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Application of zNose™ for classification of enzymatically-macerated and steamed pumpkin using principal component analysis

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Abstract: High resolution olfactory images, called VaporPrints™, derived from the frequency of a surface acoustic wave (SAW) detector, are particularly useful to human because of their ability to recognize and differentiate visual images. In this study, the VaporPrint™ of fresh pumpkin (*Cucurbita moschata*) and different products of the pumpkin including steamed pumpkin and also pumpkin purees as affected by different enzymes (Pectinex® Ultra SP-L and Celluclast®; Novozyme, Denmark) were determined using an ultra-fast GC (zNose™) based on a SAW sensor. The zNose™ fingerprints served as a potential tool for qualitative and discriminative distinction of aroma between the different pumpkin products. Principal component analysis (PCA) was used to analyse the data. Based on the results, samples were categorized into three different groups. According to the score plot of PC 2 (second component) versus PC 1 (first component), aromas of enzymatically macerated pumpkin were close together. The PC 1 and PC 2 factors resulted in the model that describe the 82.9% of the total variance and seemed sufficient to define a good model.

Keywords: zNose™, vapor print™, pumpkin, principal component analysis, enzyme

Introduction

Flavor is an important food quality attribute and is composed of two components: aroma and taste. Several analytical methods have been used for flavor analysis. Instrumental methods such as gas chromatography (GC), gas chromatography with mass spectrometry (GC-MS), sniffing GC-MS and high performance liquid chromatography (HPLC), are regularly applied for quality evaluation (Berna et al., 2004). Sensory evaluation is another approach, but it has many limitations such as being time-consuming, high cost and has a high labor requirement (Sohn et al., 2003; Oh et al., 2008a). Moreover, the above-mentioned analytical techniques are time-consuming, expensive, and require much sample preparation and skilled personnel to operate the equipment and interpret the results (Berna et al., 2004; Oh et al., 2008b). Therefore, development of a rapid, simple and low cost analytical method with clear relationship between their sensory impacts is one of the most important subjects in aroma analysis (Gan et al., 2005; Oh et al., 2008a).

Electronic nose has been successfully applied in food and beverage samples such as beer, whisky, bottled water (Staples, 2000), honey (Lammertyn et al., 2003), vegetable oils (Gan et al., 2005), tomato (Berna et al., 2005; Gómez et al., 2006), wine (Lozano et al., 2007), lilac blossom and *Thymus* species (Oh et al., 2008a and b), musk lime seed oil (Manaf et al., 2008), pear (Zhang et al., 2008), apricot (Defilippi et al., 2008) and mango (Li et al., 2009) for rapid aroma profiling. The zNose™ provides a recognizable visual image of vapor mixtures (fragrances) containing hundreds of different chemical species in 10 seconds or near real time (Staples, 2000). An electronic nose is able to simulate a sensor array containing hundreds of orthogonal (non overlapping) sensors and chemical analysis of any odor is accomplished in 10 seconds by a very fast separation of chemicals in sampled vapors with different sensitivities of part per billion for volatile compounds and part per trillion for semi-volatile compounds (Staples, 2000).

Since the raw data of the electronic nose is a fingerprint, pattern recognition techniques can be used to analyse the raw response and discriminate of materials (Sohn et al., 2003; Oh et al., 2008b). Principal component analysis (PCA) is a powerful, linear, unsupervised and non parametric pattern recognition technique which has been used by many researchers for analyzing, classifying and reducing the number of dimensions in data set without any loss of information (Gómez et al., 2006; Lozano et al., 2007). Briefly, PCA is a useful multivariate statistical

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method for classification of different products such as classification of tomato volatile profile at different stages of ripening (Gómez et al., 2006). It has been used for discrimination of various *thymus* species (Oh et al., 2008b). Discrimination capability with separate cluster, resulted from PCA score plot reported for different white wine and vegetable oils samples (Lozano et al., 2007; Gan et al., 2005).

PCA is a projection method, and dimension reduction of the data can be achieved using a smaller number of principal components compared to the original variables used in analysis of a sample. Execution of PCA results in the construction of a loading plot and it contains information about the variables, by removing small ones, which are consider unimportant for classification (Gan et al., 2005). So the information carried by the original variables is projected onto a smaller number of underlying variables called principal components, their values are the scores. The first principal component (PC 1) covers as much of the variation in the data as possible. The second principal component (PC 2) is orthogonal to the first and covers as much of the remaining variation as possible. Thus, by plotting the principal components, one can view the interrelationships between different variables, and detect and interpret sample patterns, groupings, similarities or differences.

Pumpkin is a member of the Cucurbitaceae family (Gonçalve et al., 2007) and has received considerable attention in recent years because of the nutritional and health protective values such as anti-tumor, anti-bacterial, anti-hypertensive (Caili et al., 2007). Pumpkin puree is usually thermally processed for the manufacture of many products including jam, jelly, sweets, beverage, juice blends, nectar, ice cream, preserves and others (Dutta et al., 2006), and also, it can be used directly. In food processing, there are many approaches to have a desirable product and in making puree, enzyme is another alternative to reduce the severity and adverse effect of heat. Retention of flavor during processing is a major challenge in food industry (Dutta et al., 2006), and the success of a pureed product is highly affected by the retention of the original flavor.

The objectives of this study were to evaluate the potential of zNose™ as an aroma fingerprinting tool for determination of the VaporPrint™ and discrimination of different pumpkin products as affected by different enzymes and steaming, and also application of the zNose™ for classification of pumpkin and pumpkin products using PCA.

Materials and Methods

Materials

Pumpkins (*Cucurbita moschata*) of commercial maturity, with the same size and skin color, were purchased from a local market in Serdang, Selangor, Malaysia, and kept at low temperature (10-15°C) prior to conducting the experiments. Pectinex® Ultra SP-L (pectolytic enzyme preparation) and Celluclast® 1.5 L (cellulolytic enzyme preparation) were purchased from Novozyme, Denmark.

Proximate analysis

Official methods of AOAC (1984) were used for determination of proximate analysis (ash, protein, fat, fiber and moisture content) of pumpkin.

Sample preparation

Pumpkins (a total of approximately 2 kg) were peeled, deseeded and the flesh chopped into small cubes (0.5×0.5×0.5 cm). Fresh pumpkin cubes (10 g) were used for zNose analysis without further storage. For the preparation of enzymatically-macerated pumpkin, Pectinex® Ultra SP-L at concentrations of 2.5%, 3.5%, 4.5% and 5.5% were added to 100 g of pumpkin cubes, each in separate beakers, and mixed thoroughly using a spatula followed by incubation at 50 °C in a water bath with agitation rate of 100 rpm for 1, 1.5, 2, and 2.5 hours, respectively. After the incubation period was ended, each sample was mashed manually using spatula to make the homogenous puree. For each enzyme concentration, 10 g of puree was used for zNose™ analysis without further storage. In another experiment, Celluclast® 1.5 L at concentrations of 0.5% and 1% with combination of Pectinex® Ultra SP-L (5.5%, 4.5%, 3.5% and 2.5%) (Table 1) were added to pumpkin cubes (100 g each). Preparation of enzymatically-macerated pumpkin using combination of Pectinex® Ultra SP-L and Celluclast® 1.5 L was carried out using the same procedure as described above.

The effect of steaming was examined by placing pumpkin cubes (100 g) in the basket over 95ºC water bath for 15, 30 and 45 minutes. The steamed pumpkin (10 g) was then transferred into a special bottle for zNose analysis as described below. zNose analysis was performed for each sample several times and the mean of triplicate stable measurements per sample was used for PCA analysis.

zNose analysis

The analysis was carried out using an ultra-fast GC (zNose™ 7100 Vapor Analysis System, Electronic Sensor Technology, USA) equipped with surface acoustic wave (SAW) sensor. Purified helium was

Table 1. Coded value of different raw and processed pumpkins

No.	Definition	No.	Definition
1	Fresh Pumpkin	9	Pumpkin Pu. (P 3.5%) (C 1%) (2h)
2	Pumpkin Pu. (P 2.5%) (2.5h)	10	Pumpkin Pu. (P 4.5%) (C 0.5%) (1.5h)
3	Pumpkin Pu. (P 3.5%) (2h)	11	Pumpkin Pu. (P 4.5%) (C 1%) (1.5h)
4	Pumpkin Pu. (P 4.5%) (1.5h)	12	Pumpkin Pu. (P 5.5%) (C 0.5%) (1h)
5	Pumpkin Pu. (P 5.5%) (1h)	13	Pumpkin Pu. (P 5.5%) (C 1%) (1h)
6	Pumpkin Pu. (P 2.5%) (C 0.5%) (2.5h)	14	Steamed pumpkin (95°C, 15 min)
7	Pumpkin Pu. (P 2.5%) (C 1%) (2.5h)	15	Steamed pumpkin (95°C,30min)
8	Pumpkin Pu. (P 3.5%) (C 0.5%) (2h)	16	Steamed pumpkin (95°C,45 min)

p= Pectinex® Ultra SP-L , pu=puree, C= Celluclast® 1.5 L, h=hour

Table 2. Proximate analysis of pumpkin

Analysis	Measured* (%) (wet base)	USA **** Standard (%)	Malaysian **** Standard (%)
Moisture	88.64±0.02	91.6	84.40
Fat	0.03±0.01	0.10	0.1
Protein	0.46±0.06	1.00	0.90
Ash	0.87±0.01	0.80	0.40
Crude Fiber	0.89±0.07	0.50**	0.30
Carbohydrate	9.05±0.16	6.5	13.90

*Each measured value represents the mean of triplicate samples ± standard deviation.** total dietary fiber
(****United State Department of Agriculture, 2008 and Malaysian Foods Composition Database, 2008)

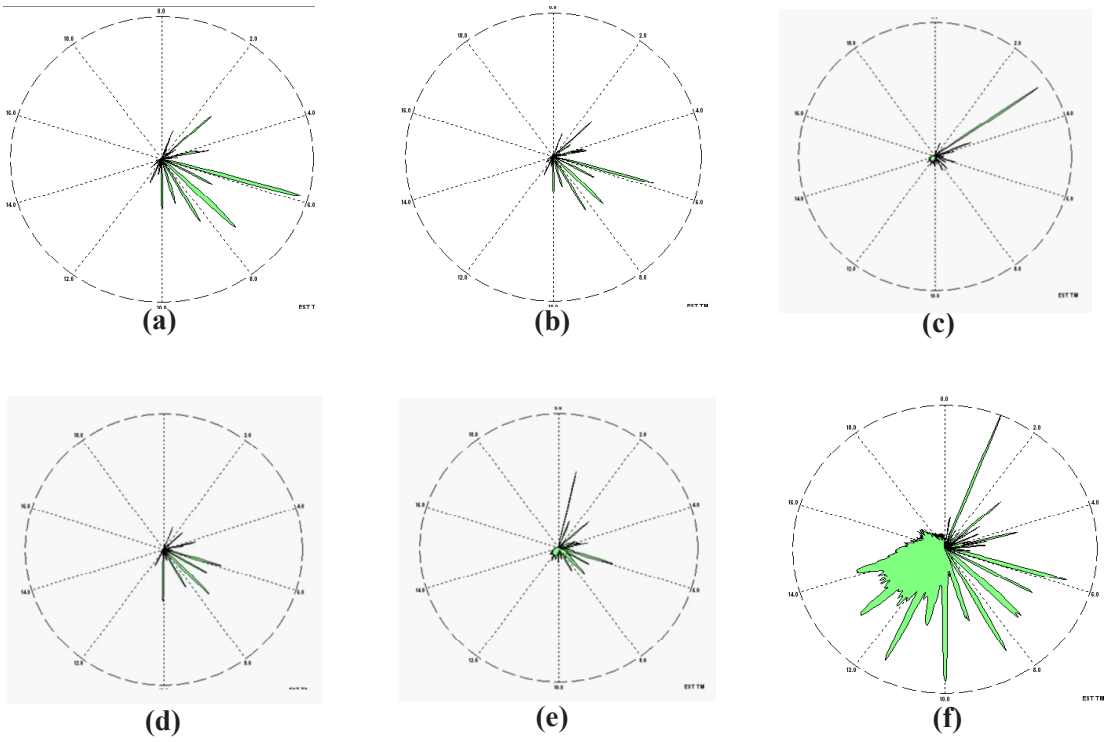

Figure 1. VaporPrints™ of fresh pumpkin (a), pumpkin puree using Pectinex® Ultra SP-L 2.5% (b), macerated pumpkin using Pectinex® Ultra SP-L 2.5% & Celluclast (1%) (c), steamed pumpkin after 15 min (d), 30 min (e) and 45 min (f)

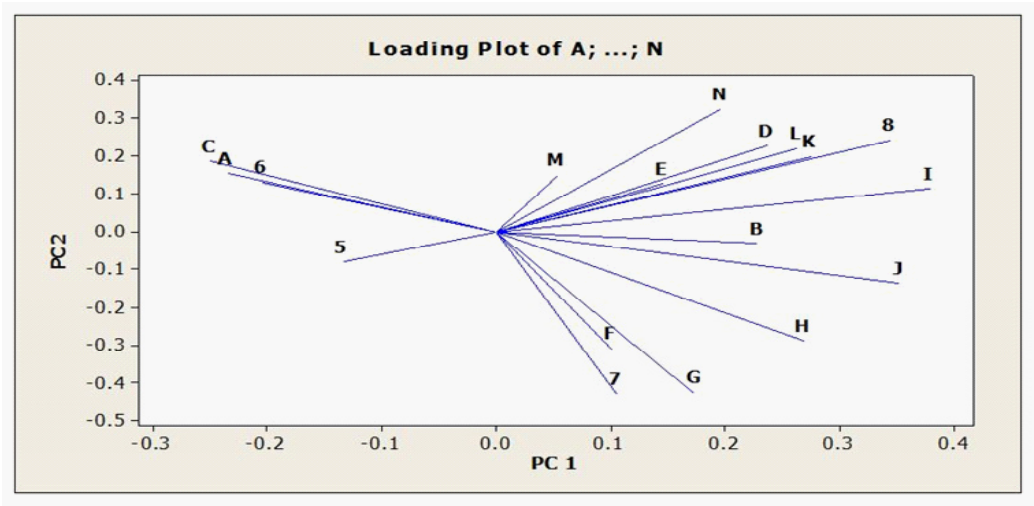

Figure 2. Loading plot of all variables (A-N)

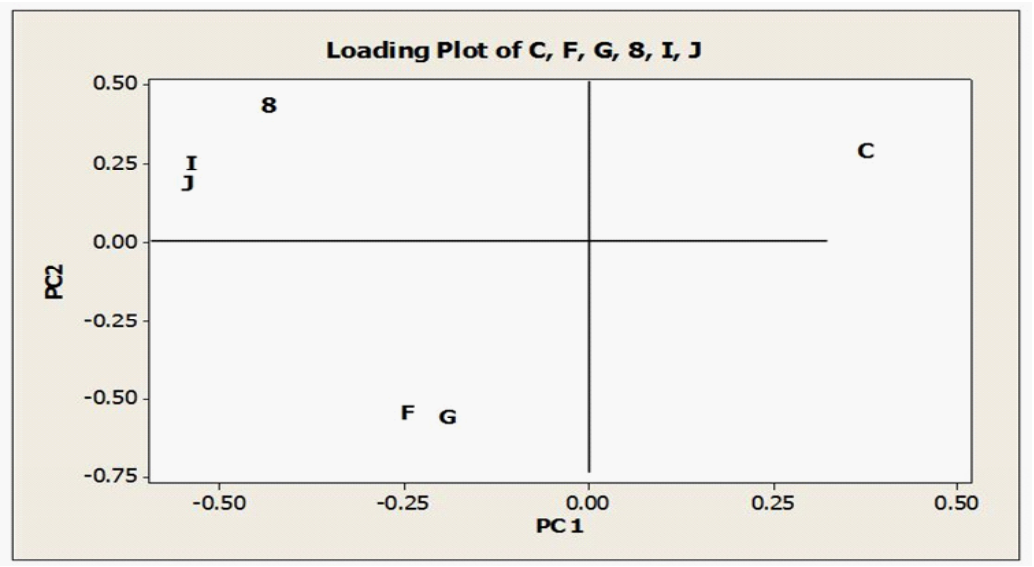

Figure 3. Loading plot of 6 important variables (C, G, F, 8, I, J)

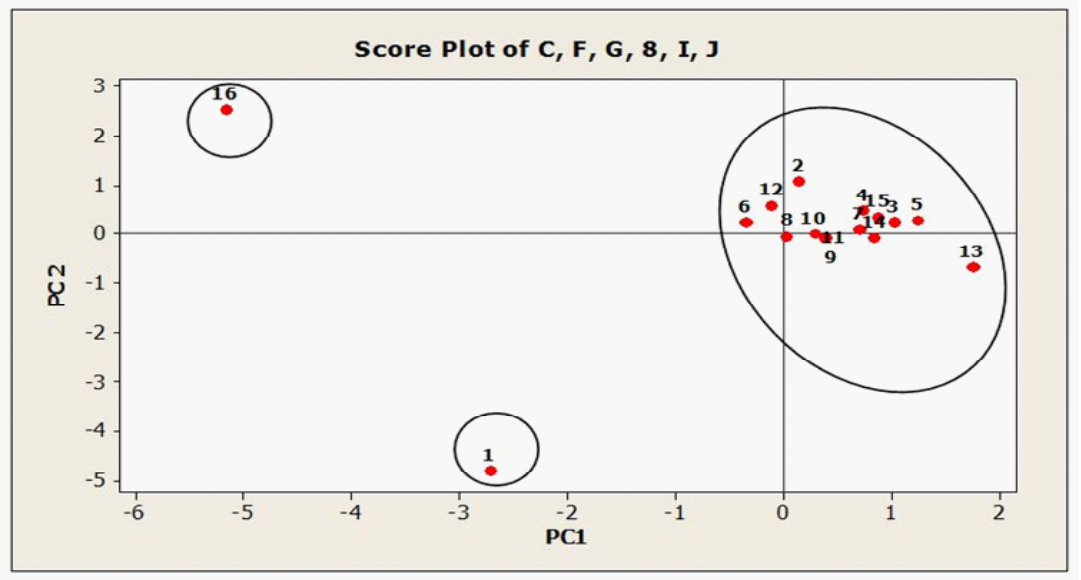

Figure 4. Score plot of raw and processed pumpkin (16 samples)

used to provide a flow rate of 3.0 cm^3. Each sample including fresh pumpkin used as the control (Table 1) was weighed (10 g) into a 40 ml bottle (98 mm height and 28 mm outer diameter) and sealed with a screw cap containing a septum. After a headspace generation of 5 min in a water bath at 60°C, the sample's vapor was introduced into the zNose using the needle as injection tool which attached to the zNose and passed through the septum of the bottle. The sampling time was 10 s, and the column temperature was programmed from 40 to 180°C, at a rate of 10°C /s, while the sensor baking temperature was 190°C. At least one air blank using an empty bottle was run between each measurement, such that baseline peaks were all under 200 Counts before resuming sample runs. Each sample was repeated many times and the mean values of three stable measurements were used for statistical analysis. PCA is then used in the construction of a loading plot containing the information about all variables (18). By removing small variables, which are consider unimportant for classification, the 6 important variables including C, G, F, 8, I, J which shows the highest total variance in PCA analysis were achieved.

Statistical analysis

Electronic nose data were analyzed using the principal component analysis (PCA) with Minitab software, release 14 (Minitab Inc., 2003).

Results and Discussions

Proximate analysis

Table 2 shows the proximate analysis of pumpkin and compared with standards of U.S.A and Malaysia. From the table it can be concluded that most of the data were in the range of the standards (Malaysian Foods Composition Database, 2008 and United State Department of Agriculture, 2008). The differences between these values may be due to the genetic variation, degree of maturity, conditions of soil, use and type of fertilizer, climate, availability of water, light (length of day and intensity) and post harvest handling.

zNose analysis

The VaporPrints™ of fresh pumpkin, pumpkin puree obtained using 2.5% of Pectinex® Ultra SP-L and macerated pumpkin using combination of Pectinex® Ultra SP-L (2.5%) and Celluclast® 1.5 L (1%) and also steamed pumpkin are shown in Figure 1(a-f). From Figure 1, it can be observed that the aromas of fresh pumpkin, enzymatically-macerated pumpkin using Pectinex® Ultra SP-L alone or in combination with Celluclast® 1.5 L were similar but were completely different from steamed pumpkin. The similarity indicated that in making puree, enzyme caused little change in aroma, while steaming, depend on its severity, may cause dramatic changes on the aroma.

The loading plots of all variables (18) and important ones (6) are given in Figures 2 and 3. Based on PCA analysis, Samples were classified in three different groups with six important compounds including C, G, F, 8, I, J while the rest are considered unimportant for classification. The PC 1 and PC 2 factors resulted in the model of PCA analysis that described the 82.9% of the total variance (47.3% and 35.6% for PC 1 and PC 2, respectively) seemed sufficient to define a good model for categorizing the samples into different groups. Based on Figure 4, the score plot of PC 2 (second component) versus PC 1 (first component), aromas of purees made from Pectinex® Ultra SP-L alone, and also a combination of Pectinex® Ultra SP-L with Celluclast® were very similar, but the aroma of steamed pumpkin after 45 minutes was very different from fresh pumpkin, steamed pumpkin after 15 and 30 minutes and enzymatically macerated pumpkin. The aromas of purees made from the combination of Pectinex® Ultra SP-L (3.5%) and (4.5%) with Celluclast® (1%) and (0.5 and 1%), respectively, which showed overlap, were very similar. For those samples that overlap on the PCA, there was no significant difference in the amount of the six influencing variables (C, G, F, 8, I, J). These results are in agreement with the results of VaporPrint™ in Figure 1. The similarity again indicated that enzyme caused little change in original aroma of pumpkin when it is turned into puree, unlike steaming which can cause a severe change to the original pumpkin aroma.

Results of this study revealed that zNose™ as an aroma fingerprinting tool has the potential for determination of the VaporPrint™ and discrimination of fresh pumpkin, steamed pumpkin and enzymatically-macerated pumpkins, but could not discriminate aromas of different enzymatically-macerated pumpkins except for the intensity of aroma based on peak heights. As can be seen in Figure 1, the finger prints of aroma compounds were largely different from those present in fresh pumpkin. The results of this study also showed that steaming for 15 and 30 minutes may retain some of the original aroma, however long steaming time (45 min) resulted very different aromas in the product compare to fresh one (Figure 4, No.16). Based on the study, it is possible to predict the over-treatment involving heating of pumpkin when the VaporPrint™ differs greatly from that of fresh pumpkin. This observation

should be used by a processer to either balance the time of steaming with aroma retention or by selecting an alternative method for puree processing such as using an appropriate enzyme for maceration.

Results of this study showed that zNose™ has been used as a useful analytical method for classification and discrimination of pumpkin and pumpkin products. Similar result was reported for different honey varieties (Lammertyn et al., 2003), vegetable oils (Gan et al., 2005) and different stages of maturity in apricots (Defilippi et al., 2008). Electronic nose data obtained from tomato, wine, pear and mango headspace allowed quality prediction (Gómez et al., 2006; Lozano et al., 2007; Zhang et al., 2008; Li et al., 2009).

Conclusion

The use of the zNose™ offered a method for discrimination of different pumpkin products. Another positive benefit of the electronic nose is that it does not need any sample pre-treatment or chemicals for analysis. In addition speed of the electronic nose method for the discrimination made it ideal for quality control purposes. This technique does not require skilled operators and is suitable for on-line application also introduce fast, non-destructive and cost-effective method for flavour analysis. The Vaporprint™ can be used by processes to monitor over-heating based on radical changes that occur to the treated sample when compared to fresh sample.

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