire as the reference electrode. The probe of the biosensor was immersed in the sample solution, and subsequently the biological recognition element (lactate oxidase, 22 U/mL) was injected into the internal buffer flow of the biosensor. After a predetermined time, the biological recognition element had reached the reaction chamber which is positioned directly in front of the amperometric transducer. At that instant the internal buffer flow was turned off, and the biological recognition element was held in direct spatial contact with the transducer by a semipermeable membrane which covers the end of the probe and separates the sample solution from the reaction chamber. Simultaneously, the target analyte, L-lactate, found in the sample matrix diffused through the membrane and reacted with the lactate oxidase, producing pyruvate and hydrogen peroxide. After 70 s the current produced (+650 mV vs silver wire reference electrode) from the electro-oxidation of hydrogen peroxide and other electroactive species found in the matrix was recorded, and the reaction chamber was washed automatically with buffer. The measurement was repeated, this time in the absence of the biological recognition element, yielding the current produced from the electro-oxidation of electroactive species found in the matrix only. This signal (matrix) was subtracted from the first signal obtained (matrix + analyte) giving a differential biosensor signal highly specific for L-lactate. The entire measuring procedure was automatic and microprocessor controlled, taking approximately 3 min per measurement. This measurement time includes an internal washing step with buffer after every measurement, which helps circumvent problems associated with deposition of the enzyme on the working electrode. The biosensor was calibrated once every day. The tomato paste and baby food samples were held at a constant temperature and homogeneity by placing them in a water bath at 25 °C and using a magnetic stirring plate. For long-term stability of the instrument the internal tubing must be washed

every six months with a washing detergent (supplied by Chemel AB), which we heated up to 60 °C before use.

Investigation of Sample Pretreatment Procedures on Biosensor Accuracy. This purpose of this investigation was to determine whether pretreating the tomato paste samples prior to dilution could help improve biosensor response. Sample pretreatment procedures investigated included filtering with filter paper, coffee filter paper, and a kitchen strainer. All samples were diluted in buffer A according to the previously mentioned dilution procedure after sample pretreatment.

RESULTS AND DISCUSSION

Effect of Enzyme Concentration on Biosensor Response. The influence of the enzyme concentration on the biosensor signal was investigated using two different lactate oxidase enzymes obtained from two different suppliers. Consequently, 0−12 U/mL recombinant lactate oxidase obtained from Genzyme and 0−12 U/mL of lactate oxidase obtained from ICN Biochemicals (Aurora, OH) were used to detect a 2 mM L-lactate sample prepared in phosphate buffer using a reaction time of 25 s. The differential signal for both enzymes increased with increasing enzyme concentration to saturation level around 8 U/mL (according to manufacturer) for the ICN lactate oxidase and 10 U/mL (according to manufacturer) for the Genzyme recombinant lactate oxidase. Although it appeared that the lactate oxidase from ICN had a greater activity per mg solid, we decided to use the recombinant enzyme from Genzyme for all further biosensor measurements because of economic incentives: New and fresh enzyme was used for every biosensor measurement and therefore we chose to use the cheaper alternative. Finally, an excess lactate oxidase concentration of 22 U/mL was chosen as the working concentration for each measurement so that the enzyme concentration would not be the limiting factor in analyte conversion when longer reaction times were used.

Biosensor Response to L-Lactate in Diluted Tomato Paste Samples. The linear range for the determination of L-lactate was 0−0.1 mM with a lactate oxidase concentration of 22 U/mL and a reaction time of 70 s. The limit of detection was 0.05 mM L-lactate. The linear range could be extended by using a shorter reaction time (i.e., 1 mM using 50 s). However, this was not advantageous because the sensitivity of the biosensor would be diminished. We should mention that the relationship between the enzymatic kinetics and the substrate and product diffusion kinetics of the system effects the detection range of the biosensor. The equation of the line obtained for 0−0.1 mM L-lactate prepared in phosphate buffer (standard curve) can be described as $y = 9367.7x$. Tomato paste samples were diluted 1:30 and 1:200 with sodium phosphate buffer and then spiked with L-lactate in the concentration range of 0−0.1 mM lactate, and the values obtained were compared with the values obtained for the calibration curve performed in buffer solution. The resulting standard addition curves are shown in **Figure 1**. The L-lactate concentration in the tomato paste was determined to be 1.02 mM ± 0.02 mM (1:30 dilution) and 1.18 mM ± 0.4 mM (1:200 dilution) by the standard addition method. However, because the L-lactate concentration of the tomato paste is around the detection limit (0.005 mM) when the sample has been diluted 200 times, the relative standard deviation is quite high (± 0.4 mM), and therefore, we chose the value 1.02 mM ± 0.02 mM given for the 1:30 dilution. Comparison of the slopes obtained for the two standard addition curves (6081.9 biosensor units/mM, 1:30; and 6488 biosensor units/mM, 1:200) with the slope of the standard curve (9368 biosensor units/mM) illustrates that the sensitivity of the biosensor decreases with increasing amounts of tomato paste present in the sample. This is probably due to matrix effects such as interferences by small particles.

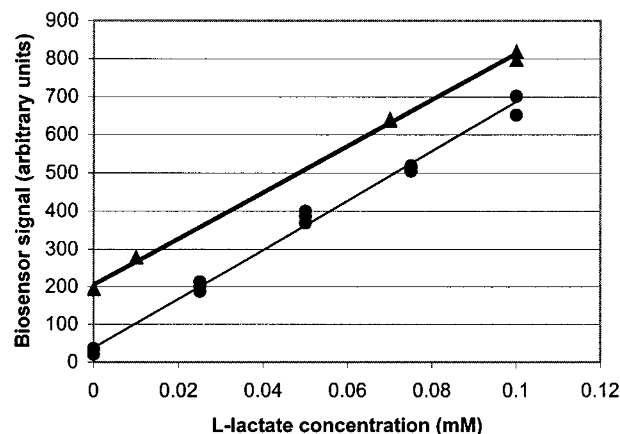

Figure 1. L-Lactate determination in tomato paste samples measured by the standard addition method using the SIRE biosensor. Tomato samples were diluted 1:30 (upper curve, $y = 6081.9x + 206.32$, $R = 0.9981$) and 1:200 (lower curve, $y = 6488x + 38.333$, $R = 0.9939$) in phosphate buffer and then spiked with L-lactate in the concentration range of 0–0.1 mM. The values obtained were compared with the standard curve obtained for buffered samples.

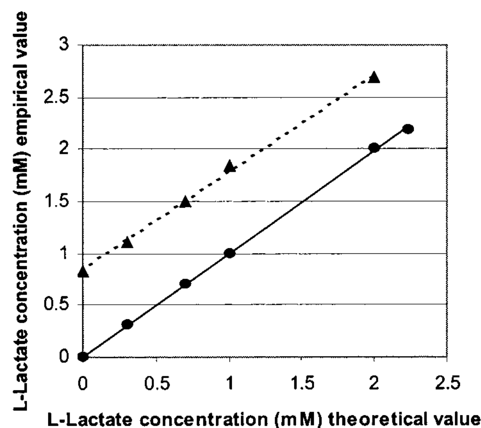

Figure 2. L-Lactate determination in tomato paste samples measured by the standard addition method using a reference spectrophotometric method. Tomato samples were spiked with L-lactate in the concentration range of 0–0.1 mM and the values obtained (upper curve, $y = 0.9339x + 0.8479$, $R = 0.9977$) were compared with the standard curve (lower curve, $y = 0.9908x$, $R = 0.9996$) performed in buffered samples.

Comparison of Biosensor Tomato Paste Measurements with Spectrophotometric Measurements. The linear range for the determination of L-lactate using spectrophotometric measurements was 0−2 mM lactate with a detection limit of 0.033 mM. Tomato paste samples were spiked with L-lactate in the range of 0−2 mM, and the values obtained were compared with the values obtained for the calibration curve performed in buffer solution. The standard addition curve and calibration curve are shown in **Figure 2**. The L-lactate concentration was determined to be 0.91 mM ± 0.05 mM by the standard addition method. The similarity between the slopes obtained for the two curves (0.9339 mM empirical/mM theoretical, standard addition and 0.9908 mM empirical/mM theoretical, calibration) illustrates that the sensitivity of the spectrophotometric assay is only slightly affected by the matrix of the tomato paste. However, the L-lactate concentration of 0.91 mM ± 0.05 mM obtained with the spectrophotometric method is in good agreement with biosensor measurements (1.02 mM ± 0.02 mM) performed on

diluted (1:30) tomato paste samples. Additionally, the biosensor measurement takes only 3 minutes to perform, whereas the spectrophotometric method requires a 35-minute measurement time. Furthermore, it has been shown that the standard addition method is a reliable method for accurately determining the L-lactate concentration of a sample with a complex matrix. Conversely, if the values obtained for unspiked tomato paste samples were applied to the equations of the lines for the calibration curves obtained with the biosensor and spectrophotometric method in pure buffer, the L-lactate concentration would be determined to be 0.63 mM ± 0.01 mM and 0.26 mM ± 0.001 mM for the biosensor and spectrophotometric method, respectively. Thus, both methods are affected by matrix interferences.

Investigation of Sample Pretreatment Procedures on Biosensor Accuracy. It was investigated whether pretreating the tomato paste sample could help improve the biosensor response. Sample pretreatment procedures investigated included filtering with filter paper, coffee filter paper, and a kitchen strainer. However, there was little or no change in biosensor response with any of these procedures. Therefore, small particles, which cannot be removed by these techniques, are likely affecting the sensitivity of the biosensor. It has been documented that tomato puree and canned tomatoes have approximately 16 mg ascorbic acid per 100 g tomato product, which corresponds to approximately 1 mM ascorbic acid (*19*). However, studies involving ascorbic acid and SIRE biosensor measurements have shown that concentrations below 0.1 mM ascorbic acid do not influence biosensor measurements.

Biosensor Response to L-Lactate on Diluted Baby Food Samples. The biosensor was calibrated according to the procedure outlined above because the baby food measurements were performed on a separate day. The equation of the line obtained for 0−0.1 mM L-lactate prepared in phosphate buffer (standard curve) can be described as $y = -0.0027565 + 7037.3x$. Baby food samples were diluted 1:50 and some were centrifuged. Subsequently centrifuged-and-diluted samples, and samples that were diluted only, were spiked with 0−0.1 mM L-lactate, and the values obtained were compared with the values obtained for the standard curve performed in buffer solution. The standard addition curves for diluted-and-centrifuged samples and diluted-only samples are shown in **Figure 3**. The L-lactate concentration was determined to be 2.18 mM ± 0.3 mM for samples that were not centrifuged, and 2.51 mM ± 0.1 mM for centrifuged samples, using the standard addition method. The L-lactate concentration of the baby food samples was nearly the same, and furthermore, centrifugation of the sample provided no significant difference in the L-lactate measurements. Thus, we can assume 2.51 mM ± 0.1 mM is the L-lactate concentration. Comparison of the slopes between the standard addition curves (7848 biosensor units/mM for diluted; and 7397 biosensor units/mM for diluted/centrifuged) and the standard curve (7037 biosensor units/mM) suggests good correlation, and thus, sensitivity of the biosensor also seems to be good with little matrix effects hindering its sensitivity. The baby food samples were not as thick and viscous as the tomato paste samples, and therefore, it was much easier for the biosensor to carry out detection of L-lactate in these samples.

Comparison of Biosensor Baby Food Measurements with Spectrophotometric Measurements. A calibration curve (0−0.1 mM L-lactate, detection limit 0.033 mM) was performed according to the procedure outlined above because the baby food measurements were performed on a separate day. The equation of the line obtained for measurements performed in phosphate buffer (standard curve) can be described as $y = 1.0053x + 0.0016$. Baby food samples were diluted 1:50 with phosphate buffer and spiked with L-lactate in the concentration range of 0−0.1 mM. The values obtained were compared with values obtained for measurements performed in buffer. The result is shown in **Figure 4**. The L-lactate concentration of the baby food sample was determined to be 2.45 mM ± 0.12 mM using the standard addition method. This value has a good correlation with 2.51 mM ± 0.1 mM (diluted/centrifuged) L-lactate value obtained with the biosensor. Comparison of slopes obtained with the spectrophotometric measurements (1.0106 mM empirical/mM theoretical, standard addition; and 1.0053 mM empirical/mM calibration) illustrates that the sensitivity of the spectrophotometric assay is only slightly affected by the matrix of the baby food. Furthermore, it has been shown once again that the standard addition method is a reliable method for accurately determining the L-lactate con-

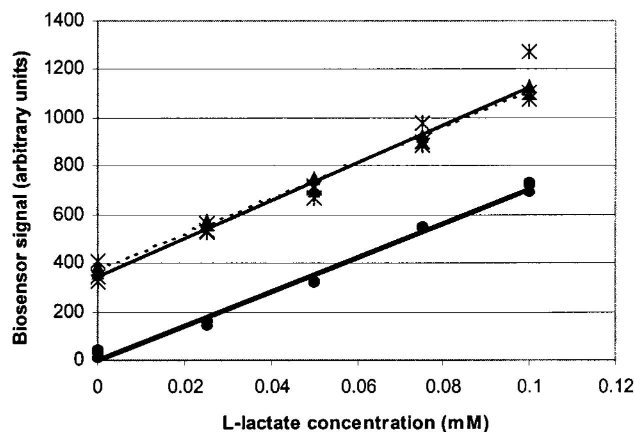

Figure 3. L-Lactate determination in baby food samples measured by the standard addition method using the SIRE biosensor. Baby food samples were diluted 1:50 in phosphate buffer and subsequently spiked with L-lactate in the concentration range of 0–0.1 mM. Values obtained for samples which were diluted and centrifuged (dotted upper curve, $y = 370.73 + 7397.3x$, $R = 0.99759$) and samples which were diluted only (thin solid upper curve, $y = 342.13 + 7848x$, $R = 0.98252$) were compared with values obtained from the standard curve (thick solid lower curve, $y = -0.0027565 + 7037.3x$) performed in buffered samples.

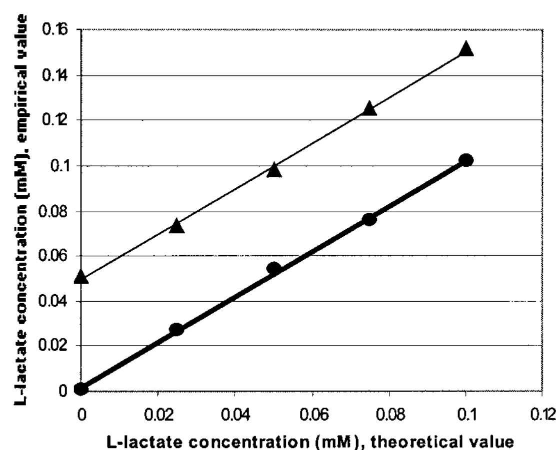

Figure 4. L-Lactate determination in baby food samples measured by the standard addition method using a reference spectrophotometric method. Baby food samples were diluted 1:50 in phosphate buffer and then spiked with L-lactate in the concentration range of 0–0.1 mM. The vales obtained (upper curve, $y = 1.0106x + 0.04$, $R = 0.9991$) were compared with values obtained for the standard curve (lower curve, $y = 1.0053x + 0.0053$, $R = 0.9986$) prepared in pure buffer.

centration of a sample with a complex matrix. Conversely, if the values obtained for unspiked baby food samples were applied to the equations of the lines for the calibration curves obtained with the biosensor and spectrophotometric method in pure buffer, the L-lactate concentration would be determined to be 2.65 mM ± 0.1 mM and 8.9 mM ± 0.3 mM for the biosensor and spectrophotometric method, respectively. Thus, the spectrophotometric measurements are most adversely affected by matrix effects. However, the biosensor can measure directly the L-lactate concentration of the baby food, quite accurately, without using the standard addition method.

Biosensor Accuracy. The repeatability was studied for four different concentrations of L-lactate (0.01, 0.03, 0.07, and 0.1 mM) and the relative standard deviation (RSD) of three independent measurements was calculated. The repeatability for L-lactate was 4.1% ($n = 12$). The reproducibility was investigated in the same manner except that the water, buffer, enzyme stock solution, and samples were changed each day. Three measurements on five different concentrations (0, 0.01, 0.03, 0.07, and 0.1 mM) of L-lactate were performed on four separate days. The slopes of the lines obtained for each day were used to calculate the reproducibility, which was 5.9% ($n = 60$).

The repeatability was also studied on diluted tomato paste (1:30) samples, which were spiked with 0–0.1 mM L-lactate. The resulting standard addition curve was used to calculate the repeatability. Three measurements were performed on five different standard addition concentrations (0, 0.025, 0.05, 0.075, and 0.1 mM) of spiked tomato paste samples. The σ_x was calculated for each of the five concentrations measured, and then the average of these values (0.026 mM) was divided by the value obtained for the L-lactate concentration of the tomato paste sample using the standard addition method (1.02 mM). The repeatability was determined to be 2.5% (RSD, $n = 15$). The reproducibility was calculated in a similar manner. However, three standard addition curves were performed on three separate days and all solutions were prepared fresh each day. Similarly, three measurements were performed on five different standard addition concentrations (0, 0.025, 0.05, 0.075, and 0.1 mM) of spiked tomato paste samples. The values obtained for the L-lactate concentration of the tomato paste using the standard addition method were used to calculate the reproducibility, which was determined to be 13.0% (RSD, $n = 45$). The large relative standard deviation can be explained by the fact that manipulation of the tomato paste during sample preparation was quite difficult due to the thickness and high viscosity of the solution.

The repeatability was also studied on diluted baby food samples (1:50), which were spiked with 0–0.1 mM L-lactate. The resulting standard addition curve was used to calculate the repeatability. Three measurements were performed on five different standard addition concentrations (0, 0.025, 0.05, 0.075, and 0.1 mM) of spiked baby food samples. The σ_x was calculated for each of the five concentrations measured, and then the average of these values (0.1 mM) was divided by the values obtained for the L-lactate concentration of the baby food samples using the standard addition method (2.51 mM). The repeatability was determined to be 4.0% (RSD, $n = 15$). The reproducibility was performed in the same manner. However, three standard addition curves were performed on three separate days and all solutions were prepared fresh each day. Similarly, three measurements were performed on five different standard addition concentrations (0, 0.025, 0.05, 0.075, and 0.1 mM) of spiked baby food samples. The values obtained for the L-lactate concentration of the tomato paste using the standard addition method were used to calculate the reproducibility, which was determined to be 3.0% (RSD, $n = 45$).

CONCLUSIONS

We demonstrated here, in this work, a food application of the SIRE biosensor: measuring the dissolved L-lactate concentrations present in tomato paste and baby food. The concentration of dissolved L-lactate can be used as a measure of freshness in the food industry. Because the food samples were thick and viscous, they were diluted in phosphate buffer and stirred in order to carry out measurements using the biosensor. It was determined that the tomato paste contained approximately 1.02 mM ± 0.02 mM L-lactate and that the baby food contained approximately 2.51 mM ± 0.1 mM L-lactate. All measurements were in agreement with measurements performed by a reference spectrophotometric method. The biosensor gave 12% higher values for the tomato paste measurements and 2.5% higher values for the baby food measurements. However, a distinct advantage of the SIRE biosensor is that it can perform L-lactate measurements within 3 minutes, whereas the spectrophotometric method requires a 35-minute measuring time. Additionally, the L-lactate concentration of the baby food can be measured directly, without using the standard addition method, when measuring with the biosensor. The repeatability for measurements performed in buffered solutions was 4.1% (RSD, $n = 12$) and the reproducibility was 5.9% (RSD, $n = 60$). The repeatability for tomato paste and baby food measurements was 2.5% (RSD, $n = 15$) and 4.0% (RSD, $n = 15$), respectively, and the reproducibility was 13.0% (RSD, $n = 45$) and 3.0% (RSD, $n = 45$), respectively. Differential measurements performed allowed for compensation of matrix effects, such as transducer fouling by electroactive species. The ability to quickly and accurately measure the L-lactate concentration in crude samples which contain a complex mixture of biomolecules makes the SIRE biosensor a good alternative for food analysis.

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