International Food Research Journal 27(5): 875 - 882 (October 2020)
Journal homepage: *http://www.ifrj.upm.edu.my*

Development of co-immobilised enzymes amperometric biosensor for the determination of triglycerides in coconut milk

Manoj, D., Auddy, I., Nimbkar, S., Chittibabu, S. and *Shanmugasundaram, S.

Indian Institute of Food Processing Technology, Thanjavur - 613005, Tamil Nadu, India

Article history

Received: 30 September 2019
Received in revised form:
8 April 2020
Accepted:
3 July 2020

Keywords

enzyme biosensor, co-immobilization, coconut milk, triglycerides

Abstract

Enzyme biosensor was developed to determine the triglycerides present in coconut milk. Lipase, glycerol-3-phosphate oxidase (GPO) and glycerol kinase (GK) were co-immobilised on the gelatine membrane, and coated on the surface of the working electrode. The standardisation of the biosensor was carried out at different standard triolein solutions. The effect of gelatine concentration, glutaraldehyde concentration, and pH of reaction buffer were optimised. It was found that 45 mg gelatine, 2.5% glutaraldehyde concentration, and pH 7.0 yielded the best results. The developed biosensor could detect the triglyceride concentration between 0.3 and 1.3 mM. Triglyceride content in coconut milk was determined using the developed amperometric biosensor method, and the results obtained were compared with the triglyceride analysis in coconut milk by ultra-high performance liquid chromatography (UHPLC) method. It was found that the correlation between the results of the two methods was 97%. Apart from these, thermal stability and sensitivity loss on storage of the working electrode was also studied. The working electrode lost 48% of its activity in 4 h when maintained at 40°C, and lost 57.7% of its activity after 30 d when stored at 4°C.

Introduction

The coconut tree is one of the most economically important palm species, cultivated in tropical regions across the globe, mainly for its endosperm. India stands as the third-largest producer with 11.1 million tons per year, out of which 90% coconut is produced in the southern states (Yong *et al.,* 2009; DebMandal and Mandal, 2011). A coconut approximately comprises of 54% kernel, 36% shell, and 11% water. The coconut kernel consists of 53% moisture, 35% fat, 4% protein, 1% ash, and 6% carbohydrate (Kwon *et al.,* 1996; Yong *et al.,* 2009). It is considered as a 'functional food' as it adds on to numerous health benefits apart from the nutritional constituents (Dendy and Timmins, 1973).

Coconut milk, extracted from the solid endosperm of coconut, is an aqueous solution, which serves as an indispensable ingredient in cuisines of south India and other parts of the world (Seow and Gwee, 1997; Tansakul and Chaisawang, 2006). It has been estimated that 25% of coconut output is consumed as coconut milk (Gwee, 1988). Coconut milk is an important ingredient in bakeries, confectionaries, and ice creams to enhance the taste and flavour. Coconut milk also reduces heart disease by reducing cholesterol level in the body (Monera and Del Rosario, 1982)..

Coconut milk has high-fat content due to which it is susceptible to chemical spoilage by oxidation. The chemical spoilage occurs due to auto-oxidation and hydrolysis of triglycerides into acylglycerol and free fatty acid (FFA). Spoilage of coconut milk depends on the oxidation kinetics of triglycerides. Therefore, the quantity of triglyceride present in coconut milk could be used as an indicator to monitor the quality of the milk. Spectroscopic methods (Monosik *et al.,* 2012), chromatographic methods (Asmis *et al.,* 1997), fluorometric method (Mendez *et al.,* 1986), and titration methods (Klotzsch and McNamara, 1990; Okazaki *et al.,* 1998) were earlier reported for the determination of triglyceride content in different samples. These methods have the disadvantages of high solvent consumption, require skilled labour, time-consuming, expensive, and non-portable. Hence, there is a need for a novel technology to overcome these disadvantages (Turner *et al.,* 1987; Rustagi and Kumar, 2013; Murugaboopathi *et al.,* 2013). An electrochemical sensor is one in which electrochemical signals is generated, and is measured by electrochemical transducers. The electrochemical-based biosensing method contains different types of detection such as electrochemical, metal oxide-based sensor, optical-based biosensor, conduct metric biosensor, and potentiometric biosensor. The electrochemical sensor was selected in the future work as the selectivity of triglyceride content is

*Corresponding author.
Email: sasu@iifpt.edu.in

high as compared to other biosensors. The present work highlights the application of biosensor in the analysis of triglyceride content in coconut milk in areas such as food industry and food safety. The novelty of the present work was to develop a biosensor which can determine triglyceride content in food sample to analyse its quality. In the present work, an enzyme biosensor was developed to instantly evaluate the triglyceride content present in the coconut milk. One enzyme cannot be used to complete the reaction process; so, three enzymes are required to generate ions which are then detected by the biosensor.

The aims of the present work were (1) to develop a three-electrode system based amperometric biosensor which has lipase, glycerol kinase (GK), and glycerol-3-phosphate oxidase (GPO) enzymes co-immobilised on the working electrode surface using gelatine membrane to find out the triglyceride content present in coconut milk, (2) to develop an optimised analytical procedure for the instantaneous measurement of triglycerides present in coconut milk using the amperometric biosensor, and (3) to study the storage and thermal stability of the immobilised enzyme electrode.

Materials and methods

Chemicals and materials

Lipase (E.C.3.1.1.3, from *Candida rugosa*, lot no. BCBV3825), glycerol-3-phosphate oxidase (GPO) (E.C.1.1.3.21, from *Aerococcus viridans*, lot no. SLBL4471V), glycerol kinase (GK) (E.C.2.7.1.30, from *Cellulomonas* sp., lot no. SLCB0815), triolein (Y0001113), Triton X-100 (9002-93-1), adenine triphosphate (ATP), and other chemicals used in the present work were purchased from Sigma Aldrich-Merck (Bengaluru, India).

Potentiostat/galvanostat/impedance analyser (Make: Palmsens 4) was purchased from Palmsens (the Netherlands). Glassy carbon electrode (working electrode), Ag/AgCl electrode (reference electrode), and platinum wire electrode (counter electrode) were purchased from Class one systems (New Delhi, India).

Preparation of standard buffer solution

A total of 15.6 g of sodium phosphate dibasic (Na2HPO4) and 14.1 g of sodium phosphate monobasic (NaH_2PO_4) were weighed and separately dissolved in 100 mL distilled water. Then, 2.89 mL of Na_2HPO_4 solution and 2.12 mL of NaH_2PO_4 solution was obtained and diluted in 45 mL of distilled water. This was then made up to 50 mL by adding distilled water, and stored at 4°C for use (Gómez *et al.,* 2001).

Preparation of triolein standard solution

Trioline was used as a substrate for lipase, GPO, and GK. Nine different concentrations (0.1 – 20 mM) of standard triolein solution were prepared to construct the standard calibration curve. The prepared standard triolein solutions were refrigerated (4°C) until further analysis (Pundir and Narang, 2013).

Preparation of mixed enzymes solution

Enzyme solutions were prepared by adding 1 mg of lipase (> 700 U), 1 mg GPO (> 70 U), and 1 mg GK (> 60 U) in 1 mL of buffer (0.1 M sodium phosphate buffer) separately. From these, 300 µL of lipase solution, 150 µL of GK solution, and 60 µL of GPO solution were added to form a mixed enzyme solution at a ratio 10:5:2. Next, 100 µL ATP in 1 mL of buffer (0.1 M sodium phosphate buffer) solution, and 1 mg of $MgCl_2$ in 1 mL of buffer (0.1 M sodium phosphate buffer) solution were prepared. From these solutions, 10 µL of ATP and 10 µL $MgCl_2$ were added to the enzyme mixture (Pundir and Narwal, 2018).

Preparation of gelatine membrane

Three different gelatine membranes were prepared with varied gelatine concentrations to study the effect of gelatine concentration in the immobilisation of enzymes. Three different portions of gelation (30, 45, and 60 mg) were weighed and mixed with 30 mg of bovine serum albumin (BSA), respectively. BSA was used as reinforcement for the solution since it prevents leakage of the enzyme through the gelatine membrane by increasing the size. Each gelatine-BSA mixture was mixed with 300 µL of buffer (0.1 M sodium phosphate buffer solution). Next, 31.5 µL of 0.0008 mg/mL of $MgCl_2$ solution was added. The solution was homogenised at 1,000 rpm for 10 min. The prepared solution was refrigerated until further analysis (Yücel *et al.,* 2016).

Sensor mechanism

Fats are made from individual fatty acid molecules attached to glycerol, a 3-carbon backbone. The most common type of fat is called a triglyceride, or triacylglycerol, which contains three fatty acids attached to the backbone, and resembles a fork without the handle. The triglyceride present in the sample will be converted into glycerol and fatty acids through catalysis by lipase. Glycerol reacts with ATP in the presence of $MgCl_2$, and will be catalysed by GK to give α-glycerol-3-phosphate and ADP. GPO is used to break the α-glycerol-3-phosphate to dihydroxyacetone phosphate and H_2O_2. Then, due to the high potential applied between the working electrode and the reference electrode, H_2O_2 under high potential

disintegrates into $2H^{+}+O^{2+}2e^{-}$, and is proportional to the triglycerides present in the sample. The electrochemical reactions involved are as follows:

$$\text{triglyceride} \xrightarrow{\text{lipase}} \text{glycerol} + \text{fatty acids}$$

$$\text{glycerol} + \text{ATP} \xrightarrow{\text{GK } MgCl_2} \propto -\text{glycerol} - 3 - \text{phosphate} + \text{ADP}$$

$$\text{glycerol} - 3 - \text{phosphate} + O_2 \xrightarrow{\text{GPO}} \underset{\text{phosphate}}{\text{dihydroxyacetone}} + H_2O_2$$

$$H_2O_2 \rightarrow 2H^{+} + O_2 + 2e^{-}$$

Fabrication of amperometric biosensor

The schematic diagram of the biosensor is shown in Figure 1. The cylindrical reaction cell made of glass with 25 mL of the total volume was filled with 10 mL of buffer (0.1 M sodium phosphate buffer) solution. The working electrode was prepared by dipping it in the solution that contained 40 µL enzyme mixture added with 10 µL of gelatine solution, and kept at 4°C for 30 min for enzyme immobilisation. The working electrode was once again dipped in freshly prepared glutaraldehyde solution for 10 min for cross-linking (Rothwell *et al.,* 2010). One –CHO group was bound to $–NH_2$ groups of BSA, and another –CHO group of glutaraldehyde linked to $–NH_2$ group of the enzyme (Schoemaker *et al.,* 1997). Then the electrode was rinsed with distilled water. All the three electrodes were fixed in the cell using Teflon holders and connected to potentiostat for measuring the current. For the immobilised sensor, when dipped into the reaction buffer added with substrate solution, the enzymes were released and catalysed the reactions involving the triglycerides present in the substrate solution. All the experiments were conducted at room temperature (28°C).

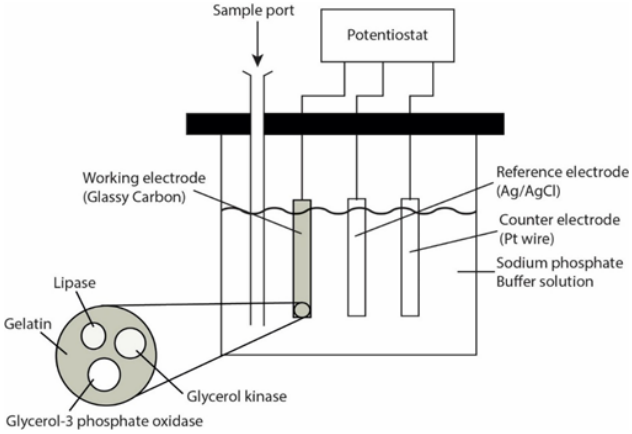

Figure 1. Schematic diagram of the biosensor.

Experimental procedure

Calibration of biosensor

Firstly, 0.1 mL of triolein solution of known concentration was added to the sodium phosphate buffer solution present in the reaction cell. The working and reference electrodes were polarised at +0.4 V using the potentiostat software PsTrace. A potential of +0.4 V was applied between the working electrode and a reference electrode to initiate the electrolysis of formed hydrogen peroxide. The output current from the counter electrode was measured using a potentiostat, and it is directly proportional to the concentration of triolein solution. Experiments were repeated for nine different triolein concentrations, and output currents were measured for each triolein solution. The calibration graph was plotted with triolein solution concentration versus measured output current. All experiments were done in triplicates, and the average value was taken for further analysis.

Effect of gelatine concentration on biosensor response

Three different gelatine membranes were prepared as explained in the preparation of gelatine membrane to study the effect of gelatine concentration on the kinetics of the immobilised enzymes. The working electrode was dipped in each membrane, and the output current was measured by the addition of 0.1 mL of seven different known triolein concentrations.

Effect of glutaraldehyde concentration on biosensor response

Crosslinking of enzymes was carried out using a glutaraldehyde solution. Three different glutaraldehyde solutions were prepared with concentrations 1.5, 2.5, and 3.5% v/v in buffer (0.1 M sodium phosphate buffer) solution to study the effect of glutaraldehyde concentration on the immobilisation of enzymes. The biosensor response was also noted for known triolein concentrations. Next, 40 µL enzyme mixture added with 10 µL of 45 mg gelatine solution were used for the preparation of the working electrode.

Effect of pH on biosensor response

Reaction buffer solutions with pH range of 4.0 to 9.0 were prepared to study the effect on biosensor response. The required quantity of citric acid solution was added to 10 mL of sodium phosphate buffer solution present in the reaction cell for maintaining the low pH range from 4.0 to 7.0 pH; and for high pH from 7.0 to 9.0 pH, glycine solution was added. A 40 µL enzyme mixture added with 10 µL of 45 mg gelatine solution and 2.5% v/v glutaraldehyde solution were used for the preparation of the working electrode. The output current for each pH condition was noted for 5 mM triolein solution.

Ultra-high-performance liquid chromatography

Three points representing three samples of coconut milk of different times were taken, and measured for triglyceride content by both HPLC standard method and the developed biosensor. Coconut oil was extracted from 15 mL of coconut milk by adding 30 mL of hexane, and mixed in magnetic stirrer for 1 h. The aqueous phase and oil phase were separated using separating funnel. The top oil layer was drained, and used for triglyceride determination using ultra-high-performance liquid chromatography (UHPLC) method. The UHPLC system (Shimadzu Corporation, Japan) was equipped with column: Shim-pack XR-ODS III (100 × 2 mm, 2.2 µm particle size), column temperature of 40°C, flow rate of 0.3 mL/min, and injection volume of 5 µL. The mobile phase was ACN/MeOH/THF (40:40:20 v/v/v) with injection volume of 40 µL, and samples were eluted at a flow rate of 1 mL/min.

Extraction of coconut milk

Fresh coconut milk was extracted from fresh coconut meat. Coconut meat was washed with water containing 100 ppm of H_2O_2 and then followed by blanching at 80°C for 10 min. Coconut meat was separated by draining with stainless steel metal strainer and grated into small pieces. The grated coconut meat was kept aside in open condition to bring down the temperature to 25°C. The grated coconut meat was ground in a domestic grinder and squeezed by using coconut milk extraction unit (Arumughan *et al.,* 1993). Next, 5 mL of milk was mixed with 10 mL of buffer (0.1 M sodium phosphate buffer) and 1 mL of 5% of Triton ×100 solution to find out the triglyceride content of coconut milk, which was used as an emulsifying agent. The output current was measured, and the amount of triglyceride was calculated using an empirical relation obtained.

Statistical analysis

Regression analysis was done to evaluate the empirical relationship between the current difference between a biosensor and triglyceride content. Mean and standard deviation were determined using Microsoft Excel 2016.

Results and discussion

Parameter optimisation

Gelatine concentration and biosensor response

Three different concentrations of gelatine were taken, and it was found that 45 mg gelatine solution yielded the best results for biosensor response based on R^2 values and linearity (Figure 2). In low gelatine amount (30 mg), the substrate may leach out easily from a bioactive layer. Thus, there is a deviation from linearity and low R^2 values. In the case of high gelatine amount (60 mg), the substrate molecules could not pass through the gelatine membrane, hence resulted in low biosensor output current. Similar results is reported in triglyceride determination in blood serum (Yücel *et al.,* 2016), immobilising the enzymes in polyvinyl chloride (Narang *et al.,* 2009), polyvinyl acrylamide (Pundir *et al.,* 2010), cellulose acetate membrane (Minakshi and Pundir, 2008), and membranes for triglyceride determination.

Glutaraldehyde concentration and biosensor response

The maximum output current was obtained for 2.5% (v/v) glutaraldehyde concentration, as shown in Figure 3. Lower biosensor response was obtained

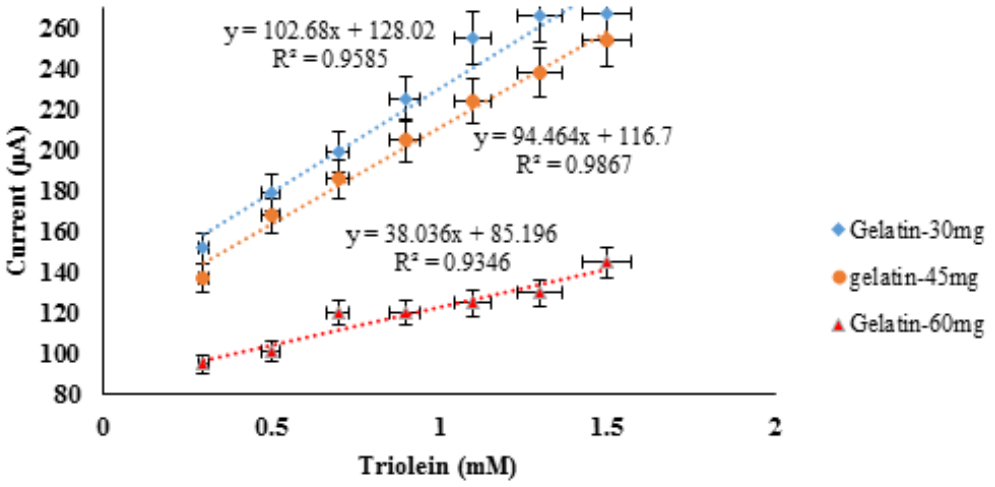

Figure 2. The effect of gelatine content on biosensor response.

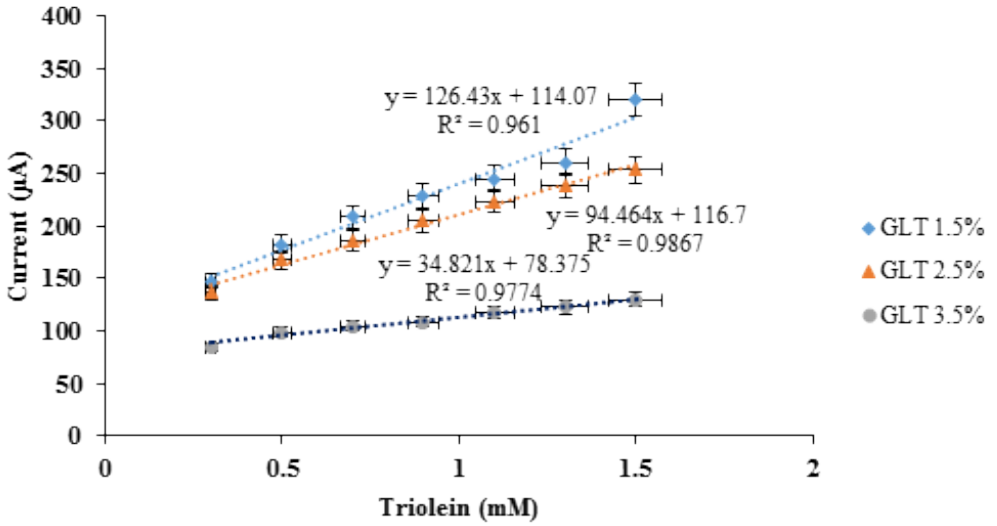

Figure 3. The effect of glutaraldehyde content on biosensor response.

for 1.5% glutaraldehyde because the substrate solution easily passed through the gelatine membrane due to weak cross-linking with the membrane. For high glutaraldehyde concentration (3.5%), the biosensor response was less due to increased crosslinking, which prevented the substrate from passing through the gelatine membrane. The above results are supported by Pundir *et al.* (2010) in the triglyceride determination in serum, and also by acrylamide determination in French fries by Tareke *et al.* (2002).

Effect of pH on biosensor response

The biosensor response increased up to pH 7.0, and then decreased for further increase in pH of reaction buffer solution (Figure 4). 5 mM substrate concentration was used to study the effect of pH. Maximum current (175 mA) was observed at pH 7.0. At low pH range, the possibility of forming ionic bonds between substrate and enzyme molecules is less, which resulted in low current output. At high pH, the output current is less due to the denaturation of enzymes (Madhavi and Lele, 2009). Similar results were obtained for oxygen meter based biosensor which was optimised for pH 7.5 (Bhambi *et al.,* 2006), potentiometric biosensor pH 7.0 (Reddy *et al.,* 2001), and oxygen meter based biosensor pH 8.0 (Kelly and Christian, 1984). Biosensor activity was calculated using Eq. 1.

$$Biosensor\ activity\ (\%) = \frac{current\ observed\ at\ specific\ pH}{Maximum\ current\ observed\ amomg\ all\ the\ pH\ range\ tester} \times 100 \quad \text{(Eq. 1)}$$

Effect of triolein concentration on the output current

The calibration curve was constructed for the

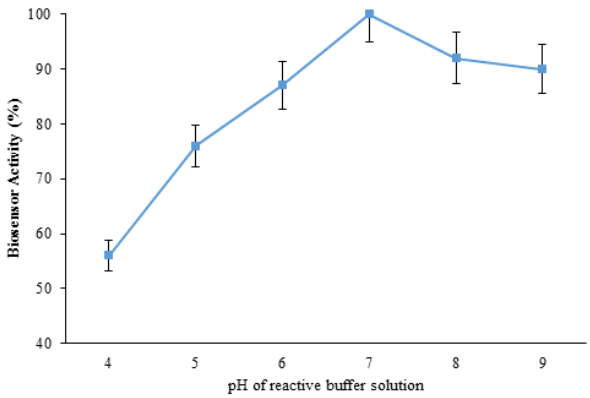

Figure 4. The effect of pH on biosensor response.

prepared biosensor, and is shown in Figure 5. The linearity obtained from 0.3 - 1.3 mM yielded the equation $y = 18.6x + 99$ ($R^2 = 0.932$). The deviation from linearity at higher concentrations may be due to less amount of oxygen or due to a smaller number of enzymes in the membrane. Similar studies were done for silicon-based biosensor (0.2 - 2.1 mM), cellulose acetate bound enzyme-based biosensor (0.2 - 3.5 mM) (Reddy *et al.,* 2001), dissolved oxygen biosensor (5 - 2.0 mM) (Bhambi *et al.,* 2006), and PVC membrane-based biosensor (5 - 2.1 mM) (Narang *et al.,* 2009).

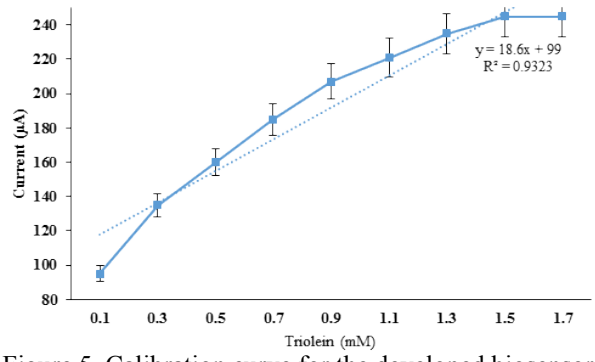

Figure 5. Calibration curve for the developed biosensor.

Validation of developed biosensor

Reproducibility

The reproducibility was tested using 0.5 mM standard triolein solution. The variation of coefficient, average value, and standard deviation were 1.97% (n = 6), 0.515 mM, and 0.043 × 10^{-3} mM, respectively. The samples were also tested for 0.7 mM triolein concentration, and the standard deviation was 0.702 mM.

Storage and thermal stability of the working electrode

The working electrode was kept in an incubator maintained at 40°C for 4 h, and experiments were performed every 1 h. The biosensor showed a response of 89, 83, 65, and 48% activity, respectively, at 40°C. At high-temperature, enzymes are denatured, thus resulting in a reduction of activities (Rangelova *et al.,* 2010).

The storage stability of the working electrode was studied for long-term usage. For this activity, the enzyme-immobilised working electrode was stored at 4°C and kept in the dark for 30 d. Experiments were conducted at every 5 d interval, and activity was noted. The biosensor response for 5th, 10th, 15th, 20th, 25th, and 30th d were 94.4, 86.9, 78.8, 72.0, 65.2, and 57.7%, respectively. A good reproducibility was shown in amperometric detection of a triglyceride of about 40% loss after 30 d (Phongphut *et al.,* 2013).

Determination of triglyceride content in coconut milk

The developed amperometric biosensor was used to detect the amount of triglyceride in coconut milk. 5 mL of milk was mixed with 10 mL of buffer (0.1 M sodium phosphate buffer). The output current was measured at 5th, 10th, and 15th h. Similarly, the triglyceride content present in the coconut milk was determined using the UHPLC method (Lee *et al.,* 2013) using the same coconut milk samples. The results obtained showed good correlation between the methods with R^2 value 0.97. Similar work has been done to detect the triglyceride content in serum, in which enzymes were immobilised to a PVA membrane. The developed system showed a good response at 25°C, 7.0 pH, and had a detection limit from 0.56 to 2.25 mM (Pundir *et al.,* 2010), which has high detection limit as compared to this method.

Conclusion

The amperometric biosensor was developed by immobilising a mixture of enzymes like lipase, GPO, and GK in a gelatine membrane for the determination of triglycerides present in coconut milk, and tested successfully. Gelatine and glutaraldehyde concentration, pH of reaction buffer, thermal, and storage stability were studied and optimised. It was found that at 45 mg of gelatine membrane with 2.5% v/v of glutaraldehyde solution concentration at pH 7.0 of reaction buffer solution gave the best biosensor response. It was also noted that the biosensor activity was reduced to 40% within 4 h at 40°C. A decrease in biosensor activity (57%) was observed after 30 d when the electrodes were maintained at 4°C and analysis was carried out at room temperature (25°C). A good correlation was found between the values of triglycerides in the samples by biosensor and by ultra-high performance liquid chromatographic method (R^2 = 0.97). Hence, this method will be very much useful for the determination of triglycerides in coconut milk. The merits of this method are: easy fabrication of biosensor, portability, instant results, detection of the triglyceride in the range of a minimum of 0.3 to 1.3 mM, a high degree of accuracy, consistency in the accuracy of the results, and ability to operate at room temperature. Other advantages include accessible to unskilled labour and lifespan of the fabricated solutions till 30 d. The demerits are high cost of the enzymes, high cost of electrodes, and maintenance of electrode at a refrigerated condition to retain the enzyme activity. The analysis of the cost is currently undertaken and will be reported in due course.

Acknowledgement

The authors would like to express thanks to the Director of Indian Institute of Food Processing Technology, Thanjavur for his unwavering support and encouragement.

References

Arumughan, C., Balachandran, C. and Sundaresan, A. 1993. Development of a process for coconut cream on commercial scale. Journal of Food Science and Technology 30: 408-412.

Asmis, R., Biihler, E., Jelk, J. and Gey, K. F. 1997. Concurrent quantification of cellular cholesterol, cholesteryl esters and triglycerides in small biological samples reevaluation of thin layer chromatography using laser densitometry. Journal of Chromatography B - Biomedical Sciences and Applications 691(1): 59-66.

Bhambi, M., Minakshi and Pundir, C. S. 2006. Preparation of oxygen meter based biosensor for determination of triglyceride in serum. Sensors

and Transducres 67(5): 561-567.

DebMandal, M. and Mandal, S. 2011. Coconut (*Cocos nucifera* L.: Arecaceae): in health promotion and disease prevention. Asian Pacific Journal of Tropical Medicine 4(3): 241-247.

Dendy, D. A. V. and Timmins, W. H. 1973. Development of a wet-coconut process designed to extract protein and oil from fresh coconut. London: Tropical Products Institute.

Gwee, C. 1988. New technologies open the passage into new usage of coconut milk products. In Maneepun, S., Varangoon, P. and Phithakpol, V. (eds). Food Science and Technology in Industrial Development, Volume 1, p. 157-162. Bangkok: Kasetsart University.

Gómez, G., Pikal, M. J. and Rodríguez-Hornedo, N. 2001. Effect of initial buffer composition on pH changes during far-from-equilibrium freezing of sodium phosphate buffer solutions. Pharmaceutical Research 18(1): 90-97.

Kelly, T. A. and Christian, G. D. 1984. Amperometric determination of glycerol and triglycerides using an oxygen electrode. Analyst 109(4): 453-456.

Klotzsch, S. G. and McNamara, J. R. 1990. Triglyceride measurements: a review of methods and interferences. Clinical Chemistry 36(9): 1605-1613.

Kwon, K. S., Bae, D., Park, K. H. and Rhee, K. C. 1996. Aqueous extraction and membrane techniques improve coconut protein concentrate functionality. Journal of Food Science 61(4): 753-756.

Lee, K. W. Y., Porter, C. J. and Boyd, B. J. 2013. A simple quantitative approach for the determination of long and medium chain lipids in bio-relevant matrices by high performance liquid chromatography with refractive index detection. AAPS PharmSciTech 14(3): 927-934.

Madhavi, V. and Lele, S. S. 2009. Laccase: properties and applications. BioResources 4(4): 1694-1717.

Mendez, A. J., Cabeza, C. and Hsia, S. L. 1986. A fluorometric method for the determination of triglycerides in nanomolar quantities. Analytical Biochemistry 156(2): 386-389.

Minakshi and Pundir, C. S. 2008. Construction of an amperometric enzymic sensor for triglyceride determination. Sensors and Actuators B - Chemical 133(1): 251-255.

Monera, O. D. and Del Rosario, E. J. 1982. Physico-chemical evaluation of the natural stability of coconut milk emulsion. Annals of Tropical Research 4(1): 47-54.

Monosik, R., Stredansky, M., Tkac, J. and Sturdik, E. 2012. Application of enzyme biosensors in analysis of food and beverages. Food Analytical Methods 5(1): 40-53.

Murugaboopathi, G., Parthasarathy, V., Chellaram, C., Prem Anand, T. and Vinurajkumar, S. 2013. Applications of biosensors in food industry. Biosciences Biotechnology Research Asia 10(2): 711-714.

Narang, J., Minakshi, Bhambi, M. and Pundir, C. S. 2009. Fabrication of an amperometric triglyceride biosensor based on PVC membrane. Analytical Letters 43(1): 1-11.

Okazaki, M., Komoriya, N., Tomoike, H., Inoue, N., Usui, S., Itoh, S. and Hosaki, S. 1998. Quantitative detection method of triglycerides in serum lipoproteins and serum-free glycerol by high-performance liquid chromatography. Journal of Chromatography B - Biomedical Sciences and Applications 709(2): 179-187.

Phongphut, A., Sriprachuabwong, C., Wisitsoraat, A., Tuantranont, A., Prichanont, S. and Sritongkham, P. 2013. A disposable amperometric biosensor based on inkjet-printed Au/PEDOT-PSS nanocomposite for triglyceride determination. Sensors and Actuators B - Chemical 178: 501-507.

Pundir, C. S. and Narang, J. 2013. Determination of triglycerides with special emphasis on biosensors: a review. International Journal of Biological Macromolecules 61: 379-389.

Pundir, C. S. and Narwal, V. 2018. Biosensing methods for determination of triglycerides: a review. Biosensors and Bioelectronics 100: 214-227.

Pundir, C. S., Singh, B. S. and Narang, J. 2010. Construction of an amperometric triglyceride biosensor using PVA membrane bound enzymes. Clinical Biochemistry 43(4-5): 467-472.

Rangelova, V., Tsankova, D. and Dimcheva, N. 2010. Soft computing techniques in modelling the influence of pH and temperature on dopamine biosensor. In Somerset, V. S. (ed). Intelligent and Biosensors. United Kingdom: IntechOpen.

Reddy, R. R. K., Chadha, A. and Bhattacharya, E. 2001. Porous silicon based potentiometric triglyceride biosensor. Biosensors and Bioelectronics 16(4-5): 313-317.

Rothwell, S. A., Killoran, S. J. and O'Neill, R. D. 2010. Enzyme immobilization strategies and electropolymerization conditions to control sensitivity and selectivity parameters of a polymer-enzyme composite glucose biosensor. Sensors 10(7): 6439-6462.

Rustagi, S. and Kumar, P. 2013. Biosensor and its application in food industry. Advances in Bioresearch 4(2): 168-170.

Schoemaker, M., Feldbrügge, R., Gründig, B. and Spener, F. 1997. The lipoxygenase sensor, a new approach in essential fatty acid determination in foods. Biosensors and Bioelectronics 12(11): 1089-1099.

Seow, C. C. and Gwee, C. N. 1997. Coconut milk: chemistry and technology. International Journal of Food Science and Technology 32(3): 189-201.

Tansakul, A. and Chaisawang, P. 2006. Thermophysical properties of coconut milk. Journal of Food Engineering 73(3): 276-280.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S. and Törnqvist, M. 2002. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. Journal of Agricultural and Food Chemistry 50(17): 4998-5006.

Turner, A. P. F., Karube, I. and Wilson, G. S. 1987. Biosensors: fundamentals and applications. United Kingdom: Oxford University Press.

Yong, J. W. H., Ge, L., Ng, Y. F. and Tan, S. N. 2009. The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. Molecules 14(12): 5144-5164.

Yücel, A., Özcan, H. M. and Sağıroğlu, A. 2016. A new multienzyme-type biosensor for triglyceride determination. Preparative Biochemistry and Biotechnology 46(1): 78-84.