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The Use of Principal Component and Cluster Analysis to Differentiate Banana Peel Flours Based on Their Starch and Dietary Fibre Components

[1]Saifullah Ramli, [2]Noryati Ismail, [3]Abbas Fadhl Mubarek Alkarkhi and [2]Azhar Mat Easa[*]

[1]Fakultas Teknik, Jurusan Teknik Kimia, Universitas Syiah Kuala, Banda Aceh, Indonesia
[2]Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia
[3]Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia

Abstrak: Tepung kulit pisang yang disediakan dari buah pisang *Cavendish* dan *Dream* (berangan) yang hijau dan masak telah dinilai jumlah kanji (TS), kanji hadam (DS), kanji rintang hadam (RS), jumlah serat dietari (TDF), serat dietari larut (SDF) dan serat dietari tak larut (IDF). Analisa komponen principal (PCA) menunjukkan hanya satu komponen yang bertanggungjawab untuk 93.74% daripada jumlah varian yang terdapat dalam komponen kanji dan serat dietari yang membezakan tepung pisang masak dan hijau. Analisa kluster (CA) dari data yang sama memperoleh dua kluster signifikan (pisang hijau dan masak) yang menunjukkan perbezaan perilaku mengikut tahap kematangan. Kesimpulannya, komponen kanji dan serat dietari boleh digunakan untuk mendiskriminasi antara tepung yang disediakan dari kulit buah yang berbeza tahap kematangannya. Keputusan juga menunjukkan potensi tepung kulit pisang hijau dan masak sebagai bahan berfungsi dalam makanan.

Kata kunci: Tepung Kulit Pisang, Pembezaan Statistikal, Kanji, Serat Dietari

Abstract: Banana peel flour (BPF) prepared from green or ripe Cavendish and Dream banana fruits were assessed for their total starch (TS), digestible starch (DS), resistant starch (RS), total dietary fibre (TDF), soluble dietary fibre (SDF) and insoluble dietary fibre (IDF). Principal component analysis (PCA) identified that only 1 component was responsible for 93.74% of the total variance in the starch and dietary fibre components that differentiated ripe and green banana flours. Cluster analysis (CA) applied to similar data obtained two statistically significant clusters (green and ripe bananas) to indicate difference in behaviours according to the stages of ripeness based on starch and dietary fibre components. We concluded that the starch and dietary fibre components could be used to discriminate between flours prepared from peels obtained from fruits of different ripeness. The results were also suggestive of the potential of green and ripe BPF as functional ingredients in food.

Keywords: Banana Peel Flour, Statistical Differentiation, Starch, Dietary Fibre

[*]Corresponding author: azhar@usm.my

INTRODUCTION

Banana peels, representing 40% of the total weight of fresh bananas (Tchobanoglous *et al*. 1993), have been underutilised. One way to utilise this waste is by converting the peel into banana peel flour (BPF) (Ranzani *et al*. 1996). This product can be exploited further into new products that have a standardised composition and functional properties for various industrial and domestic uses (Bardiya *et al.* 1996; Tewari *et al*. 1986; Annadurai *et al*. 2002; Essien *et al*. 2005). Of particular interest, is the finding that banana peel extract contains higher levels of antioxidant compounds than the pulp (Someya *et al*. 2002), promising a wider range of application of the peels in food and nutraceuticals. The potential applications of BPF depend on its chemical composition, and the important functional components of banana peel are the resistant starch (RS) and dietary fibre (Emaga *et al*. 2007). RS has attracted interest because of its positive effects in the human colon and implications for health (Langkilde *et al.* 2002). Dietary fibre mainly consists of soluble and insoluble fibre fractions (Thebaudin *et al*. 1997). Soluble fibres such as pectin and β-glucan of oats are well known to lower serum cholesterol and to help reduce the risk of colon cancer, whilst insoluble fibre such as cellulose has been shown to be beneficial for intestinal regulation and increasing the stool volume (Schneeman 1987).

Emaga *et al*. (2008) investigated the effects of the stage of ripeness on the dietary fibre components and pectin levels in banana peels. The chemical composition of banana peel, as influenced by the maturation stage and the variety, has also been studied (Emaga *et al*. 2007). Since bananas are consumed at the green, average ripe and ripe stages (Emaga *et al*. 2007), the amount of fruit peel waste is expected to increase as processing industries that utilise green and ripe bananas develop.

In its original form, it is relatively easy to differentiate between the peel of ripe and green bananas, and with experience, it is also possible to differentiate between varieties (i.e., from the skin colour, appearance, size and other characteristics). Once the peel is processed into flour, identifying the origin and stage of ripeness from the peel becomes a challenge. As far as we know, no study has been conducted on the use of the starch and dietary fibre components of BPF to discriminate flours from different varieties of bananas or from their stage of ripeness. It may be useful to devise methods that can discriminate between BPF based on their chemical composition. Statistical techniques that can be applied to this task include multivariate analysis of variance (MANOVA), cluster analysis and discriminant analysis (Forster *et al*. 2002; Rivero *et al*. 2003; Suárez *et al*. 2007).

Two of the most popular commercial varieties of banana in the Asian market are the Cavendish (*Musa acuminata* L. cv. *cavendishii*) and Dream (*Musa acuminata colla* AAA cv. *Berangan*) bananas. Discrimination of banana flour products is important, as the Cavendish banana is more expensive than the Dream banana. The objective of this study is to differentiate between the flours prepared from the peels of ripe and unripe fruits using the starch and dietary fibre components as discriminating parameters.

MATERIALS AND METHODS

Preparation of the Banana Peel Flour

Two common banana varieties, Cavendish (*M. acuminata* L. cv. *cavendishii*) and Dream (*M. acuminata colla* AAA cv. *Berangan*) bananas were purchased from 12 markets around Pulau Pinang, Malaysia. A total of 222–302 green (stage 1 of ripening: all green) and ripe (stage 6 of ripening: yellow with green tip) bananas of each variety were obtained from each market. The fruits were washed with tap water and separated into pulp and peel. To reduce enzymatic browning, the peels were then dipped in a 0.5% (w/v) citric acid solution for 10 min, drained and dried in an oven (AFOS Mini Kiln, Hull, England at 60°C overnight). The dried peels were ground in a Retsch Mill (Retsch GmbH & Co., AS200, Haan, Germany) and passed through a 40-mesh screen to obtain BPF. The yield of flour was calculated by dividing the weight of flour produced by the weight of fresh banana used, and the results are given in the units: g/kg (g of flour/kg of banana). The four types of BPF produced were: ripe Cavendish BPF (CRPe), ripe Dream BPF (DRPe), green Cavendish BPF (CGPe) and green Dream BPF (DGPe). All BPFs were stored in airtight plastic packs in cold storage (15±2°C) until analysed.

Proximate Composition

The proximate analyses of the banana pulp (BP) and peel flour (PF) were performed in triplicate following AOAC (1990) procedures and included the following: moisture by vacuum oven (method 934.06), protein by Kjeldahl nitrogen (method 920.152), and ash by direct analysis (method 940.26). The percentage of crude protein was estimated by multiplying the total nitrogen content by 6.25 (AOAC 1990). The Bligh and Dyer method (Bligh & Dryer 1959) was used to determine the lipid content. The total carbohydrates were calculated by subtracting the total percent values of the other measurements from 100. The amount of crude fibre was analysed by ceramic fibre (method 962.09E). The proximate and crude fibre analyses were expressed as grams per 100 g of flour.

Analysis of Dietary Fibre

Total dietary fibre (TDF) was measured using the 985.29 AOAC enzymatic-gravimetric method and the method of Prosky *et al*. (1988), using a TDF assay kit (Bioquant TDF-100 kit, Merck, Germany). This method is based on the isolation of dietary fibre by enzymatic digestion of the other constituents. The residue was measured gravimetrically.

Samples were suspended in MES-TRIS buffer and digested sequentially with heat-stable α-amylase at 95°C–100°C, protease at 60°C and amyloglucosidase at 60°C. The enzyme digestates were filtered through tared fritted glass crucibles. The crucibles containing insoluble dietary fibre (IDF) were rinsed with dilute alcohol and acetone, and dried overnight in a 105°C oven. The filtrates and washings were mixed with 4 volumes of 95% ethanol to precipitate materials that were soluble in the digestates. After 1 h, the precipitates were filtered through tared fritted glass crucibles. One of each set of duplicate insoluble fibre residue samples and soluble fibre residues was washed in a

muffle furnace at 525°C for 5 h. The second set of residues was used for protein determination (as Kjeldahl nitrogen 6.25). The dietary fibre values in the sample and blank fractions were calculated as the soluble dietary fibre (SDF) or IDF residues (% original sample weight) minus the % ash and the % crude protein measured in the residues. The TDF was calculated as the sum of SDF and IDF.

Analysis of the Total Starch and Resistant Starch

The total starch (TS) was determined using the method of Goñi *et al.* (1997). In brief, 50 mg of sample was dispersed in 2 M KOH (30 min) to disperse the starch fractions. The samples were then incubated with amyloglucosidase (Sigma A9913, 60°C, 45 min, pH 4.75) and the amount of glucose present was determined using the glucose oxidase assay GOD-POD (RANDOX). The TS was calculated as the glucose released (mg) x 0.9.

RS was measured by the method of Goñi *et al.* (1996). In brief, the protein was removed by incubation with pepsin (P-7012, 2500–3500 units/mg protein, Sigma-Aldrich) at 40°C and pH 1.5 for 1 h. Then, the digestible starch (DS) was removed using another incubation, this time with α-amylase (A-3176, 10–30 units/mg solid, Sigma Chemical Co.) at 37°C and pH 6.9 for 16 h. The residue was treated with 2 M KOH and incubated with amyloglucosidase (A-7255, 5000 units/g solid, Sigma Chemical Co.) at 60°C and pH 4.75 for 45 min). The amount of glucose present was determined using the glucose oxidase-peroxidase assay GOD-POD (RANDOX). The amount of RS present was calculated as glucose (mg) x 0.9. The amount of DS present was calculated as the difference between the TS and RS.

Statistical Analysis

Principal components analysis (PCA) and cluster analysis (CA) were used to analyse the data obtained from the peels from two different varieties of banana at two degrees of ripeness (green and ripe). Three samples were selected from each variety and degree of ripeness. In total, 12 samples were tested. Furthermore, each sample was measured in triplicate and the average result was recorded. Each sample consisted of 70 bananas that had been processed to banana peel flour. The total number of bananas used in this study was 840.

Principal component analysis (PCA)

PCA is designed to transform original variables into new uncorrelated variables called components, which are linear combinations of the original variables. PCA is a data reduction technique that is used to determine the number of significant variables. PCA is used to explain the variances observed in the data and to understand the relationship between the different parameters (Bryan 1991; Alvin 2002).

Cluster analysis (CA)

The starch and dietary fibre components of banana flour were analysed with CA using a linkage method. In the linkage method, the distance (similarity) between two clusters, A and B is defined as the minimum distance between a point in A and a point in B:

$$D(A,B) = \min \; d\; y_i, y_i \;, \text{for } y_i \text{ in A and } y_i \text{ in B} \quad (1)$$

where $d(y_i, y_i)$, is the Euclidean distance.

At each step, the distance was found for every pair of clusters, and the two clusters with the smallest distance (i.e., the largest similarity) were merged. After two clusters were merged, the procedure was repeated for the next step; the distances between all pairs of clusters were recalculated, and the pair with the minimum distance is merged into a single cluster. The results of a hierarchical clustering procedure can be displayed graphically using a dendrogram, which shows all the steps in the hierarchical procedure (Johnson & Wichem 2002; Alvin 2002).

RESULTS AND DISCUSSION

Proximate Composition, Starch and Dietary Fibre Components

The proximate composition of banana flour is shown in Table 1. The moisture content of the flours ranged from 9.21% to 11.20% and was higher than that reported by Rodriguez-Ambriz *et al*. (2008) for unripe BPF. This difference could be due to differences in the varieties and processing parameters used. The ash contents ranged from 17.13% to 19.17%, and these were also higher than those reported by Emaga *et al.* (2007) in unripe BPF. The protein levels of the flours (7.25%–10.04%) were similar to those reported previously (6.3%–11.2%) for unripe BPF (Emaga *et al*. 2007). It is important to note that the protein content was higher in the ripe PF than in the green PF. The crude fat contents of the flours were moderate (4.81%–5.96%), and the carbohydrate contents of the flours ranged from 54.01% to 60.81%. The higher carbohydrate content of green BPF could be related to its relatively low moisture content. The high carbohydrate content is expected, as banana is known to contain sugars, starch and dietary fibres (Rodriguez-Ambriz *et al*. 2008).

Table 1: The proximate composition (%) (mean and standard deviation) of the PF that were prepared from bananas of different varieties and stages of ripeness.

Parameter	Types of BPF			
	CGPe	CRPe	DGPe	DRPe
Moisture	9.45 ± 0.002	11.20 ± 0.002	9.21 ± 0.04	10.88 ± 0.004
Ash	17.13 ± 0.001	18.87 ± 0.003	17.92 ± 0.01	19.17 ± 0.001
Fat	5 ± 0.002	5.96 ± 0.001	4.81 ± 0.001	5.32 ± 0.001
Protein	7.09 ± 0.003	10.04 ± 0.009	7.25 ± 0.01	9.14 ± 0.01
Carbohydrate	60.52 ± 0.001	54.01 ± 0.01	60.81 ± 0.03	55.49 ± 0.01
Crude fibre	10.46 ± 0.003	11.09 ± 0.002	10.02.05 ± 0.001	10.89 ± 0.01

Notes: CGPe, Cavendish green; CRPe, Cavendish ripe; DGPe, Dream green; DRPe, Dream ripe

The mean values and standard deviations for the starch and dietary fibre components of each of the samples are shown in Table 2. The TS of green BPF were ~3.5–fold higher than that of the ripe varieties. This is expected, as the starch content is known to decrease from stage 1 to stage 7 of ripeness (Emaga *et al*. 2007). The mean values for TS were 12.31% and 11.48% for CGPe and DGPe respectively, and these were lower than those reported by Rodriguez-Ambriz *et al*. (2008) for unripe banana flour (76.8%). The TS of the green or ripe PF were comparable with the starch content of BPF prepared from various varieties of banana in Cameroon (0.1%–39.3%, Emaga *et al*. 2007). These differences could be due to differences in the varieties and processing parameters used. The average values for the RS and DS ranged from 2.30% to 8.57% and from 1.10% to 3.57% respectively, and these were higher in the green than in the ripe flours. RS has attracted interest because of its positive effects in the human colon and its implications for health (Langkilde *et al*. 2002). A high RS content has been reported in green banana flour (Rodriguez-Ambriz *et al.* 2008). However, this study also revealed the presence of RS in green and ripe BPF.

Table 2: Mean (%) and standard deviation of the dietary fibre and starch content components of BPF.

Parameter[a]	Types of BPF			
	CGPe	CRPe	DGPe	DRPe
IDF	37.01 ± 0.34	43.09 ± 0.34	36.16 ± 0.002	39.79 ± 0.34
SDF	6.66 ± 0.001	7.75 ± 0.0001	6.50 ± 0.001	7.25 ± 0.001
TDF	43.68 ± 0.34	50.84 ± 0.34	42.65 ± 0.003	47.04 ± 0.34
RS	8.57 ± 0.01	2.58 ± 0.001	8.20 ± 0.003	2.30 ± 0.003
DS	3.57 ± 0.01	1.28 ± 0.0031	3.28 ± 0.003	1.10 ± 0.005
TS	12.31 ± 0.01	3.86 ± 0.002	11.48 ± 0.004	3.40 ± 0.002

Notes: [a] IDF, insoluble dietary fibre; SDF, soluble dietary fibre; TDF, total dietary fiber; RS, resistant starch; DS, digestible starch; TS, total starch.
CGPe, Cavendish green; CRPe, Cavendish ripe; DGPe, Dream green; DRPe, Dream ripe.

Dietary fibre consists mainly of soluble (pectin, gums, etc.) and insoluble (cellulose, lignin, hemicellulose, etc.) fibre fractions (Thebaudin *et al*. 1997). Soluble fibres are known to lower serum cholesterol and to help in reducing the risk of colon cancer, and the consumption of insoluble fibre has been shown to be beneficial for intestinal regulation and increasing the stool volume (Schneeman 1987). The amount of dietary fibre in the BPF samples is shown in Table 2. It is evident that BPF contains a substantial amount of dietary fibre (Emaga *et al*. 2007). The mean values for TDF and IDF was higher in ripe BPF than in the green flours in both varieties. IDF was the dominant fibre fraction in all samples, and the IDF contents of the flours were in the following order: CRPe > DRPe > CGPe > DGPe. As CRPe had the highest IDF content, it suggests that ripe Cavendish BPF offers the potential to deliver intestinal health functions as a commercially viable product. The lower TDF content of green BPF might be related to its high residual starch content. The average value for the SDF in all

samples ranged from 6.50% to 7.75% and there were no appreciable differences between the samples. The values of the SDF obtained in all samples were comparable to those reported in BPF by Emaga *et al*. (2007).

From the descriptive statistics that are presented in Table 2, it can be noted that green banana flour contains higher levels of RS, DS, and TS than does ripe banana flour. However, the levels of IDF and TDF in ripe banana flour were higher than those found in green banana flour and the SDF content was similar among all the samples.

Principal Component Analysis (PCA)

PCA was applied on the data set (using six variables) to identify the sources of variation between the different varieties and degrees of ripeness. PCA yielded only 1 component that had an Eigen value of 5.63, and this explained 93.74% of the total variance in the data set. We performed PCA on the correlation matrix between the different parameters. The results from the PCA analysis are shown in Table 3.

Table 3: The extracted values of the PCA parameters for selected parameters.

Component	Initial Eigen values			Extraction sums of squared loadings		
	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
1	5.63	93.74	93.74	5.63	93.74	93.74

Note: Extraction method: PCA

The loadings for the component yielded from PCA are given in equation 2.

$$Z = 0.97RS – 0.96IDF – 0.96TDF + 0.97TS + 0.96DS – 0.98SDF \quad (2)$$

Equation 2 accounted for 93.74% of the total variance and the Z value was positively correlated with RS, TS and DS and negatively correlated with IDF, TDF, and SDF. This component represents the average of all selected parameters, as all of the parameters contributed highly to explain the variation in the data between the different varieties and degrees of ripeness.

The relationship between the component scores and the different varieties was studied to understand the behaviour of selected parameters in different varieties. Figure 1 represents the scores for different varieties (CGPe, CRPe, DGPe, and DRPe). It can easily be seen that selected parameters exhibited different behaviour in the green and ripe BPF. The positive contribution in green BPF is due to the high content of RS, DS, and TS, whereas the negative contribution in ripe BPF is due mainly to the high content of IDF and TDF, with some contribution from the SDF content.

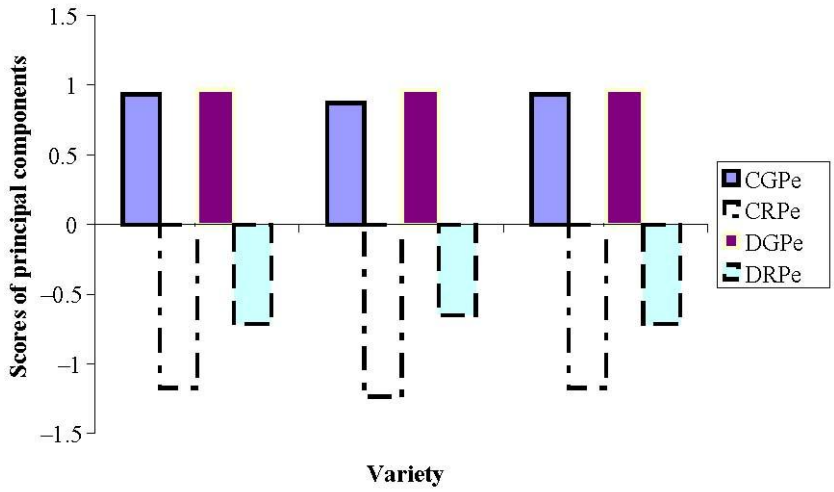

Figure 1: The scores of the principal components of PF that were prepared from bananas of different varieties and stages of ripeness.

Cluster Analysis (CA)

We used CA to identify the similarity groups between four different types of BPF (CGPe, CRPe, DGPe, and DRPe). CA resulted in a dendrogram as shown in Figure 2, grouping the 4 different types of BPF into 2 statistically significant clusters. These clusters were: cluster 1 [1–3 (CGPe) and 7–9 (DGPe)] and cluster 2 [4–6 (CRPe) and 10–12 (DRPe)]. This classification showed that green banana flour is entirely different from ripe banana flour in terms of the selected parameters. However, for both varieties, the green BPF are similar to each other. The same is true for the ripe BPF. The results are also suggestive of the potential of the green and ripe BPF as functional ingredients in food.

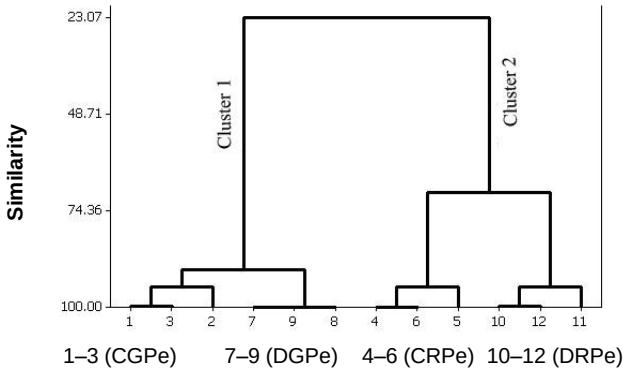

Figure 2: A dendrogram of the dietary fibre and starch components of the PF that were prepared from bananas of different varieties and stages of ripeness.

CONCLUSION

Based on the above results, we note that PCA identified 1 component that accounted for 93.74% of the total variance in the starch and dietary fibre components. This result confirmed the initial observation that the green and ripe BPF had different characteristics. CA showed two statistically significant clusters (green and ripe BPF), indicating that the different behaviour of the response depended on the stage of ripeness. In conclusion, the starch and dietary fibre components can be used to discriminate between PF prepared using fruits obtained at different stages of ripeness.

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