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QUALITATIVE ANALYSIS METHOD OF DETECTION OF WAX CONTENT IN *GORENGAN* USING SMARTPHONE

METODE ANALISIS KUALITATIF DETEKSI KANDUNGAN LILIN PADA GORENGAN MENGGUNAKAN SMARTPHONE

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ABSTRACT

Wax is one of the compounds that can be misused to be added to Gorengan, Indonesian fritter, to keep them crispy. Gorengan containing wax is difficult to identify visually, so a quick and easy method of detecting wax content is required. The purpose of this research is to develop and evaluate the analytical performance of detecting wax content in gorengan using smartphone. Gorengan sample was dissolved with hexane and then added reagent that will give discoloration followed by analysis using smartphone. Some analysis performance parameters were evaluated in terms of linearity and detection limit, qualitative analysis capability, precision, and selectivity test. The developed method was also applied in some gorengan samples. The result shows that the detection of wax content in gorengan can be conducted by using reagent consisting of NaOH, Schift, and curcumin (1 : 2 : 2). Performance analysis shows that the linearity measurement at concentration between 10% and 25% has correlation coefficient (r) of 0.9537 with detection limit at concentration of 2% and precision (%RSD) less than 3%. The developed method can be applied for the detection of wax content in gorengan in the market.

Keywords: *analysis, chemometrics, gorengan, smartphone, wax*

ABSTRAK

Lilin merupakan salah satu bahan yang dapat disalahgunakan untuk ditambahkan pada gorengan agar tetap renyah. Gorengan yang mengandung lilin sulit untuk diidentifikasi secara visual, sehingga diperlukan metode deteksi kandungan lilin yang cepat dan mudah. Tujuan penelitian ini adalah untuk mengembangkan dan menguji kinerja metode analisis kandungan lilin dalam gorengan secara kolorimetri menggunakan smartphone. Penelitian ini merupakan penelitian eksperimental menggunakan gorengan sebagai objek penelitian. Gorengan dilarutkan dengan heksan dan kemudian ditambahkan reagen yang akan memberikan perubahan warna yang selanjutnya dapat dianalisa menggunakan aplikasi smartphone. Beberapa parameter kinerja analisis dievalusi meliputi uji linearitas dan batas deteksi, uji kemampuan analisis secara kualitatif, uji presisi, serta uji selektifitas. Metode yang dikembangkan juga diaplikasikan untuk mendeteksi kandungan lilin pada beberapa sampel gorengan yang dijual di masyarakat. Hasil penelitian menunjukkan bahwa deteksi kandungan lilin dalam gorengan dapat dilakukan dengan menggunakan reagen yang terdiri dari NaOH, Schift, dan kurkumin (1 : 2 : 2). Kinerja analisis metode yang dikembangkan menunjukkan bahwa linearitas pengukuran pada konsentrasi lilin 10% hingga 25% memiliki nilai koefisien korelasi (r) 0,9537 dengan nilai batas deteksi 2%

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dengan nilai presisi (%RSD) kurang dari 3%. Metode yang dikembangkan dapat diaplikasikan untuk deteksi kandungan lilin pada sampel gorengan di pasaran.

Kata kunci: *analisis, kemometrika, gorengan, smartphone, lilin*

INTRODUCTION

Gorengan, Indonesian fritter, is one of the favorite snacks among the Indonesia society. *Gorengan* can be bought easily because it is widely sold in the roadside (Chalid et al., 2008). The people like to eat *gorengan* because of its savory and crispy taste as well as cheap price. The increasing fondness of eating *gorengan* causes the high competition among *gorengan* vendors. This situation sometimes causes the vendors to sell only some portions of their *gorengan* product in one day. This problem certainly triggers the loss among the *gorengan* vendors and some of them try to find a way to keep their *gorengan* crispy and delicious eventhough it is stored for days. One of the ways is misusing of candle as food additives. Candle contains paraffin wax and others additives (Rezaei et al., 2002). The *gorengan* vendors deliberately add wax in a heated cooking oil when frying *gorengan.* The mixture of wax in heated cooking oil affects the crispy taste of *gorengan.* Wax is not a food additive and it is difficult to be broken down by enzymes in human body. Therefore, when wax is consumed, it can harm human's digestive function and cause health problems (Griffis et al., 2010; Derudi et al., 2014; Sølling et al., 2018).

The content of wax in *gorengan* is difficult to identify visually. The analysis method of wax identification that is commonly used is gas chromatography associated with Mass Spectrometry, Near-infrared spectrometer (Palou et al., 2014), Differential Scanning Calorimetry (Chen et al., 2004; Kök et al., 2007), High-performance Liquid Chromatography (Moreau et al., 2002), and Thin-layer Chromatography (Lu et al., 2008). These methods require expensive and complex equipment, detailed preparation and particular skill on its implementation. Therefore, it is necessary to develop a simpler, cheaper, easier and more practical identification technique. A simple analysis technique commonly used in identifying a candle containing paraffin wax as the main compound is by using spot test analysis (Jungreis, 2004). Spot test analysis can be done by adding a particular reagent on the sample or by immobilizing the reagent in a membrane on the test strip and then the result is evaluated by identifying the discoloration (Ngom et al., 2010; Sutrisno et al., 2017).

The technology development has transformed smartphone not only to be a communication tool but also to be one of the identification tools of a compounds based on the color analysis of a figure or a sample. Smartphone is used in several studies as an analytical tool, such as detection of alcohol concentrations in saliva (Jung et al., 2015), detection of thiosulfate compounds using silver nanoparticles (Dong et al., 2017), formaldehyde measurement compounds in air (Yang et al., 2016), and others examinations. The potential use of smartphone for wax content detector in *gorengan* is important to be used by combining qualitative analysis of wax content based on the discoloration and the smartphone as the detector. This research aims to develop and examine methods of detection of wax content in *gorengan* by using smartphone. In addition, this research also aims to apply the developed method for qualitative analysis of wax content in *gorengan* sold in the market.

METHODS

Materials

The materials used in this research were candle, cooking oil, *gorengan*, aquadest, curcumin. The solvent and chemical compounds with pro-analysis quality were n-hexane, ethanol, NaOH, $NaNO_2$, $NaHCO_3$, Schiff's reagent (Merck Darmstadt, Germany).

Instrumentation

The equipment used in this research were plat TLC Silica Gel GF_{254} (Merck, Germany), TLC Chamber, capillary tube, vortex, micropipette 100µL-1000µL (Eppendorf, Germany), and 8 MP Smartphone camera (Oppo, China) with android operation of 5.1 ROI 32 x 32 pixel with *PhotoMetrix*® Figure analysis application version 1.2.1 (Ghelfer.net Inc., Brazil).

Selection and Optimization of Reagents

The reagent in this research was curcumin 0.5%, NaOH 10%, Schift, $NaNO_2$ 0.1% and $NaHCO_3$ 5% which were examined either single or multiple reagent combination. The test was conducted by dissolving wax in 2 mL of cooking oil that had been heated and then adding n-hexane solvent. As the negative control, the mixture of heated cooking oil and n-hexane solvent was used. On each oil solution, 500 µL of reagent was added and mixed to be homogeneous, then, the discoloration on each tube was observed. The selected reagent was a reagent giving clear discoloration when it was reacted. The selected reagent was then optimized in the form of volume of reagent and wax concentration in cooking oil in which the discoloration was observed as the next step. The test was conducted by dissolving wax in heated cooking oil and then adding n-hexane solvent and reagent. The most optimum reagent was the reagent giving proportional discoloration with the wax concentration added in cooking oil. It could be seen from the correlation coefficient (r) approaching ± 1 and providing clear and stable discoloration when it was analyzed in univariate analysis by using Photometrix® application on smartphone.

Performance Test of Wax Analysis Method with Smartphone

Several performance tests of analysis method that were conducted are linearity test and detection limit, ability test in qualitative analysis, precision test, and selectivity test. Linearity test and detection limit were conducted by making seven concentrations of wax solution in cooking oil with the concentration of 1% to 25 % with each three times replication. Two mL of wax solution from the cooking oil was poured into test tube containing 3 mL of n-hexane and 500 µL of reagent, and then, it was homogenized by using vortex. The colors formed were observed, captured by using Photometrix® application on smartphone, and analyzed through univariate analysis. The detection limit was calculated using linearity data based on the value of three times the residual deviation standard.

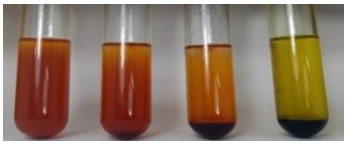
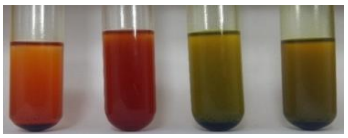
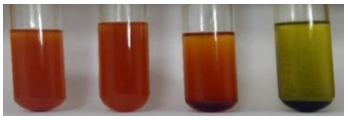
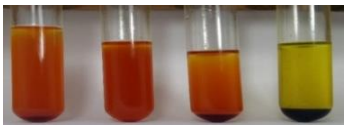
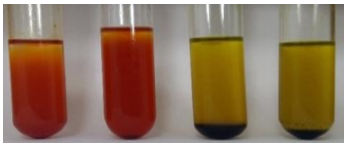
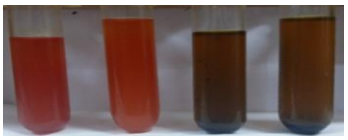
The test of method capability in qualitative analysis was conducted by examining the ability of analysis method in grouping cooking oil solution containing wax with different concentrations. In addition, the discoloration observation and capturing the figure by using smartphone was also conducted. Afterwards, it was analyzed through univariate analysis by using Photometrix® application. The precision test was conducted on self-made *gorengan* samples which is added with wax concentration of 0%, 5% and 10%. Each sample was replicated eight times. The sample was grinded and filtrated. Two mL of the sample was taken and it was mixed with 3 mL of n-hexane and 500 µL of reagent. Its discoloration was observed and the pixel value detected by Photometrix® application was evaluated. The calculation of %RSD was also conducted. Selectivity test was done by comparing cooking oil with 5% of wax concentration then fried dough was added. After it was prepared and reacted with reagent, the discoloration was observed and analyzed through univariate analysis using Photometrix® application. Afterwards, the calculation of measured wax content was done. The result was compared to the analysis of one-way ANOVA.

Application Method on *Gorengan* Sample

The developed method was applied to identify wax content on several samples of *gorengan* sold by vendors which were randomly picked in the market. The types of *gorengan* analyzed in this research were fried tofu, fried *tempe*, fried banana, fried sweet

potato and *bakwan* (vegetables fritter snack). The samples of *gorengan* were prepared with n-hexane solvent and analyzed by adding 500µL of reagent and subsequently observed. The Figure was camptured by smartphone. A multivariate analysis using Photometrix® application was conducted to each sample of *gorengan* in order to identify the sample classification into four groups; *gorengan* not containing wax, *gorengan* with low wax content, *gorengan* with medium wax content, *gorengan* with high wax content. The identification result of wax content in *gorengan* was then compared to TLC method using eluent of benzene : methanol (2.4 : 0.1).

Table I. The Result of Reagent Optimization for Wax Content Analysis in Cooking Oil

Ratio of Reagent Volume Variation				The color formed on the wax concentration in cooking oil			
Curcumin 0,5% (µL)	Schift (µL)	NaOH 10% (µL)	r	0%	1%	10%	20%
1	1	1	-0.784				
2	2	1	0.981				
1	2	2	0.637				
2	1	2	- 0.792				
3	1	1	0.863				
1	3	1	0.636				

RESULTS AND DISCUSSION

The Result of Selection and Optimization of Reagent

The examination result on several reagent which were examined either single or multiple reagent combination of curcumin 0.5%, NaOH 10%, Schiff, $NaNO_2$ 0.1% and $NaHCO_3$ 5% shows that optimal reagent can produce clear discoloration. Curcumin in the alkaline condition has a red colour (Bernabé-Pineda et al., 2004). After Schiff reagent was added, it strengthens the colour that is formed into a reddish blue. The presence of wax content will cause saponification reaction with NaOH. Therefore, it does not react to curcumin and will result curcumin reagent solution with yellow base. It is mixed with the reddish blue of Schiff reagent then resulting more concentrated green. Optimization of reagent is conducted in order to obtain reagent that can provide clearer and more stable discoloration. Optimization of reagent can be conducted by varying concentrations of curcumin, NaOH, and Schiff. The result of optimization of reagent is presented in Table I.

Based on the observation result of discoloration, there are selected ratios of reagent; curcumin 0.5%, Schiff 200 µL and NaOH 10% (2:2:1). The ratio selection causes clearer and more stable discoloration after adding cooking oil containing wax and dissolved in an n-hexane solvent. The optimization of reagent is also based on the correlation coefficient (*r*) evaluated with univariate analysis. G channel on Photometrix® application is selected as measurement channel because it has r value approaching ± 1. This shows relationship between wax concentrations in cooking oil with color intensity that is produced (Helfer *et al.*, 2017).

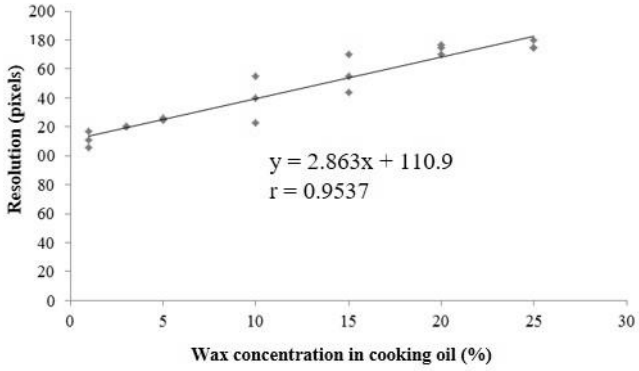


Figure 1. Linearity measurement of wax analysis in cooking oil using smartphone method

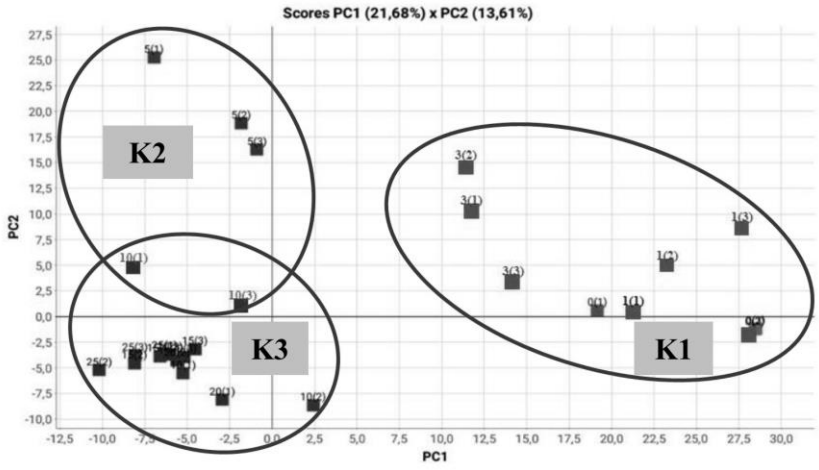


Figure 2. The analysis result of multivariative score plot of wax concentration using Photometrix® application

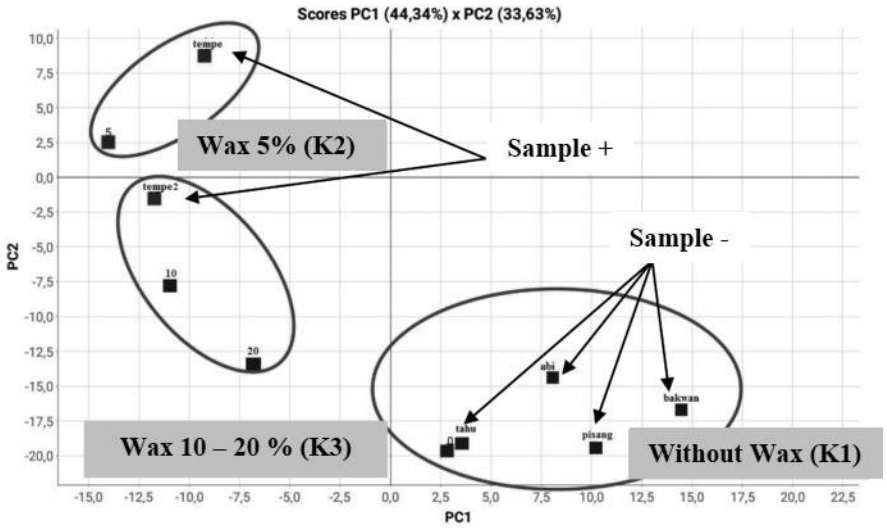


Figure 3. The result of score plot of wax content detection analysis of *gorengan* sample in the market using multivariate analysis and Photometrix® application on smartphone

The Result of Wax Analysis Method by Using Smartphone.

The result of linearity measurement of wax analysis in cooking oil was conducted to seven different wax solutions with 1% until 25% concentration detected by smartphone presented in Figure 1. Linear regression equation obtained is y = 2.863x + 110.9 with correlation coefficient of r = 0.9537. The higher wax concentration in cooking oil will provide response analysis of color resolution recorded by smartphone presented in a proportional pixel unit. The calculation of detection limit by residual standard deviation method shows that wax concentration in cooking oil can still be detected by the developed method; 2.14%.

Qualitative analysis of the classification of wax sample in cooking oil with different concentration was conducted by multivariate analysis. This analysis helps to find out whether the wax which has similar concentration will be classified based on its classification. The classification test was conducted by using Principal Component Analysis (PCA). The variable category is the difference of wax concentration and evaluation on score plot presented in Figure 2. The result of score plot shows that the first and the second field has a total variability of 35.29%. PC1 and PC2 provide visualization of the separation among wax sample cassifications in cooking oil with different concentration qualitatively. PC2 with 13.61% from the total variance is able to identify a sample into three groups; K1, K2 and K3. The first group, K1, consists of wax sample with concentration of 0%, 1% and 3%. The concentration in wax sample of K2 are 5% and 10%. While on K3, the concentration of wax sample are 10%, 20% and 25%. Due to the difference of quadrant position among wax with similar concentration, then PC selected horizontally able to separate among the groups. From the several PCs, PC1 with 21.68% of the total variance shows good result in separating among groups. K1 is on the positive side, K2 and K3 are on the negative side. However, K1 with 3% of wax concentration shows a tendency to approach the negative side. It is because the detection limit of this method is 2.14%. it can be concluded that PC1 and PC2 are proved to be able to classify wax standard based on the concentration. K1 is a group of wax standard with low concentration. K2 is a group of wax standard with medium concentration. While K3 is a group of wax standard with high concentration.

Table II. The Result of Precision Test of Analysis Method on Wax Concentration of 5% and 10%

Sample	Concentration of 0% (pixel)	Concentration of 5% (pixel)	Concentration of 10% (pixel)
1	97.50	125.00	135.00
2	102.50	128.00	138.10
3	102.00	128.00	133.50
4	95.00	125.00	141.10
5	97.50	125.10	137.00
6	97.00	127.00	141.00
7	96.50	126.00	141.20
8	100.00	124.50	133.50
Average	98.50	126.07	137.55
SD	2.70	1.41	3.33
%RSD	2.74	1.10	2.40

Table III. The Measurement Result on the Level of Wax Content in Cooking Oil Before and After Adding the Flour

Wax Concentration (%)	Measured level of wax content (%)	
	Before Adding the Flour	After Adding the Flour
5	5.27	4.58
5	4.92	4.75
5	4.92	4.84

The precision test was conducted in order to find out whether the developed method will still get the similar result if it is repeated. The determination of this test was done on self-made *gorengan* which is fried in hot cooking oil containing wax concentration of 0%, 5% and 10%, and then, the discoloration was observed by using univariate analysis on Photometrix® application. The measured pixel value on *gorengan* with no wax content shows a range of values from 95 to 102.5 pixels. This means that wax concentration measured on K1 quadrant shows 0% of wax concentration or less than the detection limit. The measured pixel value on *gorengan* with 5% and 10% of wax concentration are on a range of 124-128 pixels and 133-141 pixels which shows quadrant of K2 and K3. The %RSD obtained in 0%, 5% and 10% of wax concentration is 1.1% ; 2.4% showing that the analytical method has good degree of precision (Table II).

Selectivity is determined by comparing between cooking oil containing 5% of wax and fried dough. The flour selected as the matrix is potential to interfere the analysis result because flour is generally used in making *gorengan*. The measurement result is presented in Table III. The result of statistial data processing shows that the data is normally distributed with a significance value of 0.143. The result of one-way ANOVA analysis with 5% of α presents that the flour does not significantly show different result with p-value of 0.99. It can be concluded that adding the flour as matrix cannot significantly interfere the analysis. The developed method is selective enough to identify wax content in *gorengan* dough.

Application Method on *Gorengan* Sample

Implementation of multivariate analysis on Photometrix® application on smartphone is used to detect wax content on five different *gorengan* namely fried tofu, fried *tempe*, fried banana, fried sweet potato and *bakwan* (vegetables fritter snack). The gorengan samples were randomly picked in the market. This examination aims to identify the classifications among test samples. If the test sample containing wax, it will be classified on the similar quadrant. From the five samples that had been analyzed, the sample of fried *tempe* shows 5% and 10% of wax

concentration which is indicated on quadrants of K2 and K3 (Figure 3). The test result is in accordance with the research conducted by Helfer and other researchers who provide the ability of Photometrix® application in grouping banknotes based on their color from different countries based on the paper used (Helfer *et al.* 2017). As a comparative method, TLC test was conducted and it showed that there were spots on the sample of fried *tempe* with Rf value of 0.52 and 0.50 which is equivalent to Rf value of the sample of cooking oil which is deliberately added wax. This Rf value is almost similar with the Rf value of wax sample analyzed by the similar TLC system. Therefore, it can be ascertained that the spots appearing in the sample are wax spots. While the samples other than the sample of fried *tempe* do not show spots which indicate that the sample tested does not contain wax. The result shows that smartphone can be used to detect wax content on *gorengan*. Smartphone analysis method using Photometrix® application needs further development on the reagent and color image data processing in order to be used to detect wax content on lower concentration.

CONCLUSION

The wax content on *gorengan* can be detected by using Photometrix® application on smartphone. Parameter performance of the developed method shows good result with the correlation coefficient value (*r*) of 0.9537 and it is accurate with % RSD value less than 3%. This can be implemented to detect the wax content on *gorengan.*

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