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Fluorescence polarization method for detection of lard mixed with olive oil

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Abstract. This study is based on fluorescence polarization as a potential alternative method for an investigation of contamination of lard in food. The purpose of this study was to obtain the characteristic of fluorescence polarization in olive oil samples mixed with lard. The characteristics of fluorescence polarization were obtained by measuring changes in the polarization of light using a linearly polarized green laser pointer and observed in the direction of the scattering angle of 90° for various direction of electric field of the laser. The results showed that the critical angle of direction of electric field of the laser and the average polarization change increase as the concentration of lard is raised. It could be due by additional asymmetric triglycerides and molecular orientation that play an important role in the sample. This result shows that additional mixture of lard in olive oil can be detected, and this method seems to be developed for the detection of low concentrations of lard contamination.

1. Introduction

One of the problems relating to food in Indonesia is the violation of the use of non-halal food ingredients, for example the use of lard in food. This problem is very urgent and needs to be addressed, therefore research to detect quickly, simply, and accurately lard contamination in food needs to be conducted. Various spectroscopic methods and equipment have actually been widely used to identify lard with various claims of advantages and disadvantages [1-4]. But in our view, the method and its equipment combination are too complicated. In this study, we studied the identification of lard contamination mixed with other cooking oils using fluorescence polarization.

The use of olive oil in this study is due to the nature and benefits of olive oil which is very good in the health field [5]. The use of fluorescence polarization in this case is the ability to map vegetable and animal oils which is quite significant, by measuring only the difference of polarization change for various direction of linear polarized incoming light [6-8]. Some readers often misunderstand fluorescence polarization. We use fluorescence polarization to obtain differences in polarization character of fluorescent light from samples, instead of using fluorescence polarization spectroscopy. Contrary to spectroscopy methods of fluorescence or fluorescence polarization in evaluation olive oil composition [9-10], the polarization change in fluorescence polarization in this paper is at least much simpler than any spectroscopy methods. The method we use is very similar to the transmission



polarization method [11-13], and is likely to complement each other in future studies. Observations of fluorescence polarization here are identical to observations of Rayleigh scattering [14] but with scattering wavelengths that are greater than the incident light. In line with our future research to obtain the accuracy and effectiveness of detection of lard contamination in food, in this stage, we first study the characteristics of a mixture of lard in olive oil by measuring changes in polarization.

2. Methods

The sample used is a mixture of lard and olive oil in the ratio of volume variations presented in table 1. Data collection procedures referred to the research by Firdausi et al [14]. Changes in the polarization angle are measured using a pair of linear polarizer for various directions of polarization of the incident light. The source of light was green pointer laser with 532 nm. The design of experiment shown in figure 1 was calibrated by measured polarization change of water (aquabidest) using Rayleigh scattering. During the measurement process, the sample temperature was conditioned at 28°C - 29°C.

Table 1. Samples of mixture of olive oil and lard.

No.	Sample Code	Olive Oil Concentration	Lard Concentration
1	A	100%	0%
2	B	90%	10%
3	C	80%	20%
4	D	60%	40%

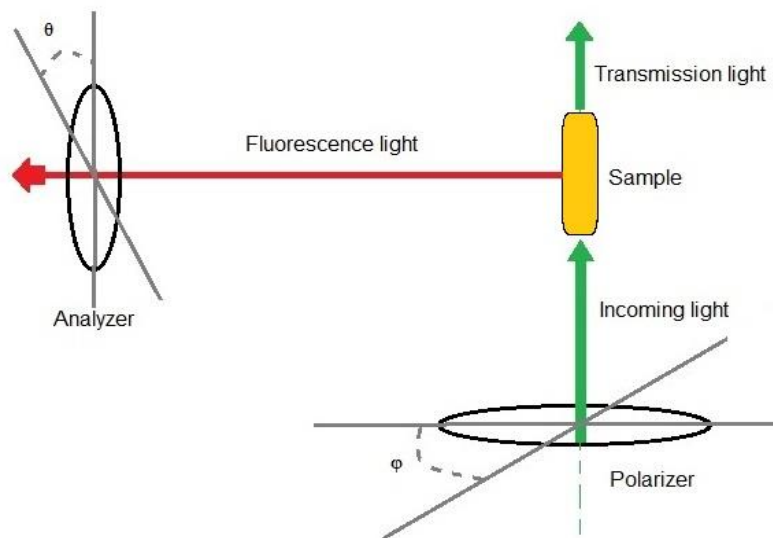


Figure 1. Design of measurement of fluorescence polarization. The characteristics of polarization change of fluorescence light θ was measured as a function of polarizer angle ϕ .

3. Results and Discussions

Figure 2 shows the profile of polarization change of scattering Rayleigh from water. The symmetric change in the interval of polarizer angle from $\phi=0^\circ$ to 180° indicates that the water liquid is excellent for calibration of the system apparatus. Figure 3 shows the characteristics polarization change against

polarizer angle for various lard concentration. The profile of oil sample shows also symmetric curves according to preceding studies [6-8], therefore in the experiment, we chose only the polarizer angle range from $\varphi=0^\circ$ to 90° .

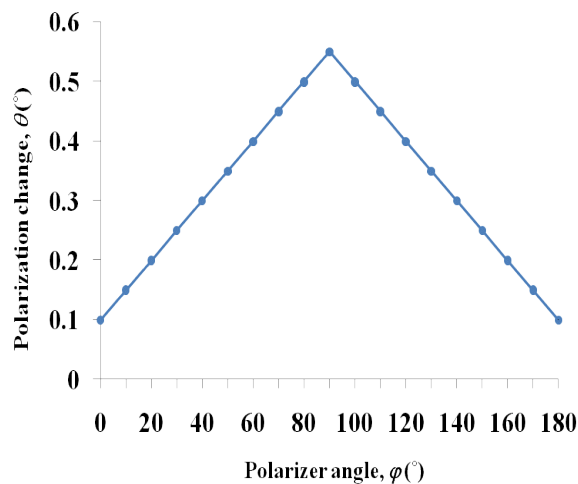


Figure 2. Change of polarization angle in water due to Rayleigh scattering.

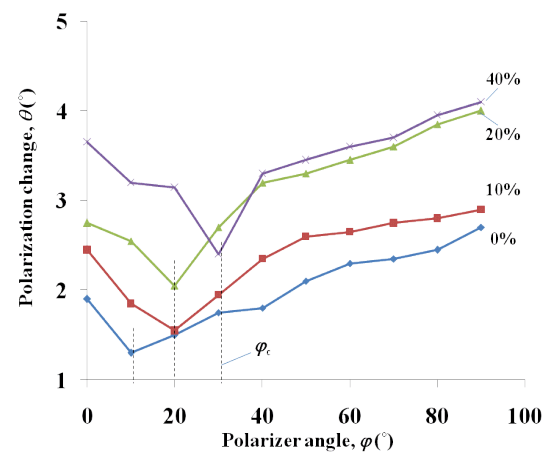


Figure 3. Change of fluorescence polarization angle in samples of mixture of olive oil and lard.

Unlike in the case of Rayleigh scattering by water molecules, the pattern of changes in polarization in oil samples on fluorescence polarization looks more complex as shown in Figure 3. Two very important things from the results shown in figure 3 are the increase of the change in polarization, and the shifting of the critical angle φ_c from 10° to 30° as the concentration increases. The increase in polarization changes with an increase in concentration indicates the number of asymmetry molecules from lard is relatively very high. Then, the shifting of the critical angle φ_c from 10° to 30° shows that the change in polarization depends on the orientation of the asymmetric molecules. Preliminary research results show that pure olives have a critical angle of 10° whereas pure lard of 40° [8]. Until now this phenomenon in detail is still a hypothesis that the difference in the number of asymmetric triglycerides with two twin monounsaturated fatty acids in olive oil is very dominant and is very homogeneous. This explains as if the orientation of asymmetric triglycerides in olive is in line with axis of analyzer at average angle of 80° and in lard at average angle of 50° . The high polarization difference and the shifting the critical angle towards 40° of lard provide that the method can be further developed as a finger print system of various types of cooking oils and as a detection of lard contamination. It is an interesting task for further investigation of lard contamination using other various cooking oils and measuring the shifting of critical angle for small concentration e.g. 0-10%. Referring to the results of Firdausi et al [8], this situation now can be also described by using a mapping model of lard mixture in olive, as shown in figure 4 and figure 5.

Figure 4 and figure 5 depict respectively average polarization change (at $\varphi = 0^\circ$, $\varphi = 90^\circ$, and $\varphi = \varphi_c$) against lard concentration and against critical angle φ_c . In figure 4, it seems that the increase of polarization angle approximately linearly dependent on lard concentration. This linear correlation is analog with the condition of an optically active substance, which has usually linear relation between polarization and its concentration. However, more precise investigation should be done for very low concentration between 0 and 10%.

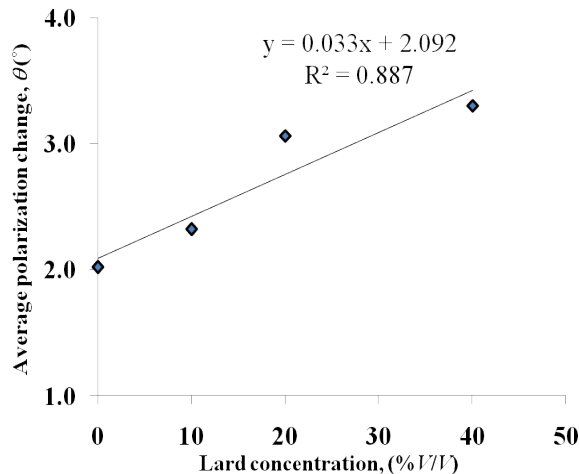


Figure 4. the average polarization change (at $\varphi = 0^{\circ}$, $\varphi = 90^{\circ}$, and $\varphi = \varphi_c$, vs. lard concentration.

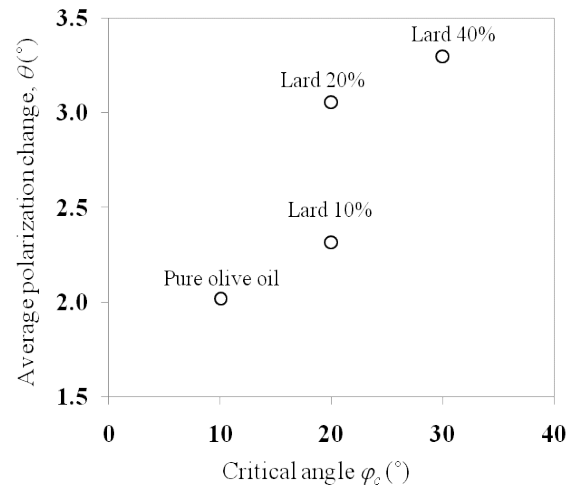


Figure 5. Mapping sample using axis average polarization change vs. critical angle φ_c .

Figures 5 show a mapping model of lard contamination in olive oil using changes in average polarization angle vs. critical angle. Although the difference polarization changes between pure olive and mixture olive with 10% of lard is relatively small, this condition can be clearly distinguished due to significant difference of the critical angle. This result can be further used to any type of oils, and therefore we define this graph as a mapping model for detection of lard contamination.

The fluorescence polarization method can be combined with the transmission polarization method to obtain a comprehensive mapping of various types of oil. The combination of these methods is very simple, however it seems very difficult to validate experimentally with existing standard methods. Because the method relies not only on optically active properties but also on the orientation of molecules when interacting with linearly polarized light. Previously we tried to validate using GC MS, which results only in the correlation between the composition of the amount of fatty acids and changes in polarization [7]. Other standard oil parameters such as peroxide numbers, free fatty acid numbers, iodine numbers and others are apparently not to be significant in relating to changes in polarization. We propose that the most suitable variable as a validation test is the triglyceride composition of oil. This is related to the increase in polarization changes related to the amount of asymmetry triglycerides. While the critical angle shift is related to the orientation of triglyceride molecules, especially triglycerides with two twin fatty acids. In the meantime, with the limitations of the experimental conditions, we have not been able to validate using the triglyceride test.

4. Conclusion

From the results of the study it was found that a significant difference in polarization changes between pure olives and olives mixed with pork oil. The increase in the average polarization which is relatively linear to the concentration of lard shows that the asymmetrical triglyceride content is quite large in lard. Another important result is a critical angle shift. This shows the difference in the orientation of triglyceride molecules, so this provides a significant advantage because it is able to distinguish lard contamination in other oils. Although validation experimentally with the standard method has not been done carefully, this fluorescence polarization method seems to provide a bright future, especially related to the investigation of types of oil. The dependence of polarization on asymmetric triglyceride molecules

and their orientation to interactions with linearly polarized light provide another idea for application in the study of molecular fields.

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