CHAPTER 6

Detection of limonin and its reduction using MgSiO$_3$ based capacitive sensor

Contents

Chapter 6: Detection of limonin and its reduction using MgSiO₃ based capacitive sensor

6.1. Introduction:

Limonin detection as well as its reduction in citrus fruit juices is essential for improving consumer's acceptability. The preceding chapter 5 explained a novel method for limonin quantification using CeO_2 as a sensor material. But at the same time reduction of it is also a vital issue for the suitable control over the bitterness. Here, a novel interdigitated capacitive sensor using magnesium silicate-poly vinyl alcohol ($MgSiO_3.xH_2O$-PVA) composite has been introduced for quantification of limonin and its reduction (debittering) through selective adsorption of limonin from the citrus fruit juices. Citrus limetta and Citrus grandis fruit juices have been used for the assessment. Limonin, as discussed in chapter 2, is a highly oxygenated triterpene derivative comprised of a furan ring and an epoxide group [1] and its interaction with compounds such as magnesium silicate is shown in Fig. 6.1.

The planar interdigitated structure used earlier has been used in this work for the capacitive sensor. It exhibits a large number of applications due to its quick response, miniature size, less fabrication cost and no harmful radiation as already stated in previous chapters [2]. The planar interdigitated sensor has access to material from a single side of the substrate in comparison to double-side access to the material [3]. The IDEs can incorporate sensitive material that can sense analyte upon its adsorption when permittivity of the same changes. Charles R. Barmore et al. [4] reported a reduction in bitterness and tartness in citrus juice by exposing the juice to magnesium silicate in batches with different concentrations (w/v) for 1 min duration at 20 °C. The $MgSiO_3$ exhibits highest reduction of limonin by 81% in citrus juice in comparison to other ingredients like naringin (51%), narirutin (57%) and total acid contents (59%).[4, 5]. Caisawadi et al. (1998) used magnesium silicate to treat lime juice
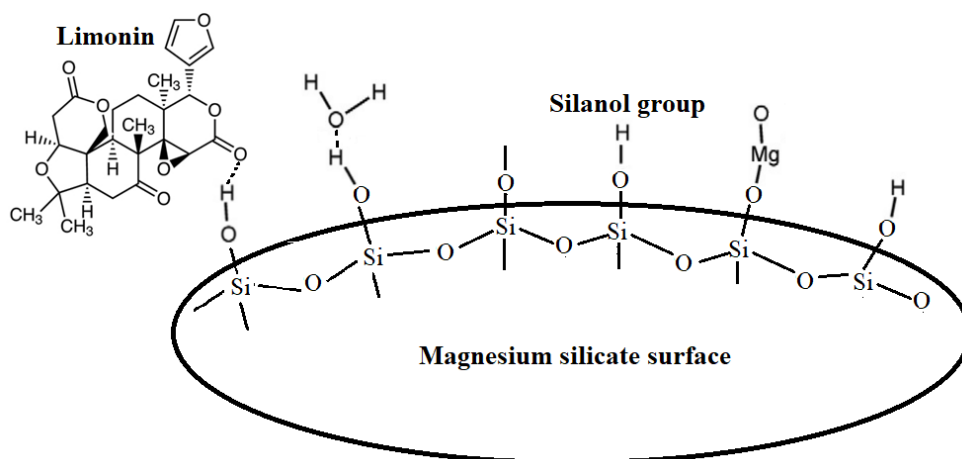

Figure 6.1: adsorption of limonin on the surface of magnesium silicate

that reduced the limonin content by 51.55% with a negligible change in pH (2.30 to 2.75), titrable acidity (7.99±0.12% to 7.03±0.32%), ascorbic acid (0.23±0.04 to 0.17±0.01 mg/ml) and total soluble solids (8 to 9 %) [6]. The activated magnesium silicate reduces limonin content without adversely affecting the nutritive quality of thai tangerine juice [6].

The magnesium silicate has therapeutic effects and it is also used for debittering citrus juices without altering the flavor, ascorbic acid and soluble solids content of treated juice [4]. According to directive 95/2/EC (E 553a), magnesium silicate is allowed as a harmless inorganic compound for food additives as it is neither acute oral toxic nor cytotoxic and nor classified as dangerous [7, 8]. In this work, the magnesium silicate material mixed homogeneously with polyvinyl Alcohol (PVA) was used for developing the sensor. The magnesium silicate was employed for the interaction with limonin whereas PVA was used as an adhesive chemical for Whatman paper-clad substrate with MgSiO$_3$.xH$_2$O. Among various polymers, PVA has been selected due to its high surface-to-volume ratio, biocompatibility, hydrophilicity, and easy availability [9, 10]. Additionally, the carbon backbone of PVA contains hydroxyl groups that facilitate hydrogen bonding interaction with other composites [11].

Here, we have focused on the development of a flexible IDE-based capacitive sensor using magnesium silicate for the detection and quantification of limonin in citrus juice with the measurement of its reduction. The sensor enables both quantifications as well as measuring the debittering of citrus juices showing a reduction in limonin content. The sensor's results were validated with HPLC analysis. The device is simple, low-cost and reusable promising easy, on-site and rapid quantification and reduction of limonin content in citrus juices without having toxicity. We have increased the sensitivity by enhancing the surface area of the sensor with the formation of nanostructure and the change of capacitance to the permittivity of sensing material is considered for the design and fabrication of the interdigital comb structure of the electrodes.

6.2. Materials and Methodology:

6.2.1 Materials:

Magnesium silicate (MgSiO$_3$.xH$_2$O), and PVA were taken from Alfa Aesar company, India. Limonin was purchased from Sigma Aldrich. For solvent and other applications, deionized water was utilized. The other interfering components such as

sugars, ascorbic acid, citric acid, naringin, etc were procured from Alfa Aeser company. The raw chemicals were of analytical graded and used without further purification.

6.2.2 Preparation of samples and chemical (HPLC) analysis:

Citrus limetta and Citrus grandis fruits were taken from the Tezpur, Assam, India. The fruits were cleaned with running water, dried and then cut into pieces. Then it was crushed and juiced. The juice was centrifuged at 1000 rpm for 5 mins. The clear solution was then filtered using whatman 1 filter paper and the filtrate was used for further investigation.

High-performance liquid chromatography (HPLC) analysis was performed to find out the content of limonin in citrus limetta and citrus grandis fruit juices analytically at room temperature using the C_{18} column. HPLC system (Water Corporation, USA) consists of two hydraulic pumps-515, an injection system, UV visible detection-2489, a C_{18} column guard and a computerized recorder/ integrator. The juice extract was diluted with methanol in the ratio of 1:2 and then filtered using a 0.45 μm nylon PTFE filter before injection into the HPLC system. The detection of limonin was performed using an isocratic system with mobile phase solvent as a mixture of acetonitrile- tetrahydrofuran-deionized water in the ratio 17.5-17.5-65 (v/v/v) for C_{18} columns with a flow rate of 0.5 ml/min and 10 mins run time. The volume of injection was 10 μl and the detection wavelength was set at 207 nm [12]. For preparing the stock solution 20 mg of limonin standard powder was mixed with 4 ml of acetonitrile and the solution was made up to 1000 ml with methanol. All other working standards (15 ppm, 10 ppm, 5 ppm, 1 ppm) were prepared from the 20 ppm stock solution. HPLC analysis was performed using the principle, $\text{Conc.}_{unknown} = (\text{Area}_{unknown}/ \text{Area}_{standard})\ \text{Conc.}_{standard}$.[13] where $\text{Conc.}_{unknown}$ is the conxentration of unknown, $\text{Area}_{unknown}$ represents area under the curve of unknown, $\text{Area}_{standard}$ is the area under the curve of standard sample and $\text{Conc.}_{standard}$ is the concentration of the known standard

6.2.3 Preparation of MgSiO₃.xH₂O –PVA composite

Here 500 mg of PVA powder was mixed with 10 ml of Deionized (DI) water. Then the mixture was vigorously stirred at 400 rpm for 3 h at 800 °C temperature until the PVA was completely dissolved. We have prepared PVA and MgSiO₃.xH₂O at different ratios to obtain 1 mM, 2 mM, and 4 mM solutions for deposition. The

$MgSiO_3.xH_2O$ powder and PVA were mixed with the aid of ultra-sonication and subsequently stirred at 400 rpm for 10 mins resulting in a homogeneous solution.

6.2.4 Sensor and its calibration

The IDE device was developed on a 6 mm x 8 mm whatman 1 filter paper substrate which is a widely available, flexible, biodegradable, environment-friendly substrate employed in the fabrication of low-cost devices [14, 15]. Moreover, Whatman brand chromatography papers are widely used as a substrate for sensors because of their wicking ability, uniform thickness, and well retention of compounds [16].

Here, the electrode fingers were patterned with Ag on top of the substrate. The width of the electrode fingers was retained at 1 mm and the gap between two adjacent electrodes was maintained to be 1 mm as suitably selected for the design stated earlier in chapter 3. Here eight fingers (digits) are taken for the sensing purpose. The overlapping area for the electrodes was kept at 5 mm. For the connection of the sensor to the signal processing circuit, wires were bonded at the two electrodes. The whole steps and process of sensor development were already discussed in chapter 4. The magnesium silicate and PVA composite was deposited on a single side of the substrate over the IDE pattern by using drop coating and dried at 60 °C for 5 mins (Fig. 4.8a and 4.8b in chapter 4).

The sensor's output signal was fed to the analog pin of the microcontroller (ATmega32) for processing the data. The embedded circuit processed the sensor's output signal and gave its output in response to the change in the property of the sensing material. The Arduino circuit with the sensor and other peripherals was already discussed in chapter 4.

The sensor was calibrated for varying concentrations of limonin from 2 ppm to 20 ppm at room temperature. The calibration curves were obtained for different compositions of magnesium silicate deposited on a substrate for the relative change in capacitance and limonin concentration. The performance of the sensor was analyzed by studying its accuracy, selectivity, reusability, sensitivity, reproducibility and time of response. As the sensing material adsorbs limonin from the citrus juice (Fig. 6.1), it subsequently reduces the amount of limonin in the juice sample. Hence an attempt has been made to measure the amount of limonin that has been reduced in the adsorption process by the fabricated sensor**.**

6.2.5: Sensory Evaluation: *Screening, and training of panelist*

The sensory evaluation of the magnesium silicate treated juice and untreated juice were performed following the established method [17] based on the panelist's acceptance using 16 points scale. For the treated juice, the juice was exposed to magnesium silicate with constant stirring for 1 min at room temperature [4]. The sweetness, tartness and bitterness of the treated and untreated juice were assessed by 10 trained panelist members consisting of students and staff. The screening and selection procedure of potential assessors was carried out to obtain a sensitive panel and a training period of 10 days was provided to develop the ability of the panel members to replicate qualitative findings. This research initially includes 20 interested participants from the department of Food Engineering and Technology and the department of Electronics and Communication Engineering, Tezpur University including both male and female participants (age group 25-40 years). The participants were also ensured with good health conditions (not impaired of primary senses, not affected by cold, allergy or any illness) before the analysis. In the screening of participants, a triangle test was conducted to detect odd samples out. Based on screening, 10 panelists were screened and oriented to give a uniform understanding of the sensory attributes. The orientation program included familiarization with reference standards and a scoring system. The panelists graded sweetness, tartness and bitterness intensity on a 0–15 numerical scale where 0 represents no difference and 15 represents extremely different. The reference standards were as follows: sweet =7 (60 g kg^{-1} sucrose), sour =7 (2 g kg^{-1} citric acid) and bitter =7 (1 g kg^{-1} caffeine). The standard solutions and juice samples were served at room temperature

We made the marking of the juice and offered it to the panellist individually. Afterward, the panelist were provided with unknown samples for evaluation. The samples were scored by the panelist on a scale of 0 to 15. The recorded mean scores from the assessors were used for sensory evaluation. The analysis of variance (ANOVAs) was performed separately for each attribute and the significance between the means was determined at the 0.05 level of significance.

6.2.6: Determination of TPC, TFC and antioxidant activity

To find out the variations in Total Phenolic Content (TPC), Total Flavonoids Content (TFC) and antioxidant properties of fresh and debittered juices, spectrophotometric analysis was performed for TPC, TFC, 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) scavenging assay of freshly extracted and magnesium silicate treated

juice (debittered). Fresh juices were centrifuged at 5000 rpm for 5 mins to get a clear solution. The clear solution was filtered (using the whatman1 filter) and 0.1 ml of the filtrate was mixed with 9.9 ml methanol (80%) for analysis of bioactive compounds. Similarly, 0.1 ml of debittered juices were mixed with 9.9 ml of methanol (80%) and the solution was used for spectrophotometric analysis.

TPC was determined using Folin– Ciocalteu reagent (FCR) [18] with minor modification. 0.1ml of the extract was mixed with 0.4ml of Na_2CO_3 and 0.5 ml of FCR and then incubated for 2 h at 22 °C. A spectrophotometer (Eppendorf BioSpectrometer, Germany) was used for the measurement of absorbance at 725 nm and from the gallic acid standard curve (Fig 6.2a), the result was expressed as mg/gm gallic acid equivalent (GAE). TFC was determined following the method with slight modification [19]. The juice sample extract was dissolved in 20 µg/ml of ethanol (95%). Five catechin standards (25 to 100 µg/ml) were prepared and 0.5 ml from the standard was aliquoted along with the sample and blank which was adjusted to 2 ml with ethanol (95%).
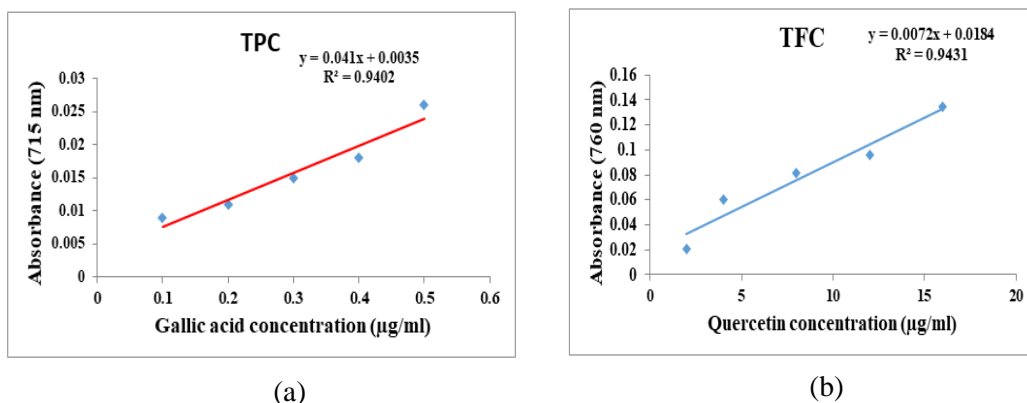

Figure 6.2: (a) Gallic acid standard curve (b) Standard regression analysis for Quercetin

0.1 ml of aluminium chloride (10%) was added to each solution followed by the addition of 0.1 ml potassium acetate (1M). Each solution was diluted with 2 ml of DI water and incubated in dark for 40 mins at room temperature. The absorbance was recorded at 415 nm and the TFC was expressed in catechin equivalent (mg CE/100 g) with the standard regression analysis as shown in Fig 6.2b.

DPPH radical scavenging activity was found according to the method of Das M., J., et al. with minor modification [18]. 0.135 mM of DPPH solution was prepared in methanol and then the solution was mixed with the juice extract (1.0 mg/ml). Absorbance was measured at 517 nm using a UV–vis spectrophotometer (Eppendorf

BioSpectrometer, Germany). The test tubes were then incubated in the dark for 20 mins at room temperature. The absorbance of mixtures was measured in a spectrophotometer at 517 nm after 30 mins of incubation in dark. The scavenging activity was calculated using DPPH (%) following Eq 6.1.

$$\text{Scavenging activity} = ((A_{control} - A_{test})/ A_{control}) \times 100, \qquad (6.1)$$

where $A_{control}$ is the absorbance of DPPH and A_{test} is the absorbance of the test sample.

6.3. Results and Discussion

6.3.1 Sensing mechanism and performance

The response of magnesium silicate based interdigitated device depends on the adsorption behaviour of limonin by $MgSiO_3.xH_2O$. Here, the free hydroxyl group (Sianol (Si-OH) groups) is present on the surface of $MgSiO_3.xH_2O$ (Fig. 6.1) which facilitates the physical adsorption of organic particles such as limonin through binding with carbonyl groups of limonin [7]. These free OH^- groups present in the sensing layer are highly reactive groups making the surface modification. Moreover, the presence of active acidic (silanol groups over silica) and basic sites (ionic oxygen ions in MgO) [20] on the $MgSiO_3.xH_2O$ surface also interact with limonin during adsorption. Hence, the high surface area of $MgSiO_3.xH_2O$ and active sites make an organic adsorbent for limonin. The surface (functional groups) composition of $MgSiO_3.xH_2O$ with the adsorption of organic analyte limonin by $MgSiO_3.xH_2O$ is shown in Fig. 6.1. Here the concept of adsorption of limonin by the surface of $MgSiO_3.xH_2O$ is exploited for the quantification of limonin and reduction of bittering (debittering) using IDE based capacitive sensor platform.

The sensor performance was carried out by using an experimental setup (Fig. 4.9 of chapter 4) consisting of a $MgSiO_3.xH_2O$-based sensing device, laptop, LCD and (ATmega32). The parameters for performance analysis such as accuracy, sensitivity, selectivity, response time, reusability, etc were already defined in chapter 5. Here the performance analysis was carried out using Citrus Limetta and citrus grandis fruit juices. In the transient curve (Fig. 6.3a) C_{sample} for all samples is saturated between 6 s to 15 s. The capacitances, C_{sample} and $C_{initial}$ were measured when exposed to the target analyte and without applying the target analyte respectively. The relative capacitance C_r is written as

$$C_r = (C^S_{sample} - C_{initial})/ C_{initial} \qquad (6.2)$$

where, C^S_{sample} is the saturated value of C_{sample} obtained from Fig. 6.3a

6.3.2 Calibration curves of IDE capacitive sensor and sensitivity

The capacitive response for the sensor using composite samples of 1 mM, 2 mM and 4 mM $MgSiO_3.xH_2O$ were studied with concentrations of limonin from 2 ppm to 20 ppm. Fig. 6.3a shows the increase in the capacitive response of $MgSiO_3.xH_2O$ –PVA (4 mM) composite-based sensor with varying concentrations of limonin with a SD of ±0.33 µF, ±1.8 µF, ±2.4 µF and ±6.7µF for 2ppm, 5pp, 10ppm and 20ppm samples respectively. The transfer characteristics of the capacitive sensors using composite samples of 1 mM, 2 mM and 4 mM $MgSiO_3.xH_2O$ were obtained with varying concentrations of limonin (2 ppm-20 ppm) (Fig. 6.3b). The curves show an almost linear increase in capacitance due to the adsorption behaviour of $MgSiO_3.xH_2O$ associated with the increase in limonin concentration. The 4 mM $MgSiO_3.xH_2O$ -PVA composite posed maximum capacitance change with respect to L_c (limonin content) as compared with the others due to having a higher number of hydroxyl groups present for the adsorption reaction with the analyte.
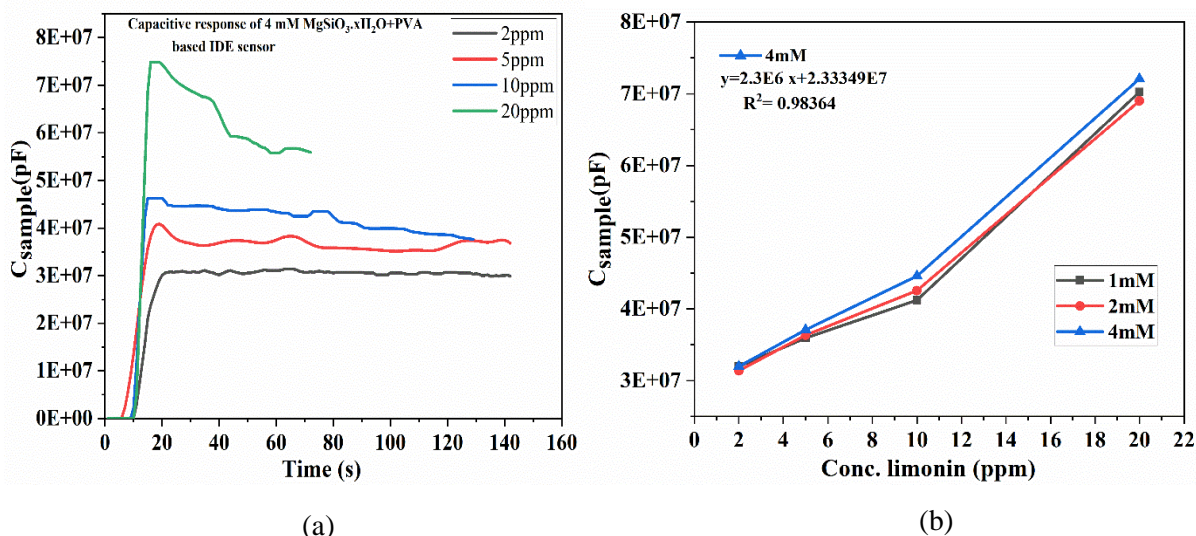

(a)   (b)

Figure 6.3: (a) capacitive response of the sensor with 4mM $MgSiO_3.xH_2O$ for different concentration of limonin (b) calibration curves of the sensor for 1mM, 2mM, 4mM $MgSiO_3.xH_2O$-PVA composite

Fig. 6.3b shows the regression analysis with the regression Eq. 6.3 for relative capacitance obtained for 4 mM $MgSiO_3.xH_2O$ composite.

$$y= 2.3E6 \; x + 2.33349E7 \tag{6.3}$$

Higher regression coefficient (R^2 =0.9836) was obtained by using a 4mM $MgSiO_3.xH_2O$ composite than that of other composites. Hence the composite of 4mM was selected as a

sensor material for further study in real time quantification and debittering process. The sensitivity from Fig. 6.3b and Eq. 6.3 is estimated as

$$(\Delta C^S_{sample} / \Delta L_C) \sim 2.39 \pm 0.3 \text{ pF/ ppm.}$$

A statistical measure of how far the data depart from the fitted regression line is the residual standard deviation, often known as the residual standard error. Eq. 6.4 is used to calculate it.

$$s(r) = \sqrt{\frac{\sum_{i=1}^{n}(y_i - y_i^*)^2}{n-2}}$$

(6.4)

where y_i^* is the value of y predicted by the Eq. 6.3 of the calibration line for a given value of x, n is the number of calibration points, and y_i is the observed value of y for a given value of x.

Table 6.1: calculation for the prediction interval

Concentration in ppm, x	Capacitance(μF), y_i	Predicted, y_i^*	Residual, $(y - y_i^*)$	Residual, $(y_i - y_i^*)^2$	S(r)
2	31.95	27.93	4.02	16.1604	3.982
5	37.07	34.83	2.24	5.0176	
10	44.59	46.33	-1.74	3.0276	
20	72.07	69.33	2.74	7.5076	

To assess the degree of uncertainty in estimated concentration values, the residual standard deviation is used rather than estimating the full standard error of prediction. Our calibration yielded a residual standard error of 3.982 (Table 6.1), which reflects the uncertainty in estimated values.

Table 6.2: Residual plot
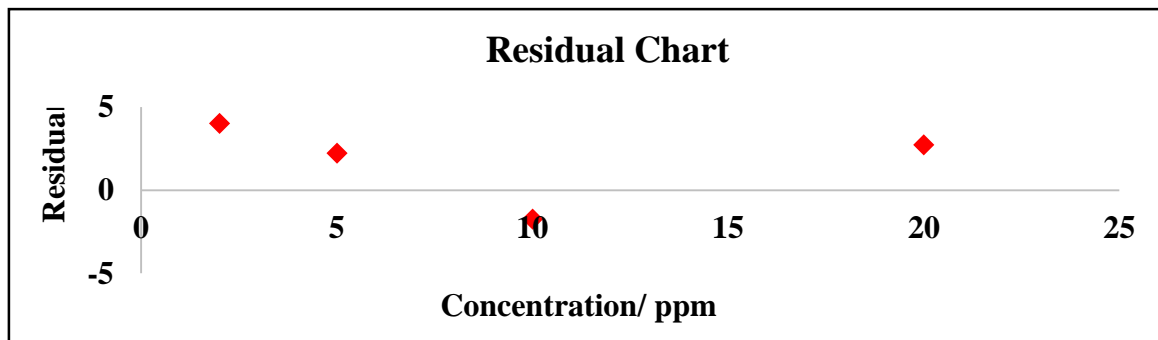
The residual plot for the residual values (y- y_i^*) obtained for various limonin standard concentrations is shown in Table 6.2. The largest deviation for 2 ppm standard limonin was shown on the chart as the difference between actual data and the expected value.

6.3.3 Selectivity and detection limit of IDE sensor

The selectivity of the sensor was performed by studying the capacitance versus time curve for the mole concentration of common non-volatile interfering compounds such as glucose, sucrose, ascorbic acid, citric acid, malic acid and naringin normally present in standard fruit juice. Accordingly, we have made 0.158 M fructose, 0.329 M glucose, 0.173 M sucrose, 26.03 mM citric acid, 2.78 mM ascorbic acid, 745.8 µM malic acid, 367.3 µM naringin aqueous solution [21]. The mole concentrations of limonin aqueous solution were taken to be 10.627 µM equivalent to 5 ppm, 21.253 µM equivalent to 10 ppm, and 42.51 µM equivalent to 20 ppm [21]. The transient curves for the relative capacitance of 4 mM MgSiO₃.xH₂O based sensing device, when exposed to these solutions (shown in Fig. 6.4a), show high saturated values (C^S_{sample}) for limonin solutions in comparison to other solutions. A maximum SD of ±0.47µF for sucrose sample and ±0.1µF for Malic acid was observed. The C_r values were obtained by using the Eq. (6.2) for the solutions that show weak dependences of the sensor's output on the interfering components in comparison to the limonin solutions (as shown in Fig. 6.4b). We have also performed the selectivity analysis under model sample aqueous solutions prepared by mixing the amount of interfering components (as stated above) where limonin was added to the sample solutions in increasing order from 5 ppm, 10 ppm and 20 ppm.
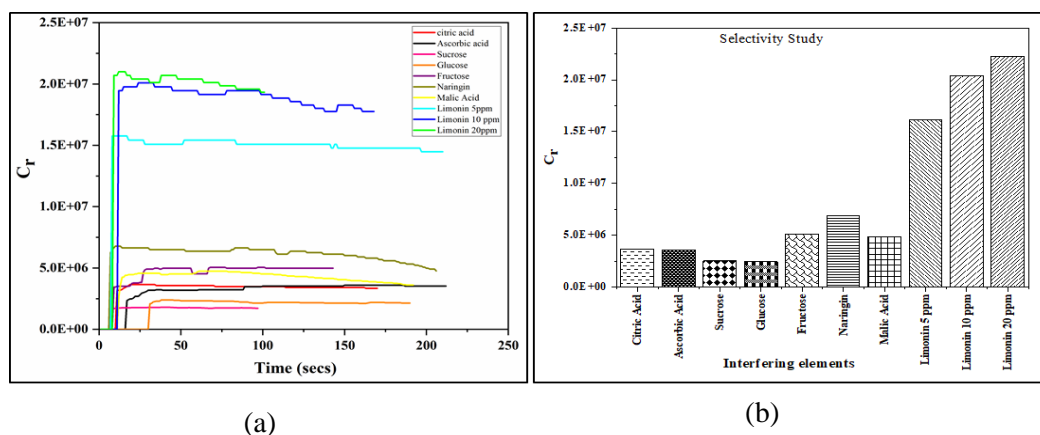

(a)   (b)

Figure 6.4: (a) Selectivity study using interfering compounds usually present in juice (b) bar graph exhibiting a gradual increase in capacitance with respect to limonin concentration with insignificance change for other interfering compounds

The transient curve of C_{sample} in Fig. 6.5a shows an increase of C_{sample} with limonin concentration (L_C) even in the model solution. Standard Deviation of the curves in Fig. 6.5a were found out to be approximately $\pm 1.72\mu F$(GSFACN) $\pm 3.81\mu F$(GSFACNL5ppm) $\pm 5.39\mu F$(GSFACNL10ppm) and $\pm 12\mu F$(GSFACNL20ppm). The values of C_r in Fig. 6.5b increase with L_C. The results confirm no noticeable changes in capacitance due to the presence of the interfering materials
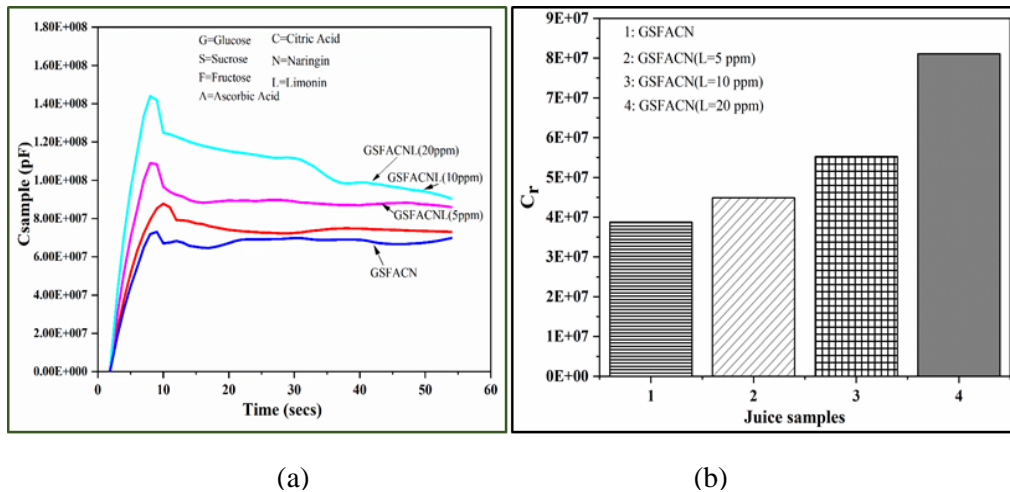

(a)   (b)

Figure 6.5: (a) Time-dependent capacitive response for model juices prepared (b) variation of sensors output with model juices

The C_r values for the model sample solution of L_C of 100 ppb in Fig. 6.6 show a noticeable change with respect to that for only DI water and the solution below L_C of 100 ppb shows a C_r value almost the same as that of DI water. Hence, the approximate threshold for detection of limonin content in the citrus juice for the sensor is assessed to be 100 ppb. The responses for the solution having a concentration of limonin such as 1 ppm and 10 ppm are also shown in Fig. 6.6 showing the increasing trend in the sensor's response with L_C. The theoretic value of

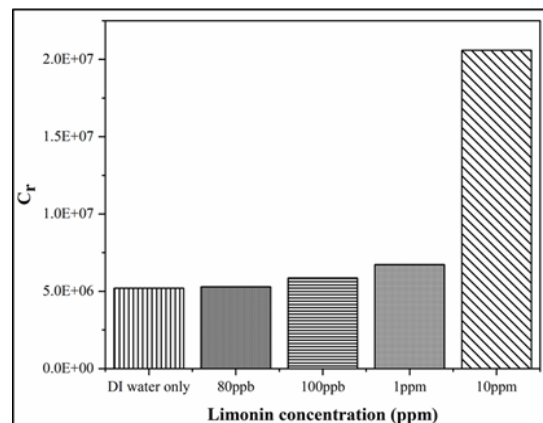

Figure 6.6: Detection limit of IDE sensor

LOD as calculated from the regression analysis using the Eq. 5.5(chapter 5) and Eq. 6.3 is ~5.542μM. In enlightening selectivity study, the sensor is fabricated with electrodes on the substrate without sensing material and a sensor with PVA is deposited over the
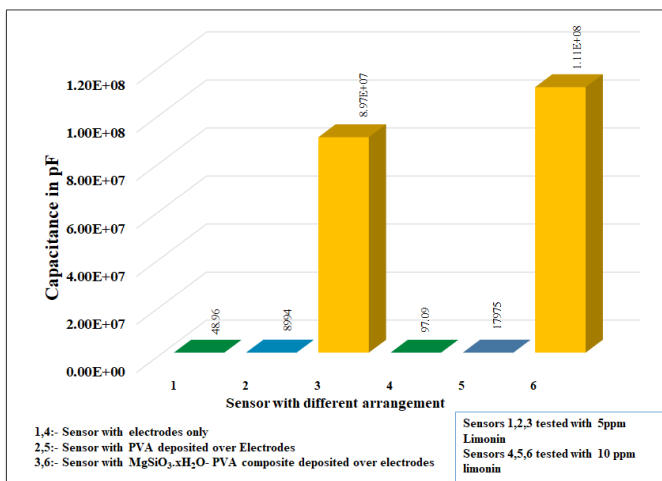
Figure 6.7: sensor's performance on the electrodes fabricated on paper with and without sensing

electrodes only without sensing material (MgSiO₃.xH₂O). Performance of these two sensors has been compared with the sensor based on MgSiO₃.xH₂O+PVA composite taking limonin standard solution of 5 ppm and 10 ppm as an analyte (Fig. 6.7). The result shows an increase in the capacitance by the sensor with MgSiO₃.xH₂O+PVA composite in comparison with the

other two sensors (Fig. 6.7). The 1.8×10^6 times increase in C^S_{sample} is observed for the sensor with MgSiO₃.xH₂O+PVA composite in comparison to the sensor without sensing material when tested with 5 ppm limonin. Similarly, a 1.14×10^6 times increase in capacitive response was observed for the same set of sensor comparisons when tested with a 10 ppm limonin standard. The sensor with MgSiO₃.xH₂O+PVA was compared with the sensor with PVA only (Fig. 6.7). A significant increase in capacitance (9.9×10^3 times when tested with 5 ppm limonin and 6.1×10^3 times when tested with 10 ppm) was found. Hence it is further proved that the sensing of limonin was only because of adsorption by magnesium silicate material.

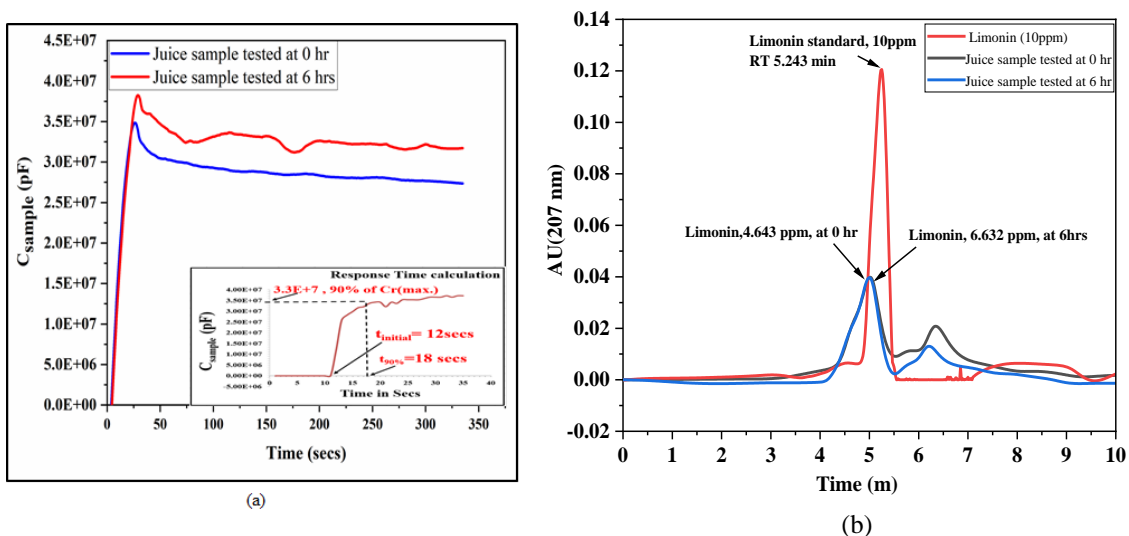## 6.3.4 Real-time analysis for Limonin quantification:

Figure-6.8: (a) real time analysis of the citrus limetta juice sample at different time intervals after juice preparation (b) quantification of limonin content in real juice sample using HPLC

The developed IDE sensor was tested in a real juice sample of citrus limetta and citrus grandis juices to quantify limonin and also it's debittering. For real-time measurement of limonin, the sample juice was dropped on the surface of the sensing layer of the device. The amount of limonin in juice increases with time enhancing delayed bitterness which leads to change in the flavor of the juice. The capacitance variation was used to detect and quantify limonin by using the present IDE capacitive sensor. Quantification of limonin is required at the time of juice preparation and during the preservation of juice
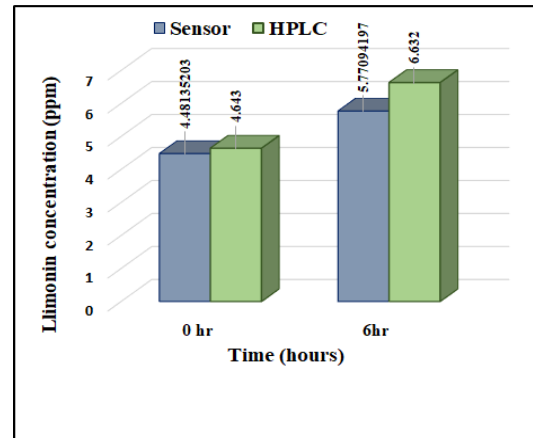

Figure 6.9: comparison of sensor's response with HPLC analysis in term of limonin quantification for citrus limetta

The transient curve of C_{sample} in Fig. 6.8a shows an increase of C^S_{sample} by 7.9 % due to limonin formation from LARL. From the calibration curve, using the regression equation as shown in Fig. 6.3b, a 19 % increase of limonin from 4.48 ppm to 5.77 ppm was obtained after 6 h times of preparation (Fig. 6.9). Moreover, the HPLC method has been applied for validation of sensor's performance. Quantification of limonin in real juice sample using HPLC analysis (Fig. 6.8b) and results are compared with the sensor's output as shown in Fig 6.9.
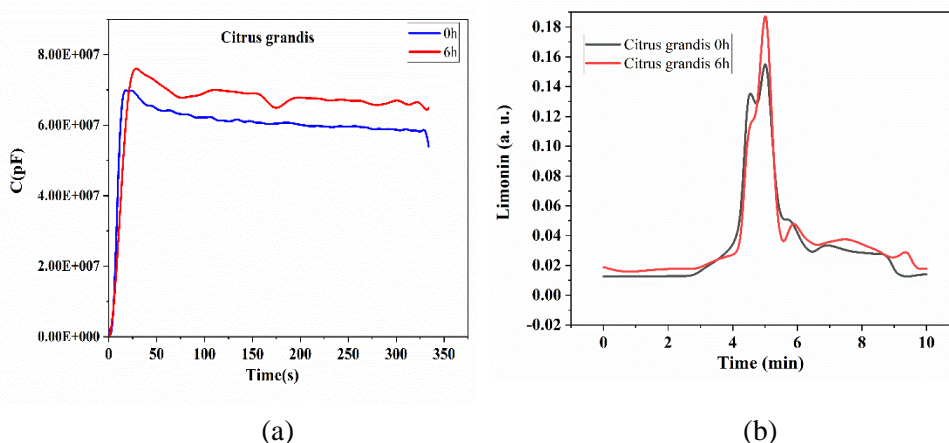

(a)

(b)

Figure-6.10: (a) real time analysis of the citrus grandis juice sample at different time intervals after juice preparation (b) quantification of limonin content in real juice sample using HPLC

The sensor's response in the quantification of limonin shows identical results as per HPLC analysis with a deviation of 0-12%.

Table 6.3: Sensor-based and HPLC analysis results for Citrus grandis fruit juice

Cycle no	Sensor based analysis For *citrus grandis*		HPLC analysis For *citrus grandis*		Accuracy (in terms of percentage error)
	Limonin conc.(ppm)	Percentage increase from initial value (approximate)	Limonin conc. (ppm)	Percentage increase (approximate)	
At 0 hr	19.42	0%	=(0.08142/0.4353)x10 =18.70	0%	3.71%
After 6 hrs of its storage	21.98	13.18 %	=(0.09369/0.4353)x10 =21.52	15.08 %	2.09%

The time domain analysis for limonin quantification in citrus grandis juice shows an increase of limonin content by 13.18 % after 6 h of storage. The limonin concentration from 17.98 ppm to 20.4 ppm was obtained after 6h times of preservation (Fig. 6.10 and Table 6.3). Moreover, HPLC results were compared with the sensor's output as shown in Fig 6.10 and Table 6.3. The sensor's response in the quantification of limonin shows identical results as per HPLC analysis with a deviation of 0-3.71%.

The response time of the sensor is determined as $t_{response} = t_{90\% total} - t_{initial}$ = 6 s, where $t_{90\% total}$ = the time required by the IDE capacitive sensor in response to the target material under test in attaining 90% of the maximum capacitance value =18 s and t_{intial}= time to get the initial value of capacitance =12 s (inset of Fig. 6.8a).

6.3.5 Reusability and Reproducibility Study

The reusability study of IDE device based on MgSiO₃.xH₂O –PVA (4mM) composite is shown in Table-6.4. The reproducibility study was also performed by analysing the sensor's (MgSiO₃.xH₂O –PVA (4mM) performance taking 10 ppm limonin standard as analyte at varying environmental conditions such as changing temperature within the laboratory set-up and performing the test at different time.

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The performance of the sensor after its complete life cycle has been shown in Table 6.4. The degradation of approximately 5.5%, 10.5%, and 22.4% in the sensor's output (in terms of the initial value of capacitance) has been observed after the 1ˢᵗ, 2ⁿᵈ and 3ʳᵈ life cycles of the sensor respectively. The performance of the sensor degrades slightly up to 2 cycles. Further, it was also observed that the sensing layer was not leached out during an entire period of analysis.

Table 6.4: Reusability of IDE sensor

Cycle no	$C_{initial}$		C^s_{sample}	
	Capacitance value (pF)	Percentage degraded (approximate)	Capacitance value(μF)	Percentage degraded (approximate)
First use of sensor	30.80	0%	255.515	0%
1ˢᵗ cycle of sample exposure	29.1	5.5 %	238.7	6.6 %
2ⁿᵈ cycle of sample exposure	26.05	10.5%	215.19	9.9 %
3ʳᵈ cycle of sample exposure	20.2	22.4 %	165.53	23.1 %

Table 6.5: Reproducibility of IDE sensor

	Temperature at which sensor is tested (°C)	Capacitance value, (pF)	Mean(pF)	% deviation(maximum) from mean
Day-1	10	100452315		
	20	112543698		
	30	108914337		
	40	120942156		
Day-2	10	111368527		
	20	111254369	111036806	9.4 %
	30	110813532		
	40	121566231		
Day-3	10	112361489		
	20	101336537		
	30	111974145		
	40	108914337		

The reproducibility of the sensor is a critical parameter in determining the consistency of the sensor with which it replicates multiple measurements under different conditions. The reproducibility study for the IDE capacitive sensor (Table 6.5) shows consistency of the results with a maximum of 9.48 % deviation from its mean value.

### 6.3.6 Debittering of juices using fabricated sensor and its measurement

The debittering of the juice plays a key role in the preservation of juice for increasing consumer's acceptability. Measurement of the reduction of limonin content by the process of adsorption of limonin in magnesium silicate surface is an important parameter of analysis for the developed sensor. In the present work, a reduction in limonin content was measured at the exposer times of the 60 s and 120 s to assess the performance of the sensor (Table 6.6). The reduction in limonin and its percentage removals were calculated and shown in Fig. 6.11.
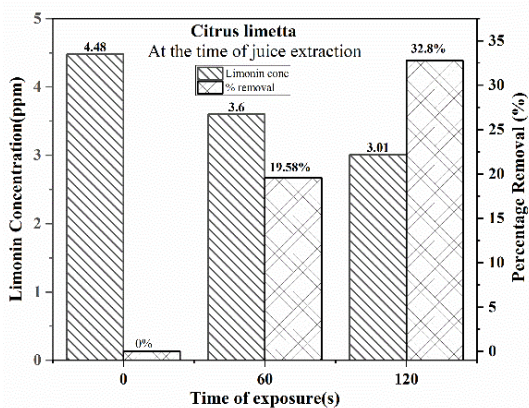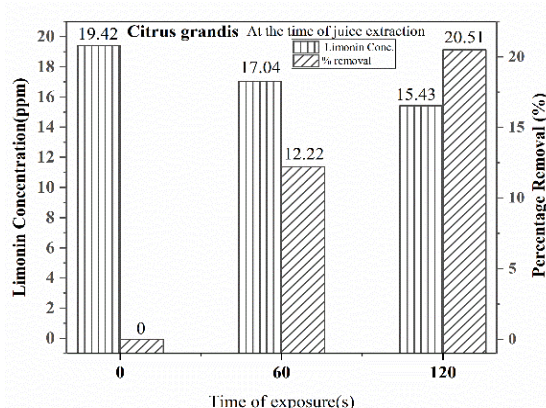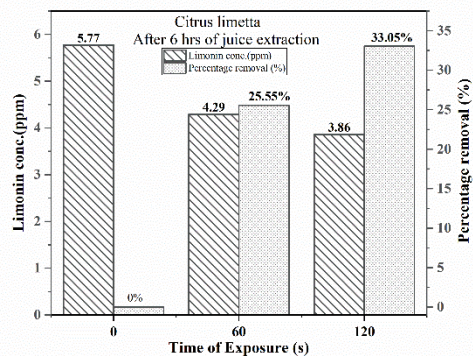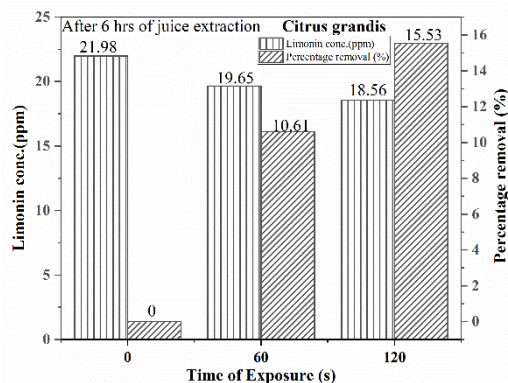
Figure 6.11: bar showing decrease in limonin content of juice (held for 6 h of its preparation) with its percentage reduction when expose to the sensor for 60 s and 120 s (a) Citrus limetta at 0 h(b) Citrus grandis at 0 h (c) Citrus limetta at 6 h (d) Citrus grandis at 6 h

A decrease in limonin from 4.48 ppm to 3.01 ppm was observed for the citrus limetta juice sample tested (exposure time of 120s) at the time of its preparation and 32.8% removal of limonin was determined for the sample (Fig. 6.11a). Likewise, the juice sample when exposed to the sensor after 6 h of its preparation, debittering of 25.55% and 33.05% was observed with a decrease in limonin level from 5.77 ppm to 4.19 ppm by 60s

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and 5.77 ppm to 3.86 ppm by 120 s respectively (Fig. 6.11a and 6.11c) for the citrus limetta juice. For Citrus grandis fruit juice a reduction of limonin content from 19.42ppm to 17.04ppm (approximately 12.22% reduction) was observed when exposed to the sensor for 60 s. The juice preserved for 6 h shows a reduction from 21.98 ppm to 19.65 ppm (Table 6.6) while exposed to the sensor for 60 s (Fig 6.11b and Fig. 6.11d). The percentage removal of a maximum of 20.51% was perceived in Citrus grandis juice with our developed sensor device.

Table 6.6: concentration of limonin, and its percentage removal in citrus limetta and citrus grandis juices treated with the fabricated device/sensor based on magnesium silicate hydrate

Juice samples Testing time	Name of Juice sample	Time of exposure of sample (Secs)	Sensor's Response (capacitance in pF)	Limonin Concentration(ppm)	Percentage Removal (%)
At time of juice sample Preparation	Citrus limetta	0	34056228	4.48	--
		60	31956648.34	3.60	19.58
		120	30539910.11	3.01	32.8
	Citrus grandis	0	69788894	19.42	0.00
		60	64112663.00	17.04	12.22
		120	60259288.00	15.43	20.51
After 6hr of Juice sample preparation	Citrus limetta	0	37139457	5.77	--
		60	33607222	4.29	25.55
		120	32570019.7	3.86	33.05
	Citrus grandis	0	75915578	21.98	0.00
		60	70338702	19.65	10.61
		120	67750338	18.56	15.53

6.3.7 Toxicity study

We have also performed toxicity verification of sensing material MgSiO₃ and PVA by using pharmacokinetics online tool 'http://biosig.un imelb.edu.au/pkcsm/prediction showing no toxicity from these materials (Table 6.7 for MgSiO₃ and Table 6.8 for PVA). This study shows no toxicity to the human body from both magnesium silicate and PVA (which are used in the sensor) as per Table-6.7 and 6.8

respectively. Hence, the toxicity study of the sensor shows easy disposal of the sensor. Moreover, the non-toxic nature of our sensor makes it environment-friendly.

Table 6.7: Toxicity study of Magnesium Silicate

Property	Model Name	Predicted Value	Unit	Remarks
Toxicity	AMES toxicity	No	Categorical (Yes/No)	Represents biological assay to assess the mutagenic potential of chemical components. Test serves as a quick and convenient assay to estimate carcinogenic potential of compound by causing mutation in DNA in organism. Test result No here may presents AMES detoxicity
Toxicity	hERG I inhibitor	No	Categorical (Yes/No)	It will not inhibit the hERG-I/II (human ether-a-go-go gene) which causes development of acquire QT syndrome by inhibiting the potassium channels encoded by hERG.
Toxicity	hERG II inhibitor	No	Categorical (Yes/No)	
Toxicity	Oral Rat Acute Toxicity (LD50)	2.928 (341530 mg/mol)	Numeric (mol/kg)	For more than 15000 mg/mol considered harmless(Hodge and Stenner scale), Since the value of LD50 is more than 15000mg/kg, hence can be considered harmless regarding its its Oral Rat Acute Toxicity
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	0.437	Numeric (log mg/kg_bw/day)	Values near 2 represents liver enzyme induction.
Toxicity	Hepatotoxicity	No	Categorical (Yes/No)	No toxic hepatitis in liver

Toxicity	Skin Sensitisation	No	Categorical (Yes/No)	No allergic response while in contact with skin
Toxicity	T.Pyriformis toxicity	-0.431	Numeric (log ug/L)	T. pyriformis toxicity predicted value log ug/L > 0.5 μg/L considered as toxic Can inhibit the 50% growth in toxicity produced by T. Pyriformis protozoa bacteria. Hence value -0.431 (less than 0.5) is non toxic
Toxicity	Minnow toxicity	2.321	Numeric (log mM)	minnow toxicity logLC50 < -0.3 considered as high acute toxicity. Lethal concentration indicates molecules necessary to cause the death of 50% of Flat head minmow and calculated value here indicates non toxicity.

Table 6.8 Toxicity study for PVA

Property	Model Name	Predicted Value	Unit	Remarks
Toxicity	AMES toxicity	No	Categorical (Yes/No)	Represents biological assay to assess the mutagenic potential of chemical components. Test serves as a quick and convenient assay to estimate carcinogenic potential of compound by causing mutation in DNA in organism. Test result No here may presents AMES detoxicity
Toxicity	hERG I inhibitor	No	Categorical (Yes/No)	It will not inhibit the hERG-I/II(human ether-a-go-go gene) which causes development of acquire QT syndrome by inhibiting the potassium channels encoded by hERG.
Toxicity	hERG II inhibitor	No	Categorical (Yes/No)	

| Toxicity	Oral Rat Acute Toxicity (LD50)	2.169(46 1042 mg/mol)	Numeric (mol/kg)	For more than 15000 mg/mol harmless(Hodge and Stenner scale), Since the value of LD50 is more than 15000mg/kg, hence can be considered harmless regarding its its Oral Rat Acute Toxicity
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	1.837	Numeric (log mg/kg_bw/day)	Values near 2 represents liver enzyme induction.
Toxicity	Hepatotoxicity	No	Categorical (Yes/No)	No toxic hepatitis in liver
Toxicity	Skin Sensitisation	No	Categorical (Yes/No)	No allergic response while in contact with skin
Toxicity	T.Pyriformis toxicity	-1.871	Numeric (log ug/L)	T. pyriformis toxicity predicted value log ug/L > 0.5 μg/L considered as toxic Can inhibit the 50% growth in toxicity produced by T. Pyriformis protozoa bacteria.hance value -01.871 (less than 0.5) is non toxic
Toxicity	Minnow toxicity	2.774	Numeric (log mM)	minnow toxicity logLC50 < -0.3 considered as high acute toxicity. Lethal concentration indicates molecules necessary to cause the death of 50% of Flathhead minmow and calculated value here indicates non toxicity.

6.3.8 Sensory:
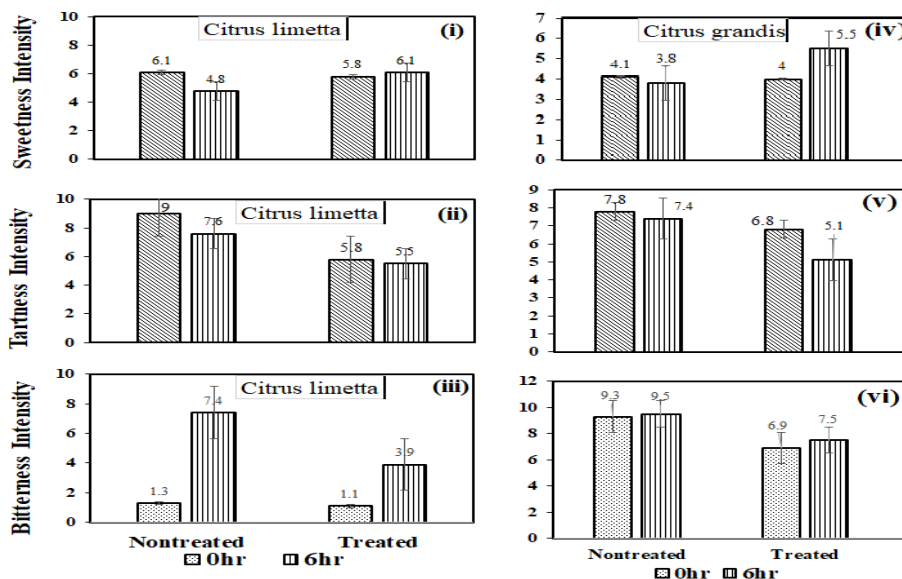
Figure 6.12: sensory evaluation of magnesium silicate treated and untreated juice.

The performance of sensory evaluation on treated and untreated samples at 0 h and 6 h (at room temperature) by 10 trained assessors is presented in Fig. 6.12. The results exhibit that storage time was not a significant factor ($P < 0.05$) affecting sweetness and tartness sensory attributes for both treated and untreated juices (Fig. 6.12 i and 6.12 vi). However, a significant increase ($P < 0.05$) in bitterness was observed when citrus limetta juice was stored for 6 hrs (Fig. 6.12 iii). The bitterness in the untreated juice sample increases with storage time. The scorings obtained from trained panelists show a similar trend as the limonin contents obtained from the sensor's output. The bitterness in citrus grandis juice shows high values for both non treated and treated juice at 0h and 6h which agrees with the results of the sensor's output. Increase in bitterness for citrus grandis is not as high as observed in citrus limetta from Fig. 6.12. From this study, it can be concluded that storage studies of extracted juice are vital regarding the acceptance of juice by consumers as fruit can have good flavor qualities when freshly juiced but develop delayed bitterness after its storage.

6.3.9 TPC, TFC and antioxidant activity

The TPC of fresh citrus limetta juice was 174.66 μg GAE mL^{-1} which was decreased to 157.83 μg GAE mL^{-1} after debittering (Fig. 6.13a). For Citrus grandis fruit juice TPC values show an increase in the value of approximately 50 μg GAE mL^{-1} for magnesium silicate-treated juice (Fig. 6.13b). The TFC value of debittered Citrus limetta

juice was found to be 21.10 µg QE mL^{-1} whereas that of fresh juice was 25.66 µg QE mL^{-1} as shown in Fig. 6.13a. There is a slight increase TFC value from 34.83 to 46.5 µg QE mL^{-1} for Citrus grandis juice (Fig. 6.13b).
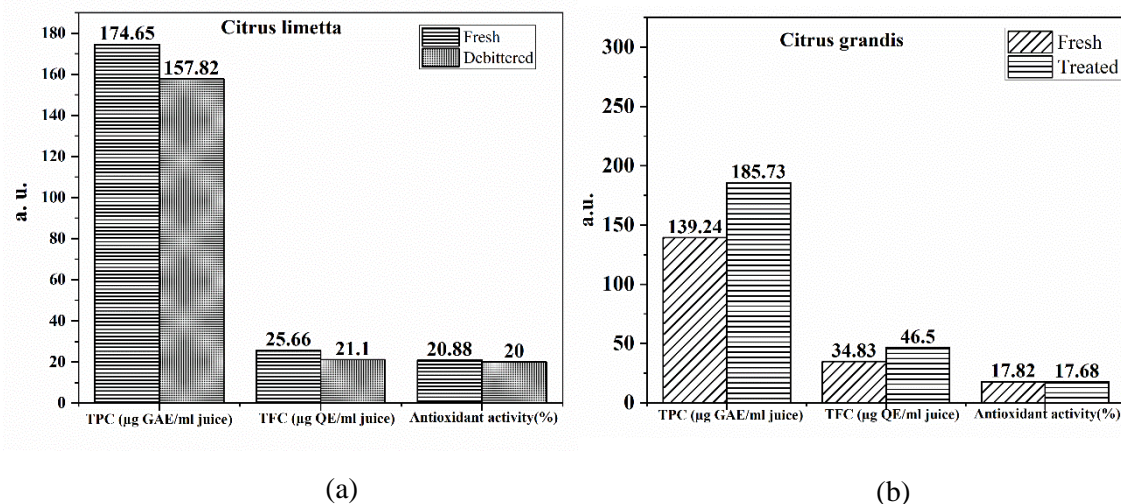

Figure-6.13: comparative study for fresh and debittered juice with respect to TPC, TFC and antioxidant activity for (a) citrus limetta (b) citrus grandis

The antioxidant property of debittered juices was nearly the same as fresh juices extract (Fig. 6.13a and 6.13b). Hence, the fresh juice when debittered through MgSiO₃.xH₂O -PVA composite exhibits no alteration of its antioxidant property with slight variation in TPC and TFC values.

6.4 Comparative study of the developed sensor with the already existing sensors/devices/methods

The present IDE-based sensor for quantification of limonin in citrus fruit juices and its debittering measurement was compared with the existing sensors (reported by previous authors [22-25] in terms of response time, detection limit, selectivity, sensitivity, reusability, flexibility (Table 6.9) The detection limit of our IDE sensor is to some extent lesser than that of the device based on electrochemical transistor [25] but our flexible IDE sensor can be reused up to 2 cycles with replacement of

Table 6.9: Comparison of some of the reported sensors/devices for limonin quantification and debittering

Device	selectivity	Sensitivity	Response/ detection time	Detection limit	reusability	flexibility

Organic electrochemical transistor (OECT) based on ceria nanoparticles integrated fibroins	selective	10.41 ± 0.35 µA/ µM	response time in seconds	10 nM=4.705ppb	recycled several times by replacing the CNPs-SF and PEDOT:PSS layers	Not flexible
Amperometric biosensor using Mutant (lim+) of a strain Pseudomonas putida G7	selective	-------	20 min for the steady-state method and 12 min for the initial slope method	----	Reusable	Not flexible
Odorant-Binding Protein-Modified Screen-Printed Electrodes (device use for Detection of Bitter Taste Molecules)	Not selective	$10-9$ mg/mL (about $10-12$ M)	---	10nM	Not reusable	Not flexible
Surface molecularly imprinted polymers (SMIPs) based debittering of limonin using SMIPs	Selective debittering	No detection but debittering time ~ 6 min				
CeO₂ based IDE sensor	selective	~ 9.62 ±0.095 µF/ ppm	response time ~ 13 s	LOD~5.462µM Minimum value practically observed ~100 ppb	Reusable up to 1st cycle	flexible

| Present work based IDE capacitive sensor using MgSiO3 | selective | ~ 2.392±0.3 µF/ppm | response time ~ 6 s and debittering time ~ 60 s | ~5.542µM (as observed from results~100 ppb) | Re usa ble | flexible |

sensor only from the set up. The set up can be used for multiple times. The amperometric biosensor [24] shows reusability property but its response time is 20 min which is much higher than our IDE sensor. The Odorant-Binding Protein-Modified Screen-Printed Electrodes is not selective to limonin and determine the overall bitterness of fruit juice with a very low LOD of 10 nM whereas our IDE sensor is highly selective to limonin detection with a fast response time. The Surface molecularly imprinted polymers (SMIPs) show only debittering with time ~ 5 min which is very slow with respect to our IDE sensor. Moreover, the IDE sensor performs both rapid quantification and debittering with an exposure time of 60 s for obtaining L_C of limonin from 5.77 ppm to 4.29 ppm in the case of citrus limetta juice (below a threshold value) and making it distinctive in comparison to other existing. The approximate cost and complexity in the fabrication of the developed and existing sensor for the quantification of limonin were compared and shown in Table 6.10

Table 6.10: Cost and complexity comparison of some of the reported sensors/devices for limonin quantification/debittering

Device	Major Development steps	Key materials used	Material cost	Synthesis and Fabrication cost	Fabrication complexity	Overall cost
organic electrochemical transistor (OECT)	E-beam evaporation deposition of PEDOT:PSS, Adherence of CNP:SF to Au gate electrodes	PEDOT:PSS, PDMS, Au, Cerium nitrate hexa., Si wafer,Limonin	High	High	Complex	High
amperometric biosensor	culture of Pseudomonas putida G7, Generation of auxotrophic mutant (lim+),	Pseudomonas putida G7 ,KH2PO4,(NH4)2SO4, FeSO4, KOH, MgSO4, TTC, limonin	High	Moderate	complex	High

	development of microbial sensor					
Odorant-Binding Protein-Modified Screen-Printed Electrodes (device use for dete Detection of Bitter Taste Molecules	Recombinant Odorant-Binding Protein-Modified Screen-Printed Electrodes (OBPs)and Reagents	Graphene oxide (rGO), HAuCl4, Au N-Hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)	High	Moderate	complex	High
debittering of limonin using SMIPs	Synthesis of molecularly imprinted polymers	Tetraethylorthosilicate, 3-aminopropyltriethoxysilane	high	moderate	---	high
CeO₂ based IDE sensor	IDE structure, drop coating, Embedded with microcontroler	CeO₂, PVA, Limonin	Moderate	Low	Relatively simple	medium
Present work	IDE patterning, drop coating, Embedded with microcontroler	MgSiO3, PVA, Limonin	Moderate	Low	Relatively simple	medium

The cost is approximately estimated as per the price of standard suppliers/companies such as sigma Aldrich, Merck.

Table 6.11: Cost estimation (approximate) of the fabricated sensor

Device	Key materials used	Company name	Material cost (in ₹)	Approximate material cost per sensor(in₹)
Present work based on IDE capacitive sensor using MgSiO3	MgSiO₃	Sigma-Aldrich	2588.00 /100g	0.5176
	PVA	Merk	4082.00/25g	4.082
	Silver(Ag)	Alfa-Aeser	2590.00/5g	25.9
	Paper substrate	Whatman, GE healthcare	1593/100pc	1.062
Total material cost per sensor(approximate)in INR				31.56
				~ 32

The developed sensor is inexpensive compared to other existing sensors [22-25] due to the use of inexpensive fabrication instruments and chemicals (Table 6.11). The cost $\approx$ ₹32 ($\approx$ $ 0.4) and fabrication complexity of the present sensor were less compared with existing ones as presented in Table 6.10. As per directive of directive 95/2/EC (E 553a), magnesium silicate is a harmless inorganic compound, Hence it provides no acute oral toxicity nor cytotoxicity in debittered fruit juice [7, 8].

6.5 Summary

Here we have designed and fabricated a novel magnesium silicate-based interdigitated electrode capacitive sensor for limonin detection and reduction in citrus fruit juices. The sensor was accomplished with a not remarkable shift in output in the presence of other elements like ascorbic acid, citric acid, sucrose, fructose, glucose, malic acid and naringin present normally in the citrus juices that interfere with the detection process. The device provides a sensitivity of nearly 2.392 ± 0.3 μF/ppm in detecting the increase in limonin in citrus juices with a fast response time of nearly 6s. The fabricated sensor shows a deviation of 0-12% (approximately) in citrus limetta juice and 0-5% in citrus grandis juice when correlated with HPLC analysis. An increase in limonin content was observed after 6 hrs of juice preparation. A reduction of limonin was achieved when juices were exposed to a magnesium silicate surface for 60 s after 6 h of its holding. The device is reused up to 2 cycles with degradation of less than 10 % by gently washing it with DI water followed by drying. As Magnesium silicate and PVA compounds are not toxic, hence no acute toxicity is generated in citrus fruit juices in the process of debittering. The present work accomplished on $MgSiO_3.xH_2O$ based IDE capacitive sensor on flexible paper substrate assures easy, rapid, onsite quantification and reduction of limonin with environmentally–friendly, low-cost, flexible and easy disposal capabilities.

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