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RESEARCH ARTICLE

Detection of adulterants in white pepper powder (*Piper nigrum* L.) using thin layer chromatography fingerprint analysis

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Abstract

Background: In the production of instant pepper powder, some manufacturers add adulterants to the pepper powder. **Objective:** This study aimed to detect the presence of corn added in instant white pepper products in the market. **Method:** The TLC Fingerprint analysis was used. Fingerprints were made using white pepper from three regions in Indonesia: Tasikmalaya, Kalimantan, and Bangka. The corn used as the adulterant came from three regions in Indonesia: West Java, Central Java, and Kalimantan. Extraction was carried out using the maceration method, where 96% ethanol was used as the solvent. TLC patterns were made and visualised under UV light at 254 nm and 366 nm. Chromatogram analysis was performed. **Result:** Loading PC-2 against PC-1 in PCA with an eigen value of 931.54 indicated the loading score of white pepper was clustered in quadrants 2 and 3, while that of the corn adulterant in quadrants 1 and 4. …. **Discussion:**The sample projection on the PCA system used showed that samples A and C are distributed between quadrants 1-2 and 3-4, while sample B is distributed in quadrant 2. **Conclusion:** Based on the loading results of PC-2 against PC-1, samples A and C contained corn adulterants, while sample B contained other adulterants.

Introduction

Pepper (*Piper nigrum* Linn) is a plant that belongs to the Piperaceae family. This plant originated in India and grows in several other Southeast Asian countries (Madhavi *et al*., 2009). Pepper is traditionally used as an analgesic, antipyretic, central nervous system depressant, anti-inflammatory, antioxidant, anti-convulsant, antibacterial, and anti-tumour, and has hepato-protective activity (Pei, 1983). White pepper contains about 5–9% of the alkaloids piperine and piperettine, about 1.2– 5% of volatile oil which included caryophyllene (max. 62.23%), 3-carene (max. 26.84%), D-limonene (max. 25.83%), caryophyllene oxide (max. 8.17%), (−)-spathulenol (max. 5.32%), copaene (max. 5.04%), and humulene (max. 4.13%), and a variety of chemical constituents, such as piperolides, propenylphenols, amides, neolignans, lignans, flavonoids, terpenes, and steroids (Hammouti *et al*., 2019; Li *et al*., 2020; Madhavi *et al*., 2009). The primary constituents of the volatile oil found in white pepper are monoterpenes of various structures and sesquiterpenoids, with 3-carene, limonene, coparene, and caryophyllene being the most prominent (Huan & Long, 2007).

Adulteration is a major concern in the global pepper trade. It refers to the addition of non-permitted substances or dilution with other materials to increase the quantity of the product. Adulteration is falsifying a product or adding harmful substances, intentionally replacing, adding, altering, or falsely representing a material and/or food product (Kunle, 2012). The most common adulterants in commercially sold pepper include starch, sawdust, papaya seeds, and other spices (Kunle, 2012; Liang *et al*., 2004). In the production of instant pepper, manufacturers often add additional ingredients such as corn, cassava starch, and others to increase weight and thus gain more profit (Kunle, 2012).

Adulteration of pepper not only affects the quality and flavour of the spice but also poses serious health risks to consumers. For example, the addition of papaya seeds to pepper has been linked to several cases of food poisoning and gastrointestinal disorders (Kunle, 2012). Therefore, quality control is necessary to maintain and direct product quality to meet the desired quality standards

Fingerprint analysis is a method used for quality control in a production process; this method can provide specific and comprehensive sample information. Fingerprint analysis can be performed using several methods, namely chromatographic techniques such as Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), and spectroscopic techniques such as UV-Vis, FT-IR, NMR, and Mass Spectrometry. The TLC Fingerprint has been widely used by the pharmaceutical industry in America, Europe, and China due to several advantages such as simplicity, selectivity and sensitivity, speed, relatively low cost, the ability to test several samples simultaneously, the ability to visualise chromatograms, and the use of minimal solvents (Liang *et al*., 2004).

This study aimed to determine whether the thin layer chromatography fingerprinting method can be used for the detection of adulterants in pepper powder and to determine the presence of corn flour mixture in white pepper preparations available in the market.

Methods

Materials

The main materials used in this research are white pepper from three regions, namely Bangka, Kalimantan, and Tasikmalaya, as well as corn from three regions: West Java, Central Java, and Kalimantan, and three instant white pepper products that are circulating in the market with different brands. Other materials include silica gel 60 F254 TLC plates, 96% ethanol, Toluene, Ethyl acetate, and Formic acid.

Procedures

The stages of this research include the preparation of raw materials, plant determination, processing of white pepper and corn raw materials, preparation of extracts, making fingerprint patterns using thin layer chromatography video densitometry, analysis of chromatogram using *ImageJ* software (Moreno, 2007; Rasband, 1997; Schneider *et al*., 2012), interpretation of results with Chemometrics using Principal Component Analysis (PCA) and testing of adulterant detection in samples.

The preparation of materials includes the procurement of white pepper (*Piper nigrum* L) from pepper markets in Bangka, Kalimantan, and Tasikmalaya. The samples were taken from three different producers. Plant identification was carried out at the School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, West Java.

The extract was prepared using the maceration method with 96% ethanol as the solvent, then concentrated using a rotary evaporator. After obtaining a concentrated extract, TLC video densitometry analysis was performed and visualised to obtain the fingerprint analysis of white pepper and corn.

Instant white pepper samples were taken from three different producers, then macerated with 96% ethanol and concentrated.

After that, the analysis method was validated using TLC with a stationary phase of silica gel 60 F254 and a mobile phase of toluene: ethyl acetate: formic acid (5:4.5:0.1 v/v). Then, the analysis was carried out using TLC video densitometry with *ImageJ* software, and the obtained chromatograms were analysed chemometrically using the PCA (Principal Component Analysis) method.

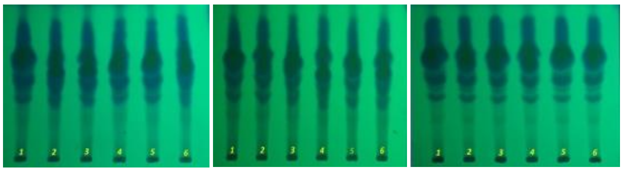
Results

White pepper and corn from three regions and three pepper powder samples were ground into powder and extracted using the maceration method, resulting in yields as shown in Table I. The chromatograms of pepper, corn, and samples can be seen in Figures 1-3.

The line and score plotting results of the principal component analysis for the extract of white pepper and corn can be seen in Figures 4 and 5. The score projection results of Samples A, B, and C to the PCA model can be seen in Figures 6-8.

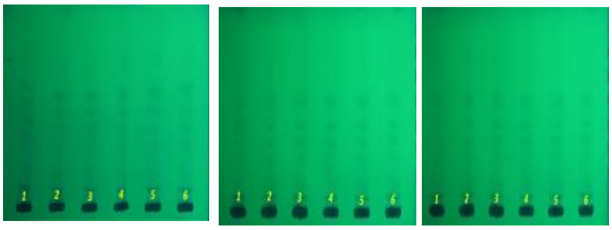
Table I: The yield of pepper, corn, and sample extract

Sample	Yield (%)
Bangka pepper	6.55
Tasikmalaya pepper	5.63
Kalimantan pepper	8.98
West Java corn	3.90
Central Java corn	4.16
Kalimantan corn	6.77
Sample A	5.57
Sample B	4.93
Sample C	5.17



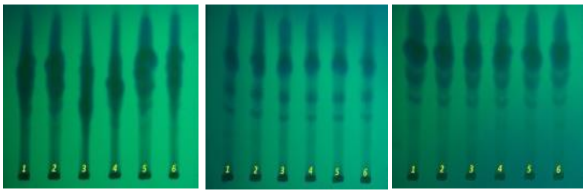
(a) (b) (c)

Figure 1: Thin-layer chromatography (TLC) pattern of (a) Bangka, (b) Kalimantan, (c) Tasikmalaya pepper extract under 254 nm UV light



(a) (b) (c)

Figure 2: Thin-layer chromatography (TLC) pattern of (a) West Java, (b) Central Java, (c) Kalimantan corn extract under 254 nm UV light



(a) (b) (c)

Figure 3: Thin-layer chromatography (TLC) pattern of (a) Sample A, (b) Sample B, (c) Sample C extract under 254 nm UV light

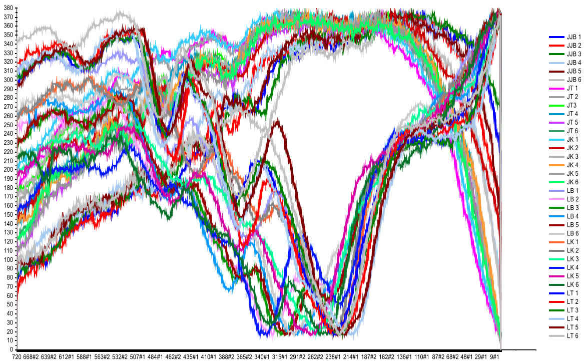


Figure 4: PC-1 *vs* PC-2 line plot of pepper and corn extracts from three regions

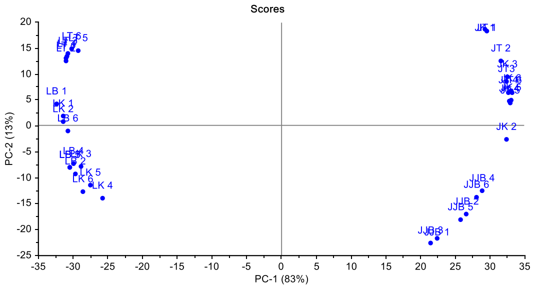


Figure 5: PC-1 vs. PC-2 scores plot of pepper and corn extracts from three regions

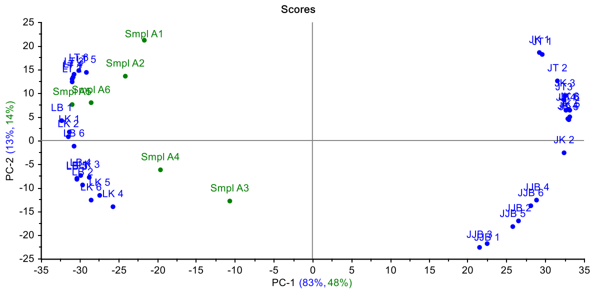


Figure 6: PC-1 vs. PC-2 scores projection of Sample A to the PCA model

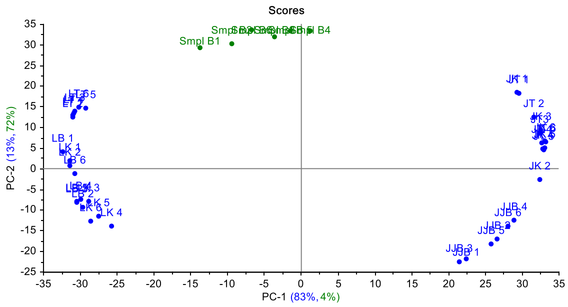


Figure 7: PC-1 vs. PC-2 scores projection of Sample B to the PCA model

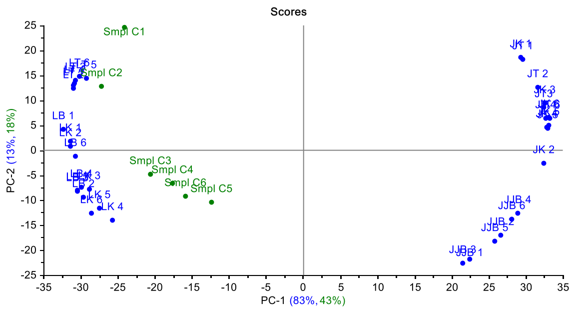


Figure 8: PC-1 vs. PC-2 scores projection of Sample C to the PCA model

Discussion

The maceration method was chosen because it is simpler and easier. The maceration process required 96% ethanol as a solvent to extract all polar, semi-polar, and non-polar chemical components. After extraction, the resulting macerate was evaporated to obtain a concentrated extract, and the percentage yield of the extract was calculated. The yield data in Table I showed that the extraction efficiency of Kalimantan white pepper was the best, with the highest yield of 8.98% w/w.

In thin layer chromatography of the extracts of pepper and corn using silica gel 60 F254 stationary phase, the mobile phase previously used for pepper by Nitin Rai (Rai *et al*., 2012), which was toluene: ethyl acetate: formic acid (5:4.5:0.5 v/v), did not provide good separation. Therefore, the composition of the mobile phase was changed to 5:4.5:0.1 v/v to obtain a good separation. This is shown in Figures 1 and 2, where the spots on the pepper chromatogram have a different pattern compared to the corn chromatogram. Different growing areas may result in different active compounds and chromatogram profiles of the same plant. Meanwhile, Figure 3 shows the chromatogram patterns of samples A, B, and C.

The chromatograms from the TLC plates were analysed using *ImageJ* software. *ImageJ* is one of the software that can be used for image analysis of spots or densities that is proven to be simple, easy, and versatile. It uses image format in the form of JPEG or TIFF. The results obtained from the chromatogram are the x and y values data (Rasband, 1997; Moreno, 2007; Schneider *et al*., 2012).

The chromatogram was further analysed using Principal Component Analysis (PCA) method. Principal component analysis (PCA) is a statistical technique that simplifies and analyses complex data sets. It is a dimensionality reduction method that identifies the most important variables in a dataset and transforms them into a new set of uncorrelated variables called principal components. These principal components are ordered by their contribution to the variation in the data, with the first component explaining the largest proportion of the variance. One of the main advantages of PCA is that it enables researchers to identify the most important variables in a dataset and reduce the dimensionality of the data without losing important information. This can be particularly useful when working with large and complex datasets (Abdi & Williams, 2010).

The line plot in Figure 4 showed a clear difference between pepper and corn chromatograms. Score plotting of PC 1 against PC 2 on the pepper and corn extracts chromatogram resulted in good data clustering. Figure 5 showed that the pepper extract from all three regions had similar characteristics and occupied the same quadrants, namely quadrants 2 and 3. The corn extracts from all three regions also had similar characteristics but differed from the characteristics of pepper, distributed in quadrants 1 and 4. This indicated that the PCA model could differentiate well between corn and pepper or vice versa. This model was then used to detect the presence of corn adulterants in instant pepper powder samples.

Figure 6 showed that the score of sample A was distributed between the groups of pepper and corn. The same was observed for sample C, as shown in Figure 8. This indicates that samples A and C were mixtures of pepper and corn.

Meanwhile, the score of sample B was not properly distributed in the pepper group region but also not in between the pepper and corn groups, as shown in Figure 7. This suggests that sample B was a mixture of pepper with adulterants other than corn.

Conclusion

The thin layer chromatography fingerprint analysis method can be used to detect corn adulterants in instant pepper preparations circulating in the market because it can distinguish the characteristics of pepper and corn adulterants in white instant pepper preparations. It can be concluded that samples A and C are suspected to contain corn adulterants, while sample B is suspected to contain adulterants other than corn.

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