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A Disposable Sensor For Assessing *Artocarpus heterophyllus L.* (Jackfruit) Maturity

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Abstract: The purpose of this work was an attempt to monitor the ripeness process and to investigate the different maturity stages of jackfruit by chemometric treatment of the data obtained from the disposable sensor. Response of the sensor strip fabricated using screen-printing technology was analyzed using Principal Component Analysis (PCA) and the classification model constructed by means of Canonical Discriminant Analysis (CDA) enable unknown maturity stages of jackfruit to be identified. Results generated from the combination of the two classification principles show the capability and the performance of the sensor strip towards jackfruit analysis.

Keywords: Jackfruit maturity, disposable sensor, chemometric

Introduction

Product quality is one of the prime factors in ensuring consistent marketing of jackfruit. However there is no precise measurement, clear definition or standard unit to identify product quality objectively since quality is always associated with the degree of acceptance by the consumers. Qualitative evaluation of agricultural products has been of interest to many and what we call organoleptic quality could be summarized as "eating pleasure". Many quality factors such as flavor or taste are actually related to ripeness [1]. For jackfruit juice manufacturing industries especially, fruits received from farm should be fully ripened as to produce good quality of fruit juice. This is because only the ripened fruit has good eating quality in terms of aroma, texture, sweetness, and taste and therefore is able to produce high quality of juice. Hence monitoring and controlling fruit ripeness has become a very important issue in the fruit industry since ripeness is perceived by customers as the main quality indicator [2]. However, until today optimal harvest dates and prediction of storage life are still mainly based on practical experience. Leaving these critical decisions to subjective interpretation implies that large quantities of fruit are harvested too soon or too late and reach manufacturers in poor condition [3]. In most industries today, human sensory panels are still the key role of QA/QC (quality assurance/quality control) in product quality monitoring. This however when used in production facilities have been time consuming and expensive to operate. This has been attributed to reliance on-plant personnel who are subjected to typical economic and personal issues such as sickness, emotions, absenteeism, scheduling conflicts, lay offs, attrition, etc. [4]. Given these challenges, a simple but reliable QA/QC analytical technique is highly desired by the fruit industries. The present paper is devoted to analytical evaluation of the disposable sensor strips performance for the description or monitoring of analysis pertaining to quality assurance of jackfruit.

A study towards the development of a simple but reliable, one-shot, disposable sensor strip which functions on a new concept of global selectivity is currently done by Universiti Sains Malaysia. The disposable strip, coupled with chemometric principle is capable of providing chemical fingerprint that represent a combination of all the chemical components, ideally suitable for simple, real-time testing and monitoring of jackfruit ripeness and taste. The basic principle behind the disposable sensor system is to combine non-specific and overlapping sensors signals with pattern recognition routines. The sensor array in this system produces signals, which are not specific for any particular components in the samples. The signal pattern generated is related to certain features or qualities of the sample. These qualities can be determined by a computer trained to recognize the class of response patterns related to the sample under study. This is thus a similar correlation to how the human sensing organs produce signal patterns to be qualitatively interpreted by the brain [5]. The disposable sensor strip is a replacement to the conventional electrodes, which is bulky and expensive. The sensor array is an integration of working electrodes and reference electrode together in a single strip based on screen-printing technology, in order to miniaturize and to simplify the instrumentation for decentralized analysis. Screen-printing technology is particularly attractive for the production of disposable sensors. The 'memory effect" between one sample to another is avoided, and the phenomenon referred to as "electrode fouling" which is one of the main drawbacks of the electrochemical sensors is overcome. As the sensor described here is targeted for one-time used, hence sterilization or cleaning to avoid contamination is no longer needed and sensor durability or usage lifetime, which is particularly important for conventional electrode, is also no longer an issue, which needed to be taken into consideration. Besides that, common problem related to conventional sensors like sensor respond drifting due to leaching of active agents in the membrane is also overcome as the membrane sensor is always new prior to use [6].

Materials and Methods

Disposable Sensor Strips with Screen-Printing Technology

The design and fabrication of the disposable strips were carried out in Universiti Sains Malaysia with the help from Scrint (M) Sdn Bhd. Screen-printing technology is a technique whereby the screens allow ink to be applied on to a substrate with a squeegee in a particular size, shape and sequence of the print. Screen-printing seems to be one of the most promising technologies allowing sensors to be placed large-scale on the market in the near future because of advantages such as miniaturization, versatility at low cost and particularly the possibility of mass production. Each of the screen-printed strips was printed in an array of eight tracks of working electrodes and a track printed with Ag/AgCl, as the reference electrode. Fig. 1 shows the screen-printed strips from left to right the layout of the sequentially printed layers. The fabrication process of the disposable sensor was carried out in four consecutive printing steps. For the first layer, nine conducting paths were printed with silver ink (Electrodag®425A) (**B**) on a polyester substrate of thickness 250 micron (**A**). Subsequently, nine conducting pads and circular working electrode areas (4mm diameter) were printed with graphite-based ink (Electrodag®440) (**C**), followed by Ag/AgCl (Electrodag®7019) as the reference electrode (4mm diameter) (**D**). Four insulating layers were printed on the polyester substrate to create circular grooves (**E**). The final layout of a single screen-printed strip is with dimension of 3.8cm x 5.7cm (**F**). Finally lipid membranes were deposited onto the eight circular grooves using high precision fluid dispenser from Musashi Engineering, Inc. The lipid material used, as listed in the Table below, are similar to those reported by Toko [7].

Sensor Channel	1	2	3	4	5	6	7	8
Lipid Types*	Decyl alcohol (DA)	Oleic Acid	Dioctyl Phosphate (DOP)	DOP: TOMA =5:5	DOP: TOMA =3:7	Trioctyl Methyl Ammonium Chloride (TOMA)	Oleyl Amine	DOP: TOMA =9:1

*Lipid Material used in the disposable sensor strips

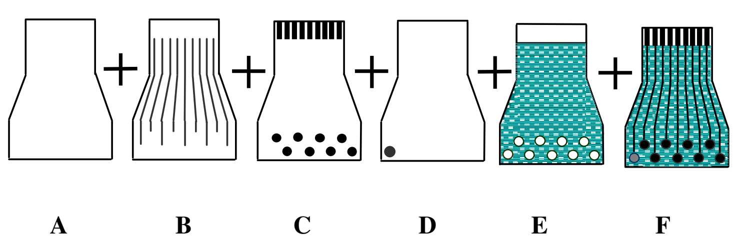

Fig.1. Scheme of a screen-printed strip preparation

Jackfruit measurement

Samples of jackfruit (*Artocarpus heterophyllus)* cultivars CJ1 and CJ3 which were used for the study was provided by Inter Crystal Argritech (ICA) farm, located in Gopeng, Perak, Malaysia. As a preliminary experiment to evaluate the response of the disposable screen-printed strips towards jackfruit, immature, unripe, and ripe jackfruit were bought from the local wet market. Seven sets of measurements consist of three different maturity stages of fruit were collected immediately upon purchase. For the second phase of experiment, immediate measurements were taken upon collection from farm for fresh CJ3 jackfruit harvested at the maturity stages of 70-80, 81-90, 91-95, 96-100 days old after anthesis. For each category, three samples of jackfruit were randomly chosen from the farm. The edible parts excluding the seed of the fruit was blended with a juice extractor in order to produce the sample in liquid form. Altogether 12 measurements were taken for each category.

The third phase of the experiment was targeted to monitor the ripeness process of the jackfruit. In this experiment, sample of CJ1 collected at the maturity stage of 82 days old after anthesis (physiologically matured but not fit for consumption) was wrapped and stored in order to enable the fruit to ripen naturally. Upon collection from the farm, one small portion of the edible part of the fruit was carefully isolated from the perianth for analysis. The remaining part of the dissected fruit were then carefully wrapped using polyetilena food wrapper and aluminum foil and was stored at ambient temperature for the next analysis at the 6^{th} hour, 12^{th} hour, 24^{th} hour, 36^{th} hour, 42^{nd} hour, 48^{th} hour, 60^{th} hour, 72^{nd} hour, 84^{th} hour, 108^{th} hour, 132^{nd} hour, 156^{th} hour, 180^{th} hour, and finally 204^{th} hour when the fruit was overripe. The disposable screen-printed strips were rinsed with distilled water before they were being immersed into the samples. One minute of conditioning was performed before data collection started and all measurements were taken for sixty seconds with ten seconds of interval with an eight-channel high impedance multi-interface data acquisition meter from Fylde Scientific, United Kingdom.

Data analysis

The raw data obtained from the experiments were treated by pattern recognition principles namely Principal Component Analysis (PCA) and Canonical Discriminant Analysis (CDA). All analyses were done with the software packaged SPSS (Statistical Packaged for Social Sciences)9.0 for Windows. PCA is a well-known method for reducing the dimensionality of a data set, while preserving most of the variance present in the data set to plots. The result is expressed in score plots, where trends, groups and outliers among the measurements can be observed, and loading plots, where relevance and similarity between variables can be seen. On the other hand, CDA is a classical statistical approach for classifying samples of unknown classes, based on training samples with known classes. In contrast to PCA, CDA needs to be trained with prior information to form a classification model [8].

Results and Discussion

Classification between immature, unripe and ripe jackfruit

At the first phase of the experiment as to evaluate the response towards jackfruit, measurements were taken from three different maturity stages of unripe, immature and ripe jackfruit samples purchased from the local wet market. Figure 2 shows the PCA plot of the first two principle components together explaining 89% of total variance, which distinguished the three different groups of jackfruit according to their maturity stages respectively. As can be observed, there's a clear distinction between the three different categories of samples. The disposable screen-printed strip response in one of the maturity stages is shown in Fig.3. Seven measurements of the same sample of fruit have been performed to evaluate and to ensure necessary repeatability of the strips. As the strip described here is used as a single-use, disposable sensor, therefore the performance of the disposable screen-printed strip in terms of reproducibility is not emphasize here. The standard deviation between the seven different measurements for channel 1 to 8 respectively is about 4mV to10mV.

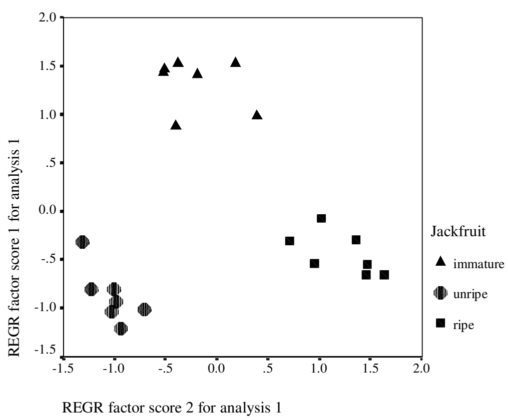

Fig. 2. PCA plot for classification of three maturity stages of jackfruit

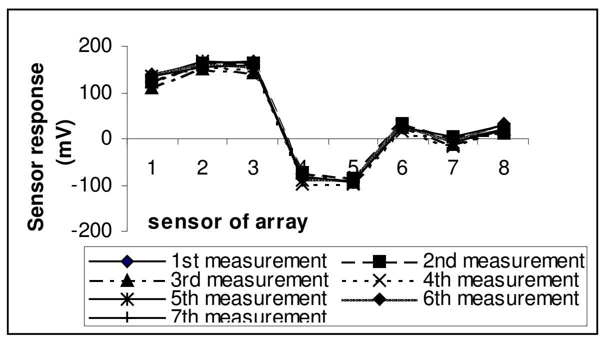

Fig. 3. Response of the disposable sensor strips for seven measurements of immature jackfruit samples

Classification of jackfruit according to different maturity stages

At the second phase of the experiment, data for four category of jackfruit i.e. 70-80 days, 81-90 days, 91-95 days, and 96-100 days were collected. Fig.4 present the PCA plot discriminating between different maturity stages of jackfruit. The first two principle components contained more than 80% of the total variance. These two principles have the most relevant information to classify the fruit according to the level of ripeness. In Fig.4, a clear distinction exists between the jackfruit with stages of 70-80 days and 81-90 days old. The first principle component discriminates the two categories. It is also worth noticing that the distance of the data point for 70-80 days are distributed further from the rest of the data groups. Fruits at this stage are still unripe. Fruits that aged from 80 days and above achieved ripeness but only in terms of its

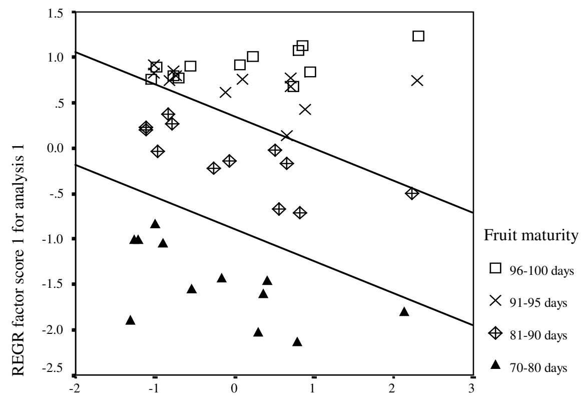

Fig.4. Discriminating abilities of the jackfruit classified according to stages

physiological state and are yet ready for consumption until it is kept at ambient temperature for 3-4 days for natural ripening. Due to this reason, it can be explained that the two categories was found to be rather easily distinguished. However, the plot does not allow a general distinction to be made between data from 91-95 days and 96-100 days. The overlapping data is possibly due to the fruit, which has achieved an optimum level of ripeness in terms of sugar and carbohydrate content, which explain the characteristic of the data points projected.

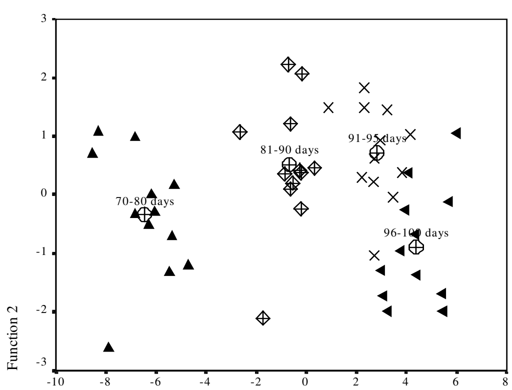

Fig. 5. CDA plot for four stages of jackfruit projected by means of CDA

Table 1: Category of maturity stages of jackfruit based on training samples of known classes in CDA

Category	Group f2_grp
70-80 days	1
81-90 days	2
91-95 days	3
96-100 days	4

Using supervised techniques such as CDA could further extend the sensor system discrimination capabilities. Unlike PCA CDA knows the class membership of each case. It is mainly used in the second step, to build a classification model, which will be applied for the identification of unknown samples [9]. Therefore to validate the method, a classification model is computed, as shown in Fig.5 and Table 1. The first discriminant function discriminates rather well the four stages of jackfruit except for samples aged 91 days and above. Four measurements which were not used in the training or learning data set of CDA are used to validate the model and are known as “unknowns”. With CDA, all the four "unknowns" which belongs to the category of 70-80 days are successfully and well classified in the corresponding group as illustrated in Fig. 6 and Table 2.

Table 2: Classification results of unknown samples constructed by means of CDA. Referring to the predicted group shown, all unknown samples are correctly classified to their respective group. It proves the performance of the strips

Unknown:Jackfruit (70-80 days)	Predicted group
Measurement 1	1
Measurement 2	1
Measurement 3	1
Measurement 4	1

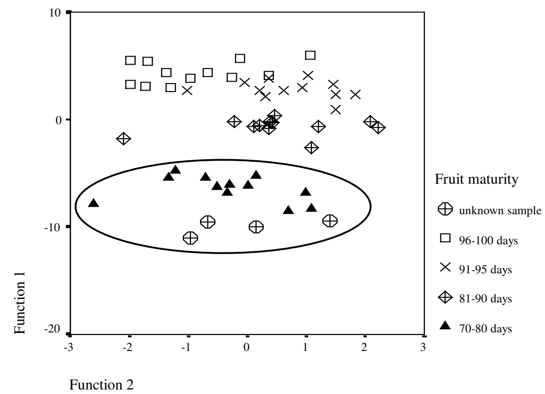

Fig.6. The construction of the classification model by CDA enable unknown maturity stage of jackfruit to be identified (with reference to Table 2)

The evolution with time of the ripeness process of jackfruit

The third phase of the experiment was designed as an attempt to monitor the whole "natural" ripeness process of the jackfruit when it is stored at room temperature. A detail study has been performed using jackfruit collected at the maturity stage of 82 days after anthesis (physiologically matured but not fit for consumption). Measurements were taken at a certain time interval and the result of the analysis is shown in Fig.7.

A dependance of the disposable sensor array data on jackfruit storage time can be observed. The principle component analysis of the jackfruit ripeness data matrix showed a characteristic development on the fruit quality dependant on storage time. The first and second principle component contributed a total variance of 86%. The plots can be divided into four parts. With incresing of time, an interesting observation can be made; the position of the samples (although partly of them are overlap) follows a path that extended from positive value to negative value of the second principle component and move right up heading to the positive region of the first principle component. The movement of the data points (36^{th} hour onwards) from positive region to the negative region of the second principle component axis can be an evidence that at this stage, the fruit has achieved its optimum level of ripeness and is ready to be eaten. Table 3 showed a side testing which was conducted with a group of human panel for physical observation of fruit. The reading of the sugar percentage from the extracted jackfruit juice was obtained using a refractometer. The results obtained supported the evidence of the disposable sensor strips that the optimum ripeness of jackfruit was achieved from the 36^{th} hour onwards.

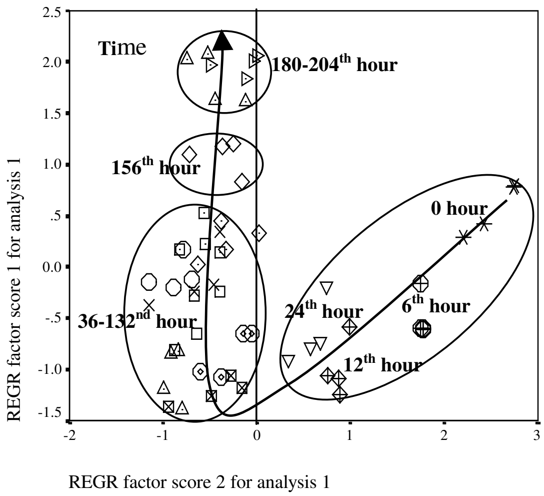

Fig.7. Score plot of a principle component analysis. First and second principle component correlated with the storage time

Table 3: Sugar percentage and physical observation of jackfruit pip for a certain time interval

Time interval (Hour)	Sugar %	Physical observation of jackfruit pip		
		Taste	Texture	Aroma
0	3.00	Tasteless	Hard	None
6	3.50	Tasteless	Hard	None
12	4.00	Tasteless	Hard	None
24	6.50	Tasteless	Partially hard	None
36	14.0	Sweet	Soft	Typical
42	14.0	Sweet	Soft	Typical
48	15.0	Sweet	Soft	Typical
60	15.0	Sweet	Soft	Typical
72	17.0	Sweet	Soft	Typical
84	18.0	Sweet	Soft	Typical
108	18.0	Sweet	Soft	Typical
132	18.0	Sweet	Soft	Typical
156	17.0	Sweet and sour	Overripe and watery	-
180	17.0	Sour	Overripe and watery	-
204	16.5	Sour	Too overripe and watery	-

In order to evaluate the sensitivity of the sensor array towards the taste of jackfruit, a calibration model for five basic taste was built (Please refer to Table 4 and Fig. 8). The CDA procedure generates a classification function, aiming at a rapid classification of unknown samples to groups.

Table 4: Category of taste based on training samples of 5 basic taste

Category	Group f2_grp
Sweet taste	1
Sour taste	2
Umami taste	3
Salty taste	4
Bitter taste	5

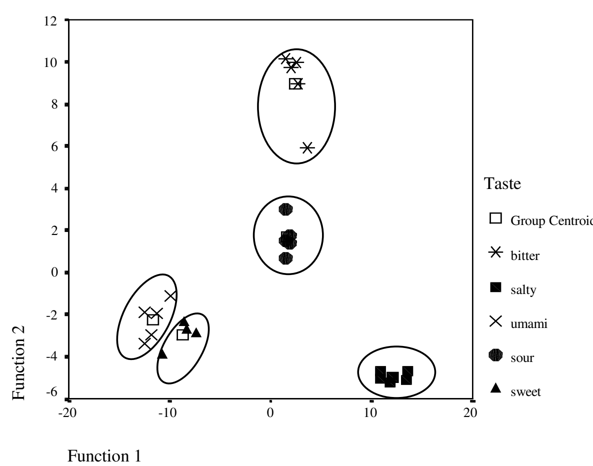

Fig. 8. Plot of CDA for five basic tatse

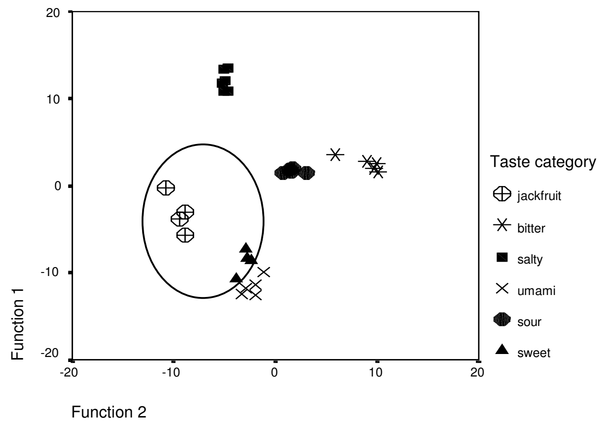

Fig.9. Identification of the "unknown" using disposable sensor strips. "Unknown" (36th hour) was correctly classified as sweet taste which corresponds to the remarks from human panel and percentage of sugar measurement. (with reference to Table 5)

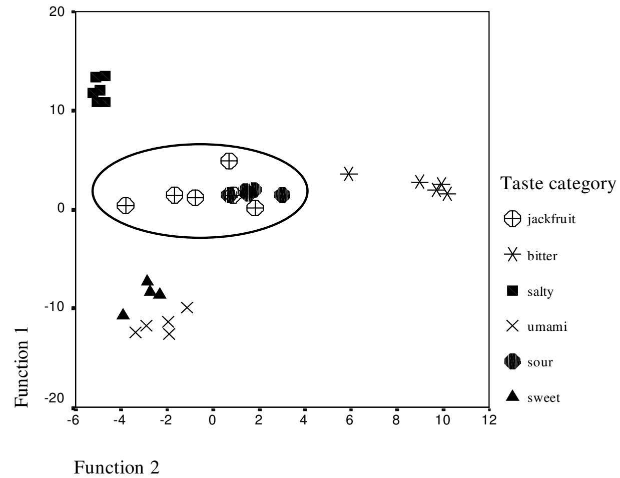

Fig.10. The construction of the classification model by CDA enables unknown taste of jackfruit (from the time interval of 156th hour onwards) to be identified (with reference to Table 5)

Table 5 : Prediction of jackfruit taste at selected interval of time using CDA

Unknown: Jackfruit	36th hour	36th hour	36th hour	36th hour	156th hour	156th hour	180th hour	180th hour	204th hour	204th hour
Predicted group	1	1	1	1	2	2	2	2	2	2

The evaluation of the jackfruit taste using disposable sensor strips was shown in Fig 9, 10 and Table 5. The taste of the jackfruit stored until the 36th hour was found correlated with the results obtained using refractometer and human sensory.

Sample at the 36th hour was sweet and from 156th hour onwards, the fruit turns sour. This can be an evidence that from the

36th hour and above, the fruit had achieved its optimum level of ripeness and in other words,it is ready to be eaten whereas sample at 156th hour onwards is no longer suitable for consumption as it is overly overipe.

4. Conclusion

The various preliminary tests conducted with the disposable sensor strips has lead to very promising results. Together with chemometric analysis, the sensor strip is able to predict an unknown sample on the basic of pattern recognition classification model. In the case of monitoring the "natural" ripening process of jackfruit when it is stored at room temperature, PCA is able to show the characteristic of the whole process which enables the optimum ripeness time of the fruit be determined. In conclusion, the disposable sensor strips incorporating chemometric analysis might become a challenging promise in the future field of analysis.

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Sample Availability: Available from the authors.